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MASS MEASUREMENTS OF NATURAL PRODUCTS BY SOLUTION PHASE SECONDARY ION MASS SPECTROMETRY EMPLOYING SILVER(I) AND THALLIUM(I) DERIVATIVES[1,2]

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ABSTRACT.—The promotion and detection of molecular ions in the mass spectra of relatively simple to complex biosynthetic products, and a large cross-section of other compounds, has been simplified by employing silver or thallium(I) tetrafluoroborate in sulfolane for solution phase SIMS. The easily recognized isotope doublets of ^{107}Ag, ^{109}Ag (52:49 natural abundance) and ^{203}Tl ^{205}Tl (30:70) combined with the generally very strong $[M+Ag]^+$ and $[M+Tl]^+$ molecular ion complex intensities provided powerful new techniques for mass measurements. Because the degree of molecular ion complex intensity was found to be a function of the specimens' structure, application of these methods augmented by lithium and/or NaI or trifluoromethanesulfonic acid (for basic nitrogen containing compounds) was recommended for all new molecular weight determinations by mass spectrometry.

Since 1981, the mass spectral analysis of complex natural products has been undergoing very rapid simplification by continuous improvements in solution-phase secondary ion mass spectrometry (SIMS, 1-3).[3] Indeed, even 5 picomoles of the peptide, human angiotensin 1 ($M^+=1296$) in glycerol solution (containing HOAc on a gold-plated stage with xenon fast atom bombardment), has been shown (4) to allow useful $[M+H]^+$ ion formation. Other recent advances have included the use of solution phase SIMS for structural studies of peptide antibiotics (5,6) and carbohydrates (7).

For the purpose of uncovering reliable and convenient techniques for detecting molecular ions in the mass spectra of various biosynthetic products, we have found that significant increases in molecular ion complex (3) intensity can be achieved by positive ion desorption from sulfolane solutions containing trifluoromethanesulfonic acid (2), a group I metal iodide (1), or either silver or thallium tetrafluoroborate [or trifluoromethanesulfonate (3)]. Except for very polar amino acids (2) of insufficient solubility in sulfolane, where glycerol-containing trifluoromethanesulfonic acid was found more useful, the cyclic sulfone proved to be an excellent solvent for solution-phase SIMS. In previous reports we summarized the considerable advantages of employing NaI in sulfolane for more routine mass spectral analyses and rubidium iodide in this solvent for recognizing (by the 72:28 isotope ratio) molecular ions and metal-specimen complexes (1,3). Meanwhile our silver(I) and thallium(I) studies, which were briefly mentioned in an earlier report (3), have been extended and the tetrafluoroborate and trifluoromethanesulfonate salts have proved to be exceptionally useful in sulfolane for promoting intense $[M+Ag]^+$ and $[M+Tl]^+$ molecular ion complexes. Because the silver 107, 109 (52:48 natural abundance ratio) and thallium 203, 205 (30:70 ratio) isotopes were found to be prominently displayed in the solution-phase SIMS mass spectra, detection and establishment of molecular ions has been greatly simplified.

The spectra of various, and quite non-polar to polar, compounds in sulfolane or even glycerol containing silver derivatives of the hexafluoroantimonate, hexafluorophosphate, tetrafluoroborate, or trifluoromethanesulfonate types were found

[1]In commemoration of Professor Carl Djerassi's 60th birthday.

[2]Part 102 of the series Antineoplastic Agents and for unit 101 see Holzapfel *et al.* (1).

[3]Well known by the less rigorous designation fast atom bombardment (FAB) mass spectrometry. A comprehensive list of references to the development and application of this technique was included in Pettit *et al.* (3).

to show strong $[M+Ag]^+$ molecular ion complexes (Table 1). While silver trifluoromethanesulfonate consistently gave better molecular ion complex enhancement, the use of silver tetrafluoroborate (0.14M in sulfolane) was preferred because of the minimal interference by background ions with masses exceeding 400. Perhaps due to a greater tendency to form ion pairs, the adding of silver acetate or trifluoroacetate proved to be considerably less effective in promoting $[M+Ag]^+$ formation.

TABLE 1. Effect of Selected Salts on the $[M+Ag]^+$ Ion Desorption Intensities[a] from Sulfolane Solution

Silver Salt (0.1M Solution)	$AgBF_4$	$AgPF_6$[b]	$AgSbF_6$	CF_3SO_3Ag
CBZ-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3 (**2**, $M^+=680$)	27,25	100,90	42,38	80,76
Phyllanthoside (**1**, $M^+=804$)	21,21	4,4	29,30	29,30

[a]Molecular ion complex intensities were measured relative to the $[M+Ag]^+$ ion equal to 100 for the hexapeptide in sulfolane containing $AgPF_6$. The two values in each column correspond to the Ag isotope doublets.

[b]Dissolution of $AgPF_6$ in sulfolane required warming, and the solution darkened.

The relative $[M+metal]^+$ forming abilities of silver and sodium were compared using a mixture of silver and sodium tetrafluoroborates in sulfolane (each 0.05M). As illustrated in Figures 1-3 the $[M+metal]^+$ ion intensity depends markedly on the specimen. Phyllanthoside (**1**, Figure 1) shows a strong preference for $[M+Na]^+$ formation, while the C-terminal hexapeptide (**2**, Figure 2) sequence of tobacco mosaic virus protein (8) and bryostatin 1 [(**3**, Figure 3, (9)] prefer $[M+Ag]^+$ molecular ion complexes. Such metal ion selectivity has been demonstrated with 2,9-dimethyl-1,10-phenanthroline and a few other substances using solid-phase SIMS (10).

Comparison (3) of $[M+H]^+$, $[M+Na]^+$, and $[M+Ag]^+$ molecular ion complexes in the positive ion solution-phase SIMS spectra of the anticancer antibiotic carminomycin 1 also showed a distinct preference for silver, and the relative impact upon fragment ion formation. Selection between various fragmentation pathways may depend on the cation-forming species and/or its position of attachment. As an aid to future structure elucidation methods, such questions will receive further attention.

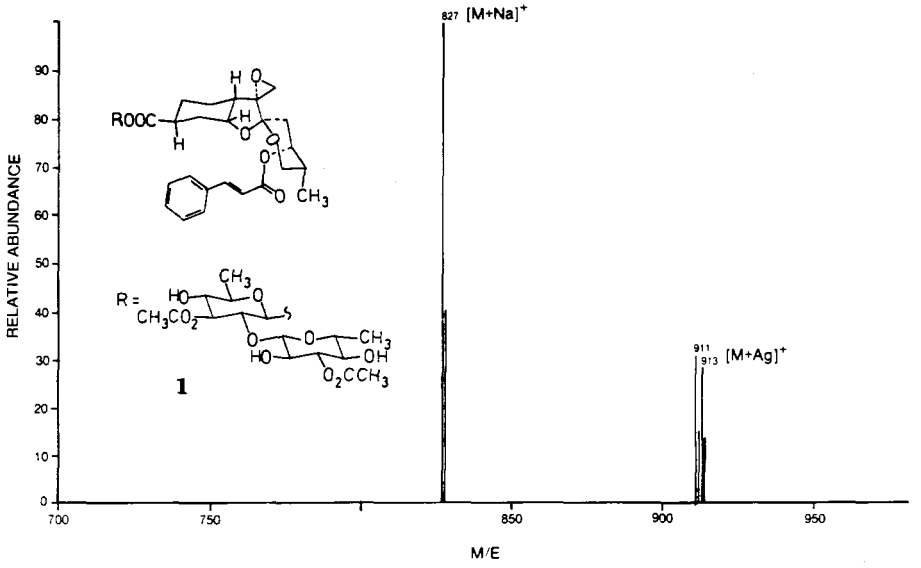

FIGURE 1. Phyllanthoside (**1**) in sulfolane containing 0.05 M sodium and silver tetrafluoroborates.

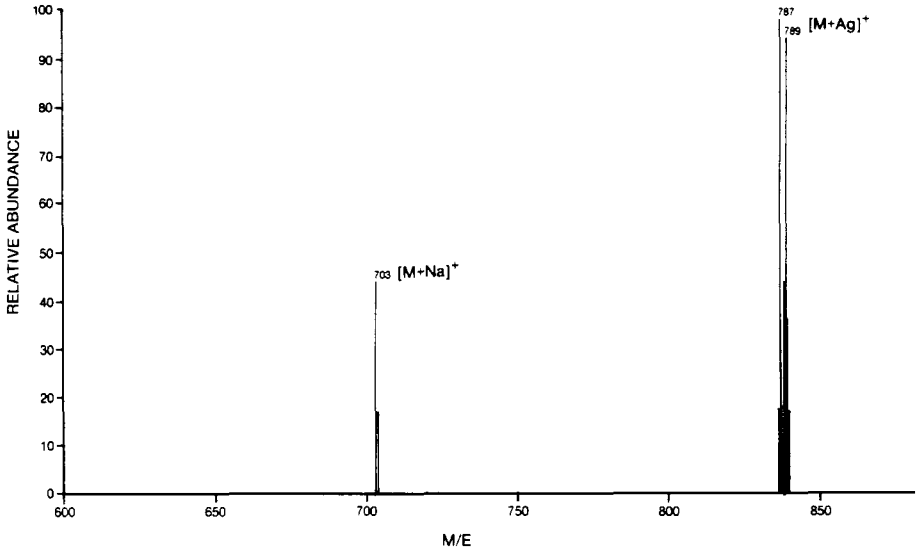

FIGURE 2. CBZ-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3 (2) in sulfolane containing 0.05M sodium and silver tetrafluoroborates.

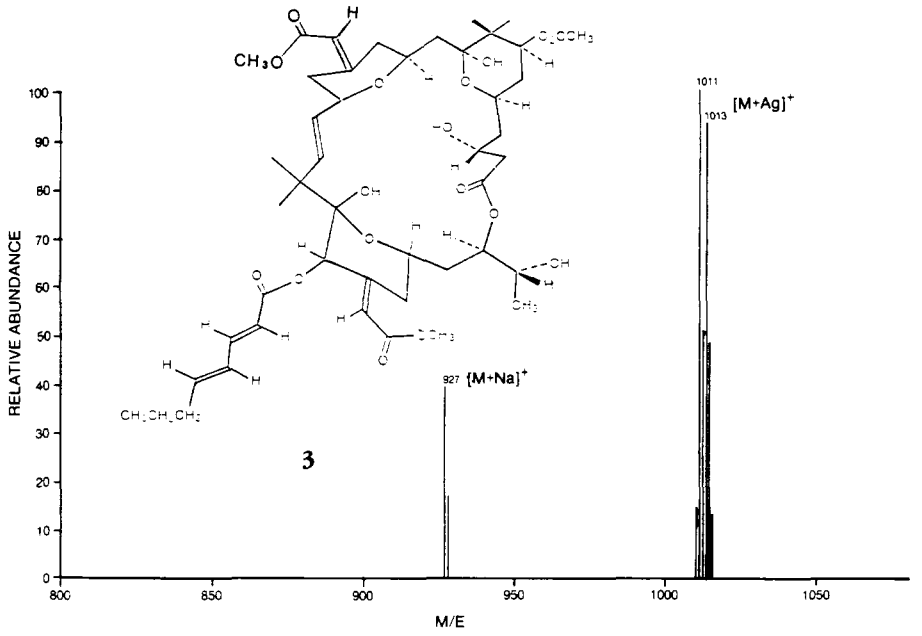

FIGURE 3. Bryostatin 1 (**3**) in sulfolane containing 0.05M sodium and silver tetrafluoroborate.

The easy recognition of the silver-containing doublets due to ^{107}Ag and ^{109}Ag isotopes in the ratio 52:48 constitutes a major advantage of $[M+Ag]^+$ molecular ion complexes. Interestingly, the few previously reported ion desorption mass spectra observed with $[M+Ag]^+$ complexes were obtained (10-14) by the bombardment of organic compounds deposited on a metallic silver surface employing solid-phase SIMS. Unlike ion desorption from liquid matrices containing silver tetrafluoroborate, which routinely yield $[M+Ag]^+$ ions, the solid-phase SIMS from silver surfaces does not yield argentated molecular ions in any predictable manner. Furthermore, under the condi-

tions of solution phase SIMS, specimens did not tend to form the $[M-H+2Ag]^+$ cluster ions observed in some solid-phase SIMS spectra (14). The same advantage applies to the solution phase SIMS spectra of $[M+Tl]^+$ molecular ion complexes, which show the characteristic ^{203}Tl and ^{205}Tl isotope (in the ratio 30:70) doublets. The attributes of this excellent complement to the silver techniques will now be viewed.

The preceding successes with silver-ion-promoted molecular ion complex formation suggested the possibility of expanding the utility of the overall procedure by using a higher mass element for analogous purposes. A substantial increase in mass due to the metal component was expected to be of particular value when analyzing certain natural products with relatively low mass (<400) and other substances. Thallium with its distinctive isotopes seemed most attractive for these purposes, and a series of monovalent derivatives were prepared from thallium(I) ethoxide. Although thallium(I) trifluoromethanesulfonate in sulfolane led to very intense $[M+Tl]^+$ ions, the background ions of mass greater than 400 were also very prominent, and thallium(I) tetrafluoroborate in the same solvent proved to be the more generally useful reagent. Indeed, strong $[M+Tl]^+$ ions were usually obtained using 0.11M solutions of thallium(I) tetrafluoroborate, trifluoromethanesulfonate, or *p*-toluenesulfonate in sulfolane. The thallium(I) acetate and trifluoroacetates gave much poorer results.

As already illustrated (Figures 1-3) for the silver and sodium derivatives, the relative molecular ion complex forming abilities of thallium(I) and silver tetrafluoroborates were found to be structure-dependent (see Table 2). For example, $[M+Tl]^+$ ion formation was strongly favored with erythromycin (**4**, Figure 4), while hexapeptide (**2**) preferentially formed $[M+Ag]^+$ ions. With phyllanthoside (**1**) bryostatin 1 (**3**), and testosterone propionate the $[M+Ag]^+$ and $[M+Tl]^+$ ion intensities were similar. Except for phyllanthoside (**1**) this series of specimens also yielded relatively intense protonated molecular ions. Bryostatin 1 (**3**) afforded fragments of the type $[M+H-(H_2O)n]^+$ resulting from loss of H_2O.

TABLE 2. Comparison of $[M+Ag]^+$ and $[M+Tl]^+$ Molecular Ion Complex Intensities[a] in Sulfolane Solution with Silver or Thallium(I) Tetrafluoroborate (0.05M)

	$[M+Ag]^+$	$[M+Tl]^+$
Testosterone propionate ($M^+=344$)	100,85	46,93
Hexapeptide[b] (**2**, $M^+=680$)	69,68	19,36
Erythromycin (**4**, $M^+=733$)	11,10	26,53
Phyllanthoside (**1**, $M^+=804$)	11,10	6,16
Bryostatin 1 (**3**, $M^+=904$)	15,17	6,14

[a]Intensities were measured relative to $[M+Ag]^+$ for testosterone propionate equal to 100. The two sets of $[M+Ag]^+$ and $[M+Tl]^+$ column numbers correspond to the silver and thallium isotope doublets.

[b]See Table 1 for structure.

The above results clearly indicate that use of silver and/or thallium(I) tetrafluoroborate in sulfolane greatly broadens the scope of positive ion solution-phase SIMS mass spectrometry in molecular weight determinations of complex natural products and a large cross-section of other compounds. The techniques (1-3) we have devised using solutions of LiI, NaI, silver tetrafluoroborate, or thallium(I) tetrafluoroborate in sulfolane

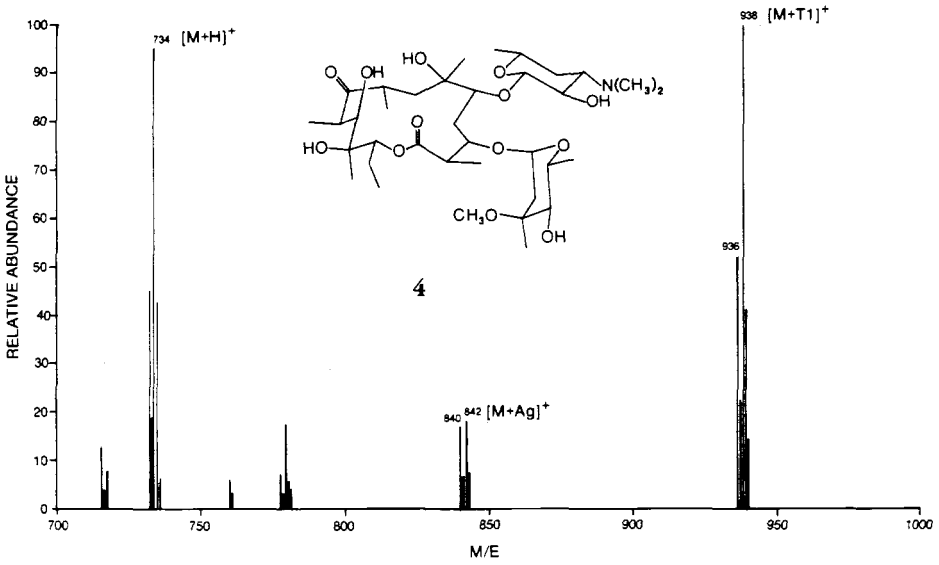

FIGURE 4. Erythromycin (4) in sulfolane containing 0.05M silver and thallium tetrafluoroborates.

provide means for greatly enriching molecular ion complex intensities for a quite broad selection of substances. To realize maximum molecular ion complex intensities, each method or a combination thereof should be utilized for a new molecular weight determination. And, if the substance for analysis proves to have a basic nitrogen or betaine unit, protonated molecular ion enhancement should prove valuable using solutions of trifluoromethanesulfonic or *p*-toluenesulfonic acid in sulfolane or glycerol (2). In summary, application of these new advances in positive ion solution phase SIMS greatly extends the versatility of this powerful approach to molecular weight determinations.

EXPERIMENTAL

Specimens of silver tetrafluoroborate, hexafluoroantimonate, hexafluorophosphate, and trifluoromethanesulfonate were employed as received from the Aldrich Chemical Co. Thallium(I) acetate, trifluoroacetate, tetrafluoroborate, *p*-toluenesulfonate, and trifluoromethanesulfonate were prepared by treating thallium(I) ethoxide with one equivalent of the corresponding acid. The resulting thallium(I) derivative was recrystallized from MeOH or EtOH. Molar concentrations of the silver and thallium salts in sulfolane ranged from 0.05 to 0.11. As before, concentrations of metal salt solutions refer to initial concentrations and are assumed to be essentially unchanged at the time of sputtering. Otherwise, the general instrument (MAT 312 mass spectrometer and capillaritron source) and other experimental details have been summarized earlier (1-3).

The main background peaks in the mass spectra arising from compounds in sulfolane solutions of silver or thallium(I) tetrafluoroborate corresponded to $[S_n+\text{metal}]^+$, where S=sulfolane and n=0, 1,2, ... With glycerol as solvent, the principal background peaks were formulated as $[G_n+\text{metal}]^+$ and weaker peaks as $[G+(G\text{-}H)_x+(x+1)\text{metal}]^+$, where G=glycerol, n=0, 1,2 . . . and x=1,2 . . . . Silver trifluoromethanesulfonate in glycerol or sulfolane gave additional cluster ions, mainly of the type $[G \text{ or } S_n+2Ag+CF_3SO_3]^+$ where n=0, 1,2, ... Only mass spectral peaks with mass of greater than 200 and intensity $\geq 15\%$ of the base peaks were included in the figures.

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