Journal of Natural Products
Vol. 52, No. 3, pp. 534-546, May-Jun 1989

MOLECULAR ION ENHANCEMENT USING SALTS IN FAB MATRICES FOR STUDIES ON COMPLEX NATURAL PRODUCTS

BIRENDRA N. PRAMANIK,* PRADIP R. DAS,

Research Division, Schering-Plough Corp., Bloomfield, New Jersey 07003

and AJAY K. BOSE

Department of Chemistry and Chemical Engineering, Stevens Institute of Technology, Hoboken, New Jersey 07030

ABSTRACT.—Production of intense pseudo-molecular ions of complex natural products in fabms can be achieved by the addition of salts (NH_4Cl, KCl, NaCl, etc.) to the glycerol-thioglycerol matrix. This simple technique produced $[M + NH_4]^+$, $[M + K]^+$, or $[M + Na]^+$ as base peaks in the fabms of such nonvolatile and/or thermolabile and multifunctional compounds as oligosaccharides, macrolides, and phospholipids. No modification of the mass spectrometer was necessary. This technique, using a mixture of $^{15}NH_4Cl/NH_4Cl$ or $NaCl/NH_4Cl$, can be used for the unambiguous determination of the molecular weights of unknown compounds.

The advent of fast atom bombardment mass spectrometry (fabms) has greatly aided the study of nonvolatile and/or thermolabile molecules (1–3). However, many of the more complex natural products with multiple functional groups (e.g., oligosaccharides and polyether antibiotics) and highly polar compounds (e.g., phospholipids) do not provide satisfactory mol wt and structural information.

The choice of matrices is an important aspect of fabms, as the nature of the matrix can influence the success of a fab analysis (4). Barber *et al.* (1) first demonstrated the value of glycerol as a matrix for enhancing the ionization of thermally labile and nonvolatile compounds. Such enhancement may sustain molecular/pseudo-molecular ions long enough in the source to permit collisional activation studies (5).

The usefulness of NH_4Cl in both eims and cims for enhancing ionization of multifunctional molecules was reported in our earlier publications (6,7). We describe now the role of NH_4Cl and other salts in facilitating fabms studies of a variety of natural products (8).

We have observed significant enhancement of the relative abundance of the pseudo-molecular ions of several types of compounds by the addition of salts (such as KCl, NaCl, NH_4Cl) to the fab matrix. We have utilized this simple technique to analyze multifunctional compounds such as oligosaccharide antibiotics, oligosaccharide-containing macrotetronolid antibiotics, phospholipids, acetylated sugars, and antiviral and antifungal natural products.

The formation of metal ion adducts has been reported by several investigators for many soft ionization techniques including sims (secondary ion mass spectrometry) (9) and laser desorption mass spectrometry (10). Fenselau and Cotter (11) and Gower (4) have reviewed the selective use of salts for different classes of compounds. A fab study on the cationization of phthalic acid has appeared (12).

RESULTS AND DISCUSSIONS

We have studied the effect on fabms of the addition of various salts to the glycerol-thioglycerol matrix. The natural products studied were such that under the standard conditions for recording fab spectra, very weak molecular or pseudo-molecular ions were observed; in some cases only fragment ions were displayed. The presence of KCl, NaCl, or NH_4Cl in the glycerol-thioglycerol matrix produced $[M + K]^+$, $[M + Na]^+$, or $[M + NH_4]^+$ as base peaks in the mass spectra. A limited number of experiments were conducted using divalent metal salts ($ZnCl_2$, $CoCl_2$, $CaCl_2$), but no $[M + Zn]^{++}$ or the corresponding divalent metal cationized species were observed.

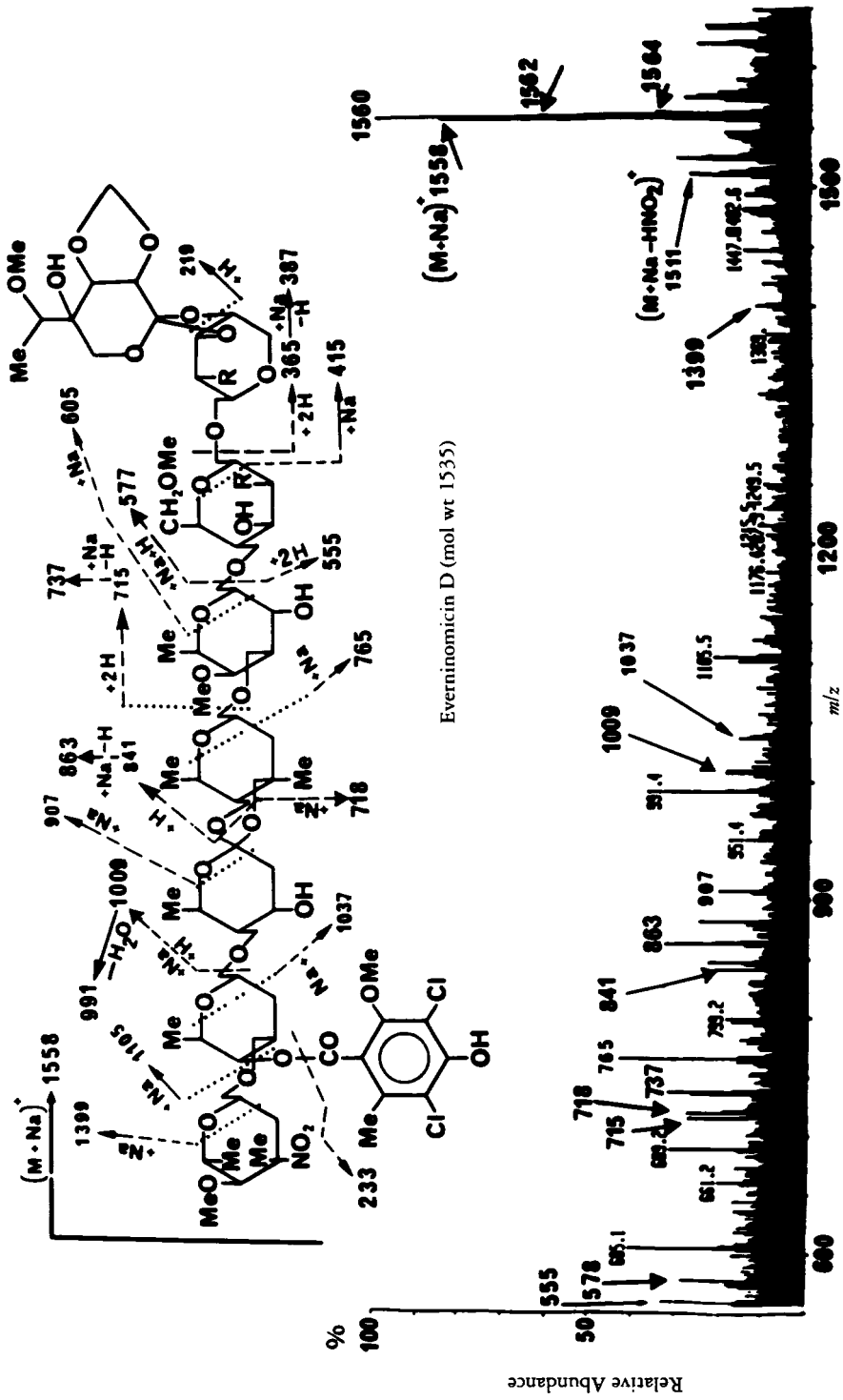


FIGURE 1. Fabms of everninomicin D (mol wt 1535) with NaCl added to glycerol-thioglycerol matrix.

Studies on a representative sampling of complex natural products of known structures are described below, and a protocol is developed for analyzing unknown compounds.

OLIGOSACCHARIDE ANTIBIOTICS.—Everninomicins, a novel class of oligosaccharide antibiotics, are produced by *Micromonospora carbonaceae* and are highly active against Gram-positive bacteria. The fabms data of everninomicins using glycerol-thioglycerol matrix has been reported (13); these compounds provide weak molecular ions in the positive ion fab spectra. In the presence of NaCl or KCl, everninomicin D provided very intense pseudo-molecular ions $[M + Na]^+$ or $[M + K]^+$ as base peaks in the spectra (Figure 1). The fragmentation pattern was similar to that reported earlier (13); however, in this case stable fragment ions with the attachment of Na or K were also observed in the spectra that were not seen earlier. Another set of cationized ions corresponding to the hemiacetals, formed from the cleavages at the anomeric carbon and at the ether linkage, was noted in the spectra (for further details, see Figure 1). Fragment ions below 500 amu are not shown in the figure.

Peracetylated oligosaccharides (lactoses), in our experience, provided weak or no pseudo-molecular ions in fabms studies. The fab mass spectrum of octa-*O*-acetyl-α-D-lactose (Figure 2a) is dominated by the intense fragment ions (*m/z* 619, 559, 457, 331), and the mol wt information is difficult to obtain from the spectrum. Salt addition, on the other hand, not only provided structurally informative fragment ions, but the cationized molecule $[M + K]^+$ was 100% in most cases. (For more details, see Figure 2b.)

POLYETHER ANTIBIOTICS.—Nigericin, along with lasolocid and antibiotic X-206, was the first polyether antibiotic reported in the literature (14). The eims of nigericin did not yield a molecular ion, and the highest mass peak observed was *m/z* $[M - 2H_2O]^+$ 688. The negative chemical ionization mass spectra (15) of this class of compounds displayed very strong $[M - H]^-$ ions. Initially, in the fab analysis of nigericin, a complex mass spectrum, difficult to interpret, was generated (Figure 3a). The usefulness of adding various salts (KCl, NaCl, NH_4Cl, $^{15}NH_4Cl$) to fab matrices in determining the mol wt is illustrated in Figure 3 (b–e). Addition of salts produced very intense ions (all base peaks) of *m/z* $[M + NH_4]^+$ 742, $[M + Na]^+$ 747, and $[M + K]^+$ 763 which corresponded to ammoniation and cationization of the molecule (Figure 3b, 3c, and 3d, respectively). By using a mixture of NH_4Cl and $^{15}NH_4Cl$, we have been able to identify unambiguously the NH_4-adduct ions (Figure 3e). The fragmentations were minimal in all cases with salt addition. The weak cationized peaks observed at *m/z* 742, 747, and 763 in Figure 3a were possibly due to the presence of very low levels of salts as contaminants in the sample. The various purification steps involved in the isolation of this compound may introduce salt contaminants.

PHOSPHOLIPID.—Figures 4a and 4b describe the fab mass spectra of 1-palmitoyl-2-oleoyl-phosphatidyl ethanol. A very weak (0.2%) molecular ion at *m/z* $[M + H]^+$ 703 was displayed in the spectrum when no salt was added to the matrix, whereas with KCl added to the matrix, very intense pseudo-molecular ions at *m/z* $[M + K]^+$ 741 and $[M + 2K - H]^+$ 779 were observed. Some major fragments are indicated in the figure. The formation of multiple cationized species could possibly be attributed to the presence of acidic protons in the molecule. The same phenomenon was observed upon addition of KCl to carboxylic or phenolic compounds. Because the two pseudo-molecular ions are intense and differ by 38 amu, they are easily recognizable. Similarly, NaCl addition produces two pseudo-molecular ions differing by 22 amu. In our studies, this phenomenon was not observed with ammonium salts.

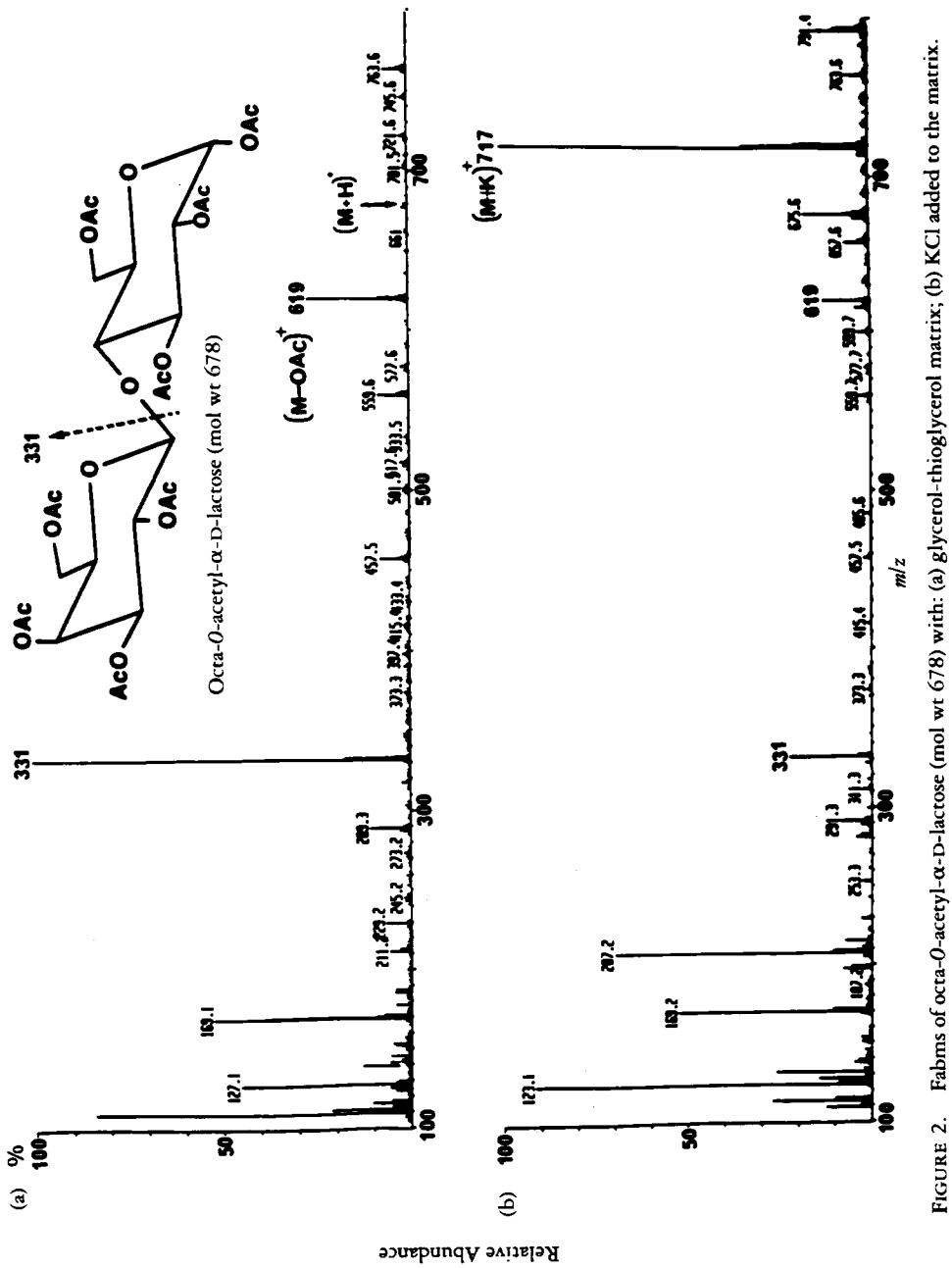


FIGURE 2. Fabms of octa-*O*-acetyl-α-D-lactose (mol wt 678) with: (a) glycerol-thioglycerol matrix; (b) KCl added to the matrix.

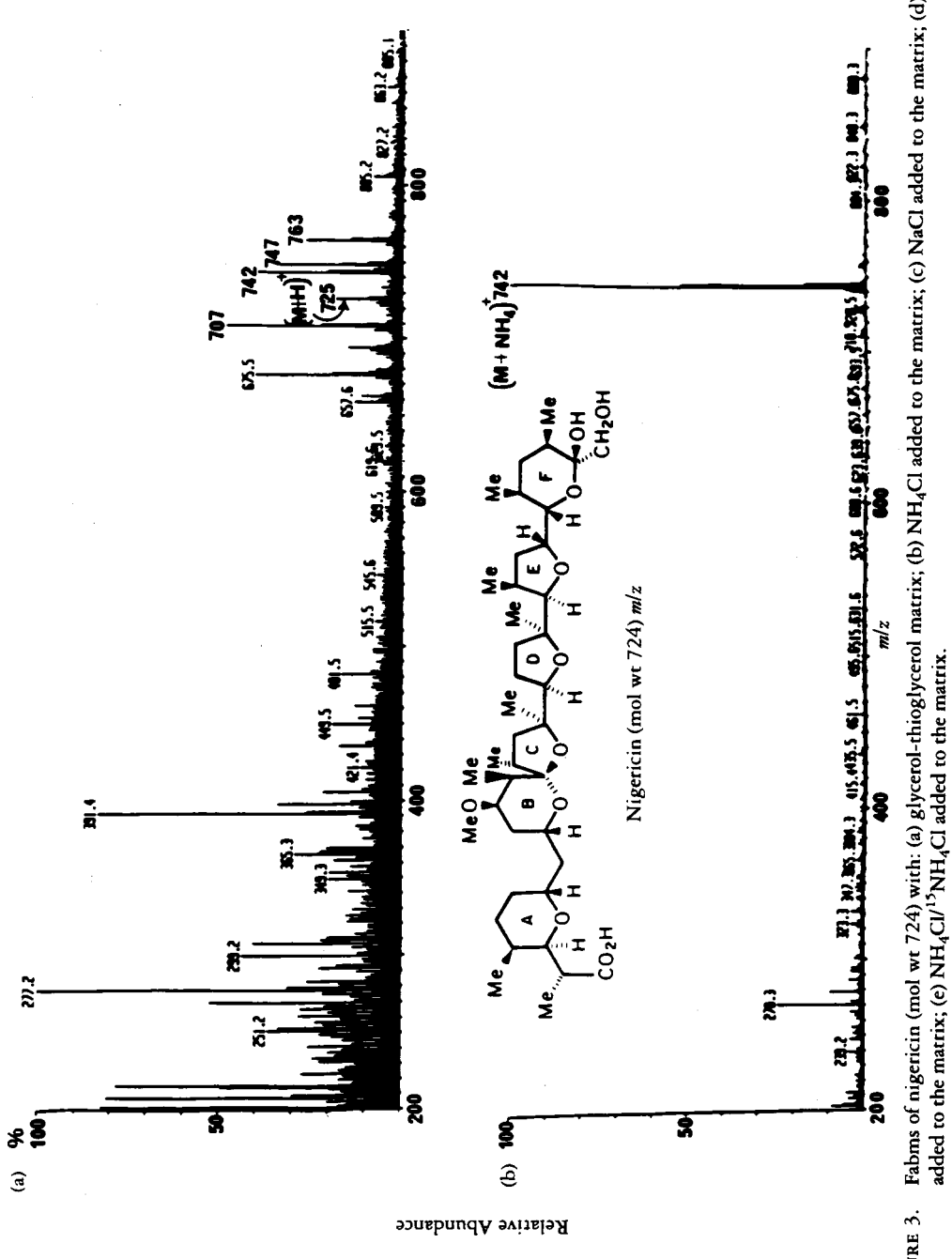


FIGURE 3. Fabms of nigericin (mol wt 724) with: (a) glycerol-thioglycerol matrix; (b) NH_4Cl added to the matrix; (c) NaCl added to the matrix; (d) KCl added to the matrix; (e) $NH_4Cl/^{15}NH_4Cl$ added to the matrix.

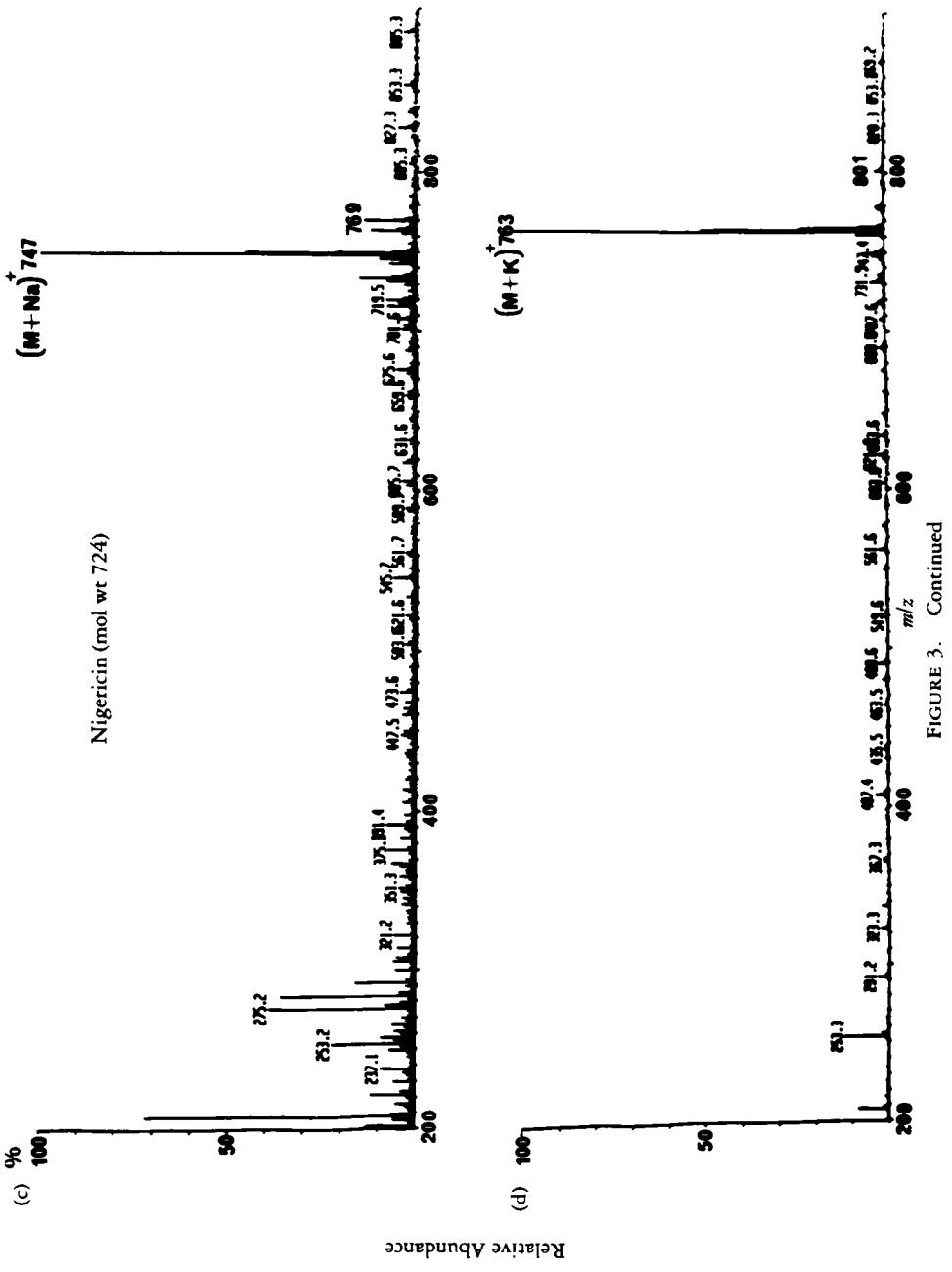


FIGURE 3. Continued

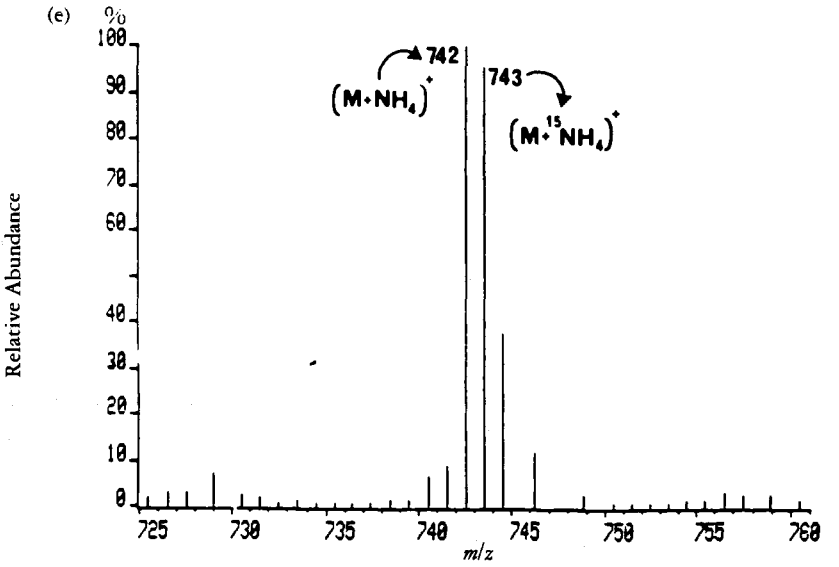


FIGURE 3. Continued

MACROLIDE ANTIBIOTIC.—Venturicidin A, an antifungal antibiotic isolated from *Streptomyces aureofaciens* strains, was analyzed by fabms in the absence of salt and with salt (NH_4Cl, NaCl). The fabms data of these analyses are presented in Figure 5. Venturicidin A, mol wt 749, produced a weak molecular ion, difficult to recognize, under fab conditions using glycerol-thioglycerol mixture as a matrix (Figure 5a). However, with addition of NaCl or NH_4Cl to the matrix, it provided *m/z* $[M + Na]^+$ 772 or *m/z* $[M + NH_4]^+$ 767 as base peaks, with characteristic fragment ions 559 $[M - \text{sugar}]^+$ and 541 $[M - \text{sugar} - H_2O]^+$ in the spectra (Figure 5b,c). These ions were also observed in the spectra with no salt added.

AMINOGLYCOSIDES.—Compounds with multiple amino functions (aminoglycosides) provided $[M + H]^+$ when the data were acquired in the presence of NH_4Cl and no $[M + NH_4]^+$ was observed in the spectrum. This observation is consistent with the fact that protonation is likely to occur for compounds with higher proton affinity than NH_3.

HIGHER-MOLECULAR-WEIGHT MACROLIDE OLIGOSACCHARIDE.—Kijanimicin, an oligosaccharide-containing macrotetronolide antibiotic, has a complex structure with high mol wt (1316). Attempts to determine the mol wt of kijanimicin by eims, cims, and fdms were unsuccessful. The ^{252}Cf-pdms (plasma desorption mass spectrometry) of 26-*O*-methyl kijanimicin (mol wt 1330) afforded a molecular ion at *m/z* $[M + Na]^+$ 1353. The fabms data of methyl kijanimicin (16) showed an intense peak at *m/z* $[M + Na]^+$ 1353, while the parent antibiotic (kijanimicin, mol wt 1316) itself displayed a moderately strong ion at *m/z* $[M + Na]^+$ 1339 when the analysis was performed in glycerol-thioglycerol matrix. The Na ion adduct *m/z* $[M + Na]^+$ 1339 appeared to be due to a low level Na salt contamination in the sample. No Na salt was added during these analyses. However, with NaCl addition to the matrix, the intensity of the cationized molecule was enhanced about threefold; the mass spectrum also contained structurally informative fragment ions (Figure 6a). With KCl or NH_4Cl added to the glycerol-thioglycerol matrix, $[M + K]^+$ or $[M + NH_4]^+$ was observed as the base peak in the spectra; weak $[M + Na]^+$ ion was also present in both cases.

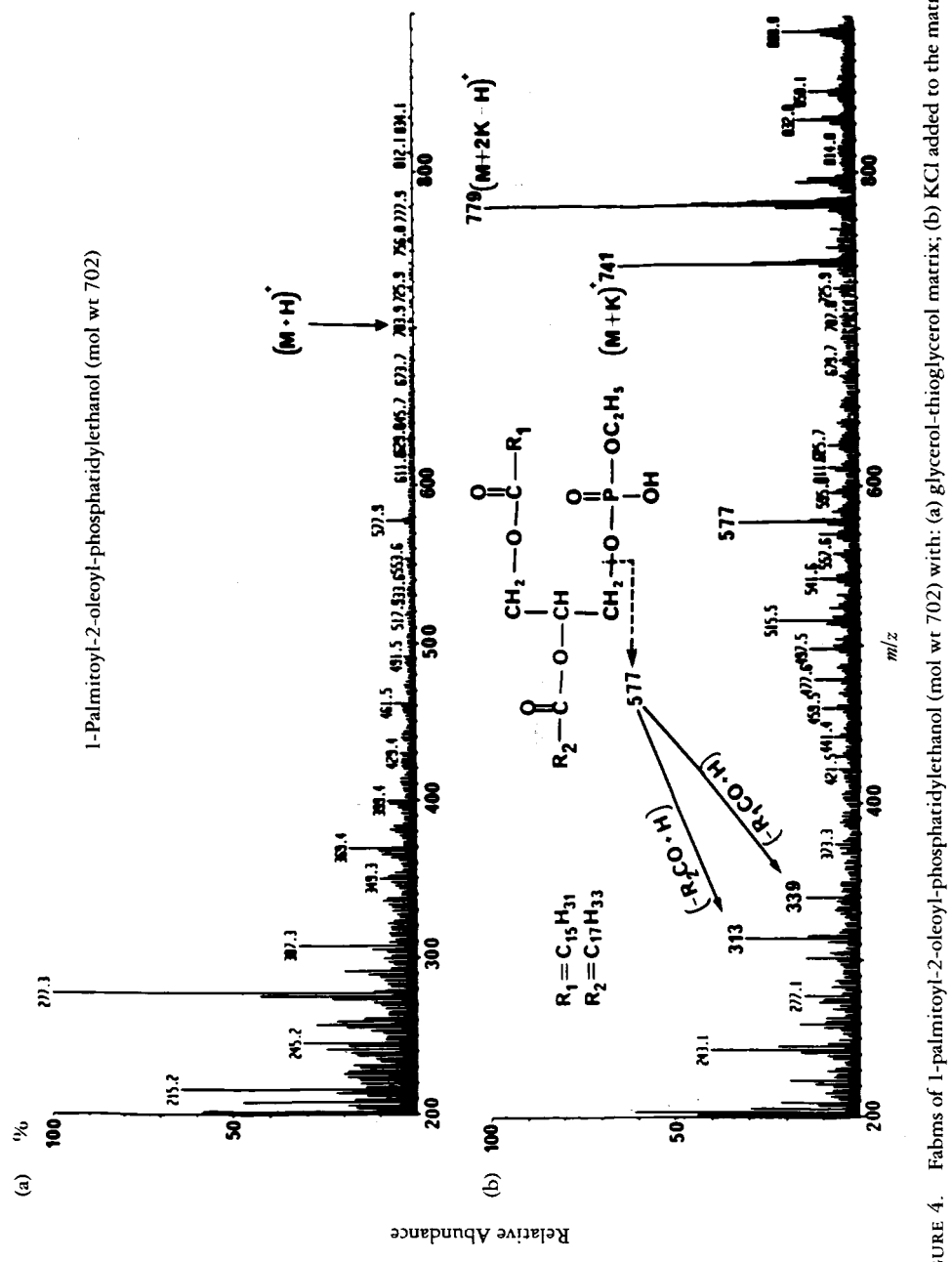


FIGURE 4. Fabms of 1-palmitoyl-2-oleoyl-phosphatidylethanol (mol wt 702) with: (a) glycerol-thioglycerol matrix; (b) KCl added to the matrix.

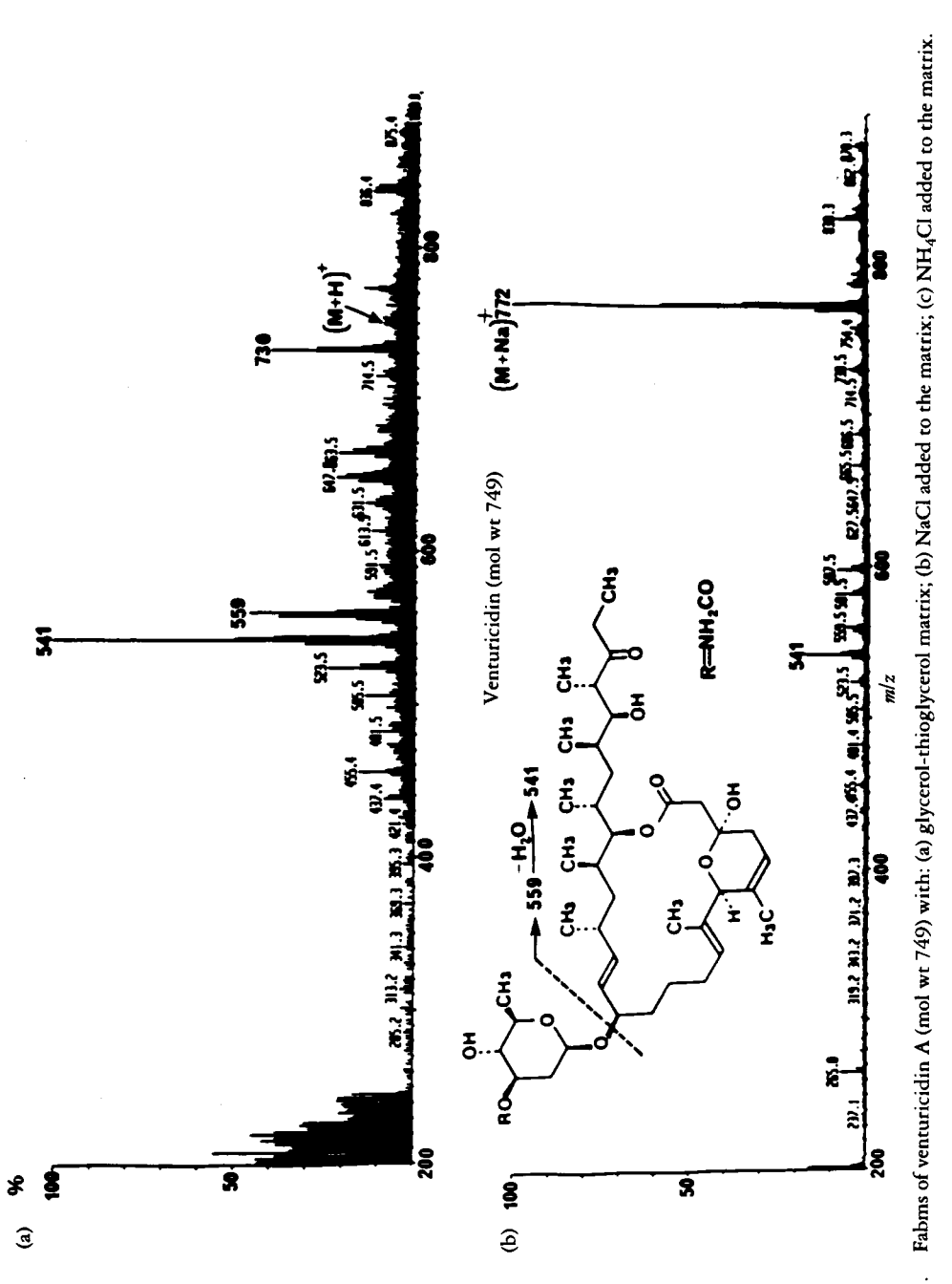
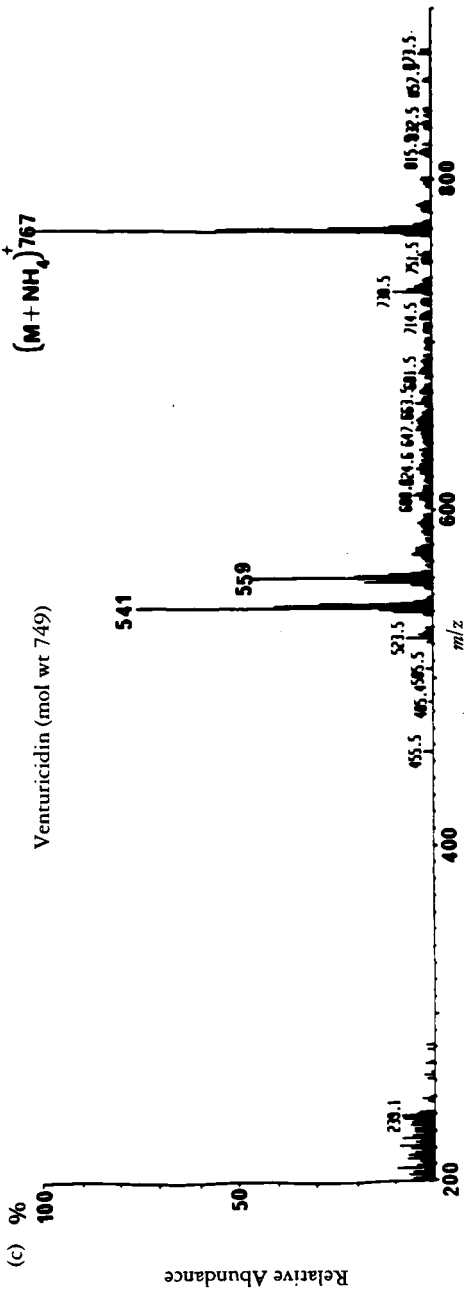


FIGURE 5. Fabms of venturicidin A (mol wt 749) with: (a) glycerol-thioglycerol matrix; (b) NaCl added to the matrix; (c) NH_4Cl added to the matrix.



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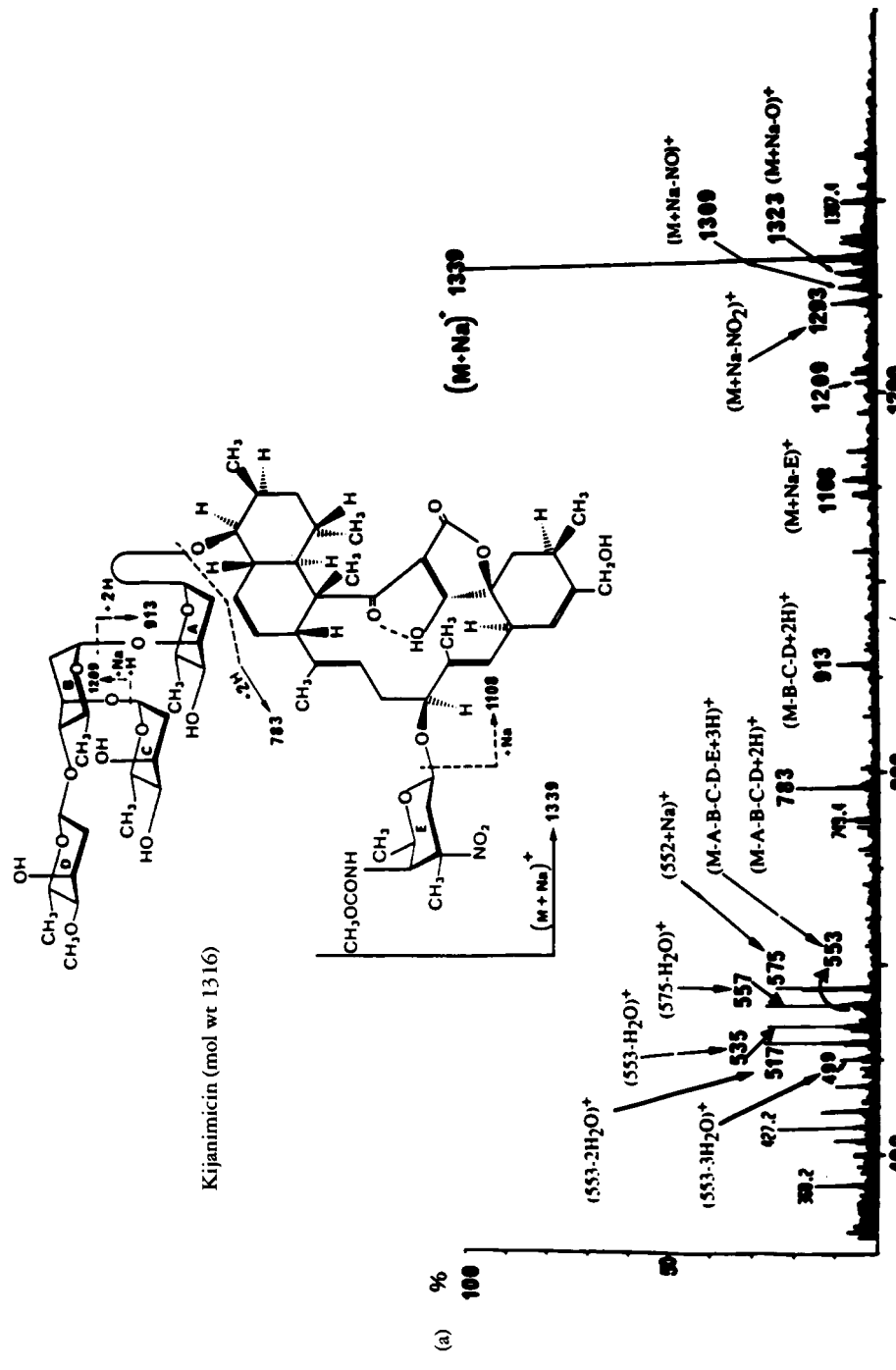

FIGURE 6. Fabms of kijanimicin (mol wt 1316) with: (a) NaCl added to the matrix; (b) NH_4Cl/NaCl mixture added to the matrix.

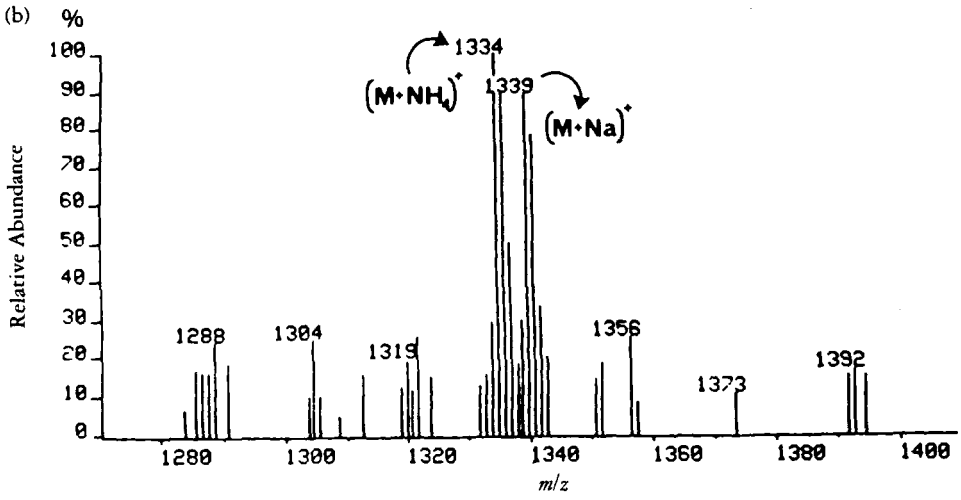

FIGURE 6. Continued

In a separate experiment, by using a 1:1 mixture of two salts, preferably NH_4Cl/NaCl, molecular ions at $[M + NH_4]^+$ and $[M + Na]^+$ were obtained 5 amu apart in a single scan in the fab spectrum. This information is valuable to the practicing natural product chemist for an unknown compound of natural origin when the quantity of material is extremely small and only one analysis can be performed. The addition of NaCl and KCl salts produces pseudo-molecular ions differing by 16 amu; for an unknown compound(s) this 16 units of difference could be mistaken for an oxygen atom. In a case study, kijanimicin, in the presence of NH_4Cl-NaCl (1:1), showed intense ions at *m/z* $[M + NH_4]^+$ 1334 and $[M + Na]^+$ 1339 and thus provided unambiguous information about the mol wt of the compound from single scan data (Figure 6b).

STUDIES ON UNKNOWN COMPOUNDS.—Our results amply demonstrate that, for a variety of natural products that are very difficult to ionize by the usual glycerol-thioglycerol matrix, addition of salts enhances the relative abundances of the pseudo-molecular ions significantly. The formation of the cationized species depends on the nature of the sample, the salt, and their relative concentrations. The fragmentation patterns, with and without salt addition, were found to be similar in most cases, with the exception of everninomicin D and related oligosaccharides, which generated additional cationized fragment ions. The use of a mixture of salts ($^{15}NH_4Cl/NH_4Cl$ or NH_4Cl/NaCl) in the fab matrices provides unambiguous mol wt information about an unknown compound from single scan data. Finally, the salt addition (fab) technique is cost effective and very simple to implement on any fab system with no instrumental modification, and the method provides valuable information for the structural studies of complex natural products without the need for prior derivatization.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All studies were conducted on a VG-ZAB-SE double focusing reverse geometry instrument using a VG 11-250J data system. Xenon gas at a source pressure of 2×10^{-6} mbar was used to generate fast atoms. This was done by an Ion Tech saddle field gun at a discharge current of 1 mA of Xe and accelerated through 8 kV. All operations were carried out at room temperature.

Everninomicin, kijanimicin, and peracylated lactose are standard Schering compounds, and the phospholipid sample was obtained from other commercial sources. Nigericin and venturicidin were obtained from Drs. R. Cooper and E. Barrrabee, respectively, of the Microbiology Department at Schering. Samples

were dissolved in DMSO (2–5 μg/μl) to which a salt (KCl, NaCl, or NH_4Cl) was added such that the sample-to-salt ratio was approximately 1:3. A thin layer of glycerol-thioglycerol (50:50) was applied to the copper probe tip to which the sample solution was added and thoroughly mixed. The probe was then introduced into the ion source operating at an accelerating potential of 8 kV.

ACKNOWLEDGMENTS

We wish to thank Mr. Peter L. Bartner for his technical assistance and Miss Karen Korec for typing the manuscript. One of us (A.K.B.) acknowledges support from the Office of Naval Research (N00014-83-C-0233).

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Received 24 October 1988