Chemical Reactivity of Penicillins and Cephalosporins. Intramolecular Involvement of the Acyl-Amido Side Chain

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The rate of degradation of 6-*epi*-ampicillin in acidic, neutral, and alkaline aqueous solutions was followed at 35 °C and an ionic strength of 0.5 mol dm^{-3} (KCl) by high-performance liquid chromatography (HPLC) and spectrophotometric assays. Pseudo-first-order rate constants were determined in a variety of buffer solutions, and the overall pH−rate profile was obtained by extrapolation to zero buffer concentration. The hydrolysis of 6-*epi*-ampicillin is subject to acid and hydroxide-ion catalysis and, for a penicillin, an unusual pH-independent reaction. Intramolecular general base-catalyzed hydrolysis by the side chain amido group is proposed to explain the enhanced rate of neutral hydrolysis of 6-*epi*-ampicillin and cephalosporins. The β-lactam of 6-*epi*-ampicillin also undergoes intramolecular aminolysis by nucleophilic attack of the 6-α side chain amino group to give a stable piperazine-2,5-dione derivative. The low effective molarity for intramolecular aminolysis of only 40 M is partly attributed to the unfavorable trans to cis isomerization about the 6-amide side chain required for ring closure. Theoretical calculations show that the intramolecular aminolysis of 6-*epi*-ampicillin nucleophilic attack occurs from the α-face of the β-lactam ring with an activation energy of 14.4 kcal/mol.

Introduction

Penicillins (**1**) and cephalosporins (**2**) are widely used β-lactam antibiotics. Both compounds contain a β-lactam ring fused to another ring, a thiazolidine in penicillins and a dihydrothiazine in cephalosporins.[1] The antibacterial activity of these compounds is, to some degree, related to their chemical reactivity, and there have been many studies on the mechanisms of their reactions.[2−16] In aqueous solution, both classes undergo acid- and base-catalyzed hydrolysis. Despite the structural similarity of penicillins and cephalosporins, their pH log−rate profiles show clear differences. While in penicillins these plots are "V" shaped, cephalosporins show "U" behavior (Figure 1) because cephalosporins, but not penicillins, show a "spontaneous" or water-catalyzed pH-independent reaction.[6,7]

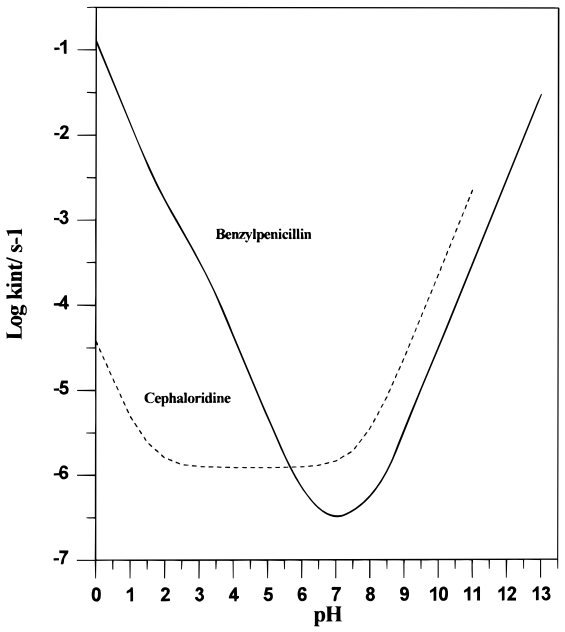

Figure 1. Plots of log k_{int}−pH profiles for benzylpenicillin and cephaloridine degradation in aqueous solution at 35 °C and an ionic strength of 0.5 mol dm^{-3}, where k_{int} is the apparent first-order rate constant (s^{-1}) for the degradation in buffer-free solutions or in buffers showing no effect on the degradation rate.

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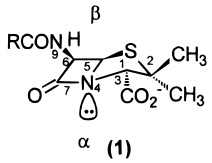

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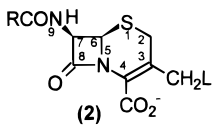

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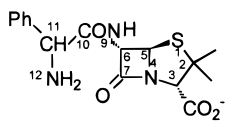

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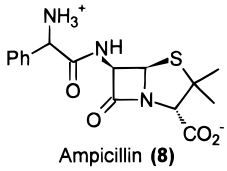

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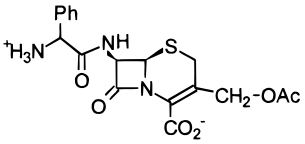

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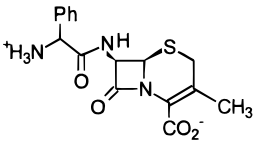

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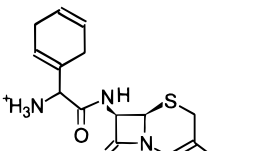

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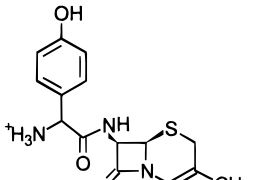

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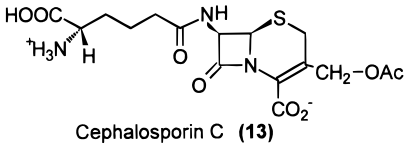

Cephalosporin C (13)

Penicillins are bicyclic [3.2.0] "butterfly-shaped" molecules, and nucleophilic attack on the β-lactam can take place from either the exo (α) side or the endo (β) face. Because of the rigid geometry of these molecules, the lone pair of electrons on the lactam nitrogen is probably located primarily on the α-face, and so the theory of stereoelectronic control[17] would predict that nucleophilic attack should occur preferentially from the β-side.[2−5] However, this direction is sterically hindered by the C3-β-hydrogen and the 2-β-methyl group, and it appears that nucleophiles probably approach from the less-hindered α-side.[1,4]

There is little evidence for a preferential direction of nucleophilic attack on the more planar cephalosporins.[1] In fact, intramolecular nucleophilic attack from the β-direction is well-known in C-7-β side chains of cephalosporins containing a primary side chain amino group, which attacks the β-lactam carbonyl carbon to give piperazine-2,5-dione derivatives.[6−16] Such intramolecular reactions do not occur in similarly β-substituted penicillins.

Herein, we report that the 6-α-epimer of ampicillin (6-*epi*-ampicillin) (**3**), which contains an amino substituent in the 6-α side chain, exhibits intramolecular aminolysis to give a piperazine-2,5-dione derivative. Kinetic studies, theoretical calculations, and molecular modeling of the intramolecular aminolysis of penicillins and cephalosporins and of the role of the acyl-amido side chain in the neutral hydrolysis are used to explain the different reactivity of these two classes of β-lactams.

Experimental Section

Materials. Hetacillin (**4**) was purchased from Sigma and used without further purification. Buffers and all other chemicals were of AnalaR grade. Deuterated solvents were obtained from SDS. Freshly boiled distilled water was used throughout.

General Procedures. High-performance liquid chromatography (HPLC) studies used an instrument equipped with a photodiode array detector, a 20 μL loop injection valve, and a reversed-phase Spherisorb ODS-2 (5 μm particles) column. The ^{1}HNMR and 2D ^{1}HNMR spectra were recorded at 300 MHz. Chemical shifts, δ, are recorded in ppm, and coupling constants, J, are recorded in Hertz. Phase sensitive NOESY spectra were acquired by using the method described by States et al.[18]

Synthesis of 6-*epi*-Hetacillin (5). A slightly modified method of that previously described[19−21] was followed. In 10 mL of aqueous sodium hydroxide solution, pH 11.5, 0.5 g of hetacillin (**4**) was dissolved. The solution was stirred at room temperature for 1 h, acidified to pH 2.0 with dilute hydrochloric acid, and filtered. The filtrate, crystalline 6-*epi*-hetacillin (**5**) (0.45 g, 92%), was washed with water and dried: mp 164−165 °C; ν_{max}/cm^{-1} 3249 (NH), 1771 (β-lactam CO); δ_H (300 MHz, 3:1 DMSO/$CDCl_3$) 1.44 (3H, s, Me), 1.47 (3H, s, Me), 1.49 (3H, s, Me), 1.58 (3H, s, Me), 4.46 (1H, s, 3-H), 4.59 (1H, d, J = 1.9, 5-H), 4.78 (1H, br s, Ph−CH), 5.4 (1H, d, J = 1.9, 6-H), 7.3−7.5 (5H, m, Ph), 8.26 (1H, br s, NH).

Synthesis of 6-*epi*-Ampicillin Trihydrate (3). In 20 mL of water at 35 °C and pH 7.0, 0.5 g of 6-*epi*-hetacillin (**5**) was dissolved by means of a pH stat. The solution was kept stirring for 4 h under these conditions, then it was acidified to pH 2.0 with dilute hydrochloric acid. The aqueous solution was washed with ether (×3). The remaining aqueous solution was filtered and freeze-dried, giving white crystalline 6-*epi*-ampicillin trihydrate in 32% yield; ν_{max}/cm^{-1} 3450 + 3300 (NH_2), 3080 (aromatic), 1750 (β-lactam CO); δ_H (300 MHz, 3:1 DMSO/$CDCl_3$) 1.54 (3H, s, Me), 1.60 (3H, s, Me), 4.51 (1H, s, 3-H), 4.90 (1H, dd, 6-H), 5.04 (1H, br d, Ph−CH), 5.20 (1H, d, 5-H), 7.5−7.6 (5H, m, Ph), 8.79 (2H, br, NH_2).

Synthesis of 2-(3,6-Dioxo-5-phenylpiperazine-2-yl)-5,5-dimethylthiazolidine-4-carboxylic Acid (6). 6-*epi*-Ampicillin trihydrate (**3**) was left stirring in neutral aqueous solution (pH 8.0) for 6 h. The solution was brought to pH 2.0 and filtered. Pure diketopiperazine (**6**) was obtained in 50% yield: mp 190−191 °C; ν_{max}/cm^{-1} 3442, 3058, 1667 (COOH), 1325 (amide), 3196, 1130 (amine), no β-lactam present; δ_H (300 MHz, 3:1 DMSO $CDCl_3$) 1.26 (3H, s, Me(α)), 1.51 (3H, s, Me-(β)), 3.75 (1H, s, 3-H), 4.32 (1H, dd br, 6-H), 5.01 (1H, s, Ph−CH), 5.25 (1H, d, 5-H), 7.30−7.65 (5H, m, Ph), 7.88 (1H, d, J = 1.6, NH(1), 8.49 (1H, d, J = 2.3, NH(2)).

Kinetic Procedures. All kinetic experiments were carried out at 35.0 ± 0.1 °C and an ionic strength of 0.5 mol dm^{-3} (KCl). The buffer solutions employed were hydrochloric acid (pH 0.5−1.0), chloroacetic acid (pH 2.0−3.0), acetic acid (pH 4.1−5.0), 3-(*N*-morpholino) propanesulfonic acid (MOPS) (pH 6.2−8.0), *N*-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS) (pH 8.5−9.2), 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) (pH 10.0), carbonate (pH 10.5−10.8), phosphate (pH 11.7), and sodium hydroxide (pH 12.0−12.5). The ionic strength of each solution was adjusted to 0.5 mol dm^{-3} by the addition of potassium chloride.

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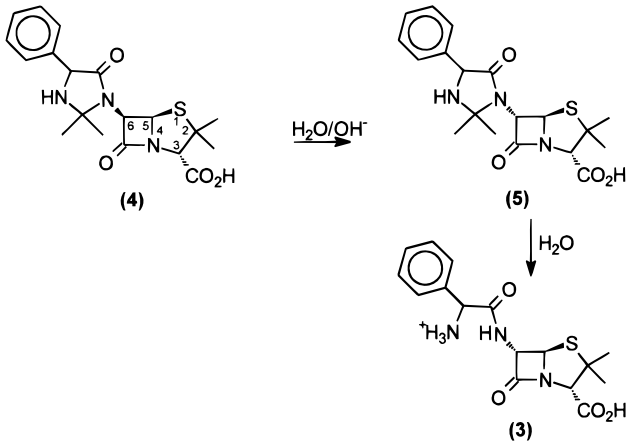
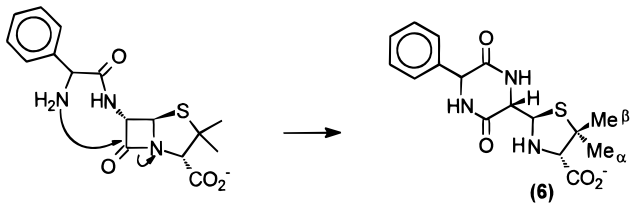
Scheme 1



Scheme 2



The degradation rate of 6-*epi*-ampicillin was followed by measuring the remaining drug concentration by a reversed-phase high-performance liquid chromatographic (HPLC) method. The liquid chromatograph was equipped with a photodiode array UV−vis detector set at 230 nm. The mobile phase was 4% acetonitrile/96% 0.01 mol dm^{-3} ammonium acetate aqueous solution. In those cases where the half-life was less than 3 h, 9 mL of buffer solution was preheated at 35 °C in a constant-temperature bath and 20 mg of 6-*epi*-ampicillin was added. At appropriate intervals, 1 mL samples were withdrawn, mixed with a small amount of a concentrated solution of sodium hydroxide or hydrochloric acid to yield a pH of about 6.5 in the mixture, and then frozen using liquid nitrogen, before analysis was undertaken.

In addition to the HPLC determination of the overall rate of 6-*epi*-ampicillin degradation, the decrease in the concentration of 6-*epi*-ampicillin was followed by measuring the change in absorbance at 230 nm using a UVIKON-941 double-beam recording spectrophotometer. Kinetic data obtained from UV measurements were identical to those determined from the HPLC experiments.

Data analysis. The nonlinear least-squares treatment of the kinetic data was undertaken using the PEAKFIT[22] computer program.

Theoretical Calculations. Semiempirical calculations were carried out using the AM1 method[23] as implemented in the AMPAC 5.0 software package.[24] The software was run on a Silicon Graphics Iris Indigo XZ4000 computer.

All calculations performed were derived from a single-determinant restricted Hartree−Fock (RHF) function. The geometries and energies of the transition states were refined by minimizing the gradient norm using the Powell or NLLSQ algorithms. The Hessian matrix had a single, negative value for all the transition states reported here.

Results and Discussion

The synthesis of 6-*epi*-ampicillin (**3**) followed a slightly modified method described previously[19−21] (Scheme 1). Hetacillin (**4**) was transformed into 6-*epi*-hetacillin (**5**) by treatment with aqueous alkali. Subsequent hydrolysis in neutral aqueous solution for several hours gave crystalline 6-*epi*-ampicillin (**3**). 6-*epi*-Ampicillin can be transformed into the diketopiperazine (**6**)[25] by nucleophilic attack of the amino group of the side chain upon the β-lactam carbonyl moiety from the unhindered exo side of the molecule (Scheme 2). All products of Schemes 1 and 2 were isolated and characterized using ^{1}HNMR and 2D ^{1}HNMR. From the NOESY experiments, a distinction could be made between the *gem*-dimethyls (α and β) and between amide NH(1) and amide NH(2).

1. Degradation of 6-*epi*-Ampicillin. The kinetics of degradation of 6-*epi*-ampicillin in aqueous solutions from pH 0.5 to 13 were determined by ultraviolet spectral measurements and HPLC. Semilogarithmic plots of the residual area of the 6-*epi*-ampicillin peak (in HPLC assays) and/or intensity (in UV) against time were linear in all cases, indicating that the degradation of 6-*epi*-ampicillin at constant pH, temperature, and ionic strength follows simple first-order kinetics with respect to 6-*epi*-ampicillin. The first-order rate constant was evaluated from the slope of these graphs.

The catalytic effect of the buffer was determined by varying the buffer concentration at constant pH, temperature (35 °C), and ionic strength (0.5 mol dm^{-3}, KCl). The observed pseudo-first-order rate constants increase linearly with buffer concentration, and extrapolation to zero buffer concentration provides, as intercepts, the values of the pseudo-first-order rate constants, k_{int}, corresponding to the buffer-independent catalyzed degradation of 6-*epi*-ampicillin. The slopes give the value of the pH-dependent second-order rate constant for the total buffer-catalyzed reaction, k_{cat}. The second-order rate constants for the different protonic forms of the buffer were determined from a plot of k_{cat} against the fraction of free base form of the buffer, α.

The pH dependence of the observed first-order rate constants, k_{int}, for the degradation of 6-*epi*-ampicillin is shown in Figure 2.

In the pH range studied, the 6-*epi*-ampicillin exists in three different ionic forms (Scheme 3), a diprotonated form (AH_2^+), a zwitterion ($AH^{\pm}$), and an anion (A^-). The values for K_1 and K_2 are expected to be similar to those for ampicillin: $pK_1 = 2.6 \pm 0.1$ and $pK_2 = 7.3 \pm 0.1$ (25 °C).[26]

The pH−rate profile (Figure 2) shows an acid-catalyzed reaction at low pH, below the pK_a of both the amine side chain and the 3-carboxylic acid substituent. This therefore corresponds to the acid-catalyzed reaction of the cationic form of the substrate (AH_2^+). At pH 2.6, there is an inflection in the profile to a pH-independent reaction, indicating that there is a change in mechanism[27] corresponding to the reaction of the zwitterionic form of ampicillin ($AH^{\pm}$), its kinetically equivalent neutral species, with the amine dissociated but the carboxylic acid residue undissociated, or even the kinetically equivalent

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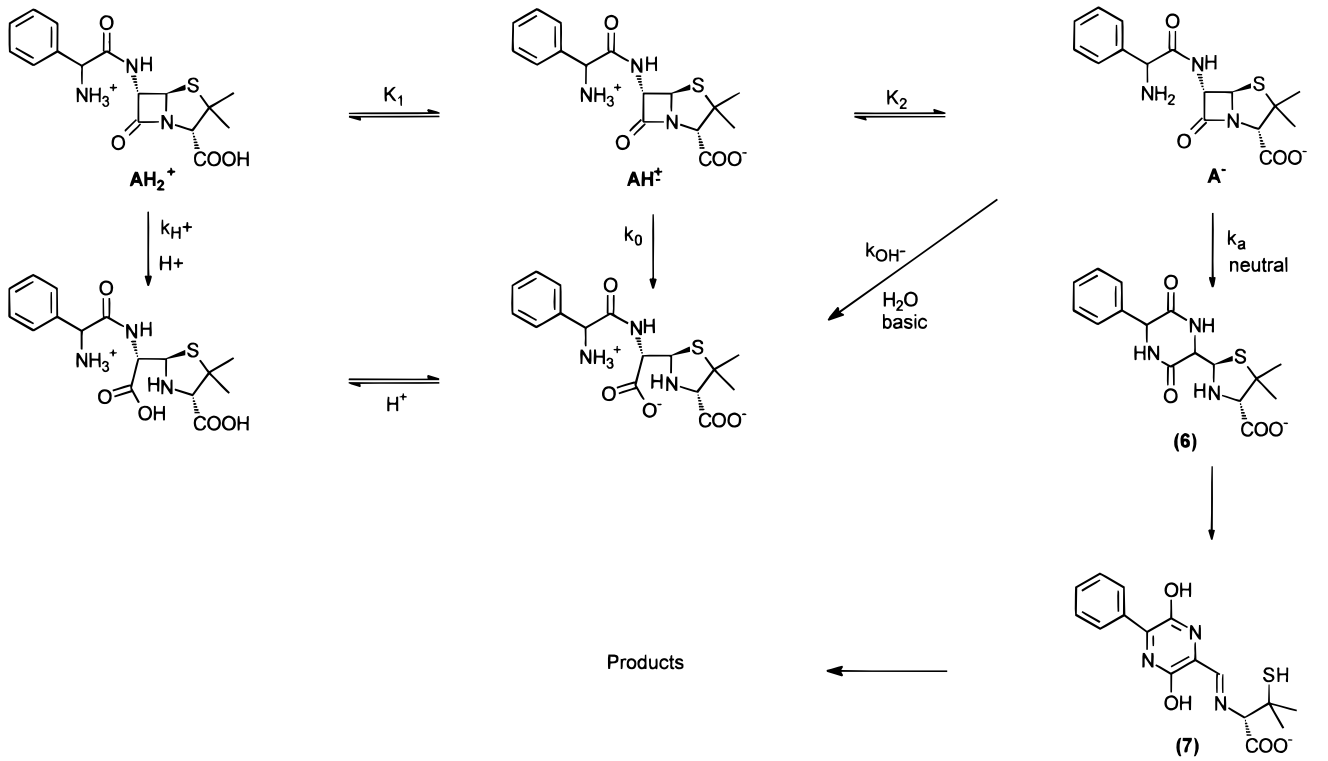
Scheme 3



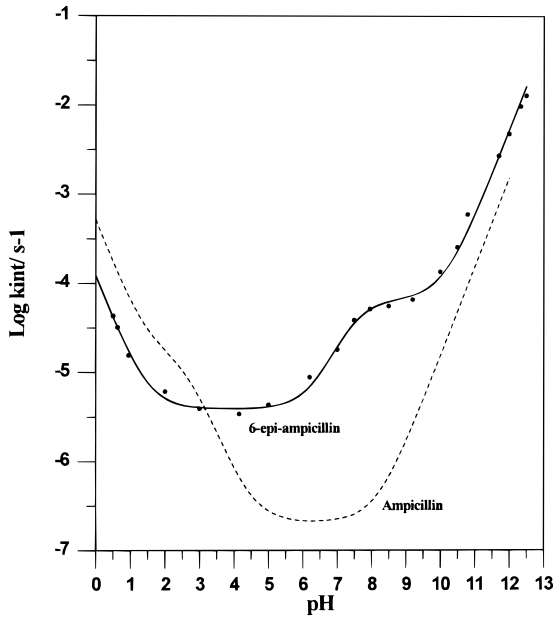


Figure 2. Plots of log k_{int}−pH profiles for ampicillin and 6-*epi*-ampicillin degradation in aqueous solution at 35 °C and an ionic strength of 0.5 mol dm^{-3}, where k_{int} is the apparent first-order rate constant (s^{-1}) for the degradation in buffer-free solutions or in buffers showing no effect on the degradation rate. The solid line represents the curve calculated from eq 2; the points are experimental values.

reaction of hydroxide ion with the undissociated acid (AH_2^+). At about pH 6, there is another increase in rate that is pH dependent and indicative of a change in mechanism[27] corresponding to the hydroxide-ion-catalyzed reaction of the zwitterion ($AH^{\pm}$) or the kinetically equivalent uncatalyzed reaction of the basic form of *epi*-ampicillin (A^-) with the side chain amine residue unprotonated. The rate then continues to increase until pH 7.3, corresponding to the pK_a of the amine side chain, when the rate again becomes pH independent. Finally, there is a change in mechanism at pH 10, when a pH-dependent reaction takes over corresponding to the hydroxide-ion catalyzed reaction of the anion (A^-).

There are thus four major reaction pathways: (i) an acid-catalyzed reaction of AH_2^+, (ii) a pH-independent reaction of $AH^{\pm}$, (iii) a pH-independent reaction of A^-, and (iv) a hydroxide-ion-catalyzed reaction of A^- or their kinetic equivalents.

Product analysis shows that all pathways, except iii, correspond to hydrolysis. The product of the reaction of 6-*epi*-ampicillin between pH 6 and 10 is the diketopiperazine derivative (**6**), the result of intramolecular aminolysis (Scheme 2).

The pH−rate profile (Figure 2) can be described by eqs 1 and 2.

$$\text{rate} = k_H[H^+][AH_2^+] + k_0[AH^{\pm}] + k_a[A^-] + k_{OH}[OH^-][A^-] \quad (1)$$

$$k_{int} = k_H(H^+)\frac{(H^+)}{K_1 + (H^+)} + k_0\frac{K_1}{K_1 + (H^+)} \times \frac{(H^+)}{K_2 + (H^+)} + k_a\frac{K_2}{K_2 + (H^+)} + k_{OH}(OH^-)\frac{K_2}{K_2 + (H^+)} \quad (2)$$

where k_H is the second-order rate constant for the acid-assisted hydrolysis of 6-*epi*-ampicillin, k_0 is the first-order rate constant for the pH-independent hydrolysis of 6-*epi*-ampicillin, k_a is the first-order rate constant for the intramolecular aminolysis of the anionic (A^-) species of 6-*epi*-ampicillin, and k_{OH} is the second-order rate constant for the hydroxide-ion-catalyzed hydrolysis of A^-.

The various rate constants of eq 1 were determined by the use of the PEAKFIT[22] computer program. The

Table 1. Rate Constants for Degradation of Some Cephalosporins and Penicillins at 35 °C and an Ionic Strength of 0.5 mol dm^{-3}

antibiotic	$10^5 k_H$ (dm^3 mol^{-1} s^{-1})	$10^7 k_0$ (s^{-1})	$10^6 k_a$ (s^{-1})	k_{OH} (dm^3 mol^{-1} s^{-1})
6-*epi*-ampicillin (**3**)	13.90	41.70	61.10	0.250
ampicillin (**7**)	38.30	≤2.78		0.540
cephaloglycin[a] (**9**)	4.17	13.90	37.50	0.364
cephalexin[a] (**10**)		3.19	2.80	0.073
cephradine[a] (**11**)		3.05	2.05	0.110
cephadroxil[b] (**12**)		2.61	4.47	0.070
cephaloridine[a] (**15**)	3.72	12.22		1.078
benzylpenicillin[c] (**16**)	13500			0.154

[a] Data from ref 7. [b] Data from ref 14. [c] Data from ref 28.

values obtained are given in Table 1 together with corresponding values for some other cephalosporins and penicillins. The good agreement between experimental and theoretical data indicates that eq 1 describes adequately the rate constant of 6-*epi*-ampicillin degradation as a function of pH (Figure 2).

The pathways for the degradation of 6-*epi*-ampicillin in aqueous solution from pH 0.5 to 13 are shown in Scheme 3. At pH less than 2, 6-*epi*-ampicillin exists predominantly as the cationic species (AH_2^+) and specific hydrogen-ion-catalyzed hydrolysis occurs. The principal reaction in the pH-independent region extending from pH 2.5 to 6 is the hydrolysis of the zwitterion ($AH^{\pm}$). Between pH 6 and 10, where the kinetic term k_a is dominant, the major product is the diketopiperazine derivative as a result of intramolecular aminolysis by the attack of the unprotonated side chain amino group on the β-carbonyl carbon. Above pH 10, the main reaction is hydroxide-ion-catalyzed hydrolysis of (A^-).

There is nothing unusual about the relative rates and magnitude of the base-catalyzed hydrolysis of 6-*epi*-ampicillin and ampicillin (Table 1). The rate constants k_{OH} are similar to each other and to that observed for benzylpenicillin, which in turn is not, in general, significantly different from the k_{OH} for cephalosporins (Table 1). The expected steric effect of 6-α substituents retarding nucleophilic attack on the β-lactam carbonyl carbon from the α-face of penicillins is not large.[1]

The rate of alkaline hydrolysis of penicillins and cephalosporins is influenced by the nature of the substituents in the two rings.[1] For example, electron-withdrawing substituents at C-6 in penicillins and at C-7 in cephalosporins facilitate nucleophilic attack of hydroxide ion on the β-lactam carbonyl carbon.[28] The 6-β-acylamido side chain in penicillins fits the Hammett plot generated by other substituents and the rate-enhancing effect of the amide substituent compared with no substituent at C-6 is due entirely to an inductive effect, and there is no evidence of neighboring group participation by this group during alkaline hydrolysis.[28]

The acid-catalyzed degradations of 6-acylamidopenicillins show rate enhancements of ca. 10^3 compared with that predicted from the Hammett plot for 6-substituents.[28] This is the result of neighboring group participation by the amide side chain probably trapping the ring opened acylium ion intermediate[28,29] to give penicillenic acid.[1,30] Interestingly, this reaction does not occur with cephalosporins which are ca. 10^4 fold less reactive than penicillins toward acid-catalyzed hydrolysis.[1,28] The second-order rate constants, k_H, for the acid-catalyzed hydrolysis of the two epimers of ampicillin are similar (Table 1). However, that for 6-*epi*-ampicillin is 10^3 fold less than that for benzylpenicillin and is similar to the values observed for cephalosporins. The lack of neighboring group participation in the acid-catalyzed degradation of the two ampicillins is almost certainly due to the reduced nucleophilicity of the amide side chain because of the adjacent protonated amine residue (AH_2^+, Scheme 3).

Studies on cephalosporins with an amino group in the 7-β side chain (cephaloglycin (**9**),[6-9] cephalexin (**10**),[6,9,11] cephradine (**11**),[6,9,12] and cephadroxil (**12**),[14]) show intramolecular attack on the β-lactam by the primary amino group to yield the piperazine-2,5-dione derivatives, but this intramolecular aminolysis does not occur in similarly 6-β-substituted penicillins.[8,31-35] Intermolecular nucleophilic attack on the β-lactam of penicillins takes place preferentially from the α-side, and so, presumably, intramolecular attack from the sterically hindered β-side is unfavorable.[20-22] For example, ampicillin (**8**) with an amino substituent in the 6-β-acyl-amido side chain does not show intramolecular aminolysis. The crystal data for ampicillin from the Cambridge Structural Database[36] (CSD code, Amcill)[37] indicates that the 2-β-methyl group and the 3-hydrogen could block nucleophilic attack at the β-lactam carbonyl carbon from the β-face.

Intramolecular aminolysis does, however, occur from the less-hindered exo face/α-side of 6-*epi*-ampicillin (**3**) to give the diketopiperazine derivative (**6**) (Scheme 2). The theory of stereoelectronic control[17] predicts that the preferred direction of attack would be from the endo face/β-side, as the lone pair of electrons on the β-lactam nitrogen is located primarily on the α-face and so would be anti to the attacking nucleophile. Which direction is actually followed depends on the balance between steric and stereoelectronic effects. A measure of the effectiveness of the intramolecular reaction is the ratio of the rate constants for intra- to intermolecular aminolysis—the effective molarity.[38] For the 6-*epi*-ampicillin, the rate constant for the uncatalyzed intramolecular aminolysis, k_a, is 6.11×10^{-5} s^{-1}, compared with a second-order rate constant of 1.6×10^{-6} mol^{-1} dm^3 s^{-1} for the intermolecular aminolysis of benzylpenicillin with an amine of pK_a 7.4, i.e., an effective molarity of only 40 mol dm^{-3}. This is similar to the effective molarity of about 20 mol dm^{-3} calculated for cephaloglycin (the rate constant for the uncatalyzed intramolecular aminolysis of cephaloglycin is 3.75×10^{-5} s^{-1}, and the estimated rate constant for the equivalent intermolecular reaction for an amine of pK_a 7.0 is 2.0×10^{-6} mol^{-1} dm^3 s^{-1}).[39]

If both the intramolecular and analogous intermolecular reactions are free of strain energy effects, then the entropy difference between the two systems can give

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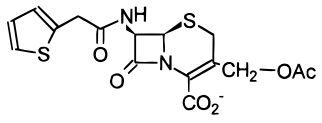
(39) Proctor, P.; Page, M. I. *J. Am. Chem. Soc.* **1984**, *106*, 3820.

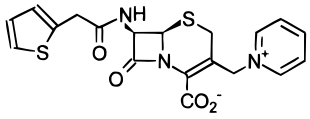
effective molarities of up to 10^8 mol dm^{-3}.[38,40] Effective molarities are less than this if ring closure in the intramolecular reaction is accompanied by an increase in strain energy in the transition state.[38,40]

If we accept that the conformation of ampicillin in solution (2-β,3-α equatorial) is the same as or not very different from the one derived from X-ray analysis,[36,37] there appears little evidence of steric hindrance to the approach of the nucleophiles from the α-side of 6-*epi*-ampicillin. This is also confirmed by theoretical calculations described later and so seems not to be responsible for the low effective molarity values obtained for intramolecular aminolysis of 6-*epi*-ampicillin.

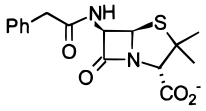
Intramolecular aminolysis in the reactions of δ-amino esters to form δ-lactams generally shows low effective molarities of 500−2500 mol dm^{-3}.[41] These values can be rationalized on the basis of the strain energy of the six-membered ring and the loss of entropy associated with internal rotation.[38,40,42] Ring closure in 6-*epi*-ampicillin does not require quite the same loss of entropy of internal rotation, and the effective molarity may therefore be expected to be greater. One obvious problem with ring closure in 6-*epi*-ampicillin is that the 6-α-amide is between the amine nucleophile and the β-lactam carbonyl carbon and exists predominantly in the thermodynamically preferred trans configuration (**17**) (see next section). Intramolecular aminolysis can only occur if the amide is converted to the less favorable cis configuration (**18**). The difference in energy between the two configurations is ca. 4 kcal mol^{-1},[43] so the concentration of the cis amide is thus $\leq$0.1%. Thus, the effective molarity for ring closure will be reduced by about 10^3, and the observed value of 40 M is in line with those of other ring closures to six-membered rings.



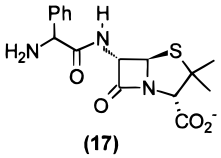
Cephalothin **(14)**



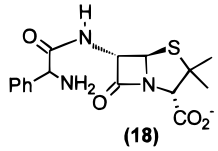
Cephaloridine **(15)**



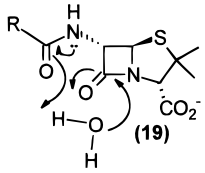
Benzylpenicillin **(16)**



(17)



(18)



(19)

Further studies on the diketopiperazine derivative (**6**) showed the appearance of a new compound that had a strong absorption at 230 nm. The diketopiperazine derivative, in neutral aqueous or basic conditions, undergoes slow oxidation of the diketopiperazine ring and ring opening of the thiazolidine to give compound (**7**) (Scheme 3). Compound **7** was assayed for thiol using the method of Ellman,[44] giving a positive test. ^{1}HNMR studies of the degradation of **6** showed the disappearance of the signals at 4.32 and 5.25 ppm (5-H and 6-H) and those of the two amine NHs (7.88 and 8.49 ppm), while a new signal appears at 6.77 ppm, attributable to the vinylic proton.

Although the rates of alkaline hydrolysis of penicillins and cephalosporins are similar (Table 1), only the latter exhibit a significant pH-independent hydrolysis (Figure 1). The hydrolytic behavior of 6-*epi*-ampicillin (Figure 2) is therefore unusual for a penicillin in that it does show a pH-independent hydrolysis with a k_0 of 4.17×10^{-6} s^{-1} (Table 1).

The spontaneous hydrolysis of cephalosporins has been attributed to the attack of the oxygen of the side chain amido group on the β-lactam carbonyl,[6,7,45,46] although there is no experimental evidence to support this mechanism. Furthermore, this proposal does not explain the different reactivity of penicillins and cephalosporins toward "spontaneous" hydrolysis and, more importantly, the pH-independent hydrolysis of 6-*epi*-ampicillin but not ampicillin. The enhanced rate of hydrolysis of 6-*epi*-ampicillin compared with that of ampicillin (Figure 2) is not due to nucleophilic participation of the 6-α-amido side chain as there is no evidence of diketopiperazine formation between pH 2 and 5. Intramolecular general acid catalysis from the protonated amino group in the 6-α side chain is conceivable by facilitating breakdown of the tetrahedral intermediate and β-lactam C−N fission. However, this requires the unfavorable isomerization of the trans to cis configuration of the 6-α-amido side chain, the formation of an eight-membered ring for proton transfer, and the formation of the conjugate acid of the carboxylic acid product to explain the kinetic pH independence. An alternative role for the amide side chain is that of an intramolecular general base catalyst (**19**), and this is supported by a kinetic solvent isotope effect of 2.1 for the pH-independent hydrolysis of 6-*epi*-ampicillin, measured at pH 3.7 and 4.7.

Nucleophilic attack on penicillins occurs from the α-side, and ampicillin is twice as reactive toward hydroxide ion compared with 6-*epi*-ampicillin (Table 1), presumably because of a small steric effect. By contrast, k_0 for 6-*epi*-ampicillin is at least 15-fold greater than that for ampicillin. This supports the idea of neighboring group participation in the α-substituted derivative where the amide side chain can facilitate the general base-catalyzed attack of water. This may be of relevance to some enzyme-catalyzed hydrolysis reactions where, for example, site-directed mutagenesis may remove the amino acid residue normally responsible for general base

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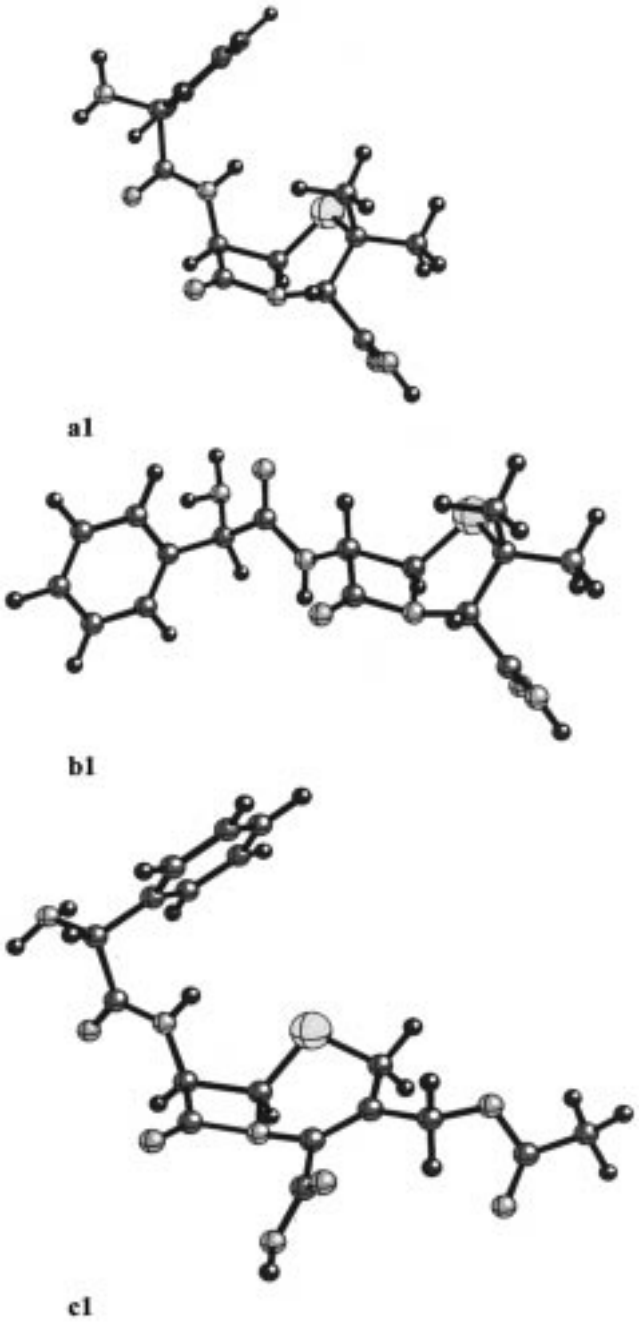

Figure 3. Minimum energy structures of ampicillin (**a1**), 6-*epi*-ampicillin (**b1**), and cephaloglycin (**c1**).

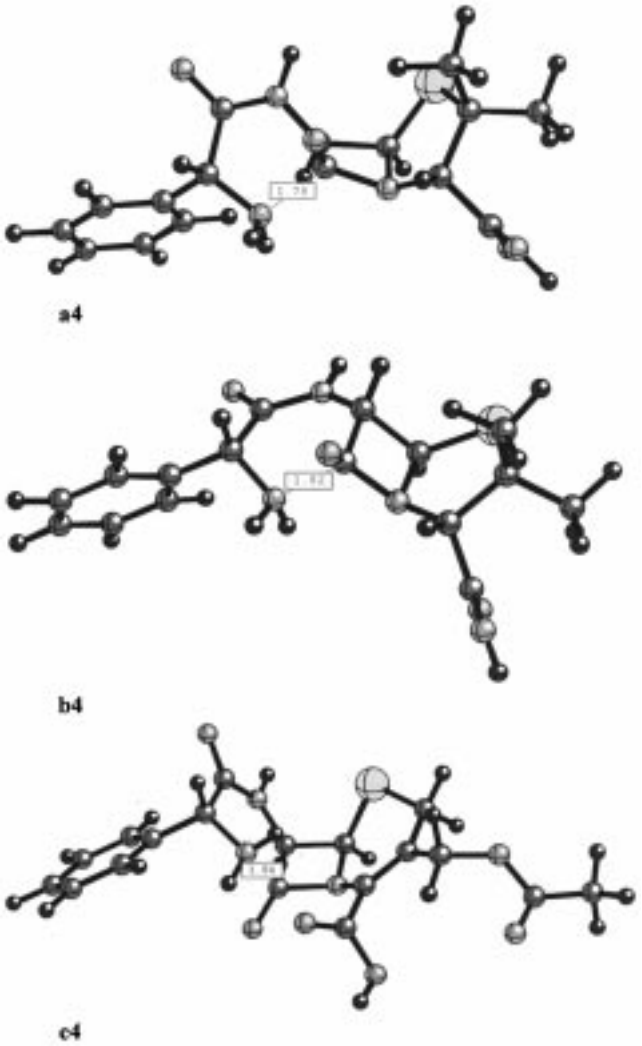

Figure 4. Structures corresponding to the transition state of the intramolecular attack to the β-lactam carbonyl group of ampicillin (**a4**), 6-*epi*-ampicillin (**b4**), and cephaloglycin (**c4**).

catalysis in say a serine protease or β-lactamase and yet the enzyme still shows residual catalytic activity.

Intramolecular general base catalysis by the amide could also explain the observation of a pH-independent hydrolysis in cephalosporins but not in penicillins, except if the latter is α-substituted, when neighboring group participation can occur. Furthermore, the possibility of observing a pH-independent hydrolysis in penicillins is overwhelmed by their enhanced rate of acid-catalyzed hydrolysis so that the latter is seen even at pH 6.

Intramolecular general base catalysis by an amido group is unusual because of the low basicity of the amide, the conjugate acids of which have a pK_a of ca. −1, and proton transfer from the attacking water, even with significant β-lactam carbonyl carbon−water oxygen bond formation in the transition state, is not thermodynamically favorable. The Bronsted β-value of general base-catalyzed hydrolysis of penicillins[47] is 0.38, and an estimated second-order rate constant for a base of conjugate acid of pK_a −1 is 5.6×10^{-7} M^{-1} s^{-1}. The effective molarity for the intramolecular reaction is therefore about 7.5 M, which is a typical value for a reaction involving proton transfer between electronegative atoms.[48]

2. Theoretical Calculations. 2.a. Intramolecular Aminolysis. Results from the previous section show that 6-*epi*-ampicillin undergoes intramolecular aminolysis, while ampicillin and other penicillins with an amino group in a 6-β side chain do not. On the other hand, cephalosporins with amino substituents in the 7-β side chain do undergo intramolecular aminolysis.

Intramolecular attack from the β-side of the β-lactam ring of a cephalosporin takes place with little steric hindrance because the dihydrothiazine ring fused to the β-lactam is quite planar and it does not present substituents which can interact with the 7 side chain.[2−5] In the case of ampicillin, the geminal 2-methyl of the thiazoli-

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Table 2. Energy and Main Bond Lengths and Dihedral Angles of Stationary States of Intramolecular Aminolysis

	penicillin (cephalosporin)[a]											
	a1	a2	a3	a4	b1	b2	b3	b4	c1	c2	c3	c4
C_7-N_4 (C_8-N_5) (Å)	1.448	1.448	1.447	1.503	1.444	1.445	1.445	1.501	1.436	1.434	1.435	1.503
$N_{12}-C_7$ ($N_{12}-C_8$) (Å)	5.746	5.353	3.146	1.778	6.084	5.623	3.136	1.819	6.010	5.941	3.157	1.840
$N_9C_{10}-C_{11}N_{12}$ ($N_9C_{10}-C_{11}N_{12}$) (deg)	−136.8	−139.0	42.9	24.9	−135.8	−138.6	−41.6	−41.3	177.0	−159.0	45.2	32.7
$C_7N_4-C_5C_6$ ($C_8C_5-C_6C_7$) (deg)	−6.5	−5.5	−6.2	−20.5	−3.9	−3.9	−3.2	−3.0	−4.2	−4.8	−4.4	−2.9
$N_{12}C_7-N_4C_5$ ($N_{12}C_8-N_5C_6$) (deg)	−44.7	10.2	43.8	112.8	27.6	4.8	−21.0	108.5	−16.4	−8.1	39.7	−102.0
ΔH_f (Kcal mol^{-1})	−89.13	−79.84	−86.69	−40.03	−89.80	−79.19	−86.19	−67.96	−155.23	−143.04	−151.20	-130.12

[a] Letters are (a) ampicillin, (b) 6-*epi*-ampicillin, and (c) cephaloglycine. Numbers are (1) *trans* conformation of the antibiotic, (2) transition state for the *trans*−*cis* conversion, (3) *cis* conformation of the antibiotic, and (4) transition state for the intramolecular aminolysis.

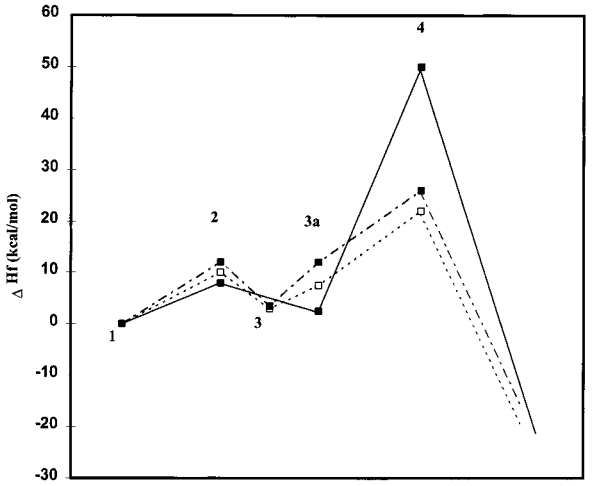

Figure 5. Reaction profile for the intramolecular aminolysis of (—) ampicillin, (···) 6-*epi*-ampicillin, and (- - -) cephaloglycin: (1) minimum for trans conformation; (2) transition state for the rotation of C−N bond (trans → cis); (3) minimum for cis conformation; (3a) structure **3** with the adequate orientation of the amino group; (4) transition state for the formation of the tetrahedral intermediate leading to intramolecular aminolysis. All heats of formation are relative to structure **1**. ΔH_f (**a1**) = −89.13 kcal/mol. ΔH_f (**b1**) = −89.80 kcal/mol. ΔH_f (**c1**) = −155.23 kcal/mol.

dine ring is placed over the β-face of the β-lactam ring, very close to the perpendicular of the carbonyl group, which generates a steric hindrance making the intramolecular aminolysis unfavorable. 6-*epi*-Ampicillin has the side chain in the 6-α-position, which allows intramolecular attack to take place from the more favorable α-direction. Molecular modeling shows that, after trans to cis isomerization of the acyl-amido side chain of 6-*epi*-ampicillin, the intramolecular amine can approach the β-lactam carbonyl relatively free from steric hindrances.

Low-level semiempirical AM1 calculations[49] were carried out to see if they supported the interpretation of the experimental observations. Figure 3 shows the molecular structures of ampicillin (**a1**), 6-*epi*-ampicillin (**b1**), and cephaloglycin (**c1**) optimized to an AM1 level. As expected, the side chain amide exists in the trans configuration of the amido group. Attack of the amine on the β-lactam carbonyl carbon requires rotation about the amide C−N bond to give the cis isomer of the amido group. The activation barrier of this process is calculated to be 9.3, 10.6, and 12.2 kcal/mol for ampicillin (**8**), 6-*epi*-ampicillin (**3**), and cephaloglycin (**9**), respectively. In these three cases, the trans amide is 3−4 kcal/mol more stable than the cis (see Figure 4 and Table 2) in agreement with experimental values.[43]

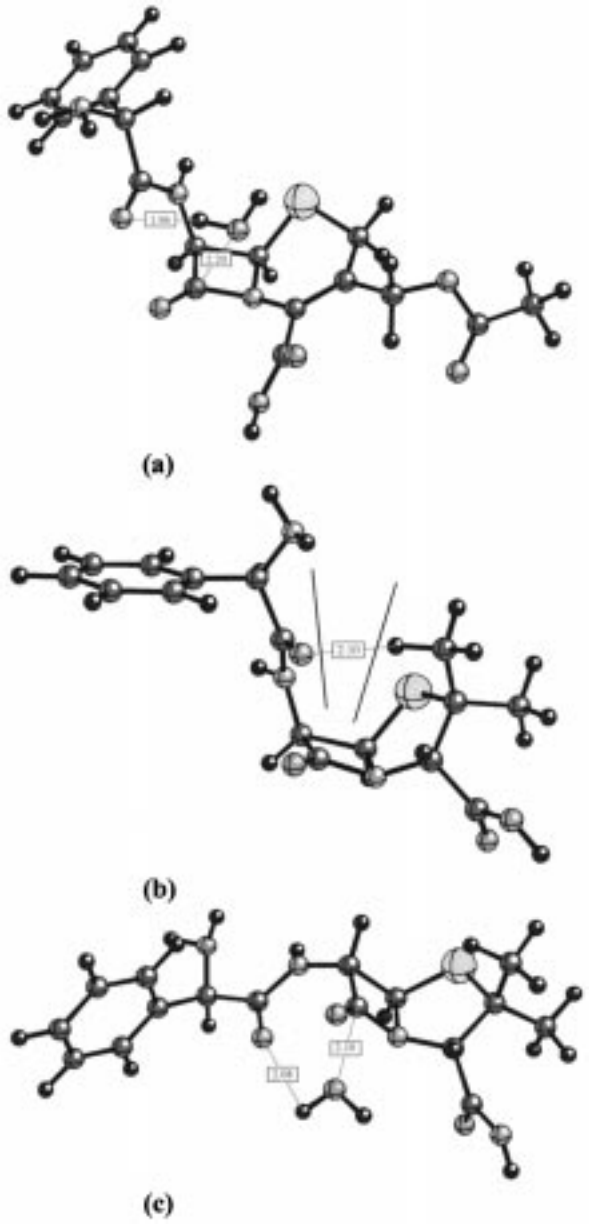

Figure 6. Structures showing the position of the water molecule involved in the catalytic process in cephaloglycin (a) and 6-*epi*-ampicillin (c) and the impossibility of this process in ampicillin (b).

(49) Frau, J.; Donoso, J.; Muñoz, F.; García-Blanco, F. *Helv. Chim. Acta* **1996**, *79*, 353; **1997**, *80*, 739. Frau, J.; Donoso, J.; Muñoz, F.; García-Blanco, F. *J. Mol. Struct. (THEOCHEM)* **1997**, *390*, 247.

Once the cis configuration has been obtained, only small conformational changes are required for intramolecular aminolysis to occur (**3a** in Figure 5). It has been demonstrated that reactions involving nucleophilic attack on the β-lactam carbonyl of penicillins and cephalosporins occur through the formation of tetrahedral intermediates.[1,47] The results of calculations on the activation energy for the formation of the tetrahedral intermediates are given in Figure 5. In the case of ampicillin, the transition state for the formation of the tetrahedral intermediate resulting from attack from the β-side could not be obtained due to the interaction of the side chain with the geminal 2-methyl group. Although intramolecular aminolysis in ampicillin from the α-side is possible (structure **a4** in Figure 4), the calculated activation energy is very high (46.6 kcal/mol) due to the formation of an energetically unfavorable trans fused ring junction.

However, with 6-*epi*-ampicillin, intramolecular nucleophilic attack from the α-side is easy and the transition state is characterized (structure **b4** in Figure 4), giving a calculated energy barrier of 14.4 kcal/mol. As with ampicillin, it is not possible to obtain the transition state for the intramolecular aminolysis from the β-side in 6-*epi*-ampicillin.

In the case of cephaloglycin (**9**), intramolecular aminolysis can theoretically occur from both sides. The activation energy for intramolecular aminolysis from the α-side is 52 kcal/mol, while for attack from the β-side, it is 13.8 kcal/mol (structure **c4**). In the case of cephalosporins, it appears that intramolecular aminolysis takes place preferentially from the β-side.

2.b. Influence of the Acyl-Amido Side Chain on Hydrolysis. Figure 6a shows the structure of the complex cephalosporin−water, which allows a direct interaction of the 6-β side chain amide carbonyl oxygen with the attacking water molecules in the hydrolysis of the β-lactam ring. Intramolecular catalysis is sterically inhibited in 6-β-substituted penicillins (Figure 6b), and hydrolysis has to occur from the α-side without any neighboring group participation. Furthermore, the enhanced rate of the acid-catalyzed reaction of penicillins compared with that for cephalosporins means that the pH range for pH-independent hydrolysis is limited. By contrast, an enhanced acid-catalyzed reaction is not seen in 6-*epi*-ampicillin for reasons discussed earlier, and intramolecular general base-catalyzed hydrolysis is possible with the α-amido side chain now α to facilitate α attack by water (Figure 6c), giving rise to the observed pH-independent hydrolysis of 6-*epi*-ampicillin.

Acknowledgment. This work was financed by the Spanish government via DGICYT Grant PB96-0596-C02-02.

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