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Mass spectrometry of β-lactam antibiotics with special reference to ionization by fast atom bombardment (FAB)

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Abstract: The mass spectral characteristics of the majority of penicillin and cephalosporin β-lactam antibiotics in world-wide clinical use are presented and reviewed. Special attention is given to the spectra recorded under fast atom bombardment (FAB) conditions and novel data on many penicillins and cephalosporins are included. Mass spectrometry features of common degradation products of benzylpenicillin and of some synthetic intermediates are also presented. The data illustrate the value of FAB mass spectrometry in identifying members of this closely related group of antibiotics without need for derivatization.

Keywords: *Mass spectrometry (MS); fast atom bombardment (FAB); penicillins; cephalosporins; degradation products and intermediates of β-lactam antibiotics.*

Introduction

Previously we have drawn attention to analytical problems posed by the ever increasing number of β-lactam antibiotics in clinical use, and reviewed the application of ^{1}H [1] and ^{13}C NMR [2] to their solution. We now present a survey of the use of mass spectrometry (MS) in this respect, another technique capable of differentiating closely related compounds. As will be clear from the literature review, the initial failure of electron impact (EI) methods to yield structural information from penicillins in free acid and alkali metal salt form (due to their involatility and thermal instability) has been overcome by use of soft ionization techniques. Since the MS spectra of relatively few penicillins (and even fewer cephalosporins) have been described we include in this account details of the FAB–MS of 25 penicillins and 20 cephalosporins, which include most β-lactams in current clinical use. The spectra of certain intermediates and degradation products also form part of this review.

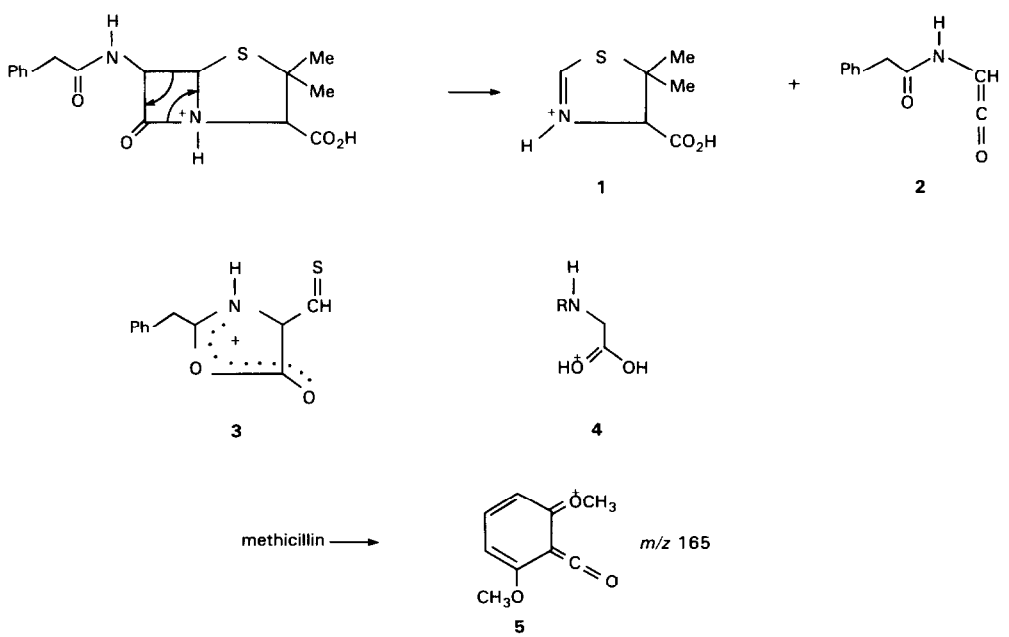
Most of the MS literature on β-lactam antibiotics published prior to 1975, concerns EI on derivatized materials [3–5] which are of greater volatility than the parent acids or alkali metal salts. Several fragmentation modes typical of the group were established from this work which subsequently guided interpretation of soft ionization spectral data; reviews are available [6, 7]. EI MS details are included in a few β-lactam monographs of

the series "Analytical Profiles of Drug Substances", e.g. ampicillin (disilyl) [8], cephradine (trisilyl) [9], cefoxitin (free acid) [10], cefaclor (free acid, field desorption) [11].

The large number of β-lactam antibiotics now in circulation together with the increasing application of liquid chromatography (LC)–MS to the analysis of urine and other biofluids have increased the demands made upon pharmaceutical analysts for the detection of such agents, and techniques which provide analytical information directly from non-derivatized materials clearly have special appeal. Although it is now known that several penicillin free acids yield diagnostic mass spectra under in-beam EI conditions [12], the value of soft ionization methods has been established for both classes of β-lactam antibiotic in either free acid or alkali metal salt form. The results of these techniques form the bulk of this review.

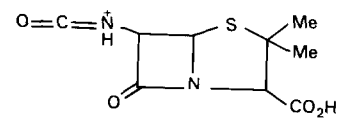
Penicillins

In 1975, Mitscher *et al.* [13] described the chemical ionization (CI) mass spectra of six penicillins in free acid and methyl ester form, using isobutane or ammonia as the reagent gas. Positive ion details for benzyl penicillin (M_r 334), typical of the set, were provided. A peak due to the protonated molecular ion ($M+H^+$) was present but of low intensity. The base peak at *m/z* 160 was shown to have the structure (**1**) characteristic of the penicillin nucleus, and believed to arise by a reverse 2+2 Diels–Alder cleavage (cycloreversion) of the protonated molecular ion. The protonated counterpart of the ketene derivative (**2**) contributed a low-intensity ion at *m/z* 176 (5%). In our hands, ion **2** was the base peak while ion **1** had negligible intensity in an isobutane CI spectrum (M+1 6%). Most of the penicillin free acid spectra [13] showed prominent peaks corresponding to $MH^+ - 115$ (220 for benzylpenicillin, intensity 10–15%) and $MH^+ - 141$ (194 for benzylpenicillin, intensity 70%) which were assigned structures **3** and **4**, respectively. The remaining ions of significant intensity were believed to result from loss of small



neutral molecules from MH^+ (H_2O, CO_2, H_2S) and fragment **1** (HNCS, CO_2, H_2S, HCN, H_2S). The isobutane CI spectrum of methicillin showed an abundant ion at *m/z* 165, assigned to **5** and characteristic of the 7-amido side chain.

Gower *et al.* [14] recorded the CI MS of five penicillin free acids using the field desorption (FD) technique in which the sample was deposited as a thin film on a coiled tungsten filament (the emitter). The reagent gases, ammonia and methane, provided positive and negative ion data, respectively. In the positive ion spectra, significant ions providing molecular weight information were observed for benzylpenicillin, phenethicillin, cloxacillin and ampicillin in the form of $[M+H]^+$ and/or $[M+NH_4]^+$ species (intensities of both were low for amoxycillin). In three cases (benzylpenicillin, phenethicillin and cloxacillin) the ketene cycloreversion ion, **2** (and appropriate analogues) formed the base peak; the thiazolidine fragments (**1**) were prominent only in the spectrum of benzylpenicillin (*m/z* 160, 60%) and amoxycillin (*m/z* 160, 20%; *m/z* 159, 100%). The $MH^+ - 115$ ion (**3**) formed the base peak in the case of ampicillin, but this ion was absent or of low intensity in the other spectra. Fragments that complexed with NH_4^+ rather than H^+ were prominent in some cases, e.g. the ketene (**2**) + NH_4 (*m/z* 193, 28%) for benzylpenicillin (*m/z* 223, 63% for phenethicillin; non-protonated **1** + NH_4, *m/z* 177, 40% for benzylpenicillin). The spectrum of cloxacillin showed an ion at *m/z* 241 (23%) attributed to **6**, formed by cleavage α to the amide carbonyl. The positive ion spectrum of ampicillin was complex and not all prominent ions were identified; that at *m/z* 216 (83%) was thought to arise from loss of $PhCHNH_2CO$ from the $[M+H]^+$ ion. *Negative ion* CI mass spectra (CH_4) also provided molecular weight information in regard to $[M-H]^-$ and $[M]^-$ ions (low intensities for amoxycillin). The relatively intense ion $[M-H_2O]^-$ of benzylpenicillin (*m/z* 316, 23%) had no counterpart in the spectra of other β-lactams. A novel pathway was proposed to account for ions *m/z* 256 (36%) and 228 (100%) present in the benzylpenicillin spectrum: loss of H_2S and CO_2 from the $[M-H]^-$ species yields **7** which then loses CO to produce the stable ion **8**. The base peak of the spectrum of phenethicillin also corresponded to an ion of type **8** (*m/z* 258). Cycloreversion ions equivalent to **1** and **2** were prominent only in spectra of benzylpenicillin (*m/z* 159, 88%; 174, 72%) and cloxacillin (*m/z* 276, 100%; 278, 57% type **2** only). Ionization by electron capture giving $[M]^-$ rather than proton loss was preferred for cloxacillin (*m/z* 435, 7%; 437, 3%) while loss of HCl from the molecular anion gave a major fragment at *m/z* 399 (28%). Amoxycillin favoured fragmentation via



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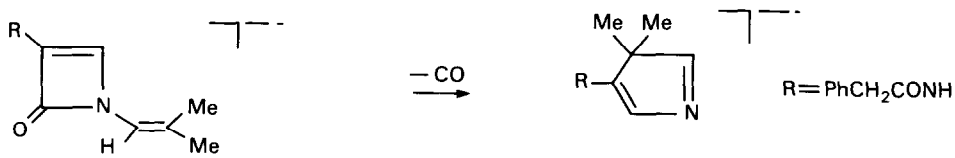


7 **8**

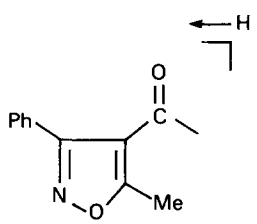
cycloreversion ($1 - H^+$) as is evident from MS features *m/z* 159 (43%), 141 (75%) — loss of H_2O, and 114 (100%) — loss of CO_2H. Likewise the base peak in the spectrum of ampicillin was *m/z* 159; the ion *m/z* 243 (20%) was assumed to be analogous to the rearrangement product **8**.

FD–MS (in-beam) spectra of penicillin free acids including 6-aminopenicillanic acid (6-APA) have also been reported using an EI source [12]. All spectra except that of amoxycillin showed low intensity $[M]^+$ ions ($[M+1]^+$ for oxacillin). Fragments due to the thiazolidine (**1**) and its breakdown products had high intensities in all spectra. Base peaks were *m/z* 91 (C_7H_7) for benzylpenicillin and carbenicillin, 77 (C_6H_5) for phenoxymethylpenicillin, 144 for oxacillin (ascribed to loss of MeCO from fragment **9**), 199 (ArCO) for nafcillin, and 75 (thiol fragment **10**) for ampicillin and amoxycillin. Fragments due to ions of type $[ArCO]^+$ and $[Ar]^+$, not observed in CI spectra, were common. The spectrum of 6-APA (base peak *m/z* 75) was unique in displaying a peak at *m/z* 116 (49%) assigned to the fragment **11**.

Alkali metal salts of penicillins proved unamenable to the MS techniques so far described. However, in 1982, Barber *et al.* [15] reported positive and negative ion spectra of such salts obtained by the then novel ionization technique of fast atom bombardment (FAB). The procedure involves depositing a mixture of the analyte and glycerol on the probe tip, and gives rise to spectra that include peaks due to glycerol which must be subtracted prior to analysis of the data (see Materials and Methods). The results of six penicillins (four salts, two free acids) were reported.

Positive ion spectra (FAB)

Molecular weight information was available from $[M+H]^+$ ions, where M is the molecular weight of the free acid or alkali metal salt, which was recorded with good intensity in every case. Salts also provided more intense $[M+X]^+$ ions, where X is the relevant alkali metal, plus low intensity cluster ions of structure $[2M+X]^+$. Most of the major fragment ions were the same or related to those identified in the earlier work, e.g. the thiazolidine, **1** (major ion in most spectra, base peak in case of ampicillin) and its



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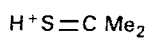

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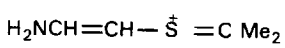

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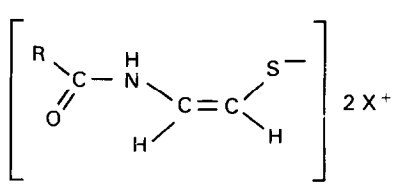

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cycloreversion partner, **2** (not prominent in free acids, base peak in spectrum of methicillin Na); both ions were associated with H^+ or X^+. Side chain information derived from ions of type $[ArCO]^+$ and $[Ar]^+$ produced by cleavage of the 7-amido substituent (only $[ArCO]^+$ ions were seen for methicillin and cloxacillin). The fragment **12** (seen in spectra of salts only) was produced by a more complex cleavage across both rings to give an anion to which are added two protons or alkali metal cations.

Negative ion spectra (FAB)

These showed molecular weight features due to ions $[M-1]^-$ (more intense for free acids) and $[M-X]^-$ for salts (ready formation of carboxylate anions) together with dimeric cluster ions of type $[2M-X]^-$ (salts only). In all samples the major (base peak) fragment was ascribed to **12** ($2X^+$ absent). In the case of phenethicillin, this ion (*m/z* 222, 100%) lost phenol to give *m/z* 128 (70%) which itself gave rise to an intense ion at *m/z* 93 (100%). In some cases a decarboxylated ion $[M-CO_2X]^-$ was observed, e.g. in the spectra of cloxacillin sodium and phenethicillin potassium.

Materials and Methods

Samples of β-lactam antibiotics and their intermediates were obtained from various pharmaceutical companies and individuals as listed in the Acknowledgements. These materials were generally of a purity suitable for pharmaceutical formulations and, in some cases, were described as analytical standards. Benzyl-penicilloic, -penilloic and -penillic acids were obtained as previously described [1]. The 7-phenylacetyl derivative of 7-amino-3-desacetoxycephalosporanic acid (7-ADCA, **14**) was obtained by catalytic hydrogenation of the corresponding *p*-nitrobenzyl ester [16], while alkaline hydrolysis of the same ester with sodium sulphide gave the ceph-2-ene isomer (**15**) [22]. Cephalothin lactone was obtained by treating the antibiotic with acid [17].

Positive and negative FAB mass spectra were obtained using a 7070E VG Analytical instrument. Mixtures of the analyte and glycerol were examined by standard procedures. In a few cases, mixtures were treated with 2 M hydrochloric acid to promote positive ion formation. The original spectra were corrected by subtraction of peaks due to matrix ions formed between glycerol (G) and protons and/or alkali metal cations. For H/Na these were:

(1) $[G+H]^+$ 93
(2) $[G-H]^-$ 91
(3) $[(G)_n+H]^+$ 185 ($n = 2$), 277 ($n = 3$) and 369 ($n = 4$)
(4) $[(G)_n-H]^-$ 183 ($n = 2$), 275 ($n = 3$) and 367 ($n = 4$)
(5) $[G+Na]^+$ 115
(6) $[(G)_n+Na]^+$ 207 ($n = 2$), 299 ($n = 3$) and 391 ($n = 4$).

Potassium, lithium, ammonium and chloride complexes were also encountered. The correction procedure involved comparing the original spectrum with one of glycerol, glycerol–sodium hydroxide or glycerol–sodium acetate, and subtracting that of most appropriate gain. Corrections were most easily made when alkali metals were absent, and in many spectra of alkali metal salts it proved impracticable to eliminate the glycerol peaks entirely. Spectra were normalized after the subtraction procedure had been carried out.

Electron impact (EI) spectra were recorded at 70 eV, and chemical ionization spectra were recorded with isobutane as the reactant gas.

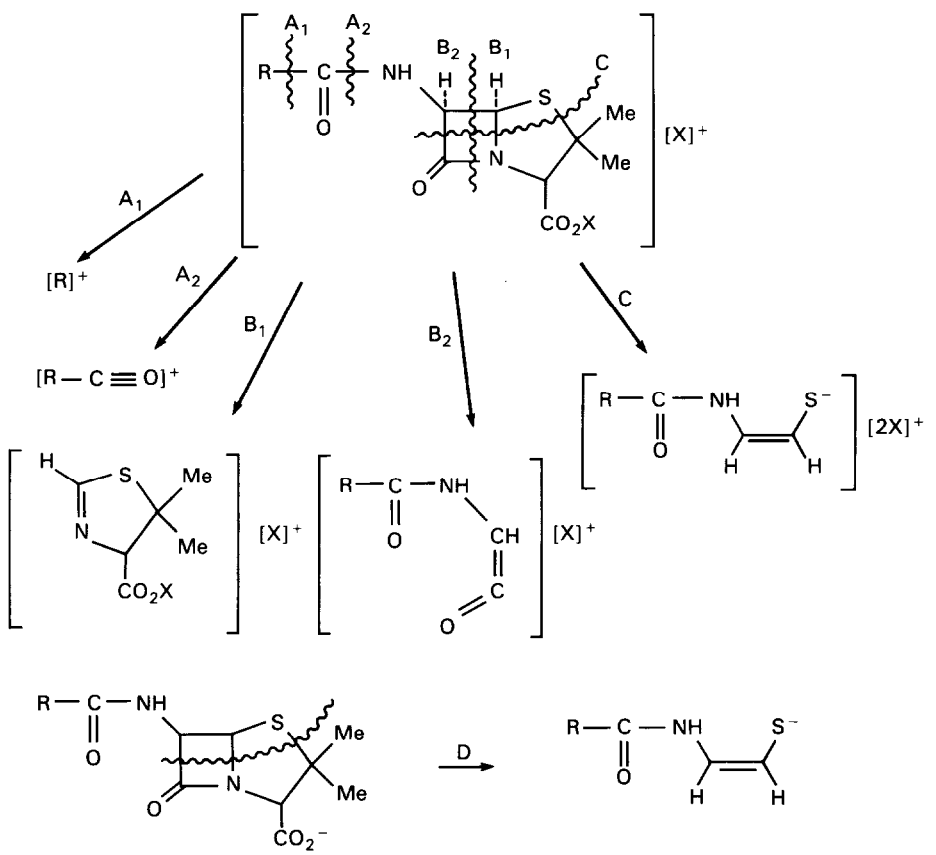
Results and Discussion

Details of the positive and negative ion FAB mass spectra of 25 penicillins (alkali metal salts, free acids and hydrochloride salts) are presented in Table 1. The data were collected at Bath and include references to the few published examples. It is convenient to denote fragment ions in the manner employed by Barber *et al.* [15] and the key is provided in Scheme 1. Corresponding data for cephalosporins follow in Table 2. The formulae of penicillins and cephalosporins are presented in Tables 3 and 4, respectively with items listed alphabetically by generic name. To facilitate presentation and discussion of the results, the penicillins are dealt with in groups which share 7-amido substituents of similar kind.

Penicillins

(a) 7-ArCH_2CO- *and* 7-ArCO-*amido derivatives.*

Positive ion spectra. In this group [Table 1(a)] which comprises benzyl, phenoxymethyl, substituted phenyl and naphthyl derivatives, molecular weight information is readily obtained from the intense (80–100%) $[M+X]^+$ ions and contributions from the weaker



Scheme 1
Positive [A–C] and negative [D] ion fragmentation pathways. (Reproduced from Barber *et al.* [15] with permission of John Wiley & Sons, Ltd.)

Table 1
FABS–MS features of some penicillin antibiotics (*m/z* values, intensities in parentheses)

Compound	Positive ion type							
	$[M+H]^+$	$[M+X]^+$	$[2M+H/X]^+$	$[A_1]^+$	$[A_2]^+$	$[B_1]^+$	$[B_2]^+$	$[C]^+$ and miscellaneous
(a) 7-$ArCH_2CO$- *and* 7-ArCO-*amido derivatives*								
Benzylpenicillin Na M_r: 356 [15]	357(10)	379(81)	735(11) Na	91(36)	119(5)	204(31) 137(39)[a]	198(7)	238(14)
Penamecillin (ester) M_r: 406	407(34) 499(4)[b]	—	—	91(100)	119(6)	232(86) ↓ $-C_3H_4O_2$ 160(46) ↓ $-CO_2H$ 115(40)	176(22)	C: abs 114(23)[c]
Phenoxymethylpenicillin K M_r: 388	389(26)	472(100)	815(14) K	107(8)	—	236(13)	230(1.4)	286(9)
Phenethicillin K M_r: 402 [15]	403(8)	441(100)	843(12) K	121(18)	149(2)	236(15)	—	300(8)
Methicillin Na M_r: 402 [15]	403(9)	425(86)	827(6) Na	137(60)	165(100)	204(61) 137(60)[a]	224(24)	284(28)
Nafcillin Na M_r: 436	437(15)	459(100)	895(8) Na	171(55)	199(80)	204(60)	278(20)	318(18)
(b) *Isoxazole derivatives*								
Oxacillin Na M_r: 423	424(14)	446(90)	869(9) Na	153(3)	186(10)	204(82)	265(9)	C: 305(11) 144(15) $A_2 - C_2H_2O$ 188(38) 217(71) 6-APA+H^+
Cloxacillin Na M_r: 457, 459 [15]	458(8) 460(5)	480(34) 482(21)	937(5) Na 929(5)	—	220(2) 222(5)	204(18)	299(3) 301(1.5)	C: 339(2.5) 341(1.5) 137(18) $B_1 - CO_2Na$
Dicloxacillin Na M_r: 491, 493, 495	492(8) 494(8) 496(3)	514(12) 516(11) 518(4)	[f]	—	254(3) 256(8) 258(4)	204(12)	333(3) 335(2.5) 337(1)	C: abs

Table 1
Continued

Compound	Positive ion type							
	$[M+H]^+$	$[M+X]^+$	$[2M+H/X]^+$	$[A_1]^+$	$[A_2]^+$	$[B_1]^+$	$[B_2]^+$	$[C]^+$ and miscellaneous
Flucloxacillin Na M_r: 475, 477	476(8) 478(5)	498(54) 500(25)	973(6) Na 975(6)	—	238(4) 204(5)	204(30)	317(4) 319(2)	C: abs 137(26) $B_1 - CO_2Na$
(c) *Ampicillin group* Ampicillin $3H_2O$ M_r: 349 (anhydrous) [15]	350(31)	—	[f]	106(100)	134(4)	160(9)	191(2)	C: abs 91(23) 118(22) $A_2 - NH_2$
Pivampicillin HCl M_r: 463 (free acid)	464(10)	—	[f]	106(100)	134(4)	275(25) ↓ $-C_6H_{11}O_2$ 160(9)	191(2)	C: abs 91(40) 118(30) 244(20)
Amoxycillin $3H_2O$ M_r: 365 (anhydrous) [15]	366(23)	—	731(1) H	122(100)	150(13)	160(34)	207(7)	C: 225(3) 94(28) 107(42) 134(52) 349(18)
Epicillin M_r: 351	352(74) ↓ $-H_2$ 350(20)	—	703(2) H ↓ $-H_2$ 701(1)	108(100) ↓ $-H_2$ 106(69)	136(9)	160(35)	193(7)	C: 211(4) 91(49)
Bacampicillin HCl M_r: 465 (free acid)	466(7)	—	931(2) H	106(100)	134(4)	276(32) ↓ 160(20)	191(6)	C: abs 91(34) 114(34) 118(23)
Talampicillin HCl M_r: 481 (free acid)	482(8)	—	[f]	106(66)	134(10.3)	292(7) ↓ 160(7)	191(1.5)	C: abs 133(100)[j] 174(5) $B_2 - NH_2$ 214(7)
Ciclacillin M_r: 341	342(11)	—	[f]	98(100)[l]	126(1.6)	160(5)	183(3.5)	C: abs 166(5) $B_2 - NH_3$

Hetacillin M_r: 389	390(75)	—	[f]	106(42)[m]	134(7)[m]	160(25)	231(18) ↓ 191(18)	C: abs 91(45) 104(42) 118(60) 146(100)
Piperacillin Na M_r: 539	540(10) ↓ +92 632(1.5)	562(17) ↓ +92 654(1)	[f]	274(2)	—	204(13)	—	C: abs 132(38) 143(68)[n] 165(100)[n] 187(72)
Azlocillin Na M_r: 483	484(17)	506(36)	989(3) Na	218(14)	—	204(10)	325(2.5)	C: abs 106(13)[m] 131(14) 137(14)[a] 207(7)
Mezlocillin Na M_r: 561	562(12)	584(15)	[f]	296(6)	—	204(6)		C: abs 106(9)[m] 132(12) 137(10)[a] 207(12)
(d)*Miscellaneous group*								
Carbencillin 2Na M_r: 422	423(2.5) ↓ $-CO_2$ 379(7) ↓ $-CO_2$ 335(2)	445(2.5) ↓ $-CO_2$ 401(5) ↓ $-CO_2$ 357(2)	[f]	157(1.5)	185(1.4)	204(17)	264(1)	C: abs 105(17) 137(52)[a]
Ticarcillin 2Na M_r: 428	429(4.5) ↓ $-CO_2$ 385(9) ↓ $-CO_2$ 341(2)	451(4) ↓ $-CO_2$ 407(9) ↓ $-CO_2$ 363(3)	[f]	163(1.5)	—	204(40)	270(1) ↓ $-CO_2$ 226(9)	C: abs 105(20) 137(71)[a]
Mecillinam HCl M_r: 325 (free base)	326(100)	—	[f]	127(47) (A_1+H_2)	153(4)	160(3)	—	C: abs 98(29) $C_6H_{12}N$ 167(44)
Pivmecillinam HCl M_r: 439 (free base)	440(100)	—	[f]	127(32) (A_1+H_2)	—	—	—	C: abs 98(25) 167(40)

Table 1
Continued

Compound	Negative ion type $[M-H]^-$	$[M-Na]^-$	$[2M-M/X]^-$, etc.	$[D]^-$	Miscellaneous
(a) 7-$ArCH_2CO$- *and* 7-ArCO-*amido derivatives*					
Benzylpenicillin Na M_r: 356 [15]	355(15)	333(10)	689(18) Na	192(100)	174(22) $[B_2]$anion[d] 255(6) [M−NA] − (H_2S+CO_2)[d]
Penamecillin (ester) M_r: 406	405(29)	—	—	192(100) ↓ −H 191(27)	89(43) $C_3H_5O_3$ (from 3-C side chain) 174(15)[d] 215(26) 6-APA − H 423(64) $[M-H]+H_2O$ 497(5) [M−H]+92
Phenoxymethylpenicillin K M_r: 388	387(18)	349(38)	737(21) K	208(100) ↓ −phenol 114(23)	93(20) PhO^- 305(8) M − CO_2K
Phenethicillin K M_r: 402 [15]	401(9)	363(10)	766(13)[e]	222(100) ↓ −phenol 128(32)	93(76) PhO^- 319(10) M − CO_2K
Methicillin Na M_r: 402 [15]	401(10)	379(7)	781(6) Na	238(100)	335(10) M − CO_2Na ↓ $-H_2S$ 301(6)
Nafcillin Na M_r: 436	435(12)	413(10)	849(12) Na	272(100)	143(20) 226(18) 266(20) 369(8) M − CO_2Na ↓ $-H_2S$ 335(5)
(b) *Isoxazole derivatives*					
Oxacillin Na M_r: 423	422(12)	—	823(8) Na	259(100)	281(23) 356(8) M − CO_2Na
Cloxacillin Na M_r: 457, 459 [15]	456(16) 458(7)	434(13) 436(4)	891(15) Na 893(12)	293(100) 295(37)	390(20) [M−Na] − CO_2

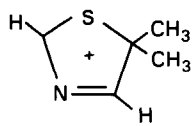
Dicloxacillin Na M_r: 491, 493, 495	490(11) 492(11) 494(4)	468(23) 470(16) 427(4)	959(14) Na 962(26)	327(100) 329(82) 331(20)	291(20) D − HCl 293(28) 295(8) 424(18) $[M-Na]^-$ − CO_2 426(11) 428(4)
Flucloxacillin Na M_r: 475, 477	474(13) 476(10)	452(9) 454(14)	927(13) Na 929(17) 931(5)	311(100) 313(43)	114(22) 138(25) 233(40) 291(71) D − HF 293(37)
(c) *Ampicillin group*					
Ampicillin $3H_2O$ M_r: 349 (anhydrous) [15]	348(48)	—	[f]	207(100)	192(22)
Pivampicillin HCl M_r: 463 (free acid) 499, 497 (HCl)	—	—	[f]	207(20)	101(100)[g] 127(40) 348[h](48)
Amoxycillin $3H_2O$ M_r: 365 (anhydrous) [15]	364(100)	—	729(7) H	223(82)	100(42) 133(37) 158(28) 206(37) 208(68)
Epicillin M_r: 351	350(85) ↓ $-H_2$ 348(36)	—	[f]	209(100) ↓ $-H_2$ 207(48)	87(30) 100(35) 192(40) D − NH_3 333(28) $[M-1]$ − NH_3
Bacampicillin HCl M_r: 465 (free acid) 501, 503 (HCl)	500(3.5) 502(2) 464(2)	—	[f]	207(22)	89(100)[i] 127(30)
Talampicillin HCl M_r: 481 (free acid) 517, 519 (HCl)	516(5) 518(2) 480(3)	—	[f]	207(56)	97(70) 127(54) 149(100)[k]

Table 1
Continued

Compound	Negative ion type				
	$[M-H]^-$	$[M-Na]^-$	$[2M-M/X]^-$, etc.	$[D]^-$	Miscellaneous
Ciclacillin M_r: 341	340(46) +92 432(9)	—	*f*	199(100)	—
Hetacillin M_r: 389	388(95) +92 480(25)	—	*f*	247(100) ↓ $-H_2$ 245(25)	89(35) 114(25) 207(5)[m]
Piperacillin Na M_r: 539	538(4) 539(22)M^-	516(25)	*f*	375(5)	233(100) 330(42) 396(43) 793(6) 815(6)[o]
Azlocillin Na M_r: 483	482(85)	460(38)	943(13) Na	319(49)	114(35) B_1(158) − CO_2 173(25) 207(26) 233(100)[p] 244(42) 255(95) 416(25) M − CO_2Na
Mezlocillin Na M_r: 561	560(8)	538(17)	*f*	397(15) ↓ −H 396(34)	163(37)[r] 233(100)[p] 255(30) 330(41) 783(8)[q] 815(9)[q]
(d) *Miscellaneous group* Carbenicillin 2Na M_r: 422	—	399(16) ↓ $-CO_2$ 355(65)	*f*	258(22) ↓ $-CO_2$ 214(53)	113(23)[s] 114(56) B_1(158) − CO_2 117(72) 174(100) 192(85)

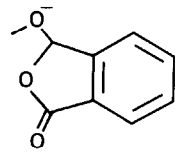
Ticarcillin 2Na M_r: 428	M − H abs ↓ $-CO_2$ 383(25)	M − Na abs ↓ $-CO_2$ 361(42)	[f]	264(5) ↓ $-CO_2$ 220(68)	89(15) 100(36) 113(22) 114(45) B_1(158) − CO_2 123(70) 180(100) 198(46)
Mecillinam HCl M_r: 325 (free base)	324(100) 360(16)[u] 362(5)	—	[f]	—	127(75)[f] 129(28)[f] 342(7) (M−H)+H_2O

[a] $[B_1]^+ - CO_2Na$.
[b] $[M+1]^+ + 92$ (glycerol).
[c]

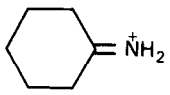


cf. Ohashi *et al.* [12].

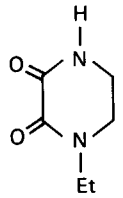
[d] cf. ref. 14.
[e] 2M−K+H.
[f] Region not scanned.
[g] $OCOCMe_3^-$.
[h] Ampicillin M−1.
[i] OCO_2Et^-.
[j] $C_8H_5O_2^+$.
[k]



[l]

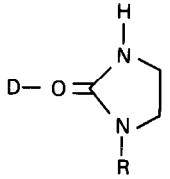


[m] Via ampicillin?
[n]



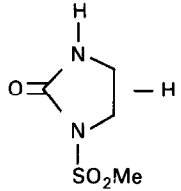
+ H^+ (143); + Na^+ (165).

[o] M+(3 × 92).
[p]



(233).

[q] Glycerol adducts.
[r]



[s] $A_1 - CO_2$ (CO_2H).
[t] $92 + {}^{35}Cl^-/{}^{37}Cl^-$.
[u] M(HCl)−1.

Table 2
FABS–MS features of some cephalosporin antibiotics (*m/z* values, intensities in parentheses)

Compound	Positive ion type							
	$[M+H]^+$	$[M+X]^+$	$[2M+H/X]^+$	$[A_1]^+$	$[A_2]^+$	$[B_1]^+$	$[B_2]^+$	$[C]^+$ and miscellaneous
(a) *3-Methylcephems*								
7-Phenylacetamido derivative (**14**) Na M_r: 354	355(50)	377(100)	731(8) Na 469(10) Na[a]	91(50)	119(5) 180(20)[b]	158(35)[b] 202(18)	198(8) 131(20)	C: 238(35) 173(35)
7-Phenylacetamidoceph-2-ene derivative (**15**) Na M_r: 354	355(40)	377(22)	[d]	91(80)	119(1–2)	158(20) 202(1–2)	198(5)	C: abs
Cephalexin (free acid) M_r: 347	348(18)	—	695(3) H	106(100)	134(3)	158(15)	191(3)	C: abs 91(16)[e] 118(22)[e] $A_2 - NH_2$ 174(10) $B_2 - NH_3$ 185(16)
Cefadroxil (free acid) M_r: 363	364(46) 456(20)[a] 548(5)[a]	—	[d]	122(69)	150(9)	158(16)	207(10) ↓ +H 208(15)	C: abs 107(26) $A_1 - 15$[f] 110(100) 134(34) $A_2 - NH_2$[f]
Cephradine (free acid) M_r: 349	350(30) ↓ $-H_2$ 348(4)	—	699(3) H ↓ $-H_2$ 697(<1)	108(100) ↓ $-H_2$ 106(44)	136(5)	158(24)	193(4)	C: abs 91(44) 176(20) $B_2 - NH_3$
Cefaclor (free acid) M_r: 367, 369	368(27) 370(11) 460(2)[a]	—	735(2.6) H 737(2.3) 739(0.6)	106(100)	134(3)	178(4) 180(1)	191(8)	C: abs 91(10) 118(20) $A_2 - NH_2$ 174(10) $B_2 - NH_3$
(b) 3-CH_2X *cephem type*								
Cephalothin Na M_r: 418	419(55) 511(4)[a]	441(46) 533(7)[a]	859(7) Na 837(3) H	97(31)	125(2)	—	204(3)	C: 244(5) 381(19) $[M+Na]-60$[h]
Cephaloridine M_r: 415	416(17) 508(2)[a]	—	[d]	97(100)	125(7)	—	204(3)	C: 244(2) 158(23) 337(9) $[M+1]-79$[h] 339(7)

Cefoxitin Na M_r: 449	450(40) 542(4)[a]	472(60) 564(5)[a]	[d]	97(100)	125(7)	—	234(5)	C: 274(30) 106(75) 110(20) 391(12) 411(30) [M+Na]−61[h]
Cefotaxime Na M_r: 477	478(90) 570(3)[a]	500(100) 592(10)[a]	955(3) H ↓ −H 954(6) 977(4) Na ↓ −H 976(13)	156(10)	184(5)	260(5)	—	C: abs 126(40) 148(20) 374(20) 440(40) [M+Na]−60[h]
Cefuroxime Na M_r: 446	447(36)	469(62) 561(4)[a]	893(2) H 915(8) Na	124(11)	152(3)	—	231(2)	C: 271(4) 88(26) 106(36) 386(5) [M+H]−61[h] 408(39) [M+Na]−61[h]
Cephamandole Li[k] M_r: 468	469(2)	475(3) 567(<1)[a]	[d]	107(2) ↓ $-H_2$ 105(16)	—	—	—	C: 222(2) 105(38) 191 $[B_2]$ − H 353(1) [M−1]−116[h] 359(2.5) [M−Li]−116[h]
O-Formyl cephamandole (free acid nafate)[j] M_r: 490	491(100) 583(10)[a]	—	[d]	—	—	—	220(18)	C: abs 118(60) 158(58) 174(22) 310(20) 375(70) [M+1]−116[h]
Cephazolin Na M_r: 476	477(55) 569(6)[a]	499(55) 591(6)[a]	953 abs H ↓ −H 952(2.5) 975(2) Na ↓ −H 974(4)	—	—	—	—	C: abs 133(100)[l] 155(5)[l] 345(18) [M+1]−132[h]
Cefatrizine[m] (propylene glycol solvate) M_r: 462 (free acid)	463(50) 555(4.5)[a]	—	[d]	122(100)	150(5)	—	207(18)	C: abs 102(40)[n] 110(49) 134(42) 363(17) [M+1]−101+H[h] 364(18)

Table 2
Continued

Compound	Positive ion type							
	$[M+H]^+$	$[M+X]^+$	$[2M+H/X]^+$	$[A_1]^+$	$[A_2]^+$	$[B_1]^+$	$[B_2]^+$	$[C]^+$ and miscellaneous
Ceftazidime[m] M_r: 546 (free acid)	547(80) 639(16)[a]	—	[d]	228(3)	256(2)	—	313(8)	C: 331(8) 110(80) 115(60) 126(100) 142(40) 170(40) 202(20) 207(25) 211(20) 218(25) 468(18) [M+1]−79[h] 470(80)
Moxalactam $2NH_4$[p] M_r: 520 (free acid)[m]	521(10)	538(4) $(M+NH_4^+)$	[d]	151(5)	—	—	—	C: abs 117(35) 134(16) 284(20) 405(2) [M+1]−116[h] 406(2.5) 407(4)
Cefixime M_r: 453 (free acid)	454(68) 546(20)[a]		—	168 abs ↓ $-C_2H_2O$ 126(100)	196(10)	170(20)	—	C: abs 85(30) 100(25) 110(40) 128(40) 192(25) 202(12) 211(30) 380(22) [M+1]−(CO_2+CH_2O)[q]

Ceftriazone 2Na M_r: 598	599(10)	621(22)	—	—	184(6)	—	—	C: abs 86(60) 182(30) 204(100)[r] 226(95) 296(30) 420(10) 440(9) $[M+Na]^+-181$[h] 441(10) 442(18)
Cefsulodin Na M_r: 554	555(8)	—	[d]	—	—	300(6)	—	C: abs 94(100) 123 − CHO 110(90) 123(80)[s] 391(30) 434(10) $[M+1]-122+H$[h] 456(5) $[M+1]-122+Na$[h]
Ceftizoxime Na M_r: 405	406(100) 498(7)[a]	428(60) 520(18)[a]	811(2) H ↓ −H 810(4) 813(2) Na ↓ −H 830(4)	156(3)	—	188(8)	—	C: abs 126(58) 299(22) 384(20)

Compound	Negative ion type $[M-H]^-$	$[M-Na]^-$	$[2M-M/X]^-$, etc.	$[D]^-$	Miscellaneous
(a) *3-Methylcephems* 7-Phenylacetamido derivative (**14**) Na M_r: 354	353(100)	331(40)	685(13)	192(80)	100(40) 127(30) 139(95) $B_1(158)-(NH_3+H_2)$ 174(70) 253(20)[c] 287(30)

Table 2
Continued

Compound	Negative ion type				
	$[M-H]^-$	$[M-Na]^-$	$[2M-M/X]^-$, etc.	$[D]^-$	Miscellaneous
Cephalexin (free acid) M_r: 347	346(100)	—	[d]	207(62)	89(35) 139(68) 156(33) B_1-H_2 189(78) B_2-H_2 ($D-H_2O$) 269(22) 302(38) $M-CO_2H$
Cefadroxil (free acid) M_r: 363	362(100) 454(56)[a]	—	[d]	abs	89(80) 109(23) 127(28) 139(70) 188(26) $D-(H_2O+NH_3)$ 205(45) $D-H_2O$
Cephradine (free acid) M_r: 349	348(73)	—	[d]	209(66) $\downarrow -H_2$ 207(20)	89(48) 98(32) 100(75) 112(25) 120(12) A_2-NH_2 135(56) 139(100) 304(73) $M-CO_2H$ $\downarrow -H_2$ 302(24)
Cefaclor (free acid) M_r: 367, 369	366(60) 368(34) 458(35)[a] 460(10)	—	733(32) H 735(30) 737(12)	207(67)	100(25) 127(95)[g] 129(22) 153(63) 176(52) 189(73) B_2-H_2 ($D-H_2O$) 286(49) 285(45) 322(100) $M-CO_2H$ 324(39)

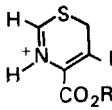
(b) 3-CH_2X *cephem type*					
Cephalothin Na M_r: 418	417(21) 509[a]	395(47)	813(19) Na 835(3) H	198(15) ↓ $-H_2O$ 180(100)	141(22) 167(36) 335(20) [M−Na]−60[h] 357(87) [M−1]−60[h] ↓ $-CO_2$ 313(25)
Cephaloridine M_r: 415	414(12) 415(16)M^- 506(12)[a] 507(20)[a]	—	[d]	198(100) ↓ $-H_2O$ 180(80)	123(20) 139(20) 155(20) 167(20) 337(35) [M−1]−79+H_2[h]
Cefoxitin Na M_r: 449	448(16)	426(30)	[d]	228(22) ↓ $-H_2O$ 210(30)	156(75) 355(100)
Cefotaxime Na M_r: 477	476(75) 568(5)[a]	454(60) 546(7)[a]	[d]	257(7)	123(40) 124(100) 151(30) 197(40) [M−Na]−[D] 205(30) 239(55) [B_2]−H 261(30) 394(18) [M−Na]−60[h] 416(45) [M−H]−60[h]
Cefuroxime Na M_r: 446	445(18) 537(5)[a]	423(63) 515(8)[a]	869(12) Na	225(47)	100(63) 125(43) 155(34) 177(30) 207(100) 362(10) [M−Na]−61[h] ↓ $-CO_2$ 318(22) 384(55) [M−H]−61[h] 402(20)
Cephamandole Li M_r: 468	—	—	[d]	208(13)	115(100)[i] 351(9) [M−1]−116[h]

Table 2
Continued

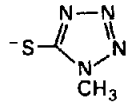
Compound	Negative ion type [M−H]⁻	[M−Na]⁻	[2M−M/X]⁻, etc.	[D]⁻	Miscellaneous
O-Formyl cephamandole (free acid nafate) M_r: 490	489(4)	—	[d]	236(2)	115(100)[i] 375(9) M−115[h]
Cephazolin Na M_r: 476	475(8) 545(2)[a]	453(12) 567(1)[a]	929(2) Na	184(4)	131(100)[l] 223(14) 245(8) 321(16) 322(4) [M−Na]−131[h] 343(5) 344(8) [M−Na]−131[h]
Cefatrizine[m] (propylene glycol solvate) M_r: 462 (free acid)	461(16)	—	[d]	223(5)	100(42)[n] 127(100) 219(40) 289(35) 311(45) 362(60) [M−1]−101+H_2[h]
Ceftazidime[m] M_r: 546 (free acid)	545(16) 546(18)M⁻ 637(35)[a]	—	[d]	329(2)	103(100) 124(37)[o] 209(20)[o] 311(55) 422(18) 468(42) [M−1]−79+H_2[h]
Moxalactam 2NH_4[m] M_r: 520 (free acid)	519(6)	—	—	—	115(100)[i] 127(65) 183(58) 219(30) 403(3) [M−1]−116[h] 475(2) M−CO_2H

Cefiximine M_r: 453 (free acid)	452(100)	—	—	—	124(38) 167(20) A_1-H 209(25) 283(22) 378(20) $[M-1]-(CO_2+CH_2O)$[g]
Cefsulodin Na M_r: 554	553(22) 554(35)M^-	531(20) $\downarrow +H_2$ 533(40)	[d]	294(75)	170(90) 171(70) 173(60) 214(40) 265(22) 337(30) 409(30) $[M-Na]-122$[h] 431(25) $[M-1]-122$[h] 432(30) 410(85)
Ceftizoxime Na M_r: 405	404(100)	382(80)	[d]	257(18)	98(28) 100(28) 123(25) 124(75) 125(80) 136(25) 139(30) 205(70) 239(60)

[a] Glycerol adduct(s).

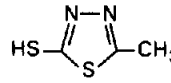
[b]  R = H, *m/z* 158; R = Na, *m/z* 180.

[c] $[M-Na]-(H_2S+CO_2)$, cf. benzylpenicillin Na.
[d] Region not scanned.
[e] cf. Ampicillin +ive FAB.
[f] cf. Amoxycillin +ive FAB.
[g] Glycerol + Cl^-.
[h] Exocyclic methylene type fragment (see **16** and **17**).

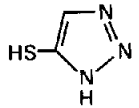
i  (115).

*j*Positive ion spectrum of Kefadol (proprietary mixture of nafate and Na_2CO_3) showed characteristics of the sodium salt of cephamandole: $[M+1]^+$ 485(30), $[M+Na]^+$ 507(40), $[M+Na]-116^h$ 391.

*k*Lithium detected by high intensity ion *m/z* 99 (Li + 92).

l  $+H^+$ (133); $+ Na^+$ (155); anion 131.

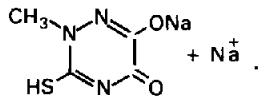
*m*Run in glycerol + HCl.

n  $+H^+$ (102); anion 100.

*o*cf. Two units higher counterparts of +ive ion spectrum.

*p*Revealed by *m/z* 110 (100) $(92+NH_4^+)$.

*q*From $NOCH_2CO_2H$ of oxime side chain.

r 

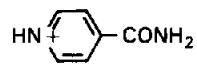
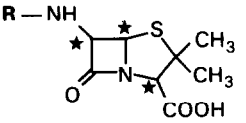
s 

Table 3

(a) *Penicillins of general formula*:



(*chiral centres starred*), *alphabetically listed by generic name*

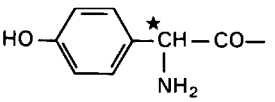
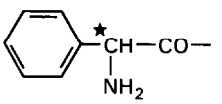
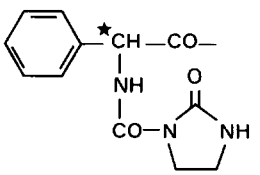
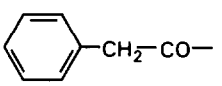
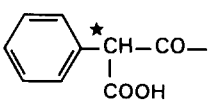
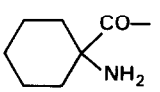
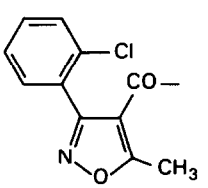
Generic name	R
Amoxycillin	
Ampicillin	
Azlocillin	
Benzylpenicillin	
Carbenicillin	
Ciclacillin	
Cloxacillin	

Table 3
Continued

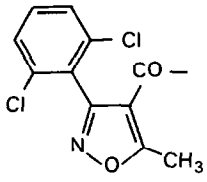
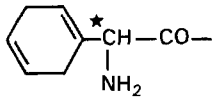
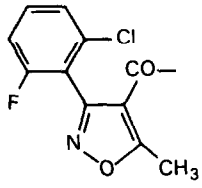
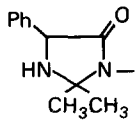
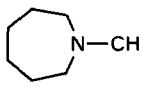
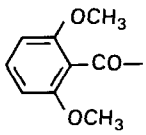
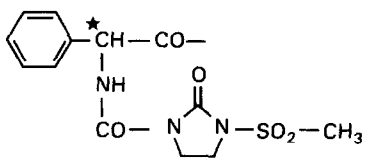
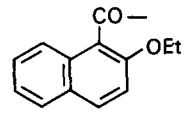
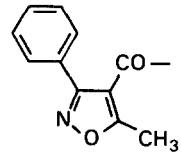
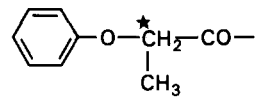
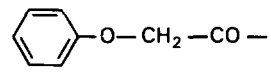
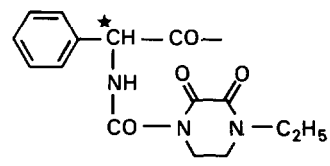
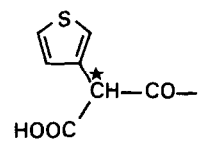
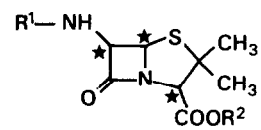
Generic name	R
Dicloxacillin	
Epicillin	
Flucloxacillin	
Hetacillin	
Mecillinam	 (−H; change in formula)
Methicillin	
Mezlocillin	

Table 3
Continued

Generic name	R
Nafcillin	
Oxacillin	
Phenethicillin	
Phenoxymethylpenicillin (penicillin V)	
Piperacillin	
Ticarcillin	

(b) *Penicillin pro-drugs with esterfied 3-carboxylate functions*



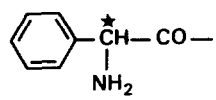
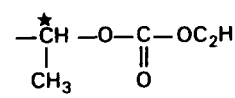
Generic name	R^1	R^2
Bacampicillin		

Table 3
Continued

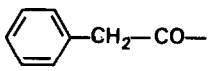
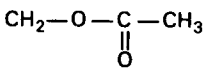
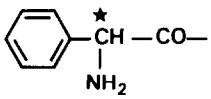
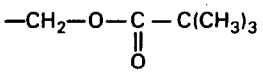
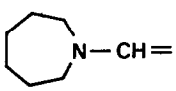
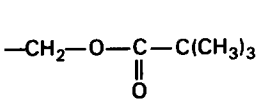
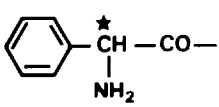
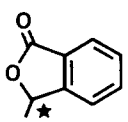
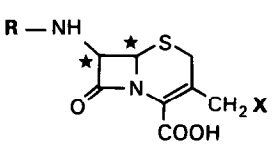
Generic name	R^1	R^2
Penamecillin		
Pivampicillin		
Pivimecillinam	 (−H; change in formula!)	
Talampicillin		

Table 4

Cephalosporins of general formula:



(*chiral centres starred*), *alphabetically listed by generic name*

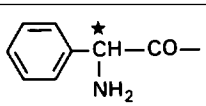
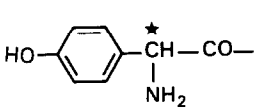
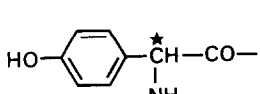
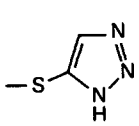
Generic name	R	X
Cefaclor		Cl (in place of CH_2X)
Cefadroxil		H
Cefatrizine		

Table 4
Continued

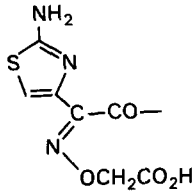
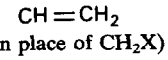
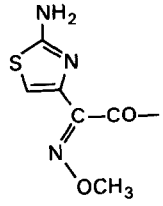
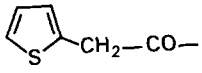
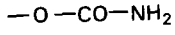
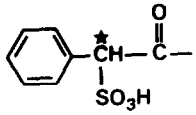
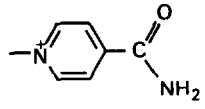
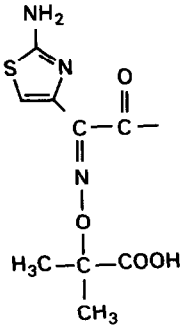
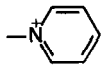
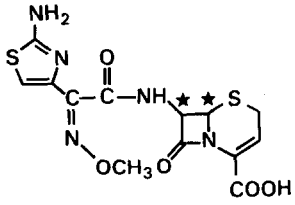
Generic name	R	X
Cefixime		 (in place of CH_2X)
Cefotaxime		
Cefoxitin (cefamycin example with 7-OCH_3 substituent)		
Cefsulodin		
Ceftazidine		
Ceftizoxime (complete formula)		

Table 4
Continued

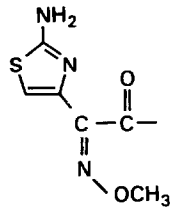
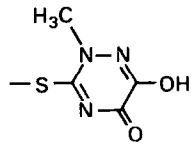
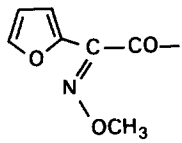
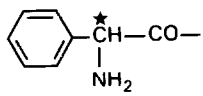
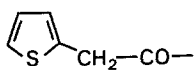
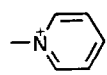
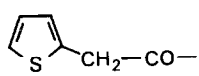
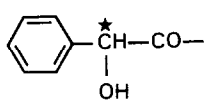
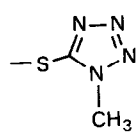
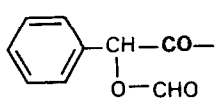
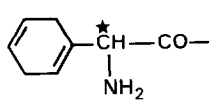
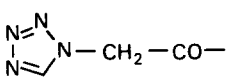
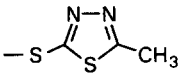
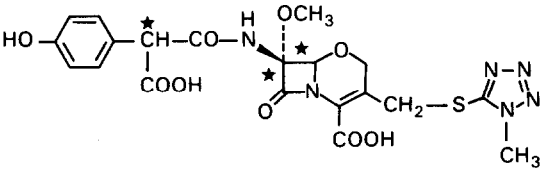
Generic name	R	X
Ceftriaxone		
Cefuroxime		$-O-CO-CH_3$
Cephalexin		H
Cephaloridine		
Cephalothin		$-O-CO-CH_3$
Cephamandole		
Cephamandole nafate		As above
Cephradine		H

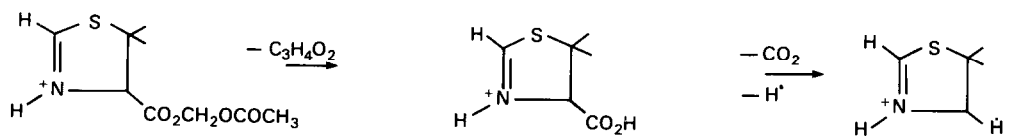
Table 4
Continued

Generic name	R	X
Cephazolin		
Moxalactam (complete formula)		

$[M+H]^+$ and $[2M+X]^+$ (cluster) ions. Side-chain evidence derives from ions of the $[A_1]^+$ and $[A_2]^+$ types: intensities are greatest in spectra of methicillin Na and nafcillin Na due probably to resonance stabilization of these $[ArCO]^+$ ions. Cycloreversion products contribute to all spectra with the thiazolidine ion $[B_1]^+$ preponderating over the ketene $[B_2]^+$. The fragmentation leading to $[C]^+$ is also common, but only a minor route for the phenoxymethyl derivative. The spectrum of penamecillin, a pro-drug form of benzylpenicillin with a labile ester function, was similar to that of the parent in regard to $[M+H]^+$, $[A_1]^+$, $[A_2]^+$, $[B_1]^+$ (notably intense, 86%) and $[B_2]^+$ ions; in addition several thiazolidine fragment ions (*m/z* 160, 115, 114) were present produced by loss of acetoxymethyl from the 3-substituent of $[B_1]^+$ (Scheme 2).

Negative ion spectra. In all cases molecular weight evidence was available from $[M-H]^-$, $[M-Na \text{ or } K]^-$ and cluster $[2M-Na \text{ or } K]^-$ ions, and also from the ion $[D]^-$ which formed the base peak. Other significant fragmentations include the $[B_2]$ anion (*m/z* 174 for benzylpenicillin sodium and penamecillin), cf. DCI study of Gower *et al.* [14], and ions produced by loss of phenol from $[D]^-$ of phenoxymethylpenicillin potassium (*m/z* 114) and phenethicillin potassium (*m/z* 128), accompanied by phenolate ions. There was also evidence of $[M-CO_2Na, K]^-$ ions. The penamecillin spectrum showed a prominent ion at *m/z* 89 (43%) which could arise from OCH_2OCOMe of the C-3 side chain.

(b) *Isoxazole group.* Positive ion diagnostic features of this group of four compounds (oxacillin, cloxacillin, dicloxacillin and flucloxacillin) were chiefly the $[M+X]^+$, $[M+H]^+$ and $[2M+X]^+$ ions; $[A_2]^+$ fragments were of low intensity with $[A_1]^+$ ions absent. A few miscellaneous ions were recorded. Negative ion spectra were more informative (molecular species plus $[D]^-$ base peak) and included some ions of special



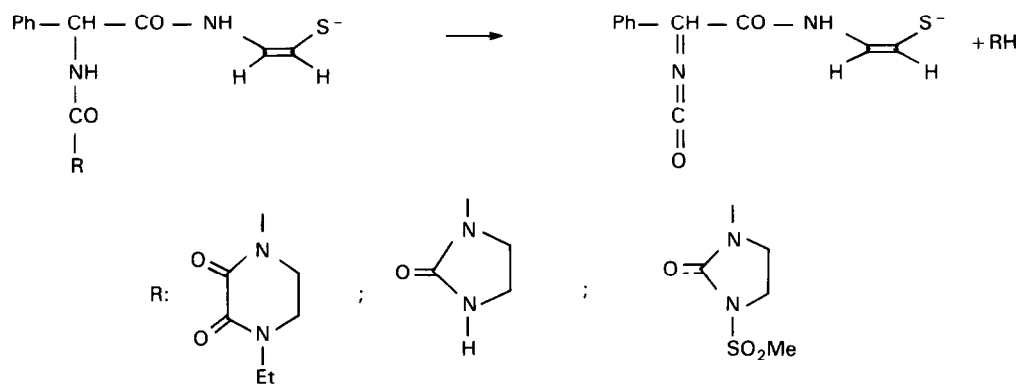
Scheme 2

interest, e.g. $[D]^-$ − HCl for dicloxacillin and $[D]^-$ − HF for flucloxacillin. In both positive and negative ion spectra, sets of signals due to chlorine isotopes provided further aids to identify.

(c) *Ampicillin group.* Positive ion spectra of this set of 11 (all containing an α-aminobenzyl feature, as such or modified) displayed $[M+H]^+$ ions which were of low intensity in the pro-drug forms pivampicillin, bacampicillin and talampicillin. Fragments $[A_1]^+$ were the base peaks in most spectra and *m/z* magnitudes were characteristic in the cases of amoxycillin, epicillin and ciclacillin. Fragments $[B_1]^+$ had intensities over 25% in most spectra, while *m/z* values recorded for bacampicillin (276) and talampicillin (292) showed the protected 3-carboxylate function to be intact; spectra of the last two examples together with that of pivampicillin showed an *m/z* ion 160 which corresponded with $[B_1]^+$ of ampicillin. Several ions of the epicillin spectrum gave evidence of its ready conversion to ampicillin by loss of H_2. Most spectra displayed additional ions which could be rationalized by separation of small molecules from the established set (see miscellaneous column of Table 1). The spectrum of hetacillin provided diagnostic $[M+H]^+$ and $[B_2]^+$ ions with an unidentified base peak (*m/z* 146).

In the negative ion spectra, the $[M-1]^-$ ions revealed molecular weights although intensities were low for the pro-drugs bacampicillin and talampicillin (that of pivampicillin was 12%). The anion $[D]^-$ was diagnostic for amoxycillin, epicillin (accompanied by a $[D]^- - H_2$ ion), ciclacillin and hetacillin. Other characteristic ions of prominence were *m/z* 101 for pivampicillin, 89 for bacampicillin and 149 for talampicillin (all base peaks), due to portions of the derivatized 3-carboxylate function. Negative ion spectra of the related trio piperacillin, azlocillin and mezlocillin all had base peaks of *m/z* 233: these ions may arise by cleavage of $[D]^-$ type ions as shown in Scheme 3.

(d) *Miscellaneous penicillins.* Of the four members of this group, mecillinam and pivmecillinam hydrochlorides were well characterized by the $[M+1]^+$ base peaks of their *positive ion* spectra. Both spectra displayed the ions *m/z* 98 (25–29%) diagnostic of the azacycloheptane substituent. In the negative ion spectrum of mecillinam (nothing was recorded for pivmecillinam) the $[M-1]^-$ ion was the base peak, while ions at *m/z* 127 (75%) and 129 (28%) probably arise from glycerol plus Cl^- isotopes. The 6-carboxylate derivatives carbenicillin and ticarcillin showed weak molecular characteristics in both positive and negative ion spectra, and were best characterized by the $[B_1]^+$ (ticarcillin,



Scheme 3

40%) and $[D]^-$ (carbenicillin, 22%) ions and some negative ions of uncertain origin (Table 1). The ion *m/z* 114 was prominent (47–56%) in both negative ion spectra (also in that of azlocillin) and is attributed to $[B_1] - CO_2Na_2$.

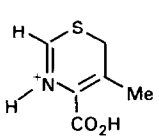
Cephalosporins

In principle, the behaviour of cephalosporin molecules under FAB–MS conditions (especially those with 3-methyl substituents) should mirror that of the penicillins. Thus molecular species together with fragment ions of the $[A_1]$, $[A_2]$, $[B_1]$, $[B_2]$ and [C] (positive) and [D] (negative) type should be detected. The $[B_1]^+$ ion for a cephalosporin has the six-membered ring structure **13** in the case of cephalexin and other 3-methyl derivatives in the free acid form. The behaviour of methyl esters of two 3-acetoxymethyl cephalosporins (one of the rare literature reports on cephalosporins) under EI conditions confirm these expectations; in addition there was evidence for fragment ions of the exocyclic 3-methylene type (see later) [6]. Our experience with underivatized cephalosporins under FAB conditions is now presented.

3-Methylcephalosporins [Table 2(a)]. Positive and negative ion spectra of the benzyl derivative (**14**) displayed the usual ions indicative of molecular weight ($[M+Na]^+$ and $[M-1]^-$ ions were base peaks) while many fragment ions were identical with those observed in spectra of benzylpenicillin sodium, a close analogue of this compound. The positive ion spectrum of the ceph-2-ene isomer (**15**) differed little from that of the ceph-3-ene (**14**) but was differentiated by absence of a $[C]^+$ ion whose formation would be blocked by a Δ^2 double bond. Spectra of cephalexin, cefadroxil and cephradine (all α-amino benzyl derivatives) provided clear evidence of molecular weight and had many features in common with those of analogous penicillins (ampicillin, etc.).

Diagnostic MS ions of cefaclor included isotopic clusters for entities retaining chlorine. Data for ions additional to the [A–D] set and of significant intensities (usually >20%) are included in Table 2; most ions appear to arise from loss of small molecules from the better defined ions but some cannot be assigned.

3-CH_2X *derivatives* [Table 2(b)]. Although spectra of this group proved more difficult to analyse than those of the β-lactam antibiotics already discussed, the majority provided clear evidence of molecular weight relevant ions ($[M \pm H]$, $[M \pm X]$, cluster ions and glycerol adducts) being of moderate to high intensities. Positive ion evidence of this kind was poor for cephamandole lithium (in contrast the $[M+1]^+$ ion was the base peak in the spectrum of the corresponding *O*-formyl ester) and cefsulodin sodium. Significant molecular ion features were also found in negative ion spectra, exceptions being those of cephamandole (lithium salt and *O*-formyl derivative), cephazolin sodium and moxalactam $2NH_4$. A 3-vinyl group or absence of a 3-substituent stabilizes the molecular ion, as is clear from the fact that $[M-1]^-$ ions were the base peaks of negative ion spectra of



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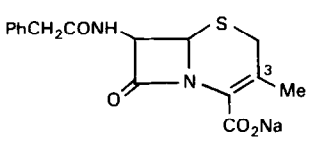

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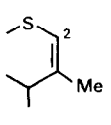


15 (partial structure)

cefixime and ceftizoxime. If [glycerol + X]$^+$ ions are unintentionally taken as the base peak all other intensities are unusually low: this problem arose in several cases, e.g. spectra of cephamandole lithium and moxalactam $2NH_4$. Ions corresponding to the [A–D] series were recorded in many spectra but intensities were low except for $[A_1]^+$ ions of the 2-thienylmethyl derivatives cephalothin sodium, cephaloridine and cefoxitin. Ions $[D]^-$ were notably intense for six to seven derivatives of this class, e.g. cephaloridine (100%) and cefsulodin (75%). Many spectra provided evidence of ions of the exomethylene type **16** which were of moderate intensity in several cases, e.g. cefoxitin sodium (positive ion), cefotaximine sodium (positive and negative ions), cefuroxime Na and cefatrizine (both negative ions). Exocyclic methylene derivatives related to **16** are well documented as products of degradation [18] and synthetic intermediates [19]. Positive ions of this kind derived from alkali metal salts may be formulated as **16** and negative ions as **17**. Exocyclic fragment ions formed from cephaloridine, cefsulodin and ceftazidime (all with 3-pyridinium methyl side chains) by loss of the pyridine moiety appear to add subsequently one or two atoms of hydrogen. Ions of type ArS^- formed the base peaks of negative ion spectra of cephamandole lithium and cephazolin sodium (42% for cefatrizine) while an ion of the same form was the base peaks of the positive ion spectrum of ceftriaxone. The ion *m/z* 123 (80%) of the positive ion spectrum of cefsulodin sodium is likewise diagnostic of the 3-CH_2X substituent.

Spectra of cephalosporins of this kind included a greater number of unassigned ions of intensities above 20% than were observed in spectra of the other classes of β-lactam antibiotic, a fact which is probably related to their more complex structure and more extensive fragmentation options.

Intermediate and degradation products

Penicillins (Table 5). Ions characteristic of molecular weight were prominent in FAB mass spectra of 6-APA (**18**), i.e. $[M+1]^+$ (46%), $[M-1]^-$ (100%) plus glycerol adducts. Of necessity only $[B_1]^+$ of the [A–D] set can arise and this structure formed the base

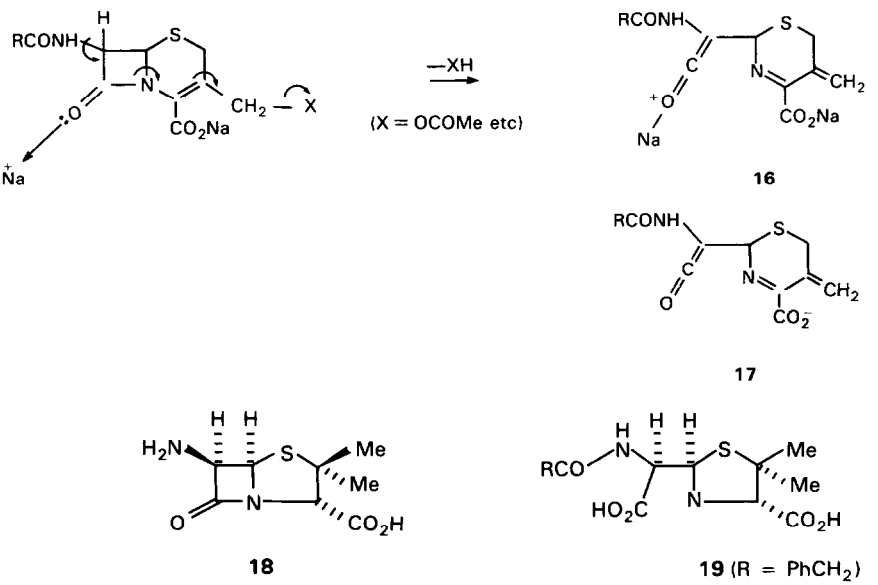


Table 5
Mass spectrometric features of intermediates and degradation products of β-lactam antibiotics recorded under FAB conditions unless otherwise stated (*m/z* values, intensities in parentheses)

Compound	Positive ions	Negative ions
6-Aminopenicillanic acid[a] M_r: 216	217(46) $[M+1]^+$ 309(25)[b] 114(45) $[B_1]^+ - (CO_2H+H)$ 160(100) $[B_1]^+$ 189(62) M−27	215(100)[c] $[M-1]^-$ 307(87)[b] 114(50)[d] 156(60) 189(40) 171(28) $M-CO_2H$
Benzylpenicilloic acid Na (**19**)[e] M_r: 374 (396 DiNa)	375(10) $[M+1]^+$ ↓ $-CO_2$ 331(3) 397(6) $[M+Na]^+$ ↓ $-CO_2$ 353(14) 419(14) DiNa salt + Na 91(19) $[A_1]^+$ 119(7) $[A_2]^+$ 204(13) $[B_1]^+$ ↓ $+CO_2$ Na 137(79) 238(11) $[C]^+$	373(11) $[M-1]$ ↓ $-CO_2$ 329(66) 351(93) $[M-Na]^-$ ↓ $-CO_2$ 307(26) ↓ $-CO_2+H_2S$ 229(14) 192(25) $[D]^-$
Benzylpenilloic acid Na (**20**, R = $PhCH_2$) M_r: 330	331(13) $[M+1]^+$ 353(69) $[M+Na]^+$ 445(1–2)[b] 683(5) $[2M+Na]^+$ 91(18) $[A_1]^+$ 119(2) $[A_2]^+$ 204(3) $[B_1]^+$ ↓ $-CO_2Na$ 137(38)	329(100) $[M-1]^-$ 307(40) $[M-Na]^-$ 659(10) $[2M-1]^-$ 637(25) $[2M-Na]^-$ 192(18) $[D]^-$ 263(6) $M-CO_2Na$ ↓ $-H_2S$ 229(71)
Benzylpenillic acid (**21**) M_r: 334	335(82) $[M+1]^+$ 427(2)[b] 91(42) $[A_1]^+$ 159(68) 301(2) $[M+1]^+ - H_2S$ ↓ $-CO_2$ 259(20) ↓ $-CO_2$ 213(80) 203(8) cf. CI 291(41) EI (70 eV) 34(50) H_2S 91(100) $[A_1]^+$ 202(25) (**22**) (nonprotonated) ↓ $-CO_2H+H_2$ 156(25) 300(35) $M-H_2S$ ↓ $-CO_2$ 256(15)	332(52) $[M-1]^-$ 425(25)[b] 74(35) $Me_2C=S^-$ 157(60) 229(100) 289(21) $M-CO_2H$ ↓ $-H_2$ 287(69) 307(88) M−27

Table 5
Continued

Compound	Positive ions	Negative ions
	CI (isobutane) 335(<2) $[M+1]^+$ 101(30) 203(80) (**22**) 301(100) $[M+1]^+ - H_2S$ $\downarrow -CO_2$ 347(80)	
7-Aminocephalosporanic acid (7-ACA) M_r: 272	164(20) 198(33)[f] 216(26) $[B_1]^+$ 363(12) $M+92-H_2$	89(100) 109(40) 124(60) 149(70) 175(75) 196(80)[f] 325(80) 345(100)
7-Aminodeacetoxycephalosporanic acid (7-ADCA) M_r: 214	215(85) $[M+1]^+$ 307(60)[b] 110(40) 158(18) $[B_1]^+$ 187(100) M−27(HCN?)	215(40) 216(50) 311(100) 427(50) $[2M-1]^-$
p-Nitrobenzylester of 7-ADCA[g] M_r: 349	350(5) $[M+1]^+$ 136(15) $CH_2C_6H_4NO_2$ $\downarrow -NO$ 106(100) CI (isobutane) 349(7) $[M]^+$ 350(4) $[M+1]^+$ 106(10) see above 136(5) see above 293(20) 322(100) M−27 EI (70 eV) 349(20) $[M]^+$ 74(100) $H_2NCH = CHS$ [D] 106(20) see above 136(35) see above 140(40) 293(70) $M-(NO+C_2H_2)$	Nothing recorded
p-Nitrobenzylester of 7-$PhCH_2CO$-ADCA[g] M_r: 467	468(5) $[M+1]^+$ 91(22) $[A_1]^+$ 103(100) 119(25) $[A_2]^+$ many ions >20% between 114 and 230	Nothing recorded

Table 5
Continued

Compound	Positive ions	Negative ions
	CI (isobutane) 91(100) $[A_1]^+$ 106(20) see above 114(50) 136(65) see above 154(55) 176(45) 289(38)	
Diphenylacetylester of 7-$PhOCH_2CO$-ADCA M_r: 514	107(50) $[A_1]^+$ 135(15) $[A_2]^+$ 167(70) $[CHPh_2]^+$ 229(50) 347(5) M−167 CI (isobutane) 514(1) $[M]^+$ 515(<1) $[M+1]^+$ 91(20) 107(12) $[A_1]^+$ 167(100) $[CHPh_2]^+$ 347(1) M−167 EI (70 eV) 514(1) $[M]^+$ 77(22) $[C_6H_5]^+$ 107(16) $[A_1]^+$ 167(100) $[CHPh_2]^+$ 347(5) M−167	Not recorded
Cefotaxime lactone M_r: 395 (**23**, general formula)	396(95) $[M+1]^+$ 488(18)[b] 126(85) 156(30) $[A_1]^+$ and/or $[B_1]^+$ 366(25) $[M+1]^+ - CH_2O$	Nothing recorded
Cephalothin lactone[g] M_r: 336	337(2–3) $[M+1]^+$ 147(100)	335(22) $[M-1]$

[a] FD–MS spectrum, ref. 8.
[b] Glycerol adduct of $[M+1]^+$ or $[M-1]^+$.
[c] In another run this ion was replaced by ions at 214(40) and 216(35).
[d] Unprotonated thiazolidine (**1**) − CO_2H.
[e] See ref. 20 for data on penicilloic acids derived from phenethicillin, carbenicillin, methicillin, propicillin, ticarcillin, phenoxymethylpenicillin, benzylpenicillin, amoxycillin, and ampicillin.
[f] M−(H_2NCH = CHS, 74); additional H_2 loss for negative ion.
[g] FAB spectra run in presence of HCl.

peak of the positive ion spectrum. FAB–MS of several penicilloic acids (alkali metal salts), formed by base-catalysed or enzyme-induced opening of the β-lactam ring of intact penicillins, have been described [20]. Both benzylpenicilloic acid (**19**) and the parent antibiotic (sodium salts) gave rise to identical $[A_1]^+$, $[A_2]^+$, $[B_1]^+$ and $[C]^+$ ions, and their spectra may be differentiated only by ions diagnostic of molecular weight. The $[D]^-$ ion (base peak of the negative ion spectrum of the penicillin) was of low intensity (25%). Other penicilloic acid salts behaved similarly under FAB conditions [20].

Likewise one must rely on ions diagnostic of molecular size to differentiate spectra of sodium salts of benzylpenicilloic acid and benzylpenilloic acid (**20**), its product of decarboxylation.

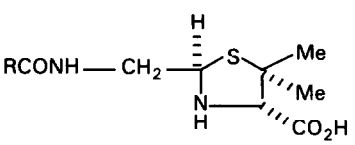
Benzylpenillic acid (**21**), an acid-catalysed rearrangement product of benzylpenicillin of radically altered structure provided useful mass spectra under several ionization conditions. Molecular ions were prominent in the two kinds of FAB spectra while the fragment, **22**, was notable in both CI and EI (*m/z* 202) spectra. Other ions of interest are tabulated which, together with the absence of a $[B_1]^+$ ion, allow differentiation of **21** and benzylpenicillin.

Cephalosporins. FAB spectra of 7-aminocephalosporaric acid (7-ACA) were difficult to interpret. Simple molecular species were absent, while positive and negative ions associated with loss of a [D] fragment were seen. Corresponding spectra of the 3-desacetoxy analogue (7-ADCA) displayed molecular ions and a $[B_1]^+$ ion typical of 3-methylcephem derivatives. Three esters encountered as synthetic intermediates were examined which gave EI and CI in addition to FAB spectra. Spectra of *p*-nitrobenzyl esters were characterized by $[M]^+$ and $[M+1]^+$ ions, and by those which revealed the nature of the ester function. Spectra of a diphenylacetyl derivative gave poor evidence of molecular weight and were dominated by the $[CHPh_2]^+$ ion.

Degradation of derivatives of 7-ADCA at acid pH often yields corresponding lactones, **23** [21]. That derived from cefotaximine gave a positive FAB spectrum which, like that of the parent, showed prominent molecular features while loss of formaldehyde from the lactone function was evident. In contrast, the spectrum of the lactone analogue of cephalothin displayed feeble [M ± 1] ions and provided none that were diagnostic.

Concluding Comments

This review demonstrates that FAB–MS may be applied directly to the identification of β-lactam antibiotics irrespective of the form in which they are presented, e.g. free acid, alkali metal salt, pro-drug or (for amino derivatives) hydrochloride salt. Apart from



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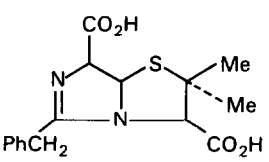

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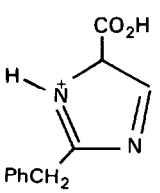

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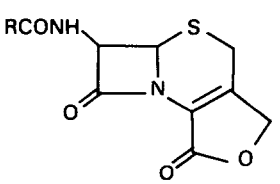

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that of pivampicillin, all spectra provided evidence of molecular weight which usually derives from a set of *m/z* values that may include cluster ion and glycerol adducts. Positive and negative ion data are complementary, and related (+):(−) *m/z* pairs (which differ by 2 mass units) may be identified with ease from initial spectral comparisons. Even artefact ions involving glycerol have the virtue of rapid identification of the nature of alkali metal cations and halide anions associated with the analyte. Most spectra include fragment ions which confirm structure, particularly in regard to the nature of the 6(7) amido substituent, the β-lactam nucleus (best for penicillins), the 3-substituent of cephalosporins and the pro-drug entities of penicillins. Such features, for example, rapidly enable differentiation of spectra of phenethicillin potassium and methicillin sodium which have identical $[M+1]^+$ and $[M-1]^-$ ions. Although the range of examples so far reported is small, the same analytical specificity is evident for degradation products of β-lactam antibiotics.

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Note added in proof — The MS of β-lactam antibiotics are included in a 1985 review (D. B. Borders, G. T. Carter, R. T. Hargreaves and M. M. Siegil, *Mass Spectrom. Rev.* **4**, 295–367 (1985)).

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