Stability in Aqueous Solution of Two Monocyclic β-Lactam Antibiotics: Aztreonam and Nocardicin A

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The catalytic effect of various buffer systems (citrates, acetates, phosphates, borates and carbonates) on the degradation of aztreonam and nocardicin A in aqueous solution was studied at 35 °C and a constant ionic strength of 0.5 mol·dm^{-3} over a pH range of 3.50 to 10.50. The observed degradation rates, obtained by measuring the remaining intact antibiotic, were shown to follow pseudo-first-order kinetics with regard to antibiotic concentrations and to be influenced by general acid and general base catalysis. The changes in the concentration of intact β-lactam antibiotic in the solutions were established by reverse-phase HPLC with UV-detection. In general the buffer systems employed in the kinetic studies showed a very weak catalytic effect on the degradation of aztreonam and nocardicin A. The pH-rate profiles for these antibiotics showed degradation minimums at pH 5.38 and 6.13, respectively. Aztreonam is slightly more reactive with hydrogen ions than nocardicin A and is much more reactive with hydroxide ions. In comparison with other β-lactamic antibiotics, aztreonam and nocardicin A are much more stable in aqueous solution, except for aztreonam in a base solution, which is just as unstable as penicillins and cephalosporins. The Arrhenius activation energies were determined for aztreonam and nocardicin A at pH's 4.23, 6.59 and 8.60.

Keywords β-lactam antibiotic; monocyclic β-lactam antibiotic; aztreonam; nocardicin A; stability; aqueous solution; degradation kinetic; catalytic effect; buffer system; pH-rate profile

The antibiotics studied, aztreonam and nocardicin A (Chart 1), belong to the monocyclic β-lactamic group, which is typified by not having a β-lactamic ring fused with a second ring with five or six members, as occurs in penicillins, cephalosporins and other β-lactamic antibiotics. Among the monocyclic β-lactamic antibiotics are the nocardicins[1,2] (including nocardicin A), derived from 3-aminocardicinic acid (3-ANA), and the monobactams[3,4] (including aztreonam), derived from 3-aminomonobactamic acid (3-AMA).

Structural elucidation of nocardicin A,[1,2] the first antibiotic isolated from the actinomycetes species *Nocardia uniformis* subsp. *tsuyamanensis* ATCC 21806, revealed its structural relationship to cephalosporins and penicillins: the configuration of the amide-carrying C-3 carbon of the β-lactam is identical, and an oxime function, which is only seldom observed in nature, is present.

The monobactams have as a characteristic feature a 3-acylamido group and the function N–SO_3H, which rarely occurs in nature. Aztreonam, a member of this group, is totally synthetic, unlike the penicillins and cephalosporins, which are products of fermentation.

As the drugs of the bicyclic β-lactam antibiotic group are generally characterized by a pronounced vulnerability to attack on the β-lactam ring by acid-base reagents, metal ions, β-lactamases, organic catalytic agents, and even water molecules and their ions,[5-19] it would be interesting to find out how susceptibile monocyclic antibiotics such as aztreonam and nocardicin A are to such attacks.

The present paper describes the stability of these monocyclic β-lactam antibiotics in various solutions as a function of catalytic components, while this study and others carried out in our laboratory[14-18] seek to provide data which will allow us to obtain a better correlation between the chemical reactivity and the biological activity of the β-lactam antibiotics, which will improve the quality of these antibiotics in the future.

Experimental

Materials Aztreonam was supplied by E. R. Squibb & Sons, Inc. (New Jersey, U.S.A.) and nocardicin A was supplied by the Fujisawa Pharmaceutical Co. (Osaka, Japan).

Buffer salts used and other chemicals were commercial products of analytical grade. All the water used was purified with a Milli-Q-reagent water system (Millipore, Bedford, MA, U.S.A.).

Buffer Solutions The buffer systems used in the kinetic studies were: citrate buffer (pH 3.50—4.75), acetate (pH 4.00—5.65), phosphate

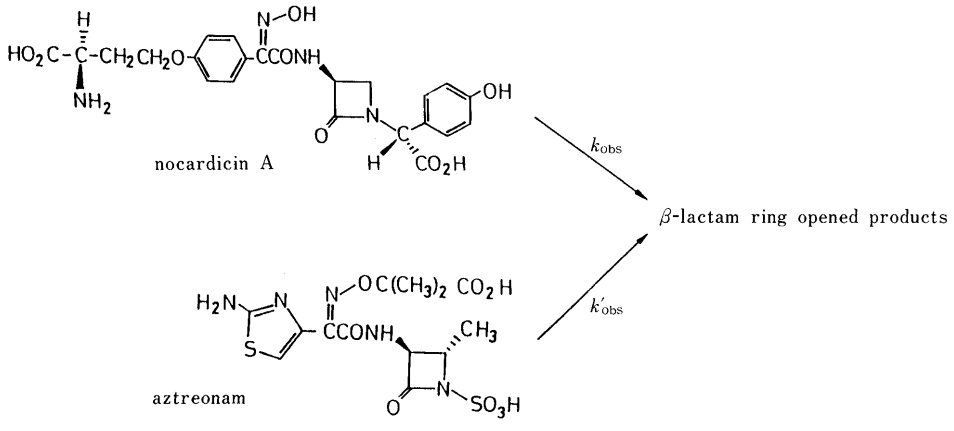

Chart 1

(6.20—7.95), borate (8.20—9.45) and carbonate (pH 10.00 and 10.50). A constant ionic strength of 0.5 mol·dm^{-3} was maintained for each buffer by adding an appropriate amount of sodium perchlorate monohydrate, and a 1×10^{-4}·mol·dm^{-3} concentration of ethylenediaminetetraacetic acid (EDTA) was used in order to avoid the catalytic effect induced by small amounts of metal ions possibly present as impurities. The buffers were prepared by dissolving the citric acid, acetic acid, sodium phosphate, boric acid and sodium bicarbonate together with sodium perchlorate monohydrate and EDTA in distilled water and adjusting the pH with concentrated sodium hydroxide. The solutions were freshly prepared and the pH's measured at 35 °C using a pH meter equipped with a combination electrode.

Analytical Procedures A reverse-phase high-performance liquid chromatographic (RP-HPLC) method was used to follow the kinetics of the degradation of aztreonam and nocardicin A. The HPLC system consisted of a Konik KNK-500-A liquid chromatograph, a Rheodyne 7125 loop injector (volume 20 μl), a Water 441 UV detector and a Varian 4290 computing integrator. The detector was set at 280 nm. The separation was carried out using a Spherisorb ODS-2 RP-18 column (10 μm; 25 cm × 0.4 cm i.d.) with the mobile phases: phosphate (0.1 mol·dm^{-3}, pH 7.0)–methanol (96:4) for nocardicin A and (90:10) for aztreonam. A pre-column (3 cm × 0.4 cm i.d.) packed with μ-Bondapak C18 (30 μm) was used to guard the main column. The flow-rate was 1.0 ml·min^{-1}. All chromatographic operations were carried out under ambient conditions.

Analyses of the experimental reproducibility and of the various time plots indicate that the relative uncertainty of the observed rate constant, k_{obs}, is 15% at the 95% confidence level.

Kinetic Procedure Weighed amounts of the antibiotic were dissolved in the buffer solutions preheated to a desired temperature to produce a final antibiotic concentration of 2×10^{-4} mol·dm^{-3}. Aliquots (10 ml) of the solutions were each sealed in a glass vial. All reactions were conducted in a constant-temperature water bath at 35.0±0.1 °C with the total buffer concentration greatly exceeding the reacting substrate concentration to maintain pseudo-first-order kinetics. Aliquots of the solution were withdrawn at appropriate time intervals and assayed immediately. The pH values of the reaction solutions were measured at the experimental temperature initially and at the end of each experiment on a pH meter. No significant changes in pH were observed.

Results and Discussion

Order of Reaction and Observed Rate Constants Aztreonam and nocardicin A have two possible sites at which degradation can occur: the amide side chain and the β-lactam ring.[19] Under conditions of pharmaceutical interest, the β-lactam ring opening predominates (Chart 1).

The degradation kinetics of aztreonam and nocardicin A were investigated at various pH values and buffer concentrations at a constant temperature (35°C) and ionic strength (μ=0.5 mol·dm^{-3}). In the presence of excess buffer at constant pH, the degradation of these antibiotics at concentrations of up to at least 2×10^{-3} mol·dm^{-3} followed pseudo-first-order kinetics and gave rate constants k_{obs} for their β-lactam ring cleavage estimated by means of the least-squares method from the slopes of time *versus* log (residual%) plots (Fig. 1).

Catalytic Effect of Buffer Systems The catalytic effect of the buffer systems used in the kinetic studies was determined by experiments at constant pH, temperature, ionic strength and antibiotic concentration, the only variation being in the buffer concentration at a given pH. This was repeated at several pH values within the effective range of the buffer employed.

Figure 2 shows the catalytic effect of citrate buffers between pH 3.50 and 4.75 for aztreonam and nocardicin A, which increased linearly with the buffer concentration. Therefore, the rates of degradation of these antibiotics in the citrate buffer at constant pH and 35 °C were shown to be affected by general acid catalysis. In addition, the

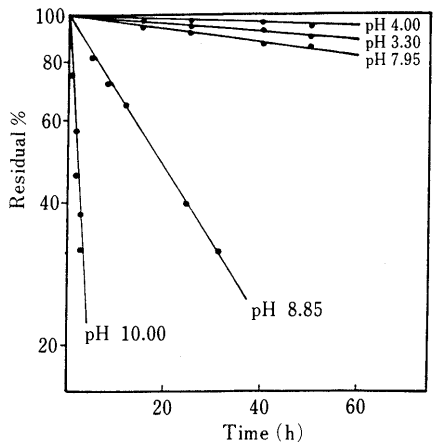

Fig. 1. Plots of the Observed Pseudo-First-Order Kinetic Degradation of Aztreonam in Aqueous Solution at Different pH's at 35 °C (μ=0.5 mol·dm^{-3})

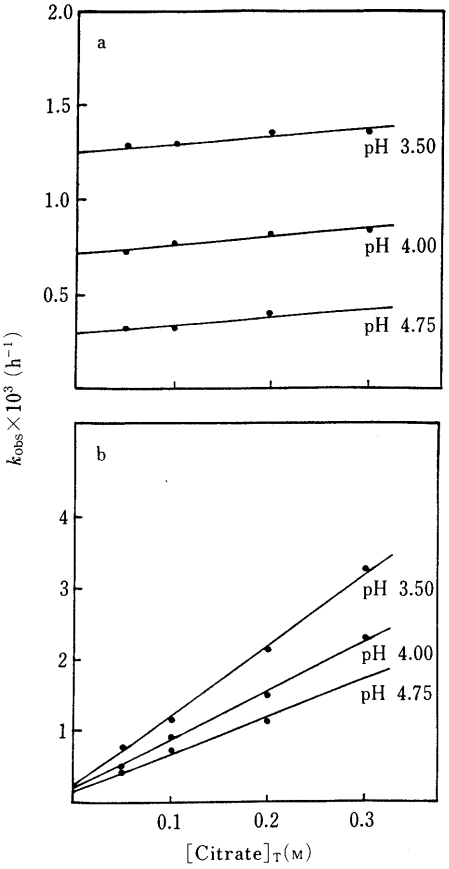

Fig. 2. Plots of the Pseudo-First-Order Rate Constants *versus* the Total Citrate Buffer Concentration at Several pH's at 35 °C and μ=0.5 mol·dm^{-3} for the Degradation of Aztreonam (a) and Nocardicin A (b)

degradation rate was greater under more acidic conditions for both antibiotics. These results indicate that the catalytic effect of the citrate species is in the order of $[H_3A]>[H_2A^-]>[HA^{2-}]>[A^{3-}]$, which are, respectively, the nondissociated, monoanionic, dianionic, and trianionic forms of citric acid.

Since the A^{3-} species exhibits the weakest catalytic effect and, in addition, since approximately only 3% of total citrate exists as this species, the observed rate constant may be expressed by the following equation:

$$k_{obs}=k_{pH}+k_{H_3A}[H_3A]+k_{H_2A^-}[H_2A^-]+k_{HA^{2-}}[HA^{2-}] \quad (1)$$

where k_{pH} is the rate constant at zero buffer concentration, and the other k's are the catalytic rate constants due to the citrate buffer species.

The total citrate concentration, c_T, is

$$c_T \simeq [H_3A]+[H_2A^-]+[HA^{2-}] \quad (2)$$

From the dissociation constants, $pK_1=2.85$ and $pK_2=4.40$

$$K_1=\frac{[H^+][H_2A^-]}{[H_3A]} \quad (3)$$

$$K_2=\frac{[H^+][HA^{2-}]}{[H_2A^-]} \quad (4)$$

and from Eqs. 1 and 2 the following overall rate expression, k_{obs}, was obtained.

$$k_{obs}=k_{pH}+c_T\frac{k_{H_3A}[H^+]^3+k_{H_2A^-}[H^+]^2K_1+k_{HA^{2-}}[H^+]K_1K_2}{[H^+]^3+[H^+]^2K_1+[H^+]K_1K_2} \quad (5)$$

A plot of k_{obs} *versus* the total citrate concentration will yield a straight line with an intercept of k_{pH} and a slope of

$$\text{slope}=\frac{k_{H_3A}[H^+]^3+k_{H_2A^-}[H^+]^2K_1+k_{HA^{2-}}[H^+]K_1K_2}{[H^+]^3+[H^+]^2K_1+[H^+]K_1K_2} \quad (6)$$

From the slopes we calculated the citrate buffer catalytic rate constants on the degradation of each antibiotic at 35 °C (Table I).

A similar treatment, described by us in a previous publication,[17] was used to study the catalytic effects of the other buffers used, including acetate buffers in the pH range of 4.00—5.65, the rate constant observed being expressed by the following equation.

$$k_{obs}=k_{pH}+c_T\frac{k_{AcH}[H^+]+k_{Ac^-}K_a}{[H^+]+K_a} \quad (7)$$

where k_{pH} is the rate constant at zero buffer concentration, c_T is the total acetate concentration, k_{AcH} and k_{Ac^-} are the catalytic rate constants due to the undissociated acetic acid and the acetate ions, respectively, and $pK_a=4.55$. Figure 3 shows the catalytic effect of acetate buffer on the degradation of aztreonam and nocardicin A. The plots allow us to obtain the k_{pH} values and the catalytic rate constants. These constants are shown in Table I.

We also studied the catalytic effects of the phosphate buffer with pH values of 6.20 to 7.95, borate buffer of pH 8.20 to 9.45 and carbonate buffer of pH 10.00 to 10.5. The observed rate constants may be expressed by the following equations[17]:

$$k_{obs}=k_{pH}+c_T\frac{k_{H_2PO_4^-}[H^+]+k_{HPO_4^{2-}}K_2}{[H^+]+K_2} \quad (8)$$

$$k_{obs}=k_{pH}+c_T\frac{k_{H_3BO_3}[H^+]+k_{H_2BO_3^-}K_a}{[H^+]+K_a} \quad (9)$$

$$k_{obs}=k_{pH}+c_T\frac{k_{HCO_3^-}[H^+]+k_{CO_3^{2-}}K_2}{[H^+]+K_2} \quad (10)$$

The plots of Eqs. 8, 9 and 10 allow us to obtain the k_{pH} values and the catalytic rate constants due to the phosphate, borate and carbonate buffer species for the degradation of aztreonam and nocardicin A. These constants are shown in Table I. Those of borate and carbonate buffers on the degradation of aztreonam are greater than on that of nocardicin A (Table I). In general the buffer systems employed in the kinetic studies showed a very weak catalytic effect in the degradation of aztreonam and nocardicin A. Analogous results have been obtained for carumonan,[20] a monobactam with a structure very similar to that of aztreonam.

pH-Rate Profile Figure 4 shows $\log k_{pH}$ *versus* pH for the degradation of aztreonam and nocardicin A at 35 °C

TABLE I. Catalytic Rate Constants of Citrate, Acetate, Phosphate, Borate and Carbonate Buffer Ions on Degradation of Aztreonam and Nocardicin A at 35°C and $\mu=0.5\,mol\cdot dm^{-3}$

Buffer solution	Catalytic rate constants ($mol^{-1}\cdot dm^3\cdot h^{-1}$)	Antibiotic	
		Aztreonam	Nocardicin A
Citrate	k_{H_3A}	6.18×10^{-4}	32.1×10^{-3}
	$k_{H_2A^-}$	3.98×10^{-4}	6.46×10^{-3}
	$k_{HA^{2-}}$	4.26×10^{-4}	4.05×10^{-3}
Acetate	k_{AcH}	3.97×10^{-4}	1.24×10^{-3}
	k_{Ac^-}	3.19×10^{-4}	0.214×10^{-3}
Phosphate	$k_{H_2PO_4^-}$	1.30×10^{-3}	1.59×10^{-4}
	$k_{HPO_4^{2-}}$	1.46×10^{-3}	5.87×10^{-4}
Borate	$k_{H_3BO_3}$	2.64×10^{-2}	3.67×10^{-4}
	$k_{H_2BO_3^-}$	4.47×10^{-2}	9.56×10^{-4}
Carbonate	$k_{HCO_3^-}$	0.754	8.56×10^{-4}
	$k_{CO_3^{2-}}$	0.671	10.7×10^{-4}

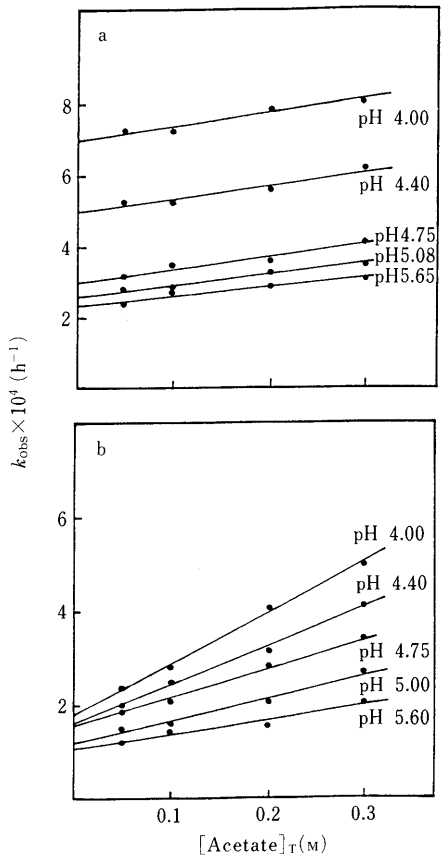

Fig. 3. Plots of the Pseudo-First-Order Rate Constants *versus* the Total Acetate Buffer Concentration at Several pH's at 35 °C and $\mu=0.5\,mol\cdot dm^{-3}$ for the Degradation of Aztreonam (a) and Nocardicin A (b)

TABLE II. Catalytic Rate Constants of the Water Species in β-Lactam Antibiotic Hydrolysis at 35 °C and $\mu = 0.5\,mol \cdot dm^{-3}$

β-Lactam antibiotic	k_H ($mol^{-1} \cdot dm^3 \cdot h^{-1}$)	k_{H_2O} ($h^{-1} \times 10^3$)	k_{OH} ($mol^{-1} \cdot dm^3 \cdot h^{-1} \times 10^{-2}$)	Ref. No.
Monocyclic				
Aztreonam	2.55±0.299	0.221±0.0189	21.17±1.19	—
Nocardicin A	0.293±0.082	0.119±0.0083	0.0781±0.0113	—
Penems				
SCH 29482	16.2±2.06	2.17±0.13	14.97±1.06	18
FCE 22101	4.24±0.66	2.25±0.15	72.80±8.95	18
Carbapenem				
Imipenem	1207±220	6.61±1.63	89.16±17.31	17
Penicillins				
Penicillin G	601	0.90	11.9	8
Carbenicillin	52.2	2.04	12.1	8
Cloxacillin	35.6	0.94	13.4	8
Propicillin	30.7	0.89	17.3	8
Cyclacillin	4.61	2.49	11.0	8
Ampicillin	1.82	0.75	25.7	8
Cephalosporins				
Cephalotin	0.172	10.9	10.6	8
Cephaloridine	0.134	4.40	38.8	8
Cephaloglycin	0.148	5.00	13.1	8

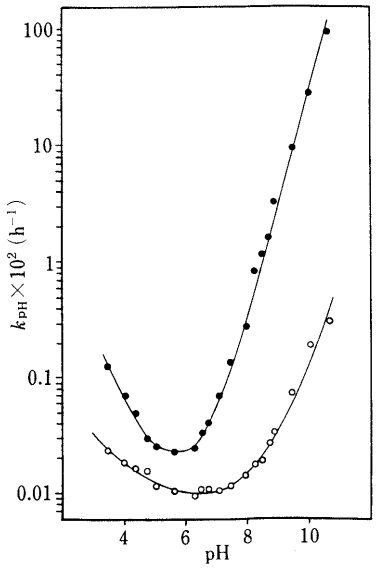

Fig. 4. $\log k_{pH}$–pH Profiles for the Degradation of Aztreonam (●) and Nocardicin A (○) in Aqueous Solution at 35 °C and $\mu = 0.5\,mol \cdot dm^{-3}$

and $\mu = 0.5\,mol \cdot dm^{-3}$. These pH-rate profiles exhibit a U-shape with three important pH regions: one where a hydrogen-ion-catalyzed reaction took place, a pH-independent region where the predominating reaction is the attack by water molecules, and a region where the reaction was hydroxide-ion catalyzed.

The apparent first-order rate constant at a given pH obeys the general rate law:

$$k_{pH} = k_{H_2O} + k_H[H^+] + k_{OH}[OH^-] \quad (11)$$

where k_H and k_{OH} represent second-order rate constants of proton- and hydroxide ion-catalyzed degradation, respectively; k_{H_2O} is the rate constant of spontaneous or water-catalyzed degradation; and $[H^+]$ and $[OH^-]$ are the hydrogen ion concentration and the hydroxide ion concentration, respectively. The values of these constants, computed by means of a non-linear least-squares method, are given in Table II. The value for the autoprotolysis constant of water, K_W, at 35 °C is 2.09×10^{-14}.[21]

The theoretical profiles generated using these constants adequately describe the behavior of each antibiotic and can be interpreted kinetically as follows: The hydrogen-ion-catalyzed reaction is important at pH 5.5 for both antibiotics, and the hydroxide-ion-catalyzed reaction occurs at pH 6.5 for aztreonam and at pH 7.5 for nocardicin A. The horizontal portion between pH 5.5 and 6.5, for aztreonam, and between pH 6.0 and 7.5 for nocardicin A, is pH-independent.

The theoretical pH minimum for the degradation of each antibiotic, obtained by taking the derivative of Eq. 11 and equating it to zero was pH 5.38 and pH 6.13 for aztreonam and nocardicin A, respectively.

If we compare the catalytic effects of water species in the degradation of the antibiotics studied in acidic and basic solutions (Table II), we find that aztreonam is more reactive with hydrogen ions than nocardicin A (*ca.* 10 fold), and much more reactive with hydroxide ions (*ca.* 300 fold).

Effect of Temperature The temperature dependence of the hydrolytic reactions of aztreonam and nocardicin A in solution was determined at three different pH values in the acidic, neutral and alkaline regions and at a constant ionic strength of $0.5\,mol \cdot dm^{-3}$. These determinations were made at pH 4.23 (acetate buffer), pH 6.59 (phosphate buffer) and pH 8.60 (borate buffer). The observed rate constants (k_{obs}) at 35, 45 and 60 °C, activation energies (E_a) and frequency factor ($\log A$) are given in Table III, and the corresponding Arrhenius plots are shown in Fig. 5. It should be noted that the values of activation energies at pH 8.60 include the heat of the ionization of water, which at 35 °C equals 13.05 kcal/mol.[21] As the Table shows, for nocardicin A the values of E_a and the frequency factor (especially the latter) are significantly higher than for aztreonam. It is also to be observed that the E_a value for aztreonam decomposition is noticeably lower in a base medium than in a neutral or acid medium. Moreover, we observed that these two parameters are considerably higher for these monocyclic antibiotics than those obtained for such bicyclic ones as penems,[18] imipenem,[17] clavulanic acid[12] or ampicillin,[6] while they

Table III. Rate Constants and Arrhenius Activation Parameters for Degradation of Aztreonam and Nocardicin A at $\mu = 0.5\,\mathrm{mol \cdot dm^{-3}}$

Antibiotic	pH	k_{obs} ($h^{-1} \times 10^3$) 60 °C	k_{obs} ($h^{-1} \times 10^3$) 45 °C	k_{obs} ($h^{-1} \times 10^3$) 35 °C	E_a (kcal/mol)	log A (h^{-1})
Nocardicin A	4.23	10.6	1.48	0.336	27.50	16.02
	6.59	6.25	0.893	0.210	27.04	15.49
	8.60	7.48	1.19	0.261	26.69	15.35
Aztreonam	4.23	11.6	2.14	0.513	24.79	14.30
	6.59	14.8	2.87	0.717	24.06	13.93
	8.60	317	68.8	19.0	22.37	14.15

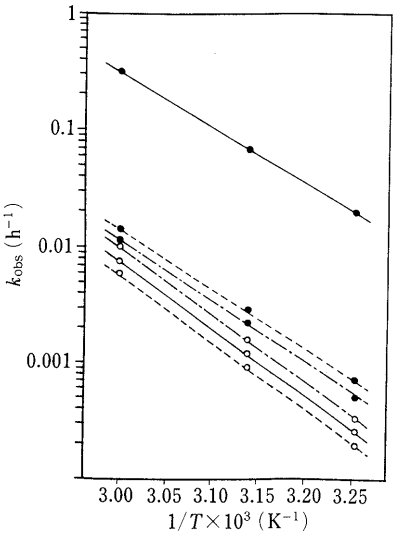

Fig. 5. Arrhenius Plots of the Degradation of Aztreonam (●) and Nocardicin A (○) at pH 4.23 (—·— Acetate Buffer), pH 6.59 (– – – Phosphate Buffer) and pH 8.60 (—— Borate Buffer)

are similar to those obtained for cephalosporins—such as cefotaxime,[13] cefazolin and cephalexin[9]—and for monobactams like carumonan.[20]

Stability Comparison of Aztreonam and Nocardicin A and Other β-Lactam Antibiotics If we compare the catalytic effects of buffer solutions on the degradation of the antibiotics studied (Table I) with those found in the literature for other β-lactam antibiotics, we find that the catalytic constants of the antibiotics studied (monocyclic β-lactams) are much lower than those of other β-lactam antibiotics (bicyclic β-lactams).

The reactivities of the antibiotics studied, monocyclic β-lactam antibiotics, and other β-lactam compounds with hydrogen ions, water, and hydroxide ions are listed in Table II. A comparison of the catalytic constants in the hydrolysis of the monocyclic β-lactam antibiotics with those obtained from other β-lactams shows that aztreonam is more reactive with hydroxide ions (k_{OH}) than the penicillins and cephalosporins, and less reactive than penems and carbapenems, while the reactivity of nocardicin A is much lower than that of these β-lactam antibiotics. The reactivities of the antibiotics studied with water (k_{H_2O}) are lower than those of the rest of the β-lactamic antibiotics. Aztreonam and nocardicin A are less reactive with hydrogen ions (k_H) than are the penicillins (with the exception of ampicillin), penems and carbapenems, but they exhibit greater reactivities than cephalosporins. In an acid medium, the reactivity of carumonan and aztreonam is

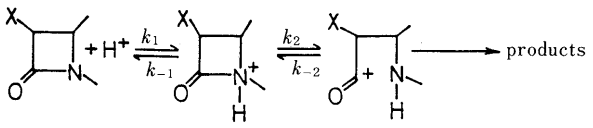

Chart 2

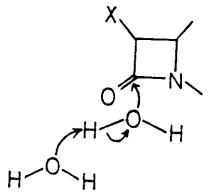

Chart 3

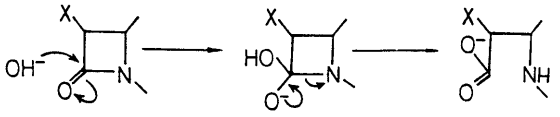

Chart 4

similar, while in the neutral or basic medium, the degradation constant k_{pH} of carumonam is four times higher than that of aztreonam.[20]

The hydrolysis mechanisms of the β-lactam ring on the monocyclic antibiotics studied must be analogous to those proposed for bicyclic systems. Chart 2 shows the unimolecular mechanism proposed for acid-catalysed hydrolysis of the β-lactam ring with protonation occurring on nitrogen. The profile for the hydrolysis of the β-lactams is unbroken, indicating that there is no change in the mechanism and that Chart 2 is operative throughout the acid region studied.[22]

The most likely mechanism of spontaneous hydrolysis or water-catalysed degradation involves general base catalysis by water or nucleophilic attack by water on the β-lactam as indicated in Chart 3.[22]

The mechanism proposed for alkaline hydrolysis is represented in Chart 4, the rate-limitor being the formation of the tetrahedral intermediate.[19]

The high reactivity of the β-lactam system antibiotics has been explained by a reduction in amide resonance coupled with a fusion of rings giving rise to pyramidal geometry of the β-lactam nitrogen[23-25] and by a strain of the nuclei as shown by the X-ray crystallographic data (despite the obvious criticism that solid state conformations may not be relevant to solution conformations) (Table IV). The table includes values for the sum of the bond angles about the β-lactam ring nitrogen, $\sum N$, and the distance of the nitrogen from the plane of the three attached carbon atoms, D.[26,27] The reactivity of the bicyclic systems is also influenced by the substitutes possessed by the β-lactam skeleton.

In monocyclic β-lactam antibiotics, reactivity must be related to the tension of the β-lactam ring and the presence of a substituent. Substituent attracting electron joined to the β-lactam ring such as the sulphonic group ($-SO_3H$) in aztreonam, cause a significant increase in reactivity to nucleophilic attack by hydroxide ions, as may be observed by comparing the catalytic constants k_{OH} for aztreonam and nocardicin A.

All this allows us to state that the degradation of β-lactam

TABLE IV. ΣN and D Values Determined from X-Ray Crystallographic Data

Compound	ΣN (°)	D (Å)	Ref. No.
Carbapenem			
N-Acetylthienamycin methyl ester	325.9	0.49	25
Penicillins			
Ampicillin	339	0.38	25
Penicillin V	337	0.40	25
6-Aminopenicillanic acid	343	0.32	25
Cephalosporins			
Cephalosporin C	345	0.32	26
Cephaloridine	350.7	0.24	25
Δ^2-Cephem	359.3	0.06	26
Monocyclic β-lactams	*ca.* 360	0	
Amides	*ca.* 360	0	

antibiotics in aqueous solution is complex and that, consequently, comparisons are difficult to make. We can, however, conclude that when the results obtained for the monocyclic antibiotics studies are compared with those of bicyclics, the reactivity of the bicyclic systems must be determined mainly by the greater tensions of the β-lactam ring when this is fused to another five-member ring, and by the inhibition of amide resonance, while in the monocyclic systems, it is the substitutes of the β-lactam ring that have the most influence on reactivity.

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