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# BROAD SCOPE SECONDARY ION MASS SPECTROMETRY[1]

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ABSTRACT.—The general technique of secondary ion mass spectrometry has been substantially improved by the discovery that use of sulfolane as solvent containing, *e.g.*, lithium iodide, sodium iodide, silver tetrafluoroborate, or thallium tetrafluoroborate, will routinely provide easily detectable molecular ion complexes.

Reliable mass measurements are a fundamental requirement for structural elucidation of important animal and plant biosynthetic products. Indeed, rapid developments in mass spectrometric techniques and instrumentation over the past decade have greatly assisted in structural analysis of, for example, new and potentially useful antineoplastic agents of natural origin. Among these newer techniques (2-17), the desorption ionization methods such as field (2) and plasma (with $^{127}I$ and $^{252}Cf$) desorption (3,4) and variations of secondary ion mass spectrometry (SIMS) (5) with atomic ions or neutral atoms (fast atom bombardment, FAB) (6-17) have proved to be especially useful for obtaining molecular ions from complex natural products. Fast atom mass spectrometry was pioneered by Devienne (6) and advanced by Vickerman (7,9) and Barber and Bordoli (8), who developed the technique of sputtering ions from glycerol solution by fast atom or ion impact.

In our experience, mass spectrometry of ions sputtered from solvents, mainly glycerol, has been the single most important advance in determining the mass of natural products in recent years. While a good advance, the glycerol approach has not proved routinely successful. Therefore, we evaluated a great variety of biosynthetic products (more than 200, including derivatives in a mass range $<2000$), such as alkaloids, antibiotics, carbohydrates, nucleotides, peptides, terpenes, and steroids, while investigating the possibility of discovering uniformly successful techniques for obtaining unambiguous molecular weight data through enhancement of molecular ion complexes[2] [quasi-molecular ions (13)] of the type $[M+C]^+$ where C is an incorporated metal ion.

We now report that 0.14 molar solutions of lithium or sodium iodides (or trifluoromethanesulfonates) in tetramethylene sulfone (sulfolane)[3] as dispersant affords optimal molecular ion complex enhancement. Attachment of metal to a particular ion was verified, using a mixture of both iodides. Alternatively, the $[M+Rb]^+$ ions arising from use of the somewhat less effective rubidium iodide were easily detected due to the distinctive $^{85}Rb$ and $^{87}Rb$ isotope pattern (ratio of 72:28). We also report that a majority of relatively nonpolar and polar compounds in sulfolane, containing silver tetrafluoroborate (or trifluoromethanesulfonate, 0.16 molar solution), gave strong $[M+Ag]^+$ molecular ion complexes that were easily recognized by the $^{107}Ag$ and $^{109}Ag$ isotopes (ratio of 52:48).[4]

[1]Dedicated to Professor Carl Djerassi on the occasion of his 60th birthday. Contribution 92 of the series Antineoplastic Agents; for Part 91 see reference (1).

[2]We propose the designation molecular ion complex as a more chemically meaningful alternative to the terms pseudo- or quasi-molecular ion.

[3]After completion of this study sulfolane was mentioned in a review among a list of seven solvent types that included crown ethers and polyethylene glycols as possible useful alternatives to glycerol [see reference (14)]. To our knowledge there is no prior published report using sulfolane in FAB procedures.

[4]Ion sputtering from a silver surface has been used to obtain $[M+Ag]^+$ ions in the SIMS spectra of kasugamycin and lividomycin. See references (18) and (19-21) for related studies.

Comparison of glycerol and a variety of other low vapor pressure solvent possibilities, such as dimethylsulfoxide, hexamethylphosphoramide, and thioglycerol, showed sulfolane to be more generally useful for both polar and relatively nonpolar substances. Addition of trifluoromethanesulfonic acid (0.03-0.07 molar solution) to sulfolane or *p*-toluenesulfonic acid[5] to glycerol generally yielded only weak enhancement of protonated molecular ions $[M+H]^+$, except with fairly nonpolar and weak bases such as yohimbine (**1**). Here the increase in $[M+H]^+$ was greater than tenfold. The beneficial effects decreased at higher acid concentrations with a concommitant increase in fragmentation and a decrease in $[M+H]^+$ intensity. A survey of divalent metal salts (0.05-0.5 molar solutions) such as magnesium (II) chloride gave detectable (weak) molecular ion complexes of the type $[M-H+Mg]^+$ and $[M+Mg+Cl]^+$. The former were exhibited by substances containing readily exchangeable hydrogen atoms,[6] but were usually less abundant than the $[M+H]^+$ ions. No example of $[M+Mg]^{++}$ type ions were observed, possibly because of the relatively high heat of their formation in the gas phase.

The most useful and consistent molecular ion complex enhancements were obtained by addition of group IA metal ions, and this leads to a significant reduction in fragmentation. Good spectra were obtained with group IA metal iodides in concentrations near 0.14 molar in sulfolane. Lithium and sodium iodides gave comparably excellent results that decreased, proceeding to cesium iodide (*ca.* 50% less effective). In glycerol, thioglycerol, and tetraglyme, the group IA metal iodides gave optimal results at concentrations near 0.26, 0.22, and 0.20 M, respectively. However, the use of protic solvents with certain polar compounds required up to 0.5-M concentrations to suppress $[M+H]^+$ formation. The quasimolecular ions obtained with sulfolane were considerably more intense than those detected employing, *e.g.*, glycerol (up to five times less), thioglycerol (up to three times less) and tetraglyme (up to ten times less). Most importantly, the use of sulfolane with a group IA metal iodide led to good molecular ion complexes derived from, for example, 2,4,6-trichlorophenol esters of amino acids, other such active esters of some peptides, diazoketones, and important antineoplastic biosynthetic products such as bryostatin 1 (**2**) (figure 1) (24) that otherwise failed to yield molecular ions.

A study of other monovalent cations indicated that silver tetrafluoroborate was especially useful in sulfolane and about equal to lithium or sodium iodide. With some types of compounds such as carminomycin 1 (**3**, figure 2) (25), a silver ion led to a greater than fivefold increase in the quasimolecular ion over that recorded for the sodium ion (figure 2). And this may be due to a very favorable chelation reaction with silver. The isotopes of silver produce remarkably useful mass spectra bearing easily recognized doublets that specifically identify those ions containing the metal cation and greatly simplify detection of molecular ions. Also, the silver ion mass spectra were not complicated by $[M-H+2\ Ag]^+$ cluster ions observed in some SIMS spectra.[4] While thallium (I) tetrafluoroborate or trifluoromethanesulfonate were found somewhat less effective than silver tetrafluoroborate, the $[M+Tl]^+$ ions (in a ratio of 30:70 for $^{203}Tl$ and $^{205}Tl$) proved similar to $[M+Na]^+$ ions in respect to showing little tendency to fragment. The $[M+Ag]^+$ ions appear in this regard to reside between $[M+H]^+$ and $[M+Na]^+$. The utility of such fragmentation reactions and their application in structural determinations (*cf.*, figure 1) is presently under study.

Results of the preceding investigation clearly point out that solution phase secondary ion mass spectrometry, employing sulfolane containing the lithium, sodium,

[5]In a series of SIMS experiments *p*-toluenesulfonic acid led to > fivefold enhancement of $[M+H]^+$ [see reference (22)].

[6]Bleomycin complexes of the type $[M-H+Cu]^+$ have been observed by FAB; see reference (23).

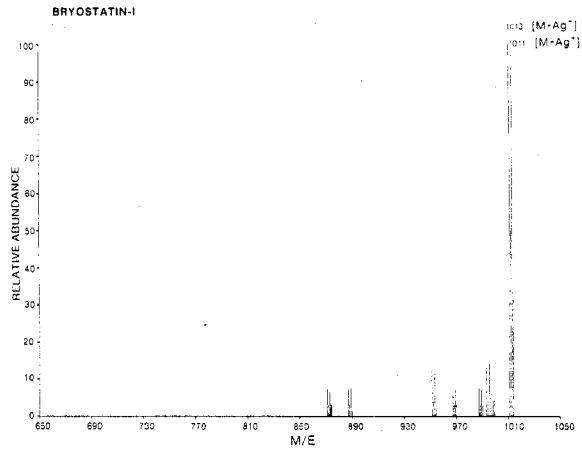



FIGURE 1. Positive ion spectrum of bryostatin 1 (0.5 mg/ml$^{+1}$ in 0.15 M $AgBF_4$) in sulfolane.

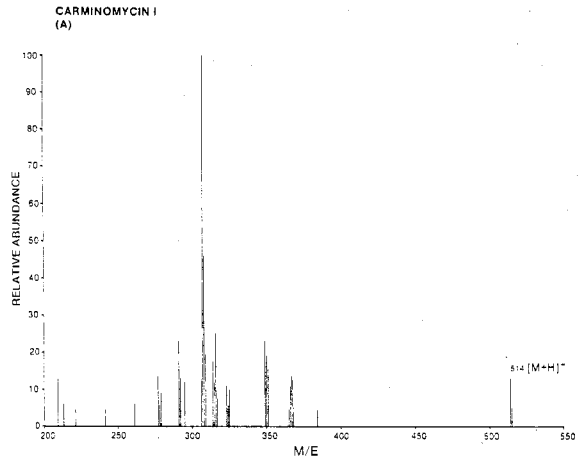



FIGURE 2A. Positive ion spectrum of carminomycin I hydrochloride (0.5 mg/ml$^{-1}$) in sulfolane.

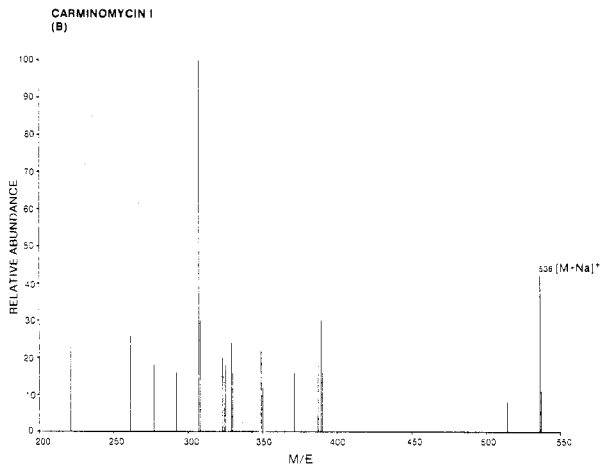



FIGURE 2B. Positive ion spectrum of 0.5 mg/ml$^{-1}$ in 0.14 M NaI in sulfolane.

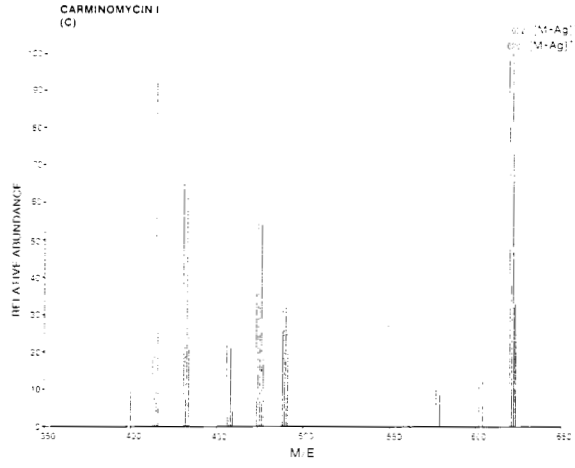
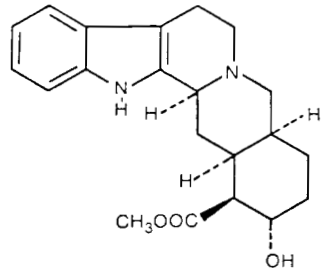
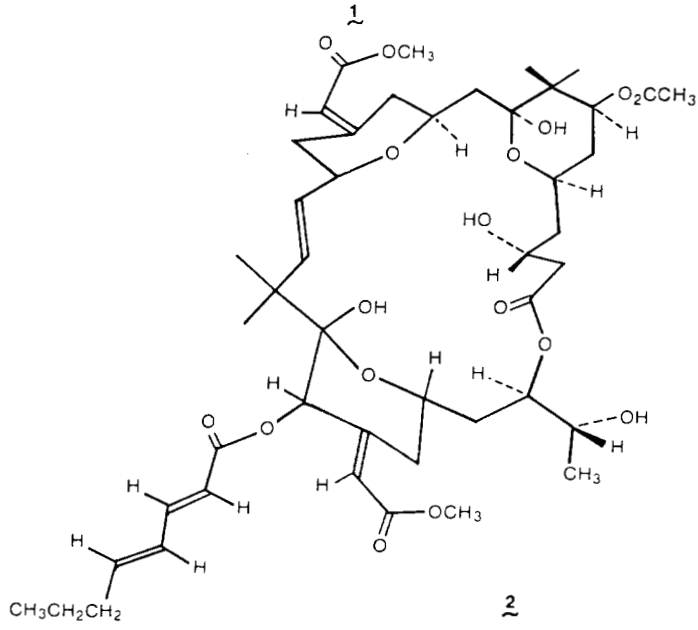



FIGURE 2C. Positive spectrum of 0.5 mg/ml$^{-1}$ in 0.15 M $AgBF_4$ in sulfolane.

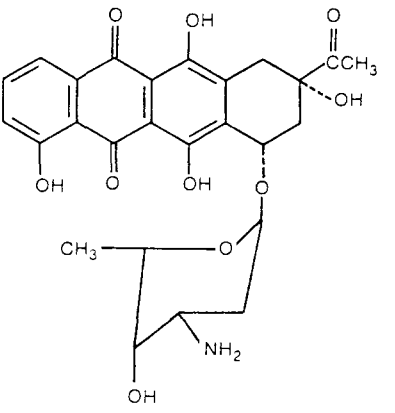
silver, and/or thallium salts recommended above, provides a simple, reliable, and most effective means for obtaining the accurate mass of polar and nonpolar compounds in the very important molecular weight range of about 400-2000 (26). Presumably the same method can be extended to determining much higher molecular weights, and this aspect will also be evaluated in future studies.




1




2



3

## EXPERIMENTAL

Specimens from the collection of antineoplastic agents and other natural products in the Cancer Research Institute, Arizona State University, were employed in this study. Positive secondary ion mass spectra were obtained from 50-100 μg samples dissolved in 30 μl of the respective nonvolatile solvent (usually sulfolane or glycerol) or in solutions of known concentration containing an acid or metal salt. Concentrations of the acid solutions were generally 0.1 M or less, and those of the metal salt solutions were 0.14-0.15 M. Exact concentrations at the time of sputtering are unknown, but presumably under the experimental conditions used they were not significantly altered due to evaporation of the dispersant.

Solutions of the sample were deposited onto a stainless steel probe tip (*ca.* area of 0.3 $cm^2$) and introduced into the source through a vacuum lock. Immediately after a vacuum of $10^{-4}$ torr had been attained, irradiation was performed by a mixed (no ion suppression) beam of fast argon atoms (energies of 0-8 KeV) and ions (energies of 0-5 KeV) at a beam current of approximately 100 μamp. The beam was generated in a modified (27) capillaritron (28) ion source operated at an accelerating potential of 8 KeV in the ionization chamber of a MAT 312 double-focusing mass spectrometer having an accelerating potential of 3 KeV. The spectra were recorded with a Varian MAT Oscillofil uv recorder. Masses were measured against perfluorokerosene and octa(ethylamino)cyclotetraphosphazene. The spectra shown have been corrected by the substraction of peaks due to the presence of the supporting medium. For example, in addition to peaks due to sulfolane, solutions of the group IA metal iodides (*e.g.*, NaI) in sulfolane gave rise to additional background peaks, mainly $[S_n+Na]^+$, $[S_n+Na+NaI]^+$, weaker $[S_n+Na+2NaI]^+$, and $[Na+(NaI)_n]^+$ peaks (n=1,2,3, etc.; S=sulfolane). Only ions with intensity ≥4% of the base peak are shown.

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## LITERATURE CITED

1. M.L. Niven, L.R. Nassimbeni, G.M. Cragg, and G.R. Pettit, *Acta Crystollogr.*, (in press).
2. H.R. Schulten and H.M. Schiebel, *Naturwissenschaften*, **65**, 223 (1978).
3. P. Håkansson, I. Kamensky, B. Sundqvist, J. Fohlman, P. Peterson, C.J. McNeal, and R.D. Macfarlane, *J. Am. Chem. Soc.*, **104**, 2948 (1982).
4. R.D. Macfarlane, *Biomed. Mass. Spectrom.*, **8**, 449 (1981).
5. K.L. Busch and R.G. Cooks, *Science*, **218**, 247 (1982).
6. F.M. Devienne and J.C. Roustan, *Org. Mass Spectrom.*, **17**, 173 (1982).
7. D.J. Surman and J.C. Vickerman, *J. Chem. Soc. Chem. Comm.*, 324 (1981).
8. M. Barber, R.S. Bordoli, R.D. Sedgwick, and A.N. Tyler, *J. Chem. Soc. Chem. Comm.*, 325 (1981).
9. D.J. Surman, and J.C. Vickerman, *J. Chem. Res.*, 170 (1981).

10. M. Barber, R.S. Bordoli, G.J. Elliott, R.D. Sedgwick, and A.N. Tyler, *Anal. Chem.*, **54**, 645 (1982).
11. M. Barber, R.S. Bordoli, R.D. Sedgwick, A.N. Tyler, G.V. Garner, D.B. Gordon, L.W. Tetler, and R.C. Hider, *Biomed. Mass Spectrom.*, **9**, 265 (1982).
12. R. Steinauer and U.P. Schlunegger, *Biomed. Mass Spectrom.*, **9**, 153 (1982).
13. R. Stoll, U. Schade, F.W. Röllgen, U. Giessmann, and D.F. Barofsky, *Int. J. Mass Spectrom. Ion Phys.*, **43**, 227 (1982).
14. K.L. Rinehart, *Science*, **218**, 254 (1982).
15. M. Barber, R.S. Bordoli, R.D. Sedgwick, and A.N. Tyler, *Biomed. Mass Spectrom.*, **8**, 492 (1981).
16. M. Barber, R.S. Bordoli, R.D. Sedgwick, A.N. Tyler, and E.T. Whalley, *Biomed. Mass Spectrom.*, **8**, 337 (1981).
17. S.S. Wong, R. Stoll, and F.W. Röllgen, *Z. Naturforscher*, **37a**, 718 (1982).
18. H. Kambara, S. Hishida, and H. Naganawa, *J. Antibiot.*, **35**, 67 (1982).
19. R.J. Day, S.E. Unger, and R.G. Cooks, *Anal. Chem.*, **52**, 557A (1980).
20. S.E. Unger, R.J. Day, and R.G. Cooks, *Int. J. Mass Spectrom. Ion Phys.*, **39**, 231 (1981).
21. A. Vincze, K.L. Busch, and R.G. Cooks, *Anal. Chim. Acta*, **136**, 143 (1982).
22. K.L. Busch, S.E. Unger, A. Vincze, R.G. Cooks, and T. Keough, *J. Am. Chem. Soc.*, **104**, 1507 (1982).
23. M. Barber, R.S. Bordoli, R.D. Sedgwick, and A.N. Tyler, *Biochem. Biophys. Res. Commun.*, **101**, 632 (1981).
24. G.R. Pettit, C.L. Herald, D.L. Doubek, D.L. Herald, E. Arnold, and J. Clardy, *J. Am. Chem. Soc.*, **104**, 6846 (1982).
25. G.R. Pettit, J.J. Einck, C.L. Herald, R.H. Ode, R.B. von Dreele, P. Brown, M.G. Brashnikova, and G.F. Gause, *J. Am. Chem. Soc.*, **97**, 7387 (1975).
26. R.A. Kerr, *Science*, **216**, 163 (1982).
27. P. Williams and D. Adams, 30th Annual Conference on Mass Spectrometry, Abstract. Honolulu, Hawaii, 1982, p. 53: See also S.A. Martin, C.E. Costello, and K. Biemann, *Anal. Chem.*, **54**, 2362 (1982).
28. J.F. Mahoney, J. Perel, and A.T. Forrester, *Appl. Phys. Lett.*, **38**, 320 (1981).

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