Bordwell and Boyle[41] suggested that a localized carbanion is the initial intermediate resulting from a base-catalyzed proton removal from nitroalkanes. Therefore, electron delocalization to form a more stable intermediate would lag behind proton transfer. The PKIE results for the methoxide-catalyzed exchange reactions of 9-PhFl, 9-MeFl, and fluorene[11a] are consistent with an internal-return mechanism.[42] These results also suggest that electron delocalization lags behind proton transfer and that Scheme IV can best describe a possible mechanism.

Concluding Remarks

A major goal for the study of chemical reaction mechanisms is to understand not only the detailed pathway of a reaction but also the timing of various steps along that pathway. Proton-transfer reactions are of fundamental importance to many organic and biochemical reactions, yet many details regarding that process are still unknown. The role of solvent reorganization associated with proton-transfer reactions is a complex subject and is currently under investigation by others.[43] Careful studies of product distributions and PKIE can give meaningful insight regarding solvent reorganization during the course of nucleophilic reactions of alkenes[25] and we continue studies in the area.

The consequences of a hydrogen-bonded carbanion, H, as the initial intermediate generated by a proton transfer from carbon to oxygen has important mechanistic implications. When a leaving group can depart from H, the experimental results for that elimination reaction will be similar to those ascribed to an E2 mechanism. The hydrogen bond can preserve the stereochemistry of the carbanion, and elimination occurs with complete streospecificity.[44] Since k_2 is rate limiting for the exchange reaction, the leaving group departs faster than exchange can occur with solvent molecules. Therefore elimination is not accompanied by exchange even though reaction proceeds via a carbanion intermediate.[15] The measurement of sizable isotope effects coupled with observed leaving group element effects can be associated with either an E1cB or E2 mechanism.[13,17b]

Since polar C–F bonds are capable of stabilizing localized carbanions, there are definite advantages to using highly fluorinated compounds in studies of carbanions. The reactivity of fluorinated alkenes toward nucleophiles allows for the generation of carbanions in protic solvents. Therefore we have generated carbanions that do not require π delocalization for stability and can compare their behavior to that of highly delocalized anions.[25] There is the added advantage to generating carbanions in situ by the reaction of alkoxide with either alkyltrimethylsilanes or fluoroalkenes that reactions occur at lower temperatures than those required for a base-promoted proton abstraction, and the problem of internal return is eliminated. One should not study the chemistry of carbocations using only fluorocarbons, and for similar reasons, one should not predict the behavior of localized carbanions from model compounds that generate delocalized carbanions.

Some idea discussed in the Account are not new but are often overlooked. The ideas proposed by Richie,[37] Bordwell,[41] Kresge,[29] and Streitwieser[31] have greatly influence our thoughts. More recent studies by Jencks and co-workers[45] and Bernasconi et al.[46] are also relevant.

It is a pleasure to acknowledge the hard work and contributions of all co-workers whose work has been mentioned in the references and especially to Gerrit Lodder and Bill Tumas who made specific suggestions regarding this Account. Particularly valuable comments and suggestions have also been provided by Drs. R. D. Chambers,[47] W. P. Jencks, A. J. Kresge, and A. Streitwieser. Financial support by the Research Corporation and the Petroleum Research Fund, administered by the American Chemical Society, is gratefully acknowledged.

(41) Bordwell, F. G.; Boyle, W. J., Jr. *J. Am. Chem. Soc.* **1975**, *97*, 3447–3452.
(42) Discussed in ref 13, pp 6105–6106.
(43) Bernasconi is active in this area and has recently published an excellent summary (Bernasconi, C. F. *Pure Appl. Chem.* **1982**, *54*, 2335–2348) with pertinent references.
(44) Root, K. S.; Touchette, N. A.; Koch, J. G.; Koch, H. F. Abstracts of Papers, Euchem Conference on Mechanisms of Elimination Reactions, Assisi, Italy, Sept 12–16, 1977.
(45) Alunni, S.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 2052–2060. Keeffe, J. R.; Jencks, W. P. *Ibid.* **1981**, *103*, 2457–2459. Gandler, J. R.; Jencks, W. P. *Ibid.* **1982**, *104*, 1937–1951.
(46) Bernasconi, C. F.; Carre, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 1698–2707. Bernasconi, C. F.; Kanavarioti, A. *J. Org. Chem.* **1979**, *44*, 4829–4832.
(47) We gratefully acknowledge a NATO grant that has made these discussions possible.

The Mechanisms of Reactions of β-Lactam Antibiotics

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It is now more than half a century since Fleming observed the antibacterial action of penicillin and 40 years since the work of Florey and Chain led to the widespread use of penicillin and its congeners in medicine. Today there is a large family of antibiotics whose single common structural feature is the possession of a β-lactam ring. As a class they consist of penicillin **1**, the cephalosporins **2**, and the "nonclassical" β-lactam antibiotics such as clavulanic acid **3**, thienamycin **4**, nocardicin A **5**, and the monobactams **6**. All the novel β-lactam antibiotics identified over the last 10 years are

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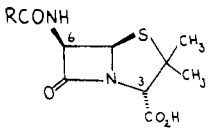
products of the actinomycetes although recently bacteria have been shown to produce both β-lactam- and β-lactone-containing molecules.[1]



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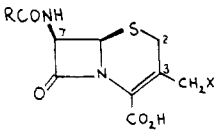

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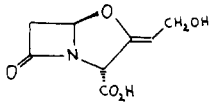

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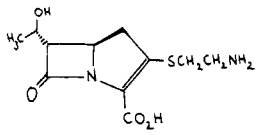

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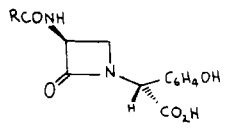

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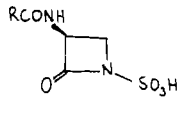

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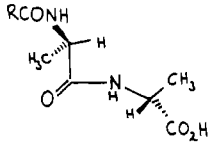
The penicillins and cephalosporins show bacteriocidal effects because they disrupt bacterial cell wall synthesis by inhibiting the enzymes that catalyze the cross-linking reactions of D-alanyl peptides on peptidoglycan strands of the growing cell wall[2] (eq 1).

$$\text{X-D-Ala-D-Ala-CO}_2\text{H} + \text{Y-NH}_2 \rightarrow \text{X-D-Ala-CONHY} + \text{D-Ala-CO}_2\text{H} \quad (1)$$

It has been proposed that a β-lactam antibiotic inhibits transpeptidase activity because it is a structural analogue of the D-alanyl-D-alanine portion of the nascent peptidoglycan 7.[2-8] However, there are multiple



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receptor sites within the plasma membrane of bacteria as several proteins can covalently bind penicillin and then be separated and identified.[7,8] Some penicillin-binding proteins (PBPs) show enzymic behavior and exhibit carboxypeptidase, transpeptidase, endopeptidase, and even transglycosylase activity.[8] Unfortunately the exact role and necessity for the PBPs during cell growth is not yet clear and hence the molecular mechanism for the killing action of penicillin remains speculative.

The mechanism of the enzyme- and nonenzyme-catalyzed reactions of the β-lactam antibiotics are of obvious interest. It is somewhat surprising that more effort seems to have been expended in generating apparently random synthetic or semisynthetic analogues of the naturally occurring β-lactams than in understanding the mechanism of their reactions. Considering the relative rarity of the β-lactam ring in nature, it is probably not surprising that the biological activity of these compounds should be attributed to their chemical reactivity. Shortly after the introduction of penicillin to the clinicians' armory, it was suggested that the antibiotic activity was due to the inherent strain of four-membered rings[9] or to reduced amide resonance because the butterfly shape of the molecule prevents the normal planar arrangement assumed necessary for delocalization of the nitrogen lone pair.[10] Because these ideas are intuitively appealing, they have remained unchallenged for the last few decades although the evidence to support them is ambiguous.

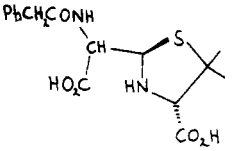
In penicillins 1 the nitrogen atom is 0.4 Å out of the plane defined by its substituents,[11] whereas in the cephalosporins 2 it deviates by 0.2–0.3 Å.[12] Amides are less reactive toward nucleophiles than are ketones because of the resonance stabilization energy resulting from delocalization of the lone pair of electrons on nitrogen over the carbonyl group. It is estimated[13] that this resonance stabilizes amides by ca. 18 kcal mol^{-1}. If delocalization is inhibited, a reaction that involves the loss of resonance in the transition state could proceed up to 10^{13}-fold faster than the analogous stabilized system. The strain energy of a four-membered ring[14] is 26–29 kcal mol^{-1} and therefore a reaction involving ring opening could occur up to 10^{20}-fold faster than an analogous acyclic system. The total possible rate enhancement of a resonance-inhibited β-lactam is therefore 10^{33}, so if these effects are even slightly significant they should be easily observable.

The β-lactam of penicillins is generally more reactive toward nucleophiles than are normal amides, but the exact rate enhancement is not easy to estimate. Substituent effects on both the acyl and amine portions of the β-lactam should be considered. For example, carbon-β-lactam nitrogen bond fission in penicillins involves the expulsion of a much more weakly basic amine than that normally found in amides. A simple way to determine the reactivity of the β-lactam antibiotics is to examine their rates of hydrolysis.

(1) J. C. Wells, J. C. Hunter, G. L. Astle, J. C. Sherwood, C. M. Ricca, W. H. Trejo, D. P. Bonner, and R. B. Sykes, *J. Antibiot.*, **35**, 814 (1982).
(2) P. M. Blumberg and J. L. Strominger, *Bacteriol. Rev.*, **38**, 291 (1974).
(3) D. J. Tripper and J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **54**, 1133 (1965).
(4) B. Lee, *J. Mol. Biol.*, **61**, 463 (1971).
(5) D. B. Boyd, *J. Med. Chem.*, **22**, 533 (1979).
(6) D. J. Waxman, R. R. Yocum, and J. L. Strominger, *Philos. Trans. R. Soc. London, Ser. B*, **289**, 257 (1980).
(7) H. Amanuma and J. L. Strominger in "β-Lactam Antibiotics", S. Mitsuhashi, Ed., Japan Sci. Soc. Press, Tokyo, 1981, p 185; J. L. Strominger, in "β-Lactam Antibiotics", M. R. J. Salton and G. D. Shockman, Ed., Academic Press, New York, 1981, p 123; M. Matsuhashi, J. Nakagawa, F. Ishino, S. Nakajima-Iijima, S. Tomioka, M. Doi, and S. Tamaki in "β-Lactam Antibiotics", S. Mitsuhashi, Ed., Japan Sci. Soc. Press, Tokyo, 1981, p 203.
(8) B. G. Spratt, *Philos. Trans. R. Soc. London, Ser. B*, **289**, 273 (1980).
(9) J. L. Strominger, *Antibiotics*, **1**, 706 (1967).
(10) R. B. Woodward in "The Chemistry of Penicillin", H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, 1949, p 443.
(11) R. M. Sweet and L. F. Dahl, *J. Am. Chem. Soc.*, **92**, 5489 (1970).
(12) R. M. Sweet in "Cephalosporins and Penicillins, Chemistry and Biology", E. H. Flynn, Ed., Academic Press, New York, 1972, p 280.
(13) A. R. Fersht and Y. Requena, *J. Am. Chem. Soc.*, **93**, 3499, 3502, (1971).
(14) M. I. Page, *Chem. Soc. Rev.*, 295 (1973).

Alkaline Hydrolysis of the β-Lactams

The alkaline hydrolysis of benzylpenicillin ring opens the β-lactams to give benzylpenicilloic acid (**8**). The



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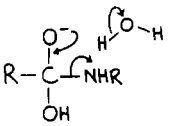
pK_a of the protonated amine in the product thiazolidine derivative **8** is 5.2, and because of this weakly basic nitrogen, the amine's ability as a leaving group is expected to be improved. However, the effect of substituents on the hydrolysis of amides and β-lactams is different. The logarithms of the second-order rate constants for the hydroxide-catalyzed hydrolysis of N-substituted acyclic amides[15] and anilides[16] plotted against the pK_a of the leaving group amine give a straight line, the slope of which is the Brønsted β_{lg} value of −0.07. This small dependence of the rate upon the basicity of the amine indicates that in the transition state the nitrogen has more positive charge than it does in a tetrahedral intermediate with a neutral nitrogen but less than in one with a positively charged nitrogen, which is compatible with water acting as a general-acid catalyst in the rate-limiting breakdown of the intermediate **9**.[17] By contrast the rates of alkaline hydrolysis



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of β-lactams show a larger dependency upon substituents and increase with decreasing basicity of the amine. These show a Brønsted β_{lg} value of −0.44, indicative of rate-limiting formation of the tetrahedral intermediate.[18] β-Lactams of the more basic amines show a positive deviation from this line and exhibit a smaller dependence upon basicity of the leaving group, which may signify a change in rate-limiting step to breakdown of the intermediate. The monocyclic β-lactam antibiotics, nocardicin **5** and the monobactams **6** hydrolyze with the reactivity predicted from the basicity of the amine leaving group.

A consequence of the different dependency upon leaving group basicity is that the rate enhancement of β-lactams compared with acyclic amides depends on the basicity of the leaving group amine. β-Lactams of weakly basic amines are ca. 500-fold more reactive than an acyclic amide of the same amine. However, β-lactams of basic amines are only slightly more reactive than an analogous acyclic amide. Crystallographic[19] and spectroscopic evidence[20] show that monocyclic N-substituted β-lactams are planar. The rate enhancement of 30–500-fold shown by β-lactams of amines of $pK_a \leq 6$ may be adequately rationalized by the change in coordination number and hybridization of the carbonyl carbon as the tetrahedral intermediate is formed.[14,21] The magnitude is similar to the 500-fold faster rate of reduction of cyclobutanone by borohydride compared with that of acetone.[22] There is no evidence of the significant strain energy of the four-membered ring being released in the transition state for the hydrolysis of β-lactams.

What is the effect of making the β-lactam part of the bicyclic system? Most β-lactam antibiotics are derivatives of 1-azabicyclo[3.2.0]heptan-2-ones and 1-azabicyclo[4.2.0]octan-2-ones, and it is conceivable that geometrical constraints brought about by the bicyclic systems could increase their reactivity compared with monocyclic β-lactams. The bicyclic antibiotics are, in fact, ca. 100-fold more reactive than β-lactams of amines of similar basicity. The Brønsted β_{lg} value for the hydrolysis of the bicyclic systems is −0.55, indicative of rate-limiting formation of the tetrahedral intermediate, similar to that for monocyclic β-lactams.[15] Although the rate enhancement is significant, it is hardly of the magnitude expected from the release of strain in opening a four-membered ring or from a system in which amide resonance is significantly inhibited. Even the penem system **4** shows little sign of significant strain.[15] Monocyclic β-lactams of weakly basic amines may be as reactive as the 1-azabicyclo[3.2.0]heptan-2-ones of the more basic amines.[15,21] This has now been highlighted by the discovery of the monobactams **6**. The sulfamic acid leaving group of **6** has a pK_a of ca. 2 ($H_2N^+RSO_3^-$) and has a similar reactivity (within a factor of 2) to benzylpenicillin toward alkaline hydrolysis.[24] There is no evidence of the 3-carboxylate group facilitating the hydrolysis of penicillins—the methyl ester derivatives are 10–20-fold more reactive.[15,25]

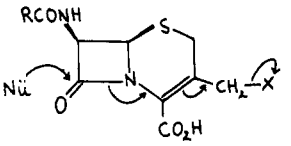
The degree of amide resonance that is lost by distortion of the normal planar geometry is not immediately apparent. The angular dependence of resonance interactions, particularly steric inhibition of resonance, has been of long-term interest. Resonance stabilization may occur even when the interacting p orbitals are orthogonal. For example, nearly 50% of the possible charge donation from oxygen to the ring carbons in phenol is calculated to occur in the perpendicular conformation.[26] However, the energy barrier to rotation about the C–N bond in amides of 20–30 kcal mol^{-1} [27]

(15) P. Proctor, N. P. Gensmantel, and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1185 (1982).
(16) M. L. Bender and R. J. Thomas, *J. Am. Chem. Soc.*, **83**, 4183 (1961); R. L. Schowen, H. Jayaraman, and L. Kirschner, *ibid.*, **88**, 3373 (1966); V. Gani and P. Viout, *Tetrahedron Lett.*, 3663 (1974).
(17) J. J. Morris and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 679, 685 (1980).
(18) G. M. Blackburn and J. D. Plackett, *J. Chem. Soc., Perkin Trans. 2*, 1366 (1972).
(19) J. L. Luche, H. B. Kagan, R. Parthasarathy, G. Tsovcaris, C. De Rango, and C. Zeliver, *Tetrahedron*, **24**, 1275 (1968).
(20) M. S. Manhas, S. Jeng, and A. K. Bose, *Tetrahedron*, **24**, 1237 (1968).
(21) N. P. Gensmantel, D. McLellan, J. J. Morris, M. I, Page, P. Proctor, and G. Randahawa in "Recent Advances in the Chemistry of β-Lactam Antibiotics"; G. Gregory, Ed., Royal Society of Chemistry, London, 1981, p 227.
(22) H. C. Brown and K. Itchikawa, *Tetrahedron*, **1**, 221 (1957).
(23) R. B. Sykes, D. P. Bonner, K. Bush, and N. H. Geogopapakou, *Antimicrob. Agents Chemother.*, **21**, 85 (1982).
(24) A. Rous and M. I. Page, unpublished observations.
(25) N. P. Gensmantel, E. W. Gowling, and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 335 (1978).
(26) W. J. Hehre, L. Radom, and J. A. Pople, *J. Am. Chem. Soc.*, **94**, 1496 (1972).
(27) H. Kessler, *Angew. Chem., Int. Ed. Engl.*, **9**, 219 (1970); W. E. Stewart and T. H. Siddall III, *Chem. Rev.*, **70**, 517 (1970).

indicates a significant angular dependence for resonance. The lone pair on a α-nitrogen adjacent to a carbocation can significantly stabilize the electron-deficient center even when the orbital geometry is apparently unfavorable.[28] Although a carbocation, because of its lower stability, probably makes a greater and less stereospecific demand upon adjacent nitrogen lone pairs than a carbonyl group, a small distortion of amide geometry probably does not cause a significant loss of amide resonance.

Electron-withdrawing substituents at C-6 increase the rate of alkaline hydrolysis of penicillins **1** by a purely inductive effect[15] although the biological activity is greatly dependent upon the nature of this substituent.

The major structural differences between cephalosporins **2** and penicillins **1** are that the five-membered thiazolidine ring of penicillins is replaced by a six-membered dihydrothiazine ring in cephalosporins and that the deviation of the β-lactam nitrogen from the plane defined by its three substituents is smaller in the cephalosporins. Many of the cephalosporins have a leaving group, e.g., acetate, pyridine, at C-3 so that expulsion of these groups occurs during the hydrolysis of the β-lactam.[29] There have been many suggestions,[30] supported by theoretical calculations,[31] that nucleophilic attack upon the β-lactam carbonyl carbon is concerted with expulsion of these leaving groups **10**. Furthermore it has been proposed that biological activity is related to the leaving group ability of the C-3 substituent.[32]



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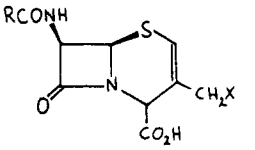
The second-order rate constants for the hydroxide ion catalyzed hydrolysis for cephalosporins are similar to those for penicillins.[15,33] This fact alone is enough to indicate that the nonplanarity of the β-lactam cannot significantly affect the chemical reactivity of these systems. In penicillins the nitrogen atom is 0.4 Å out of the plane defined by its substituents,[11] whereas in the cephalosporins it deviates by 0.2–0.3 Å.[12] The β-lactam nitrogen of the Δ^2-cephalosporins **11** (inactive antibiotics) is almost coplanar with its substituents[12] and yet these are only 2–3-fold less reactive toward alkaline hydrolysis than are the analogous Δ^3-isomers (active antibiotics).[15,34]

As in penicillins, electron-withdrawing substituents attached to the β-lactam nitrogen increase the rate of



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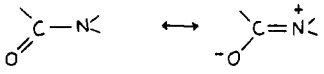
alkaline hydrolysis of cephalosporins with a Brønsted β_{lg} of ca. −0.6, indicative of rate-limiting formation of a tetrahedral intermediate.[15] The variation in rates with substituents at C-3 generate a Brønsted β_{lg} of −0.06, which is consistent with a purely inductive effect of these substituents transmitted to the β-lactam nitrogen[15,35] and little change in charge on the leaving group in the transition state. There is no evidence for expulsion of the leaving group at C-3 occurring during the rate-limiting step of hydrolysis. As shown later for the aminolysis reaction there is evidence that it makes no difference to the chemical reactivity of the β-lactam whether a group at C-3 is expelled or not. If the C-3 substituent is CH_3, the mechanism equivalent to **10** would involve the expulsion of hydride ion, which, of course, does not occur. However, the hydrolysis of cephalosporin β-lactams that also involve expulsion of acetate at C-3 occur only 3 times faster than the hydrolysis of the β-lactam in which no leaving group is expelled at C-3.[15] Substituents that might be and those that cannot be expelled are correlated by the *same* linear free energy relationship.

A number of parameters have been proposed to characterize the chemical reactivity of the β-lactam of penicillins and cephalosporins. These include the following: variations in the IR carbonyl stretching frequency,[35,36] C—N and C=O bond length differences,[12] theoretical calculations of charge distribution[32,37] and conformation,[38] and NMR chemical shifts of ^{13}C and ^{15}N.[39] Reduced amide resonance in a β-lactam will enhance the contribution of canonical form **12** compared with **13** and may be expected to increase the



12 **13**

carbonyl stretching frequency, increase the C–N bond length, decrease the C–O bond length, decrease the negative charge density on oxygen and the positive charge on nitrogen and move their chemical shifts downfield and upfield, respectively, increase the positive charge density on the carbonyl carbon and move its chemical shift downfield. The β-lactam C–O distance is the same (±0.01 Å) in penicillins, Δ^2- and Δ^3-cepha-

(28) H. A. Kranbenhoft, J. R. Wiseman, and C. B. Quinn, *J. Am. Chem. Soc.*, **96**, 258 (1974); W. P. Meyer and J. C. Martin, *ibid.*, **98**, 1231 (1976); P. G. Gassman, R. L. Cryberg, and L. Shudo, *ibid.*, **94**, 7600 (1972).

(29) J. M. T. Hamilton-Miller, E. Richards, and E. P. Abraham, *Biochem. J.*, **96**, 739 (1970); C. H. O'Callaghan, S. M. Kirby, A. Morris, R. E. Waller, and R. E. Duncombe, *J. Bacteriol.*, **110**, 988 (1972).

(30) H. Bundgaard, *Arch. Pharm. Chemi., Sci. Ed.*, **3**, 94 (1975).

(31) D. B. Boyd, R. B. Hermann, D. E. Presti, and M. M. Marsh, *J. Med. Chem.*, **18**, 408 (1975); D. B. Boyd and W. H. W. Lunn, *ibid.*, **22**, 778 (1979).

(32) D. B. Boyd, D. K. Herron, W. H. W. Lunn, and W. A. Spitzer, *J. Am. Chem. Soc.*, **102**, 1812 (1980).

(33) T. Yamana and A. Tsuji, *J. Pharm. Sci.*, **65**, 1563 (1976).

(34) P. Proctor and M. I. Page, unpublished observations.

(35) J. M. Indelicato, T. T. Norvilas, R. R. Pfeiffer, W. J. Wheeler, and W. L. Wilham, *J. Med. Chem.*, **17**, 523 (1974).

(36) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **91**, 1401 (1969).

(37) D. B. Boyd, *J. Med. Chem.*, **16**, 1195 (1973); D. B. Boyd and W. H. W. Lunn, *J. Antibiot.*, **32**, 855 (1979); C. Petrongolo, E. Prescatori, G. Ranghino, and S. Scordamaglia, *Chem. Phys.*, **45**, 291 (1980); C. Petrogolo and G. Ranghino, *Theor. Ch. Acta*, **54**, 239 (1980).

(38) T. K. Vasudevan and V. S. R. Rao, *Curr. Sci.*, **51**, 402 (1982).

(39) R. Mondelli and P. Ventura, *J. Chem. Soc., Perkin Trans. 2*, 1749 (1977); K. Tori, J. Nishikawa, and Y. Takeachi, *Tetrahedron Lett.*, **22**, 2793 (1981); A. Schauk, B. Coene, M. Van Meerssche, and J.-M. Dereppe, *Org. Magn. Reson.*, **12**, 337 (1979); J. Nishikawa and K. Tori, *J. Antibiot.*, **34**, 1641.

losporins and β-lactams, while the C–N distance is the same (±0.01 Å) in penicillins and Δ^3-cephalosporins but 0.03 Å shorter in β-lactams and Δ^2-cephalosporins.[12] The carbonyl stretching frequency does increase (up to 20 cm^{-1}) as the β-lactam nitrogen becomes less planar, but what does this mean? The direct interpretation of carbonyl stretching frequencies in terms of bond strengths or electron-density distributions is not straightforward. For an X—C=O system the C=O stretching frequency can be increased by decreasing the C—X bond length or by increasing the C—X stretching or CCX and OCX bending force constants even if the force constant for C=O stretching remains constant.[40] The β-lactam carbonyl carbon ^{13}C chemical shifts in penicillins resonate about 10 ppm to lower field than in cephalosporins but show the same shift in nonplanar Δ^3- and planar Δ^2-cephalosporins.[34,39] Steric inhibition of resonance should make the carbonyl carbon more positive. Furthermore, the ^{15}N chemical shifts of the β-lactams shows a 30-ppm *downfield* shift on going from the planar Δ^2-cephalosporins to the nonplanar penicillins.[41] This is in the opposite direction expected for reduced amide resonance.

Probably the most important point that is missed when chemical reactivity of the β-lactam, nonplanarity, or inhibition of amide resonance is unthinkingly correlated with biological reactivity is that a reactive β-lactam is not the sole or most important criterion required.[21] Successful inhibition of the bacterial transpeptidase enzyme in the presence of natural substrate depends upon the second-order rate constant, k_I/K_I, for the reaction between enzyme and inhibitor[42] and the formation of a stable antibiotic-enzyme derivative, EI*, which does not regenerate the free enzyme, i.e., low k_r (eq 2). If the initially formed intermediate is an acy-

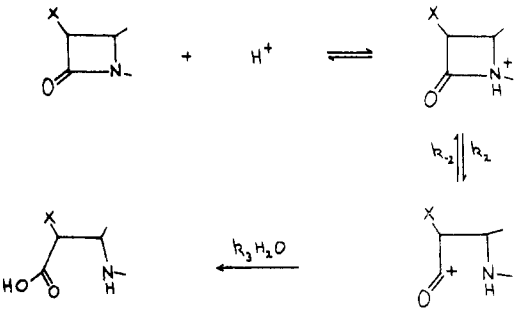
$$E + I \underset{K_I}{\rightleftharpoons} EI \xrightarrow{k_I} EI^* \xrightarrow{k_r} E + I^* \qquad (2)$$

lated enzyme, this could be sterically prevented from subsequent hydrolysis by the intrinsic structure of the antibiotic or because, unlike the natural acyclic amide substrate from which D-alanine is expelled,[3] ring opening of the cyclic β-lactam generates an acyl enzyme with the leaving group amine still covelently linked to the acyl portion of the antibiotic. Alternatively the acyl enzyme could be converted to another derivative that is stable to hydrolysis.[21]

Acid Hydrolysis

If amide resonance in penicillins is significantly impaired, the β-lactam nitrogen should be more basic than a normal amide nitrogen and the β-lactam oxygen less basic than a normal amide oxygen. The pK_a of N-protonated amides is normally ca. −7 to −8,[43] whereas that for the resonance-inhibited amide 1-azabicyclo-[2.2.2]octan-2-one is 5.[44] Thermodynamically the most basic site for the protonation of normal amides is oxy-

Scheme I



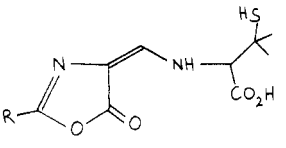
gen and the pK_a of O-protonated amides is 0 to −3,[45] although it is claimed that N-protonation occurs in dilute acids and O-protonation in strong acid.[46]

The logarithm of the pseudo-first-order rate constants for the hydrolysis of some β-lactam antibiotics and derivatives increase linearly with decreasing H_0 values up to −5.[15] This is quite unlike the behavior of other amides for which the rate of hydrolysis passes through a maximum, which is attributed to complete conversion of the amide into its O-conjugate acid and to decreasing water activity.[47] β-Lactams are thus less basic than normal amides for O-protonation (cf. the reduced basicity of cyclobutanone), and a different mechanism of hydrolysis is operating. The nitrogen of the bicyclic β-lactams is insufficiently basic for complete or partial conversion to the N-protonated amide. The pK for N-protonation must be <−5. This behavior is not peculiar to bicyclic β-lactams as *monocyclic* β-lactams show similar reactivities and behavior.[15]

The slopes of the logarithms of the pseudo-first-order rate constants for hydrolysis against H_0 are −1 to −1.3, and as water activity decreases with increasing acidity, it appears that water is not involved in the transition state. This can be explained by a unimolecular A-1 mechanism with N-protonation of the β-lactam (Scheme I), which is supported by the effect of substituents.[15] That N-protonation takes place is not the result of reduced amide resonance in penicillins and cephalosporins but is simply an intrinsic property of all β-lactams.[15] The A-1 mechanism is probably favored because of the enhanced rate of C–N bond fission that occurs in β-lactams resulting from the relief of ring strain.[15] Because of the similarity of the rate constants for the degradation of benzylpenicillin and its methyl ester, there is *no* evidence for neighboring-group participation of the carboxy group in the fission of the β-lactam ring.

The rate enhancement for the acid hydrolysis of penicillins lacking an acylamido group at C-6 is ca. 10^2 compared with that of an analogous amide.[15] As the mechanisms of their hydrolysis are different, the rate enhancement for the bicyclic β-lactam proceeding by the same mechanism as that for an acyclic amide is much less than this. Penicillins with an acylamido side chain at C-6 give penicillenic acid 14 and other products in acidic solution.[45] This can be incorporated into the proposed mechanism (Scheme I) as the intermediate

(40) A. J. Collings, P. F. Jackson, and K. J. Morgan, *J. Chem. Soc. B*, 581 (1970).
(41) J. P. Paschal, D. E. Dorman, P. R. Srinivasan, and R. E. Lichter, *J. Org. Chem.*, **43**, 2013 (1978).
(42) M. I. Page, *Int. J. Biochem.*, 11, 331 (1980); *Chem. Ind.*, 144 (1981); M. I. Page in "The Chemistry of Enzyme Action", M. I. Page, Ed., Elsevier, Amsterdam, 1984, Chapter 1.
(43) A. Williams, *J. Am. Chem. Soc.*, **97**, 6278 (1975); A. R. Fersht, *ibid.*, **93**, 3504 (1971).
(44) H. Pracejus, M. Kehlen, H. Kehlen, and H. Matscheiner, *Tetrahedron*, 21, 2257 (1965).
(45) M. Liler, *J. Chem. Soc. B*, 385 (1969); K. Yates and J. B. Stevens, *Can. J. Chem.*, **43**, 529 (1965).
(46) M. Liler, *J. Chem. Soc., Perkin Trans. 2*, 71 (1974).
(47) C. J. O'Connor, *Q. Rev., Chem. Soc.*, **24**, 553 (1970); B. C. Challis and J. Challis "The Chemistry of Amides", J. Zabicky, Ed., Interscience, New York, 1970, p 371; C. R. Smith and K. Yates, *J. Am. Chem. Soc.*, 94, 8811 (1972).



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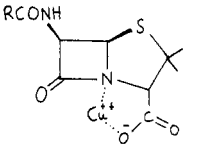

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acylium ion can be trapped by the intramolecular amido group rather than by water. However, the rate of penicillin degradation is ca. 10^3 faster than predicted from the σ value for RCONH.[15] The implication is that the acylamido group participates in the rate-limiting step, which is acceptable if k_3 is rate limiting (Scheme I), i.e., the formation of the acylium ion is reversible.

The acid hydrolysis of cephalosporins **2** shows similar behavior to that of penicillins, but they are about ca. 10^4-fold less reactive. Electron-withdrawing substituents at C-7 in cephalosporins **2** decrease the rate of acid hydrolysis and, as for penicillins, the Hammett ρ_I value is ca. 5. There is no evidence for neighboring-group participation by the 7-acylamido group as postulated for the penicillins. There seems no obvious explanation of the difference in behavior between the cephalosporins and penicillins. Similar to alkaline hydrolysis, there is no evidence for a leaving group at C-3 in cephalosporins significantly affecting the rate of reaction. In fact, the 3-methyl derivative is more reactive than the cephalosporins with acetate or pyridine at C-3, which indicates that explusion of these groups is not important in the rate-limiting step.[15]

Metal Ion Catalyzed Hydrolysis

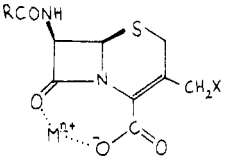
Transition-metal ions cause an enormous rate increase in the rate of aminolysis and hydrolysis of penicillins and cephalosporins.[25,49] For example, copper(II) ions can enhance the rate of hydrolysis of benzylpenicillin 100 million fold. In the presence of excess metal ions, the observed apparent first-order rate constants for the hydrolysis of the β-lactam derivatives are first order in hydroxide ion but show a saturation phenomenon with respect to the concentration of metal ion, which is indicative of the formation of an antibiotic metal ion complex. A kinetic scheme is shown in eq 3

$$M + L \overset{K}{\rightleftharpoons} ML \xrightarrow{k_2(OH)} \text{products} \qquad (3)$$

where M is the metal ion and L is the β-lactam. The rate of hydroxide ion catalyzed hydrolysis of benzylpenicillin bound to metal ion shows the following rate enhancements compared with the uncoordinated substrate: Cu(II), 8×10^7; Zn(II), 4×10^4; Ni(II), 4×10^4; Co(II), 3×10^4. The analogous data for cephaloridine are as follows: Cu(II), 3×10^4; Zn(II), 2×10^3.

Copper(II) ion coordinates to the carboxylate group and the β-lactam nitrogen of benzylpenicillin **15**. Coordination occurs to the carboxylate group because esterification of this group decreases the rate enhancement by ca. 5×10^3. It has been suggested that copper(II) ions coordinate to the 6-amido side chain and the β-lactam carbonyl group,[50] but replacement of the 6-amido side chain by the more basic amino group or by hydrogen has little effect upon the binding constant or upon the rate enhancement, indicating that metal ion coordination does not occur to the 6-amido side chain of penicillins.[25,49] Copper(II) ions bind 10-fold *more* tightly to cephalosporins than to penicillins, which would be surprising if the sites of coordination are similar.

Molecular models indicate that one of the conformations of cephalosporins would be very suitable for metal ion coordination between the carboxylate group and the β-lactam carbonyl oxygen **16**. This has been



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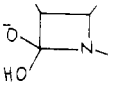
confirmed by IR and NMR studies of the solid complexes although, of course, these may not be relevant to solutions.[21,25] For example, the kinetic data in solution indicate only a 1:1 complex, whereas cephalosporins form 2:1 solid complexes. Also the thermodynamically favored binding site is not necessarily the kinetically important one. Copper(II) ion stabilizes the transition state for benzylpenicillin hydrolysis by 13.9 kcal mol^{-1} at 30 °C and that for cephaloridine by 11.2 kcal mol^{-1}. This enormous stabilization is attributed to the large change in basicity of the β-lactam nitrogen in the transition state. If the transition state for benzylpenicillin hydrolysis resembles the tetrahedral intermediate **17**, coordination of copper(II) ion would



17

stabilize the system by 9.8 kcal $mol,^{-1}$ based on the calculated basicity of the bridgehead nitrogen.[25]

It is not known if the coordination site of these model studies is relevant to the enzyme-catalyzed reactions. Metal ion binding to an amide nitrogen makes chemical sense for amide hydrolysis, but it is thought that carboxypeptidase A, for example, uses it zinc(II) to bind to the amide oxygen although there are arguments against this. The zinc(II)-dependent β-lactamase from *Bacillus cereus* 569/H catalyzes the hydrolysis of benzylpenicillin to benzylpenicilloic acid. At pH 7.0 and 30 °C the half-life for benzylpenicillin bound to β-lactamase II is ca. 5×10^{-4} s compared with one of 100 s

(48) M. A. Schwartz, *J. Pharm. Sci.*, **54**, 472 (1965); J. L. Longridge and D. Timms, *J. Chem. Soc. B*, 852 (1971); J. P. Degelaen, S. L. Loukas, J. Feeney, G. C. K. Roberts, and A. S. V. Burgen, *J. Chem. Soc., Perkin Trans. 2*, 86 (1979).

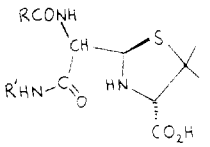
(49) N. P. Gensmantel, P. Proctor, and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 1725 (1980).

(50) W. A. Cressman, E. T. Sugita, J. T. Coluisio, and P. J. Niebergall, *J. Pharm. Sci.*, **58**, 1471 (1969).

when it is bound to zinc(II). This comparison involves the effectiveness of 10^{-7} mol L^{-1} hydroxide ion with an unknown intramolecular nucleophile in the enzyme. It is not known whether benzylpenicillin is coordinated to the zinc(II) of β-lactamase II. β-Lactamase II increases the rate of hydrolysis of benzylpenicillin by a factor of ca. 10^{13} L mol^{-1} at pH 7 compared with a factor of 2.5×10^7 brought about by zinc(II) alone. Although the zinc(II)-dependent D-alanyl-D-alanine peptidase is thought to be an important target of β-lactam antibiotics,[51] it is very ineffective at catalyzing the hydrolysis of benzylpenicillin. In fact, zinc(II) ions are 10^2–10^3 *more* efficient than the zinc(II)-bound protein in catalyzing the hydrolysis of pencillins!

Aminolysis of the β-Lactams

Aminolysis of the penicillins to give penicilloyl amides **18** is a stepwise process catalyzed predominantly by



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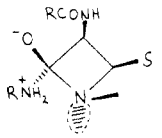
bases that remove a proton from the attacking amine. The tetrahedral intermediate ($T^{\pm}$) **19** is formed reversibly, and products are formed when this is trapped by diffusion-controlled encounter with a base (Scheme II).[52–54] With a constant amine nucleophile there is a nonlinear dependence of the rate upon the basicity of the catalyst. The Brønsted plot generates an Eigen-type curve consistent with a stepwise process involving rate-limiting diffusion-controlled encounter of the tetrahedral intermediate and base when proton transfer is thermodynamically favorable. When the latter is thermodynamically unfavorable, the rate-limiting step occurs after proton transfer.[54] The Brønsted values of $\beta_{nuc} = 0.96$ for the hydroxide ion catalyzed, $\beta = 1.09$ for the amine-catalyzed, and $\beta_{nuc} = 1.0$ for the uncatalyzed aminolysis of penicillin are all consistent with this proposal.[53]



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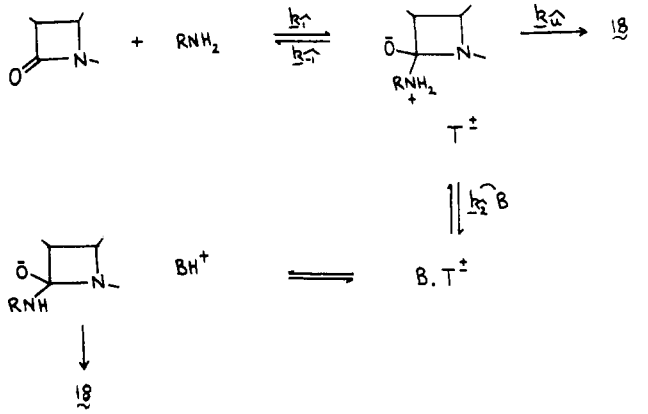
More convincing, perhaps, is the nonlinear dependence of the rate of the hydroxide ion catalyzed aminolysis upon hydroxide ion concentration.[52] At low concentrations of hydroxide ion the rate of collapse of the tetrahedral intermediate to reactants is faster than its reaction with hydroxide ion, $k_{-1} >> k_2[OH^-]$, 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 values for the protonated amine of the tetrahedral intermediates ($T^{\pm}$) are well below that for water. Proton transfer from the tetrahedral intermediate to hydroxide ion is therefore in the thermodynamically favorable direction. 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 hydroxide ion concentration and k_1, the rate of formation of the tetrahedral intermediate, is rate limiting. Assuming that the diffusion-controlled step k_2 has a value of 10^{10} L mol^{-1} s^{-1}, values of k_{-1} and the equilibrium constants for the formation of the tetrahedral intermediates have been obtained.[52] The rates of expulsion of the attacking amine from the tetrahedral intermediate to regenerate the reactants, k_{-1}, are very rapid, ca. 10^9–10^{10} s^{-1}. The equilibrium constants for formation of the tetrahedral intermediates are 10^{-8}–10^{-11} L $mol.^{-1}$

Increasing the basicity of the amine increases the stability of ($T^{\pm}$) as is reflected by the Brønsted β value of 0.9, consistent with the development of a unit charge on nitrogen.[52] The mechanism of general-base–acid catalysis of acyl-transfer reactions depends critically upon the lifetime of the intermediates involved.[55] The aminolysis of penicillin is subject to general-base catalysis because the initially formed tetrahedral intermediate, $T^{\pm}$, breaks down rapidly to reactants and at a rate that is faster than proton donation to water (Scheme II,k_2[B] with B = H_2O), which is a relatively slow process (ca. 1–10^4 s^{-1}). Catalysis occurs because the intermediate is *unstable*;[55] if $T^{\pm}$ was more stable, proton transfer would occur to water and if $k_{-1} < k_2[H_2O]$, no catalysis would be observed. Except in the presence of strongly acidic or intramolecular catalysts, general-acid-catalyzed breakdown of $T^{\pm}$ by proton transfer to the β-lactam nitrogen is a relatively unimportant pathway to products. This is expected because of the weak basicity of the β-lactam nitrogen in $T^{\pm}$.[53]

The behavior of cephalosporins **2** toward amines is similar to that shown by the penicillins.[57] Despite suggestions[31] that nucleophilic attack upon the β-lactam carbonyl carbon is concerted with expulsion of the leaving group at C-3, **10**, a tetrahedral intermediate, is clearly formed. The stability of this intermediate increases with electron-withdrawing groups at C-3. From a knowledge of the equilibrium constant for formation of these intermediate and the observed rate constant for the uncatalyzed aminolysis, the rate constant, k_u (Scheme II), for the uncatalyzed breakdown of the intermediate to products may be estimated. Interestingly these rate constants are similar whether or not a leaving group is expelled at C-3, e.g., 1.0×10^6 s^{-1} for cephaloridine where pyridine is expelled at C-3 compared with 4.5×10^5 s^{-1} for a 3-methylceph-3-em where no leaving group is expelled.[57] Having a leaving group at C-3 in cephalosporins thus has little or no effect upon the rate

(51) J. M. Ghuysen, J.-M. Frere, M. Leyh-Bouille, and O. Dideberg in "Molecular Basis of Drug Action", T. P. Singer and R. N. Ondarza, Eds., Elsevier, North Holland, 1981, p 3.
(52) N. P. Gensmantel and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 137 (1979).
(53) J. J. Morris and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 212 (1980).
(54) J. J. Morris and M. I. Page, *J. Chem. Soc., Perkin Trans. 2*, 220 (1980).
(55) W. P. Jencks, *Acc. Chem. Res.*, **9**, 425 (1976).
(56) A. F. Martin, J. J. Morris and M. I. Page, *J. Chem. Soc., Chem. Commun.*, 298 (1979).
(57) M. I. Page and P. Proctor, *J. Am. Chem. Soc.*, in press.
(58) P. Deslongchamps, *Tetrahedron*, **31**, 2463 (1975).

Scheme II



of cleavage of the β-lactam carbon–nitrogen bond; the effect of such groups upon chemical reactivity is mainly their inductive effect upon the electrophilicity of the β-lactam carbonyl carbon.

According to the theory of stereoelectronic control,[55] the breakdown of tetrahedral intermediates is facilitated by the lone pairs of the heteroatoms attached to the incipient carbonyl being antiperiplanar to the leaving group. Application of this theory to the microscopic reverse steps predicts that the direction of the nucleophilic attack on the carbonyl carbon to be such that the lone pairs on the heteroatoms will be antiperiplanar to the attacking group. Nucleophilic attack on the pencillins occurs from the least hindered α-side to generate an intermediate with stereochemistry as shown in **19**.[21,53,56] It is unlikely that α-attack would give the stereoisomer predicted by stereoelectronic control because this would introduce a highly strained transfused bicyclic system. Although stereoelectronic control therefore favors β-attack, this is not observed.

Conclusion

The bicyclic β-lactam antibiotics do not exhibit exceptional chemical reactivity or reaction mechanisms. Monocyclic β-lactams with suitable electron-withdrawing substituents may be as reactive as the bicyclics. There is no significant reduction of amide resonance in the penicillins. Nucleophilic attack upon the β-lactams carbonyl carbon may be reversible, for it is easier to expel an attacking amine than to break the β-lactam C–N bond. Expulsion of a leaving group at C-3 in cephalosporins is not concerted with nucleophilic attack at the β-lactam carbonyl carbon and makes little difference to the rate of β-lactam C–N bond fission. The unusual mechanism for the acid-catalyzed hydrolysis of the antibiotics is not due to their bicyclic nature but is characteristic of all β-lactams. A pyramidal geometry of the β-lactam nitrogen is not necessary for either high chemical reactivity or antibiotic activity. An important contribution to antibacterial activity must be transport, the binding of the antibiotic to its receptor site, and inhibition of the relevant enzyme. Strained β-lactam systems are not necessarily better antibiotics. A logical extension of these arguments is that the β-lactam itself if not a necessary requirement for antibacterial activity—we are currently pursuing this hypothesis!

Note added in proof: We have recently shown that β-lactam ring opening and expulsion of the leaving group at C-3 occur in separate steps in the β-lactamase I catalyzed hydrolysis of cephaloridine.

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