An Easy and Powerful Technique of Negative Ion Fast Atom Bombardment Mass Spectrometry employing a Crown Ether Matrix

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The fast atom bombardment (F.A.B.) mass spectrometry of free adenosine triphosphate [ATP, (**4**)] as well as glutathione has been examined, employing a crown ether, (**2**), in the search for a good matrix material.

The development of fast atom bombardment (F.A.B.) mass spectrometry has enabled the molecular weight determination of large non-volatile organic molecules, particularly those of biological interest.[1] Generally, glycerol has been used as a matrix because it facilitates the production of sample ions in high abundance for relatively long periods. Molecular weight information is usually obtained from the $(M + H)^+$ and $(M - H)^-$ ions by positive and negative ion spectra, respectively. It is believed that the $(M + H)^+$ and $(M - H)^-$ ions are formed by proton transfer reactions in the glycerol matrix.[2] In the course of our studies on 3,4-crowned morphinanone (**1**),[3] we have found that the positive ion F.A.B. mass spectrum of (**1**) clearly shows the intensive molecular ion species, $(M + H)^+$, with negligibly small amounts of background ions derived from glycerol. This indicates the effective proton transfer from glycerol to the crown ether moiety of (**1**). We report here the easy and powerful method of molecular ion determination by negative ion F.A.B. mass spectrometry employing a crown ether matrix.

At first, we examined the mass spectral properties of 15-crown-5 (**2**) on a glycerol matrix: the molecular ion and its fragments were detected as the significantly intense ion peaks in the positive ion spectra, and no background ion species derived from glycerol were detected. However, when the spectrum of (**2**) was measured in the presence of sodium ions, the intense $(M + 23)^+$ ion peak instead of the $(M + H)^+$ ion peak and a number of the glycerol background ion species were detected.† On the other hand, in the negative ion spectra, no ion species derived from (**2**) were detected, indicating that (**2**) cannot be a proton donor in the F.A.B. ionization mechanism. In accordance with this, tetrodotoxin (**3**), which can be regarded as an oxo-cage compound

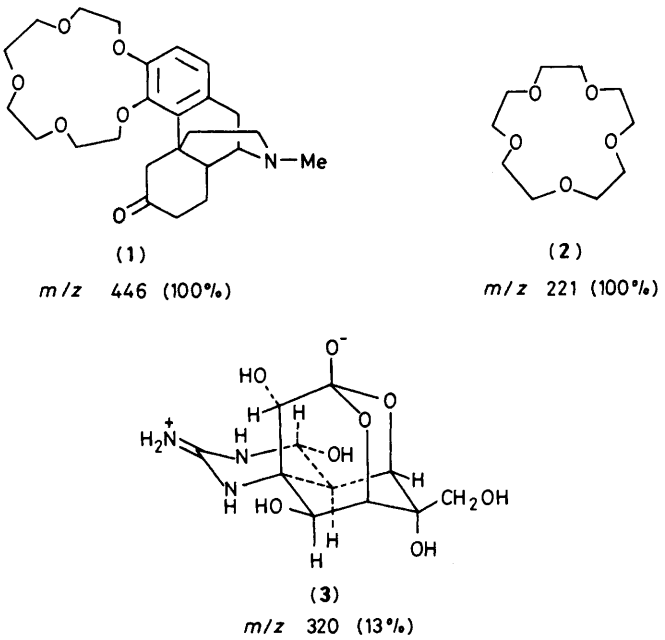

Figure 1. Relative intensities of protonated molecular ions above *m/z* 1.0 obtained from the positive ion F.A.B. mass spectra of 3,4-crowned morphinanone (**1**), 15-crown-5 (**2**), and tetrodotoxin (**3**).

† These results agree with the well-known properties of crown ethers which have the capacity of selectively binding alkali metal ions in their endopolarophilic cavity, ref. 4.

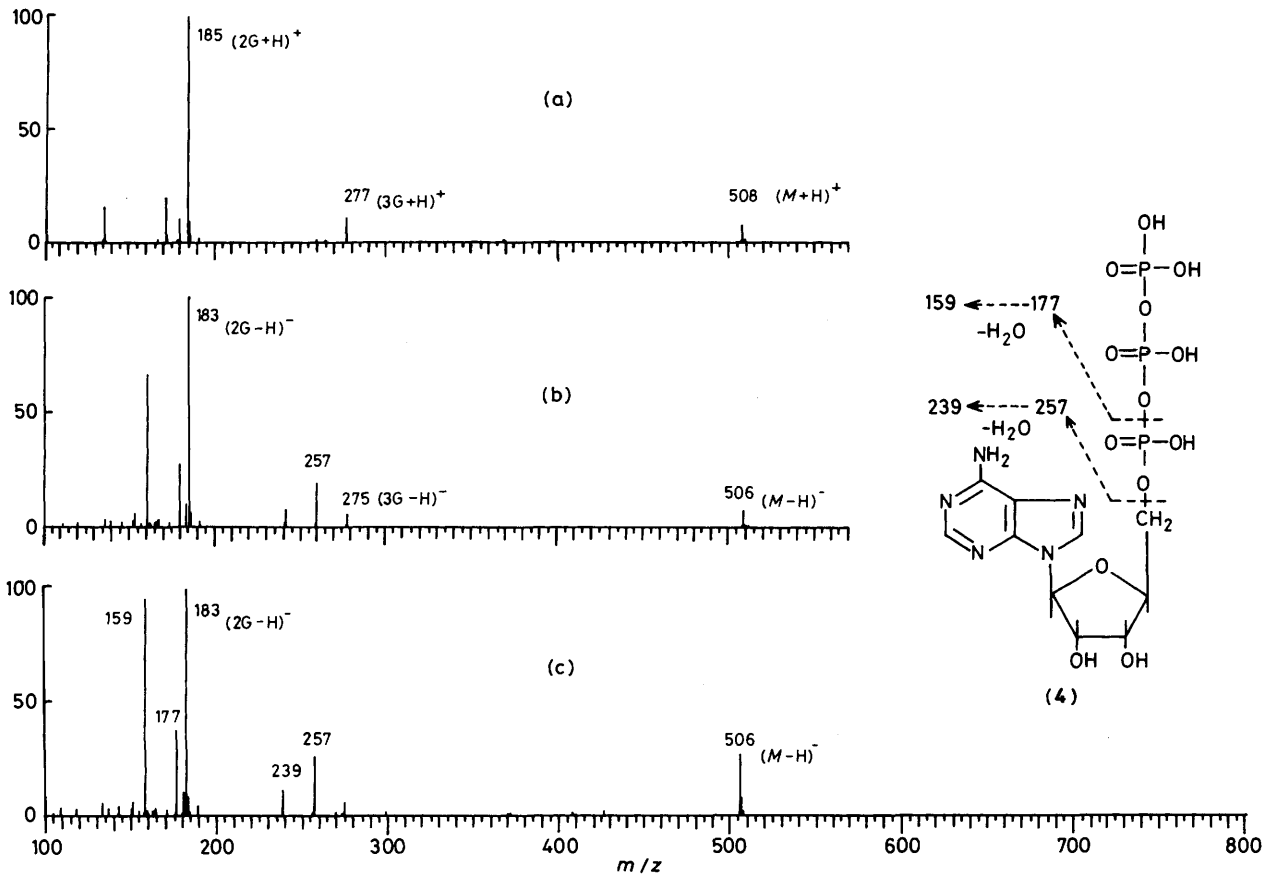

Figure 2. F.A.B. mass spectra of free ATP: (a) positive ion spectrum, glycerol matrix; (b) negative ion spectrum, glycerol matrix; (c) negative ion spectrum, crown ether–glycerol matrix.

possessing an analogous structure to crown ethers, showed the intense molecular ion species at *m/z* 320 $(M + H)^+$ in the positive ion spectra.

These results led us to employ crown ethers as a new matrix for negative ion F.A.B. mass spectrometry. Thus, the F.A.B. mass spectra of free adenosine triphosphate [ATP, (**4**)] and glutathione were measured using the following operation conditions: an aqueous solution of the sample was mixed with glycerol and a drop of (**2**) was added to the sample which was then analysed.‡ As shown in Figure 2, the negative ion spectrum of (**4**) in the presence of crown ether (**2**), Figure 2(c), displayed clearly the molecular ion species at *m/z* 506, $(M - H)^-$, with an intensity three times that of the same species in the case of the glycerol-only matrix, and the fragment ion species at *m/z* 257, 239, 177, and 159. In the case of glutathione, addition of (**2**) to glycerol increased the intensity of the molecular ion species five times compared with using glycerol alone.§

Although a matrix (1,1,3,3-tetramethylurea and triethanolamine)[5] has been developed for further application of F.A.B. mass spectrometry to the characterization of labile and non-volatile organic molecules, the new method reported here is more convenient in practice and superior in detecting the molecular ion and its fragment ion particularly for acidic compounds. The interesting effect of crown ether as a matrix can be attributed to its ability to capture protons from acidic samples.

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‡ FABMS: JMS DX-300/JMS-3500 data system, postive and negative ions were produced by bombardment of 6 keV accelerated Xe^0 atom beam.

§ The effect of the matrix on the intensity of glutathione was as follows: in the case of glycerol alone, *m/z*(%), 91(100), 183(61), 275(6), 306(14); in the case of crown ether–glycerol, *m/z*(%), 91(100), 183(87), 275(7), 306(74).

References

1 R. G. Finke, M. W. Droege, J. C. Cook, and K. S. Suslick, *J. Am. Chem. Soc.*, 1984, **106**, 5750; M. Barber, R. S. Sedgwick, and A. N. Tyler, *Nature*, 1981, **293**, 270.
2 D. H. Williams, C. Bradley, G. Bojesen, S. Santikarn, and L. C. E. Taylor, *J. Am. Chem. Soc.*, 1981, **103**, 5700.
3 I. Fujii, K. Hayakawa, and K. Kanematsu, *Tetrahedron Lett.*, 1984, **25**, 3335.
4 F. Vogtle and E. Weber, 'The Chemistry of Ethers, Crown Ethers, Hydroxyl Groups and their Sulphur Analogues,' ed. S. Patai, Wiley, Chichester, 1980, Part 1, Chapter 2.
5 M. Arita, M. Iwamoto, T. Higuchi, and Y. Nagai, *J. Biochem.*, 1983, **93**, 319.