Fast Atom Bombardment Mass Spectra of Azthreonam and Its Salts

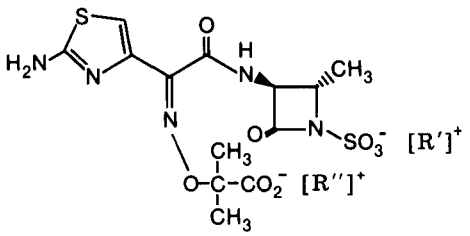
Keyphrases □ Fast atom bombardment—mass spectra of azthreonam and its salts □ Azthreonam—biologically active azetidinesulfonic acids, monobactams, fast atom bombardment mass spectra □ Mass spectrometry—fast atom bombardment of azthreonam and its salts □ Monobactams—biologically active azetidinesulfonic acids, azthreonam, fast atom bombardment mass spectra

To the Editor:

Recently, Cimarusti *et al.* (1) reported the synthesis of a new class of biologically active azetidinesulfonic acids, termed monobactams. One of these monobactams, azthreonam[1], is highly selective against aerobic Gram-negative bacteria (2), including *Pseudomonas aeruginosa* (3).

This communication reports the results of the mass spectra of the named compound and its monosodium and dipotassium salts by the fast atom bombardment technique pioneered by Barber *et al.* (4) and applied by various investigators in the analysis of vexatious polar compounds (5–12).

Mass spectra were obtained on a double-focusing magnetic sector instrument[2] equipped with a fast atom bombardment source using 4–8 kV xenon neutral atoms. Azthreonam (I) gave a very prominent $[M—H]^-$ ion (base peak) in the negative-ion detection mode[3] (Table I) and a significant MH^+ ion in the positive-ion detection mode (Table II). Weak, but perceptible, dimeric ions were also observed. Based upon the expected losses, the fragmentation patterns in each ion detection mode yielded diagnostic information.



	R′	R″	MW	MH^+	$[M·H]^-$
I Azthreonam	H	H	435	436	434
II	Na	H	457	458	456
III	K	K	511	512	510

In the negative ion spectrum, the ions resulting from the direct N—O bond cleavage yields the $[m/z\ 332]^-$ ion and its $[m/z\ 103]^-$ ion complement (Table I). The principal high mass fragment ion in the positive-ion mass spectrum results from the loss of sulfur trioxide from the MH^+ ion (Table II). Cleavage of the monobactam ring of I gives rise to the $[m/z\ 313]^+$ ion in the positive-ion detection mode and its $[m/z\ 122]^-$ complement in the negative-ion detection mode.

Because polar acids are often isolated as alkali salts, the fast atom bombardment spectra of the monosodium (II) and dipotassium (III) salts (13) were compared to the spectra of azthreonam (I). The negative-ion spectra produced readily assignable $[M—H]^-$ and $[M—R']^-$ ions (Table I). The positive-ion spectra yielded complimentary information, although ions generated from mono- and dicationization were more intense than the MH^+ ions. Consequently, care should be taken to introduce as minimum extraneous inorganic salts, or, if unavoidable, only a single species of cation. Fast atom bombardment mass spectrometry has been used in the characterization of related naturally occurring monobactams, synthetic intermediates for azthreonam, and impurities.

Table I—Negative-Ion Fast Atom Bombardment Mass Spectra [a,b]

Negative-Ion Compound Assignment	I R′ = R″ = H	II R′ = Na, R″ = H	III R′ = R″ = K
	[mass/charge (Relative Intensity)]		
2M+R′—2H	—	935(5)	—
2M—H	869(1)	913(5)	—
2M—R′	—	891(8)	983(2)
M+R′—2H	—	478(4)	548(3)
M—H	434(100)	456(76)	510(20)
M—R′	434(100)	434(76)	472(39)
M—R″$O_2CC_3H_6O$	332(16)	354(11)	370(24)
M—R′$O_2CC_3H_6O$	332(16)	332(13)	370(24)
$HO_3SNC_4H_4NCO$	205(16)	205(65)	205(100)
O_3SNCO and/or $O_3SNC_2H_4$	122(58)	122(100)	122(80)
$HO_2CC_3H_6O$	103(10)	103(18)	103(59)
SO_3NH_2	96(26)	96(39)	96(20)
SO_3	80(48)	80(87)	80(85)

[a] Xenon neutral gas. [b] Glycerin matrix.

Table II—Positive-Ion Fast Atom Bombardment Mass Spectra [a,b]

Positive-Ion Compound Assignment	I R′ = R″ = H	II R′ = Na, R″ = H	III R′ = R″ = K
	[mass/charge (Relative Intensity)]		
2M+2R′—H	—	959(9)	1099(1)
2M+R′	871(1)	937(24)	1061(4)
2M+H	871(1)	915(3)	1023(2)
M+2R′—H	—	502(44)	588(14)
M+R′	436(30)	480(100)	550(47)
MH	436(30)	458(21)	512(16)
M+R′—SO_3	356(44)	400(14)	432(11)
MH—SO_3	356(44)	378(?)	—
M—H—SO_3	354(3)	377(83)	—
MH—HO_3SNCO and/or MH—$HO_3SNC_2H_4$	313(65)	335(22)	389(11)
MH—$O_2CC_3H_6O$	334(4)	356(5)	410(13)
$C_4H_6N_3S$	126(100)	126(93)	—

[a] Xenon neutral gas. [b] Glycerin matrix.

[1] {2*S*-[2α,3β(*Z*)]}-3[[2-amino-4-thiazolyl-[(1-carboxy-1-methyl-ethoxy)imino]-acetyl]-amino]-2-methyl-4-oxo-1-azetidinesulfonic acid; CAS 78110-38-0, SQ 26,776.

[2] Model ZAB 1F, VG Analytical Ltd., Altrincham, U.K.

[3] A similar negative-ion mass spectrum of I was obtained on the Kratos MS 50 mass spectrometer, with xenon gas, at the Middle Atlantic Mass Spectrometry (MAMS) Laboratory, Baltimore, Md.

(1) C. M. Cimarusti, H. Breuer, T. Denzel, D. K. Kronenthal, U. D. Treuner, D. P. Bonner, and R. B. Sykes, Abstract 487, 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., November 4–6, 1981.

0022-3549/82/0900-1065$01.00/0

(2) R. B. Sykes, K. Bush, and J. S. Freudenberger, Abstract 479, *ibid.*

(3) P. B. Fernandes, D. P. Bonner, R. R. Whitney, B. H. Miller, C. O. Baughn, and R. B. Sykes, Abstract 490, *ibid.*

(4) M. Barber, R. S. Bardoli, R. D. Sedwick, and A. N. Tyler, *J. Chem. Soc. Chem. Commun.*, **1981**, 325.

(5) C. Fenselau and R. J. Cotter, *Pure Appl. Chem.*, in press.

(6) D. H. Williams, C. Bradley, G. Bojesen, S. Santikarn, and L. C. E. Taylor, *J. Am. Chem. Soc.*, **103**, 5700 (1981).

(7) M. Barber, R. S. Bardoli, R. D. Sedwick, and A. N. Tyler, *Biomed. Mass. Spectrom.*, **8**, 492–495 (1981).

(8) A. Dell, H. R. Morris, M. D. Levin, and S. M. Hecht, *Biochem. Biophys. Res. Commun.*, **102**, 730 (1981).

(9) M. Barber, R. S. Bardoli, R. D. Sedwick, and L. W. Tetler, *Org. Mass Spectrom.*, **16**, 256 (1981).

(10) C. Fenselau, R. Cotter, G. Hansen, T. Chen, and D. Heller, *J. Chromatogr.*, **218**, 21 (1981).

(11) C. Fenselau, *Anal. Chem.*, **54**, 105A (1982).

(12) C. J. McNeal, *ibid.*, **54**, 43A (1982).

(13) H. Breuer, C. M. Cimarusti, Th. Denzel, W. H. Koster, W. A. Slusarchyk, and U. D. Treuner, *J. Antimicrob. Agents Chemother.*, **8**, Suppl. E, 21 (1981).

Allen I. Cohen[x]
Phillip T. Funke
Department of Analytical Research
Squibb Institute for Medical Research
New Brunswick, NJ 08903

Brian N. Green
VG Analytical Ltd.
Altrincham, U.K.

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Effect of Initial Conditions and Drug–Protein Binding on the Time to Equilibrium in Dialysis Systems

Keyphrases □ Drug–protein binding—effect of initial conditions on the time to equilibrium in dialysis systems □ Equilibrium dialysis—effect of initial conditions and drug–protein binding

To The Editor:

A major disadvantage of using equilibrium dialysis methods for determining *in vitro* plasma protein binding of drugs is the time needed to reach equilibrium. Recently, Øie and Guentert (1) mathematically showed that equilibration is more rapid when the drug is initially added to the plasma side as opposed to the buffer side. The approach to equilibrium was described by integrated equations for the concentration of drug on the buffer side as a function of time. The relative time to equilibrium for the two initial configurations was found to be:

$$R = \frac{t_B}{t_P} = \frac{\ln \delta + \ln \alpha}{\ln \delta} = 1 + \frac{\ln \alpha}{\ln \delta} \qquad \text{(Eq. 1)}$$

where t_B and t_P are the times to reach some fraction (δ) from the equilibrium concentration when the drug is initially added to the buffer or plasma side, respectively, and α is the unbound fraction in plasma which is assumed to be constant during the dialysis. For example, a δ value of 0.05 indicates a deviation of 5% from the true equilibrium value.

The authors indicated that the closer to the true equilibrium value one wants to be, the closer the ratio is to unity. Furthermore, a smaller α (stronger binding) or a larger δ increases the advantage of spiking the plasma side. While we are in complete agreement with these conclusions, some interesting and practical information may be lost if the examination of this system is limited only to the ratio of the times to equilibrium. In this communication the concept of approach to equilibrium in dialysis systems will be further developed and factors affecting comparative equilibration times will be discussed.

When drug is initially added to the plasma side, the concentration on the buffer side (C_B) at any time (t) is:

$$C_B = \frac{C_0\alpha}{1+\alpha}(1 - e^{-K_T(1+\alpha)t}) \qquad \text{(Eq. 2)}$$

where C_0 is the initial concentration, and K_T is the rate constant governing the transfer of drug across the membrane (1).

When drug is initially placed in the buffer side, a similar equation is obtained:

$$C_B = \frac{C_0}{1+\alpha}(\alpha + e^{-K_T(1+\alpha)t}) \qquad \text{(Eq. 3)}$$

Although the actual concentration on the buffer side is the variable of interest, a more useful relationship for examining the influence of α on equilibrium times would be an expression of C_B in relative terms. A fraction away from the equilibrium concentration in the buffer side (δ) is defined as:

$$\delta = \frac{\text{Absolute Value } (C_B^\infty - C_B)}{C_B^\infty} \qquad \text{(Eq. 4)}$$

where C_B^∞ is the concentration of C_B as $t \to \infty$ and $C_B^\infty = C_0\alpha/(1+\alpha)$ for both cases (buffer or plasma spiked).

In a form analogous to Eq. 2, the time course of this fraction when drug is initially on the plasma side is then described by:

$$\delta_P = e^{-K_T(1+\alpha)t} \qquad \text{(Eq. 5)}$$

When drug is initially placed on the buffer side, the time course of δ is described by:

$$\delta_B = \frac{e^{-K_T(1+\alpha)t}}{\alpha} \qquad \text{(Eq. 6)}$$

A hypothetical semilog plot for the time course of these fractions from equilibrium concentration is shown in Fig. 1. As dictated by Eqs. 5 and 6, the δ values decline exponentially with time. For a given value of α, $\ln \delta_P$ and $\ln \delta_B$ decrease at the same rate with slope $= -K_T(1+\alpha)$. As $\alpha \to 0$, the slopes become $-K_T$ and as $\alpha \to 1$, the slopes become $-2K_T$. At $t = 0$, $\delta_P = 1$, whereas the $\delta_B = 1/\alpha$. Thus, δ_P values are always ≤ 1 whereas δ_B has no limit. Although the actual rate constants for approach to equilibrium are the same for both buffer and plasma spiked systems, the buffer spiked system requires more time to reach comparable δ values, because a greater amount of drug must be

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