POSITIVE AND NEGATIVE ION FAST ATOM BOMBARDMENT MASS SPECTRA OF SOME PENICILLOIC ACIDS

JOHN LEVESON GOWER, GERALD D. RISBRIDGER and MICHAEL J. REDRUP

Beecham Pharmaceuticals Research Division, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey RH3 7AJ., England

(Received for publication October 22, 1983)

The technique of fast atom bombardment mass spectrometry has been shown to be capable of producing molecular weight and useful fragmentation information from a selection of penicilloic acids. In addition, the technique has been shown to give similar information on alkali metal salts of penicilloic acids.

Penicilloic acids are the major degradation products of penicillins and are formed by alkaline hydrolysis of the β-lactam ring as shown in Scheme 1. Further degradation can occur and penicilloic acids can readily be decarboxylated under acidic conditions to give rise to the corresponding penilloic acids.

The preparation of penicilloic acids from the corresponding penicillins has been described in detail by MUNRO and co-workers[1].

We have previously reported in detail on the fast atom bombardment mass spectra of penicillin free acids and alkali metal salts[2,3]. With all the penicillins reported and many others studied since those reports, molecular weight and useful fragmentation information has been obtained from the fast atom bombardment mass spectra.

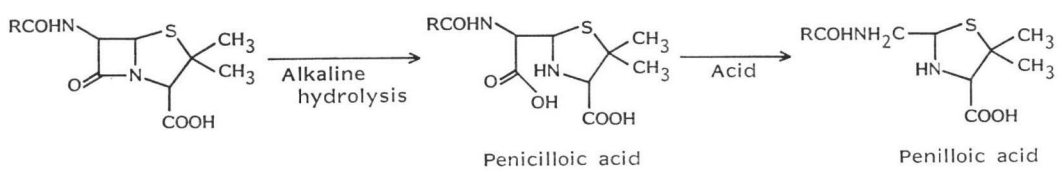
There is very little published mass spectral data on penicilloic acids. MITSCHER and co-workers[4] have reported the isobutane chemical ionization mass spectra of benzylpenicilloic acid dimethyl ester and phenoxymethylpenicilloic acid dimethyl ester. In the spectra of both of these compounds the protonated molecular ion was the base peak in the spectrum with major fragmentation ions being restricted to the thiazolidine cation at m/z 174. No mass spectra on free penicilloic acids or penicilloic acid alkali metal salts have been published.

Experimental

Mass spectra were obtained using a VG Analytical ZAB reverse geometry mass spectrometer fitted with an Ion Tech Fast Atom Bombardment gun. The fast atom beam was of xenon atoms.

Samples were prepared by dissolving the penicilloic acid in methanol and then mixing the solution with glycerol on the target. Stable ion currents were produced for several minutes and in a number of cases both positive and negative ion spectra were recorded from the same sample loading.

Scheme 1. Degradation of penicillin to penicilloic and penilloic acids.



Penicilloic acid Penilloic acid

The mass spectrometer was scanned over a mass range of 80~1,400 atomic mass unit at a scan rate of 15 seconds per mass decade. The mass spectra were recorded into a VG Multispec Data System calibrated in the fast atom bombardment mode using glycerol. Background subtraction of the glycerol ions has been carried out. The purity of the penicilloic acids was confirmed by ^{1}H NMR.

There was some variation in the spectra during data acquisition with the ratio of the intensity of molcular ion species to other ions decreasing with time. The spectra reproduced here were taken when this ratio was at its greatest.

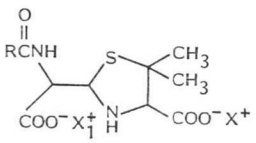
Results

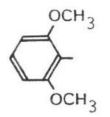
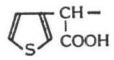
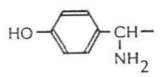
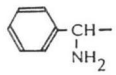
Six commercially available penicilloic free acids and three penicilloic acid sodium salts have been studied. The structures of these compounds are shown in Fig. 1 and the positive and negative ion fast atom bombardment mass spectra are shown in Figs. 2~10.

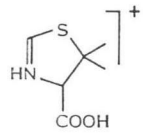
Positive Fast Atom Bombardment Mass Spectra

All the free penicilloic acids studied showed intense protonated molecular ions $[MH]^+$. In most cases this was the base peak in the spectrum. Dimeric species were usually observed *i.e.* $[2M+H]^+$. Fragment ions involving simple losses *e.g.* $[M-COOH]^+$ were present as was the thiazolidine cation at *m/z* 160.

Fig. 1. The structures and molecular weights of the penicilloic acids studied.



Penicilloic acid	MW	R	X	X_1
Phenethicillin	382	PhOCH(CH$_3$)–	H	H
Carbenicillin	396	PhCH(COOH)–	H	H
Methicillin	398		H	H
Propicillin	396	PhOCH(CH$_2$CH$_3$)–	H	H
Ticarcillin	402		H	H
Penicillin V	368	PhOCH$_2$	H	H
Benzylpenicillin	374	PhCH$_2$	Na	H
Amoxycillin	405		Na	H
Ampicillin	389		Na	H



Phenethicillin Penicilloic Acid (Fig. 2a)

The protonated molecular ion $[MH]^+$ at *m/z* 383 showed an intensity greater than 90% with the base peak in the spectrum being the R group cation $[PhOCH(CH_3)]^+$ at *m/z* 121. Loss of carbon dioxide from the protonated molecular ion gave a major fragment ion at *m/z* 339 with the only other major fragment ion being the thiazolidine cation at *m/z* 160. The protonated dimeric species $[2M+H]^+$ was observed at *m/z* 765.

Carbenicillin Penicilloic Acid (Fig. 3a)

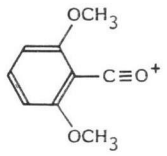
The protonated molecular ion $[MH]^+$ at *m/z* 397 was the base peak in the spectrum with losses of carbon dioxide giving rise to major fragment ions $[MH-CO_2]^+$ at *m/z* 353 and $[MH-2CO_2]^+$ at *m/z* 309. The remaining significant fragment ions include loss of formic acid from the protonated molecular ion to give the ion at *m/z* 351, the thiazolidine cation at *m/z* 160 and the benzyl cation at *m/z* 91.

Spectra acquired after the probe had been in

the instrument for a few minutes showed a base peak at m/z 91 with correspondingly lower intensity high mass ions at m/z 351, 353 and 397.

Methicillin Penicilloic Acid (Fig. 4a)

The base peak in the spectrum was the protonated molecular ion $[MH]^+$ at m/z 399. Loss of carbon dioxide gave rise to an intense fragment ion $[MH-CO_2]^+$ at m/z 355. Cleavage across the side-chain amide bond gave rise to the major fragment ion at m/z 165 *i.e.*



The thiazolidine cation at m/z 160 was observed but was not of great intensity. Other significant fragment ions were observed at m/z 323, 240, 151, 135 and 114.

The protonated dimeric species $[2M+H]^+$ was observed at m/z 797. As with carbenicillin penicilloic acid the ratio of the intensity of molecular ion species to fragment ions decreased with time.

Propicillin Penicilloic Acid (Fig. 5a)

The base peak was the protonated molecular ion $[MH]^+$ at m/z 397. Intense fragment ions due to

Fig. 2. Fast atom bombardment mass spectra of phenethicillin penicilloic acid.
a) Positive ion, b) negative ion.

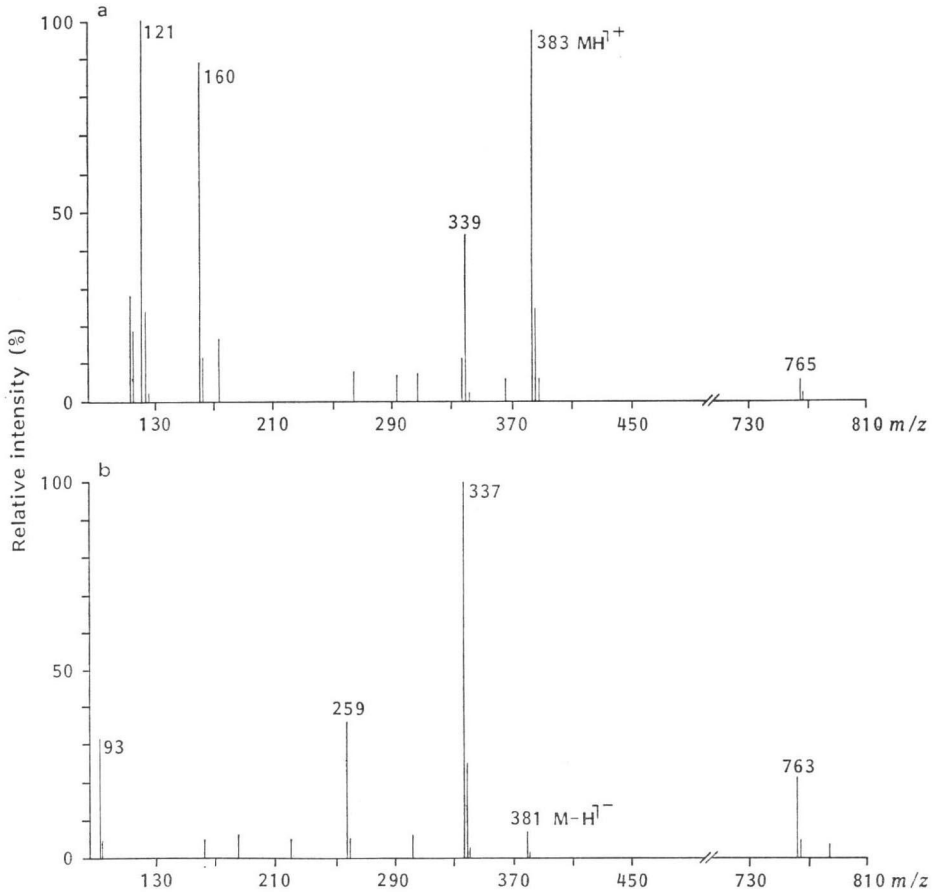
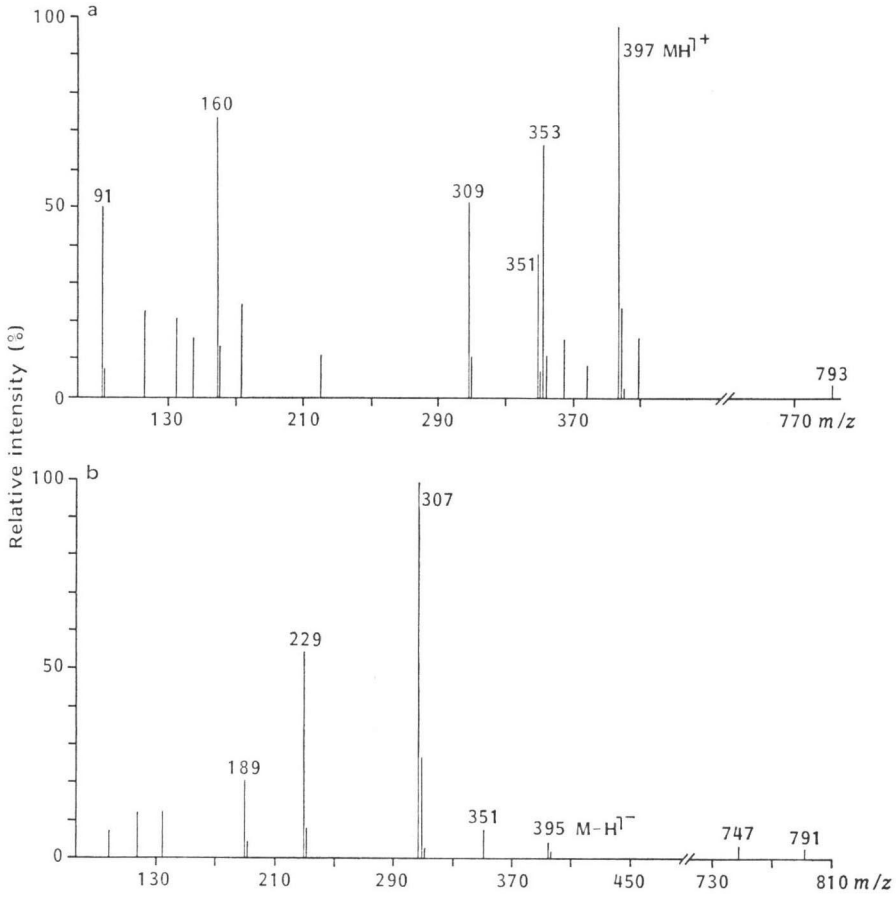


Fig. 3. Fast atom bombardment mass spectra of carbenicillin penicilloic acid.
a) Positive ion, b) negative ion.



loss of carbon dioxide from the protonated molecular ion *i.e.* $[MH-CO_2]^+$ at *m/z* 353 and the thiazolidine cation at *m/z* 160 were observed. The only other major fragment ion is the R group cation *i.e.* $[PhOCH(CH_2CH_2)]^+$ at *m/z* 135. Low intensity fragment ions were observed at *m/z* 221, 174, 114 and 107.

The protonated dimeric species $[2M+H]^+$ at *m/z* 793 was observed.

Ticarcillin Penicilloic Acid (Fig. 6a)

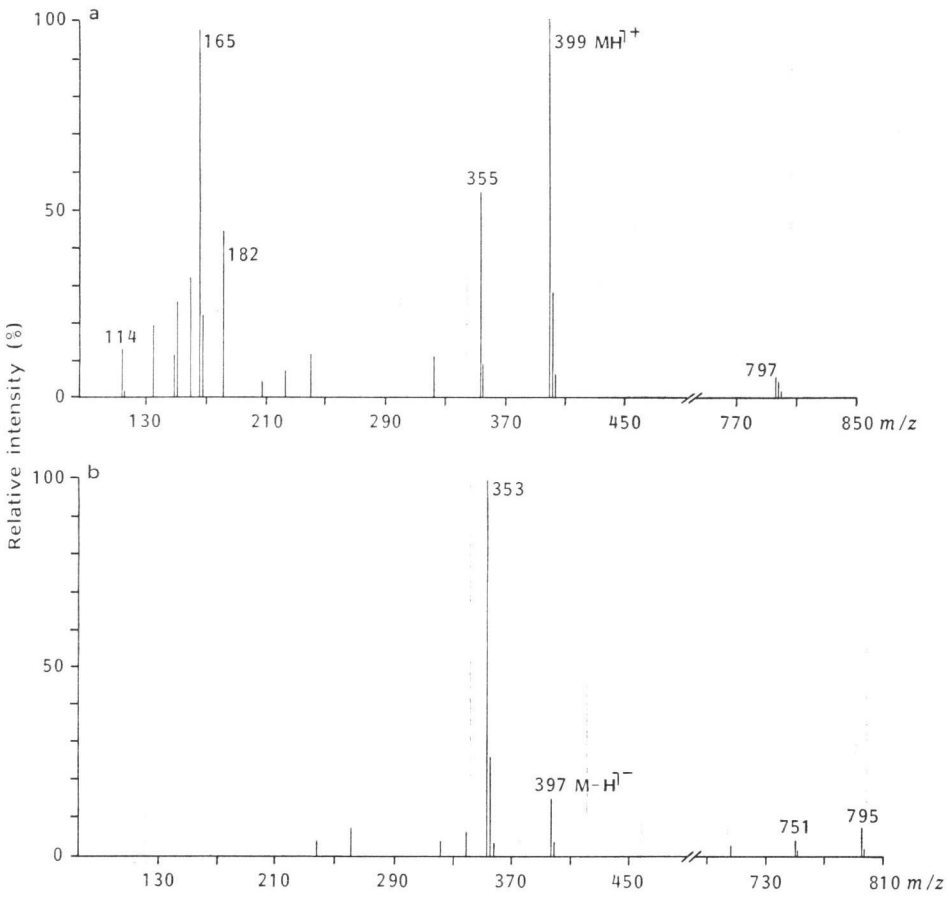
The thiazolidine cation at *m/z* 160 was the base peak in the spectrum with the protonated molecular ion $[MH]^+$ at *m/z* 403 having a relative intensity greater than 80%. Losses of carbon dioxide from the protonated molecular ion gave rise to intense fragment ions at *m/z* 359 $[MH-CO_2]^+$ and *m/z* 315 $[MH-2CO_2]^+$. Low intensity fragment ions were observed at *m/z* 174 and 97.

The protonated dimeric ion $[2M+H]^+$ was observed at *m/z* 805 as were dimeric species corresponding to $[(2M+H)-CO_2]^+$ at *m/z* 761 and $[(2M+H)-2CO_2]^+$ at *m/z* 717.

Phenoxymethylpenicilloic Acid (Fig. 7a)

The protonated molecular ion $[MH]^+$ at *m/z* 369 was the base peak in the spectrum. Fragment

Fig. 4. Fast atom bombardment mass spectra of methicillin penicilloic acid.
a) Positive ion, b) negative ion.



ions due to loss of carbon dioxide from the protonated molecular ion *i.e.* $[MH-CO_2]^+$ at *m/z* 325 and loss of formic acid from the protonated molecular ion *i.e.* $[MH-HCOOH]^+$ at *m/z* 323 were observed. The thiazolidine cation at *m/z* 160 was the major fragment ion with the phenoxymethyl cation at *m/z* 107 also being observed. Low intensity fragment ions were observed at *m/z* 250, 174 and 114.

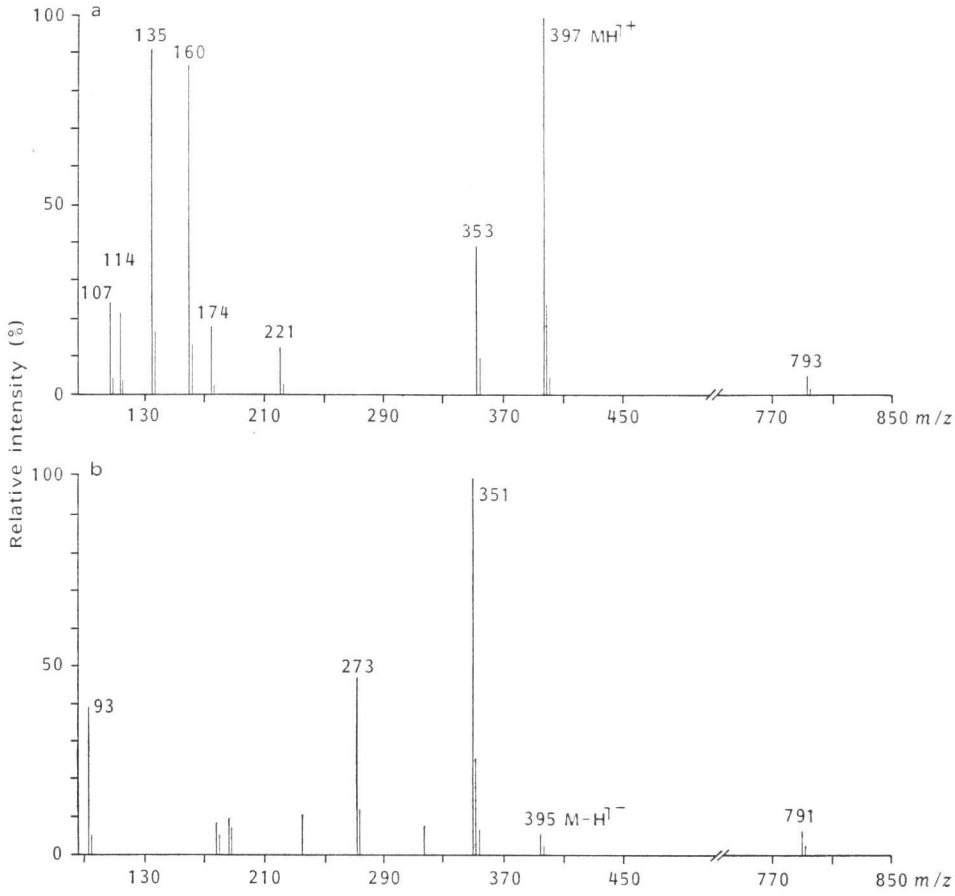
Benzylpenicilloic Acid Sodium Salt (Fig. 8a)

Intense protonated cationized molecular ions were observed at *m/z* 375 $[MH]^+$ and *m/z* 397 $[MNa]^+$. Loss of carbon dioxide from both of these ions was observed at *m/z* 331 and *m/z* 353 respectively. The ion at *m/z* 353 may well have a large contribution from the protonated molecular ion of the free penicilloic acid. The base peak in the spectrum was the benzyl cation at *m/z* 91 with other fragment ions being observed at *m/z* 320, 319 and 253. Several dimeric species were observed at *m/z* 683, 705, 727, 749 $[2M+H]^+$, 771 $[2M+Na]^+$, 793 and 815. The presence of these higher mass dimeric species together with an intense ion at *m/z* 419 suggests the presence of some disodium salt of benzylpenicilloic acid.

Amoxycillin Penicilloic Acid Sodium Salt (Fig. 9a)

Both the protonated molecular ion $[MH]^+$ at *m/z* 406 and the protonated molecular ion for the free

Fig. 5. Fast atom bombardment mass spectra of propicillin penicilloic acid.
a) Positive ion, b) negative ion.



acid at *m/z* 384 were observed. The base peak in the spectrum was the R group cation $[Ph(OH)CH(NH_2)]^+$ at *m/z* 122 with the thiazolidine cation at *m/z* 160 being the next most intense ion. Other fragments were observed at *m/z* 367, 322, 189, 150, 134 and 107. Dimeric species were observed at *m/z* 811 $[2M+H]^+$, 789 and 767.

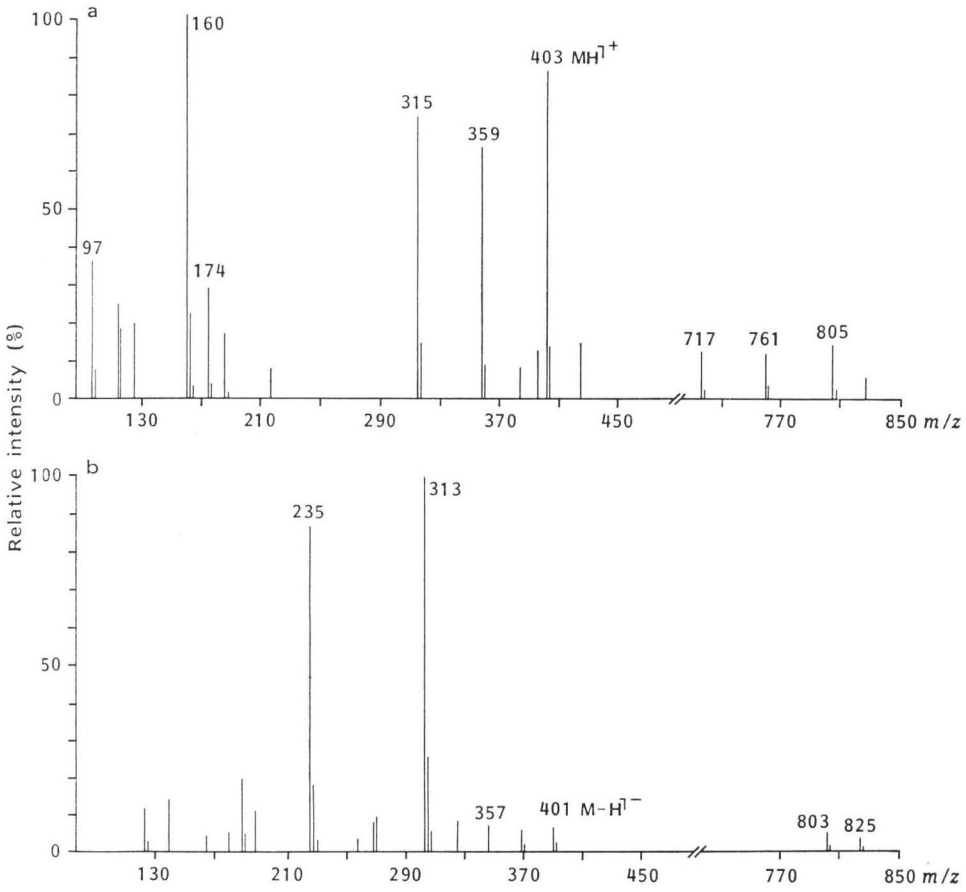
Ampicillin Penicilloic Acid Sodium Salt (Fig. 10a)

An intense protonated molecular ion $[MH]^+$ was observed at *m/z* 390 together with the cationized molecular ion $[MNa]^+$ at *m/z* 412 and the protonated molecular ion of the free penicilloic acid at *m/z* 368. The aminobenzyl cation $[PhCH(NH)_2]^+$ at *m/z* 106 was the base peak in the spectrum with other fragment ions being observed at *m/z* 324, 160 and 137. A dimeric protonated molecular ion was present at *m/z* 779 [2M+H].

Negative Ion Fast Atom Bombardment Mass Spectra

As with the penicillins previously published the negative ion fast atom bombardment mass spectra were more intense than the positive ion spectra. Intense $[M-H]^-$ ions were observed with all the penicilloic acids together with dimeric ions and structurally significant fragment ions.

Fig. 6. Fast atom bombardment mass spectra of ticarcillin penicilloic acid.
a) Positive ion, b) negative ion.



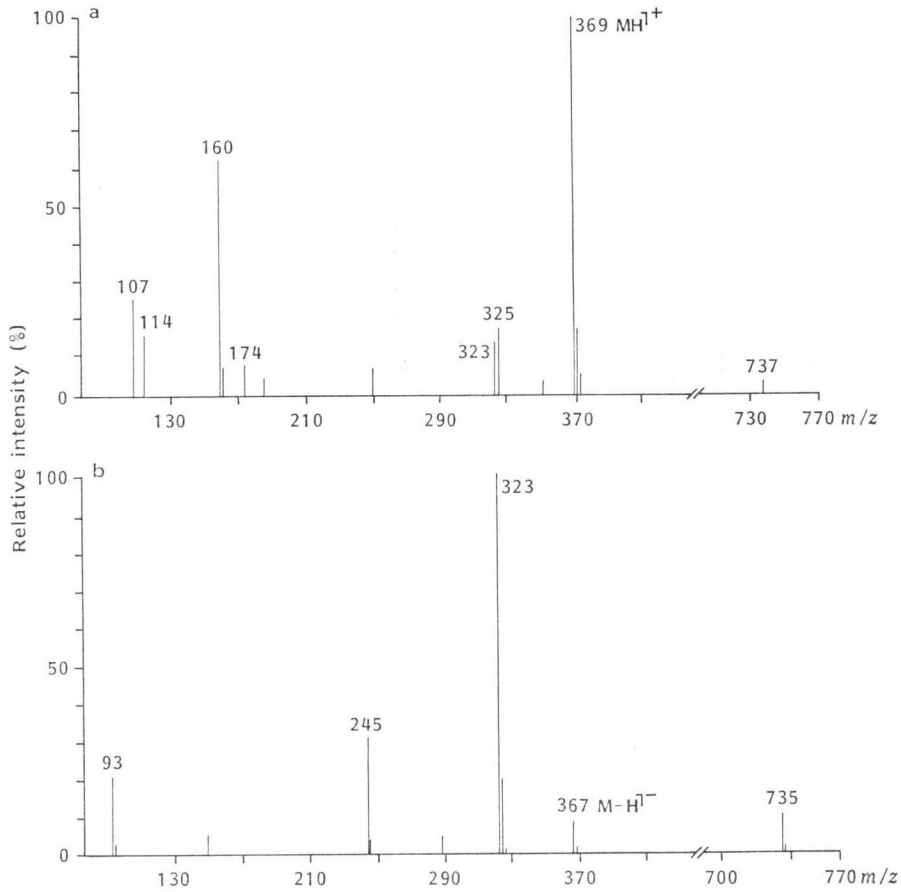
Phenethicillin Penicilloic Acid (Fig. 2b)

The base peak in the spectrum corresponds to the decarboxylated anion $[M-COOH]^-$ at *m/z* 337. An ion corresponding to $[M-H]^-$ was present at *m/z* 381 and an intense dimeric anion $[2M-H]^-$ was present at *m/z* 763. Two major fragment ions were observed at *m/z* 93 $[PhO]^-$ and *m/z* 259. The identity of this last fragment ion is uncertain but may well be a fragment ion due to loss of carbon dioxide and hydrogen sulfide from the decarboxylated anion $[M-COOH]^-$ at *m/z* 337. This type of fragmentation pathway has already been postulated as occurring in the negative ion desorption chemical ionization mass spectra of penicillins although it did not occur to any great extent in the negative ion FAB spectra of penicillins.

Carbenicillin Penicilloic Acid (Fig. 3b)

Low intensity ions for the $[M-H]^-$ anion at *m/z* 395 and the $[M-COOH]^-$ anion at *m/z* 351 were present with the base peak in the spectrum being $[M-(COOH+CO_2)]^-$ at *m/z* 307. The dimeric species $[2M-H]^-$ at *m/z* 791 and $[2M-COOH]^-$ at *m/z* 747 were observed. Several fragment ions were present with that at *m/z* 229 being due to loss of carbon dioxide and hydrogen sulfide from the ion at

Fig. 7. Fast atom bombardment mass spectra of phenoxymethylpenicilloic acid.
a) Positive ion, b) negative ion.



m/z 307.

Methicillin Penicilloic Acid (Fig. 4b)

The molecular anion $[M-H]^-$ was observed at *m/z* 397 with the base peak being the decarboxylated anion $[M-COOH]^-$ at *m/z* 353. Dimeric ions at *m/z* 795 $[2M-H]^-$ and *m/z* 751 $[2M-COOH]^-$ were also observed.

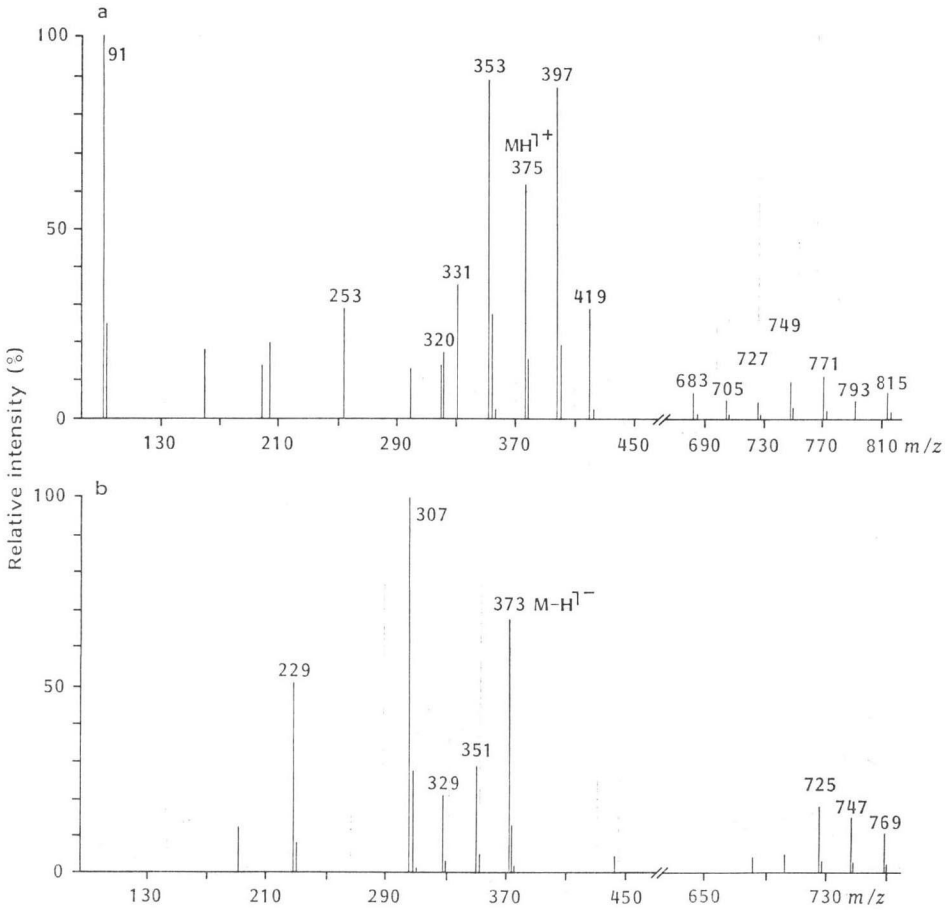
Propicillin Penicilloic Acid (Fig. 5b)

A low intensity $[M-H]^-$ anion was observed at *m/z* 395 with the base peak in the spectrum being the decarboxylated anion $[M-COOH]^-$ at *m/z* 351. The phenoxy anion at *m/z* 93 and the fragment ion due to loss of carbon dioxide and hydrogen sulfide from the decarboxylated anion $[M-COOH]^-$ at *m/z* 273 were also present. The dimeric anion $[2M-H]^-$ at *m/z* 791 was relatively intense.

Ticarcillin Penicilloic Acid (Fig. 6b)

Low intensity ions were observed at *m/z* 401 $[M-H]^-$ and *m/z* 357 $[M-COOH]^-$. The base peak in the spectrum was the decarboxylated anion $[M-(COOH+CO_2)]^-$ at *m/z* 313. Loss of carbon dioxide and hydrogen sulfide from this ion gave rise to the fragment ion at *m/z* 235. Dimeric ions were

Fig. 8. Fast atom bombardment mass spectra of benzylpenicilloic acid sodium salt.
a) Positive ion, b) negative ion.



present at *m/z* 803 $[2M-H]^-$ and *m/z* 825 $[(2M+Na)-H]^-$.

Phenoxymethylpenicilloic Acid (Fig. 7b)

The decarboxylated anion $[M-COOH]^-$ at *m/z* 323 was the base peak in the spectrum with the $[M-H]^-$ anion at *m/z* 367 also being prominent. An intense dimeric anion $[M-H]^-$ was observed at *m/z* 735. Fragment ions included the phenoxy anion at *m/z* 93 and the ion due to loss of carbon dioxide and hydrogen sulfide from the decarboxylated anion $[M-COOH]^-$ at *m/z* 245.

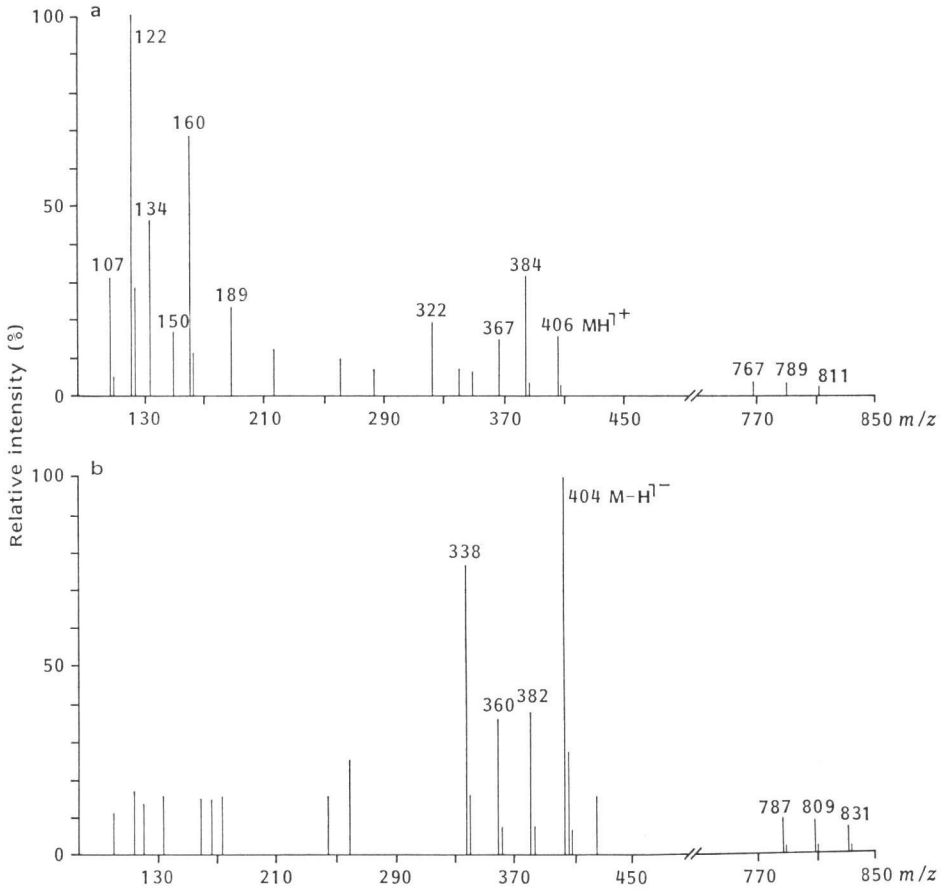
Benzylpenicilloic Acid Sodium Salt (Fig. 8b)

Intense ions were observed at *m/z* 373 $[M-H]^-$, *m/z* 351 $[M-Na]^-$, *m/z* 329 $[M-COOH]^-$ and *m/z* 307 $[M-(Na+COOH)]^-$. An intense fragment ion at *m/z* 229 due to loss of carbon dioxide and hydrogen sulfide from the ion at *m/z* 307 was also observed. Dimeric anions were present at *m/z* 747 $[2M-H]^-$, *m/z* 725 $[2M-H]^-$ for the di-acid and *m/z* 769 $[2M-H]^-$ for the disodium salt.

Amoxycillin Penicilloic Acid Sodium Salt (Fig. 9b)

The base peak in the spectrum was the $[M-H]^-$ anion at *m/z* 404 with the other major ions being at

Fig. 9. Fast atom bombardment mass spectra of amoxycillin penicilloic acid sodium salt.
a) Positive ion, b) negative ion.



m/z 382 [M−Na]⁻, 360 [M−COOH]⁻, and *m/z* 338 [M−Na+COOH]⁻. Dimeric anions were observed at *m/z* 809 [2M−H]⁻, *m/z* 787 [2M−H]⁻ for the di-acid and 831 [2M−H]⁻ for the disodium salt.

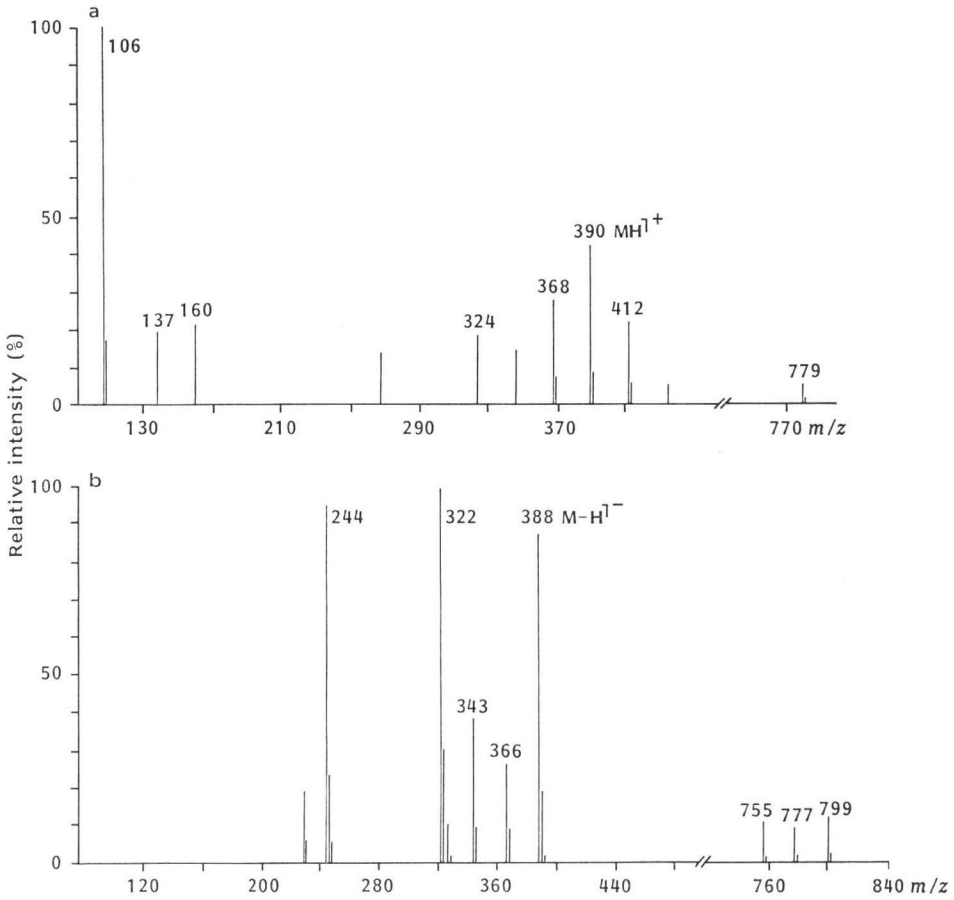
Ampicillin Penicilloic Acid Disodium Salt (Fig. 10b)

Intense ions were observed at *m/z* 388 [M−H]⁻, *m/z* 322 [M−COOH]⁻, and *m/z* 244, this last ion arising by loss of carbon dioxide and hydrogen sulfide from the ion at *m/z* 322. Dimeric anions were observed at *m/z* 777 [2M−H]⁻, *m/z* 755 [2M−H]⁻ for the di-acid and *m/z* 799 [2M−H]⁻ for the di-sodium salt.

Conclusions

Fast atom bombardment has been shown to be an important mass spectrometric technique for the molecular weight and structural determination of penicilloic acids both as free acids and as sodium salts. A combination of the information available from both positive and negative ion spectra has been shown to be important in studying this class of compounds by fast atom bombardment mass spectrometry.

Fig. 10. Fast atom bombardment mass spectra of ampicillin penicilloic acid sodium salt.
a) Positive ion, b) negative ion.



Penicilloic acids, as with most samples where glycerol has been used as the matrix compound, gave spectra that lasted for several minutes which is potentially important in that accurate mass measurement and metastable studies can be performed.

References

1) MUNRO, A. C.; M. G. CHAINEY & S. R. WORONIECKI: Preparative immunological cross-reactions of penicilloic and penilloic acids. J. Pharm. Sci. 67: 1197~1204, 1978
2) BARBER, M.; R. S. BORDOLI, R. D. SEDGWICK, A. N. TYLER, B. N. GREEN, V. C. PARR & J. L. GOWER: Fast atom bombardment mass spectrometry of some penicillins. Biomed. Mass Spectrom. 9: 11~17, 1982
3) GOWER, J. L.: Fast atom bombardment mass spectrometry of β-lactam antibiotics. Int. J. Mass Spectrom. & Ion Physics 46: 431~434, 1983
4) MITSCHER, L. A.; H. D. H. SHOWALTER, K. SHIRAHATA & R. L. FOLTZ: Chemical-ionization mass spectrometry of β-lactam antibiotics. J. Antibiotics 28: 668~675, 1975