

FIELD DESORPTION MASS SPECTRA OF ANTIBIOTICS

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Field desorption mass spectrometry has been shown to be the method of choice in determining molecular weights of a number of non-volatile or thermally unstable antibiotics. Examples are neomycin, streptolydigin, and novobiocin, which do not give molecular ions by electron impact or chemical ionization mass spectrometry. In addition, field desorption mass spectrometry provides good indication of the composition of complexes of antibiotics, as illustrated by streptovaricin, filipin, and dermostatin.

Field desorption¹⁾ has rapidly become recognized as a technique *ne plus ultra* for the ionization and mass spectral investigation of organic compounds of low volatility or thermal instability. Since these are precisely the characteristics of many antibiotics it seemed appropriate, in view of our long-standing interest in antibiotics,²⁾ to investigate the applicability of the field desorption technique to a number of antibiotics of several classes, most of whose structures we had assigned in our laboratory.

Methods

Except as noted, all field desorption measurements were carried out on a Varian MAT CH5 DF mass spectrometer (double focussing), equipped with a combination field desorption-field ionization-electron impact ion source. Experimental conditions included 10~20 ma of emitter wire current optimized to give best anode temperature (BAT) for molecular ions, 60° source temperature, 1,000 resolution, 3 kV accelerating potential, and 4~10 sec/decade scanning rate. Approximately 0.5~2 μg of sample (obtained by drying the adsorbed quantity from a solution of 10~30 $\mu\text{g}/\rho\text{l}$) was used. Spectra were recorded oscillographically, measured manually, and plotted on a STATOS 21 recorder. Peak positions were determined by comparing the positions of the mass marker with those for a recorded electron impact spectrum of perfluorokerosene.

Electron impact spectra were obtained, using a line of sight solid inlet probe, on a Varian MAT CH5 DF spectrometer, with data reduction³⁾ employing a Varian Data Systems 620i computer and STATOS 21 recorder. High resolution mass measurements, indicated by four decimal places, were carried out by the peak matching procedure on a Varian MAT 731 mass spectrometer. Chemical ionization spectra were obtained on CEC 21-110 mass spectrometers, employing the direct probe technique and the ionizing gases indicated.*

Results

Antibiotics of a variety of classes were studied—macrolides (erythromycin), polyenes (filipin, dermostatin), ansamycins (streptovaricins), aminocyclitols (neomycin), acyltetramic acids (streptolydigin), and others (novobiocin). Electron impact (EI) and field desorption (FD) mass spectra were determined for all the antibiotics and chemical ionization (CI) mass spectra for those which gave

* See footnote on page 2.

CI spectra.* Spectra determined by two or three of these methods are presented in Figs. 1 (erythromycin), 2 (streptovaricin A), 3 (streptovaricin complex), 4 (novobiocin), 5 (streptolydigin), 6 (filipin complex), 7 (dermostatin complex), and 8 (neomycin B).

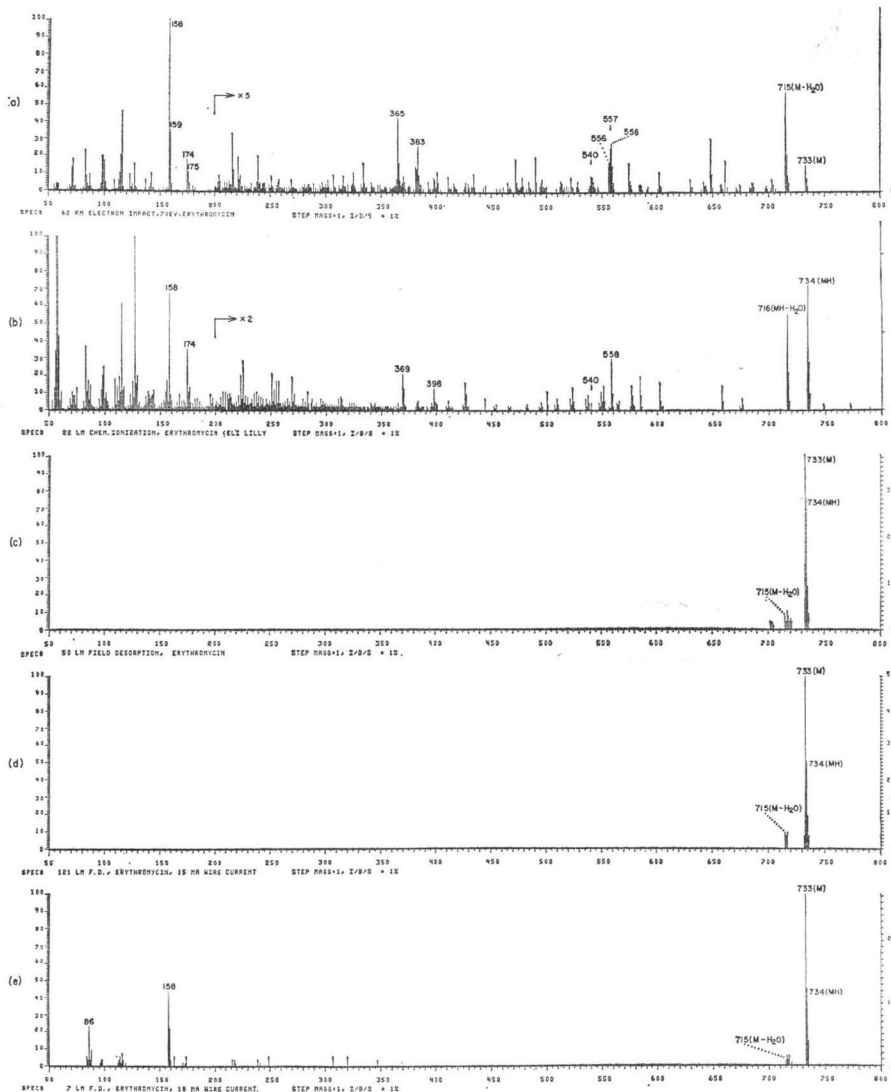
Discussion

Erythromycin

Although the mass spectrum of erythromycin itself ($M=733$, $C_{27}H_{47}NO_{13}$) does not seem to

Fig. 1. Mass spectra of erythromycin.

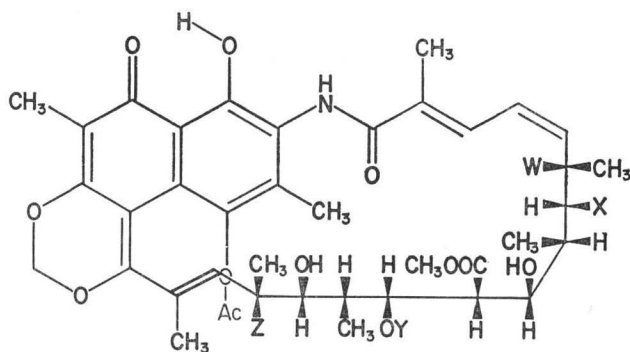
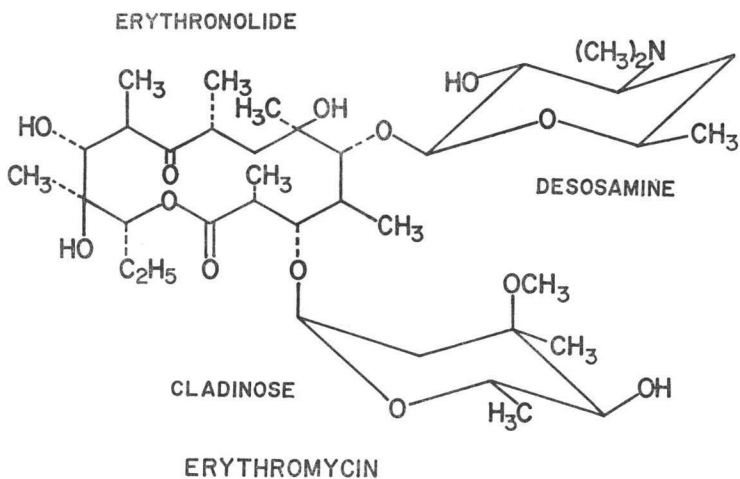
(a) Electron impact (EI) spectrum; (b) chemical ionization (CI) spectrum (isobutane ionizing gas); (c) field desorption (FD) spectrum, best anode temperature (BAT); (d) field desorption spectrum, 15 mA wire current, 731 spectrometer; (e) field desorption spectrum, 18 mA wire current, 731 spectrometer.



* Attempts to determine chemical ionization mass spectra of antibiotics other than erythromycin, novobiocin, and streptolydigin were unsuccessful. This presumably represents failure of the compounds to volatilize in the probes employed rather than failure of the ionization method *per se*.

have been reported, it would be expected to present relatively few difficulties in view of the reported electron impact⁴⁾ mass spectra of numerous neutral macrolides and the chemical ionization mass spectra of both neutral and basic macrolides,^{5,6)} including erythromycin B,⁶⁾ which lacks one hydroxyl group of erythromycin itself. The expectation of success was indeed justified, as can be seen from the electron impact spectrum* shown in Fig. 1a. Although the molecular ion is observed at m/e 733 it is weak (*ca.* 1 % of the base peak), presumably due to low volatility (five hydroxyl groups) and ready fragmentation at the glycosidic bonds of the sugars. Other weak ions are found at m/e 715 ($M-H_2O$), 557.3538 ($C_{29}H_{51}NO_6$, M -cladinose), 556.3475 ($C_{29}H_{50}NO_6$), 540.3519 ($C_{29}H_{50}NO_5$, M -cladinose-OH), and 382.2340 ($C_{21}H_{34}O_6$, M -cladinose-desosamine).

Much stronger peaks are observed at m/e 158.1181 ($C_8H_{16}NO_2$, desosaminyl) and 159.1256 ($C_8H_{17}NO_2$). Although the molecular ion (actually $M+H$, m/e 734) and the ions due to loss of water (m/e 716) and cladinose (m/e 558) from the $M+H$ ion were somewhat more intense than those in the electron impact spectrum, the chemical ionization mass spectrum (Fig. 1b, isobutane ionizing gas)** has much the same appearance as the electron impact spectrum. A dramatic contrast is provided, however, by the field desorption mass spectrum (Fig. 1c), in which the molecular ion at m/e 733 is almost the only ion found. Thus, even for this relatively simple mass spectral problem, field desorption mass spectrometry provides an ionization technique which is not only gentle, stressing the molecular ion, but is also fully



STREPTOVARICIN	<u>W</u>	<u>X</u>	<u>Y</u>	<u>Z</u>
A	OH	OH	Ac	OH
B	H	OH	Ac	OH
C	H	OH	H	OH
D	H	OH	H	H
E	H	O=	H	OH
G	OH	OH	H	OH
J	H	OAc	H	OH

* Dr. M.I. LEVENBERG, Abbott Laboratories, has informed us of a similar electron impact spectrum (50 eV, 155°C source temperature, direct probe) determined on an AEI MS-902 spectrometer.

** Determined by Dr. J.L. OCCOLWITZ, Lilly Research Laboratories.

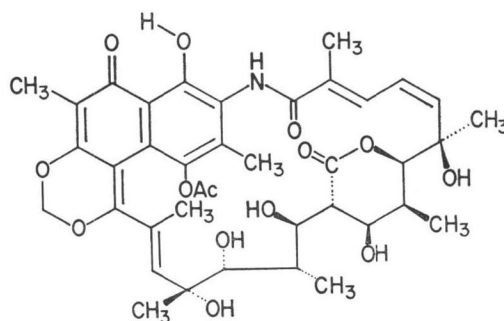
complementary with *both* the electron impact and chemical ionization techniques.

In order to study the effect of anode temperature, FD spectra of erythromycin were also measured at 15 and 18 mA wire current on a Varian MAT 731 spectrometer (Fig. 1d and 1e, respectively). The 15-mA spectrum is like the FD spectrum discussed above, showing only the molecular ion ($M=733$) together with traces of a cluster of ions near m/e 715 ($M-H_2O$). The 18-mA spectrum, however, shows a relatively intense ion at m/e 158 (desosaminyl) and a slightly less intense ion at m/e 86 (unidentified).

Some additional information on fragments is, therefore, supplied by the FD spectrum at higher anode current but still not as much as that in the EI or CI spectrum.

Streptovaricins

The electron impact mass spectra of the streptovaricins⁷³ and their O-methyl and O-acetyl derivatives have provided a great deal of useful information in structural studies of these novel antibiotics.



STREPTOVARICIN F

Fig. 2. Mass spectra of streptovaricin A.
(a) EI spectrum; (b) FD spectrum, BAT.

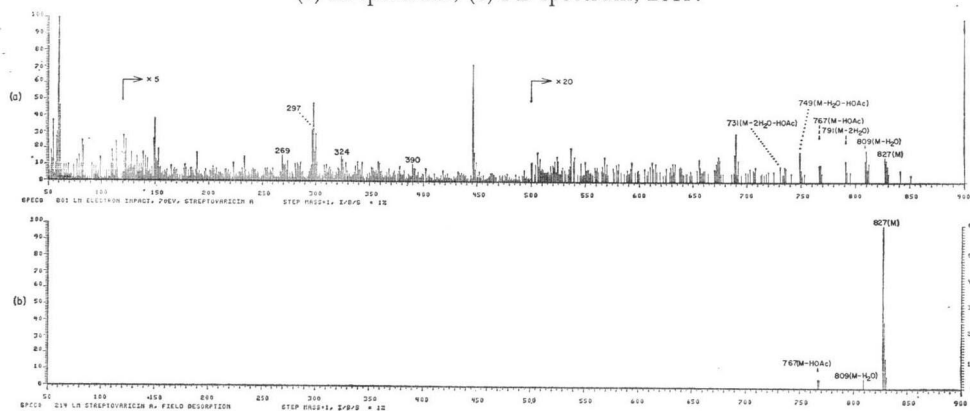
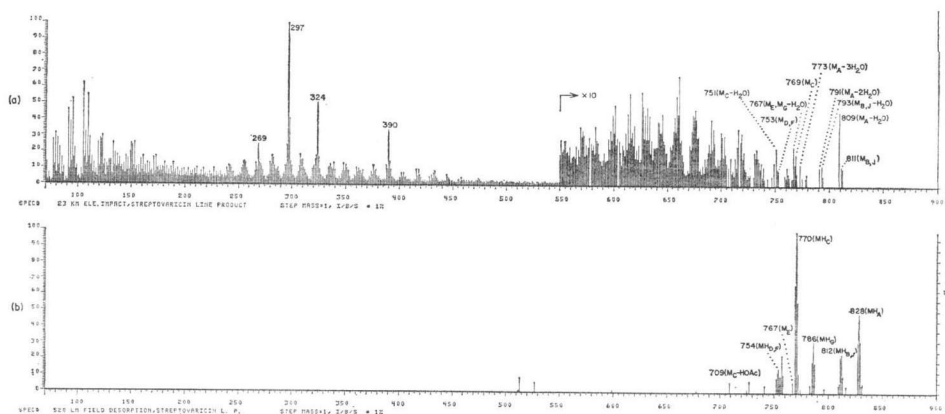


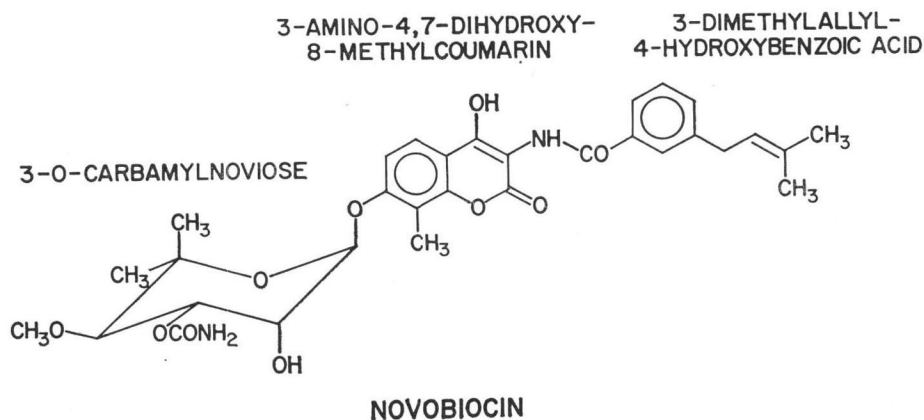
Fig. 3. Mass spectra of streptovaricin line product.
(a) EI spectrum; (b) FD spectrum, BAT.



Streptovaricin A is the component of highest molecular weight ($M=827$, $C_{42}H_{53}NO_{16}$), but even its electron impact spectrum shows a weak molecular ion (Fig. 2a), in spite of its five aliphatic hydroxyl groups and its amide and enol functions.* Nevertheless, the field desorption mass spectrum (Fig. 2b) provides a dramatic contrast, showing only the molecular ion and weaker ions at $M-H_2O$ (m/e 809) and $M-HOAc$ (m/e 767).

A more interesting study involves the streptovaricin complex, which has been shown^{7,9} to consist of the characterized streptovaricins B,C,E,F,G, and J in addition to streptovaricin A. The electron impact mass spectrum (Fig. 3a) of streptovaricin line product (Upjohn, 11,560-3) shows weak molecular ions for streptovaricins B and/or J (m/e 811), C (m/e 769), E (m/e 767), plus D and/or F (m/e 753), but none for streptovaricins A (m/e 827) and G (m/e 785). In addition, the intense diagnostic peaks⁷ at m/e 390, 324, 297 and 269, found in spectra of nearly all the streptovaricins, are very prominent since they are additive for the antibiotics. The field desorption mass spectrum (Fig. 3b), however, shows molecular ions for all of the presently recognized streptovaricins (A, m/e 827; B,J, 811; C, 769; D,F, 753; E, 767; G, 785), together with ions for loss of water from some of the components.** Curiously, the $M+H$ ions are more intense for the components of the streptovaricin complex than the true molecular ions (M), while the reverse was true in the spectrum of streptovaricin A (Fig. 2). It should be noted, however, that M and $M+H$ ions were found in nearly all field desorption spectra of the present study, with the two ions being of comparable intensity.

It is of interest to compare the relative amounts of the streptovaricins indicated by their mass spectra with the best available estimates obtained by other methods. Thin-layer chromatography on silica gel suggests that streptovaricin line product (Upjohn 11,560-3) contains *ca.* 18 % streptovaricin A, 8 % streptovaricin B, 28 % streptovaricin C, and 13 % streptovaricin G, with other streptovaricins being present to only a lesser extent.⁹ Countercurrent distribution indicates 42 % A, C and F, 4 % B, 4 % D, 1 % E, 12 % G, and 3 % J (P.V. DESHMUKH, University of Illinois, unpublished observations). The electron impact spectrum (Fig. 3b) gives a totally incorrect picture. The field desorption spectrum indicates 22 % A, 11 % B (or J), 44 % C, 7 % D (or F), 2 % E, 7 % F (or D), 14 % G, and 11 % J (or B). Although the field desorption figures are about 1~1½ times as high as those indicated by thin-layer chromatography and countercurrent distribution, since the latter allow for additional materials (not streptovaricins) present in the line product, the ratios are roughly the same (*e.g.*, A:C:G, 3:5:2). In fact, the field desorption spectrum may provide as good an estimate as any other method of the actual composition of the streptovaricin complex.



* See footnote on page 2.

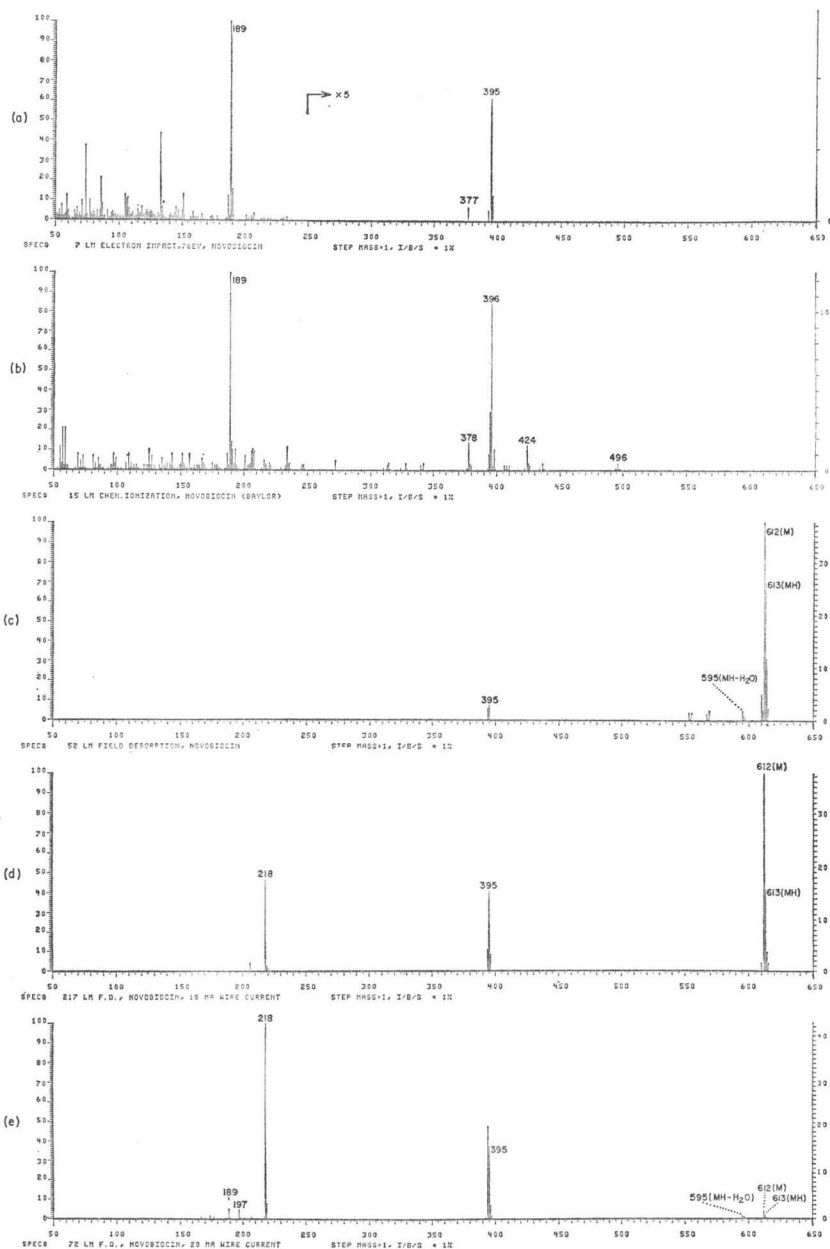
** This spectrum was determined on a Varian MAT 731 high resolution mass spectrometer, using the low resolution program³) to assign peak heights and positions directly.

Novobiocin

The mass spectrum of novobiocin ($M=612$, $C_{31}H_{38}N_2O_{11}$) seems not to have been recorded previously. Polar features include hydroxyl, amide, and enol groups. Neither the electron impact (Fig. 4a) nor the chemical ionization spectrum (Fig. 4b) shows a molecular ion, though the latter (isobutane* or methane**) contains a weak ion for $M-CH_3$ at m/e 597. Other recognizable, more

Fig. 4. Mass spectra of novobiocin.

(a) EI spectrum; (b) CI spectrum; (c) FD spectrum, BAT; (d) FD spectrum, 15 mA, 731 spectrometer; (e) FD spectrum, 18 mA, 731 spectrometer.



* Determined by Dr. J.L. OCCOLOWITZ, Lilly Research Laboratories.

** Determined by Dr. J.A. McCLOSKEY, Baylor College of Medicine.

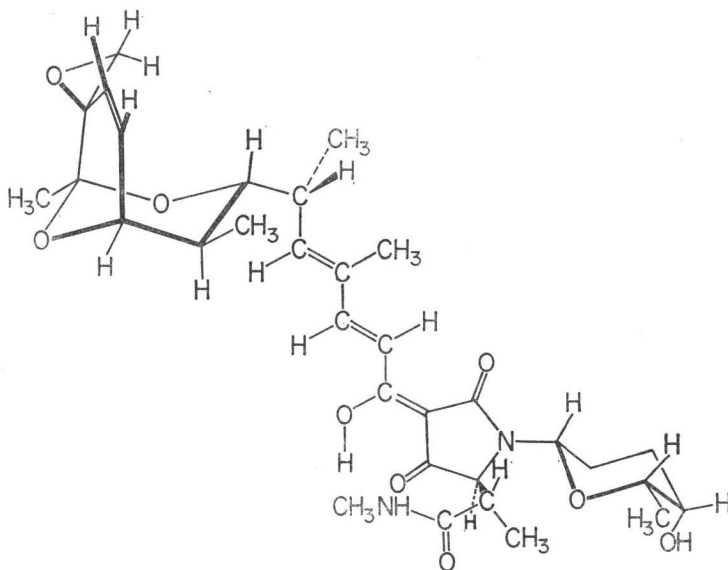
intense, ions are found in the electron impact spectrum at m/e 395.1342 ($C_{22}H_{21}NO_6$, M-carbamyl-noviosyl+H), 189.0912 ($C_{12}H_{13}O_2$, ring A, due to fragmentation at the NH-CO bond), 151.0388 ($C_8H_7O_3$) and 133.0288 ($C_8H_5O_2$), the last two due to cleavage within the coumarin system. Corresponding ions are found at m/e 396 and 189 in the chemical ionization spectra. By contrast, the field desorption spectrum (Fig. 4c) of novobiocin contains a very intense molecular ion, once again illustrating the complementary nature of field desorption with either electron impact or chemical ionization.

Field desorption spectra of novobiocin were also determined at 15 and 20 mA on a Varian MAT 731 spectrometer (Fig. 4d and 4e, respectively). At 15 mA the molecular ion ($M=612$) still provided the base peak, but it was joined by intense peaks at m/e 395 and 218. The former peak is found in the EI and CI spectra, but the latter, due to the carbamylnoviosyl ion is not. Thus, the FD spectrum at 15 MA provides a diagnostic fragment ion lacking in the EI and CI spectra. At 20 mA the ion at m/e 218 provides the base peak, with the peaks at m/e 395

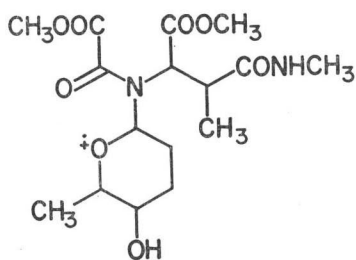
and 612 (M) being the second and third most intense. Other ions in the 20-mA spectrum, not found in the 15-mA spectrum, are at m/e 595 ($MH-H_2O$), 197 (unidentified), and 189 (from ring A).

Streptolydigin

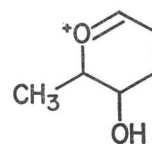
As with novobiocin, neither the electron impact (Fig. 5 a) nor the chemical ionization mass spectrum (Fig. 5 b, c) of streptolydigin ($M=600$, $C_{32}H_{44}N_2O_6$) shows a molecular ion, although



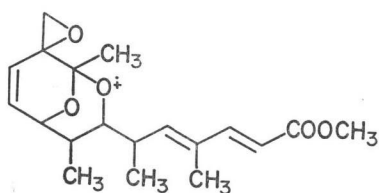
STREPTOLYDIGIN



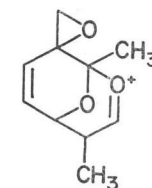
DIMETHYL DIGINATE
 $M = 374$ (13%)



m/e 115 (100%)



METHYL STREPTOLATE
 $M = 334$ (2%)



m/e 181 (100%)

Fig. 5. Mass spectra of streptolydigin. (a) EI spectrum; (b) CI spectrum (methane ionizing gas); (c) CI spectrum (isobutane ionizing gas); (d) FD spectrum, BAT.

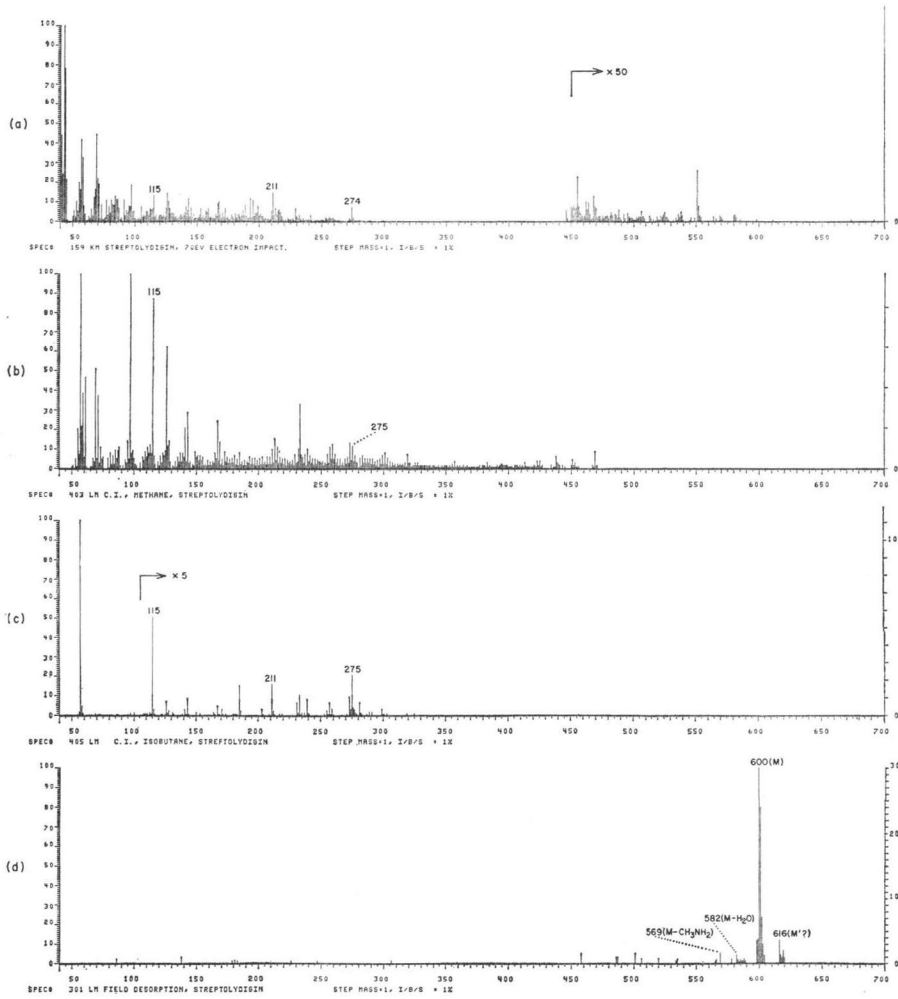


Fig. 6. Mass spectra of filipin. (a) EI spectrum; (b) FD spectrum, BAT.

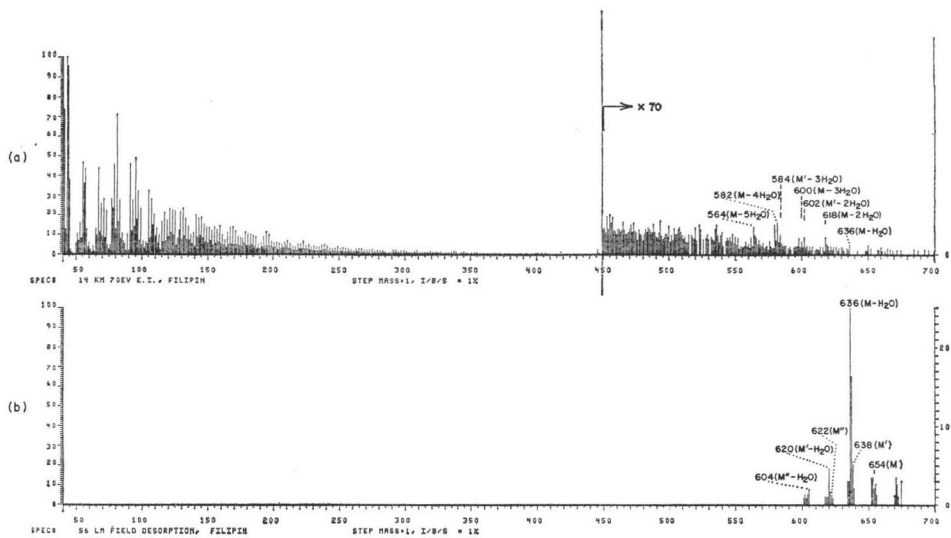


Fig. 7. Mass spectra of dermostatin acetate.
(a) EI spectrum; (b) FD spectrum, BAT.

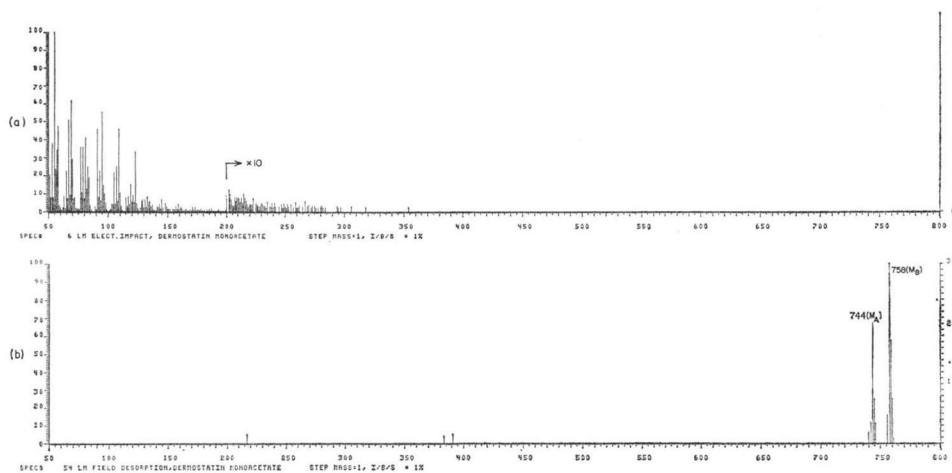
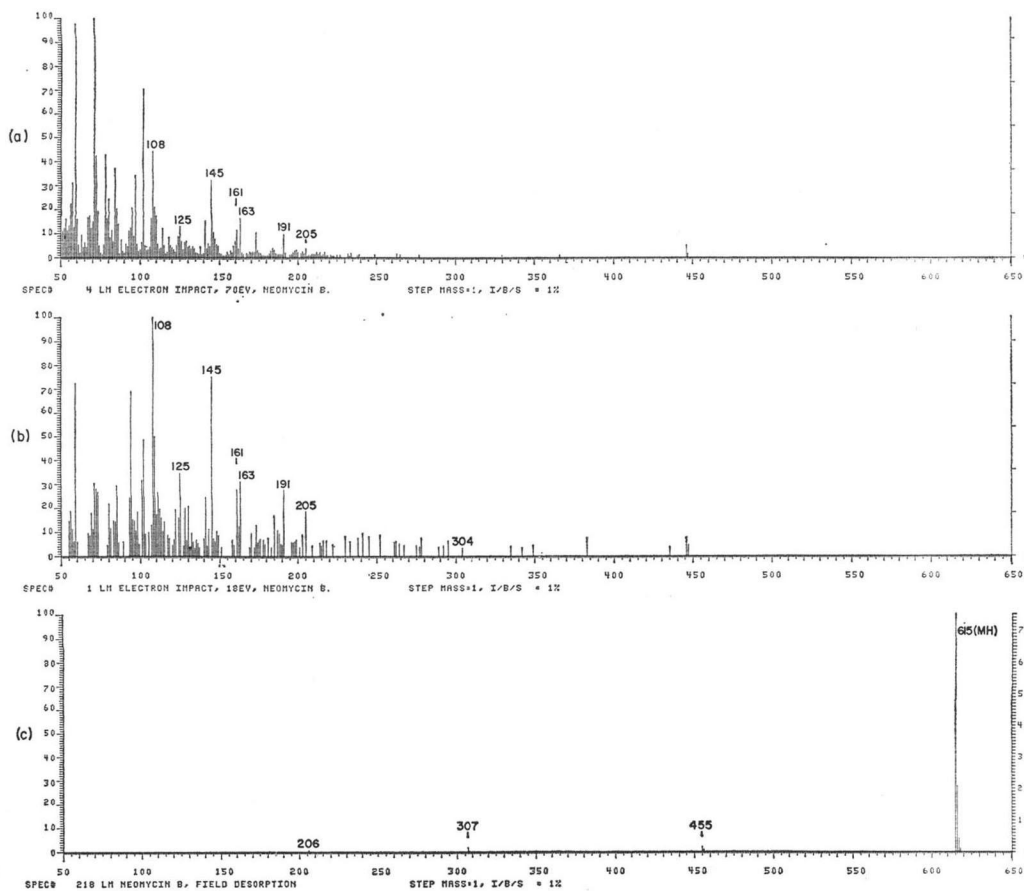


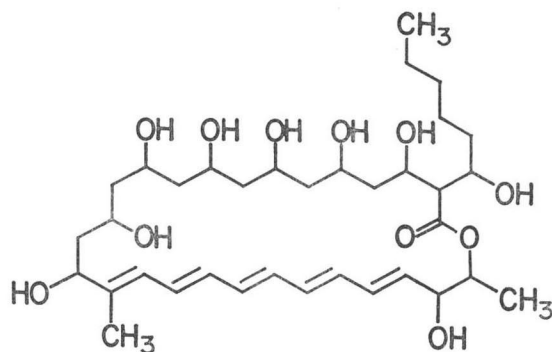
Fig. 8. Mass spectra of neomycin B.
(a) EI spectrum; (b) EI spectrum, 18 eV ionizing voltage; (c) FD spectrum.



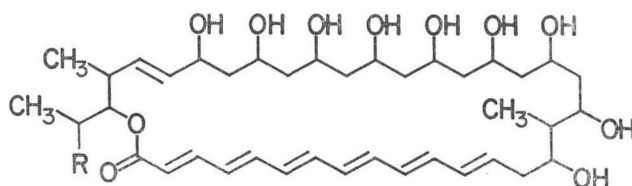
ions at m/e 582 ($M-H_2O$), 569 ($M-CH_3NH_2$), and 551 ($M-H_2O-CH_3NH_2$) are found in the EI spectrum. More surprisingly, few strong ions are found due to the characteristic fragmentations¹⁰⁾ of the degradation products of streptolydigin. Some of those ions are present in the electron impact spectrum (Fig. 5a) but are weak and not prominent: those at m/e 211 and 274 are from the streptolic acid portion of the molecule, that at m/e 115 from the sugar portion of streptolydigin,^{10b)} but the expected ion at m/e 181 from the streptolic acid portion^{10c)} is lacking. This is also true for the EI spectrum at reduced ionizing voltage.

The CI spectra (isobutane* or methane**) are also less characteristic than hoped, though the isobutane CI spectrum (Fig. 5c) is perhaps the most interpretable of the four. Once again, however, the molecular ion is the most prominent in the field desorption spectrum

(Fig. 5d). Additional, weaker ions at somewhat higher masses (near m/e 616) can be attributed to minor components recently observed (H. A. WHALEY, The Upjohn Co., personal communication to K.L. RINEHART) in the fermentation product.

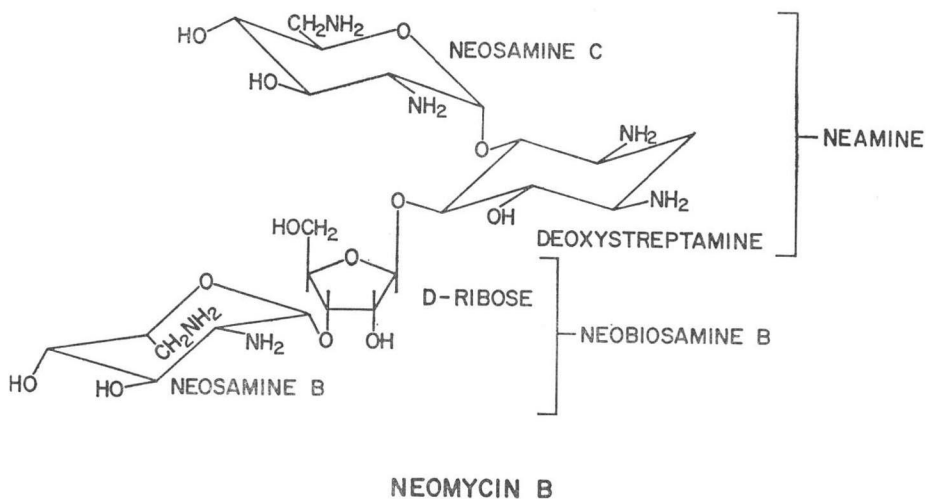


FILIPIN III (IV)



DERMOSTATIN A : R = CH₃

DERMOSTATIN B : R = C₂H₅



NEOMYCIN B

* Determined by Dr. J.L. OCCOLOWITZ, Lilly Research Laboratories.

** Determined by Dr. J.A. MCCLOSKEY, Baylor College of Medicine.

Filipin

The structure of filipin was assigned (most recently) in 1964.¹¹⁾ Shortly thereafter, it was shown to be a mixture of four components,¹²⁾ which differed¹³⁾ in the number of hydroxyl groups (seven, eight, or nine). These components give good EI spectra as their acetate derivatives.¹³⁾ Thus, the spectrum of filipin is the spectrum of a mixture, with filipins III and IV ($M=654$, $C_{88}H_{88}O_{11}$) the major components. This is not clear from the EI spectrum of the antibiotic (Fig. 6a),* which contains no molecular ions and no characteristic fragmentations, as might be expected for a molecule with nine hydroxyl groups (in the major components) and no recognizable sub-units. It does contain peaks for loss of water, but these are not very prominent. By contrast, the field desorption spectrum of filipin (Fig. 6b) contains peaks for the three expected molecular ions, at m/e 654, 638 and 622. However, considerably more intense ions are observed at positions corresponding to loss of water from the three molecular ions, *i.e.*, at m/e 636, 620 and 604. The intensities of these $M-H_2O$ ions suggest the composition of the filipin complex to be 5 % filipin I, 15 % filipin II, and 80 % filipins III and IV (isomers). These values compare reasonably well with those obtained from chromatographic separation by BERGY and EBLE¹²⁾: 4 % filipin I, 25 % II, 53 % III, 18 % IV. However, an even closer fit is obtained by summing the M and $M-H_2O$ ion intensities for each component, which gives 7 % I, 25 % II, and 68 % III and IV.

Dermostatin

The antibiotic dermostatin consists of two components, dermostatins A and B ($M=720$ and 734 , $C_{40}H_{64}O_{11}$ and $C_{41}H_{66}O_{11}$, respectively), whose structures have recently been assigned.¹⁴⁾ The polyacetates of dermostatin give good EI spectra.¹⁴⁾ Like filipin, these antibiotics have no discrete sub-units and, with nine hydroxyl groups, the electron impact mass spectra of the underivatized antibiotics would be expected to give no molecular ions and no recognizable fragments. This is true for the EI spectrum,* shown in Fig. 7a. By contrast, the FD spectrum (Fig. 7b) contains two very intense ions in the molecular ion region at m/e 744 and 758, indicating clearly a mixture of homologs. These two ions do not correspond to the molecular formulas previously assigned to dermostatins A and B, but they would correspond to the masses expected for the ions $M+C_2H_2O-H_2O$, *i.e.*, to ions resulting from loss of water from the mono-acetates of dermostatins A and B. The composition of the complex would be estimated from the FD spectrum to be 40 % dermostatin, A, 60 %, B, which is in good agreement with that estimated (43 % A, 57 % B)¹⁴⁾ from glc of reduction products derived from the complex.

Neomycin

Neomycin is a complex of antibiotics, of which neomycin B ($M=614$, $C_{23}H_{46}N_8O_{13}$) is the principal component.¹⁵⁾ With seven hydroxyl groups and six primary amino groups it represents the least volatile compound in the present series. In the past it has been necessary to convert it to its hexa-*N*-acetyl-hepta-*O*-trimethyl derivative for a direct inlet spectrum.¹⁶⁾ The EI spectrum of underivatized neomycin B (Fig. 8a) shows, as expected, no molecular ion, but weak ions are observed for ions characteristic of the deoxystreptamine ring (plus attached carbons of neosamine C and D-ribose) at m/e 145, 163, 191, and 205.¹⁷⁾ In the EI spectrum (Fig. 8b) determined at lower ionizing potential (18 eV vs. 70 eV) these characteristic fragment ions are enhanced and an ion at m/e 304 for loss of neobiosamine is observed, but still no molecular ion is found. As with the other com-

* See footnote on page 2.

pounds in the present group, the FD spectrum of neomycin (Fig. 8c) gives an intense molecular ion, here at m/e 615 ($M+H$), together with far weaker ions at m/e 455, 307 and 206, due to loss of sugar fragments.

Comments

From the spectra shown in Figs. 1~8 it is clear that field desorption mass spectrometry is the method of choice for determining the molecular weights of complex non-volatile antibiotics. Indeed, with new antibiotics of unknown structures this should be one of the first determinations made. For new compounds it would be especially important to make the determinations at high resolution, which can distinguish molecular ions M from $M+H$ ions. One may still wish to obtain the complementary EI and/or CI spectra to make use of the information usually found in these spectra, but field desorption spectra determined at different emitter wire currents (temperatures) may provide much of the information found in EI and CI spectra.

The use of FD mass spectrometry in determining the composition of mixtures of related antibiotics also appears to be very promising since many antibiotics are found as complexes. Results described for a mixture of dermostatins A and B, homologs, are very good. Results for filipins I~IV and streptovaricins A~J are not quite as close to chromatographic results as those for the dermostatins, but the filipins and streptovaricins differ in the number of hydroxyl groups they contain; thus, volatility and the degree of dehydration should be different for the components. An attempt to sum all ions (M and $M-H_2O$) derived from each component yielded improved results for the filipin complex.

Acknowledgment

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