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BIOSYNTHETIC PRODUCT MOLECULAR WEIGHT DETERMINATIONS BY SOLUTION PHASE SECONDARY ION MASS SPECTROMETRY EMPLOYING GROUP 1A METAL SALTS[1,2]

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ABSTRACT.—Sulfolane was found to be an excellent solvent for solution phase SIMS mass measurements of natural products employing group 1A metal iodides for molecular ion complex enhancement. Lithium iodide generally afforded maximum molecular ion complex formation. Although this very important effect was found to decrease through the series sodium↦potassium↦rubidium↦cesium iodides, it remained quite useful. The ^{85}Rb and ^{87}Rb isotopes (ratio of 72:28) proved especially effective for detection of molecular ions and recognizing other metal-complex ions. A mixture of lithium and sodium iodides was also found helpful for the latter purpose. Application of, e.g., the sodium iodide-sulfolane technique for increasing the intensity of molecular ion complexes proved to be a very simple and reliable method for conducting natural product molecular weight determinations.

Experiments, directed at the discovery of very reliable and rapid techniques for establishing molecular weights of naturally occurring substances in the approximate mass range 400-2000 by solution phase secondary ion mass spectrometry (SIMS), led us to study molecular ion enhancement using proton or metal ion sources in the sample solvent (2, 3). Sulfolane was selected (2) as the most generally effective solvent because of its excellent solvating properties [a notable exception was found to be amino acids (3)], weak attraction for metals, and a low background level. The principle advantages of molecular ion recognition by protonation (promoted by trifluoromethanesulfonic acid in sulfolane or glycerol) were generally found to be limited to nitrogen bases and betaines, especially amino acids (3). When the acid (relatively weak to strong) concentration was increased beyond about 0.1 M, the protonated molecular ion intensity decreased or disappeared due to increased fragmentation. And with certain complex biosynthetic products, such as the remarkable marine animal anticancer constituent bryostatin 1 (**1**) (4), the presence of even trace amounts of acid caused complete disappearance of the protonated molecular ion and formation of $[M+H-nH_2O]^+$ ions, where n=1, 2, or 3. Considerably more versatile techniques were revealed by a concurrent and detailed investigation of metal cation sources on the intensity of molecular ion complexes (2) formed from a wide range of natural products. In a preliminary summary of this study, we reported that a sulfolane solution of the specimen containing an alkali metal iodide or silver or thallium tetrafluoroborate (or trifluoromethanesulfonate) afforded easily recognized molecular ion complexes (2). The present contribution summarizes our extended investigation of the Group 1A metal salt methods.[4,5]

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[1]Dedicated to Professor Carl Djerassi in recognition of his sixtieth birthday.

[2]Antineoplastic Agents Contribution 101. For part 100 of the series, see Pettit *et al.* (1).

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[4]Subsequent to completion of our investigation of solution phase SIMS with alkali metal salts in sulfolane, the use of; sodium and potassium salt derivatives of a β-lactam (azthreonam) in glycerol (5) or estrogen sulfates in glycerol-H_2O or glycerol-DMSO (6); lithium, sodium, or potassium chlorides in glycerol-5% HOAc-H_2O with a glucose polysaccharide (7) or in glycerol with an aflatoxin (8); sodium and potassium molecular ion complexes to confirm the mass of a polyene antibiotic (9); and a negative ion technique to eliminate sodium ion from the spectrum of testosterone sodium sulfate (10) were recorded.

[5]Interestingly, after completion of this manuscript, a 3:1:1 mixture of sodium, rubidium, and cesium iodides was noted as the primary instrument calibration reference in a FAB (solution phase SIMS) study of anthocyanins in thioglycerol (11).

Generally, the strongest and most consistent enhancements of molecular ion complex intensities with neutral compounds were observed with Group 1A metal derivatives. Greater stability of $[M+metal]^+$ ions in the gas phase as compared to the corresponding $[M+H]^+$ ions probably accounts for the greater utility of the Group 1A cations in increasing molecular ion complex intensities. As we reported earlier (2), the significantly reduced fragmentation of $[M+Na]^+$ as compared to $[M+H]^+$ in the spectra of carminomycin I in sulfolane containing NaI compared to carminomycin HCl in sulfolane appears to nicely illustrate this point. Under optimal conditions, the intensity of the molecular ion complex containing sodium was at least four times that of the protonated aminoglycoside molecular ion complex $[M+H]^+$. Comparison of the corresponding $[M+H]^+$ and $[M+Na]^+$ spectra from erythromycin (**2**) (Figures 1 and 2) and from phyllanthoside (**3a**) (Figures 3 and 4) provides another illustration. With NaI in sulfolane considerably better (up to fiftyfold higher) enhancement of $[M+Na]^+$ ion

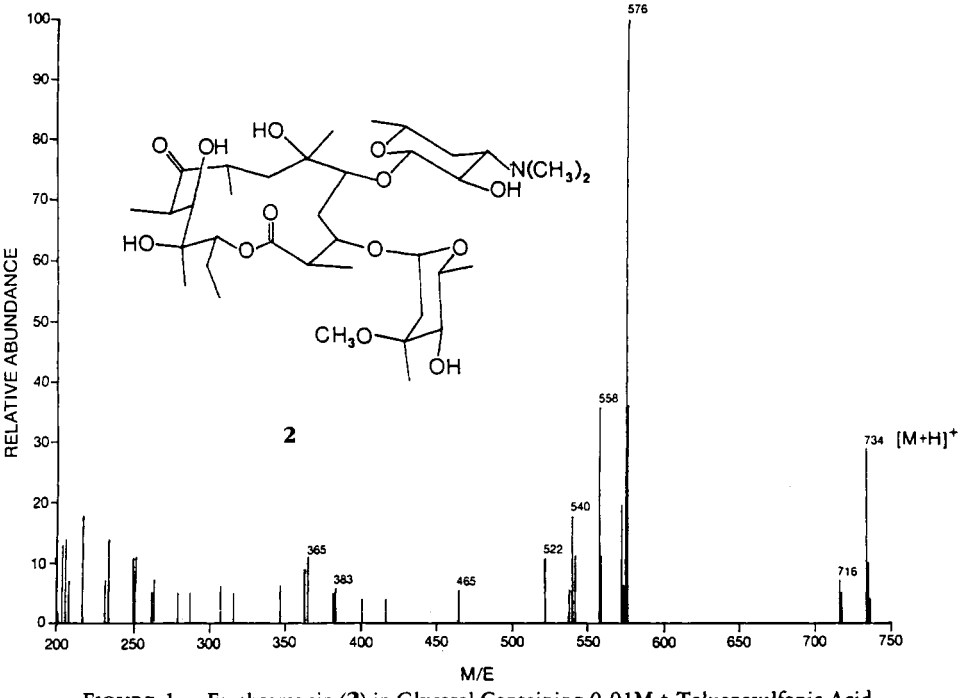


FIGURE 1. Erythromycin (**2**) in Glycerol Containing 0.01M *p*-Toluenesulfonic Acid.

intensities over those of $[M+H]^+$ was realized with less polar compounds, such as, phyllanthoside peracetate (**3b**), pleniradin acetate (**6**), fastigilin C (**7**), and *Pimelea* factor P_2 (**10**) which do not readily yield protonated molecular ions in positive solution phase SIMS spectra. Selection of iodide as the favored anion for Group 1A metals in sulfolane was based on very favorable solubility properties and a comparison with other anions, such as chloride, tetrafluoroborate, and trifluoromethanesulfonate, in promoting molecular ion complex formation. The alkali metal fluorides were found insufficiently soluble in sulfolane and glycerol to be of any value. Such a comparison using phyllanthoside (**3a**) and the hexapeptide, Cbz-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3[6] has been summarized in Table 1. In glycerol, sodium tetrafluoroborate and trifluoromethanesulfonate gave better (M+Na]+ enhancements, but this advantage was offset by more complex backgrounds compared to the corresponding iodides. Also in glycerol, sodium

[6]The C-terminal hexapeptide unit of tobacco mosaic virus protein was synthesized as recorded by Pettit, *et al.* (12).

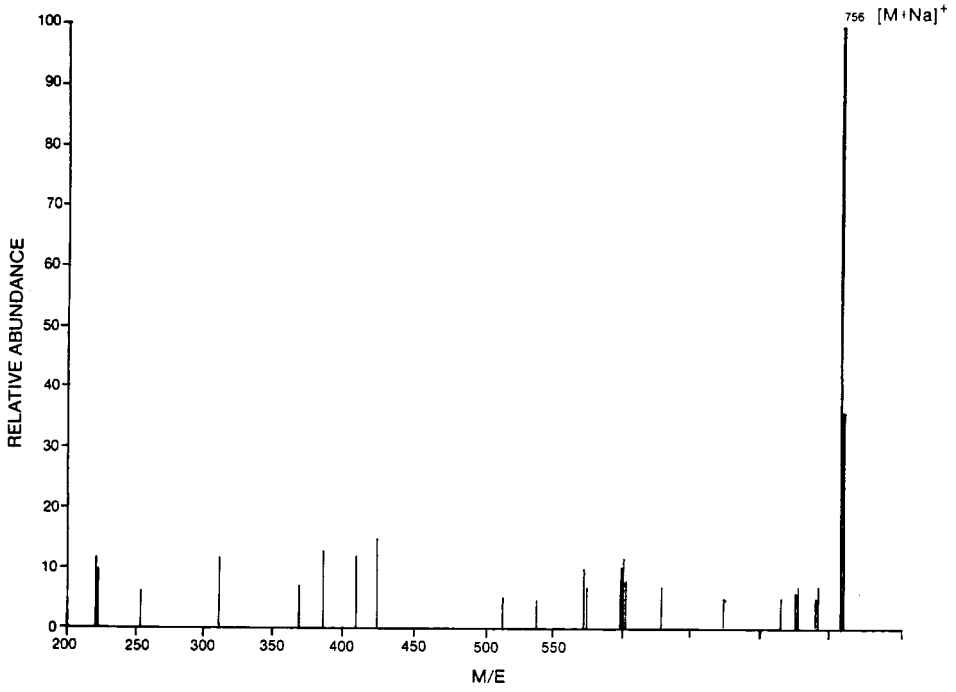


FIGURE 2. Erythromycin (**2**) in Sulfolane Containing 0.14M NaI.

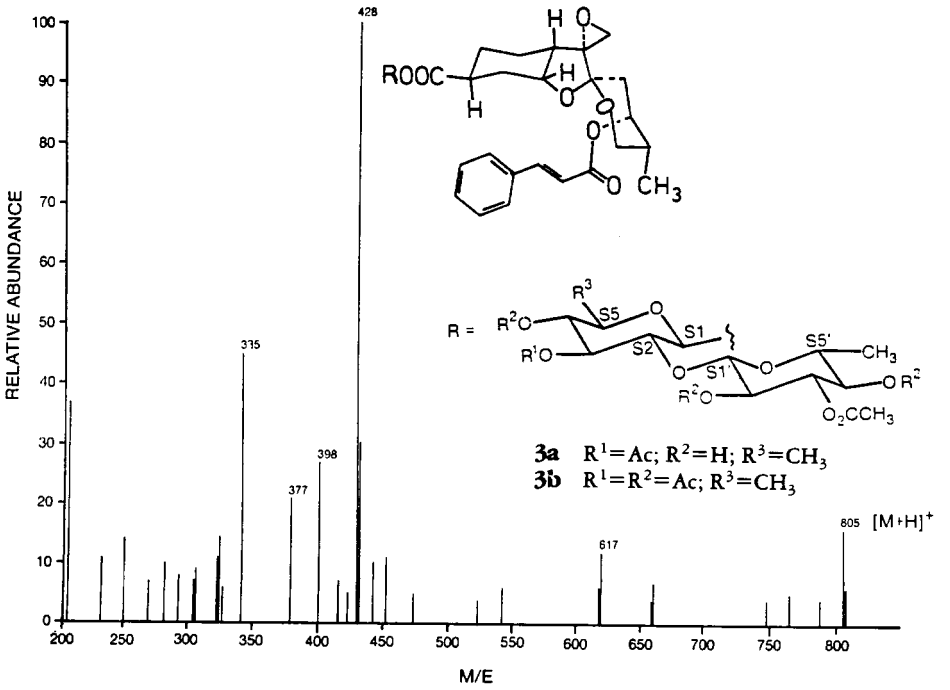


FIGURE 3. Phyllanthoside (**3a**) in Sulfolane Containing 0.02M *p*-Toluenesulfonic Acid.

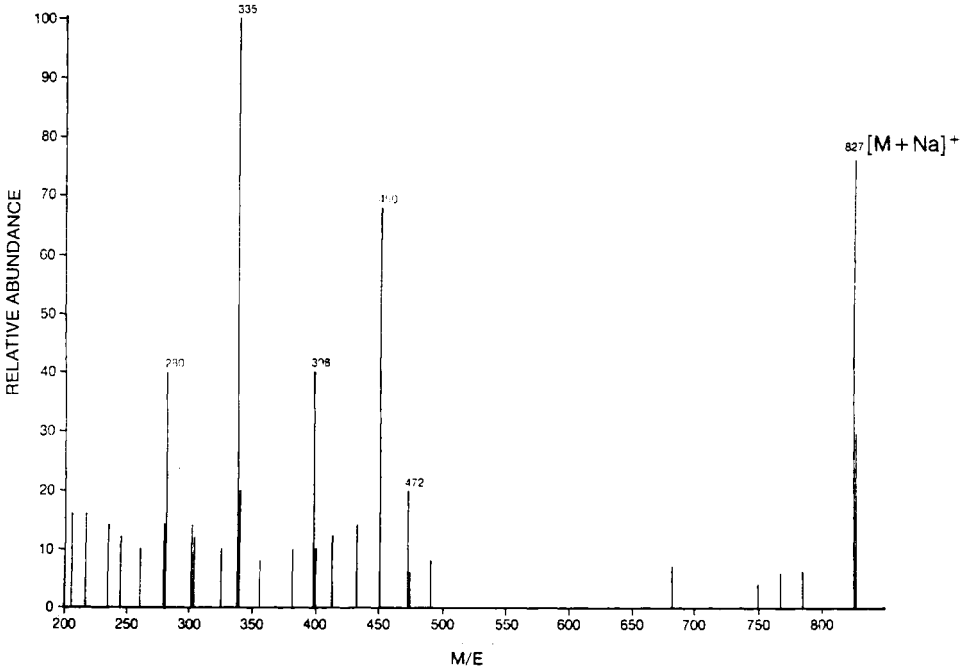


FIGURE 4. Phyllanthoside (**3a**) in Sulfolane Containing 0.14M NaI.

trifluoromethanesulfonate allowed less $[M+H]^+$ formation than any of the other sodium salts explored. But the increase in background ions compared to those resulting from NaI resulted in selection of the latter anion. With NaI, the $[M+H]^+$ ions were suppressed and hardly detectable in the spectra of phyllanthoside in glycerol or sulfolane and appeared only very weakly in spectra of the hexapeptide (12) in solfolane. In general, NaI in sulfolane was found to provide uniformly satisfactory molecular ion complexes and optimal results were obtained with concentrations near 0.14 M. Sulfolane also proved to be a practical solvent for relatively nonpolar compounds.

Molecular ion complex abundance was reduced to approximately half of the maximum value by decreasing the concentration of Group 1A metal iodide to 0.055 M or by increasing the concentration to 0.3 M. The decrease in $[M+metal]^+$ abundance at higher metal iodide concentration was considered due to increased ion pair formation and increased sputtering (desorption) of background ions. In glycerol, thioglycerol, and tetraglyme, the optimal concentrations of Group 1A metal iodides for molecular ion complex enhancements were found to be near 0.26, 0.22, and 0.20 M, respec-

TABLE 1. Comparison of Selected Sodium Salts in Promoting $[M+Na]^+$ Formation in Glycerol and Sulfolane Solutions

Sodium Salt (0.14M solution)	$NaBF_4$	NaCl	NaI	CF_3SO_3Na
Phyllanthoside (**3a**)				
$[M+Na]^+$ in sulfolane			13[a]	14
$[M+Na]^+$ in glycerol	17[a]	4	6	5
CBZ-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3 (**19**)				
$[M+Na]^+$ in sulfolane			12[a]	40
$[M+Na]^+$ in glycerol	56[a]	57	57	100

[a]Molecular ion complex intensities expressed relative to $[M+Na]^+$ equal to 100 for the hexapeptide in CF_3SO_3Na-glycerol solution.

tively. But, complete suppression of $[M+H]^+$ ions of polar compounds in protic solvents in some cases (e.g., erythromycin and carminomycin I) required metal iodide concentrations of up to 0.5 M.

Under conditions of optimal molecular ion complex formation, spectra resulting from sulfolane solutions showed less interference from background ions due to the lower alkali iodide concentration requirement, and this was of considerable advantage in detection of molecular ions $\leq$450. In addition, with nonpolar compounds, the molecular ion complexes $[M+metal]^+$ obtained (optimal conditions) from sulfolane solution were more intense ($\leq 3\times$) than those resulting from the same compound in glycerol or thioglycerol, and up to ten times that obtained from tetraglyme. Thus glycerol, thioglycerol, or tetraglyme probably function as weak chelating agents and compete more favorably than sulfolane for the metal ions. Evidence for this assumption has been illustrated in Figures 5 and 6 which show the higher mass regions of the phyllanthoside peracetate (**3b**) spectra arising from solutions of NaI in sulfolane and tetraglyme, respectively. In Figure 5, the strong substrate molecular ion complex and weak sulfolane background peaks become apparent. Conversely, in Figure 6 the weaker substrate molecular ion complex and strong tetraglyme-sodium cluster ions become obvious.

Clearly sulfolane offered distinct advantages over other solvents evaluated, and a number of reactive electrophiles which did not furnish molecular ion complexes from protic solvents were detected when dispersed in sulfolane containing an alkali iodide. Examples included the 2,4,5-trichlorophenol and other active ester derivatives of polypeptides (12) and amino acids (13) as well as diazoketones such as *N*-trifluoroacetylazotomycin dimethyl ester (**18**) (13). A selection of some of the natural products and related compounds which have been investigated using 0.14M NaI in sulfolane is given in Table 2. In many cases, protonated molecular ions $[M+H]^+$ were observed in addition to the $[M+Na]^+$ molecular ion complexes and the ratios of the intensities of these two ions are recorded where relevant. As noted below, the $[M+H]^+$

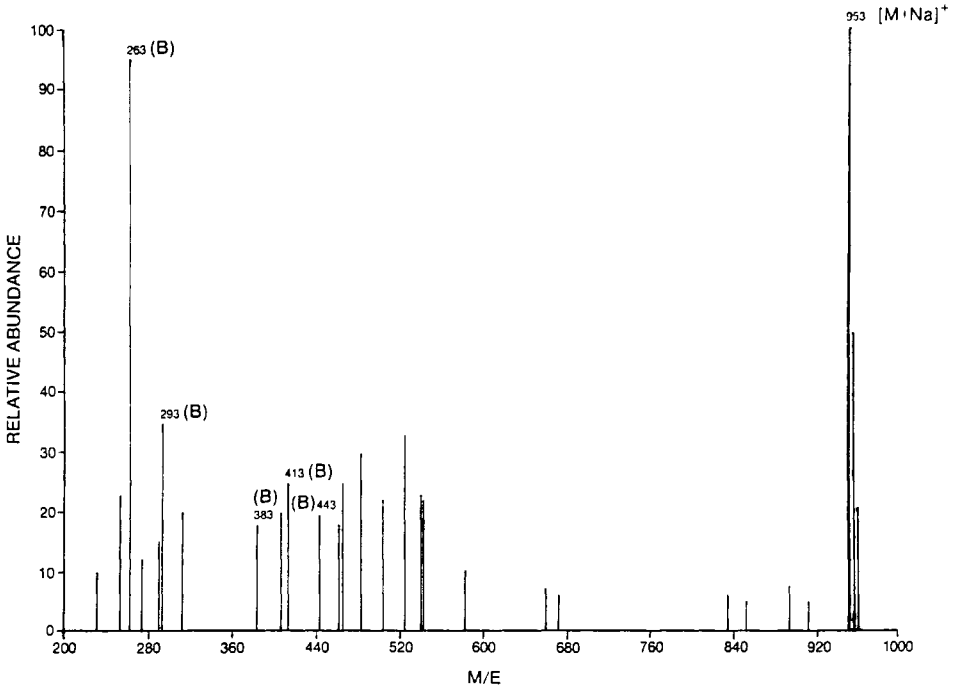


FIGURE 5. Phyllanthoside Peracetate (**3b**) in Sulfolane Containing 0.18M NaI (B=background).

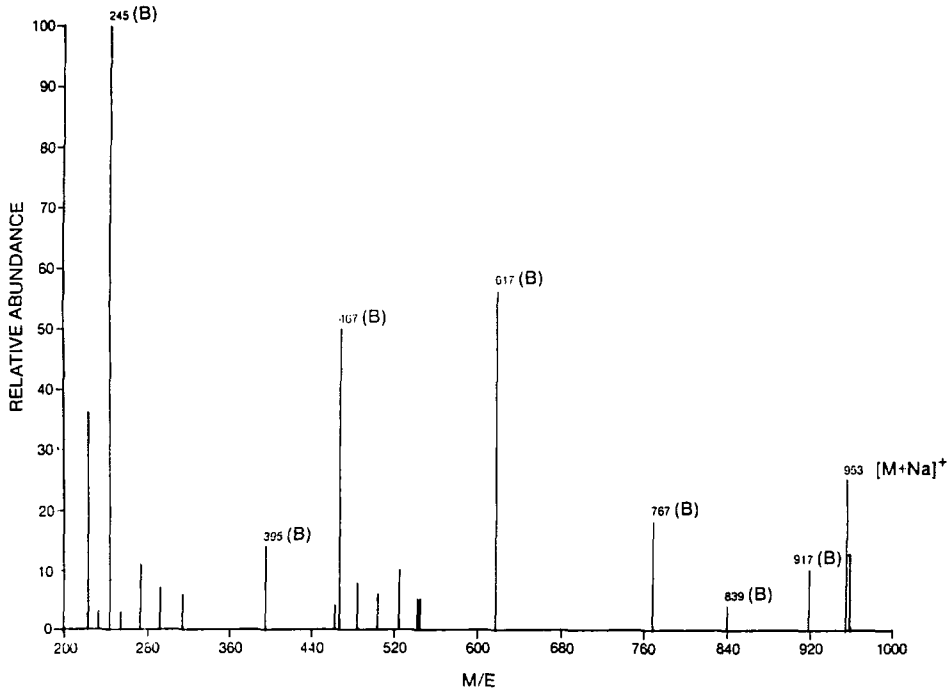


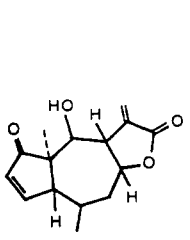
FIGURE 6. Phyllanthoside Peracetate (**3b**) in Tetraglyme Containing 0.18M NaI (B=background).

peak intensities could be decreased by rigorously drying the sulfolane prior to use. But in most instances, strong $[M+Na]^+$ ions were observed without special preparation of the solvent. A notable exception proved to be the limonoid, prieurianin (**11**) (14), where only a strong $[M+H]^+$ ion was obtained.

The molecular ion complex promotion studies were extended to a variety of natural products (*cf.*, Table 3) in protic and aprotic solvents containing equal amounts (0.05M) of lithium, sodium, potassium, rubidium,[5] and cesium[5] iodides. Generally, lithium ion was found to offer superior,[7] complex forming properties which underwent a gradual decrease from sodium to potassium to rubidium and cesium (Table 3). The general trend has been illustrated in the spectrum of bryostatin 1 (**1**, from the marine bryozoan, *Bugula neritina* L.) shown in Figure 7. When dry sulfolane was used as solvent, $[M+H]^+$ ions were not usually produced, and this allowed the attachment of metal to a particular ion to be verified by using a mixture of lithium and sodium iodides. Alternatively, rubidium iodide proved especially effective for this purpose, and molecular ions (as $[M+Rb]^+$) were very easily recognized by their distinctive isotope pattern (^{85}Rb and ^{87}Rb in the ratio 72:28). In fact, application of the rubidium isotopes were considered an especially important advance with many potential advantages for future studies, e.g., with rubidium tetrafluoroborate and trifluoromethanesulfonate. A representative summary of results with the alkali metal iodides in sulfolane has been presented in Figure 7 employing bryostatin 1 (**1**) (4). Prior to these advances, a molecular ion corresponding to bryostatin 1 could not be obtained by ei or fdms.

The relative simplicity of molecular ion complex fragmentation in solution phase SIMS spectra obtained from sulfolane-specimen-alkali metal iodide solutions suggests

[7]Contamination of the mass spectrometer by lithium proved to be a disadvantage. After using a lithium salt $[sulfolane)_n+Li]^+$ or $[glycerol)_n+Li]^+$ peaks were found in the spectra arising from experiments with other metal salts. However, a "baking out" procedure routinely eliminated the lithium residues.



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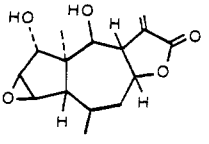

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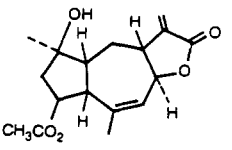

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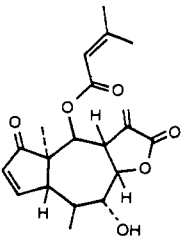

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TABLE 2. A Selection of Solution Phase SIMS Experiments Using 0.14M NaI in Sulfolane[a]

			Terpenes		
4	Helenalin (16) $C_{15}H_{18}O_4$ (262) 3/1	**5**	Autumnolide (17) $C_{15}H_{20}O_5$ (280) 6/1	**6**	Pleniradin acetate (18) $C_{17}H_{22}O_5$ (306) 12/1
7	Fastigilin C (19) $C_{20}H_{26}O_6$ (362) >20/1	**8**	Methyl ursolate (20) $C_{31}H_{50}O_3$ (470) 1/1	**9**	Epilimonol (14) $C_{26}H_{32}O_8$ (472) 1.2/1
10	Pimelea Factor P_2 (21) $C_{37}H_{50}O_9$ (638) No $[M+H]^+$	**11**	Prieurianin (14) $C_{40}H_{50}O_{16}$ (786) Only strong $[M+H]^+$		
			Steroids		
12	Progesterone $C_{21}H_{30}O_2$ (314) 0.3/1	**13**	Testosterone propionate $C_{22}H_{32}O_3$ (344) 0.4/1	**14**	Resibufogenin (22) $C_{24}H_{32}O_4$ (384) 2/1
15	3β,7α-Diacetoxy-5α-pregn-20-one $C_{25}H_{36}O_5$ (416) >20/1	**16**	3β-Tetrahydropyranyl-5α-card-20(22)-enolide $C_{28}H_{42}O_4$ (442) 0.7/1		
			Peptides		
17	$RO_2C(CH_2)_2CHCONHCH(CH_2)(CH_2)_2CO_2R$; R=$CH_3$ (12) $NHCO(CH_2)_2CHCO_2CH_3$ $NHCOCF_3$ $C_{21}H_{30}F_3N_3O_{11}$ (557) No $[M+H]^+$	**19**	Cbz-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3 (12) $C_{30}H_{44}N_6O_{12}$ (680) No $[M+H]^+$		
18	**17**; R=CHN_2 (13) $C_{21}H_{26}F_3N_7O_9$ (577) No $[M+H]^+$	**20**	N-Boc-(gly)Thz-(gln)Thz-Val-Leu-Pro-OCH_3 (13) $C_{35}H_{52}N_8O_9S_2$ (792) 3/1		
			Antibiotics		
21	Lincomycin (23) $C_{18}H_{34}N_2O_6S$ (406) 1/1	**22**	Carminomycin I (24) $C_{26}H_{27}NO_{10}$ (513) 6/1		

[a]Structure number, Compound (Reference) Molecular Formula (Molecular Weight), and Ratio $[M+Na]^+/[M+H]+$. A selection of the structures have been illustrated.

TABLE 3. Comparison of the Group 1A Metal Iodides (0.05M Solutions) in Promoting Molecular Ion Complex Formation in Sulfolane

Molecular Ion Complex[a]	$[M+Li]^+$	$(M+Na]^+$	$[M+K]^+$	$[M+Rb]^{+b}$	$[M+Cs]^+$
Erythromycin (**2**, M+=733)	23	17	16	9 5	9
Cbz-Thr-Ser-Gly-Pro-Ala-Thr-OCH_3 (**19**, M^+=680) .	100	31	26	13 7	13
Phyllanthoside (**3a**, M^+=804)	43	23	30	20 13	21
Bryostatin 1 (**1**, M^+=904)	35	21	18	8 5	9

[a]Molecular ion complex intensities are expressed relative to $[M+Li]^+$ equal to 100 for the Table 3 hexapeptide in sulfolane containing 0.1M lithium iodide.

[b]Two peaks due to the isotopes ^{85}Rb and ^{87}Rb.

that eventual interpretation of these cleavage pathways by high resolution mass measurements and isotope labeling experiments will prove to be very productive. And further simplification of background peaks by collisionally activated dissociation (15) methods with a tandem mass spectrometer should also be helpful.

EXPERIMENTAL

The alkali metal salts were employed as received from Aldrich Chemical Co., (lithium and cesium iodides and NaCl), Fischer Scientific Co., (sodium and potassium iodides) and Alfa Products (rubidium iodide). The specimens of sodium tetrafluoroborate and sodium trifluoromethanesulfonate were prepared by reaction of NaOH with the corresponding acid.

Other general experimental and instrument (MAT 312 mass spectrometer and capillaritron source) details have been summarized previously (2, 3). All specimen concentrations for mass determinations were 1 mg/0.1 ml of solvent containing 0.14-0.15M amounts of the Group 1A metal salt.

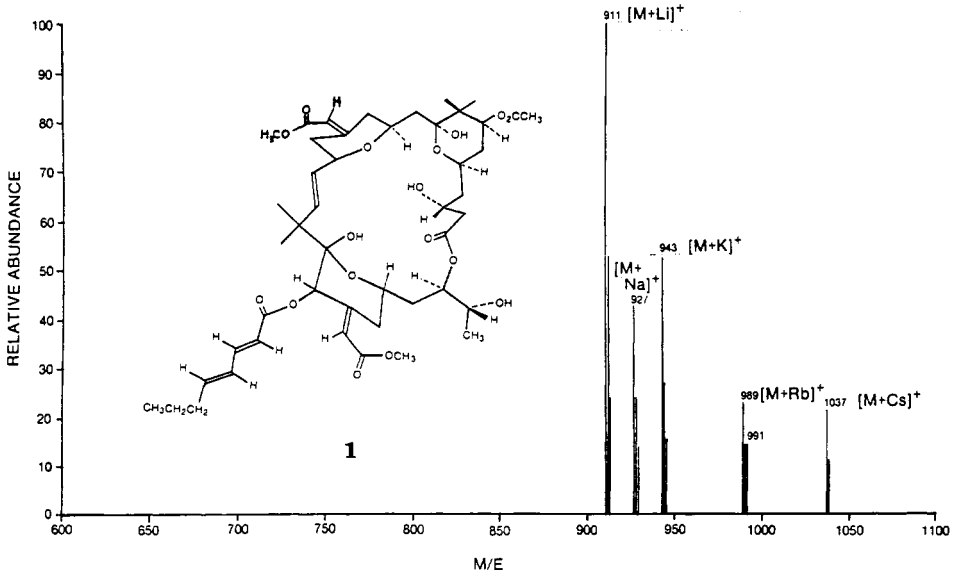
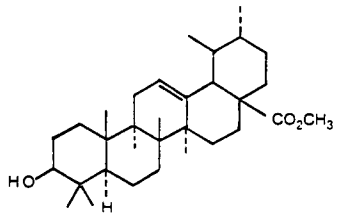


FIGURE 7. Bryostatin 1 (**1**) in Sulfolane Containing 0.05M Lithium, Sodium, Potassium, Rubidium, and Cesium Iodides.



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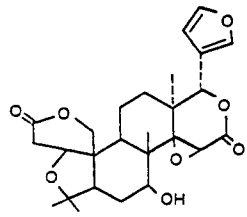

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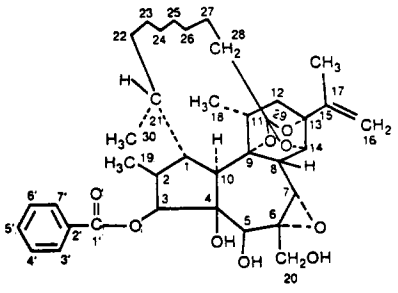

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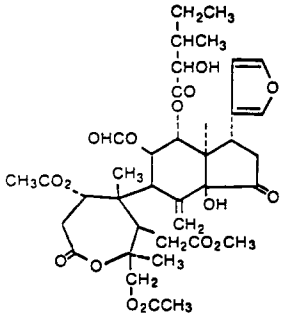

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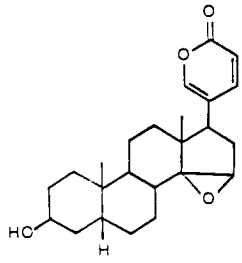

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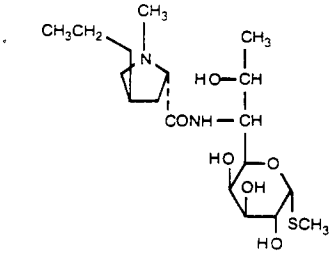

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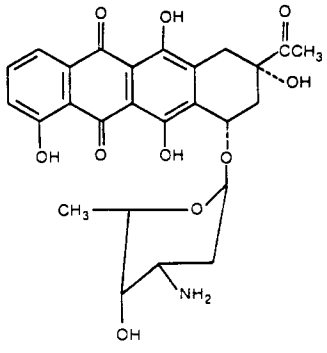

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The original positive ion mass spectra have been corrected by subtraction of peaks due to the solvent and solvent-metal complexes. The sulfolane(S)-Group 1A metal iodide (e.g., NaI) background peaks were of the type; $[S_n+Na]^+$, $[S_n+Na+NaI]^+$, and weaker $[S_n+Na+2NaI]^+$ and $[Na+(NaI)_n]^+$ where n=1,2,3, etc. The corresponding peaks appeared in matrixes based on glycerol (G) with additional weak peaks corresponding to $[G_n+2Na\text{-}H]^+$, $[G_n+2Na\text{-}H+NaI]^+$ and $[G_n+2Na\text{-}H+2NaI]^+$ where n=1,2,3, etc.

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