Supplementary Material Available: Listings of observed and calculated structure factor amplitudes for **1** and **2**; Table A, anisotropic thermal parameters for **1**; Table B, hydrogen atom parameters for **1**; Table C, additional bond lengths and angles for **1**; Table D, anisotropic thermal parameters for **2**; Table E, hydrogen atom parameters for **2**; and Table F, additional bond lengths and angles for **2** (27 pages). Ordering information is given on any current masthead page.

Mechanism of β-Lactam Ring Opening in Cephalosporins

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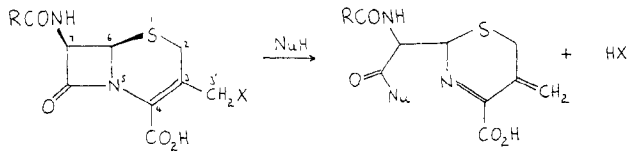
Abstract: There is a nonlinear dependence of the rate of aminolysis of some cephalosporins upon hydroxide ion concentration which is interpreted in terms of formation of a tetrahedral addition intermediate. At high concentrations of hydroxide ion the rate-limiting step is formation of the tetrahedral intermediate but at low concentrations it is the diffusion-controlled encounter of the intermediate and hydroxide ion. Rate constants for the formation and breakdown of the intermediate are reported. The equilibrium constants for formation of the intermediates are between 10^{-8} and 10^{-10} L mol^{-1}. Contrary to other suggestions nucleophilic attack on the β-lactam carbonyl carbon is not concerted with the expulsion of a leaving group at C3′. The rate of breakdown of the tetrahedral intermediates to products in the uncatalyzed aminolysis reaction is ca. 10^6 s^{-1} and not dependent upon whether or not a leaving group is expelled at C3′. Expulsion of a leaving group at C3′ does not significantly enhance the rate of β-lactam carbonyl carbon–nitrogen bond fission.

Cephalosporins **1** belong to the β-lactam class of antibiotics.[1] They differ from the penicillins by characteristically having a potential leaving group, eg., acetate or pyridine, at C-3′ which is expelled during the reaction of nucleophiles with the β-lactam (eq 1).[2] It has been suggested, on the basis of MO calculations,



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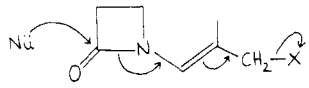
that nucleophilic attack on the β-lactam carbonyl is concerted with expulsion of the leaving group **2**.[3] Furthermore it has been



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proposed that the biological activity of the cephalosporins is related to the ease of expulsion of the leaving group at C-3′.[4]

A reaction is concerted when it proceeds in one step because all "intermediate" species that would be formed are too unstable to exist. Substitution reactions at the carbonyl group proceed by mechanisms that are often enforced by the lifetime of the addition intermediates that may be formed during the reaction.[5,6] Although it has been suggested[7] that certain conformations of tetrahedral intermediates have lifetimes which are short compared with the times for intramolecular rotations (ca. 10^{-12} s),[8] a number of stable derivatives are known.[9] The reaction of amines with cephalosporins **1** (eq 1, Nu = RNH_2) requires proton removal from the attacking amine. Because of the relative time required for proton transfer and that for bond fission between heavy atoms this type of reaction may provide an experimental method for detecting and measuring the lifetime of intermediates.[10,11] The aminolysis of cephalosporins is also of interest because the primary allergic response is probably due to the formation of a protein conjugate resulting from the reaction of the ε-amino groups of lysine residues in proteins with the β-lactam.[12]

We present here evidence that the mechanism of the reaction of amines with cephalosporins is not concerted but a stepwise process involving the formation of a tetrahedral intermediate which then breaks down to products with little or no assistance from a potential leaving group at C3′.

Experimental Section

Materials. The cephalosporins were the generous gift of Glaxo Group Research (UK). The amines were purified by crystallization of the hydrochlorides or by distillation. Freshly boiled deionized water was used throughout.

Kinetics. The ionic strength was maintained at 1.0 M by the addition of potassium chloride unless otherwise stated. The reactions were initiated by the addition of 25 μL of aqueous 10^{-2} M cephalosporin to 2.5 cm^3 of the aqueous amine solution, preincubated at 30.0 ± 0.05 °C, with thorough mixing. The disappearance of the β-lactam was followed spectrophotometrically on a Gilford 240 spectrophotometer at 260 nm. The output from the spectrophotometer was fed into a Solarton data

(1) Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144.
(2) Hamilton-Miller, J. M. T.; Newton, G. G. F.; Abraham, E. P. *Biochem. J.* **1970**, *116*, 371. Hamilton-Miller, J. M. T.; Richards, E.; Abraham, E. P. *Ibid.* **1970**, *116*, 385. Bundgaard, H. *Arch. Pharm. Chemi., Sci. Ed.* **1975**, *3*, 94.
(3) Boyd, D. B.; Hermann, R. B.; Presti, D. E.; Marsh, M. M. *J. Med. Chem.* **1975**, *18*, 408. Boyd, D. B.; Lunn, W. H. W. *Ibid.* **1979**, *22*, 778.
(4) Boyd, D. B.; Herron, D. K.; Lunn, W. H. W.; Spitzer, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 1812.
(5) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345.
(6) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161.
(7) Deslongchamps, P. *Tetrahedraon* **1975**, *31*, 2463.
(8) Goldammer, E.; Zeidler, M. D. *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 4.
(9) Rogers, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1973**, *95*, 4452. Hine, J.; Ricard, D.; Perz, R.; *J. Org. Chem.* **1973**, *38*, 110. Capon, B.; Ghosh, A. K. *J. Am. Chem. Soc.* **1981**, *103*, 1765. Guthrie, J. P. *Can. J. Chem.* **1976**, *56*, 202.
(10) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425.
(11) Page, M. I.; Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 8828.
(12) Assem, E. S. K.; Vickers, M. R. *Immunology* **1974**, *27*, 255. Mine, Y.; Mishida, M.; Goto, S.; Kuwahara, S. *J. Antibiot.* **1970**, 23, 195. Shibata, K.; Atsumi, Y.; Horiuchi, Y.; Mashimo, K., *Nature (London)* **1966**, *212*, 419.

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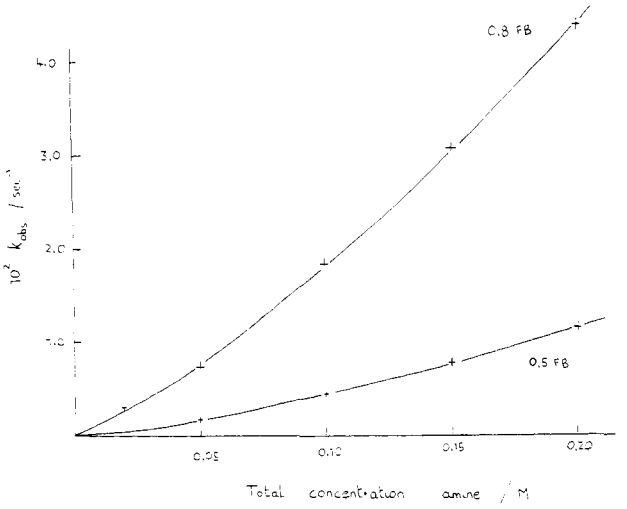


Figure 1. Observed pseudo-first-order rate constants for the reaction of propylamine with cephalothin (**4**) in water at the indicated fraction of free base (FB) in the amine buffer at 30 °C, ionic strength = 1.0 M. The lines are calculated from the rate constants in Table I.

logger equipped with a Facit tape punch, thus enabling voltages proportional to the absorbance to be punched at constant time intervals. Rate constants were calculated from the results on an IBM 1130 or ICL 2960 computer by using a generalized least-squares method which treated the absorbances at time zero and infinity and the first-order rate constant as disposable parameters.

Some kinetic measurements were collected on a Gilford 2600 spectrophotometer which fed the data directly into an Apple II Europlus microcomputer and the calculated parameters were then displayed on a video display unit while an Epson printer gave a permanent record of calculated and observed absorbances and the calculated rate constants.

Fast reactions were followed with a Nortech SF-3A stopped-flow spectrophotometer. Optical density changes were determined at 262.5 nm. The signal from the photomultiplier was fed into a Datalab DL901 transient recorder which was automatically triggered and simultaneously triggered the display on a Gould Advance OS-Z50B oscilloscope. The change in absorbance with time was output from the transient recorder to a chart recorder. The slopes and intercepts of linear relationships were determined using a linear least-squares method. The pH of all solutions was checked before and after each kinetic experiment and if it had changed by more than 0.03 the experiment was rejected.

Results

(a) Reactions in Buffered Solutions. The aminolysis of cephalosporins at 30 °C in aqueous solutions of the amine follows the rate of law of eq 2, where k_{obsd} is the observed pseudo-first-order

$$\text{rate}/[\text{ceph}] = k_{obsd} = k_0 + k_u[RNH_2] + k_b[RNH_2]^2 + k_{OH}[RNH_2][OH^-] \quad (2)$$

rate constant for the disappearance of cephalosporin, k_0 is that for the hydroxide ion catalyzed hydrolysis reaction, k_u is the second-order rate constant for uncatalyzed aminolysis, and k_b is the third-order rate constant for the general base catalyzed and k_{OH} that for the hydroxide ion catalyzed reaction. The evaluation of these rate constants is illustrated by reference to the aminolysis of cephalothin **4**. The observed pseudo-first-order rate constants for propylamine (Figure 1) exhibit a sharp upward curvature in plots against amine concentration which is indicative of a term which is second order in amine; ie., one molecule of amine acts as the nucleophile and the other as a catalyst. The individual rate constants were determined by using the slopes and intercepts of plots of the observed first-order rate constants, corrected for hydrolysis, divided by the total amine buffer concentration against the total amine buffer concentration, as shown in Figure 2. The steep slopes in this figure illustrate the large contribution of the catalyzed reaction to the observed rate, even at low amine concentration. The slope of these plots, k_{cat}, is equal to $k_b\alpha^2$ (eq 3),

$$\frac{k_{obsd} - k_0}{[RNH_2]_{tot}} = k_u\alpha + k_b\alpha^2[RNH_2]_{tot} + k_{OH}\alpha[OH^-] \quad (3)$$

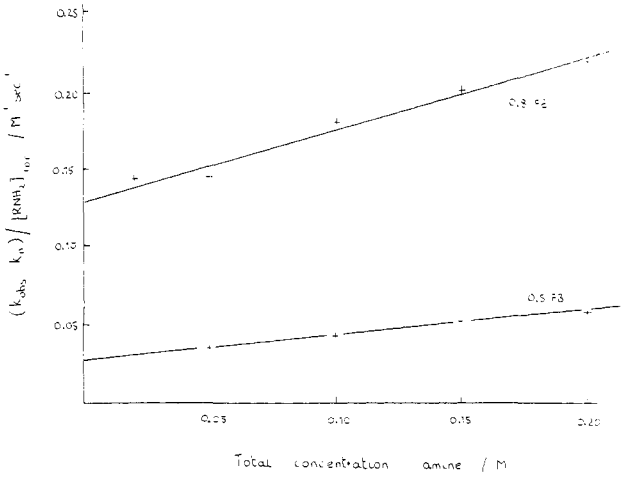


Figure 2. Observed second-order rate constants for the reaction of propylamine with cephalothin (**4**) in water at the indicated fraction of free base as a function of total amine concentration at 30 °C, ionic strength = 1.0 M.

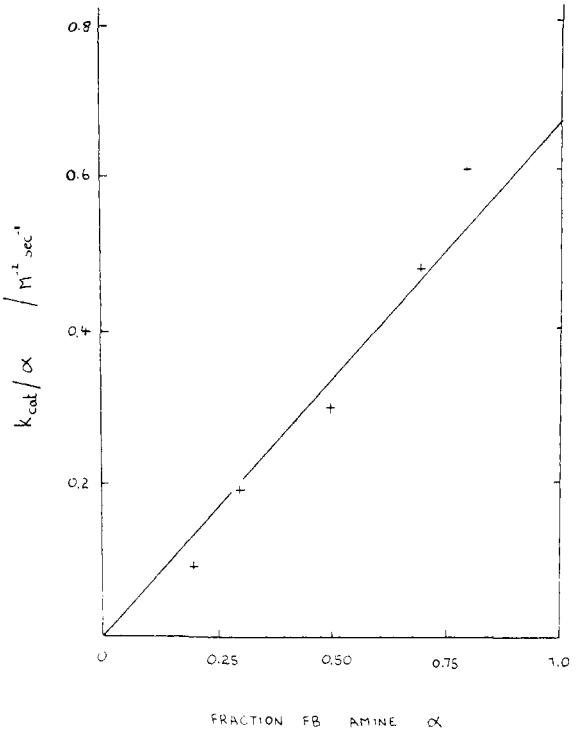


Figure 3. The dependence of the third-order rate constants, k_{cat}, divided by the fraction of free base, α, for the reaction of propylamine with cephalothin (**4**) upon the fraction of free base. The left and the right intercepts give the catalytic constants for the acidic and the basic species of the buffer.

where α is the fraction of the free base of the amine, and a plot of k_{cat}/α against α gives a straight line as illustrated in Figure 3. The intercept of these plots at $\alpha = 0$ would give the rate constant for any term in the rate law proportional to $[RNH_2][RNH_3^+]$ but it was indistinguishable from zero. The general acid catalyzed aminolysis of the cephalosporins therefore makes a negligible contribution to the observed rate. The intercept of plots of k_{cat}/α against α at $\alpha = 1.0$ gives the rate constant for the term in the rate law proportional to $[RNH_2]^2$, ie., k_b. The dominant form of buffer catalysis in the aminolysis is, therefore, general base catalysis.

The intercept of plots of $(k_{obsd} - k_0)/[RNH_2]_{tot}$ against $[RNH_2]_{tot}$ (Figure 2) represent the terms which are first order in amine concentration k_u and k_{OH} and are designated k_{int}. Plots of k_{int} against α are nonlinear and show upward curvature as is illustrated in Figure 4. This indicates that the hydroxide ion

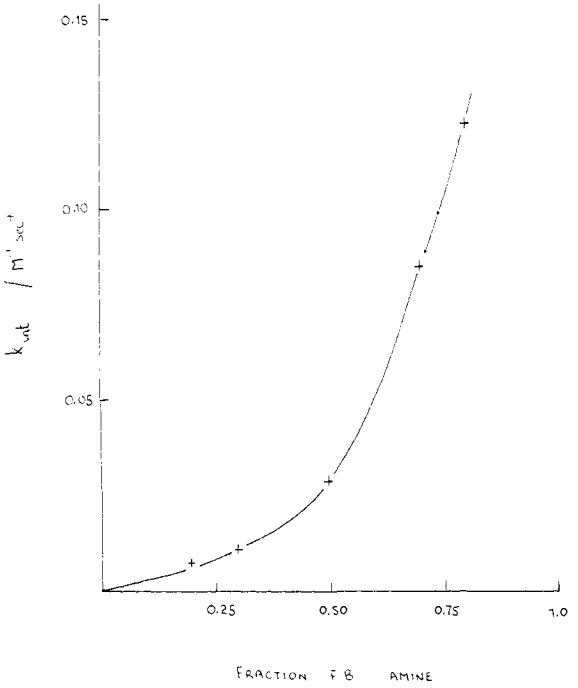
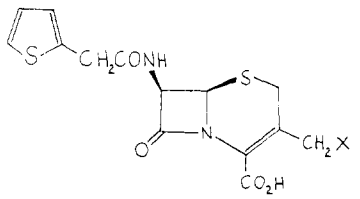
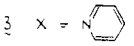


Figure 4. The dependence of the second-order rate constants, k_{int}, for the reaction of propylamine with cephalothin (**4**) upon the fraction of free base of the buffer.

catalyzed reaction makes a significant contribution to the observed rate even in buffer solutions. A plot of k_{int}/α against the concentration of hydroxide ion is linear for the reaction of propylamine with cephalothin and the intercept gives the rate constant k_u and the slope gives the rate constant k_{OH}, as shown in Figure 5.

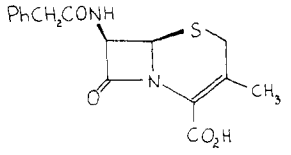
However, a similar graph for the aminolysis of cephaloridine **3** is nonlinear (Figure 6) and is taken to indicate a change in the kinetic dependence upon hydroxide ion and a change in the rate limiting step of the reaction. This nonlinear dependence upon



3 X =  **4** X = OAc

hydroxide ion was also observed for cephalothin (**4**) and other cephalosporins when the aminolysis was carried out in aqueous solutions of sodium hydroxide.

(b) Reactions in Hydroxide Ion Solutions. The observed pseudo-first-order rate constants for the reaction of the cephalosporin **5** with propylamine at different concentrations of hy-



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droxide, ion are illustrated in Figure 7 as a function of the concentration of amine. There is apparently a first-order dependence of the observed rate constants upon the concentration of amine although a small correction due to the contribution of the k_b term (eq 2), general base catalysis, to the rate of aminolysis was made for subsequent calculations on the basis of the known values of

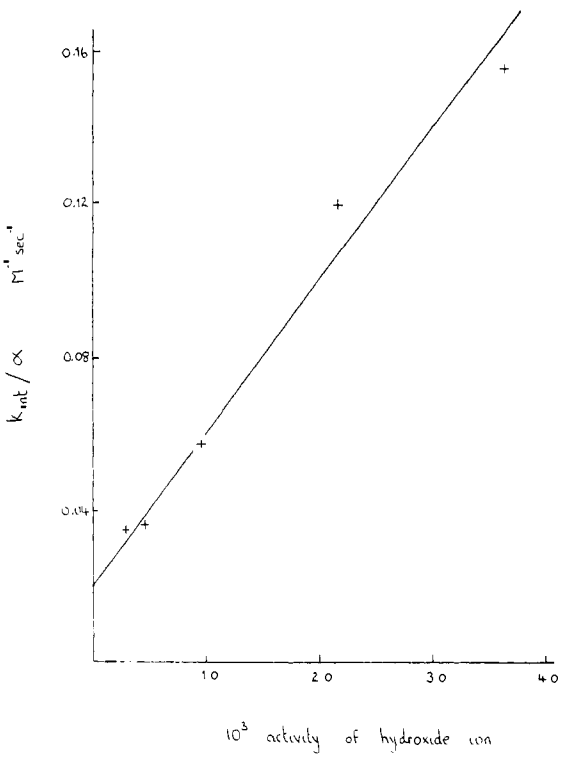


Figure 5. The dependence of the second-order rate constants, k_{int}/α, for the reaction of propylamine with cephalothin (**4**) upon the activity of hydroxide ion (antilog (pH − pK_w)). The slope gives k_3 and the intercept k_1.

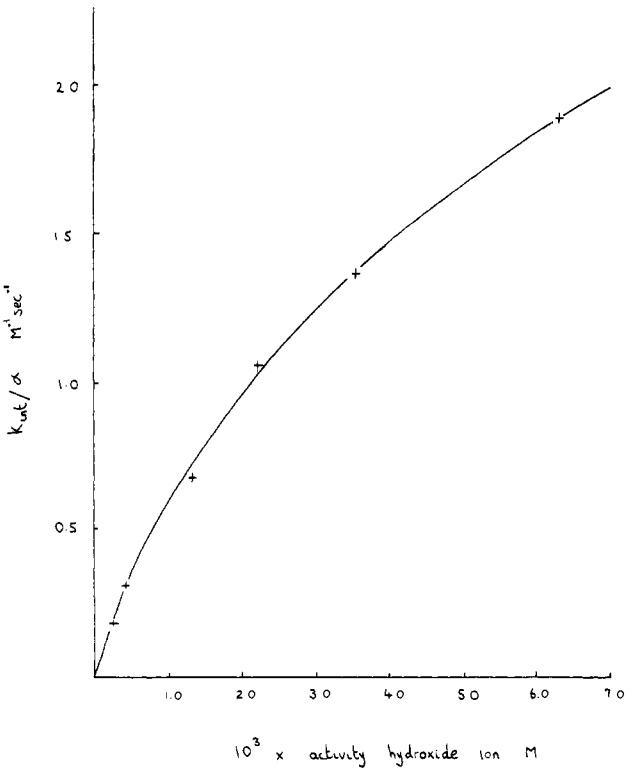


Figure 6. The apparent second-order rate constants, k_{int}/α, for the reaction of propylamine with cephaloridine (**3**) as a function of the activity of hydroxide ion. The curved line is calculated from the constants given in the text.

these rate constants and the concentrations of amine used. There is a nonlinear dependence of the apparent second-order rate constants $(k_{obsd} - k_0)/[RNH_2]$ obtained from the slopes of lines in plots such as those shown in Figure 7 upon the concentration of hydroxide ion (Figure 8). At low concentrations of hydroxide

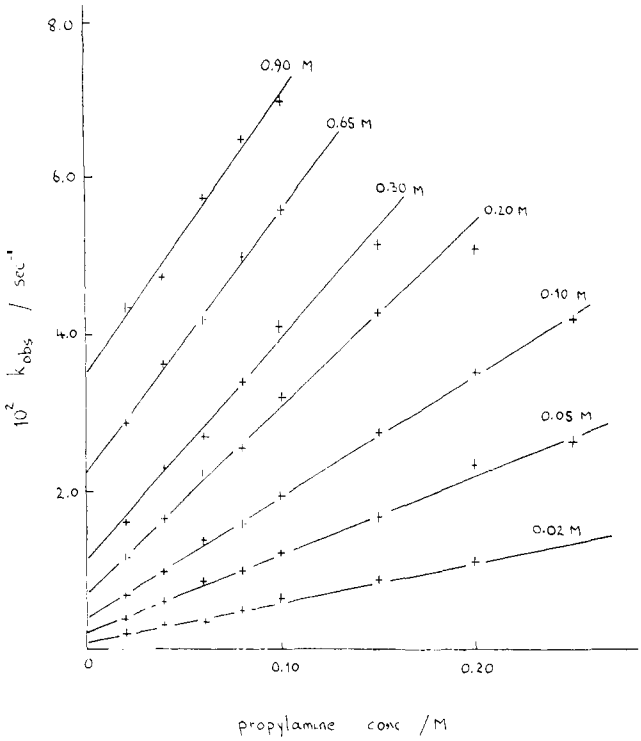


Figure 7. The observed pseudo-first-order rate constants for the reaction of propylamine with 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid (**5**) in water as a function of the concentration of amine at the concentration of hydroxide ion stated at 30.0 °C and ionic strength = 1.0 M.

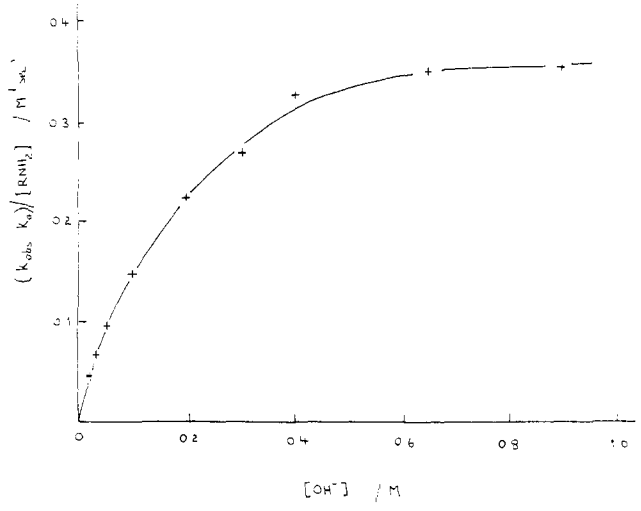


Figure 8. The apparent second-order rate constant $(k_{obsd} - k_0)/[RNH_2]$ as a function of hydroxide ion concentration for the reaction of propylamine with the cephalosporin **5**. The curved line is calculated from the constants given in the text.

ion the rate is first order in hydroxide ion and the initial slopes give values of k_{OH} which agree well with those determined at lower pH in buffer solutions. At high concentrations of hydroxide ion the rate becomes independent of the concentration of hydroxide ion.

A plot of the reciprocal of the apparent second-order rate constant, $(k_{obsd} - k_0)/[RNH_2]$ against the reciprocal of the hydroxide ion concentration gives a straight line (Figure 9). The slopes and intercepts of these graphs may be used to calculate the equilibrium constants for formation of the tetrahedral intermediate and the rate constants for its formation and breakdown.

Discussion

The rate law for the aminolysis of cephalosporins is given in eq 2, which is similar to that observed for the reaction with

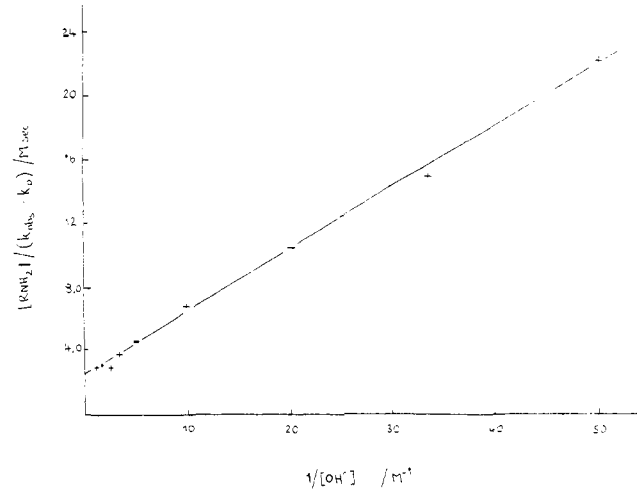


Figure 9. The reciprocal of the apparent second-order rate constant for the aminolysis of **5** with propylamine as a function of the reciprocal of the hydroxide ion concentration.

Table I. Summary of the Rate Constants for the Reaction of Cephalosporins with Propylamine[a]

cepholosporin	k_u, M^{-1} s^{-1}	k_b, M^{-2} s^{-1}	k_{OH}, M^{-2} s^{-1}
cephalothin (**4**)	2.19×10^{-2}	6.67×10^{-1}	38.8
cephaloridine (**3**)	6.14×10^{-2}	7.68	469
3-methyl-7-(β-phenylacetamido)-ceph-3-em-4-carboxylic acid (**5**)	1.13×10^{-4}	6.25×10^{-2}	1.35

[a] At 30 °C in water and ionic strength 1.0 M (KCl) as given in eq 2.

penicillins.[13] A minor pathway at all pH's is the uncatalyzed aminolysis represented by k_u. The dominant term in the rate law in buffered solutions is the general base catalyzed reaction represented by k_b for which a second molecule of amine acts as the catalyst. In solutions of sodium hydroxide the major term is k_{OH} where hydroxide ion is the catalyst. Four cephalosporins were studied: cephaloridine, **3**, with a pyridine leaving group at C3′; cephalothin, **4**, with an acetate leaving group at C3′; 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid (**5**), which has no leaving group at C3′; and 7-aminocephalosporanic acid, with an amino group at C7 and an acetate leaving group at C3′. The rate constants for the aminolysis of these cephalosporins with propylamine are summarized in Table I. Compared with benzyl penicillin,[13] all three rate constants for the aminolysis of cephaloridine, **3**, are about 7-fold greater whereas those for cephalothin, **4**, are similar. These ratios are similar to those observed for the hydroxide ion catalyzed hydrolysis of the β-lactam derivatives.[14]

The C3 substituent in **5** is CH_3 and the mechanism equivalent to **2** would involve the expulsion of hydride ion which does not occur. However, substituents at C3 which might be and those which cannot be expelled are correlated by the *same* linear free energy relationship for alkaline hydrolysis.[14] Electron-withdrawing substituents at C3 in cephalosporins increase the rate of hydrolysis, but the rate diffuerences are those expected purely by an inductive effect with a Brønsted β_{lg} of −0.06 and a ρ_I of 1.50.[14]

Similar conclusions may be drawn from the aminolysis data. For example, a plot of the logarithm of the rate constants for general base catalyzed aminolysis, k_b, against the inductive σ_I values for the C3′ substituent[15] generates a ρ_I of 2.1 with no deviations shown by leaving groups at C3′ compared with non-leaving groups. Electron-withdrawing substituents at C3′ exert

(13) Morris, J. J.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1980**, 212.
(14) Proctor, P.; Gensmantel, N. P.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1185.
(15) Wells, P. *Chem. Rev.* **1963**, *63*, 171.

Table II. Summary of the Rate and Equilibrium Constants for the Aminolysis of Some Cephalosporins at 30 °C[a]

cephalosporin	amine	k_1, M^{-1} s^{-1}	k_{-1}, s^{-1}	K, M^{-1}	k_2K, M^{-2} s^{-1}	k_3, s^{-1}
3-methyl-ceph-3-em (**5**)	propylamine	4.17×10^{-1}	1.65×10^{9}	2.53×10^{-10}	2.53	4.47×10^{5}
cephalothin (**4**)	propylamine	1.52	2.60×10^{8}	5.85×10^{-9}	58.5	3.74×10^{6}
cephaloridine (**3**)	propylamine	4.45	7.54×10^{7}	5.92×10^{-8}	592	1.04×10^{6}
7-aminocephalosporanic acid	propylamine	8.40×10^{-1}	8.57×10^{8}	9.80×10^{-10}	9.80	
3-methyl-7-(β-phenylacetamido)-ceph-3-em-4-carboxylic acid (**5**)	2-methoxy-ethylamine	1.76×10^{-1}	3.67×10^{9}	4.80×10^{-11}	4.80×10^{-1}	2.29×10^{5}

[a] I = 1.0 M; as given in Scheme I.

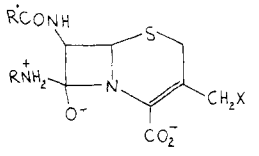
their effect by stabilizing the tetrahedral intermediate formed during aminolysis, the presence of which is indicated by the nonlinear dependence of the apparent second-order rate constant $(k_{obsd} - k_0)/[RNH_2]$ upon the hydroxide ion concentration (Figure 8).

The change in the kinetic dependence on hydroxide ion in the aminolysis of cephalosporins is indicative of a change in the rate-limiting step of the reaction which, in turn, requires that there be at least two sequential steps in the reaction. The second of these steps is rate limiting at low concentrations of hydroxide ion and the transition state for this step contains hydroxide ion, or its kinetic equivalent. The first step is rate limiting at high concentrations of hydroxide ion but the transition state for this step does not contain hydroxide ion. The existence of two sequential steps demands that there be an intermediate in the reaction,[16] which is probably the tetrahedral intermediate, **6**.

A mechanism compatible with the observations involves formation of a tetrahedral intermediate **6** followed by diffusion of hydroxide ion into the same solvent cage as the intermediate. This step is followed by rapid proton transfer from the tetrahedral intermediate to hydroxide ion pursued by rapid collapse to products (Scheme I). The observed first-order rate constant for the aminolysis of cephalosporins in solutions of sodium hydroxide, under conditions where k_u and k_b are unimportant, will then be given by eq 4.

$$k_{obsd} - k_0 = k_1k_2[RNH_2][OH^-]/(k_{-1} + k_2[OH^-]) \quad (4)$$

Similar schemes have been proposed previously for the aminolysis of penicillins[17] and acetylimidazole.[18] At low concentrations of hydroxide ion the rate of collapse of the tetrahedral intermediate to reactants must be faster than its reaction with hydroxide ion, $k_{-1} >> k_2[OH^-]$, and the observed rate constant is dependent upon the concentration of hydroxide ion with k_2, the diffusion-controlled step, being rate limiting. The calculated pK_a value for the protonated amine of the tetrahedral intermediate **6** is well below that for water. Proton transfer from the tetrahedral



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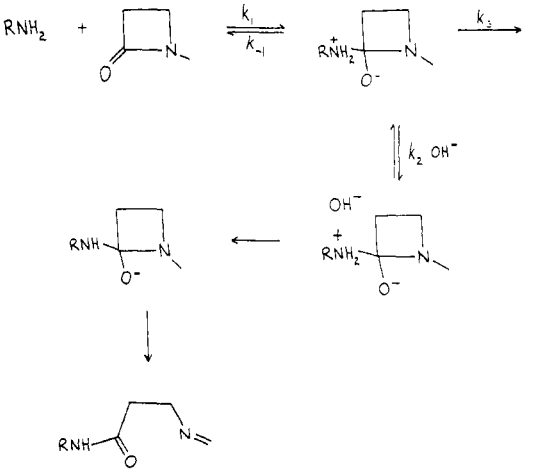
intermediate to hydroxide ion is therefore in the thermodynamically favorable direction, and it is to be expected that the rate-limiting step for this process is the diffusion-controlled encounter of the proton donor and acceptor.[19]

At high concentrations of hydroxide ion the tetrahedral intermediate and hydroxide ion diffuse together faster than the intermediate collapses back to reactants, $k_2[OH^-] >> k_{-1}$. Under these conditions the observed rate constant is independent of

Scheme I



hydroxide ion concentration and k_1, the rate of formation of the tetrahedral intermediate, is rate limiting.

We therefore consider that there is good experimental evidence to support the prosposal that the addition of amine nucleophiles to the β-lactam carbonyl of cephalosporins is usually reversible. It is easier to expel the attacking amine from the tetrahedral intermediate than it is to break the β-lactam carbon–nitrogen bond. Reaction occurs when the intermediate encounters a base to remove a proton from the attacking amine. The presence of the intermediate shows that nucleophilic attack on the carbonyl carbon and expulsion of the leaving group at C3′ *cannot* be concerted. Furthermore, accepting the mechanism proposed in Scheme I, it may be shown that expulsion of a leaving group at C3′ is not concerted with fission of the β-lactam carbon–nitrogen bond.

When eq 4 is used the individual rate constants of the proposed mechanism may be elucidated. From eq 5, a plot of the reciprocal of the apparent second-order rate constant, $(k_{obsd} - k_0)/[RNH_2]$, against the reciprocal of the hydroxide ion concentration gives

$$[RNH_2]/(k_{obsd} - k_0) = 1/k_1 + k_{-1}/k_1k_2[OH^-] \quad (5)$$

a straight line (Figure 8) of intercept $1/k_1$ and of slope k_{-1}/k_1k_2. If the reasonable assumption[14] is made that the diffusion-controlled step, k_2, has a value of ca. 10^{10} L mol^{-1} s^{-1}, then values of k_{-1} and the equilibrium constants, $K = k_1/k_{-1}$, for the formation of the tetrahedral intermediates may be obtained. Values of these constants for the reaction of propylamine with the cephalosporins, **3**, **4**, and **5**, and 7-aminocephalosporanic acid, and for 2-methoxyethylamine with **5** are given in Table II. The rate of expulsion of the attacking amine from the tetrahedral intermediate to regenerate the reactants, the values of k_{-1}, are very rapid, ca. 10^8–10^9 s^{-1}. Although these rate constants are very large they are of the order of magnitude that have been postulated for the breakdown of tetrahedral intermediates formed in acyl-transfer reactions.[9,17,18]

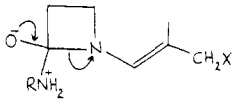
The tetrahedral intermediates **6** formed from amine attack upon the cephalosporins are relatively stable, the equilibrium constants for their formation being 10^{-8}–10^{-10} L mol^{-1} (Table II). Tetrahedral intermediates formed from β-lactams are expected to be more stable than the analogous derivatives formed from acyclic amides because of the favorable conversion from a three-coordinate carbon in a four-membered ring to a four-coordinate one.[20] For

(16) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1968; p 467.
(17) Gensmantel, N. P.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2* **1979**, 137.
(18) Page, M. I.; Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 8828.
(19) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1.

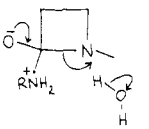
example, cyclobutanone is reduced by borohydride 5×10^2 faster than is acetone.[21]

The aminolysis of cephalosporins is catalyzed by bases other than hydroxide ion, but the rates of these reactions doe not show a nonlinear dependence upon the concentration of the base up to 0.5 M base. By analogy with the penicillin system[13,14,22] it is thought that the mechanism of the general base-catalyzed aminolysis is similar to that for the hydroxide ion catalyzed reaction (Scheme I) except that the general base B replaces hydroxide ion as the proton acceptor. Because no nonlinearity is observed in the general base-catalyzed reaction, k_2[B], the rate of reaction of the tetrahedral intermediate with the base B, must be less than k_{-1}. This is not surprising in view of the smaller rates of proton transfer from acids to bases compared with those to hydroxide ion.[19] Also because of the lower basicity of bases other than hydroxide ion, proton transfer from the tetrahedral intermediate to the base may be thermodynamically unfavorable and the rate-limiting step may not be diffusion-controlled encounter of the base and the intermediate.[18]

The uncatalyzed pathway, k_u, could represent either a purely uncatalyzed reaction of amine and cephalosporin or solvent catalysis with water acting as a general base, removing a proton from the attacking amine, or as a general acid, donating a proton to the β-lactam nitrogen.[13] The k_u pathway cannot represent rate-limiting formation of the tetrahedral intermediate because the observed rate constants are much smaller than the microscopic rate constants calculated for this step at high hydroxide ion concentration (Table II). Furthermore it is known that water does not act as a proton acceptor in the uncatalyzed aminolysis of penicillin[13] so it is reasonable to assume that this is also the case for cephalosporins. This is substantiated by a Brønsted β_{nuc} of 1.05 for the uncatalyzed aminolysis of cephaloridine,[23] which indicates that the reaction behaves as if a unit positive charge is developed on the amine nitrogen in the transition state. The uncatalyzed pathway therefore represents a rate limiting breakdown of the tetrahedral intermediate **7** or general acid catalyzed breakdown of the same intermediate by water, **8**.

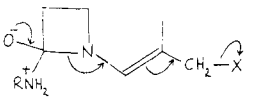


7



8

The observed rate constant, k_u, for the uncatalyzed aminolysis must be given by $k_3K = k_3k_1/k_{-1}$ (Scheme I). As the values of K, the equilibrium constant for formation of the tetrahedral intermediate, are known (Table II), the values of k_3, the rate constant for the breakdown of the intermediate to products, can be calculated. These are given in Table II. The interesting observation is thus made that whether or not a leaving group is expelled at C3′ the rate constants for breakdown of the tetrahedral intermediate are of similar magnitude. This therefore argues against expulsion of the leaving group at C3′ being concerted with fission of the carbon–nitrogen bond of the β-lactam **9**. At least



9

this is true in the sense that there can be no significant coupling of the processes so that there is a significant lowering of the activation energy for carbon–nitrogen bond fission in the β-lactam *because* a group at C3′ is expelled. It could be the case that the intermediate formed by β-lactam carbonyl carbon–nitrogen bond fission is so unstable and that its lifetime is so short as to preclude its existence and therefore the breakdown of the tetrahedral intermediate is enforced to be concerted.[5] However, the important conclusions is that *expulsion of a leaving group at C3′ does not significantly enhance the rate of β-lactam carbon–nitrogen fission.*

The rate of protonation of the β-lactam nitrogen in the tetrahedral intermediate **6** by water may be estimated from its estimated[13] pK_a of 5 to be ca. 10^3 s^{-1}. As this is less than the rate constant for the uncatalyzed breakdown of the intermediate (10^5–10^6 s^{-1}) the latter cannot proceed by stepwise proton transfer from water to the β-lactam nitrogen. Breakdown could therefore occur either by a concerted mechanism—proton transfer from water occurring synchronously with carbon–nitrogen bond fission, **8**—or by uncatalyzed expulsion of the nitrogen anion, **7**. The rate of expulsion of the imidazole anion from the tetrahedral intermediate formed in the aminolysis of acetylimidazole is $<10^6$ s^{-1}.[18] If the strain energy of the β-lactam ring of *ca.* 120 kJ mol^{-1} is relieved upon ring opening, then expulsion of the nitrogen as the anion could be treated as a leaving group of pK_a ca. 10 rather than the normal value of 30 for ordinary amines. It is conceivable therefore that carbon–nitrogen bond fission occurs without protonation of the β-lactam nitrogen, **7**. Such a step could, of course, be reversible and in the aminolysis of acetylimidazole it has been suggested that this is the case with rate-limiting diffusion apart of the ion pair.[24]

Finally, other possible interpretations of the nonlinear dependence of the rate upon hydroxide ion (Figures 7 and 8) deserve consideration but may be excluded. Salt effects or self-association of hydroxide ion could be causes of the observed nonlinearity.[25] The best argument against these interpretations is that in buffer solutions linear plots are observed for the aminolysis of cephalothin but, under identical conditions, nonlinear plots are observed for the reaction with cephaloridine. Furthermore, in solutions of sodium hydroxide linear plots are observed for the aminolysis of 6-aminopenicillanic acid with 2-cyanoethylamine under conditions identical with those in which benzylpenicillin yields data showing curvature.[17] These conditions are identical with those used for the other amines which gave a nonlinear dependence of the rates upon hydroxide ion concentration for both cephalosporins and penicillins.

Conclusion

The mechanism of the aminolysis of cephalosporins is a stepwise process. A tetrahedral intermediate is formed by the reversible addition of the amine to the β-lactam carbonyl carbon. Expulsion of the attacking amine from the tetrahedral intermediate occurs faster than fission of the β-lactam C–N bond. The reaction proceeds by trapping the intermediate with base. Expulsion of a leaving group at C–3′ in cephalosporins is not concerted with nucleophilic attack of the amine on the β-lactam carbonyl carbon and makes little difference to the rate of β-lactam C–N bond fission.

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Registry No. 3, 50-59-9; **4**, 153-61-7; **5**, 27255-72-7; propylamine, 107-10-8.

Supplementary Material Available: Tables of rate constants for aminolysis in the following systems: cephalothin, sodium cephalothin, 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid, acid, cephaloridine, and 7-aminocephalosporanic acid with propylamine; cephalothin, 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid, acid, cephaloridine, and 7-aminocephalosporanic acid with propylamine in aqueous sodium hydroxide; 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid with methoxyethylamine; 3-methyl-7-(β-phenylacetamido)ceph-3-em-4-carboxylic acid with methoxyethylamine in aqueous sodium hydroxide (18 pages). Ordering information is given on any current masthead page.

(20) Page, M. I. *Chem. Soc. Rev.* **1973**, 295.
(21) Brown, H. C.; Itchikawa, K. *Tetrahedron* **1957**, *1*, 221.
(22) Morris, J. J.; Page, M. I. *J. Chem. Soc., Perkin Trans.* **1980**, 220.
(23) Proctor, P., unpublished observations.
(24) Page, M. I.; Jencks, W. P. *J. Am. Chem. Soc.* **1972**, *94*, 3263.
(25) Hand, E. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 6221.