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Fast Atom Bombardment Mass Spectrometry

Kenneth L. Rinehart, Jr.

Summary. Fast atom bombardment mass spectrometry has become a powerful structural tool since the first reports of its use in 1981. Samples are ionized in the condensed state, usually in a glycerol matrix, by bombarding the matrix with xenon or argon atoms with energies of 5000 to 10,000 electron volts. This yields both positive and negative secondary ions, which are sputtered from the surface. The technique has been used to detect inorganic ion clusters to mass 25,800 and biologically active peptides to mass 5700, and it gives molecular ions of such highly polar or labile organic compounds as glycosphingolipids and polyene antibiotics. It can be especially valuable in determining the sequences of amino acids in polypeptides.

Recent developments in mass spectrometry have made it possible to ionize molecules of interest to biologists and biologically oriented chemists and to provide answers to vexing biological problems (*1, 2*). It is now possible to obtain mass spectra of compounds with molecular weights above 12,500 (*3*) and to obtain detailed structural information on compounds with molecular weights as high as 2800 (*4, 5*). In addition to being of high molecular weight, many of these compounds are highly polar and of generally low volatility.

The new developments in mass spectrometry which have achieved this success partly involved building larger magnets to deal with the larger molecules, but mainly involved designing methods for ionizing molecules in the solid state and desorbing them into the vapor state, where they can be detected. A number of these methods are discussed elsewhere in this issue by Busch and Cooks (*6*).

Among the recently developed ionization methods, fast atom bombardment (FAB) is the newest (*7–9*) and in several respects the most successful. Not only can it provide precise masses for molecular ions of biologically active peptides with high molecular weights, but it can provide nearly complete amino acid sequences of many of these peptides and structural information deduced from their fragmentation loci on other classes of compounds as well. FAB mass spectrometry and its applications to peptides and other biologically important compounds will be the subject of this article.

Fast Atom Bombardment Mass Spectrometry

The overall diagram of the equipment required for FAB mass spectrometry is shown in Fig. 1. This method is closely related to another condensed state ionization method, secondary ion mass spectrometry (*9a*), but different from SIMS in that the accelerated inert gas ions (preferably xenon) (*5, 10, 11*) employed in SIMS undergo charge neutralization by electron capture or charge exchange, giving rise to accelerated inert gas atoms. The residual ions may be removed by deflector plates (Fig. 1) and the accelerated atoms allowed to bombard the sample, which is usually dispersed in a glycerol solution or matrix, giving rise to both "molecular" ions, which include M+H and M+Na ions, and fragment ions. As with SIMS, a reasonable mechanism may involve "sputtering" or "splashing" the sample

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into the vapor state with attached residual debris from molecules destroyed by the atom beam. This desorption is presumed to take place from the surface monolayer and often with dispersant (glycerol) molecules attached as well.

The heart of the FAB equipment is the ion gun, in which the inert gas ions are accelerated to high energy before undergoing neutralization (Fig. 1). Normally, the ions are accelerated to 3 to 10 kiloelectron volts. Although argon is a satisfactory inert gas, xenon gives about a threefold increase in sample ion intensity, perhaps because of its greater mass and enhanced momentum.

Once the atoms emerge from the ion gun, they enter the ion source of the spectrometer (Fig. 1) and are directed onto the glycerol matrix containing the sample. An approximately 70° angle of incidence (20° angle vis-à-vis the sample) appears optimal. Ribbons of various metals and nonmetals have been employed for the solid surface on which the glycerol matrix is placed; more substantial solid-metal probes are also satisfactory. It is not necessary to use a liquid dispersant to produce ions from the sample, but it does assist greatly in maintaining the intensity of sample ions by regenerating the surface so that fresh sample ions can be extracted from it. With neat solid samples, ion current lifetimes are usually short and depend on the rate of destruction of the sample, in part by polymerization (*12, 13*). Neat liquid samples are satisfactory, but large samples are required. Glycerol is not the only useful dispersant, but it is one of the best because it is able to dissolve many of the highly polar compounds of greatest interest and its low volatility ensures a relatively long matrix lifetime. Other useful matrices are thioglycerol, polyethylene glycols such as tetragol (tetraethylene glycol) and its dimethyl ether tetraglyme, sulfolane (tetramethylene sulfone), diethanolamine, diamylphenol, crown ethers, and substituted ureas. Ethylene glycol and water are, unfortunately, too volatile.

The types of analyzer and detector used in the mass spectrometer appear not to be critical for success; single- and double-focusing magnetic instruments as well as triple analyzers (*14*) have been employed, as have quadrupole (*8, 15*) mass spectrometers. Time-of-flight instruments should be suitable, since they are successfully employed with SIMS (*16*). Indeed, one of the appealing aspects of FAB mass spectrometry is its simplicity. New ion sources can be purchased for old spectrometers, or a FAB ion gun can be attached with relatively little modification and expense to an existing FD, EI, or CI ion source (*17*).

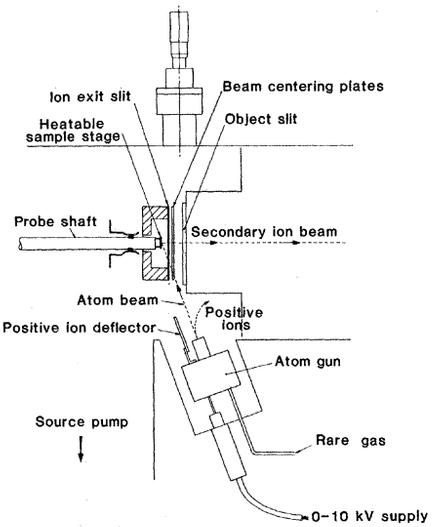

Fig. 1. Diagram of fast atom bombardment ion source. The secondary ion beam provides the mass spectrum. [Courtesy of VG Analytical, Altrincham, England]

The other standard operations of a modern mass spectrometer can also be carried out while measuring FAB mass spectra. Metastable studies to assign ion fragmentation pathways are possible (*9*), as are collisional activation experiments to enhance fragmentation (*15*). A recent study showed that mass spectra (including relative intensities) obtained by FAB are reproducible and the mass spectra are very similar for samples analyzed with widely different instrumentation (*18*).

Relation of FAB to SIMS. Almost from the first announcement of FAB, there has been considerable discussion of how closely related it is to SIMS (*19*). On a theoretical level it has been argued that the bombarding ions in SIMS, through the Auger effect, become, effectively, bombarding atoms (*19*). Experimentally, the same ion gun is used in both methods, and it was demonstrated recently that the beam from the ion gun used in SIMS contains many neutral atoms (*20*). On the other hand, turning off the deflector plates, which are installed to remove ions from the FAB beam (Fig. 1), still allows a spectrum much like the FAB spectrum to be produced. In addition, it has been noted that Devienne and Roustan (*21*), in the course of SIMS studies, earlier carried out FAB experiments on organic compounds. Perhaps most important, recent SIMS studies demonstrate that results can be obtained with SIMS that are very similar to those obtainable with FAB (*22*).

The announcement of the FAB technique by Barber *et al.* (*7*) and Surman and Vickerman (*8*) stimulated efforts throughout the world with respect to the process, as well as a number of symposia concerned in whole or in part with its explication or application (*23–25*). The major contribution of these workers was the demonstration that large, highly polar organic molecules can be profitably studied by the technique. A second contribution was the introduction of the use of a liquid matrix for solid samples, and a third was the use of a glancing angle (70° angle of incidence) for the bombarding atom beam.

Characteristics of FAB

Mass Spectrometry

Fast atom bombardment mass spectrometry has been reported to be a highly sensitive technique, capable of detecting samples as small as 5 nanograms (*26*). However, until recently quantitative data were not available to substantiate this claim. The measurements needed are difficult to make, since they are greatly dependent on sample concentration in a heterogeneous mixture, salt impurities, and other factors. Nevertheless, it now seems clear that the sensitivity of FAB is comparable to that of FD, when one takes into account the considerably higher abundance of fragment ions obtained with the FAB technique (*17*). One would probably not choose FAB for molecules that give good ion intensities in EI mass spectrometry; however, for the intractable, barely volatile compounds for which FAB was designed, it is a sensitive procedure.

Both the molecular size and the complexity of the compounds amenable to FAB study are impressive. Palytoxin, a potent marine toxin (*27, 28*), is one recent example of a compound whose molecular ion in FAB was sufficiently intense for accurate mass measurements to be carried out (*29*).

The study of peptides has been especially successful (*5, 26, 30–32*), but oligosaccharides (*33, 34*), trinucleotides (*35*), nucleotide adducts (*36, 37*), adenosine triphosphate (*18*), glycosphingolipids (*38, 39*), steroid conjugates (*40, 41*), leukotrienes (*42, 43*), antibiotics (*31, 35, 44, 45*), and organic salts (*46*) as well as inorganic (*47*) and organometallic compounds (*48*) have also been amenable to FAB mass spectrometry. Similar results have recently been obtained for several of the same classes of compounds with SIMS. In our laboratory we have attempted to obtain the FAB spectra of more than 400 compounds, many of which do not give useful spectra by other

methods. We employed four different FAB ion sources on three different mass spectrometers, and more than 90 percent of the samples gave useful spectra (*17*). We now consider FAB to be a routine technique.

Spectra can be obtained equally well for positive and negative ions and the two modes often complement one another. Low-resolution spectra of these compounds can be recorded by most of the procedures employed for other types of mass spectrometry. Computerized data reduction can be employed (*49*) with masses calibrated against standards such as perfluorokerosene, trisperfluoroheptyltriazine, trisperfluorononyltriazine, or Fomblin. For high masses, oscillographic recording has been the standard procedure, with masses assigned by either peak matching (for instance, against phosphazenes or alkali halide clusters) or manual counting, employing one of the characteristics of FAB mass spectra: the occurrence of weak peaks at nearly every mass in a sort of ubiquitous low-level background spectrum. In addition to those low-intensity peaks, there are much stronger peaks (sometimes more intense than sample peaks) at masses corresponding to multiples of glycerol molecules plus some added cation. For example, one series of such ions appears at mass-to-charge ratio (*m/z*) 93, 185, 277, . . . for $[(C_3H_8O_3)_n + H]^+$; another series at *m/z* 115, 207, 299, . . . for $[(C_3H_8O_3)_n + Na]^+$; and another at *m/z* 75, 167, 259, . . . for $[(C_3H_8O_3)_n + H - H_2O]^+$. A similar series, at *m/z* 91, 183, 275, . . . for $[(C_3H_8O_3)_n - H]^-$ is prominent in negative-ion FAB spectra. The intensities of these peaks diminish with mass but increase with the energy and abundance of the bombarding rare gas atoms. The presence of these matrix ions is an advantage in providing mass markers, but a disadvantage in obscuring sample peaks. For this reason, we first study total unknowns by FD mass spectrometry, which provides simpler spectra that lack such glycerol peaks and contain a preponderance of molecular ions rather than a mixture of molecular and fragment ions.

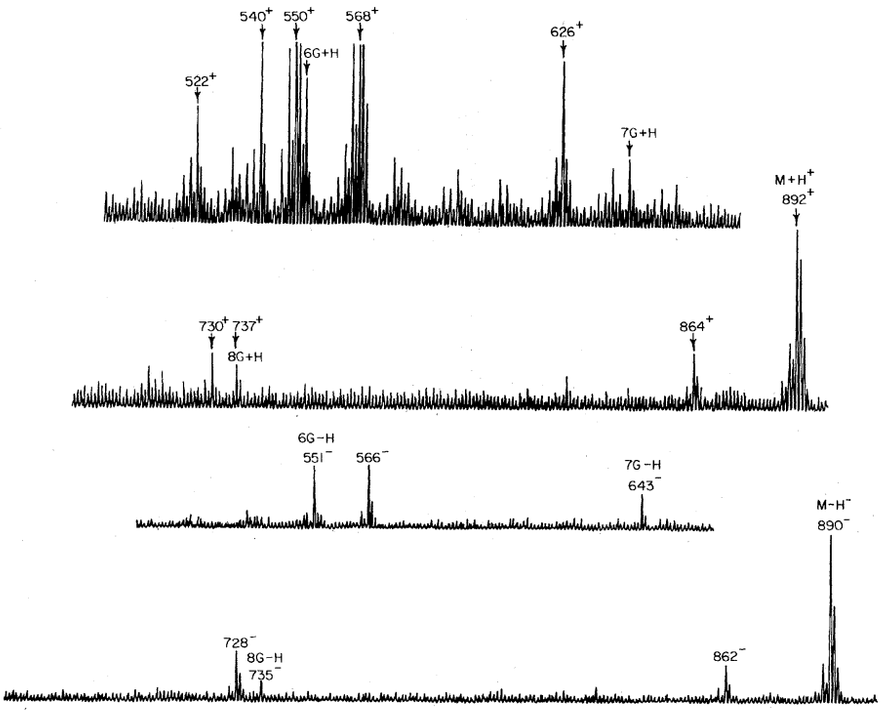

Fig. 2. Fast atom bombardment mass spectra of lactosylceramide (**1** in Fig. 3). The top two charts show the positive ion spectrum; the bottom two charts the negative ion spectrum. Ions labeled $nG \pm H$ are due to the glycerol matrix.

High-resolution mass spectrometry is also feasible with FAB. Precise mass measurements have been carried out with peak matching to masses above 2800 (*29*) and with photoplates to masses around 1100 (*50*). Photoplates may be the method of choice for recording a very large number of ions at high resolution in this low mass range, where glycerol background peaks from the FAB spectra can be employed as standards (*50*).

Peak matching is more laborious, since each accurate mass must be determined separately, but it is amenable to the determination of higher masses with substances such as phosphazenes (*51*), rubidium or cesium iodide clusters (*29*), or Fomblin (*50*) used as standards. For these measurements a multichannel signal averager (MSA) (*52*) has proved quite useful. In this procedure the sample is ionized by FAB and the standard is ionized by EI (or another method) and the two peaks are overlaid (matched) by the computer. Measurements of more than 40 compounds by the MSA technique have recently been reported (*17*). The precision with this technique varied but was usually within 5 parts per million, which is adequate for distinguishing between competing isobaric possibilities, although inadequate for assigning molecular formulas de novo at higher masses.

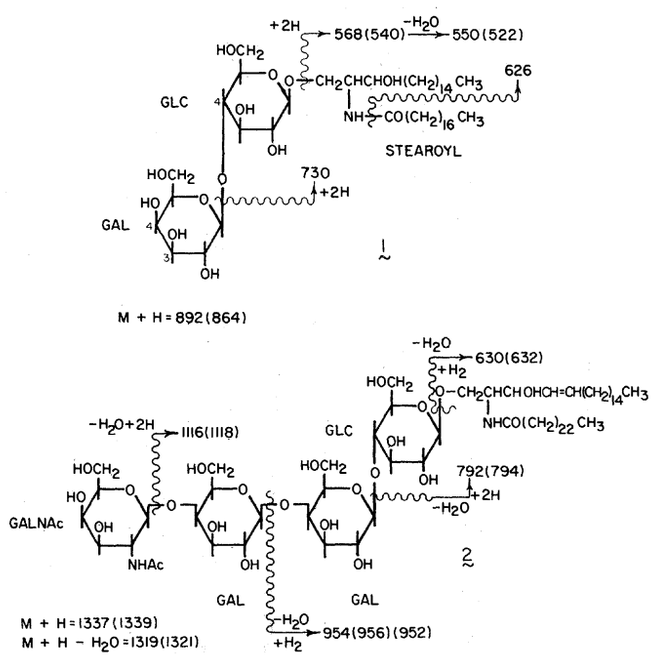

Fig. 3. FAB fragmentation patterns of lactosylceramide (**1**) and human erythrocyte globoside I (**2**).

Application of FAB to Structural Problems

The best measure of the success of FAB mass spectrometry is its ability to provide solutions to otherwise intractable problems. To illustrate this, examples have been chosen from studies in our laboratory of three classes of compounds—glycosphingolipids, polyene antibiotics, and polypeptides.

Glycosphingolipids. Glycosphingolipids consist of mixtures of naturally occurring compounds of the general formula R–CH(OR′)–CH(NHCOR″)–CH_2OH, where R and R″ are long-chain saturated or unsaturated alkyl groups and R′ is an oligosaccharide substituent (*53*). In the past, mass spectrometry has contributed to the characterization of glycosphingolipids in two ways. Hydrolysis or methanolysis of the compounds gives mixtures of sugars, fatty acids, and sphingosines, all of which can be characterized in the form of appropriate derivatives by gas chromatography–mass spectrometry. Posthydrolysis characterization does not, however, link particular R, R′, and R″ groups. Such linking has been attempted with some success by derivatization of the intact glycosphingolipids, followed by CI mass spectrometry (*53*), but every chemical modification introduces an element of uncertainty into the characterization. The FAB spectra of glycosphingolipids, on the other hand, provide intense ions without derivatization, including molecular ions and a number of fragment ions (Fig. 2). Similar results have been obtained elsewhere with both FAB (*52*) and FD (*54*) mass spectrometry.

Study of the FAB fragmentations of the intact, underivatized glycosphingolipids is revealing. The fragmentations follow a general rule in FAB, that major cleavages occur predominantly at carbon-heteroatom (C–X) bonds. In this case, these are within the oligosaccharide unit and at the amide (N–CO) bond. The former identify the sequence of oligosaccharide units (Fig. 3), although, of course, they cannot distinguish between isomeric possibilities such as glucose and galactose. The latter link specific R and R″ groups and provide an estimate of the proportional compositions of mixtures of glycosphingolipids. Two examples will serve to illustrate the fragmentations. In the first and simpler example, that of lactosylceramide (**1** in Fig. 3), successive losses of galactosyl and glucosyl units are observed in one series and loss of stearoyl in another (Fig. 2). The occurrence of peaks 28 mass units lower (values in parentheses in Fig. 3) for the former series but not for the latter argues for the existence of homology in the fatty acyl group—that is, partial replacement of stearoyl by palmityl.

In the second example, human erythrocyte globoside I (**2** in Fig. 3), no cleavage of the ceramide bond was observed, but successive cleavages in the tetrose chain indicate that the oligosaccharide sequence is terminated by *N*-acetylgalactosamine. While these two glycosphingolipids are known compounds and are thus examples of spectral characterization rather than structure proof, spectra of other underivatized glycosphingolipids indicated that much more complex, but readily characterizable, mixtures were present than was previously suspected (*39*).

Polyene antibiotics. The polyene antibiotics have long posed a challenge to mass spectrometry. None of them yields meaningful mass spectral data by EI or CI techniques. Until recently, FD was the principal source of useful mass spectral information, giving molecular ions

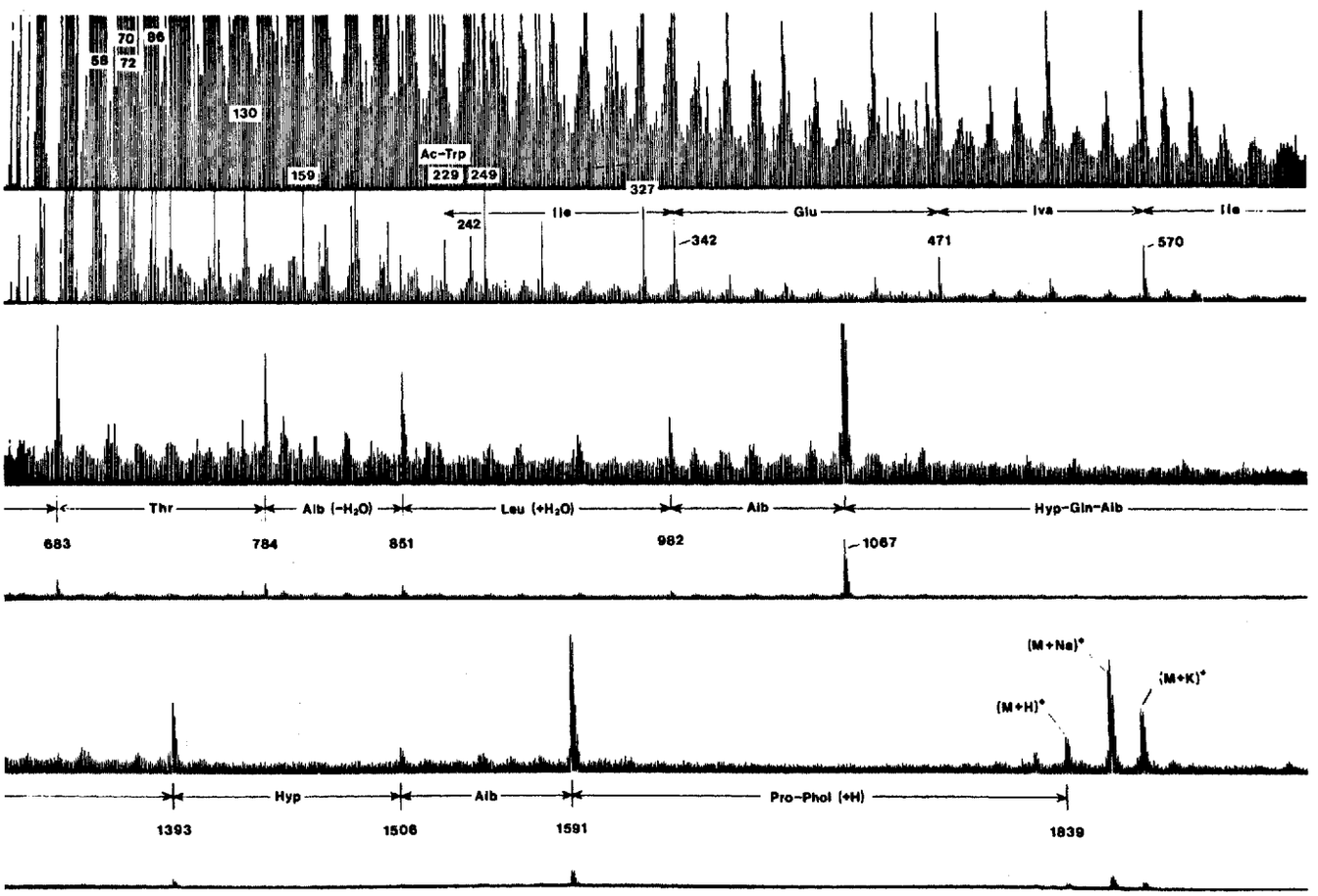

Fig. 4. Positive ion FAB mass spectrum of zervamicin IC. The peptide antibiotic's sequence (structure) is also shown in Fig. 5.

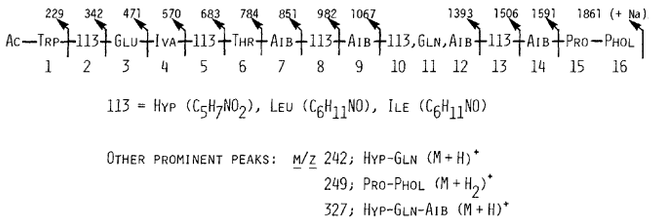

Fig. 5. FAB fragment ions for zervamicin IC.

for the neutral, non-ionic pentaenes and heptaenes like filipin and dermostatin (*55*). However, the more intractable tetraenes and heptaenes—massive zwitterions with carboxyl and amino sugar functions—gave no information even with FD, although some simpler members gave molecular ions with PD mass spectrometry. Thus it was of great help when FAB was shown to give molecular ions in both the positive- and negative-ion modes, as well as fragment ions indicative of loss of the mycosamine sugar unit (*44*). For example, the tetraene antibiotic pimaricin, used clinically for the treatment of ocular fungal infections, gives positive FAB ions at *m/z* 666, 648, 503, 485, and 467 (M + H, M + H − H_2O, M + H − S, M + H − S − H_2O, and M + H − S − $2H_2O$, respectively, where S is the sugar moiety) and negative FAB ions at *m/z* 664 and 502 (M − H and M − S, respectively). Similarly, partricin A (*56*), a constituent of mepartricin (*57*), which is used clinically, gives a positive FAB ion at *m/z* 1127 (M + H) and negative FAB ions at *m/z* 1125 and 963 (M − H and M − S, respectively). As with the glycosphingolipids, the major points of cleavage are the C–X bonds, with the fragmentations serving to characterize the sugar component of the antibiotic.

The examples given above are of polyene antibiotics with previously assigned structures, which the FAB spectra served to confirm. In another example, however, FAB was employed to characterize a number of amphotericin B derivatives with unknown structures by making use of the preferred loss of the sugar unit. Amphotericin B methyl ester (AME) has been proposed as a less toxic clinical substitute for amphotericin B, the present drug of choice for treating systemic mycoses (*58*). In clinical trials of AME a number of cases of neuropsychiatric aberrations and leukoencephalopathy were observed and the drug was implicated. A sample of the AME employed was subjected to high-performance liquid chromatography, which yielded a number of heptaene-containing peaks (*59*). These chromatographic peaks were individually examined by FAB, with the results found in Table 1. It was found that peak 3 contained a monomethylated amphotericin, presumably the expected AME, but that the other peaks were the results of overmethylation of amphotericin B, with up to four methyl groups being introduced in addition to the expected methyl ester. In each case, the FAB spectrum revealed that the overmethylation took place on the mycosamine unit, since the same M − sugar peak was observed at *m/z* 775, with related peaks due to loss of water.

Polypeptides. Of all the classes of compounds investigated by FAB, polypeptides have provided the most abundant examples of useful information. Judging from recent reports, this also appears to be true of SIMS (*60*). Three types of information can usually be extracted from a peptide's FAB spectrum (*31*, *61*): (i) the molecular ion provides the molecular weight or composition of the peptide, (ii) ions at low masses are characteristic of individual amino acids, and (iii), most important, ions at intermediate masses provide details of the sequence of the amino acids in the peptide.

A particularly good example is the zervamicin complex of peptide antibiotics (*31*). Zervamicins belong to the peptaibophol (*62*) antibiotics, but did not yield to the FD-based general procedure developed (*63*) for dealing with this class of antibiotics, due, in part at least, to the presence of tryptophan. However, the FAB mass spectra provided almost all the information sought, as can be illustrated for the major acidic component of the zervamicin complex. Zervamicin IC gives a molecular ion (M + H) at *m/z* 1839 in the positive-ion mode (Fig. 4) and a corresponding M − H ion at *m/z* 1837 in the negative-ion mode. Ions at *m/z* 58, 70, 72, 86, and 130 were observed, characteristic of Aib, Pro, Iva, Leu (and Ile, and Hyp), and Trp, respectively (*64*). The spectrum also contained sufficient fragment ions for a nearly complete assignment of the sequence of zervamicin IC, as shown in Fig. 4 and Fig. 5. High-resolution data allowed a distinction between the isobaric Leu (or Ile) and Hyp units at positions 10 and 13. Although the

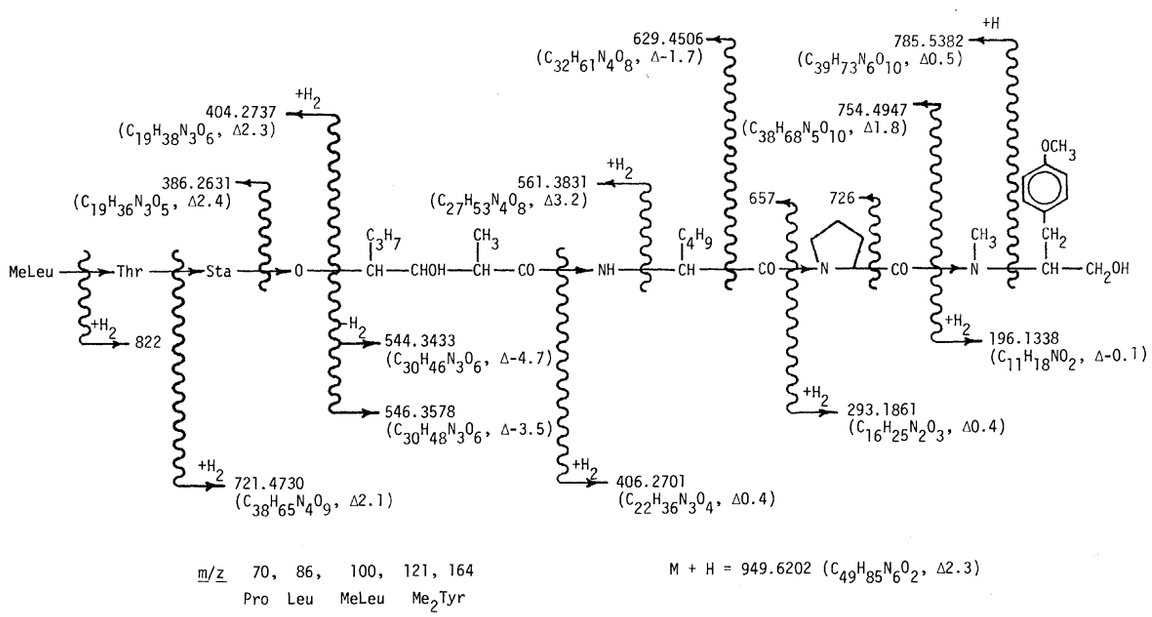

Fig. 6. FAB fragmentation pattern of hexahydrodidemnin A.

remaining few pieces of sequence information came from the identification of oligopeptides obtained by chemical degradation, the molecular formula and nearly the entire sequence were determined from the FAB spectrum (*31*).

Similar arguments were applied to the other components of the zervamicin complex, based on their FAB spectra, to assign their structures (*31*). Particularly significant in the zervamicin work was the rather clean, regular fragmentation at the CO–N bonds of the polypeptide, with charge retention on the protected NH_2-terminal fragment (Fig. 5).

Exclusive charge retention on the NH_2-terminal fragments is not always found, even for linear peptides. An example is hexahydrodidemnin A (Fig. 6), a sodium borohydride reduction product of didemnin A, the simplest of a family of tunicate-derived antiviral and antitumor cyclic depsipeptides (*65*). The FAB fragmentation pattern of hexahydrodidemnin A shown in Fig. 6 gives charged fragments from both the NH_2 and reduced COOH termini, which would be adequate for unequivocal assignment of the structure of the peptide, especially when bolstered by the high-resolution data shown.

With cyclic peptides even more complex fragmentations are found. Thus, didemnins A and B (the latter slated for early clinical trial as an antitumor agent) fragment in the manner shown in Fig. 7. Since their structures had been assigned before they were investigated by FAB, they are not true examples of de novo structure assignment. However, by use of the high-resolution data shown, their structures could have been assigned from their FAB mass spectra.

The examples shown here and in the two preceding sections demonstrate the power of FAB mass spectrometry, especially when high-resolution data are included, in assigning structures to nonvolatile organic compounds, and suggest that FAB may be the method of choice for dealing with novel peptides.

Table 1. Results of FAB mass spectral analysis of AME chromatographic peaks (S = mycosamine, M = 163).

AME peak	CH_3	Positive ions									Negative ions				
						M + H − S − nH_2O									
		M + K	M + Na	M + H	M + H − H_2O	$n = 0$	$n = 1$	$n = 2$	$n = 3$	$n = 4$	M − H	M − H + H_2O	M − H − COOH	M − S	M − S − H_2O
1*	5	1032	1016				757				992			774	756
2*	3	1004	988			775	757	739			964			774	756
3*	1		960	938	920	775	757	739	721	703	936†	918		774	756
3‡	1		960	938	920		757								
4‡	1			938	920§	775	757	739	721	703 ‖					
5‡	2			952	934		757								
6‡	3			966	948										
6B‡	3			966									919		
7‡	3			966			757								

*Determined on a ZAB mass spectrometer at VG Analytical, Altrincham, England. †Also M − H + HCl at *m/z* 972. ‡Determined on a Finnigan MAT311A mass spectrometer at the University of Illinois, Urbana. §Also M + H − $2H_2O$ at *m/z* 902. ‖ Also M + H − S − $5H_2O$ at *m/z* 685.

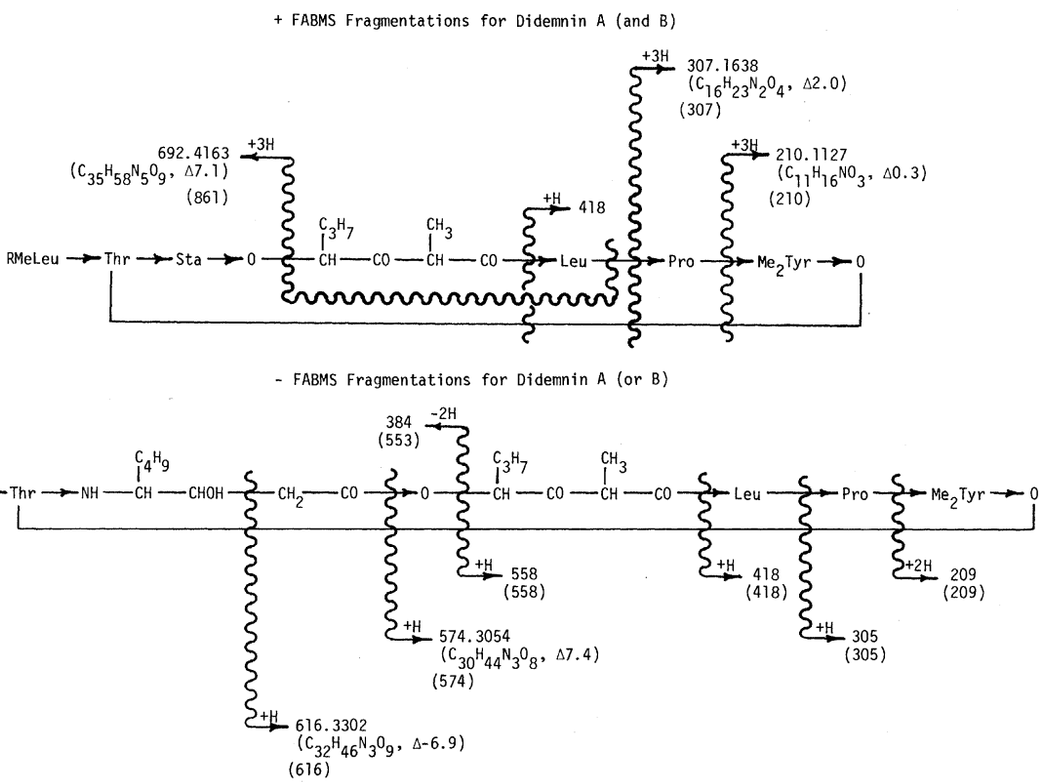

Fig. 7. FAB fragmentation patterns of didemnins A and B.

Future Developments

For such a new technique, FAB has made a remarkable impact on organic mass spectrometry. Much of its future development lies in refining and extending the present technique. For example, it is still not known how large a molecule can be lifted from the matrix surface by FAB—that is, whether it can compete with PD. This question can be answered properly only with time-of-flight mass spectrometers (or perhaps with Fourier transform mass spectrometers) with their great mass range, although it can be approached with the larger magnets now available, which have already yielded results above *m/z* 5700 for biologically interesting molecules (*4*) and above *m/z* 25,800 for inorganic ion (cesium iodide) clusters (*66*).

The question of sensitivity is still not completely resolved and remains difficult since FAB depends so much on the matrix. The same is true of isotope ratio measurements, though these appear promising on the basis of our results with diacetyl and bis(trideuterioacetyl) didemnins to quantitate crude extracts (*67*) and results elsewhere [Gaskell *et al.* (*40*)] on steroid sulfates. A universal dispersant remains a desirable, though elusive, goal, and new matrices will continue to be tested. Similarly, mass markers for high-resolution FAB will continue to be a high priority, especially for computerized data reduction of high-resolution data. New bombarding atoms—perhaps mercury (*68*) or cesium (*69*)—may become important.

Aside from these extensions of the present technique, perhaps the most exciting prospect is for a linking of FAB with HPLC, since the ability of FAB to ionize polar molecules in the solid state seems particularly suited to the moving-belt approach to liquid chromatography–mass spectrometry. Some preliminary results have been reported, both for FAB (*70*) and SIMS (*71*), and many more attempts will certainly be made to ionize (and vaporize) polar molecules from a moving belt (such as Mylar) containing the HPLC effluent. Some of these attempts should succeed. Even if they do not, FAB has become a routine mass spectrometric tool in the space of 1½ years, and its future prospects should mirror its past success.

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64. Abbreviations (used in text and figures): Gal, galactose; Glc, glucose; GalNAc, *N*-acetylgalactosamine; Aib, α-aminoisobutyric acid; Pro, proline; Iva, isovaline, α-ethylalanine; Leu, leucine; Ile, isoleucine; Hyp, hydroxyproline; Glu, glutamic acid; Gln, glutamine; Trp, tryptophan; Phol, phenylalaninol; MeLeu, *N*-methylleucine; Thr, threonine; Sta, statine, 4-amino-3-hydroxy-6-methylheptanoic acid; and Me_2Tyr, *N*,O-dimethyltyrosine.
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