β-Secondary and Solvent Deuterium Kinetic Isotope Effects and the Mechanisms of Base- and Acid-Catalyzed Hydrolysis of Penicillanic Acid

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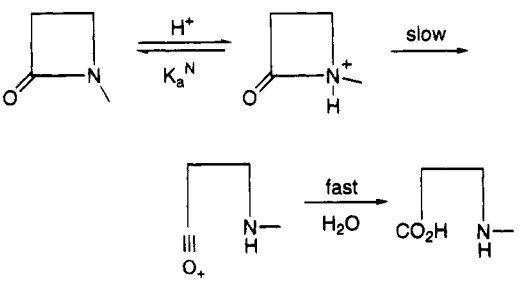
β-Secondary and solvent deuterium kinetic isotope effects have been determined at 25 °C for the alkaline and acid-catalyzed hydrolysis of penicillanic acid. In order to determine the former isotope effect, [6,6-$^2H_2$]dideuteriopenicillanic acid has been synthesized. In alkaline solution, the former isotope effect (corrected to the effect of a single hydrogen orthogonal to the plane of the carbonyl group and for the inductive effect of deuterium) was found to be 0.95 ± 0.01 and the latter 0.76 ± 0.01. These values support the $B_{AC}2$ mechanism of hydrolysis with rate-determining formation of the tetrahedral intermediate that has been proposed for other β-lactams. The measured β-secondary kinetic isotope for the acid-catalyzed reaction was 1.00 ± 0.01. This represents a value averaged from experiments in 0.45 M HCl, 0.97 M HCl, 4.5 M HCl, and 33.3 wt % H_2SO_4. All precedent suggests that this result would be very unlikely for an associative mechanism, such as that commonly observed ($A_{AC}2$) for amide hydrolysis at these acid concentrations. Semiempirical AM1 calculations suggest that bicyclic β-lactams are not only very weakly basic (in accord with previous experiment) but also protonate preferentially on nitrogen. This likelihood, taken with the secondary isotope effect, indicates that a likely pathway of acid-catalyzed hydrolysis would be that of an $A_{AC}1$ mechanism with an intermediate acylium ion. If this were so, the calculated β-secondary isotope effect per hydrogen coplanar with the breaking C–N bond and corrected for the inductive effect of deuterium would be 1.06 ± 0.01. This suggests an early $A_{AC}1$ transition state, which would be reasonable in this case because of destabilization of the N-protonated amide with respect to the acylium ion because of ring strain. The absence of specific participation by solvent in the transition state, as would be expected of an $A_{AC}1$ but not an associative mechanism, is supported by the strongly inverse solvent deuterium kinetic isotope effect of 0.25 ± 0.00 in 1 M HCl and 0.22 ± 0.01 in 33.3 wt % H_2SO_4.

The various mechanisms of amide hydrolysis have long been a subject of interest and research among chemists and biochemists, and the fine points are still being investigated both experimentally[1] and by means of modern theoretical methods.[2] The β-lactams are atypical amides, but are of particular interest because of their activity as antibacterial agents. Much bacterial resistance against β-lactam antibiotics arises through their destruction by hydrolytic enzymes, the β-lactamases.[3] Thus, β-lactam hydrolysis has been much studied.[4]

In alkaline solution, where most information is available, a $B_{AC}2$ mechanism, typical also of acyclic amides and esters, has been proposed for β-lactam hydrolysis. It appears that nucleophilic attack by hydroxide ion to form the tetrahedral intermediate is commonly the rate-determining step.[5–7] In acid solution, where fewer and less detailed studies have been made, it has been suggested that an $A_{AC}1$ mechanism obtains.[6,8] Here (Scheme 1), unimolecular fission of the protonated β-lactam would be rate-determining, in a reaction that proceeds by way of an acylium ion intermediate. An N-protonated amide seems likely to be another required intermediate.

Scheme 1



The evidence for this mechanism, which is unusual for an amide in dilute acid where an $A_{AC}2$ mechanism is normally obtained,[9] derives from studies of the effects of high acidity (and concurrent low water activity)[8] and substituents[6] on acid-catalyzed hydrolysis rates. There is some uncertainty in the interpretation of these observations, particularly in the case of bicyclic β-lactams whose low basicity[4,6] and high rates of hydrolysis precluded studies in regimes of acidity where a major proportion of the amide is proton-

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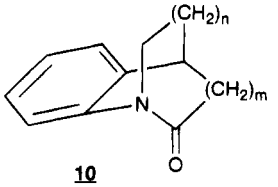
ated. There is also uncertainty as to whether O or N protonation of the β-lactam is more favorable. It is possible that the site of protonation and the mechanism of hydrolysis may be medium-dependent, different, for example, in solvents of low and high acidity; the latter phenomenon has in fact been observed with certain amides.[10,11]

The mechanism and the nature of transition states of acyl-transfer reactions have been conveniently determined with little ambiguity from β-secondary deuterium isotope effects.[12] The results of such studies are usually interpreted in terms of changes in the extent of hyperconjugation of β-hydrogen atoms with the carbonyl group of the amide on proceeding to the transition state.[13] Mechanisms of hydrolysis involving a tetrahedral intermediate where much of the β-hydrogen hyperconjugation present in the starting amide will be lost in the transition state, e.g., in the $B_{AC}2$ mechanism of alkaline hydrolysis and the $A_{AC}2$ mechanism of acidic hydrolysis of simple esters and amides, therefore give inverse β-secondary isotoped effects.[12] In contrast, an $A_{AC}1$ mechanism of acyl transfer, with a strongly hyperconjugating acylium ion intermediate, would be expected to yield a normal isotope effect. For example, Bender and Feng[14] obtained a β-secondary deuterium isotope effect of 1.51 for the hydrolysis of acetyl chloride at −22 °C which they interpreted in terms of an $A_{AC}1$ mechanism. More relevant to the matter at hand, Congdon and Edward[11] have studied the hydrolysis of an amide, 1-acetyl-5,5-dimethylthiohydantoin, in sulfuric acid. In 39.6% sulfuric acid at 25 °C, an isotope effect of 0.93 was obtained, while in 96.3% sulfuric acid the effect was 1.22 at 20 °C. These observations were interpreted in terms of $A_{AC}2$ and $A_{AC}1$ mechanisms, respectively. This precedent appears to be a good one for distinguishing these bimolecular and unimolecular mechanisms of amide hydrolysis.

Since we hoped to employ β-secondary deuterium kinetic isotope effects in an investigation of the nature of the rate-determining steps and transition states of β-lactamase catalysis, we thought it important to first establish their behavior in nonenzymic hydrolyses where the mechanisms and rate-determining steps can be more readily established. To date, this type of isotope effect has not been employed in studies of β-lactam hydrolysis. This paper describes the determination of β-secondary deuterium kinetic isotope effects for the specific acid and base-catalyzed hydrolysis of penicillanic acid, **1**, the parent bicyclic β-lactam of the penicillin class of β-lactam antibiotics. Solvent deuterium kinetic isotope effects were also obtained to provide corroborating evidence of mechanism.



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Experimental Section

Penicillanic Acid (1a). 6,6-Dibromopenicillanic acid was prepared from commercial (Aldrich Chemical Co.) 6β-aminopenicillanic acid as described by Volkman et al.[15] and purified by recrystallization from ethyl acetate. It was converted to penicillanic acid by catalytic hydrogenation over Pd/$CaCO_3$, essentially according to Clayton.[16] The product, a colorless viscous liquid, was converted into its potassium salt by treatment with 1 equiv of aqueous potassium bicarbonate. The solution was freeze-dried to obtain the solid salt. Purification of the latter was achieved by its elution with water from a Sephadex G10-120 column at 4 °C. Appropriate fractions, detected by UV absorption spectra, were pooled and freeze-dried. The ^1H NMR spectrum of the product, a colorless solid, was essentially identical to that reported by Ikeda et al.:[17] (200 MHz, [$^2H_6$]DMSO) δ 5.08 (dd, 1H, J = 4, 2 Hz), 3.80 (s, 1H), 3.39 (dd, 1H, J = 16, 4 Hz), 2.78 (dd, 1H, J = 16, 2 Hz), 1.52 (s, 3H), 1.41 (s, 3H).

[6,6-$^2H_2$]Penicillanic Acid (1b). This compound was prepared in essentially the same way as described above for the dihydro compound with precautions to ensure maximal deuteration. Thus, 2.0 g of 6,6-dibromopenicillanic acid and 1.4 g of sodium bicarbonate were dissolved in 23 mL of 99.9 atom % ^2H_2O (Aldrich Chemical Co.) and the solution freeze-dried. This procedure was repeated. The residue was dissolved in 40 mL of ^2H_2O and transferred to a twice ^2H_2O-rinsed and dried glass hydrogenation vessel. Reaction with $^2H_2$ gas (99.8 atom %, Cambridge Isotope Labs.) at 33 psi at room temperature over 0.8 g of Pd/$CaCO_3$ (Aldrich) in a Parr hydrogenation apparatus then took place as described above. The same procedure for the isolation and purification of the potassium salt was then followed. The ^1H NMR spectrum of the 6,6-dideuterio compound was as expected from the dihydro analog: (300 MHz, $^2H_6$-DMSO) δ 5.09 (s, 1H), 3.83 (s, 1H), 1.51 (s, 3H), 1.41 (s, 3H). The NMR spectrum indicated that the amount of protium present at C-6 was around 1% at both the α and β− positions.

Kinetic Methods. The hydrolyses of **1a** and **1b** were followed spectrophotometrically by means of either a Hewlett-Packard HP8452 or a Perkin-Elmer Lambda 4B spectrophotometer at either 220 or 230 nm. All reactions were carried out at 25.0 °C where a constant temperature was maintained by either a Peltier junction accessory (HP 89090A) or a thermostated water bath; thermistor probe measurements indicated that the temperature remained constnat during and between runs to within ±0.1 °C. Reactions were followed for at least 6 half-times and the computer-acquired readings of absorbance *vs* time used to calculate pseudo-first-order rate constants by means of a nonlinear least-squares program which treated the final absorbance and the rate constant as adjustable parameters. Second-order rate constants for acid- and base-catalyzed hydrolysis were obtained by dividing the pseudo-first-order rate constants by the relevant acid or base concentration.

Deuterium chloride (37 wt %, 99.5 atom % ^2H), [$^2H_2$]sulfuric acid (98 wt %, 99.5+ atom % ^2H), and sodium deuteroxide (40 wt %, 99.9 atom % ^2H) solutions in ^2H_2O were purchased from Aldrich Chemical Co. They were appropriately diluted with 99.9 atom % ^2H_2O to provide solutions for solvent isotope effect measurements. Final acid and base concentrations were determined by titration against standard base and acid solutions, respectively.

Concentrated stock solutions of **1a** and **1b** in either H_2O or, for the solvent isotope effect measurements, 1/1 v/v H_2O/^2H_2O, were prepared directly prior to kinetic runs. To initiate the reactions, aliquots (100 μL) of these stock solutions at 25 °C were delivered by thermostated syringe to thermostated cuvettes containing the appropriate acid or base solutions. Appropriate allowance was made for dilution of the acid or

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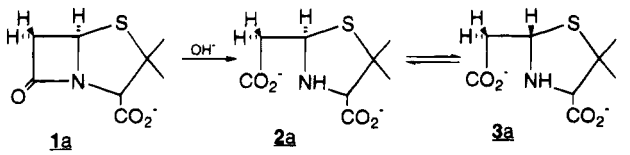
Scheme 2



1a 2a 3a

base. Final concentrations of **1a** and **1b** ranged between 0.5 and 1 mM. Generally, kinetic runs were alternated between the two isotopes, and isotope effects were calculated from successive pairs.

Theoretical Calculations. Molecules and ions were constructed by means of the Builder module of INSIGHT II, version 2.2.0 (Biosym Technologies, San Diego, CA) run on an IBM RISC/6000 Model 530H computer. Initial structural relaxation was performed by the DISCOVER Molecular Simulation Program, version 2.9. Final energy and structural minimization was achieved by semiempirical AM1 calculations (MOPAC 6.0).

Results

The hydrolysis of β-lactams is well-known to be specific-acid and base catalyzed.[4] Accordingly, penicillanic acid was observed to undergo reaction in both acidic and basic solution. Observation of **1a** in 0.1 M NaO^2H by ^1H NMR spectroscopy showed the rapid disappearance of the starting material and the appearance of a product P_1 [(^1H NMR, $^2H_2O/O^2H^-$) δ 4.92 (dd, 1H, J = 10, 5 Hz), 3.51 (s, 1H), 2.75 (dd, 1H, J = 15, 5 Hz), 2.52 (dd, 1H, J = 15, 10 Hz), 1.59 (s, 3H), 1.24 (s, 3H)]. At an only slightly slower rate a second product P_2 [(^1H NMR, $^2H_2O/O^2H^-$) δ 4.7–4.8 (dd, 1H, 9, 5 Hz, not observed, presumably obscured by the water peak), 3.42 (s, 1H), 2.90 (dd, 1H, J = 16, 5 Hz), 2.56 (dd, 1H, J = 16, 9 Hz), 1.62 (s, 3H), 1.26 (s, 3H)] was formed, presumably through further reaction of P_1. When no further reaction was observed, the ratio of P_1 to P_2, presumably an equilibrium mixture, was approximately 1/3. These spectral changes are in accord with alkaline hydrolysis of the β-lactam of **1a** to the penicilloate **2a** (Scheme 2), where upfield shifts of the 3-H, 5-H, and 6-H protons would be expected, accompanied by an increase in vicinal coupling constants as the rigid geometry of the β-lactam ring is lost. The conversion of P_1 to P_2 most likely represents the process of epimerization at C-5, commonly observed in penicilloates[4] via reversible opening of the thiazolidine ring, and likely to be rapid in **2a** where no electron-withdrawing groups are present on C-6.[18] Thus, P_1 and P_2 are probably **2a** and **3a**, respectively.

This conclusion is supported by the observation that the same mixture of **2a** and **3a** is also generated by the action of the TEM β-lactamase (which would of course be expected to catalyze β-lactam hydrolysis) at neutral pH (20 mM MOPS buffer). The conversion of **2a** to **3a** was slower than in alkaline solution, however, and equilibrium was reached in about 2 h.

In 1 M ^2HCl and in 1–34 wt % $^2H_2SO_4$, a similar two-step process was observed. The same products were generated by addition of a mixture of **2a** and **3a** (a freeze-dried enzyme-catalyzed hydrolysis mixture) to $^2H_2SO_4$. They thus must be **2a** and **3a** in protonated, presumably, cationic form. The reaction of **1a** in acid solution is therefore also β-lactam hydrolysis, followed by epimerization of the product.

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Table 1. β-Secondary Deuterium Kinetic Isotope Effects for Hydrolysis of Penicillanic Acid

medium	k_H 10^4 (s^{-1})	k_D 10^4 (s^{-1})	no. of detn.	k_H/k_D
0.45 M HCl[a]	1.298 (0.010)[a]	1.308 (0.008)	6	0.993 (0.009)
0.971 M HCl	2.939 (0.015)	3.006 (0.018)	6	0.978 (0.008)
4.5 M HCl	4.326 (0.161)	4.315 (0.173)	8	1.003 (0.020)
33.3% H_2SO_4	5.364 (0.120)	5.519 (0.161)	6	1.029 (0.015)
		average	26	1.000 (0.014)
0.1 M NaOH	3.781 (0.025)	3.987 (0.044)	7	0.948 (0.012)

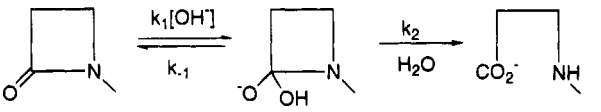
[a] Standard deviations in parentheses.

Table 2. Solvent Deuterium Kinetic Isotope Effects for Hydrolysis of Penicillanic Acid

medium	k_H 10^4 (s^{-1})	k_D 10^4 (s^{-1})	no. of detn.	k_H/k_D
0.971 M HCl	2.939 (0.015)[a]		6	
1.11 M ^2HCl		12.43 (0.035)	6	0.249 (0.002)[b]
33.3% H_2SO_4	5.347 (0.177)	20.24 (0.32)	5	0.215 (0.009)[b]
0.0971 M NaOH	3.781 (0.025)		7	
0.139 M NaOH		3.987 (0.044)	7	0.757 (0.009)[b]

[a] Standard deviations in parentheses. [b] Corrected where necessary for the different acid and base concentrations and linearly extrapolated from 95% to 100% H_2O and ^2H_2O.

Scheme 3



As with other penicillins,[19] the hydrolysis reaction could be followed spectrophotometrically at wavelengths between 220 and 240 nm as the β-lactam chromophore disappeared. From measurements of absorbance *vs* time at such wavelengths, pseudo-first-order rate constants for specific-acid- and base-catalyzed hydrolysis of **1a** were determined as described in the Experimental Section. The derived second order rate constants were 3.0×10^{-4} s^{-1} M^{-1} and 3.9×10^{-3} s^{-1} M^{-1} respectively. These values are in good agreement with those reported by Proctor et al.[6] at 30 °C, 7.56×10^{-4} s^{-1} M^{-1} and 7.40×10^{-3} s^{-1} M^{-1}, respectively.

From determinations of the rate constants of hydrolysis of **1a** and **1b** in acid and base and in H_2O and ^2H_2O, β-secondary and solvent deuterium isotope effects were obtained. These are presented in Tables 1 and 2, respectively. Since no systematic trend in the β-secondary isotope effects with acid concentration is evident, all of the values obtained were averaged as shown in Table 1.

Discussion

The alkaline hydrolysis of β-lactams, like that of acyclic amides, is believed to proceed by way of a $B_{AC}2$ mechanism involving a tetrahedral intermediate (Scheme 3). The formation of this intermediate is generally rate-determining for β-lactams.[4-7] Among the evidence in support of this proposition is the solvent deuterium isotope effect. Gensmantel et al.[20] report a value for k_{OH}/k_{OD} of 0.65 for benzylpenicillin at 30 °C. Bowden and Bromley have determined values of 0.69, 0.78, and 0.84 for *N*-(*m*-nitrophenyl)azetidinone at 70 °C in 50% dioxane/water,[7a] *N*-methylazetidinone at 100 °C,[7b] and benzylpenicillin at 30 °C[7b] respectively. These inverse

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isotope effects are believed to reflect the greater nucleophilicity of O^2H^- in ^2H_2O than OH^- in H_2O. They are thought to largely reflect the solvent isotope effect on k_1. In contrast, in cases where water-catalyzed decomposition of the tetrahedral intermediate, accompanied by some degree of proton transfer, is thought to be rate-determining, such as in anilides and *N,N'*-dimethylacetamide,[1,7b] the isotope effect is larger and generally normal. The value for the alkaline hydrolysis of **1a**, 0.76, determined as part of the present work, is thus in good agreement with precedent and appears to indicate that nucleophilic attack by OH^- to form the tetrahedral intermediate is rate-determining to alkaline hydrolysis.

The β-secondary isotope effect for alkaline hydrolysis of **1** can now be evaluated in the light of the above conclusion. That the isotope effect obtained, 0.95, is inverse, is appropriate for a transition state where nucleophilic attack is occurring or has occurred on the β-lactam carbonyl group. In such a transition state the hyperconjugation of the hydrogens at the C-6 position with the carbonyl group would be reduced leading to an inverse effect.[12] Although there seems to be no precedent in β-lactam hydrolyses, β-secondary isotope effects have been measured for a few amide hydrolyses. Stein et al.[21] have measured a value of 0.94 for nucleophilic attack of hydroxide (k_1) on *p*-nitroacetanilide, and values of 0.95 and 0.97 for acetamide[12] and 1-acetyl-5,5-dimethylthiohydantoin[11] hydrolysis have been reported, respectively; in the two latter cases, it is not possible to decide from these values alone whether formation or breakdown of the tetrahedral intermediate is rate-determining, but the interpretation of solvent isotope effects described above indicates that breakdown may be.

Direct comparison of the amide precedents with the present data for **1** cannot immediately be made, however, because the former involve substitution of ^2H for ^1H in a presumably close to freely rotating methyl group, while the latter result arises from such substitution in a rigid methylene group. Direct comparison can be made by calculation of the contribution to the effect for a single deuterium. Since β-secondary isotope effects arise through hyperconjugation of the electrons of the α-CH bond with the π orbital of the carbonyl group, the effect will depend on overlap of the C−H bond with the π orbital, i.e., on the dihedral angle θ between the C−H bond and the carbon p orbital. An equation to calculate the β-secondary isotope effect $(k_H/k_D)_0$ for one hydrogen of a freely rotating methyl group at the optimal zero value of θ was presented by Sunko et al.[22] (eq i).

$$\ln\left(\frac{k_H}{k_D}\right)_{CH_3} = \frac{3}{2}\ln\left(\frac{k_H}{k_D}\right)_0 + 3\ln\left(\frac{k_H}{k_D}\right)_i \qquad \text{(i)}$$

In this equation, $(k_H/k_D)_{CH_3}$ represents the experimentally measured methyl group effect and $(k_H/k_D)_i$ the inductive effect of a deuterium substitutent which is assumed to have no angular dependence. The inductive effect can be estimated from application of the Taft equation (eq ii) where σ^* and ϱ^* are the usual Taft substituent constant, reflecting in this case the inductive effect of the

$$\ln\left(\frac{k_H}{k_D}\right)_i = -2.303\varrho^*\sigma^* \qquad \text{(ii)}$$

deuterium substituent and the reaction constant, here for alkaline hydrolysis of acetamides, respectively. The latter parameter has a value of +2.7.[23] The small negative (electron-donating with respect to hydrogen) inductive effect of deuterium, which is thought to arise from the anharmonicity of a C−H(^{2}H) bond,[24] has not been extensively studied. The inductive effect of a β-deuterium and its hyperconjugative effect will necessarily be opposing influences. Probably the best estimate of the magnitude of the inductive effect arises from measurements of Streitweiser and Klein[25] on the isotope effect on the pK_a of perdeuterated pivalic acid where all of the deuterium is β to the carboxyl group and thus unable to hyperconjugate. From this value of the isotope effect, $pK(9D) - pK(9H) = 0.018$, and given that the transmission coefficient of the inductive effect is 2.8 per carbon[26] and that ϱ^* for the dissociation of substituted acetic acids is −1.72,[27,28] a value for σ^* for deuterium can be calculated to be −0.0033. Thus, from eq ii, $\ln(k_H/k_D)_i = 0.0202$ and, thence, from eq i, taking the value of $(k_H/k_D)_{CH_3} = 0.95$ for acetamide, $(k_H/k_D)_0 = 0.93$. The correction for reduction to one hydrogen and the inductive effect have essentially cancelled each other in this case.

A similar calculation for **1**, using eqs ii and iii[22] and values of 0.948 for $(k_H/k_D)_{CH_2}$ (Table 1), $\theta = 26°$,[29] σ^* as above, and $\varrho^* = +2.0$ for the alkaline hydrolysis of β-lactams,[6] yielded $(k_H/k_D)_0 = 0.96$.

$$\ln\left(\frac{k_H}{k_D}\right)_{CH_2} = 2\cos^2\theta\ln\left(\frac{k_H}{k_D}\right)_0 + 2\ln\left(\frac{k_H}{k_D}\right)_i \qquad \text{(iii)}$$

These values of $(k_H/k_D)_0$ for acetamide and **1** indicate very similar degrees of tetrahedrality in the transition state, although as indicated above, they may represent different steps of the reaction. Stein et al. observed the same secondary isotope effects, within experimental error, 0.94 ± 0.02, for the formation and breakdown of the tetrahedral intermediate in the alkaline hydrolysis *p*-nitroacetanilide.[21] The present result supports the proposition by Page[4] that nucleophilic addition to acyclic amides and β-lactams represents very similar processes, largely unaffected by β-lactam ring strain.

The acid-catalyzed hydrolysis of **1**, like that of other β-lactams, has, as noted in the Introduction, unusual features.[4,6,8] Likely correlated with this is the unusual

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(29) Conveniently, the hydrogens at the 6-position of the β-lactam ring of **1** are likely to be essentially equivalently oriented with respect to the β-lactam ring and its carbonyl group. Crystal structures of penicillins[30] display an average dihedral angle between the C6−N bond and the p-orbital of the carbonyl group (i.e., and a line through the carbonyl carbon orthogonal to the carbonyl plane) of 32.4°; AM1 calculations on benzylpenicillin give a value of 31.6° for this angle. The structure of penicillanic acid from AM1 calculations yields a value for the above angle of 28.4° and an angle of 23.5° for the α-hydrogen; an average value of 26° was used for θ in eq iii. The similarly obtained average value of the H−C_6−C_7−N dihedral angle for penicillanic acid was 64°.
(30) Domiano, P.; Nardelli, M.; Balsamo, A.; Macchia, A.; Macchia, B.; Macchia, F. *Acta Crystallogr. B* **1979**, *35*, 1363.

Table 3. AM1 Heats of Formation[a] of N- and O-Protonated Amides and Lactams and of Acylium Ions

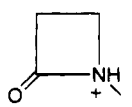
compd	ΔH_N[b]	ΔH_O[c]	$\Delta H_{N\rightarrow O}$[d]	$\Delta H_{N\rightarrow AC}$[e]
formamide	184.5	167.5	−17.0	40.5
N-methylacetamide	181.9	157.7	−24.2	22.0
ϵ-caprolactam	172.5	152.1	−20.4	23.5
2-azetidinone	170.9	168.6	−2.3	−8.6
penicillanic acid	166.3	180.0	+13.7	−7.9

[a] Units are kcal/mol. [b] Difference between the standard heats of formation of the neutral amide and the N-protonated amide. [c] Difference between the standard heats of formation of the neutral amide and the O-protonated amide. [d] Standard heat of formation of the O-protonated amide from the N-protonated; $\Delta H_O - \Delta H_N$. [e] Standard heat of formation of the acylium ion plus amine from the N-protonated amide.

protonation behavior of β-lactams. 2-Azetidinone itself, when protonated, has a significantly lower pK_a than acyclic amides and larger ringed lactams.[8] This has been likened[4] to the reduced basicity of cyclobutanone with respect to acyclic ketones.[31] All previous indications, from the kinetics of acid-catalyzed hydrolysis of **1**,[6] suggest that the pK_a of its protonated form is even lower than that of 2-azetidinone—no evidence of significant protonation was observed at H_0 values above −2. We saw no changes in the chemical shift of **1a** in $^2H_2SO_4$ solutions at acidities as high as $H_0 = -0.75$. Not only is the pK_a of protonated bicyclic β-lactams unknown, but no evidence seems available as to the preferred site of protonation. Acyclic amides are preferentially protonated on oxygen[32] but this may not be true for β-lactams, especially bicyclic β-lactams where the amide nitrogen is firmly nonplanar.

The results of AM1 calculations which may bear on this point of protonation are presented in Table 3. This shows the calculated heats of formation of O- and N-protonated amides and lactams. AM1 calculations have previously been used to acceptable effect with β-lactams.[33] The results on protonation of acyclic amides seem qualitatively and semiquantitatively reasonable. Formamide is seen to protonate preferentially, by 17 kcal/mol, on oxygen. Higher level theory has yielded the same preference by 14−15 kcal/mol.[2a] The AM1 calculations may therefore be useful in indicating relative stabilities. The results shown in Table 3 show that the formamide result applies also to *N*-methylacetamide and to ϵ-caprolactam.

The calculations reported in Table 3 for β-lactams suggest that 2-azetidinone itself should be more difficult to protonate on O than acyclic and unstrained cyclic amides (in accord with experiment), that O and N protonation may be of comparable ease, and that N-protonation should be thermodynamically more facile than in acyclic amides. Finally, the data for **1** indicate again that protonation should be more difficult than for similar acyclic amides and unstrained lactams but that the thermodynamically favored site of protonation is likely to be at N (**4**) rather than O (**5**). The relatively greater accessibility of N-protonated forms of β-lactams



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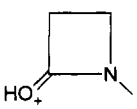

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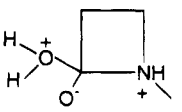
may be relevant to and at least partly responsible for their much higher rates of acid-catalyzed hydrolysis than higher lactams. For example, the data of Wan et al.[8] indicate that 2-azetidinone hydrolyzes some 500 times faster than ϵ-caprolactam and 5000 times faster than *N*-ethylacetamide in 5.1% H_2SO_4. Williams' experiments with *N*-acetyltrialkylammonium ions[34] suggest that an N-protonated acyclic amide would react with water some 10^5 times faster than an O-protonated species. Other classes of strained amide, which also hydrolyze rapidly, are also thought to protonate preferentially on nitrogen.[35,36]

If the dominant protonated form of **1** has the proton on N, it is unlikely that the hydrolysis proceeds by way of the O-protonated form, i.e., by the $A_{AC}2$ mechanism-favored by acyclic amides. The measured secondary isotope effect of 1.00 supports this conclusion. Inductive effects of acyl substituents in acid-catalyzed amide hydrolysis by the $A_{AC}2$ mechanism essentially cancel out[23] so that if this mechanism obtained for **1**, the above figure would represent the hyperconjugative contribution to the secondary kinetic isotope effect of the two deuteriums at C-6. The $A_{AC}2$ mechanism, with a tetrahedral transition state, would be expected to generate a significant inverse β-secondary isotope effect. One example from the literature, the acid-catalyzed hydrolysis of 1-acetyl-5,5-dimethylthiohydantoin in 36% H_2SO_4 at 25 °C, yielded a value of (0.93 ± 0.01) for three deuteriums. The acid-catalyzed hydrolysis of alkyl esters, which is thought to proceed by a closely analogous mechanism, yields similar values.[37] The conclusion is also in agreement with that of Wan et al.[8] and Proctor et al.[6] from their studies of water activity effects in sulfuric acid solutions and substituent effects, respectively, on acid-catalyzed β-lactam hydrolysis. β-Lactam hydrolysis under these conditions therefore seems likely to proceed by way of **4**. There is no indication from the β-secondary isotope effects of a change in mechanism in acid solutions of H_0 values lying between −0.2 and 2.0.

As discussed by others, Yates and co-workers[38] and Williams,[34] for example, one can imagine at this stage two extreme mechanistic possibilities for this reaction and a range of intermediate variants. At one extreme, the A_N^{T2} mechanism,[38] addition of water occurs prior to any C−N bond breaking and a tetrahedral intermediate **6** is generated. Provided that proton transfer were sufficiently fast, however, or concerted with nucleophilic attack, **7** would be generated as the most stable tetrahedral species. At the other pole, the A_N^{D1} mechanism,[38]



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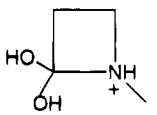

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C−N bond cleavage would be complete prior to any C−O bond formation, yielding an acylium ion intermediate **8**. Between these a spectrum of single step mechanisms involving concerted C−O bond formation and C−N cleavage **9** is conceivable (an A_N^{D2} mechanism[38]). Concerted

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(32) (a) Homer, R. B.; Johnson, C. D. In *The Chemistry of Amides*; Zabicky, J., Ed.; John Wiley: London, 1970; p 187. (b) Giffney, C. J.; O'Connor, C. J. *J. Chem. Soc., Perkin Trans. 2* **1975**, 706.

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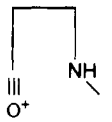
(34) Williams, A. *J. Am. Chem. Soc.* **1976**, *98*, 5645.

mechanisms of acyl transfer have recently been advocated for derivatives with very good leaving groups,[39] as in fact is present in **4**.



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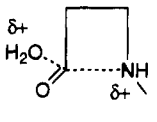

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Prior evidence,[6,8] as described in the Introduction, has been interpreted in terms of the A_N^{D1} mechanism with the intermediate acylium ion **8**. It is not evident, however, from the relatively small amount of available data to what extent various shades of **9** might also be viable possibilities. It is not clear, for example, what the effects of water activity[8] on the transition states relevant to **6** and **9** might be. Nor are the acyl substituent effects [6] clearcut since the steric effects of C-6 substituents on the rates were not taken into account—that they may be substantial is clear from the data for 6-α-chloro and 6-α-bromopenicillanic acid. The behavior of the phenylacetamido side chain also appears anomalous.[4] The present results can be used to address these uncertainties.

It seems likely from the experiments of Proctor et al.[6] that electron-withdrawing substituents at the 6-position hamper the acid-catalyzed hydrolysis of **1**. Thus, ϱ^* will be negative, and therefore, from eq ii and iii, $(k_H/k_D)_0$ will be normal. If a value of -2.0 from ϱ^* is taken from Proctor et al.,[6] for example, the equations (taking $\theta = 64°$;[29] note that the relevant dihedral angle in an A_N^{D1} mechanism is between the C−H bond and the β-lactam C−N bond, the latter orthogonal to the p-orbital of the β-lactam carbonyl group) yield a value for $(k_H/k_D)_0$ of 1.07. Again, the opposing effect of the inductive ability of the deuterium is observed, leading to observation of a lower apparent normal β-secondary isotope effect. Another example of such cancellation of effects in an A_N^{D1} mechanism is seen in the acid-catalyzed hydrolysis of methyl *p*-methylbenzoate.[40] It is assumed in the above calculation that all changes in hyperconjugation leading to the observed isotope effect are along a reaction coordinate orthogonal to the p-orbital of the β-lactam carbonyl group. Although there might also be a change in the extent of hyperconjugation with the carbonyl p-orbital itself, such orthogonal effects would presumably be smaller and also to a large extent counterbalanced by the complementary change in electron density at carbon in the direction of bond-breaking.

The transition states corresponding to the high energy species **6**−**9** of the mechanisms under consideration would yield characteristic β-secondary isotope effects. Tetrahedral transition states adjacent to **6** or **7** would, presumably, like those of the $A_{AC}2$ mechanism, yield inverse isotope effects. The A_N^{D1} mechanism whose transition state would resemble **8** should yield a normal isotope effect since hyperconjugation of the C-6 hydrogens with the acylium ion should be more pronounced than with the original carbonyl group of **1**. That this is true is demonstrated by the examples quoted in the Introduction.[11,14]

From the value of the β-secondary isotope effect for the hydrolysis of 1-acetyl-5,5-*bis*(methylthio)hydantoin in 96.3% H_2SO_4[11] and an assumption of ϱ^* of -2.0, eq i and ii yield a value of $(k_H/d_D)_0$ of 1.16. This is clearly larger than the value for **1**, indicating, if the mechanism is indeed A_N^{D1}, a significantly earlier transition state in the hydrolysis of **1**, with a smaller change in hyperconjugation of the C-6 protons, presumably arising from less C−N bond cleavage. This could stem from the relief of ring strain on C−N fission in **4** creating a more exoergonic reaction. Although it might be suggested that the difference in bonding involved in the β-lactam than in the acyclic amide might make direct comparison of the isotope effects invalid, the apparently reasonable response of the β-lactam to β-deuterium substitution in the $B_{AC}2$ mechanism of alkaline hydrolysis, described above, provides no support to this position.

The concerted A_N^{D2} mechanism via transition state **9** could also generate a normal isotope effect if the extent of C−N bond fission exceeded that of C−O bond formation. This would be an A_N^{D1}-like mechanism where the free acylium ion is not fully formed prior to nucleophilic attack and would presumably yield a smaller normal β-secondary isotope effect than a pure A_N^{D1} mechanism. Such a mechanism could therefore also accommodate the observed β-secondary effect.

The remaining piece of evidence to fit into the picture is the deuterium solvent isotope effect. This was found to be strongly inverse (Table 2). This is indicative of preequilibrium protonation of **1** prior to reaction, as would be anticipated of most acid-catalyzed mechanisms, and of very little solvent participation. Analysis by fractionation factors[41] suggests that the observed isotope effect can arise largely from the ground state fractionation factors of the reactant H_3O^+ with little or not contribution from transition state fractionation factors.[42] This situation could arise in the A_N^{D1} mechanism, but mechanisms such as A_N^{T2} and A_N^{D2} with participation of water in the transition state should have less inverse solvent isotope effects. For example, the hydrolysis of acetamide in dilute acid, presumably by way of an A_N^{D2} mechanism, is reported to have a deuterium solvent isotope effect of 0.67.[44] Brown et al.[1] have recently reported a number of examples of transition states for $A_{AC}2$ mechanisms with signifiant water participation and weakly inverse solvent isotope effects (>0.5). These include examples of strained amides[36] (see below).

(35) (a) Pracejus, H. von; Kehlen, M.; Kehlen, H.; Matschiner, H. *Tetrahedron* **1965**, *21*, 2257. (b) Blackburn, G. M.; Skaife, C. J.; Kay, I. T. *J. Chem. Res., Miniprint* **1980**, 3650.

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(37) (a) Kovach, I.; Hogg, J. L.; Raben, T.; Halbert, K.; Rodgers, J.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 1991. (b) Kovach, I.; Elrod, J. P.; Schowen, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 7530.

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(41) (a) Schowen, R. L. *Prog. Phys. Org. Chem.* **1972**, *9*, 275. (b) Schowen, K. B. J. In *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum, New York, 1978; p 225.

(42) The contribution to the solvent isotope effect from H_3O^+ alone would be $(0.69)^3 = 0.329$.[41] That the observed value is lower than this may result from a combination of the generally small, but perhaps here, in strong acid solutions, significant, medium effects on all of the species involved, both ground and transition state.[43]

(43) (a) Kresge, A. J.; More O'Ferrall, R. A.; Powell, M. F. In *Isotopes in Organic Chemistry*; Bunsell, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Chapter 4. (b) Quinn, D. M.; Sutton, L. D. In *Enzyme Mechanisms from Isotope Effects*; Cook, P. F., Ed.; CRC Press: Boca Raton, FL, 1991; Chapter 3.

(44) Reitz, O. Z. *Physikal. Chem.* **1939**, *183*, 371.

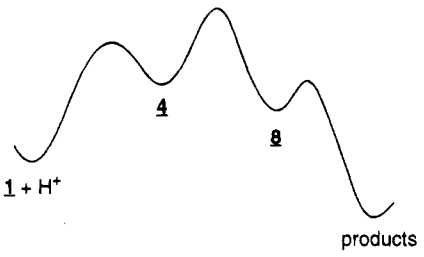
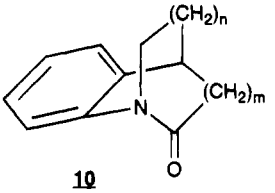


Figure 1.

The results of the new experiments reported in this paper support previous suggestions of an A_N^{D1} mechanism for the acid-catalyzed hydrolysis of β-lactams and provide further details of the transition state. Something close to a "pure" $A_{AC}1$ mechanism is indicated with little or no participation of water in the transition state. The isotope effects suggest that this mechanism applies in acid solutions with the H_0 value lying between −0.2 and 2.0. The transition state appears to be an early one with respect to C−N bond cleavage, presumably because of release of ring strain. This suggests that the acylium ion may be of energy comparable to or lower than that of the N-protonated β-lactam **4** (Figure 1). The results of AM1 calculations on this point are given in Table 3. These results show that although the acylium ion is enthalpically less stable than N-protonated forms of simple amides and an unstrained lactam, it is distinctly more stable than **4** in the case of β-lactams.

Wang et al.[36] have described the kinetics of hydrolysis of **10** (n, m = 1 or 2) which are also strained amides and which hydrolyze unusually rapidly. They found a solvent



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deuterium kinetic isotope effect of 0.63 for acid-catalyzed hydrolysis. Exchange of oxygen from the amide carbonyl group into solvent water was also observed.[45] These results suggest a mechanism involving reversible formation of a tetrahedral intermediate, arising from attack of water on either the O-protonated ($A_{AC}2$) or N-protonated (A_N^{T2}) amide. The A_N^{T2} (or perhaps an A_N^{D2}) mechanism had earlier been suggested by Blackburn et al.[36b] Our AM1 calculations on these systems suggest that N-protonation is favored but that the corresponding acylium ions are less stable than the N-protonated amide. It is possible that ring strain in these systems is insufficient for a dissociative mechanism to be favored.

The results described above suggest that the combination of β-secondary and solvent kinetic isotope effects are as useful in determining reaction mechanisms in acyl-transfer reactions of β-lactams as they are in acyclic systems. In the evaluation of the β-secondary isotope effects, it is important to take into account the inductive effect of deuterium and the angular dependence of the hyperconjugative interaction. The results indicate that, in accord with all precedent, alkaline hydrolysis of **1** proceeds by way of a $B_{AC}2$ mechanism with formation of the tetrahedral intermediate rate-determining. The mechanism of acid-catalyzed hydrolysis seems best described by an A_N^{D1} (a classical $A_{AC}1$) mechanism involving N-protonated β-lactam and acylium ion intermediates. The transition state is early (little C−N cleavage) and requires little or not specific solvation. The unique structure of a bicyclic β-lactam is responsible for the unusual features of the acid-catalyzed reaction—the very weak basicity of the β-lactam, the preference for N-protonation, and the preference, presumably because of ring strain, for a dissociative mechanism.

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