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SOME RECENT APPLICATIONS OF FIELD IONIZATION/FIELD DESORPTION MASS SPECTROMETRY TO ORGANIC CHEMISTRY

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INTRODUCTION

Field desorption mass spectrometry (FDMS) is just short of 13 years old[1] and a precocious teenager indeed. Considering the wide acceptance of the older sibling, chemical ionization mass spectrometry (CIMS), and the apparent vitality at maturity of electron impact mass spectrometry (EIMS), it seems safe to forecast a bright future for this relative newcomer. This prediction is made in the face of constant challenge from younger offspring of the singularly productive marriage of physics and chemistry called mass spectrometry. An appreciation of the place of mass spectrometry in molecular analysis which appeared recently[2] is recommended for any reader who has not been a participant in recent developments in this area. A more extensive review of recent work in mass spectrometry in organic and biological chemistry has recently been published.[3]

The aim of this review is to bring to the attention of organic chemists some of the newer applications of FDMS judged to be of relevance. Since the subject has been reviewed from various perspectives, chiefly by the group at Bonn, an effort will be made not to duplicate their work. Among the latter are the book by Prof. Beckey,[4] who originated the technique, and a review by his colleague Dr. Schulten.[5] Although organic chemistry will be defined broadly in this review, readers with special interests in analytical, inorganic, physical chemistry, or biochemistry will find much additional material of interest in the earlier works and in other periodic reviews of mass spectrometry.[6,7]

2. TECHNIQUES OF FDMS

Commercial instruments for FDMS are distinguished by a number of modifications to the source. An anode with very small radius of curvature, comparable to the razor blade edge widely used in early experiments, is introduced into the ion source on an insulated push rod through a vacuum lock. The most common anode now in use is a 10 μm tungsten wire carrying many sharp points in the form of a dense growth of dendritic needles about 30 μm in length.[8] A slotted cathode separated from this anode by 1–2 mm is mounted so that a potential of 10 kV may be established between these elements. Experiments in which the sample is vaporized and flows past the anode are called field ionization mass spectrometry (FIMS). Those in which the anode is withdrawn, coated with the sample, and replaced in the source prior to turning on the voltage, are called FDMS. Since vapor may condense on the anode in the former, and liquid or solid may vaporize in the latter, there is no sharp distinction between these techniques. In fact, it is largely the physical characteristics of the sample, not the method of its introduction, which determine the dominant process.

Volatile samples of low polarity which are amenable to introduction by batch inlet for EIMS study no doubt approach the limit of "pure" FI, especially if the anode is heated. These molecules of gas give up an electron upon close approach to the anode (field gradients of 10^7–10^8 V/cm are sufficient to overcome the ionization potential of most organics). By this electron loss a molecular ion of low internal energy is generated and very quickly (*ca.* 10^{-11} s) accelerated out of the ionization region. Both the low energy and short time contribute to the high probability of molecular ions reaching the detector. Indeed, many common organic molecules yield $M^{+\cdot}$ as the only ion in the normal FI spectrum. Decompositions occurring between 10^{-12} and 10^{-9} s may be identified by the defocussing techniques known as field ionization kinetics. Leading references to this unique (in mass spectrometry) method for obtaining a time-resolved view of fragmentation at extremely short times may be found in the reviews quoted[4–7] and in more specialized works.[9,10]

For samples of intermediate volatility the spectrum usually arises in part from molecules in the condensed phase whether introduced in the gas phase or not. Since such molecules tend to desorb rapidly from the anode, and there is no means of replacing them conveniently by FD technique, they are studied by FI (gas phase introduction), if at all.

Compounds which are too involatile for successful vaporization from a direct probe, a criterion also related to thermal stability, are generally candidates for FD. The standard procedure for sample loading consists of dipping the anode in a solution of the sample (1–10 mg/ml of $CHCl_3$, CH_3OH, H_2O, CH_3SOCH_3 is typical). Clearly, most of the sample remains after dipping, but if insufficient material is available for 200 μl of solution, direct syringe loading may be substituted. In that procedure quantities of 1 μg or less are added in solution with a microsyringe.

Some experience is required before reasonable predictions can be made about desorption rate under FD conditions. Polarity no doubt plays a role as well as vapor pressure under normal conditions. Thus DMSO is a common solvent for FD and it evaporates sufficiently slowly to produce an m/z 78 peak for several minutes following removal of bulk solvent by the mechanical pump. On the other hand, phenylalanine zwitterion desorbs smoothly without anode heating (t = 50–60°) over several minutes as well. Although these examples desorb as odd-electron molecular ions and therefore are presumed to ionize in a manner similar to FI, this is by no means the rule in FD (condensed phase) experiments. Before going to a discussion of ionization/desorption processes in FD, a brief mention of anode heating is needed. The 10 μm tungsten anode is mounted in the source so that a current may be passed through it. The usual range, 0–50 mA, covers temperatures from ambient (50–60° in the source) to red heat. Calibration has established that in the usual working range (10–25 mA) Celsius temperature is approximated by adding one digit to the mA current. Below 10 mA (*ca.* 100°) and above 25 mA (*ca.* 250°) deviations are significant.[11]

3. IONS IN THE FD SPECTRUM

Generation of ions from condensed phase is at once the *raison d'etre* and the main weakness of FDMS. Some of the most common ion formation processes are presented in Fig. 1 for a hypothetical sample with both covalent (M) and ionic (C^+A^-) components.

In the applications which follow, it will be noted that the abundance of the odd-electron $M^{+\cdot}$ species parallels in a general way the ease of ionization of the molecule. Thus benzene, condensed aromatics, amines, divalent sulfur compounds, and other easily ionized structures often yield this ion. On the other extreme, sugars, aliphatic amino acids, and other molecules lacking low ionization potential groups yield mainly even-electron ions such as $(M+H)^+$. As will be seen later, the abundance of these ions may be enhanced by addition of suitable donors to the sample, but virtually all samples and/or ion sources contain enough H^+ donors for ready protonation. Further, the ubiquitous presence of Na^+, even in carefully purified samples and solvents, came as an unpleasant shock to the early workers in this field. An early biochemical sample in our laboratory which was said to contain "small amounts of residual sodium ion from buffers" was found to be over 50% NaCl by weight! Here and elsewhere, the emphasis is on Na^+ because it is the most common contaminant. However, K^+ is not uncommon, and Li^+ has been observed. All of these alkali metal ions and higher members of the series behave similarly.

A sample containing a preformed cation, C^+, will often give that ion as base peak at a fairly low anode temperature. Clearly, no ionization is required here, and ease of desorption will be determined by a complex set of factors such as lattice energies, more general (matrix) binding, rate of evaporation of neutrals, fluidity and interaction with the anode surface.

Appearance of sample anions, A^-, as A^+ is most commonly seen with I^-. Clusters of salts, starting with $2C^+A^-$ and often continuing to the limit of the mass scale, are very common. Less general, but to be expected whenever one component of the salt is readily ionized, is the odd-electron $(C\cdot A)^{+\cdot}$. No charges are shown inside the parentheses for this general formulation; allocating the charge is far from a trivial question. The remaining ions, $(M+C)^+$, and clusters of molecular ion with fragments will be encountered in various examples to be cited. Also to be presented are some cases where ions found in FD spectra must be regarded as artifacts. One simple example[12] would be the presence of *m/z* 59 and 142 in the FD spectrum of $(CH_3)_4N^+I^-$, ions that arise from nucleophilic attack by I^- on carbon to form CH_3I and expel $(CH_3)_3N$. Ionization of these neutral reaction products then produces the ions in question.

A central fact which limits the usefulness of FDMS is the relatively low yield of focussed ions. In the early development of the technique, this was overcome in part by integrating all ions collected over 10–30 min by means of a photoplate. Although this technique has some advantage over electrical recording for samples which desorb in erratic bursts, for most samples electrical recording of relatively slow scans (10 s/decade) is now preferred since it allows one to average several scans for improved ion statistics and still retain the individual scans to follow changes in the spectrum as desorption proceeds. Since anode temperature is the most sensitive adjustable parameter, correlation of a certain ion with the temperature at which it desorbs may be extremely important in judging its origin and structural significance. Most laboratories now use some form of anode temperature controller which has improved reproducibility of scanning. With this device a series of scans taken during rapid heating of the anode (0–25 mA in 5 min or less) yields the heating current at which $M^{+\cdot}$ or $(M+H)^+$ is most abundant. This is called the best anode temperature (BAT). A second sample loading then allows rapid heating until this value is approached, followed by slower increments during desorption of the key ions. The most sophisticated versions of this device incorporate a feedback circuit which establishes the heating rate as a function of total ionization.

In the previous discussion the implicit standard for ion yield was of course EIMS. For very rough comparison between these techniques, one may take an EIMS detection limit of pg in favorable cases. FDMS usually requires ng for complete scans, and for convenience, about 1 μg is placed on the anode. This crude comparison overlooks the fundamental point that FD is properly used for samples which give unsatisfactory EI spectra. Thus, even 100 ions in a molecular ion peak and a few minor peaks with poor reproducibility is of inestimable value when the sample fails to yield structural information by EIMS.

$$\left.\begin{matrix} M \\ C^+A^- \end{matrix}\right\} \xrightarrow{\text{FDMS}} \begin{matrix} M^{+\cdot};\ (M+H)^+,\ (M+Na)^+ \\ C^+,\ A^+,\ 2C^+A^-,\ (C\cdot A)^{+\cdot} \\ M+C^+,\ M+(M-m)^+ \end{matrix}$$

Fig. 1. Generalized list of ions to be expected from an FD sample containing both molecules, M, and salts, C^+A^-. Molecules are neutral overall, but may be zwitterionic. Salts are commonly partly organic: e.g. $R_4N^+X^-$, or Na^+RCOO^-, but such materials as NaCl and even $CuSO_4$ have been recorded successfully.

4. APPLICATIONS

(a) *Mixture analysis by FIMS*

Since FIMS requires sample vaporization prior to analysis it offers no advantage over EIMS except for those few molecules which vaporize without decomposition, but which subsequently are extensively fragmented by electron impact. This fact, coupled with the relatively low sensitivity compared to EI or CI, limits applications of the technique. The one example to be presented here makes use of the simplicity of FI spectra of single compounds in the analysis of complex mixtures.

The low level of fragmentation in FIMS gives it a tremendous advantage over EIMS in characterization of complex mixtures, such as are commonly found in the petroleum industry. Thus, whereas GC/EIMS is largely limited to mixtures of moderate complexity, GC/FIMS has been shown to give a good deal of information from mixtures much too complex for GC separation.[13] An homologous series of hydrocarbons, such as C_nH_{2n-8}, C_nH_{2n-10}... generates a corresponding set of ions separated by 14 amu. For any one of these series, the data system can be programmed to plot a simulated GC trace. Since this trace is effectively limited to molecular ions of this single homologous series, it appears as a set of well-separated clusters, each representing isomeric structures of a certain carbon number within the homologous series chosen. Sample plots for C_nH_{2n-6} are shown in Fig. 2(a) along with the uninformative total ion current trace (Fig. 2b). The former plots show that there are three isomeric C_9H_{12} compounds, and at least five $C_{10}H_{14}$ isomers in this benzenoid fraction. At higher carbon numbers the substituted benzene series declines and two other series of longer retention times appear. Series B was identified as $C_nH_{2n-10}S$ (carbon number 2 less than label), and Series C is C_nH_{2n-20} (carbon number 1 more than label). That is, series B starts with C_9H_8S on the C_{11} line and series C starts with $C_{16}H_{12}$ on the C_{15} line.

Other recent publications from this group demonstrate the use of this technique in characterization of oil-sand bitumens and its potential for other similarly complex mixtures.[14,15]

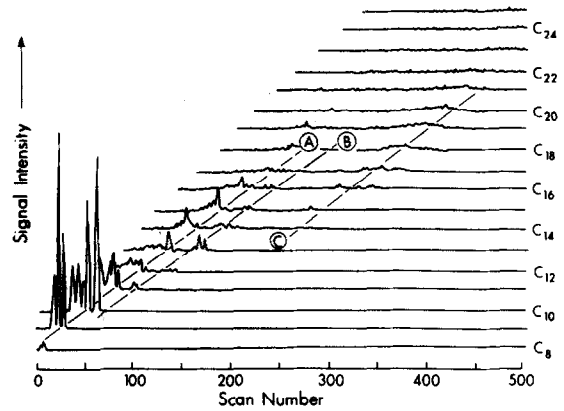


Fig. 2(a). Aromatic fraction. Individual molecular ion intensities for the mass series corresponding to the formula C_nH_{2n-6}. Line A is drawn through the homologous series of benzenes. Line B is drawn through the series of benzothiophenes, $C_nH_{2n-10}S$, and line C through material of formula C_nH_{2n-20}. Most intense peak is 13.5 units.

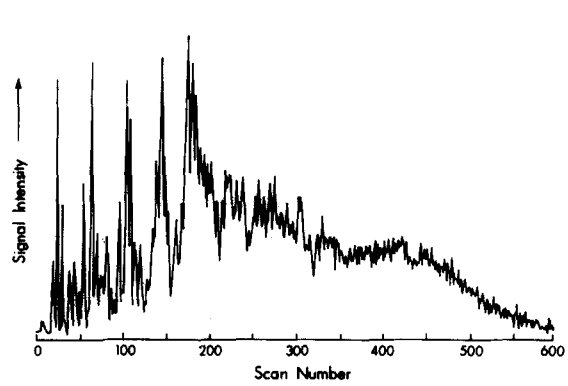


Fig. 2(b). Aromatic fraction. Total ion current (sum from $m/z = 28$–600). Most intense peak is 18 arbitrary units. The scan number is proportional to GC elution time.

(b) *Structural information from FDMS*

Brucine adduct. A neat demonstration of the power of FDMS for structure elucidation of labile ionic species is provided by Rastetter and Frost.[16] It had been thought for some time that brucine resolution of enantiomeric oxaziridines in methylene chloride was accompanied by oxygen transfer to produce brucine N-oxide. However, the present authors showed that the same white crystalline solid was formed by refluxing brucine with methylene chloride. This product was shown to be a 1:1 adduct whose structure was confirmed largely by FDMS.

The even-electron ions *m/z* 443, 445 are to be expected in FDMS from any preformed cation. That this structure gives such an array of odd-electron species may be attributed to the ease of ionization of the brucine nucleus, perhaps in the dimethoxybenzene region. The base peak, *m/z* 394, may be formulated as electron loss from the tertiary amine site, a product which is attributable to fragmentation only when the sample has been scrupulously purified to remove brucine.

Prostaglandin salt. The synthetic prostaglandin salt, Estrumate, is characterized quite satisfactorily by its FD spectrum.[17] Figure 4 shows an expected cluster ion, *m/z* 469 and the base peak results from complexation of a sodium ion with the free carboxylic acid, a not uncommon process. The minor peak at *m/z* 246 helps to define the nominal mass of the carboxylate ion, providing it is recognized as a doubly-charged ion.

Enterobactin. Evidence of general acceptance of the FD method may be found in the increasing frequency with which its use is buried in experimental sections of publications along with other analytical techniques. For example, the recent communication on synthesis of enterobactin and enantioenterobactin included FDMS determination of the molecular weights of this microbiol iron-binding ionophore and its synthetic antipode based on D-serine (Fig. 5).[18]

Myricoside. This potent insect antifeedant, collected in East Africa with the assistance of a local medicine man, was isolated by droplet countercurrent chromatography[19] in modest amount (10 mg). FDMS figured among the identification techniques, chiefly for the sequence of sugars and aglycone. These data and the structural assignments are shown in Fig. 6.

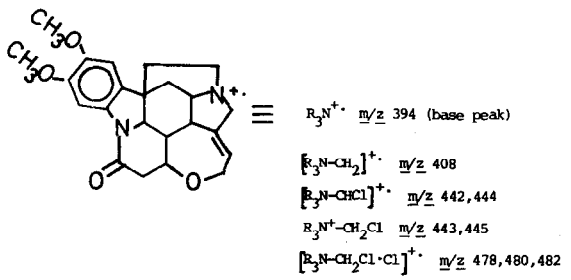


Fig. 3. Ions found in FDMS of adduct of brucine with methylene chloride, R_3N^+–$CH_2Cl{\cdot}Cl^-$.

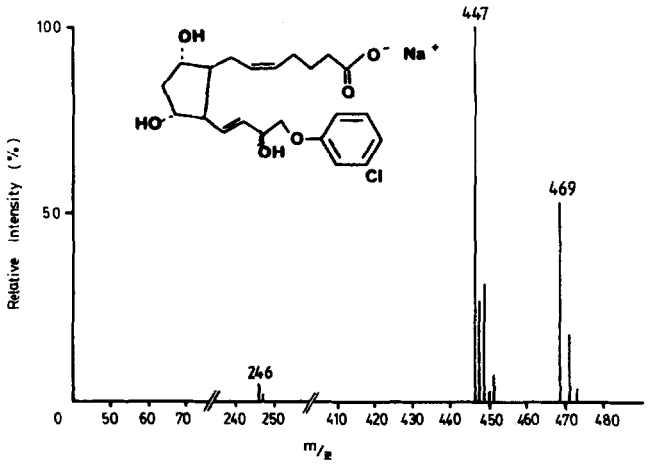


Fig. 4. Field desorption mass spectrum of Estrumate, $RCOO^-Na^+$, at 16 mA. Ions at *m/z* 469, 447, 246 are assigned to $RCOO^-{\cdot}2Na^+$, $RCOOH{\cdot}Na^+$ and $RCOO^-{\cdot}3Na^+$.

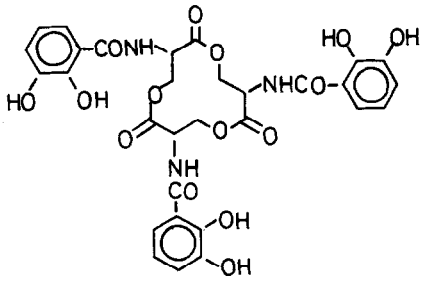


Fig. 5. Enterobactin, $C_{30}H_{27}N_3O_{15}$, M = 669.

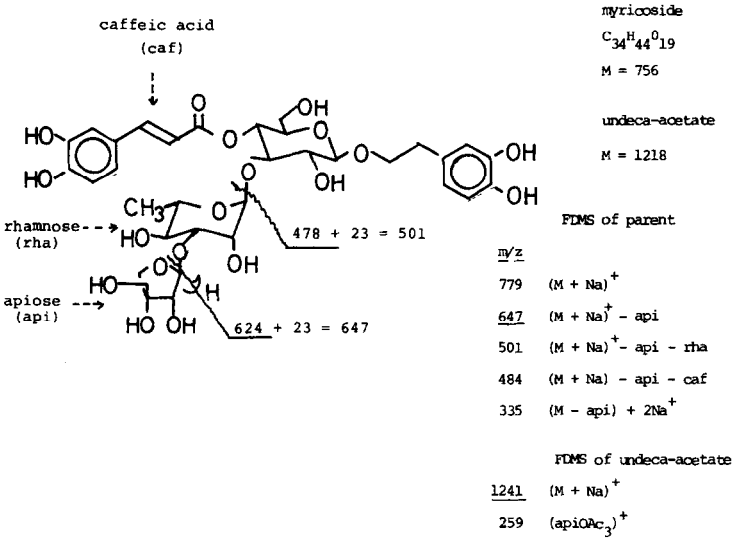
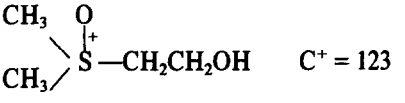


Fig. 6. Structural assignments of major ions in FD mass spectrum of myricoside.

The base peak (underlined) at *m/z* 647 suggests apiose (api) as the terminal sugar, as does the undecaacetate peak at *m/z* 259 and the doubly-charged peak in the original compound at *m/z* 335. The location of rhamnose (rha) in the penultimate position is revealed by the peak at *m/z* 501. There is some ambiguity about the *m/z* 484 peak, which may represent loss of apiose and cleavage of caffeic acid (caf) as indicated or an alternate fragmentation of the api-rha chain from glucose.

Dogger Bank Itch allergen. Another anonymous application of FDMS was in the identification of the allergen which causes Dogger Bank Itch.[20] Extraction of the marine animals which cause this contact dermatitis allowed the isolation of the (2-hydroxyethyl)dimethylsulfoxonium ion



which gave *m/z* 108 ($CH_2S(O)CH_2CH_2OH$), *m/z* 90 ($CH_3C(O)CH{=}CH_2$), *m/z* 78 ($CH_3S(O)CH_3$), by FDMS of the chloride. Unfortunately, the absence of the intact cation from this spectrum was not mirrored in the FDMS behavior of trimethylsulfoxonium iodide which was chosen as a model compound, perhaps because of the difference in accompanying anion. This speculation, based on some of our published work,[21,22] would be more firmly based had the present results been accompanied by information on anode heating.

The crucial technique in this identification appears to have been NMR, since EIMS gave no ions of *m/z* greater than 90, and high resolution measurement was not achieved above *m/z* 78.

Immonium salt. A brief report on the FDMS characterization of an immonium salt which is readily hydrolyzed[23] reveals yet another facet of the technique. The compound in question was prepared by alkylation with an excess of triethyloxonium hexafluorophosphate (Fig. 7). FDMS of the reaction

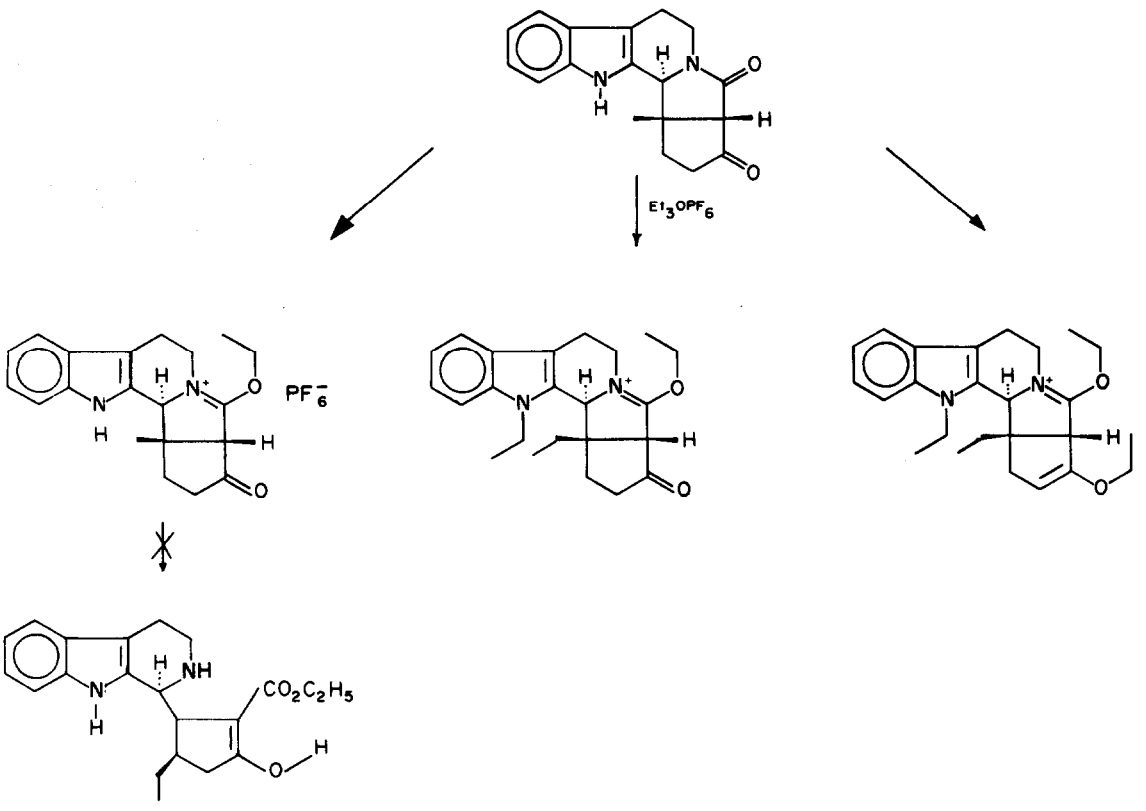


Fig. 7. Reaction products from alkylation of 5-membered lactam to mono-, di- and tri-ethylated level by triethyloxonium hexafluorophosphate.

Table 1. FD mass spectrum from the solution of the alkylated 5-membered lactam

m/z	%	m/z	%
103	48	393	3
337	24	585	<1
338	8	599	<1
351	100	613	<1
365	12	641	<1

product (Table 1) showed some evidence for polyalkylation, but no sign of the β-ketoester which would result from hydrolysis. Ignoring the very substantial abundance of reagent ions (*m/z* 103, Et_3O^+; 351, $2Et_3O^+ \cdot PF_6^-$; 599, $3Et_3O^+ \cdot 2PF_6^-$), the mixture is seen to consist of the desired monoalkylated product (*m/z* 337), with decreasing amounts of dialkylated (*m/z* 365) and trialkylated (*m/z* 393) material. Minor ions at *m/z* 585, 613 and 641 are attributed to complexation of the three cation products with the overall neutral $Et_3O^+ \cdot PF_6^-$ ($C^+A^- = 248$).

With respect to the original report, it is important to note that none of these ions arise from electron removal at the field anode, and that they are not odd-electron ions. Thus, failure to detect the neutral β-ketoester (as the odd-electron $M^{+\cdot}$ at *m/z* 354) in the presence of the several preformed cations is far from conclusive evidence for its absence from the mixture. In fact, one of the major weaknesses of FDMS is the dramatic sensitivity difference often observed between preformed cations (even-electron) and radical cations formed by electron removal. It is not uncommon to note that these two types of ions are maximized at different focus points, which further complicates the picture.

Dimeric disulfide. An illustration of FDMS as a "soft" ionization method has been provided by Parfitt *et al.*[24] who studied the product of LAH reduction of 3H-1,2-benzodithiole-3-thione. In spite of some confusion in the earlier literature, these authors were able to present strong evidence that their crystalline material was the dimeric reduction product as shown in Fig. 8.

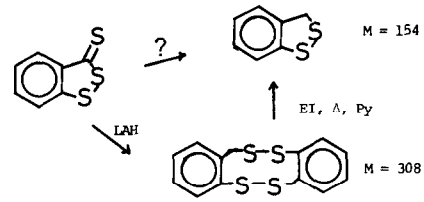


Fig. 8. Interconversions between reduction products of dithiol-thione.

The key elements in their assignment were the presence of *m/z* 308 as base peak in FD spectra from benzene and in FI spectra (cold source). The ion *m/z* 154, which was present in these spectra (5 and 20%, respectively), became more abundant in FDMS from chloroform, and was the base peak in FDMS from pyridine as well as in EIMS (70 eV and 10–20 eV). The protonated form of this ion, *m/z* 155, was the base peak in CI. Several fragment ions in these latter spectra were consistent with the dimer structure as formulated. Further evidence for the ease of conversion of dimer to monomer was presented by samples freeze-dried from pyridine which gave oils similar to those in a previous report on the presumed monomer.

C- *and* O-*glycosides of anthraquinones, anthrones.* Although common anthraquinone and anthrone aglycones give molecular weight information by EIMS, their C- and O-glycosides do not.[25] Examples of comparative results for EI and FD spectra for these latter compounds are shown in Fig. 9. It may be worth noting that only one of these examples, the C-glycoside barbaloin, gave CI(NH_3) spectra with molecular weight information.

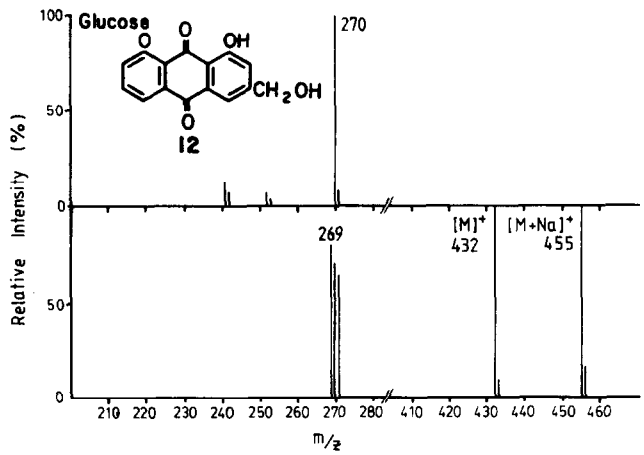


Fig. 9(a)

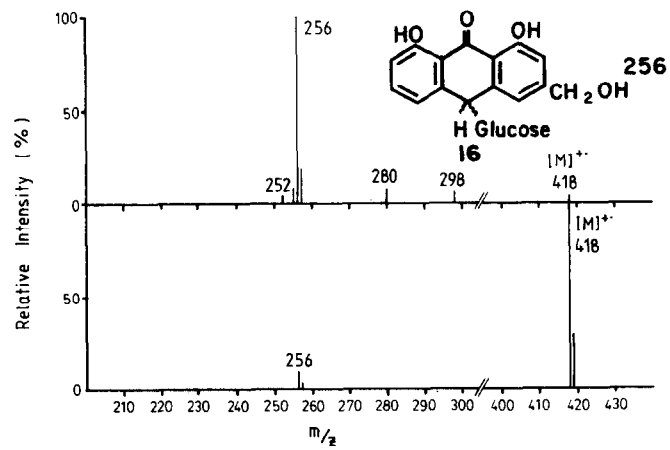


Fig. 9(b)

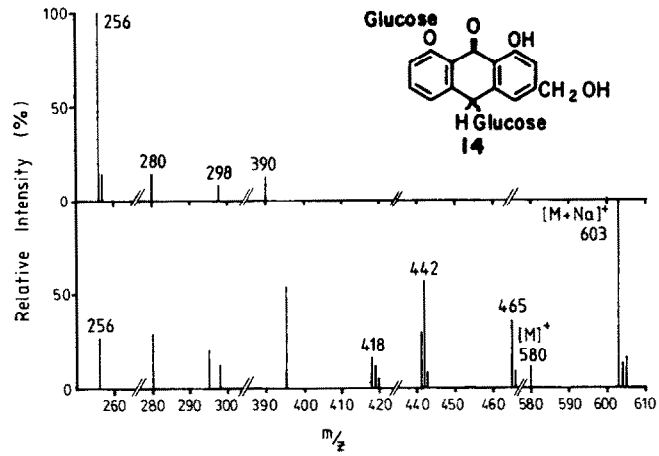


Fig. 9(c)

Fig. 9. Comparison of EI spectra (upper panel) and FD spectra (lower panel) for O-glycoside (top), C-glycoside barbaloin (middle), C-, O-digylcoside (bottom).

Phthalocyanine pigments. Phthalocyanine pigments, another class of compounds of low volatility, have been studied by FD.[26] Phthalocyanine with no complexed metal atom gave only a singly-charged $M^{+\cdot}$. However, Ni, Co, Fe and Cu complexes all produced, in addition to the molecular ion as base peak, some evidence of M^{2+} (Fig. 10). A sample of Cu "mononitrophthalocyanine" was shown to be a 3-component mixture containing almost equal quantities of mono-, di- and non-nitrated material. Al chlorophthalocyanine was shown to contain 1–4 atoms of chlorine, with $AlCl^{2+}$ complexed and 0–3 atoms substituted on the aromatic rings. Curiously, Cu tetraphenylphthalocyanine showed a weak ion at *m/z* 204 corresponding to M^{4+} for the empty phthalocyanine. The only other sample reported to give ions with charge greater than 2^+ was Co phthalocyanine, which had *m/z* 190.4 ($M^{3+\cdot}$).

Sulfonic acids and salts. Among the compounds accessible to study by FDMS are complex sulfonic acids and sulfonate salts, including dyestuffs.[27] Two monosodium salts which gave relatively straightforward results are those of naphthalene-2-sulfonic acid and anthraquinone-1-sulfonic acid. Even here, only some of the major ions represent corresponding structures (Table 2).

Both of these salts show base peaks for the same cluster, (A^-2Na^+), but higher clusters of this series are prominent for naphthalenesulfonate (*m/z* 483, 713, 943, 1173) in contrast to their modest contribution (*m/z* 643) to the anthraquinonesulfonate spectrum. The remaining prominent peaks in the naphthalenesulfonate spectrum (*m/z* 208, 230, 460) are all attributable to one electron ionization from the naphthalene moiety, a process which one might well expect to be of lower energy than in the anthraquinone compound.

A disulfonic acid and two disulfonate salts were also reported in this study, but here interpretation becomes more difficult. The authors suggest a number of structure assignments for which alternatives exist. For example, naphthalene-1,6-disulfonic acid shows, in addition to the base peak $(M+H)^+$, several peaks which were attributed to loss of portions of both sulfonic acid groups. The most important peaks at *m/z* 433, 434, 435 (40, 9, 6% respectively) could represent $(3M+2H)^{2+}$ as an alternative to the original workers' suggestion of $(2M+1-SO_3-SO_2)+$. This latter assignment implies extensive decomposition of this molecule at 10 mA. The alternative fits reasonably the intensities demanded by ^{34}S isotopes. Thus, if *m/z* 433, 434, 435 represent $m = 866, 868, 870$ and $z = 2$, the 6 S atoms would generate 868 of 10% and 870 of 1.4 compared to the 9% and 6% reported.

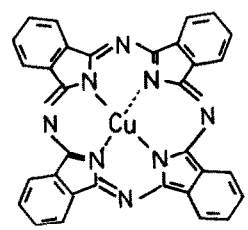
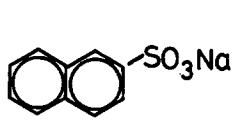


Fig. 10. Copper phthalocyanine, $M^{+\cdot} = 575$ (based on ^{63}Cu), 100%; $M^{2+} = 287.5$, 1%.

Table 2. Comparison of ions in FD spectra of two sulfonate salts

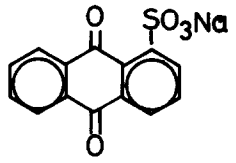


$A^- \cdot Na^+$

(207 · 23)

$A=C_{10}H_7SO_3=ArSO_3$

23 mA



$A^- \cdot Na^+$

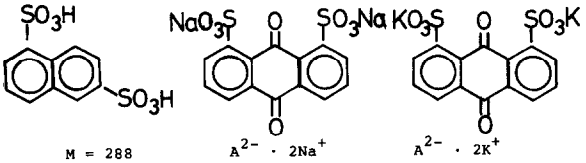
(287 · 23)

$A=C_{14}H_7O_2SO_3=ArSO_3$

22 mA

%	m/z		m/z	%
		$ArSO_2^+$	271	5
11	208	$ArSO_3H^{+\cdot}$		
19	230	$ArSO_3Na^{+\cdot}$		
		$ArSO_3H \cdot Na^+$	311	1
100	253	$ArSO_3^- \cdot 2Na^+$	333	100
			334	7
			335	4
3	460	$ArSO_3Na \cdot ArSO_3Na^{+\cdot}$		
86	483	$2ArSO_3^- \cdot 3Na^+$	643	11
			644	1
			645	2
80	713	$3ArSO_3^- \cdot 4Na^+$		
20	943	$4ArSO_3^- \cdot 5Na^+$		
3	1173	$5ArSO_3^- \cdot 6Na^+$		

The results for anthraquinone-1,8-disulfonic acid, disodium and dipotassium salts, are discouraging for a number of reasons. The fact that the two salts show only a superficial resemblance is disconcerting. More serious is the fact that the dipotassium salt shows *m/z* 39, K^+, as the base peak (with no *m/z* 41 recorded for the 7% ^{41}K isotope!) along with *m/z* 483 (14%) as the only ion of structural significance ($Ar(SO_3)_2^{2-} \cdot 3K^+$). In short, these results point to a potential application for FDMS, but further work on improving ion yields and thus reproducibility is required.



M = 288

10 mA

$A^{2-} \cdot 2Na^+$

(366 · 46)

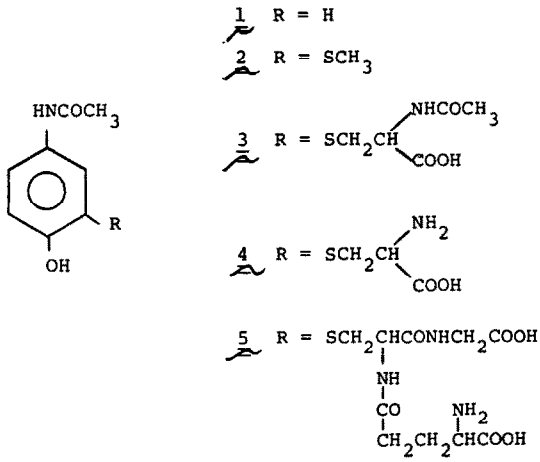
25 mA

$A^{2-} \cdot 2K^+$

(366 · 78)

20 mA

Acetaminophen conjugates. Study of derivatives and conjugates of acetaminophen by FD and comparison with EI/CI spectra provides some interesting results on the power of FD.[28] Thus, compounds **1**, **2**, **3** gave abundant $M^{+\cdot}/(M+H)^+$ ions in EI and CI (isobutane) as well as generally satisfactory results in FD (**3** was difficult) while compounds **4** and **5** were increasingly intractable to the two former methods but gave ions related to the intact molecules by FD. Compound **4**, M = 270, gave EI *m/z* 226, 197(1–2%), 183(70%), 141(100%); CI *m/z* 271(30%); FD *m/z* 270(80%), 293(30%). Compound **5**, M = 456, gave EI *m/z* 368, 225, 197, 183(1–2%), 43(100%); CI *m/z* 411(1%), 148(100%); FD *m/z* 457 (M + H, 70%), 479(M + Na, 20%), 412(MH–CO_2H, 10%), 152(100%).



A further point of comparison for spectra of **4** and **5** was the sample required. For example, 10–20 μg of **4** was required for EI or CI spectra, while only 100–200 ng was required for FD of the more difficult **5**. This 100-fold lower sensitivity for EI and CI suggests that much of the sample is consumed in ways that produce no relevant ions at the detector which certainly is a handicap when sample quantity is limited.

These authors also investigated the collision-induced dissociation (CID) of the molecular ion species of **4** and **5** detected by FDMS. These FD/CID spectra consumed 1–2 μg of sample and revealed fragmentation comparable to CI in terms of its use for structure identification. For those laboratories with the appropriate set-up FD/CID spectra are a valuable complement to the sometimes featureless (beyond molecular ion) FD spectra.

In this same study glucuronide and sulfate conjugates were attempted by FD only. The glucuronide gave $M^{+\cdot}$ (*m/z* 327) as the base peak in FD along with the fragments *m/z* 176 (glucuronic acid) and 151 (aglycone). The sulfate failed, giving only *m/z* 151 ($M-SO_3$). Perhaps this should not be surprising because of the notorious difficulty in obtaining mass spectra of intact sulfates. As far as FD is concerned, it is not clear whether this difficulty truly applies to pure acids, such as monosubstituted sulfates and phosphates, or if it relates to the difficulty in obtaining these samples free of alkali metal ions.

Gluthathione conjugate of p-benzoquinone. Metabolic activation of benzene, phenol and related compounds by rat liver microsomes was shown to be followed by covalent binding to gluthathione and the conjugate identified by FDMS.[29]

Figure 11 shows the FD spectrum and structure of the model conjugate formed by reaction of glutathione and *p*-benzoquinone. Comparison with spectra obtained on the appropriate HPLC fraction from microsomal incubation of *p*-benzoquinone, hydroquinone, and phenol allowed identification of these products. Perhaps the most notable aspect of this FDMS spectrum is the wealth of structural information available in the fragment ions. This spectrum was recorded at 14 mA, slightly above the BAT for *m/z* 416.

Oregonin, a diarylheptanoid. An early, and still interesting, contribution of FDMS to structure proof came from the Barofskys.[30] In this work a compound dubbed oregonin, implicated in the staining of freshly cut red alder, was assigned a novel diarylheptanoid structure (Fig. 12).

The role of FD in this identification may be summarized as follows: Treatment of a fraction of bark extract yielded compound **7** which gave no ions above *m/z* 384 by EIMS. In contrast, FDMS yielded *m/z* 534 which was subsequently assigned to the molecular ion. Either FDMS pyrolysis or acid hydrolysis yielded the same products **9** and **10**, with structures confirmed by independent synthesis. Acetylation of the tetramethyl ether **7**, yielded an apparent triacetate which permitted exact mass measurement (EIMS) for $M^{+\cdot}$ of 600.278. As usual, the other spectroscopic tools contributed to the assignment of the structure of oregonin as the bis(dihydroxyphenyl) relative of **7**. There may be independent evidence for the introduction of all four methyl ethers during methylation, although the communication at hand does not present it.

Tetrahydrofuranyl derivatives of 5-fluorouracil. In the context of a study on new variants of the antitumour drug 5-fluorouracil[31] comparative EI, CI(NH_3) and FD mass spectra were obtained. For

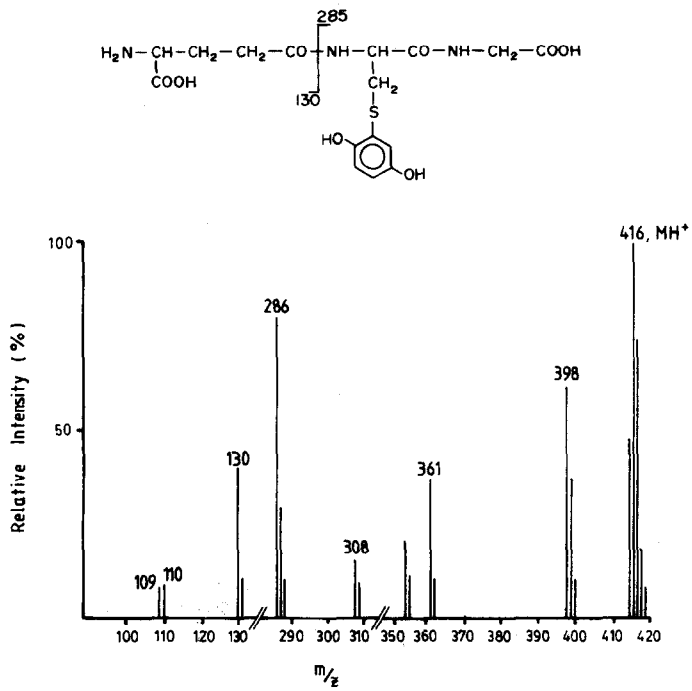


Fig. 11. Field desorption mass spectrum of the conjugate isolated by HPLC from the reaction mixture of *p*-benzoquinone and glutathione (reduced form).

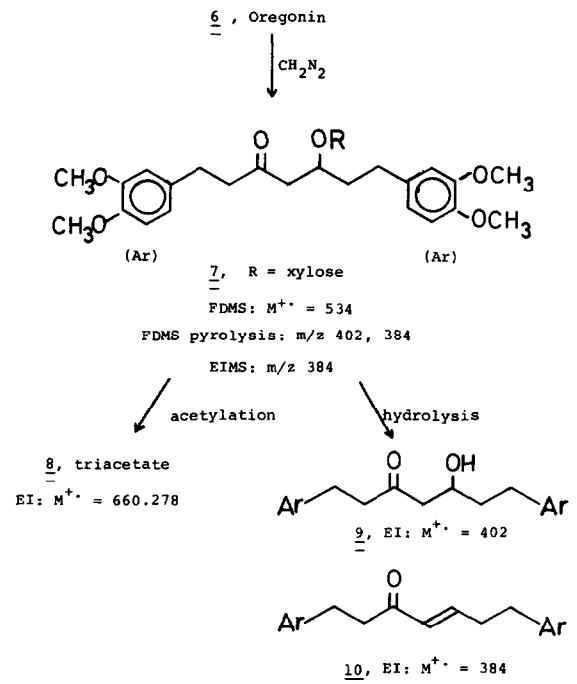


Fig. 12. Summary of structures and mass spectral data for oregonin and related compounds.

purposes of this review, these spectra will be used to give an indication of the relative behaviour of the three techniques for some moderately complex but volatile organics (Fig. 13).

For the parent compound, 5-fluorouracil, EI yields the molecular ion *m/z* 130 as base peak along with significant fragments. Both CI and FD yield spectra dominated by molecular ion species, although in the former *m/z* 130 is dwarfed by the complex ion $(M + NH_4)^{+\cdot}$.

The isomeric monotetrahydrofuranyl derivatives show readily identified molecular ions by EI, as well

as rich and distinctive fragmentation patterns. Qualitative differences at *m/z* 157, near *m/z* 130 and *m/z* 100 in addition to major quantitative differences reveal considerable sensitivity to detailed structure. No such distinction is present in the CI and FD spectra, although the latter show major peaks corresponding to both fragments form direct cleavage of the furanyl ring.

The disubstituted compound continues to yield a distinct molecular ion and numerous fragments related to structure in the EI spectrum. Here the CI spectrum has an intense $(M+H)^+$ ion (no doubt reflecting increased basicity of the molecule with increasing tetrahydrofuran substitution), and three other ions involving complexation with NH_3 (*m/z* 218, 88 represent complexes with monosubstituted fluorouracil and tetrahydrofuranyl, respectively). The FD spectrum of this compound is generally uninformative, save for the molecular ion and the tetrahydrofuranyl fragment. As will be seen in other examples, even the unambiguous appearance of the molecular ion is not assured in FDMS. Many difficultly ionized samples give an $(M+H)^+$ ion, even in the absence of an obvious source of the proton, which can cause confusion about the molecular weight.

Monolayer films of surfactants. Analysis of monolayer films at the air-water interface is a novel FD application suggested to have broad potential interest.[32] Dipping the FD anode in a surfactant film transferred enough surfactant to give an FD spectrum. Whereas *n*-octadecanol was too volatile for reliable mass measurement, *n*-octadecanoic acid, N,N-dimethyl *n*-octadecylamine, vitamin K, and chlorophyll b gave satisfactory FD spectra. Chlorophyll b in contact with a neutral sub-phase (borate buffer pH 8.2) gave an intense ion at *m/z* 907.5; and after 10 min contact with an acidic sub-phase (aqueous 10^{-3} M HCl), an additional signal at *m/z* 885.5, corresponding to magnesium-free phenophytin b. These chlorophyll b FD spectra do not show hydration ions in contrast to a recent FD report on chlorophyll a.[33]

Some of these experiments on monolayer films were also used to estimate the amount of sample transferred to the FD anode. For a ruthenium complex this figure was estimated at 1.1–75 ng, from which the instrumental sensitivity was bracketed between 2.1×10^{-12} and 1.6×10^{-10} A · s/μg for this compound. Deposition of cholesterol from a mixed monolayer gave an estimated sensitivity of 3.6×10^{-9}–4.8×10^{-11} A · s/μg, suggesting that an earlier determination[34] represents a lower limit of sensitivity.

Polypropylene glycols. An impressive demonstration of the potential of FDMS for polymer studies has been presented by Derrick and co-workers. A home-built grand-scale mass spectrometer,[35a] capable of detecting ions to at least *m/z* 7400, was used for FDMS of several polypropylene glycol samples of nominal molecular weight as high as 4000 Daltons.[35b] The spectrum for mass-1000 polypropylene (Fig. 14) illustrates these results. The major series of ions in the molecular weight region correspond to M + H (*m/z* 1121, the base peak, represents M = 1120, a 19-mer where $M = 19\times58+18$). Other major ions in this series span the range *m/z* 947–1411 (n = 16–24). Although this envelope of ions clearly corresponds approximately to the molecular weight of this mixture, the envelope centroid shifts according to the anode heating current such that a scan at 11.5 mA yields *m/z* 947 as base peak. As the authors point out, this effect of "distillation" means that applications to determination of molecular weight distributions would require either integration of all scans or some form of calibration. One approach to these problems has been published.[36]

Other series of even-electron ions found include M-31 and M-17, which are responsible for most of the ion current below *m/z* 500, and a second series in the molecular weight region labelled M-103. Minor odd-electron ions at M-18, M-104 may be a by-product of $M^{+\cdot}$ formation.

Another recent publication[37] reported interesting FDMS results on oligomer mixtures, although in this work some of the polyether samples were contaminated with sodium ions to the extent that $(M+Na)^+$ ions were dominant.

Cyanogenic glycosides. Cyanogenic glycosides are constituents of a number of foods which may release significant amounts of hydrogen cyanide. Difficulties in analysis of these glycosides led to the evaluation of FDMS as a screening method.[38] The FD spectra shown in Figs. 15–17 are representative of the results obtained. The aglycone generally gives rise to the base peak (*m/z* 68, 116 and 131) although in the case of *p*-hydroxymandelonitrile an apparent oxidation of this fragment has occurred. Molecular weight information is generally present as $(M+H)^+$ and $(M+Na)^+$ or $(M+K)^+$, but once again the *p*-hydroxymandelonitrile is anomalous in producing $M^{+\cdot}$ with $(M+K)^+$. Although the authors attribute the observation of $M^{+\cdot}$ to "loss of a hydrogen", it appears likely that this molecule simply loses an electron very readily as the result of an unusually high electron density on the phenolic ring. Weak ions representing the sugar were found, but characterization of this fragment is probably best accomplished by subtraction of the aglycone from the molecular weight. In the case of vicianin this calculation gives

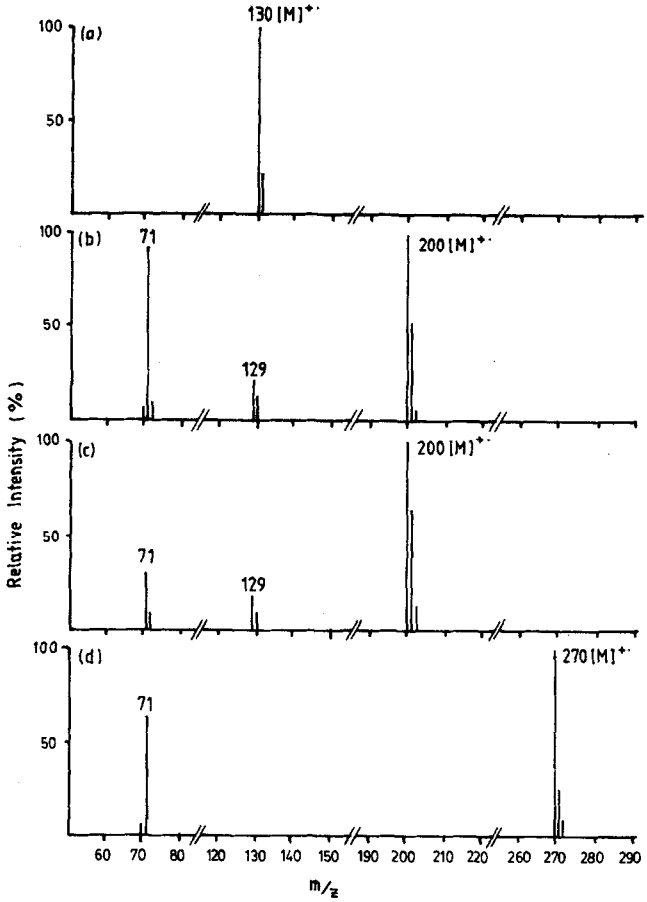


Fig. 13(a)

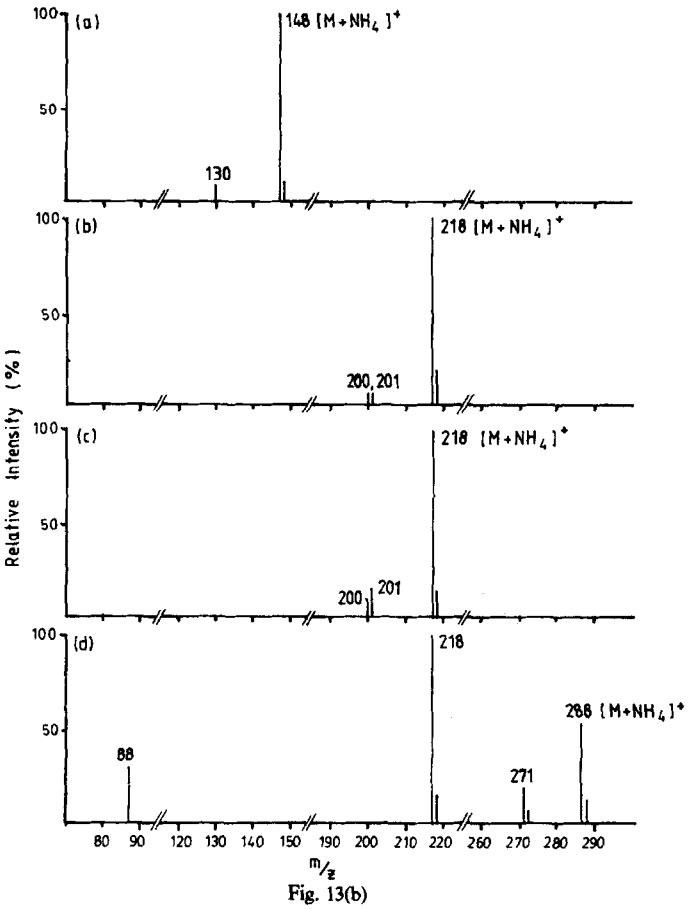


Fig. 13(b)

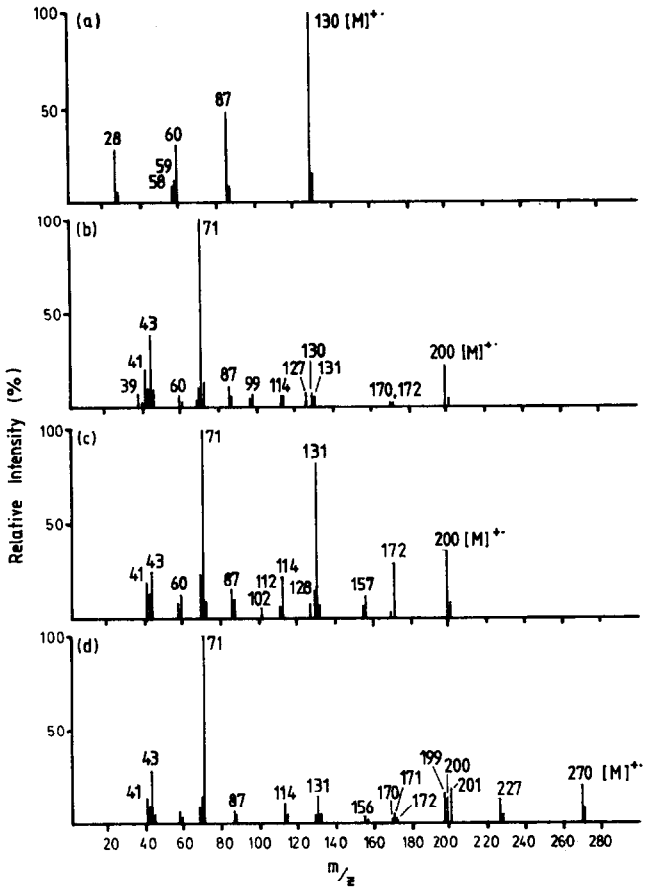


Fig. 13(c)

Fig. 13. Mass spectra of (a) 5-fluorouracil (FU), (b) 1-tetrahydrofuranyl FU, (c) 3-tetrahydrofuranyl FU, (d) 1,3-bis(tetrahydrofuranyl) FU. Panel on left: FD spectra, centre panel: CI(NH_3) spectra, right panel: EI spectra.

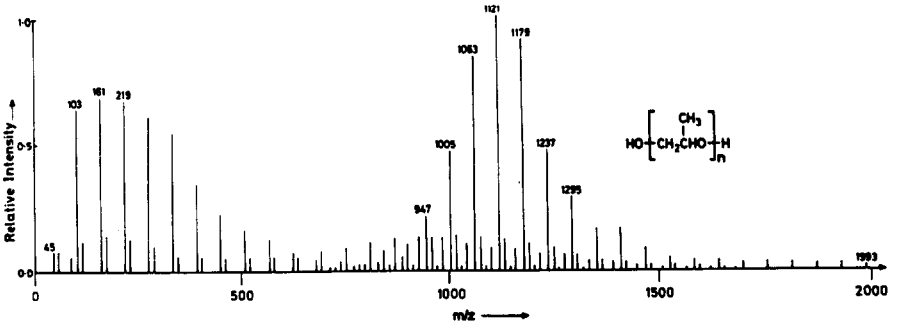


Fig. 14. The field desorption mass spectrum of the mass-1000 polypropylene glycol. Emitter heating current was 13.0 mA. ^{13}C isotope peaks have been omitted.

M = 312 for the sugar which is then taken to be the dissacharide vicianose. In this spectrum the peak at *m/z* 133 may represent in part the terminal monosaccharide since amygdalin shows the comparable glucose cleavage at *m/z* 163.[39]

An example of the utility of FDMS is provided by comparison of the spectrum from a compound isolated from the seeds of *Vicia sativa*, a plant known as a contaminant of seed grain. The purified compound had a spectrum essentially identical to Fig. 16 and the plant extract prior to purification showed most of the key peaks (Fig. 18). To make an identification from this spectrum, one would have to recognize that higher levels of Na^+ and K^+ give *m/z* 450 and 466 whereas $(M+H)^+$ is absent. Attribution of the *m/z* 140 peak would also reduce the apparent differences from the standard spectrum.

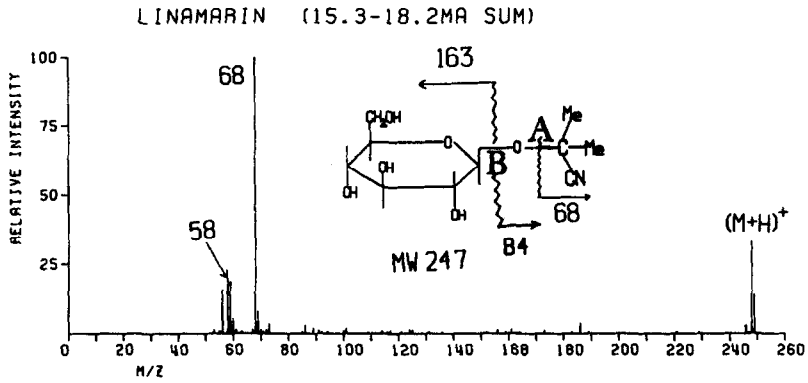


Fig. 15. FDMS of linamarin.

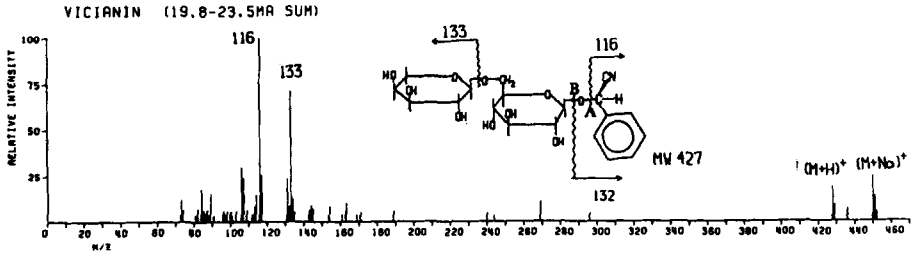


Fig. 16. FDMS of vicianin.

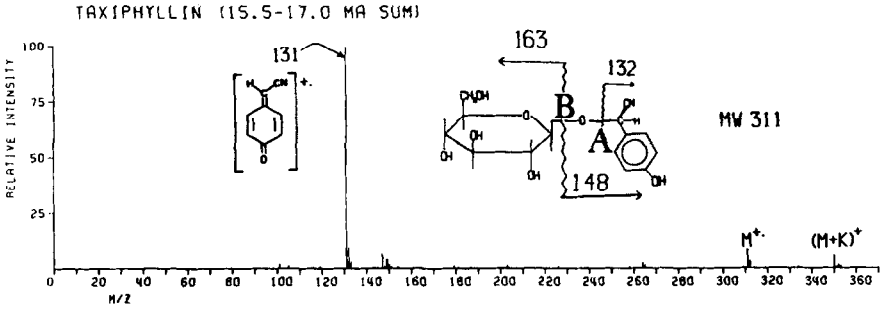


Fig. 17. FDMS of taxiphyllin.

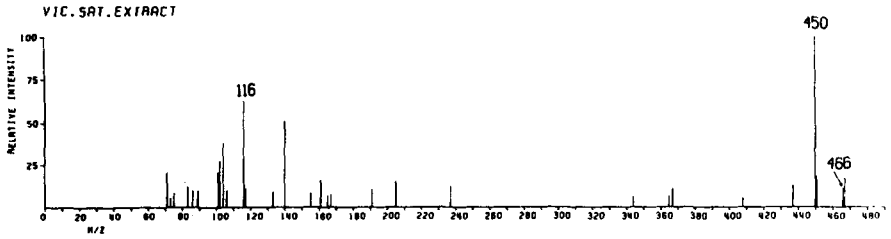


Fig. 18. FDMS of crude extract from the seeds of *Vicia sativa*.

Polyether antibiotics, A current review on mass spectrometry of polyether antibiotics[40] presents a good deal of interesting information previously unpublished, and provides a new slant on comparisons between EI and FD results. As pointed out in that review, the first comparative mass spectral data on a family of polyether antibiotics published in 1970[41] contained some interesting observations on EIMS of alkali metal salts. For example, whereas the ion of highest mass in the free acid form of monensin was *m/z* 634, representing RCOOH–$2H_2O$, the sodium salt of the acid yielded *m/z* 692, representing $(RCOONa)^{+\cdot}$. Further, conversion of the free acid to K and Rb salts gave mass shifts appropriate for

$(RCOOK)^{+\cdot}$ and $(RCOORb)^{+\cdot}$, thus serving to confirm the assignment. It should be noted that this confirmation was necessary because the mass doublet 2C:Na + H (24 amu) is separated by less than 3 mmu. The K and Rb salt spectra showed sodium salt peaks at least equal in intensity to the expected intact salt ions (not truly *molecular* ions!) at *m/z* 708 and 754. Analysis of these salts showed the presence of Na salt (10 and 3% respectively), presumably a reflection of the presence of this impurity in the original free acid. The enhanced abundance of the Na-containing species is attributed to volatility differences or to exchange of alkali metal ions within the mass spectrometer source. Reading this account in the context of intervening experience with the ubiquitous Na ion in FDMS produces a strong sense of *deja vu*, although more recent experiments[42] supporting the notion of alkali metal ion exchange in the ion source provide no encouragement for attribution of this process to the short pathlength set-up typical of FDMS. The superior behavior of Na salts to that of free acids in EIMS has since been generalized and on occasion salt formation has been used as a derivatization procedure![43] The sodium salts generally give FD spectra containing molecular weight information although here the lack of fragmentation is often a problem and methyl esters or other derivatives may be used to provide more structural information. For example, the structure of K-41 (Fig. 19) was deduced from a combination of NMR, EIMS and FDMS.[44] FDMS of the mixed Na/K salt yielded dominant $(RCOOH + Na)^+$ and $(RCOOH + K)^+$ ions, and the sodium salt provided a cationized peak of sufficient intensity for accurate mass measurement (969.5373 for $C_{48}H_{82}O_{18}Na$). Derivatization of the carboxylic acid and tertiary alcohol functions (see Fig. 19) yielded the important fragments "a" and "b" as confirmed by high resolution measurement and deuteromethylation. These particular fragments, representing almost half of the structure were not represented in any of the EI spectra except for an ion *m/z* 129 (fragment c) resulting from cleavage of the sugar group.

The Saponin ginsenoside Rb_2. A number of physiologically active saponins, including some associated with Ginseng root, have been characterized by FDMS.[45] As an example of the structural information provided, the FD spectrum of ginsenoside Rb_2, M = 1078.6, is presented in Fig. 20.

In common with other spectra in this work, the base peak is $(M + Na)^+$. As noted by the authors, sodium is present as contaminant in virtually all these compounds even after thorough purification. Indeed, almost every ion in the spectrum contains one or more alkali metal ions. Thus singly-charged sodium-containing ions are found at *m/z* 1083, 969, 939, 807 and 777. The four latter peaks are related to loss of various neutral sugar units from the complex, always with transfer of 1H to the remaining complex ion. The ion at *m/z* 1233 could result from complexation of the terminal C-5 sugar with the base peak, although the original authors suggest other origins. Most of these singly-charged ions are echoed by corresponding potassium-containing ions, starting with *m/z* 1117 $(M + {}^{39}K)^+$. One of the most interesting features of the spectrum is the presence of significant doubly-charged ions of two kinds. Comparison with other spectra in the original paper suggests that the presence of two independent sugar moieties is necessary for double complexation $(M + 2Na)^{2+}$. It is important to note that the exact masses

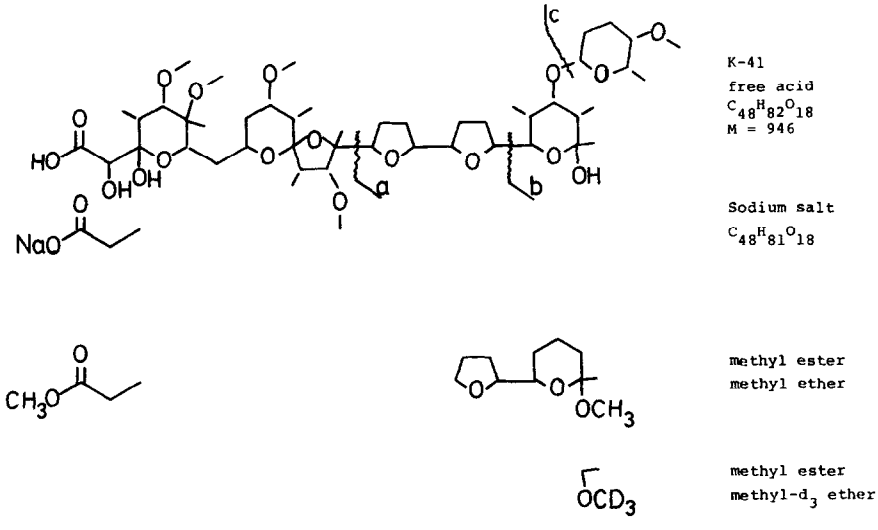


Fig. 19. Structures of antibiotic K-41 and derivatives with identification of key ions "a" and "b" in FD spectrum.

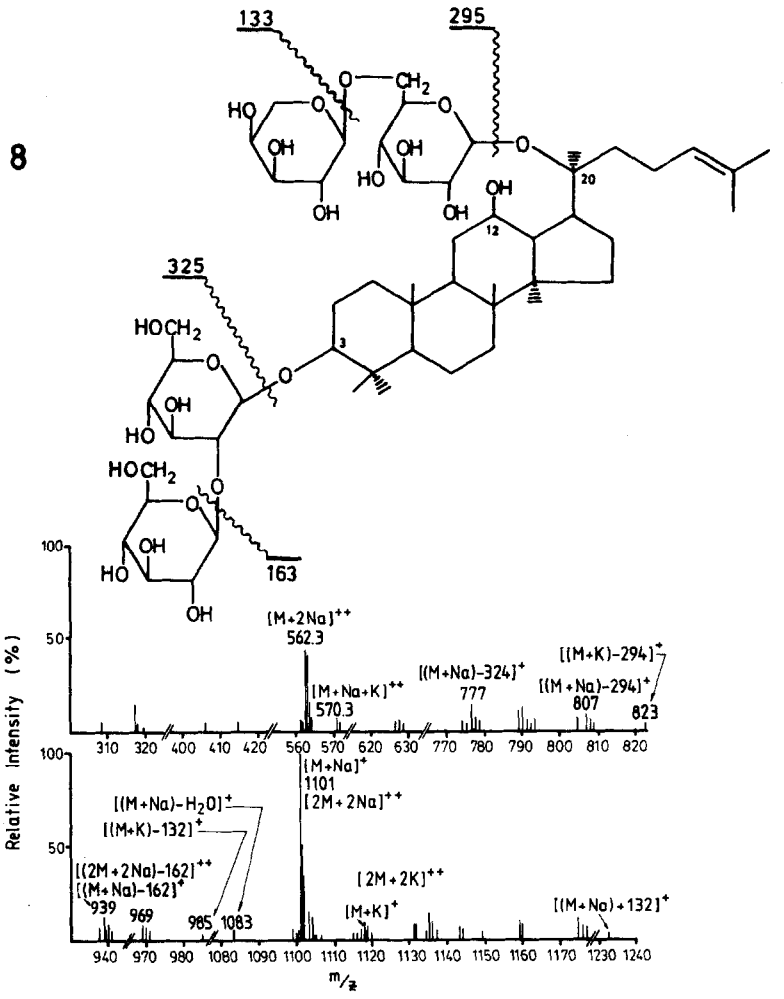


Fig. 20. FD mass spectrum of ginsenoside Rb_2.

recorded for doubly-charged peaks do not imply any mass difference from the corresponding singly-charged species. That is, the base peak *m/z* 1101 $(M+Na)^+$ and the peak $(2M+2Na)^{2+}$ ***both*** have *m/z* 1101.582. The distinguishing feature marking the presence of $(2M+2Na)^{2+}$ would be the first isotope peak at *m/z* 1102.1 [1/2(2 × 1101.6 + 1)], whereas the normal isotope peak occurs at *m/z* 1102.6.

True double complexation is revealed by the peak at *m/z* 562.286 $(M+2Na)^{2+}$, a mixed Na/K complex at *m/z* 570.273, and perhaps a very small peak at *m/z* 578 for $(M+2K)^{2+}$.

One sidelight on this work is the fact that several of the spectra were recorded in Bonn (on an 8 kV instrument) and Fukuoka (on a 3 kV instrument) with qualitatively similar results. This agreement is rendered even more significant when one realizes that the 3 kV instrument was operated at 2 kV to extend the mass scale (i.e. the spectrum shown would no doubt exceed the normal mass scale and therefore be recorded at 2 kV accelerating voltage to obtain a 3/2 scale expansion. For similar reasons the 8 kV instrument was operated at 6 kV). Further examination of the experimental parameters indicates that whereas the scan rates employed in Bonn were normal for FD (4, 8 s/decade), the scan rates in Fukuoka were much slower (120, 300 s full range). In the latter case, one might have anticipated some difficulty in obtaining a full scan with comparable intensities at both ends. However, the sample quantity specified is 1×10^{-5} g applied by syringe, a rather heavy loading. One cannot help but speculate that these facts are connected.

Polyene macrolide antibiotics.† Elucidation of the structure of rimocidin ($C_{39}H_{61}NO_{14}$, M = 767) relied heavily on FD along with other spectroscopic techniques. Although ^{13}C NMR played a crucial role

†In conformity with the notation introduced earlier (p. 5), neutral molecules are represented by M and salts by (C^+A^-). Thus rimocidin sulfate is $(2C^+A^{2-})$.

in correcting errors in an earlier partial structure, molecular weight information was obtained largely by FDMS after several false starts on various derivatives.[46] For example, rimocidin sulfate yielded *m/z* 731 and 714 by FDMS, whereas EIMS produced no ions above *m/z* 580. At the same time, the FD result was less than definitive not only because the observed peaks represented ions which had lost H_2O, but also because peaks were recorded at masses as high as *m/z* 815.

Several derivatives gave some structural information by EI or FD or both, but the most informative compound turned out to be perhydro-rimocidin, $C_{39}H_{71}NO_{13}$ and derivatives thereof. This reduction product was thought to have gained 2H across the C-11 carbonyl as well as each of the C=C bonds of the tetraene system. At the same time, hydrogenolysis apparently removed an O from C-3 (Fig. 21). These changes appeared to be very significant for FD behavior since the perhydrorimocidin sulfate gave a C^+ (perhydrorimocidin cation) ion of sufficient abundance for accurate mass measurement (*m/z* 762.5008, calc. for $C_{39}H_{72}NO_{13}$, 762.5004).

Confirmation of this assignment was found in the polyacetylperhydro derivative, where FD yielded *m/z* 1056 (M + H for heptaacetyl) and EI yielded *m/z* 1055. With the structure of rimocidin solved by bringing these results together with considerable additional data, it may be worth noting the ambiguous behavior of the original rimocidin sulfate under FD conditions. Most notably, the ions *m/z* 731 and 714, which are now assigned to $(M-2H_2O)^+$ and $(C-3H_2O)^+$, reveal that this sample gives both desorption of a rimocidin cation (presumably protonated at 1° amine) *and* ionization of the neutral organic, perhaps in the tetraene or at the neutral 1° amine. There is a similar chance for confusion in the doubly-charged ions, since *m/z* 815 appears to lack 2H ($2C^+ \cdot A^{2-}$ contains 1632 amu and would therefore appear at *m/z* 816 after loss of 2 electrons) by comparison with *m/z* 798 $(2C \cdot A-2H_2O)^{2+}$, 780 $(2C \cdot A-4H_2O)^{2+}$.

Another example of the application of FDMS to polyene macrolide antibiotics and commentary on the role of FD in this area of structure proof comes in a recent publication from the Illinois group[47] (Fig. 22). The molecular weight and the presence of the pentaene moiety were confirmed by the FD spectra of the antibiotic and its reduction product. Combustion analysis, IR and UV spectra led to the same conclusion, but EIMS yielded no ions above *m/z* 652 $(M-H_2O)$. The significance of the FD scan at 17 mA (BAT; defined in Section 3) is difficult to overestimate. Two major peaks separated by 22 amu (*m/z* 671, 693) is a signature for $(M+H)^+$ and $(M+Na)^+$, in this case further supported by *m/z* 653 $(M+H-H_2O)^+$. Indeed, there is some evidence of a peak near *m/z* 675 which would represent H_2O loss from the sodium complex. At higher anode temperature the peaks representing loss of 1–4 molecules of H_2O become important. The perhydro derivative appeared to be more heavily contaminated by Na^+ and K^+ since most of the major high mass peaks included one of these ions. Thus the pair of ions *m/z* 719, 703

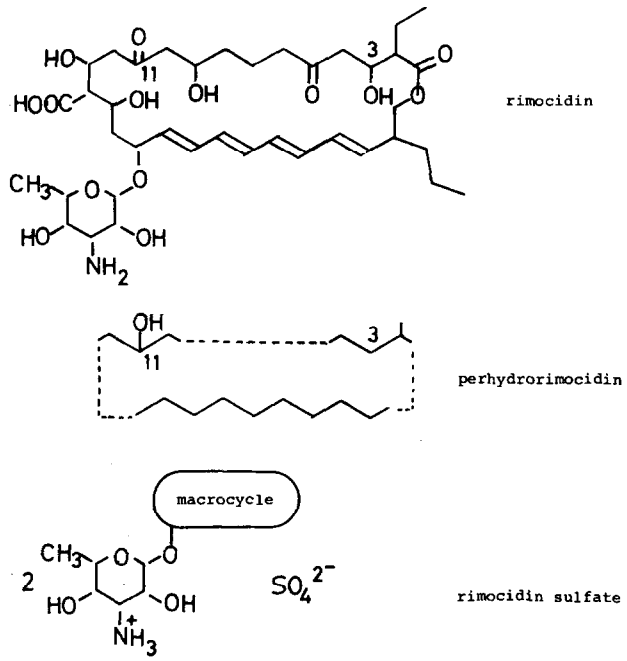


Fig. 21. Structures of rimocidin, perhydrorimocidin and rimocidin sulfate.

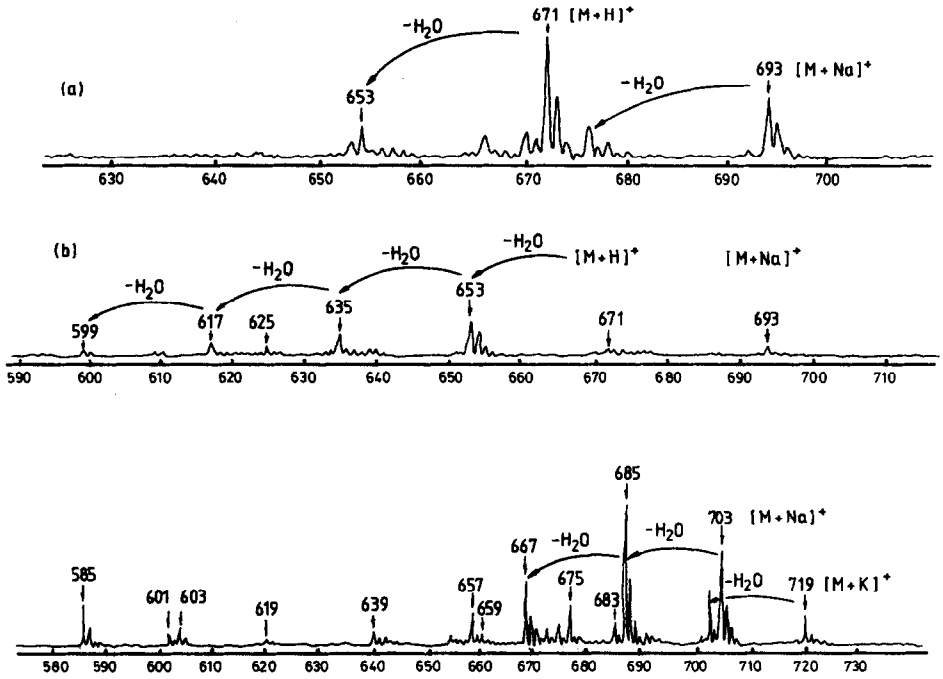


Fig. 22. (top) FD mass spectra of pentaene (a) 17 mA; (b) 19 mA. (bottom) FD mass spectra of decahydropentaene (12 mA).

have the appropriate separation for $(M+Na)^+$, $(M+K)^+$ as do the set *m/z* 701, 685 and 667, representing loss of H_2O from their respective precursors. The remaining labelled peaks in the diagram seem to fit best with H_2O loss from a partially hydrogenated sample, e.g. *m/z* 585, 639 and 657 could represent a molecule which had taken up 4H ($M+H=675$) and subsequently lost 1, 2, 4 or 5 molecules of H_2O.

By combination of this information with other results and knowledge of the polyene antibiotic field, a structure was proposed (Fig. 23).

A neat combination of chemistry with FDMS provided further evidence on the substitution pattern of adjacent hydroxyl groups (Fig. 24). The mixture of 3 products obtained from a periodate oxidation/borohydride reduction sequence of the decahydropentaene was submitted to FDMS.

The simplicity of these 3 overlaid spectra (Fig. 25) makes it possible to pick out 3 series $(M+H)^+$, $(M+H-xH_2O)^+$: I, *m/z* 411, 393, 375; II, *m/z* 397, 379; III, *m/z* 261. Although the latter molecule shows no H_2O loss, the ions *m/z* 215, 197 are correlated with its structure. Likewise, the important ion *m/z* 101 could arise from either I or II. Each of the $(M+H)^+$ ions was confirmed by high resolution FDMS with an error of about 1 mmu.

Phosphatidyl cholines. Phosphatidyl cholines have proven to be a challenging and relatively rewarding application for FDMS. Since these zwitterions are the most widely occurring class of phospholipids,

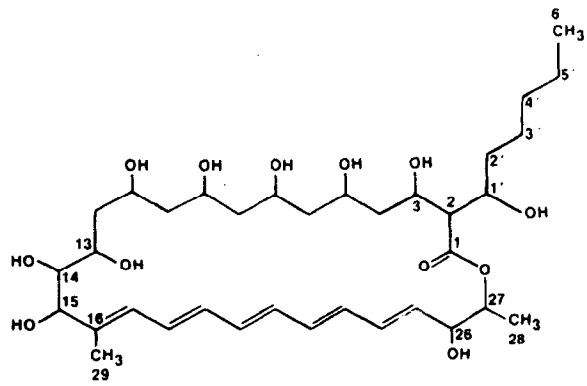


Fig. 23. Structure of pentaene macrolide antibiotic.

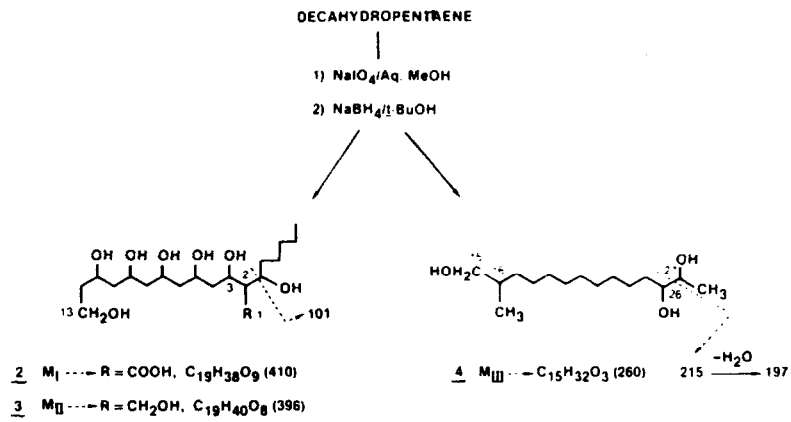


Fig. 24. Products of periodate cleavage/reduction.

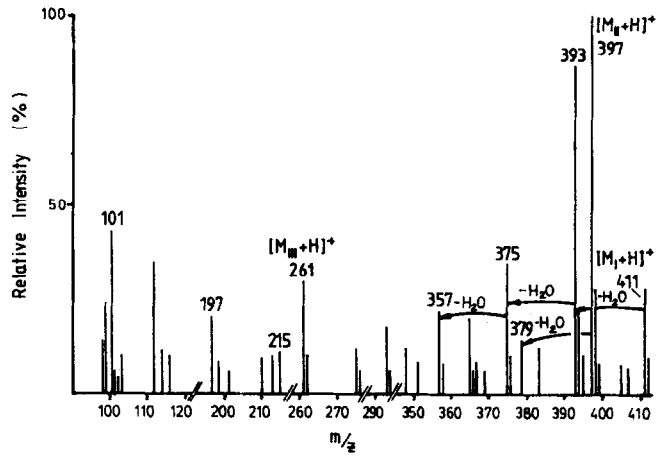


Fig. 25. FD mass spectrum of periodate oxidation products of decahydropentaene (18–20 mA).

a variety of mass spectrometric approaches have been taken to their analysis.[17,48] In brief, EIMS analysis usually involves some form of hydrolysis and derivatization of the separated portions. For example, phospholipase C removes the phosphoryl ester headgroup leaving a diacyglycerol which is readily derivatized for GC/MS analysis. One is then left to assume that the original sample contained only molecules with the phosphorylcholine headgroup, or to attempt a separate derivatization and analysis of the headgroup portion.

Various attempts to simplify the problem by EIMS analysis of intact phosphatidyl cholines culminated in work by Klein[49] in which careful heating from the direct probe was shown to produce metastable ions corresponding to molecular ion decomposition, and on occasion, very weak molecular ions at the detector.

Much of the early work in FDMS of phosphatidyl cholines was concerned with difficulties in sample purity and FD methodology rather than mass spectrometry problems. Techniques have now advanced to the stage where a number of laboratories have been successful with FDMS of a variety of complex lipids.

One example of the early work on phosphatidyl cholines is shown in Fig. 26. The base peak for dimyristoylphosphatidyl choline is $(M+H)^+$ and all of the significant ions above M are likewise even-electron. The ion $(M+CH_3)^+$ represents intermolecular transfer of CH_3^+ presumably to form phosphoryl methyl ester. The small $(M+Na)^+$ ion indicates a very low level of sodium ion contamination in this sample. In other samples this complex may be the base peak. Ions $(M+86)^+$ and $(M+104)^+$ are assigned to complexation of choline, $(CH_3)_3N^+CH_2CH_2OH$, and its dehydration product, $(CH_3)_3N^+CH{=}CH_2$, with M. The ion *m/z* 269, usually present in phosphatidyl cholines above the BAT, has been assigned to dicholine phosphate, $[(CH_3)_3N^+CH_2CH_2O]_2P(O)O^-$.

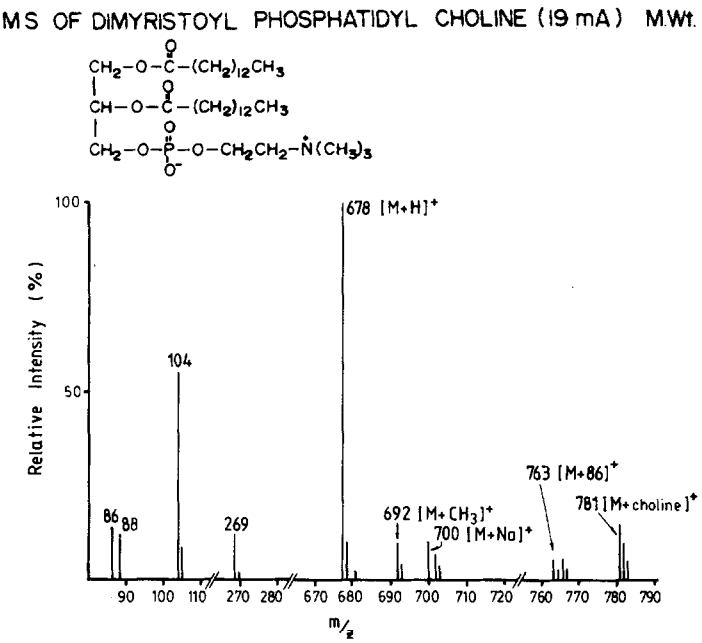


Fig. 26. FDMS of dimyristoylphosphatidyl choline, M = 677.5, at 19 mA.

This spectrum shows no *m/z* 495, indicating that sample heating has been cautious enough to avoid the otherwise prominent C–O cleavage to yield diacylglyceryl ions (the usual high mass ion in EI).

A survey of FDMS of complex lipids with various headgroups attached to diacylglycerol has been published,[50] and more recent evidence indicates that phosphatidic acids and other especially difficult samples yield good spectra after careful purification.[51] The phosphatidyl sulfocholines, in which choline is replaced by $(CH_3)_2S^+CH_2CH_2OH$, were also amenable to FDMS,[52] as was a series of 1-alken-1-yl-2-acyl-*sn*-glycero-3-phosphorylethanolamines.[53]

Membrane phospholipids. An application of FDMS to phospholipid-derived products has been presented as a collaborative effort from MIT.[54] The molecular arrangement within membranes may be inferred from noting the points of cross-linking between molecules submitted to photochemical reaction *in situ.* In this study phosphatidyl cholines were prepared with a photolabile diazo group as shown in Fig. 27. After irradiation and basic hydrolysis, two main products, A′ and B′, were isolated. These materials showed simple FD spectra with ions related to the intact molecule. The A′ products showed mainly $M+H^+$ whereas B′ products have weak $M+H^+$, dominant $M^{+\cdot}$, and some $M-H^+$. An attractive rationale for the difference between these products is that the presence of the CH_3O—C=C– in B′ introduces a readily ionizable site to a molecule which otherwise has high ionization potential ester sites which are subject to protonation. This work constitutes another example where FDMS contributed to structure elucidation, but other techniques, here chiefly EIMS and ^{19}F NMR, were also very valuable.

Nucleosides and nucleotides. A good deal of work has been reported on FDMS of nucleosides, and much of it has been reviewed.[5] Although these samples typically do not yield an intense FD spectrum, they generally give a reasonably abundant molecular ion (or M + H) and some fragmentation. As a measure of the reliable FD behavior of nucleosides, it may be mentioned that in the early days of the technique in North America a sample of adenosine was circulated in a form of round-robin to assure that the various laboratories had their technique up to standard. Nucleosides are still popular demonstration samples with manufacturers.

Of more current interest are the various combinations of phosphate with nucleosides to form nucleotides. Some of the problems encountered with these compounds were discussed in a preliminary paper by Budzikiewicz.[55] Such points as anode deterioration, desorption temperature and heating rate, variations in anode quality, low and erratic rates of ionic production, formation of clusters including doubly-charged ions, sample decomposition, and formation of artifacts, are all potential problems with non-volatile polar compounds, but problems which come together in disconcerting synergism in nucleotides. Careful reading of this paper[55] is recommended for anyone contemplating experimental

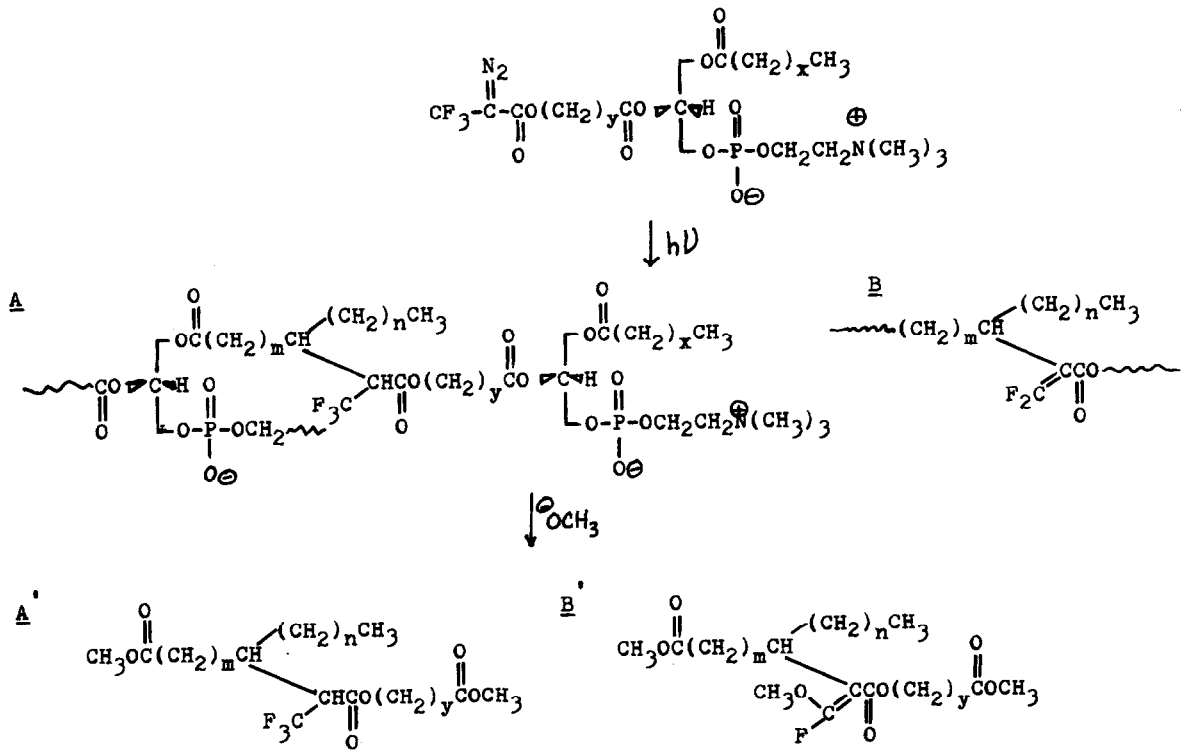


Fig. 27. Transformations of phosphatidyl choline containing photolabile group.

work in this area. While the litany of problems encountered here is still very much current, not everyone would subscribe to all of the explanations offered for their origins.

As early as 1973 an FD spectrum of adenosine-5′-monophosphate had been reported.[56] Integration of ions collected on a photographic plate over a range of anode temperatures yielded the peaks tabulated below:

m/z	%	assign
60		$(CHOH)_2^+$
61		$(CHOH)_2 + H^+$
99		$H_3PO_4 + H^+$
136	100	$B + H(C_5H_5N_5)$
137		$B + 2H$
267	29	adenosine
348	73	$M + H^+$

Since the compositions of these ions were confirmed by accurate mass measurements, the assignments are secure.

A definitive study of the dinucleoside phosphate CpA provided new insight on FDMS of these compounds.[57] Among the ions that were sufficiently abundant for accurate mass measurements (Fig. 28) were $(M+H)^+$, $(M+H)^+$-adenine, $(AMP+H)^+$, $(CMP+H)^+$, $(A+H)^+$, $(C+H)^+$, $(adenine+H)^+$ and $cytosine+H)^+$. The most obvious omission from this list of fragments is $(M+H)^+$-cytidine. Interesting ions found include (*cyclo* $AMP+H)^+$, (*cyclo* $CMP+H)^+$ and $(M+2H)^{2+}$.

The inverse dinucleotide, ApC, gave a substantially different FD spectrum, but the presence of Na^+ contamination leaves open the possibility that this factor is at least partially responsible for the different behavior.

Naturally occurring glycolipids. In what is claimed to be the first use of FDMS in structure proof of naturally occurring glycolipids and nucleosides, Komori, Schulten and co-workers[58] identified a cerebroside mixture and two nucleosides from the starfish, *Acanthaster planci.* The cerebroside mixture is

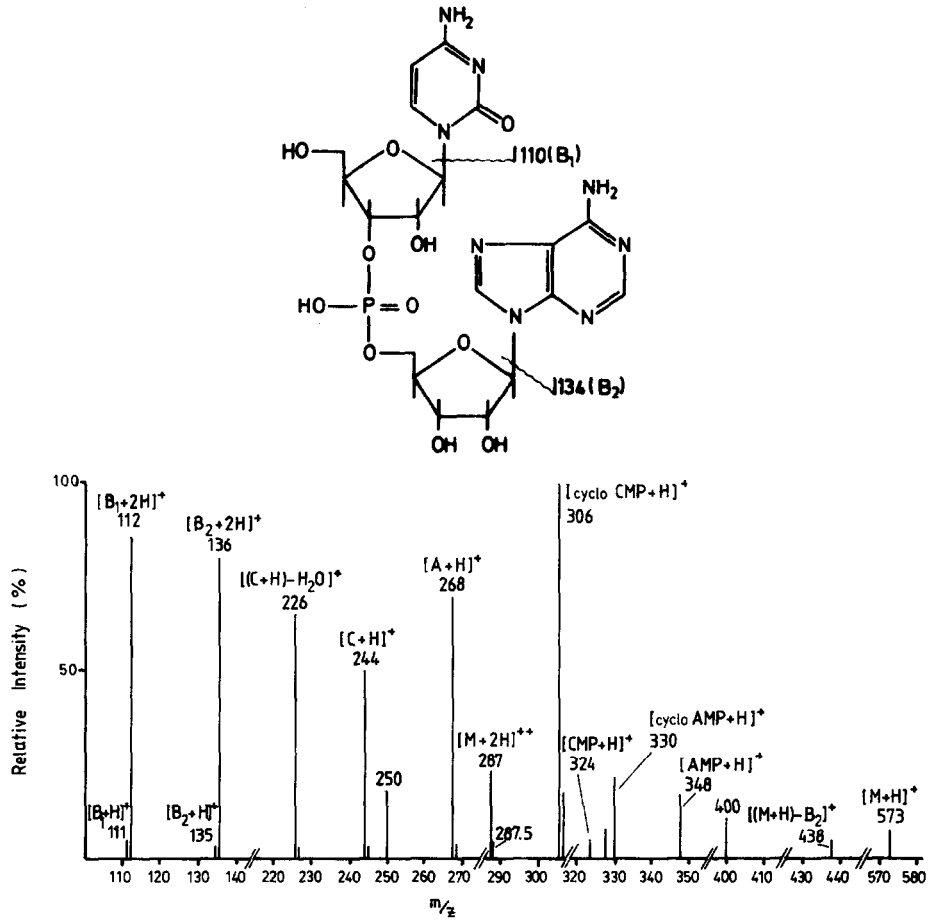


Fig. 28. FD mass spectrum of CpA.

represented by the structure shown where several homologues of the long chain bases are included (Fig. 29).

Major peaks in the FD spectrum in order of decreasing intensity were complexed with sodium:

m/z	long chain bases (m + n)	assigned formula
840	20 + 12, 19 + 13	
812		
826	19 + 12	
854	21 + 12, 20 + 13	$C_{47}H_{93}NO_{10} + Na$
798	16 + 13	
784	16 + 12	

Relative abundances of these ions do not reflect the amounts of long chain bases as determined by GC/MS. This discrepancy is most noticable for *m/z* 812 which must represent a long chain base not picked up by the independent analysis. Peracetylation of the mixture produced an FD spectrum in which only one ion was significant, *m/z* 1112.7, corresponding to the protonated heptaacetate of $C_{46}H_{91}NO_{10}$. This latter ion, in the form of its sodium complex, is the base peak (*m/z* 840) in the underivatized mixture.

High resolution FDMS measurements on the molecular ion of one of the nucleosides yielded *m/z* 242.0900, corresponding to $C_{10}H_{14}N_2O_5$. That result, plus fragments at *m/z* 117 (S), and 126 (B + H), led to the assignment of thymidine. The other nucleoside gave *m/z* 228, 117, 112 by FDMS corresponding to deoxyuridine. For this compound the high resolution measurement was carried out in the EI mode on the diacetate.

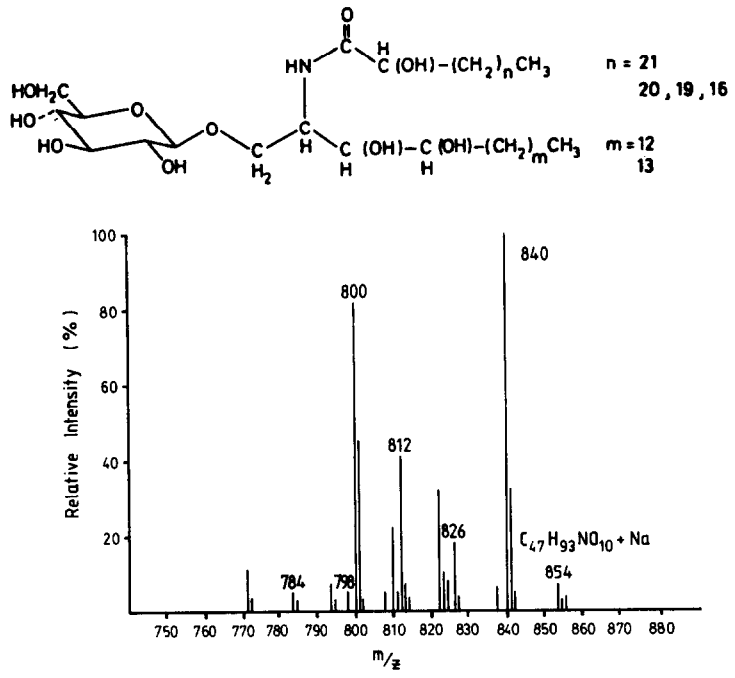


Fig. 29. FD mass spectrum of glycolipid mixture. The major component has n = 21, m = 12.

Peptide antibiotics. The presence of $(M+Na)^+$ peaks in an FD spectrum is often considered a nuisance, and indeed it may be. However, there are numerous cases where formation of alkali metal ion complexes with molecular ion has aided the investigation. A particularly instructive example was published by the Rinehart group[59] where these complex ions were formed by the deliberate addition of LiCl, NaCl, KCl or RbCl to the sample of a peptide antibiotic before analysis.

Antiamoebin I, from the FDMS molecular weights of tripropionate and tributyrate forms, should have had a molecular weight of 1692. While this weight did not fit with any of the compositions considered likely, it did correspond to a sodium ion complex of one of them ($C_{82}H_{127}N_{17}O_{20}Na$). This hypothesis was confirmed by addition of solutions of LiCl and KCl to samples of the triacetate (M = 1795). As shown in Fig. 30, Li^+ addition largely displaced Na^+ from the complex to yield a dominant peak at *m/z* 1802 $(M+{}^7Li)^+$, while K^+ similarly yielded a dominant peak at *m/z* 1834 $(M+{}^{39}K)^+$. Addition of Na^+ enhanced the *m/z* 1818 peak $(M+{}^{23}Na)^+$ and permitted a confirming

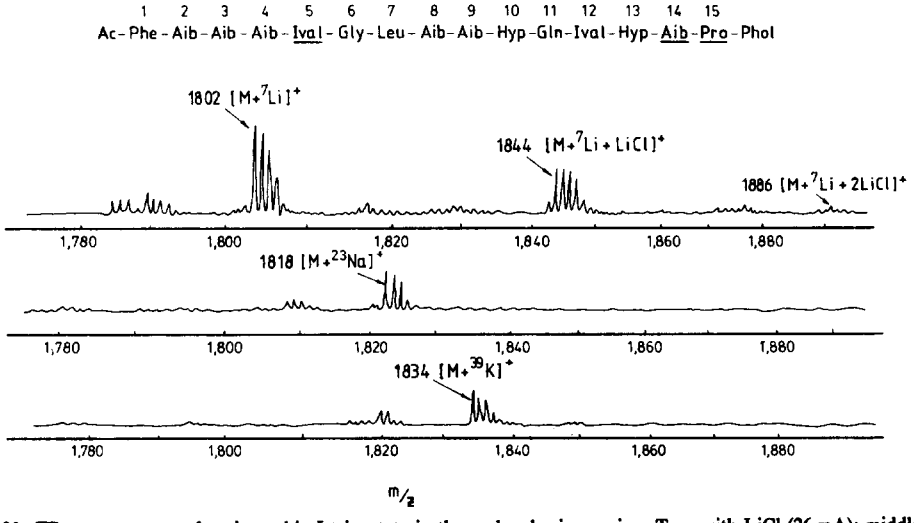


Fig. 30. FD mass spectra of antiamoebin I triacetate in the molecular ion region: Top, with LiCl (26 mA); middle, with NaCl (23 mA); bottom, with KCl (21 mA).

accurate mass measurement. The point was made by these workers, among others, that in addition to confirming the molecular weight by mass difference, this technique provides more abundant ions, and more stable ion production. It seems clear now that this advantage is to be expected whenever complexation of a difficultly ionizable molecule can be achieved, since this form of ion production obviates the necessity for electron removal. It will be interesting to see whether the potential for still further improvement in this technique by use of low lattice energy salts (e.g. $LiBPh_4$ as in Ref. 60) in place of chlorides will be realized.[22] A recent report in which eight quinomycin antibiotics were characterized by FDMS following cationization by lithium as the chloride[61] emphasized the value of having both $M^{+\cdot}$ and $(M+Li)^+$ ions. It is possible that more complete cationization by a lower lattice energy salt would thus be counter-productive.

Sugars. The monosaccharide D-glucose was the first compound used to evaluate FDMS[1] and sugars, especially sucrose, have since been used as test substances for virtually every alternative ion-forming process put forward. Much of the earlier work on FDMS of sugars has been reviewed,[5] and a number of compounds containing sugar moieties are discussed elsewhere in this review. In spite of the early and continued attention to these molecules, FD spectra of sugars are generally of low intensity and their desorption characteristics are not ideal. Among the techniques proposed for improving these spectra, cationization is the most promising.[62] In common with other classes of compounds which ionize with difficulty, sugars form even-electron ions by cation (H^+, Na^+, etc.) attachment to the neutral sugar molecule. These positive complexes desorb readily and give important structural information providing their nature is understood.

A recent investigation[63] has uncovered fragmentation differences among isomeric sugars complexed with K^+. Thus, field anodes pre-treated with KH_2PO_4 gave significantly different FD spectra for D-glucose and D-fructose (Fig. 31). A rationale for these differences, as well as comparative results for D-altrose, is presented in Fig. 32.

Cationization by divalent ions has been shown to result in doubly-charged ions for several sugars, including raffinose.[64] The spectrum presented in Table 3 has $(M+Ba)^{2+}$ as base peak with a manifold from m/z 318.9–322.0 representing the various barium isotopes.

Oligopeptides. Early work on FDMS of amino acids and oligopeptides has been reviewed,[5] and integration of this technique into a general procedure for elucidation of peptide structures has been described.[65]

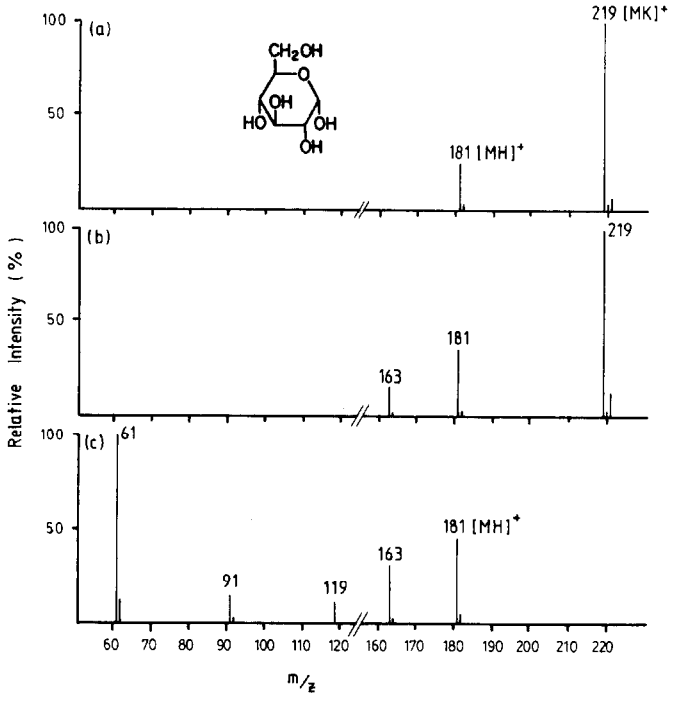


Fig. 31(a)

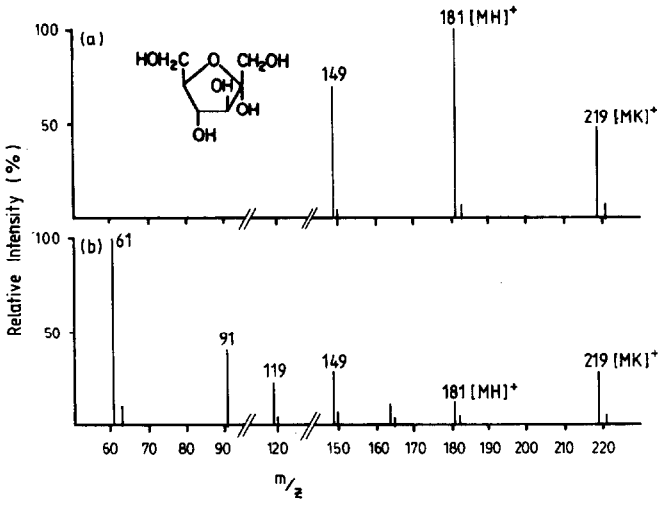


Fig. 31(b)

Fig. 31. Left panel: FD mass spectra of D-glucose at (a) 10 mA; (b) 15 mA; (c) 20 mA. Right panel: FD mass spectra of D-fructose at (a) 10 mA; (b) 20 mA.

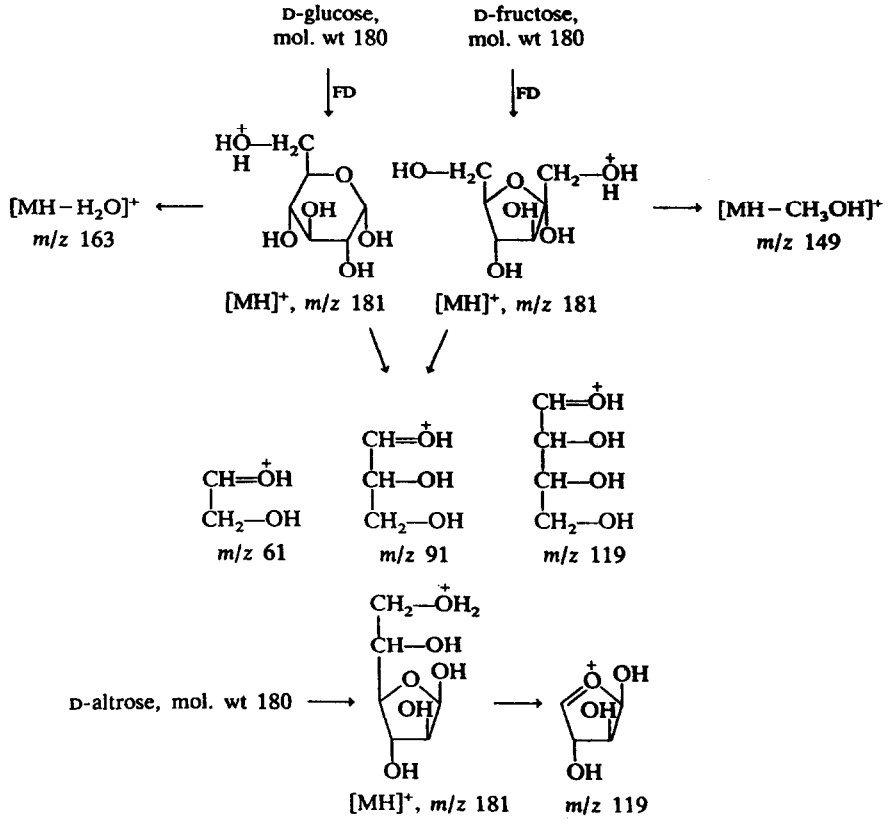


Fig. 32. Fragmentation scheme for FDMS of D-glucose, D-fructose and D-altrose.

The variety of pendant groups possible on an oligopeptide gives rise to difficulties for an analytical technique dependent on chemical interactions, such as FDMS. An extended, and at times heated, debate over the merits of FDMS for analysis and/or sequencing of peptides has been inconclusive. Recent studies using roughened but unactivated anodes,[66] derivatization,[67] collision induced dissociation,[68] pulsed FD[69] and linked scanning[70] reflect the considerable activity in this area, and no doubt some dissatisfaction with conventional FDMS results. These references provide an entry for anyone wishing to evaluate the state-of-the-art.

Table 3. FD mass spectra of raffinose ($C_{18}H_{32}O_{16}$, mol. wt = 504.2) with equimolar $Ba(NO_3)_2$ added (anode current = 20.2 mA)

m/z	%	Assignment	m/z	%	Assignment
240.1	1.5		541.8	9.4	$[M+K?]^+$
312.0	1.3		542.8	2.4	
318.9	4.0		563.8	3.0	
319.4	9.2		564.4	1.6	
320.0	14	$[M+Ba]^{2+}$	566.0	1.6	
320.5	26		571.1	2.9	
321.0	100		571.9	6.7	$[2M+Ba]^{2+}$
321.5	21		572.8	23	
322.0	5.2		573.9	2.3	
490.1	1.9		587.0	3.9	
490.7	7.4		594.0	4.8	
491.3	17		702.8	3.2	
491.8	43	$[2M-162+Ba]^{2+}$	703.7	18	
492.0	33		704.4	12	$[M+Ba(NO_3)]^+$
492.3	27	(^{138}Ba =492.1)	705.3	15	
492.8	11		775.4	2.7	
493.5	1.4		823.3	1.3	
526.9	7.1	$[M+Na]^+$	824.3	5.3	$[3M+Ba]^{2+}$
527.9	3.3		825.3	12	
			827.2	3.2	

Quantitation of trimethylammoniumbenzenesulfonate. A paper with an early demonstration of the quantitative capabilities of FDMS, as well as the proclivity for methyl transfer, involved analysis of trimethylammoniumbenzenesulfonate zwitterions for deuterium content.[71] The zwitterions arose in a novel solid-state reaction where molecular orientation in the crystal is responsible for significant rate enhancement of the conversion from methyl *p*-dimethylaminobenzenesulfonate (Fig. 33). In order to gain evidence for an intermolecular attack of N on ester CH_3 (sulfonate ion is the leaving group), it was desired to analyze the product zwitterion resulting from an equimolar mixture of d_0 and CD_3-d_9 labelled starting esters.

The FDMS data is presented in Table 4. From spectra of pure d_3 and d_6 zwitterions (entries 2, 3), it was apparent that FD was accompanied by a methyl transposition in the sense opposite from the original reaction. In spite of this interfering back-reaction, comparison of the reaction product (entry 5) with various known mixtures established that the original solid state reaction was at least 76% intermolecular (entry 10). These data were among the first demonstrations of the potential for quantitative measurements by FDMS. It may be noted that a number of scans were averaged to achieve this result. This procedure had been used earlier to demonstrate that precision of a set of measurements in FDMS improved with total ion collection time in a manner consistent with ion statistical considerations.

Table 4. Results of FDMS on trimethylammoniumbenzenesulfonates

Entry	No. Scans	Relative molar ZWT comp d_0	d_3	d_6	d_9	Observed peaks[a,b] 215	218	221	224
1		1	0	0	0	100			
2	9	0	1	0	0	46	100	45	3
3	9	0	0	1	0	3	41	100	51
4		0	0	0	1				100
5	24	MSE-d_0 and MSE-d_9 reaction mix				43	100	101	46
6	9	1	1	1	1	44	100	98	46
7	14	1	0	0	1	61	100	94	65
8	8	2	1	1	2	49	100	101	54
9	12	1.74	1	1	1.74	47	100	98	52
10	18	1.6	1	1	1.6	46	100	99	47
11	10	0.7	1	1	0.7	44	100	96	39
12	12	0.3	1	1	0.3	37	100	98	34

[a] Intensities normalized so that peak at 218 = 100 (except entries 1, 3, 4). [b] Average standard deviation = ±5 units.

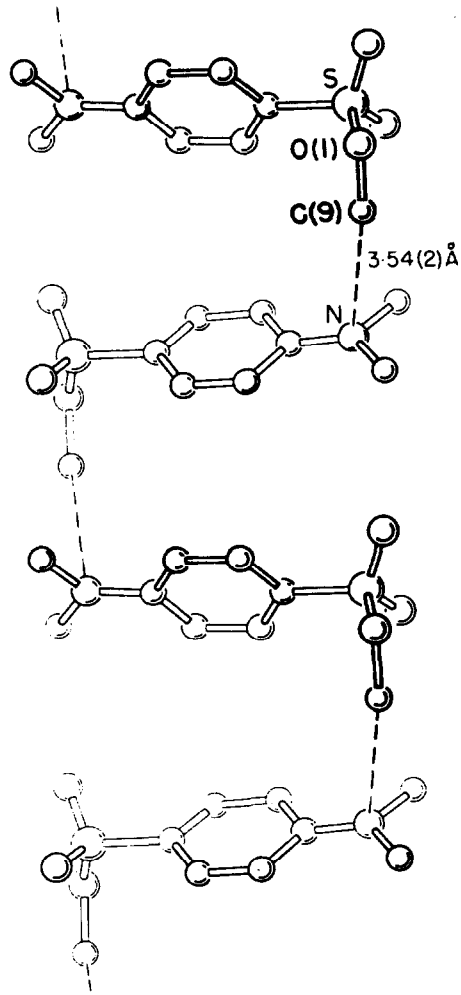


Fig. 33. A view of the stacking along one chain of molecules in crystals of methyl *p*-dimethylaminobenzenesulfonate.

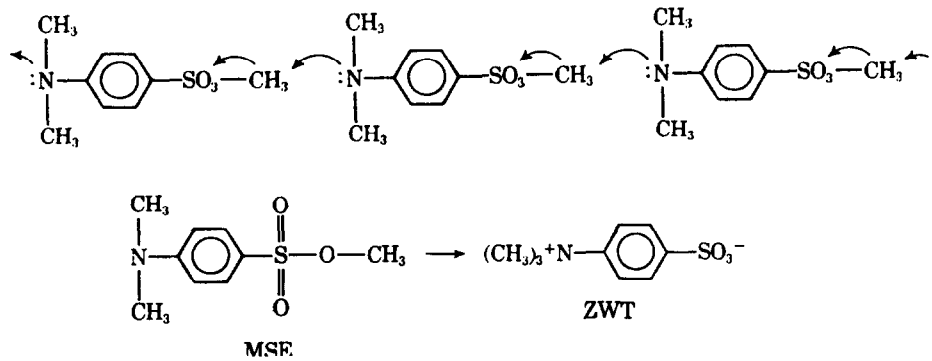


Fig. 34. Schematic representation of conversion of sulfonate ester to zwitterion.

Anode chemistry and pre-treatment. Not surprisingly, the technique of FD has developed in a number of directions with little serious attention being paid to mechanisms. Recent contributions have made a beginning in this area, particularly in bringing together a number of divergent experiments. One important contribution to this thrust was made by Ligon[72] who reported on some experiments with polyphosphorphoric acid (PPA) coated bare wires as FD anodes. Under these conditions FI (electron loss to anode) is eliminated so that "pure" FD spectra result in spite of the fact that the sample is introduced in the gas phase. For example, isopropyl alcohol, M = 60, yields *m/z* 60, 45 (base peak) under

FI conditions. The FD experiment (PPA-coated bare wire) yields *m/z* 121, 61 (base peak), 43, corresponding to $(2M+H)^+$, $(M+H)^+$ and $(M+H-H_2O)^+$. Several such experiments contrast the dependence of FI sensitivity on ionization potential and the dependence of FD sensitivity on ease of protonation as well as the difference between ionization (FI) and cation extraction (FD). This paper is recommended for anyone wishing to establish a context for many of the earlier FD experiments where the FI/FD overlap was less clearly defined. Comparisons with the related technique of electrohydrodynamic ionization are also illuminating.

For an organic chemist, one of the intriguing aspects of FDMS is that the anode may be considered a micro-reactor in which chemical transformations are taking place before, during and after desorption. To some extent these reactions might be predictable from a knowledge of temperatures (50–250°), exposure time, and surface condition of the anode. However, other factors, especially the high field strength, are often invoked to rationalize otherwise unexpected behavior. In this section, some examples will be presented of chemistry observed on normal anodes as well as some attempts at anode modification for a particular purpose.

Certainly the simplest and most widespread technique for influencing FD behavior is the addition of cations, usually from one of the alkali metals, to the sample.[73] Applications of this technique have been described in this review for polyether and peptide antibiotics as well as sugars, and further discussion will not be presented here, except to underline the opinion stated earlier that the influence of the counter-ion accompanying the added cation has probably not been adequately recognized.[22]

One of the earliest attempts to influence physicochemical interactions on the anode by addition of covalent compounds involved a mixture of polyvinyl alcohol, sucrose and NaCl.[74] This paper no doubt provided the impetus for much of the subsequent work to be presented here.

Phosphatidyl cholines and other zwitterionic compounds produce dominant $(M+H^+)$ ions under favorable FDMS conditions.[50] Keough and DeStefano have shown that formation of this key ion can be enhanced by addition of p-toluenesulfonic acid (*p*TSA) to the sample solution.[75] As an illustration of the value of this technique to mixture analysis and quantitation, an equimolar mixture of five ammoniocarboxylates $C_nH_{2n+1}\overset{+}{N}Me_2(CH_2)_5CO_2^-$ (n = 8, 10, 12, 14, 16) was field-desorbed before and after addition of *p*TSA. Whereas the original mixture desorbed at 20 mA and showed fragments corresponding to decarboxylation and methyl transfer, the acid-doped mixture desorbed at 14 mA to generate the 5 expected $(M+H)^+$ peaks with equal intensity (± 1%) and no other peaks above 10% relative abundance.

Bare wire anodes (unactivated) coated with polyphosphoric acid were found to be powerful agents of protonation by Ligon.[72] Thus, not only were bases such as pyridine protonated to give intense $(M+H)^+$ ions, but much weaker bases such as benzene also yielded $(M+H)^+$ ions. Recognition from this work that effective acidity appears to be increased in the high-field, solvent-free environment has led to a variety of protonation experiments using weaker acids.

Tartaric acid is still another matrix material demonstrated to influence FD spectra.[76,77] Treatment of a silicon anode with tartaric acid in anhydrous ethanol and solvent removal prior to addition of a peptide sample was shown to enhance protonated ions, such as $(M+H)^+$, and especially multiply protonated ions, $(M+2H)^{2+}$, etc. (Table 5).

Table 5. Peptides examined on tartaric acid-treated anodes

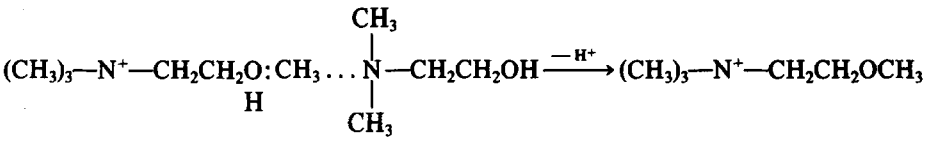
Peptides	n_+	M. W.	Observed ions
H-His-His-OH	2	292	$(M+H)^+$, $(M+2H)^{2+}$
H-Lys-Lys-Met-(Pro-Leu-Gly)$_3$-OH	2	1206	$(M+H)^+$, $(M+2H)^{2+}$
H-Lys-Lys-Met-(Pro-Leu-Gly)$_4$-OH	2	1473	$(M+H)^+$, $(M+2H)^{2+}$
H-Lys-Lys-Met-(Pro-Leu-Gly)$_6$-OH	2	1740	$(M+H)^+$, $(M+2H)^{2+}$
H-Lys-Lys-Met-(Pro-Leu-Gly)$_8$-OH	2	2541	$(M+H)^+$, $(M+2H)^{2+}$
H-Arg-Pro-Pro-Gly-Phe-Ser -Pro-Phe-Arg-OH	2	1059	$(M+H)^+$, $(M+2H)^{2+}$
H-Lys-Lys-Gly-Lys-Lys-Gly-OH	4	644	$(M+H)^+$, $(M+2H)^{2+}$, $(M+3H)^{3+}$, $(M+4H)^{4+}$

n_+: number of basic side chains.

One of the more interesting aspects of this report is the effective expansion of the mass scale illustrated by the fifth entry where an oligopeptide of molecular weight 2541 would be registered as $(M+2H)^{2+}$ near *m/z* 1271.5.

A recent report from Bursey's laboratory[78] shows that chemically bonded carboxyl groups yielded FD anodes which are strongly acidic. Considering the results just discussed for unbonded tartaric acid, it may be necessary to re-examine the necessity of covalent binding of the acid surface group to obtain the advantage of enhanced acidity.

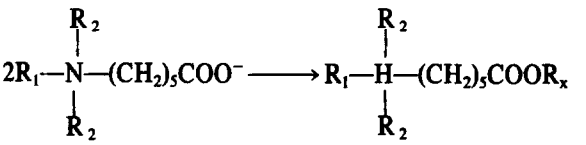
Several references have been made in this review to methyl transfer reactions accompanying FDMS. This process was first documented in choline halides where, by labelling experiments, the transfer was shown to be an intermolecular process involving hydroxyl oxygen attack on ammonium methyl.[21]



m/z 104 *m/z* 118

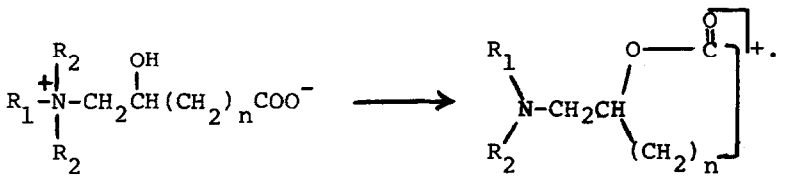
Similarly, the source of the $(M+14)^+$ peak in phosphatidyl cholines was shown by labelling to be CH_3 from the trimethylammonium headgroup.[79] In this case the transfer terminus (attacking nucleophile) is presumed to be phosphate oxygen.

A series of quaternary ammoniohexanoates studied by Keough *et al.* showed intermolecular transfer of methyl, ethyl, propyl, and even decyl groups.[80]



As might be expected, alkyl transfer is most important for CH_3 ($R_1 = C_{10}H_{21}$; $R_2 = CH_3 > R_1 = C_{22}H_{45}$; $R_2 = CH_3$), but $(M+29)^+$ and $(M+43)^+$ are also major ions when $R_1 = C_{22}H_{45}$ and $R_2 = C_2H_5$ or C_3H_7, respectively. The $(M+141)^+$ peak, corresponding to transfer of R_1 when $R_1 = C_{10}H_{21}$, $R_2 = CH_3$, is of very low intensity, but is qualitatively reproducible.

Another chemical reaction accompanying FDMS is lactonization of hydroxyammoniocarboxylates.[81] This reaction is favored by high anode temperature, and generates the base peak from a variety of structures of the type



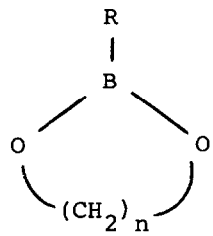
[M] $[M - R_2OH]^{+\cdot}$

The mechanism for this process is suggested to involve alkyl transfer to form a dialkylaminoester, which then cyclizes with loss of alcohol. Infrared evidence for lactone formation during pyrolysis of these samples suggests that the process is not field-dependent.

Earlier experiments with several hydroxycarboxylic acids also produced ions that appeared to be esters or lactones, depending on chain length.[82] The most interesting molecule in this study was 3-hydroxypropanoic acid which generated a series of polyester ions, $xM-(x-1)H_2O+H^+$, extending to the limit of the mass scale (x = 12). Influence of the field could not be ruled out, but it appeared that the dominant effect was thermal since the base peak shifted with anode temperature in the expected way. That is, at 0 mA the base peak was $(M+H)^+$ corresponding to x = 1, and starting at 10 mA the base peak shifted up with increasing anode current through x = 6 at 18 mA to x = 11 at 23 mA. At each temperature an envelope of 2–6 adjacent members of the series was found.

Bursey *et al.* have undertaken a wide-ranging series of experiments on anode modification for FI/FD. In one approach[83] anodes were coated with boric acid prior to introduction of alcohols through the heated inlet (FI) or by coating the anode (FD). Some evidence of borate ester ions, $(RO)_3B$, was found for the more volatile alcohols (e.g. $PhCH_2OH$) under FI conditions, but esterification proceeded more completely under FD conditions. For example, $n\text{-}C_{20}H_{41}OH$ yielded triester peaks of intensity equal to those of ionized alcohol at 0 mA. At 30 mA only the triester ions were seen. Surprisingly, in none of these experiments were peaks found corresponding to the presumed intermediate mono- and di-esters.

This work was extended to diols and polyols by the use of the involatile 1-naphthalenylboronic acid for coating the anode.[84] A variety of diols differing in separation between the hydroxyl groups and in stereochemistry were shown to form cyclic boronate esters.



n = 2–6

Some differences in relative intensity for this ion were observed, but in general the reaction was not sensitive to arrangement of the diol hydroxyls. However, the strained medium rings (n = 5, 6) formed from 1,5-pentanediol and 1,6-hexanediol are of modest intensity compared to corresponding ions with twice their mass, suggesting the formation of unstrained 16- and 18-membered rings.

5. CONCLUSIONS

The technique of field desorption mass spectrometry has progressed through introduction in one academic laboratory in 1969, commercialization by one instrument manufacturer in 1972, widening availability and application since 1974, and general acceptance as the method-of-choice for many problems (since 1976). Recently, several innovations in methods for mass spectrometry of non-volatile compounds have challenged the supremacy of FDMS in this area, the most recent being fast atom bombardment (FAB). Of all the alternatives proposed, this last one appears to have attracted the most attention. Whatever the outcome of this challenge, at the present time FDMS has established itself as an important tool for solution of a broad range of organic chemistry problems. A sampling of these problems has been presented in this review, along with a frank assessment of the pitfalls inherent in this technique.

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