Table IV—Statistical Analysis of Digoxin 0.25-mg Tablet Content by HPLC and RIA with Paired t Test at 95% Confidence Level

Lot[a]	Method	Average Digoxin Content, μg	p
A	HPLC	235.6 ± 9.5	<0.05
A	RIA	252.6 ± 3.8	
B	HPLC	238.2 ± 13.0	<0.05
B	RIA	250.8 ± 6.6	

[a] $n = 10$.

that the digoxin would inhibit the radiolabeled antigen from binding with the antiserum in the same manner as if the digoxin was present in human serum. Since the drug was dissolved in USP dilute alcohol, there were no other steroid molecules present in the solutions which might cross-react with the antiserum. The filtering step prior to the assay procedure eliminates most of the excipient ingredients in the tablet dosage form.

As mentioned previously, the content uniformity test for tablets in USP XX required that each tablet must contain not <85% or not >115% of the average of the limits specified in the drug monograph. Digoxin tablets must contain <90% or not >105% of the label claim. Thus, a conforming tablet must fall within 82.9 and 112.1% of the average of the digoxin monograph limits[18].

All digoxin tablets assayed by both HPLC and RIA met the requirements of the content uniformity test.

This study showed that RIA is an accurate alternative to HPLC for content uniformity of digoxin tablets. The data showed the range of digoxin content determined by RIA was narrower than the range of digoxin content determined by HPLC. The significant difference at the 95% confidence level with the paired t test between the assay results of both methods showed that the RIA method appeared to be more precise and closer to the labeled amount than HPLC in the determination of digoxin content in the two lots of digoxin tablets.

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In-Beam Electron Ionization Mass Spectra of Penicillins

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Abstract □ The characteristics of in-beam electron ionization mass spectra of 6-aminopenicillanic acid and several penicillins, which yield no detectable molecular ion peaks using a conventional direct-insertion probe, have been established. The spectra of all compounds studied, with the exception of amoxicillin, exhibited molecular ion or (M+1) peaks with spectral features similar to the reported methyl ester or amide derivatives of the compounds. The fragmentation of penicillin G on electron impact under in-beam conditions can be described on the basis of data from mass analyzed ion kinetic energy spectrometry. A desorption technique utilizing polyethylene glycol 4000 was used as a means of obtaining satisfactory spectra of ampicillin and amoxicillin.

Keyphrases □ Penicillin—in-beam ionization mass spectra β-lactam antibiotics, amoxicillin, ampicillin □ Electron ionization mass spectra, in-beam—penicillin, β-lactam antibiotics, amoxicillin, ampicillin □ Amoxicillin—in-beam electron ionization mass spectra of penicillins, β-lactam antibiotics □ Ampicillin—in-beam electron ionization mass spectra of penicillins, β-lactam antibiotics

Because of their low vapor pressure and thermal instability, penicillins, a class of β-lactam antibiotics, have generally required chemical pretreatment with formation of their esters or amides prior to mass spectrometric investigations (1, 2). Recently, isobutane and ammonia chemical ionization (CI) mass spectrometric data were published on the free acids of penicillins G and V (3), and the ammonia CI mass spectrum of the potassium salt of penicillin G was reported (4). Pyrolysis mass spectrometry was investigated (5) as a means of characterizing the compounds. The use of in-beam or extended-probe techniques to study apparently nonvolatile and thermally unstable compounds is now commonplace and well documented in the literature (6–14). Using this technique, ammonia-positive and methane-negative ion desorption CI of penicillins was reported (15); however, this report did not include any electron ionization (EI) data. Other researchers[1] have developed a technique using a mixture of the respective potassium salt and ammonium chloride to obtain the EI mass spectra of several penicillins. Accordingly, results obtained in this laboratory are presented on in-beam EI mass spectra of several penicillins, as their free acids, and their fragmentation processes based on mass analyzed ion kinetic energy spectrometric studies (16).

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Table I—In-beam EI Spectra [a] of Penicillins (Based on Cleavages in Scheme I)

Compound	$M^{+\cdot}$	a	b	c	d	e 160	f 115	g 100	h 114	i 75	Others [b]		
6-Amino-penicill-anic acid	216(9)	57(31)	(−)	(−)	116(40)	(91)	(9)	(17)	(69)	(100)			
I	334(2)	175(14)	119(18)	91(100)	234(−)	(20)	(16)	(40)	(32)	(44)	141(16)	118(47)	
II	350(2)	191(14)	135(20)	107(85)	250(3)	(68)	(13)	(42)	(55)	(66)	77(100)	94(85)	179(25)
											209(33)	211(48)	
III	402(1) (M + 1)	242(18)	186(8)	158(−)	301(−)	(12)	(14)	(31)	(19)	(36)	187(55)	144(100)	77(62)
											103(22)	217(18)	
IV	414(1)	255(25)	199(100)	171(98)	314(−)	(10)	(84)	(80)	(60)	(44)	69(68)	170(67)	
V	378(1)	220(−)	164(−)	136(9)	278(−)	(24)	(6)	(14)	(7)	(45)	91(100)	92(52)	102(24)
											135(10)	217(5)	
VI	349(0.3)	190(5)	134(−)	106(48)	249(−)	(53)	(12)	(30)	(37)	(100)	91(58)	104(49)	147(31)
											332(5)		
VII	365(−)	206(−)	150(−)	122(36)	265(−)	(67)	(24)	(60)	(80)	(100)	107(64)	120(41)	122(36)
	348(3) [c]										134(32)	163(24)	

[a] Unless otherwise noted, the numbers in parentheses represent percent relative intensity with (−) indicating not observed; all other numbers represent m/z values above m/z 50. [b] All spectra exhibit an intense peak at m/z 44. [c] No molecular ion was observed; the highest ion observed was M—OH at m/z 348.

EXPERIMENTAL

Apparatus—All spectra were recorded on a double-focusing mass spectrometer[2] equipped with a modified field-desorption (FD)/EI source. The emitter heating current contact of the FD source was modified for in-beam purposes similar to that reported previously (17). An unactivated tungsten wire (10-μm diameter) or stainless-steel wire (125-μm diameter) was substituted for an activated emitter, and no high voltage was applied to the source except the usual 3K accelerating voltage. The sample on the FD wire was placed at a distance <3 mm from the ionizing electron beam and quickly heated by a maximum of 50 mA of the heating current (17–19) supplied by a standard heating current supply unit. The spectra were recorded under the following conditions: ionizing energy, 80 eV; source temperature, 280–300°; sample heater, 50 mA; emission current, 500 mA; accelerating voltage, 3 kV. Mass analyzed ion kinetic energy spectra, using identical parameters, were recorded by electric sector scanning.

Reagents—The following commercially available penicillins or precursors were used without additional purification: 6-aminopenicillanic acid[3] (*a*) and the penicillins (*b*), penicillin G potassium (I)[4], penicillin V potassium (II)[4], sodium oxacillin (III)[3], sodium nafcillin (IV)[5], disodium carbenicillin (V)[6], ampicillin (VI)[5], amoxicillin trihydrate (VII)[6] (Fig. 1). The metallic salts of the particular penicillins (~1 mg) were dissolved in 0.5 ml of water and acidified by addition of a drop of 88% aqueous formic acid solution. Immediately after formation of the precipitated free acid, 0.5 ml of ethanol was added to make a clear solution. Then 1–2 μl of the solution was deposited on the tungsten wire from a microsyringe[7].

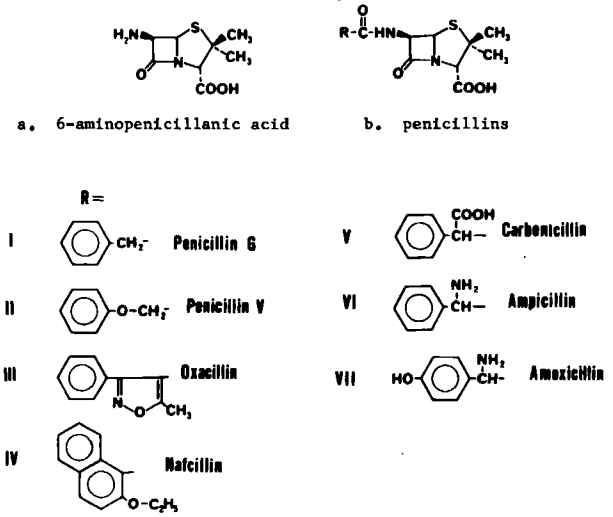


Figure 1—*Structures of pencillin analogues.*

[2] Varian MAT 311A, Varian Associates, Palo Alto, CA 94303.
[3] Bristol Lab., Syracuse, NY 13201.
[4] Pfizer Inc., Groton, CT 06340.
[5] Wyeth Lab., Philadelphia, PA 19101.
[6] Beecham Lab., Piscataway, NJ 08854.
[7] Unimetrics Corp., Anaheim, CA 92801.

The solvent was evaporated in the mass spectrometer before final insertion of the sample into the ion source.

To obtain more reproducible results with the free acids of ampicillin and amoxicillin, ~5 mg of polyethylene glycol 4000 was added to the aqueous solution of the sample and stirred until dissolved. An aliquot of the solution was deposited on the wire, and spectra were obtained as previously described. The addition of low levels of polyethylene glycol to the sample did not contribute markedly to the sample spectrum and could easily be subtracted from the spectrum by computer techniques. One explanation for this lack of contribution of the polyethylene glycol is the greatly varying vaporization temperatures between the desorption enhancer and the sample, similar to the best emitter temperature effect observed in field desorption mass spectrometry.

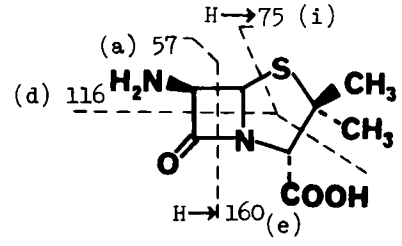
RESULTS AND DISCUSSION

Previous studies (1, 2) reported the electron ionization-induced fragmentation of penicillins G and V as the methyl ester or amide. An adaptation of these findings is summarized in Scheme I. Based on the ions in the spectrum, the nature of the R substituents could easily be assigned. In this work, the in-beam EI spectra of 6-aminopenicillanic acid, I, II, IV, V, and VI exhibit molecular ions, or MH ions in the case of III; VII, however, did not give $(M^{+\cdot})$ but gave instead $(M—OH)^+$ or $(MH—H_2O)^+$. The general fragmentation patterns of these compounds are similar to those reported for penicillin methyl esters or amides (1, 2) (Scheme I). The results are summarized in Table I in a format similar to that of previous work (2, 20).

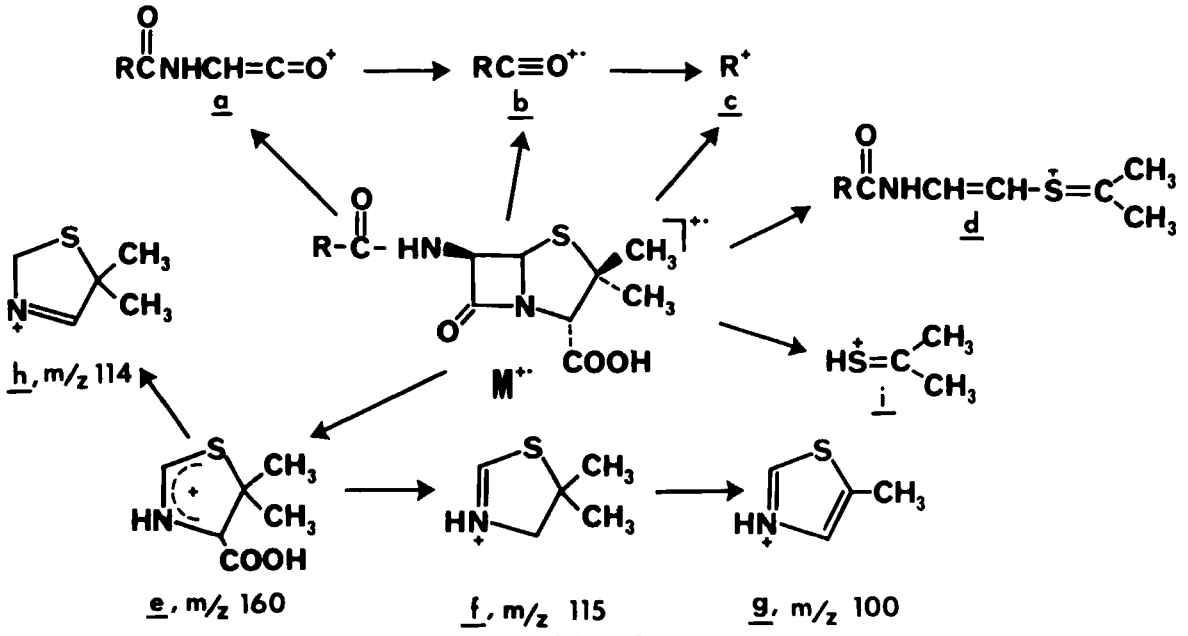
The simplest compound, 6-aminopenicillanic acid, is an amino acid thought to be difficult to vaporize without decomposition, but the in-beam EI technique routinely gave useful spectra, including the molecular ion at m/z 216. The abundant peaks are explained on the basis of fragmentation of the methyl esters presented previously (1). The electron ionization-induced fragmentation by the in-beam technique of the β-lactam ring system having the free acid moiety is shown below.

An important rearrangement ion at m/z 160 containing the thiazoline moiety is commonly observed in all spectra studied to date (Scheme I, *e*).

The fragmentation processes originating from the molecular ion of I have been examined by the mass analyzed ion kinetic energy spectrometric technique and are shown in Fig. 2A and 2B. The first spectrum (Fig. 2A) was recorded immediately after insertion of the sample into the ion source; the second spectrum (Fig. 2B) was recorded after a few min-

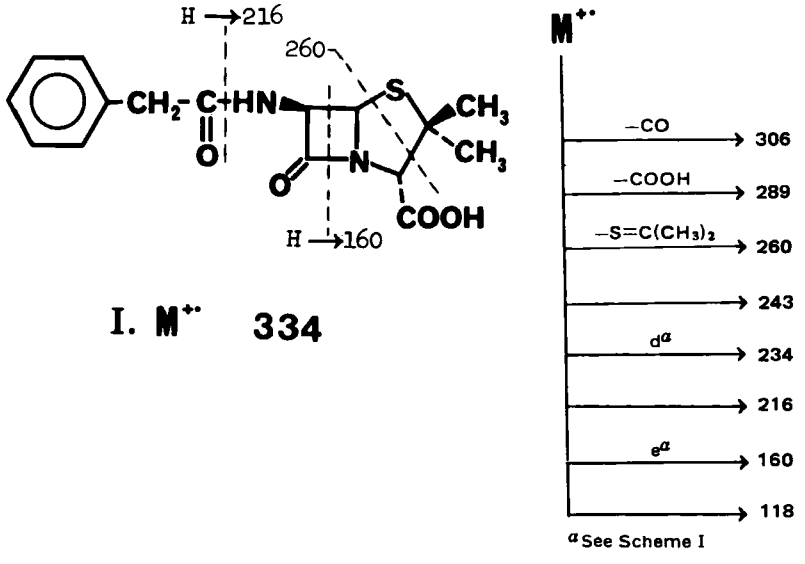


6-Aminopenicillanic Acid
$M^{+\cdot}$ 216



Scheme I

utes. This change in mass analyzed ion kinetic energy spectrometric spectral features suggests that the structures of the molecular ions undergo isomerization with time, *i.e.*, thermal alterations of the compound prior to electron ionization in the mass spectrometer. The major cleavages and metastable transitions from the molecular ion of I are summarized in Scheme II:



Scheme II

Eight transitions were clearly recognized. The transitions leading to the ions at *m/z* 289, 260, 234, 216, 160, and 118 have been suggested by previous investigators (1, 2) on the basis of accurate mass measurements and are confirmed by the mass analyzed ion kinetic energy studies. The transitions leading to the ions at *m/z* 234 and 160 correspond to processes d and e in Scheme I, respectively. In the mass analyzed ion kinetic energy spectra (Fig. 2A and 2B), one of the most abundant transitions is the rearrangement process with cleavage leading to the ion at *m/z* 216, corresponding to the molecular ion of 6-aminopenicillanic acid. Alternatively, the loss of carbon dioxide from the ion at *m/z* 260 could also conceivably yield *m/z* 216. However, since the methyl ester of I exhibits the same rearrangement ion with the empirical formula of 6-aminopenicillanic acid methyl ester confirmed by accurate mass measurement, the former process would be more probable. Additionally, transitions leading to the ion at *m/z* 293 corresponding to the loss of benzyl from the molecular ion and the ion at *m/z* 118 were observed. Since the contribution of the fragment ions to the in-beam spectra is negligibly small, successive transitions from these ions could not be investigated to further clarify the fragmentation scheme.

The in-beam EI spectrum of II exhibits a base peak at *m/z* 77 and abundant ions at *m/z* 94 and 107. These ions are commonly observed in the spectra of the methyl esters of I and II and are derived from the phenoxymethyl group based on accurate mass measurements (1). The mass analyzed ion kinetic energy spectrum of II (Fig. 2C) exhibits features suggesting that many isomerized forms of the molecular ion exist before and/or after electron ionization. The most intense transition in the spectrum at *m/z* 256 arises by the loss of phenol. The transitions leading to ions at *m/z* 216 and 160 (Scheme III) represent the rearrangement ions involving the β-lactam ring system; the fragment ions at *m/z* 276 and 250 are the phenoxy analogues of the ions at *m/z* 260 and 234, respectively. The other transitions are difficult to analyze because of the extensive thermal alterations of the molecule suggested earlier (Scheme III).

In the case of III, the $(M + 1)^+$ peak was observed at *m/z* 402 instead of $(M^{+\cdot})$. A series of peaks corresponding to $(MH—H_2O)^+$, $(MH—H_2O—CO)^+$, and $(MH—H_2O—CO_2)^+$ were detected at *m/z* 384, 356, and 340, respectively. The base peak at *m/z* 144, shown below, is believed to be derived from the *m/z* 187 ion, based on data of the well-known electron-impact fragmentation of the isoxazole moiety (21–25).

The in-beam EI spectrum of IV exhibits very intense peaks due to the naphthalene moiety at *m/z* 199 (base peak), 171, and 170 as well as the

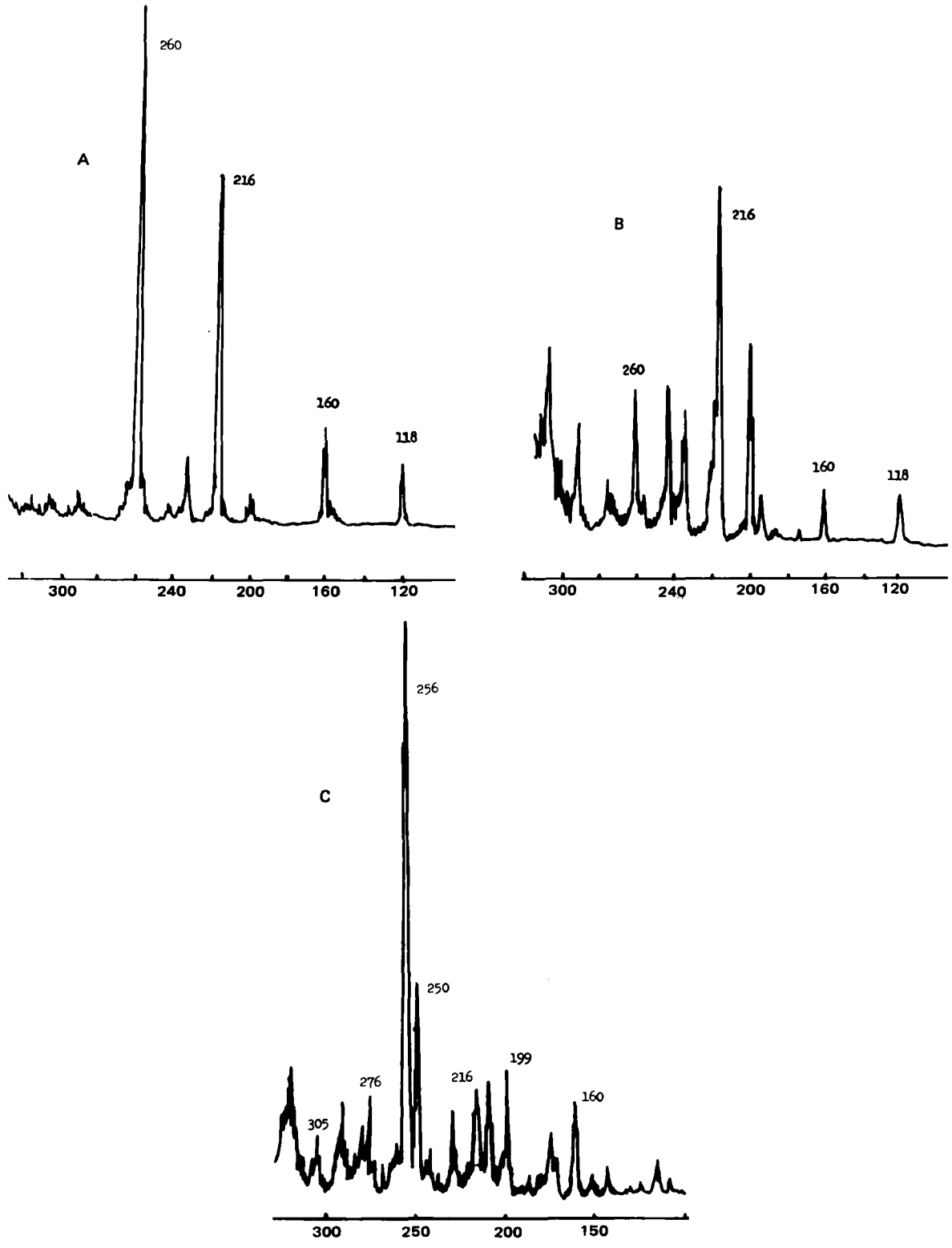


Figure 2—*Mass analyzed ion kinetic energy spectra. Key: from penicillin G molecular ion (m/z 334): (A) immediately after insertion, (B) after elapsed time, (C) from penicillin V molecular ion (m/z 350).*

peaks due to the general fragmentation at *m/z* 115(*f*), 114(*h*), and 100(*g*) as delineated in Scheme I. The abundance of fragment *e*, Scheme I, becomes rather weak.

Since V has a β-carbonyl carboxylic acid moiety, electron-impact or thermal-induced decarboxylation must take place easily, producing the abundant ions at *m/z* 91 and 92 in its spectrum. The peak corresponding to ion *c*, Scheme I, which is diagnostic of the R substituent, was also observed. Additionally, the spectrum exhibited peaks similar to those observed in the spectrum of I (*m/z* 334, 316, 288, 272, 160, 118, 114, 100, 92, and 91).

Ampicillin (VI) is an amino acid and as such is rather difficult to vaporize. Nevertheless, satisfactory in-beam EI spectra were obtained that exhibited $(M + 1)^+$, $(M^{+\cdot})$, and $(M - 17)^+$ at *m/z* 350, 349, and 332, respectively, when the compound was mixed with polyethylene glycol and examined under in-beam EI conditions (Fig. 3). Without polyethylene glycol, the in-beam spectral features were variable and were lacking characteristic peaks in the molecular weight region. The mechanism by which polyethylene glycol acts is not clear, but it appears to absorb the sample, and under quick-heating conditions, the sample was efficiently desorbed intact from its surface as in the case of polyimide- (26),

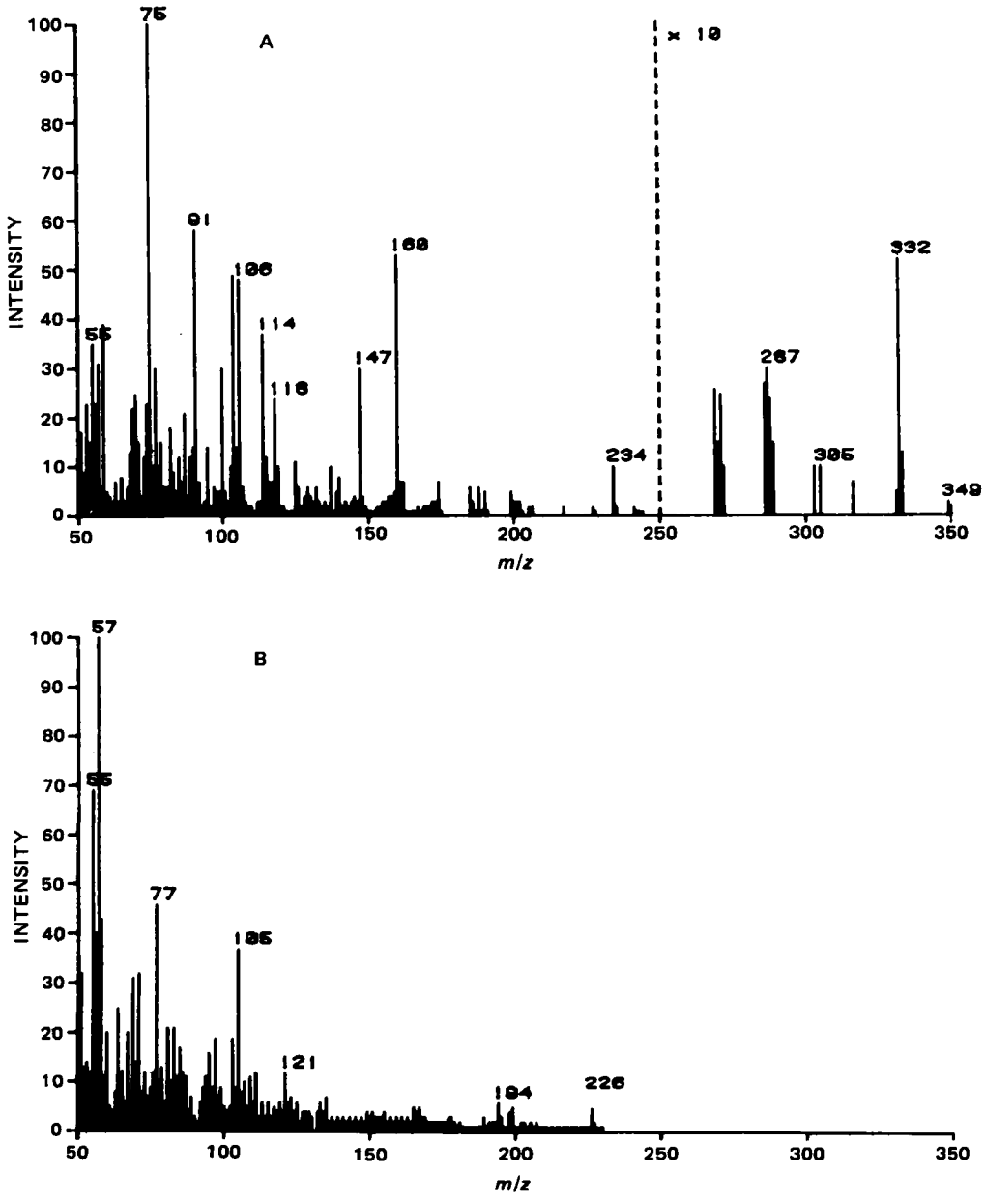
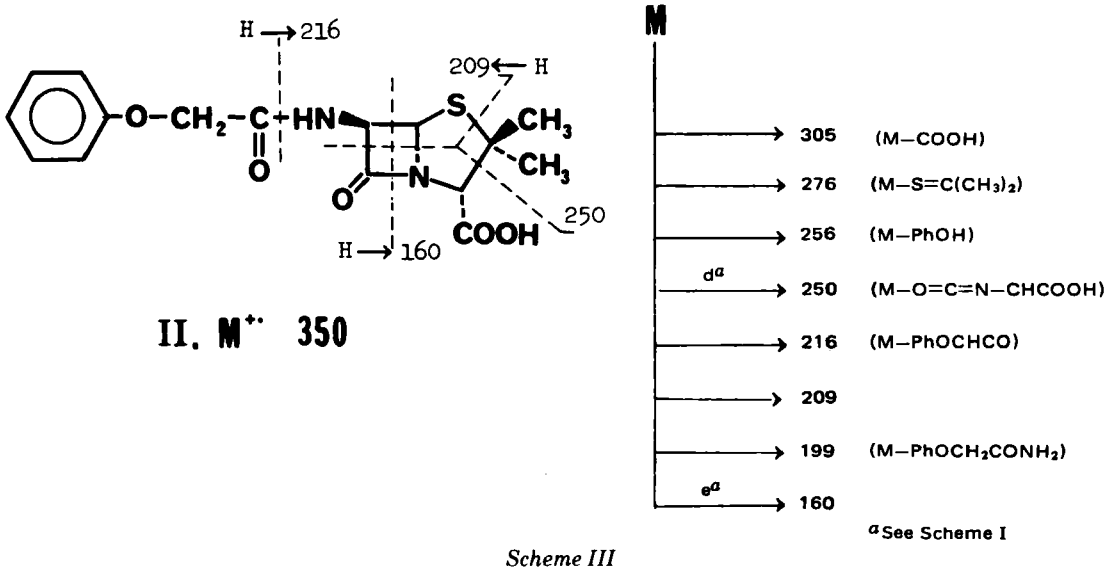
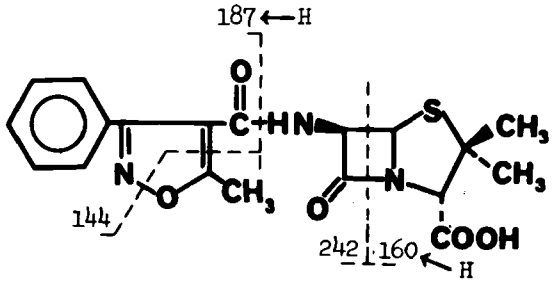


Figure 3—*Mass spectra of (A) ampicillin with polyethylene glycol 4000; (B) polyethylene glycol.*



Scheme III



III. MH^+ 402

polytetrafluoroethylene- (27), or silicone- (28) coated tips. It should be noted that previous researchers have demonstrated the utility of polyethylene glycol in negative ion FD mass spectrometry (29, 30). Figure 3A shows the in-beam EI spectrum of VI mixed with polyethylene glycol and Fig. 3B shows the spectrum of polyethylene glycol. A comparison of these spectra indicates that polyethylene glycol contributes negligibly to the spectra of VI.

Amoxicillin trihydrate (VII) has a similar structure to VI. Mass spectra of this compound can be interpreted by the classical mass shift technique (31). Peaks at *m/z* 75, 100, 114, and 160 were commonly observed in the spectra of both compounds; the peaks at *m/z* 91, 104, 106, 118, and 147 in the spectrum of VI shift to *m/z* 107, 120, 122, 134, and 163, respectively, in the spectrum of VII[8]. It is clear that the common peaks are derived from the penicillanic acid moiety and the shifted peaks involve the aromatic moiety. The major fragmentation ions of these compounds are summarized below.

In conclusion, it has been demonstrated that EI mass spectra of intact, unmodified penicillins can be obtained by the in-beam EI technique. Although the contributions to spectra of thermal degradation products formed during sample heating cannot be excluded, the spectral features proved more than satisfactory for obtaining structural information on the samples. When the sample was difficult to vaporize, as in the case of VI, the desorption technique from polyethylene glycol 4000 was useful to obtain the spectra. The general fragmentation of free acid forms of penicillins under in-beam conditions were in accord with those reported for methyl esters and amides, further indicating the usefulness of the in-beam electron-impact technique.

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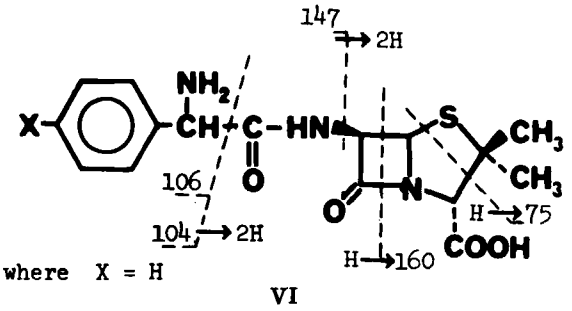
[8] When the in-beam EI spectrum of VII was obtained by use of a Finnigan 4023 mass spectrometer (Finnigan Corp., Sunnyvale, CA 94086) equipped with a direct insertion probe tip coated (32) with Pyre M.L. (DuPont) under the in-beam conditions (6–12), a simpler and clearer spectrum was obtained than described in the text: *m/z* 75(11), 79(16), 106(100), 1145(9), 118(8), 160(20), 271(0.8), 332(0.1), 350(0.01).



VI

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