The Aminolysis of Penicillin Derivatives. Rate Constants for the Formation and Breakdown of the Tetrahedral Addition Intermediate

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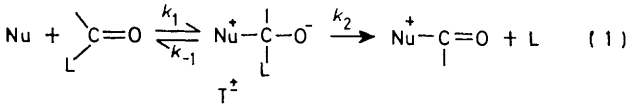
There is a non-linear dependence of the rate of aminolysis of benzylpenicillin and 6-β-aminopenicillanic acid upon hydroxide ion concentration which is interpreted in terms of formation of a tetrahedral addition intermediate. At high concentrations of hydroxide ion the rate-limiting step is formation of the tetrahedral intermediate but at low concentrations it is the diffusion-controlled encounter of the intermediate and hydroxide ion. Rate constants for the formation of the intermediate and its breakdown to reactants are reported for a variety of amines. The dependence of these rate constants upon the pK_a of the conjugate acid of the amine yield Brønsted β values of *ca.* 0.3 and −0.6 for the formation and breakdown of the intermediate, respectively. There is thus quite a large dependence of the rate of expulsion of the amine from the intermediate upon the basicity of the amine despite the rate constants for this step being *ca.* 10^9—10^{10} s^{-1}. Possible stereoelectronic control in the breakdown of the tetrahedral intermediate is discussed. There is no evidence for intramolecular general base catalysis in the formation of the tetrahedral intermediate from 6-β-aminopenicillanic acid.

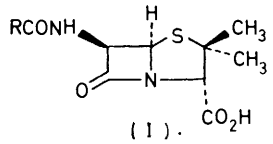
THE reactions of nucleophiles with carbonyl groups often proceed through tetrahedral addition intermediates. The stepwise mechanism appears to be true for reactions of derivatives of carboxylic acids [1] and of aldehydes and ketones.[2] Although it has been suggested [3] that certain conformations of tetrahedral intermediates have lifetimes which are short compared with the times for intramolecular rotations (*ca.* 10^{-12} s [4]) a number of stable derivatives are known.[5]

When nucleophilic substitution at the carbonyl centre occurs through the formation of a tetrahedral addition intermediate, $T^{\pm}$ [equation (1)], the bond to the attacking group is made before the bond to the leaving group L is broken. The experimental observation of the inter-

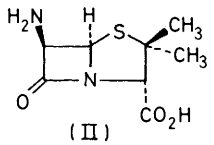


(1)

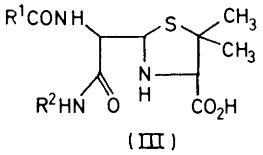
mediate depends on its relative stability and its rates of breakdown to reactants and products. The rate-limiting step of these nucleophilic substitutions is



(I).



(II)



(III)

determined by the relative rates of partitioning of the tetrahedral intermediate to reactants and products, k_2/k_{-1}. The existence of a tetrahedral intermediate is often deduced from the observation of a change in the rate-limiting step as the reaction conditions are varied which induce a change in the ratio k_2/k_{-1}.[6] Such a change in the rate-limiting step requires that there be at least two sequential steps in the reaction which demands that there be an intermediate in the reaction. Herein is reported kinetic evidence of a tetrahedral intermediate in the aminolysis of benzylpenicillin (I; R = $C_6H_5CH_2$) and 6-β-aminopenicillanic acid (II).[7]

EXPERIMENTAL

Materials.—Benzylpenicillin and 6-aminopenicillanic acid were of general reagent grade and other materials of AnalaR grade.

Kinetic Measurements.—The ionic strength was made up to *I* 1.0M by adding potassium chloride. The rates of reaction of benzylpenicillin and 6-aminopenicillanic acid were determined by following the change in absorbance at 235 nm on a Gilford 240 recording spectrophotometer having the cell compartment controlled at 30 ± 0.05°. The reactions followed a first-order course with the initial penicillin and 6-aminopenicillanic acid concentration being 5—10 × 10^{-4}M and the corresponding rate constants were calculated using a generalised least squares program which treated the first-order rate constant and the absorbances at time zero and infinity as disposable parameters.[8] Straight-line relationships were analysed using a linear least-square method.

RESULTS AND DISCUSSION

The aminolysis of penicillin (I) involves opening of the β-lactam ring and formation of the corresponding penicilloylamide (III). A similar reaction occurs in the formation of the major antigenic determinant of penicillin allergy when penicillin reacts with amino groups on proteins.[9] The rate law for the aminolysis of benzylpenicillin in buffered aqueous solutions below pH 11 at 30.0 °C has been previously described [10] and the observed pseudo first-order rate constant k_{obs} is given by equation

$$k_{obs} = k_{OH}[OH^-] + k_1[RNH_2] + k_2[RNH_2]^2 + k_3[RNH_2][OH^-] \quad (2)$$

(2), where k_{OH} is the first-order rate constant for the hydroxide-ion catalysed hydrolysis reaction.

A similar rate law is observed for the aminolysis of

6-aminopenicillanic acid (II) under the same conditions. The individual rate constants are normally determined using the amine as both buffer and reactant.[10,11] The rate constant k_3 for the hydroxide-ion catalysed reaction may also be determined in aqueous solutions of sodium hydroxide, in which more than 90% of the aminolysis reaction occurs through the k_3 pathway.

The observed pseudo first-order rate constants for the reaction of benzylpenicillin and 6-aminopenicillanic acid with various amines at different concentrations of hydroxide ion are given in Supplementary Publication No. SUP 22410 (3 pp.) * and are illustrated in Figure 1 as a function of the concentration of amine. There is a first-order dependence of the observed rate constants upon the concentration of amine. This is consistent with a negligible contribution of the k_2 term [equation (2)], general base catalysis, to the rate of aminolysis which is predicted from the known values of these rate constants and the concentrations of amine used.

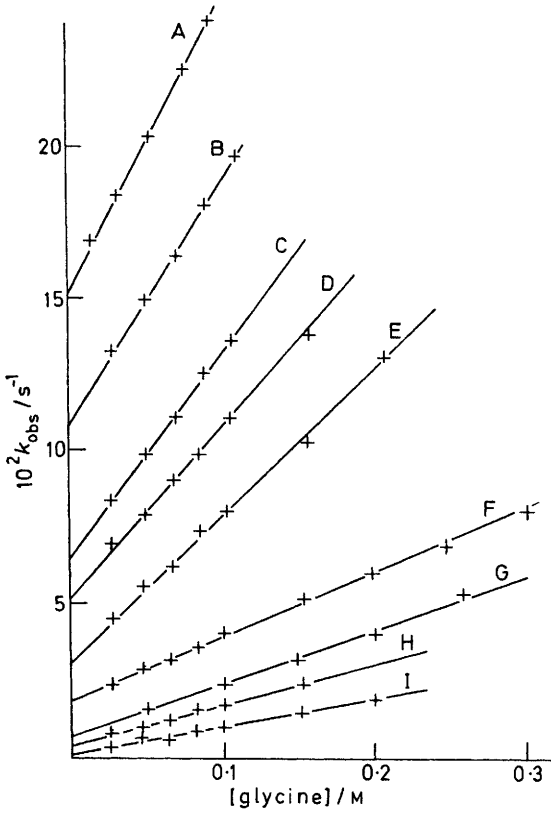


FIGURE 1 Plot of the observed pseudo-first-order rate constants for the reaction of glycine with benzylpenicillin in water as a function of the concentration of glycine at the concentration of hydroxide ion stated. 30.0°, ionic strength = 1.0 (KCl) unless otherwise stated. [NaOH]: A, 1.0 (I 1.1); B, 0.65; C, 0.40; D, 0.30; E, 0.20; F, 0.10; G, 0.05; H, 0.03; I, 0.02M

There is a non-linear dependence of the apparent second-order rate constants $(k_{obs} - k_o)/[RNH_2]$ obtained from the slopes of lines in plots such as those shown in Figure 1, upon the concentration of hydroxide ion (Figure 2). At low concentrations of hydroxide ion the

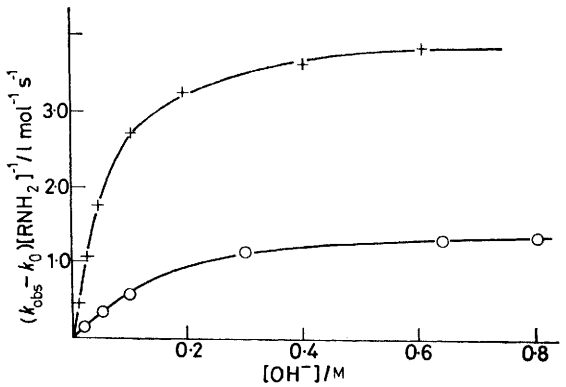
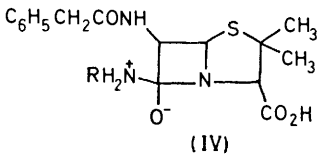


FIGURE 2 Plot of the apparent second-order rate constant $(k_{obs} - k_o)/[RNH_2]$ as a function of hydroxide ion concentration for the reaction of propylamine with benzylpenicillin, +, and 6-aminopenicillanic acid, ○. 30.0°, I = 1.0 (KCl). The curved lines are calculated from the constants given in the text

rate is first order in hydroxide ion and the initial slopes give values of k_3 which agree well with those determined at lower pH in buffer solutions.[10,11] At high concentrations of hydroxide ion the rate becomes independent of the concentration of hydroxide ion. This change in the kinetic dependence on hydroxide ion is indicative of a change in the rate-limiting step of the reaction which, in turn, requires that there be at least two sequential steps in the reaction. One of these steps is rate limiting at low concentrations of hydroxide ion and the transition state for this step contains hydroxide ion, or its kinetic equivalent. The other step is rate limiting at high concentrations of hydroxide ion but the transition-state for this step does not contain hydroxide ion. The existence of two sequential steps demands that there be an intermediate in the reaction,[6] which is probably the tetrahedral intermediate (IV).



(IV)

A mechanism compatible with the observations involves formation of a tetrahedral intermediate (IV) followed by diffusion of hydroxide ion into the same solvent cage as the intermediate. This step is followed by rapid proton transfer from the tetrahedral intermediate to hydroxide ion pursued by rapid collapse to

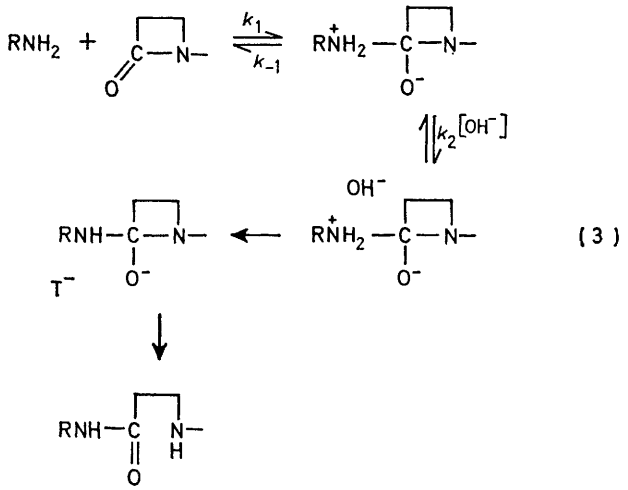
$$k_{obs} - k_o = k_1 k_2 [RNH_2] [OH^-]/(k_{-1} + k_2[OH^-]) \quad (4)$$

products † [equation (3)]. The apparent first-order rate constants for such a scheme is given in equation (4). At

* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

† It is conceivable that the proton transfer and carbonyl carbon β-lactam nitrogen bond fission occur in a concerted manner. This mechanisn depends upon the lifetime of the intermediate, T^-.[1] We have no evidence at present for or against this pathway.

low concentrations of hydroxide ion the rate of collapse of the tetrahedral intermediate to reactants must be



(3)

faster than its reaction with hydroxide ion, $k_{-1} \gg k_2[OH^-]$, 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 [12,13] pK_a values for the protonated amine of the tetrahedral intermediates (IV) are well below that for water. Proton transfer from the tetrahedral intermediate to hydroxide ion is therefore in the thermodynamically favourable direction and it is to be expected that the rate-limiting step for this process is the diffusion-controlled encounter of the proton donor and acceptor.[14] Support for the proposed mechanism comes from the Brønsted β value of 1.1 for the dependence of the hydroxide ion catalysed aminolysis of benzylpenicillin, the k_3 term in equation (2), upon the basicity of the amine.[10,11] This value indicates that the reaction behaves as if approximately a unit positive charge has been developed on the attacking amine in the transition state and this is consistent with rate-limiting diffusion together of the tetrahedral intermediate (IV) and hydroxide ion.

At high concentrations of hydroxide ion the tetrahedral intermediate and hydroxide ion diffuse together faster than the intermediate collapses back to reactants, $k_2[OH^-] \gg k_{-1}$. 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. The aminolysis of penicillin is catalysed by bases other than hydroxide ion but the rates of these reactions do not show a non-linear dependence upon the concentration of the base up to 0.5M-base.[10,11]

It is thought that the mechanism of the general base catalysed aminolysis is similar to that for the hydroxide ion catalysed reaction [equation (3)] except that the general base B replaces hydroxide ion as the proton acceptor.[10,11] Because no non-linearity is observed in the general base catalysed reaction, $k_2[B]$, the rate of reaction of the tetrahedral intermediate with the base B, must be less than k_{-1}. This is not surprising in view of the smaller rates of proton transfer from acids to bases compared with those to hydroxide ion.[14] Also because of the lower basicity of bases other than hydroxide ion proton transfer from the tetrahedral intermediate to the base may be thermodynamically unfavourable and the rate-limiting step may not be diffusion-controlled encounter of the base and the intermediate.[12]

Using equation (4) the individual rate constants of the proposed mechanism may be elucidated. From equation (5), a plot of the reciprocal of the apparent second-order

$$\frac{[RNH_2]}{k_{obs} - k_o} = \frac{1}{k_1} + \frac{k_{-1}}{k_1 k_2 [OH^-]} \quad (5)$$

rate constant, $(k_{obs} - k_o)/[RNH_2]$, obtained from the slopes of graphs such as those shown in Figure 1, against the reciprocal of the hydroxide ion concentration should give a straight line (Figure 3) of intercept $1/k_1$ and of slope k_{-1}/k_1k_2. If the reasonable assumption [14] is made that the diffusion-controlled step, k_2, has a value of *ca.*

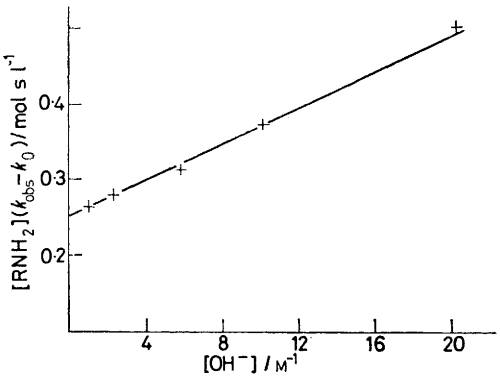
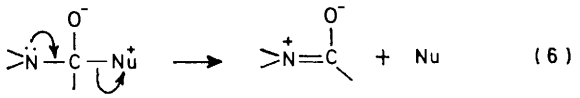


FIGURE 3 Plot of the reciprocal of the apparent second-order rate constant for the aminolysis of benzylpenicillin with propylamine as a function of the reciprocal of the hydroxide ion concentration

10^{10} l mol^{-1} s^{-1} then values of k_{-1} and the equilibrium constants for the formation of the tetrahedral intermediates may be obtained. Values of these constants for a variety of amines reacting with both benzylpenicillin and 6-aminopenicillanic acid are given in the Table. The rates of expulsion of the attacking amine from the tetrahedral intermediate to regenerate the reactants, the values of k_{-1}, are very rapid, *ca.* 10^9—10^{10} s^{-1}. Although these rate constants are very large they are of the order of magnitude that have been postulated for the breakdown of tetrahedral intermediates formed in acyl transfer reactions.[12,15] Normally changes in the rate-limiting step of acyl transfer reactions are difficult to detect. There are several reasons why such changes have been detected in the reactions of benzylpenicillin and 6-aminopenicillanic acid. The β-lactam of penicillins is an unusual amide. The susceptibility of penicillins to attack by nucleophiles has been attributed to strain in the β-lactam ring [16] and to the nonplanarity of the system which inhibits the usual amide resonance.[17] Carbonyl carbon–amine bond fission in the tetrahedral intermediate formed from penicillin (IV) to expel the attacking nucleophile is expected to be a relatively slower

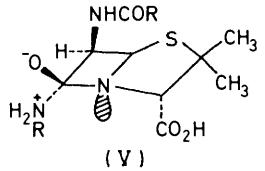
process than that found in normal tetrahedral intermediates. There are two reasons for this: (i) three-co-ordinate approximately sp^2 hybridised centres in four-membered rings are more strained than four-co-ordinate approximately sp^3 hybridised centres [18] and (ii) a significant driving force for the collapse of tetrahedral



(6)

intermediates is the conjugation of the amide nitrogen lone pair with the incipient carbonyl group [12] [equation (6)]. This resonance interaction, *ca.* 80 kJ mol^{-1} in normal amides,[19] is severely reduced in penicillins.[17]

An alternative, but broadly equivalent, interpretation of the reduced leaving group ability of the attacking amine is in terms of stereoelectronic control in the breakdown of tetrahedral intermediates.[3] It has been suggested that the ease of breakdown of a conformer of a tetrahedral intermediate depends upon the orientation of the lone pair orbitals of the heteroatoms. In particular, rapid expulsion of a group from a tetrahedral intermediate requires a lone pair on the heteroatoms attached to the central carbon atom to be antiperiplanar to the bond to be cleaved.[3] It is possible, because of the shape and the configuration of the penicillin molecule, that the incoming amine attacks the β-lactam carbonyl group preferentially from the least hindered side. The configuration of the tetrahedral intermediate would then be as shown in (V) in which the lone pair on the ring nitrogen is almost periplanar to the newly formed carbon-nitrogen bond. As the molecule cannot undergo a conformational change to make the lone pair on the ring nitrogen antiperiplanar to the carbon-attacking amine nitrogen bond the only driving force for rapid expulsion of the attacking amine from the tetrahedral intermediate



(V)

is an antiperiplanar lone pair on oxygen (V). The rate of expulsion of the nucleophile RNH_2 in (V) is thus expected to be slower than that in normal tetrahedral intermediates. Alternatively, it could be argued that if stereoelectronic control is sufficiently important to outweigh unfavourable steric interactions then attack should take place from the β-face as this is the microscopic reverse of having the β-lactam nitrogen lone pair antiperiplanar to the leaving group. Expulsion of the nucleophile RNH_2 would then be a slower process because of steric hindrance.

Summary of rate and equilibrium constants for reactions of amines with benzylpenicillin and 6-aminopenicillanic acid in water at 30.0° and ionic strength = 1.0M (KCl) [see equation (3)]

	Amine	pK_a	k_1/ l mol^{-1} s^{-1}	$10^{-8}k_{-1}$/ s^{-1}	$10^{11}K$/ l mol^{-1}	k_2K/ l mol^{-1} s^{-1}
Benzylpenicillin						
	2-Cyanoethylamine (CEA)	8.20	0.724	167	4.34	0.434
	2-Methoxyethylamine (MEA)	9.66	1.54	19.3	79.8	7.98
	Glycine (gly)	9.81	1.24	26.7	46.4	4.64
	Propylamine (PA)	10.89	4.07	4.59	886	88.6
6-Aminopenicillanic acid						
	2-Cyanoethylamine	8.20	0.43	360	1.20	0.120
	2-Methoxyethylamine	9.66	0.730	53.0	13.8	1.38
	Glycine	9.81	0.398	40.0	9.95	0.995
	Propylamine	10.89	1.64	10.2	161	16.1

The dependence of the rates of formation of the tetrahedral intermediates formed from benzylpenicillin and 6-aminopenicillanic acid and their rates of breakdown to reactants upon the basicity of the attacking amine are shown in Figures 4 and 5, respectively. The Brønsted-type β_{nuc} values for the formation of the tetrahedral intermediate are 0.30 and 0.29 for benzylpenicillin and

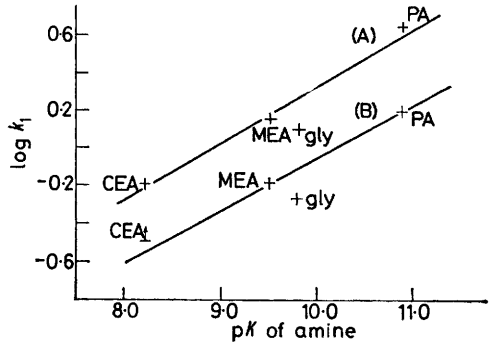


FIGURE 4 Brønsted plot for the formation of the trahedral intermediates (IV) from the reaction of amines with benzylpenicillin (A) and 6-aminopenicillanic acid (B) at 30°. A lower limit for the rate constant is indicated by an arrow. The abbreviations are identified in the Table

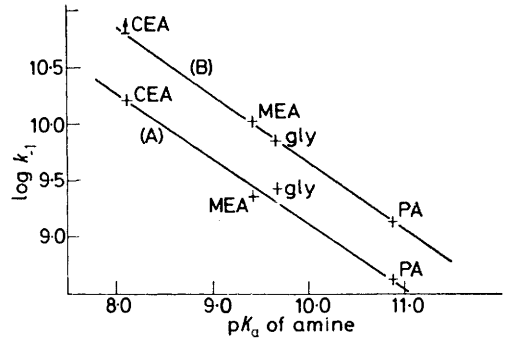
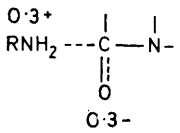


FIGURE 5 Brønsted plot for the breakdown of the tetrahedral intermediates (IV) of benzylpenicillin (A) and 6-aminopenicillanic acid (B) at 30°. A lower limit for the rate constant is indicated by an arrow. The abbreviations are identified in the Table

6-aminopenicillanic acid, respectively.* This indicates that the reaction behaves *as if* there is a *development* of *ca.* 0.3 of a positive charge on the attacking nitrogen in the transition state (VI). Similarly, the Brønsted-type β_{lg} values for the breakdown of the tetrahedral intermediate to reactants, k_{-1}, are −0.59 and −0.62 for



(VI)

benzylpenicillin and 6-aminopenicillanic acid, respectively. This indicates that the reaction behaves *as if* there is a *removal* of *ca.* 0.6 of positive charge on the amine nitrogen on going from the tetrahedral intermediate (IV) to the transition state (VI). It is, perhaps, a little surprising that even for such fast reactions as the expulsion of the attacking amine from the tetrahedral intermediate there is a significant dependency upon the basicity of the leaving group. If it is energetically important to have lone pairs on heteroatoms attached to the central carbon atom of a tetrahedral intermediate antiperiplanar to the bond to be cleaved [3] then perhaps the late transition state for expulsion of the attacking amine (VI) is due in part to the unfavourable arrangement shown in (V).

The dependences of the equilibrium constants for the formation of the tetrahedral intermediates from benzylpenicillin and 6-aminopenicillanic acid upon the basicity of the attacking amine give Brønsted β values of *ca.* 0.89 and *ca.* 0.91 respectively. This provides experimental support for the Brønsted β value of 1.0 that is often postulated [12,20] for the formation of the tetrahedral intermediate from amines and carbonyl groups, in which the amine nitrogen develops a unit positive charge, and presumably resembles the conjugate acid of the amine in structure and in its stability dependence upon substituents.

Possible other interpretations of the non-linear dependence of the rate upon hydroxide ion (Figure 2) deserve consideration, but may be excluded. Salt effects or self-association of hydroxide ion could be causes of the observed non-linearity.[21] The best argument against these interpretations is that linear plots are observed for the aminolysis of 6-aminopenicillanic acid with 2-cyanoethylamine under conditions identical to those in which benzylpenicillin yields data showing curvature. These conditions are also identical to those used for the other amines which gave a non-linear dependence of the rates upon hydroxide ion concentration for both 6-aminopenicillanic acid and benzylpenicillin. The reason why the rate of the hydroxide ion catalysed reaction of 6-aminopenicillanic acid with 2-cyanoethylamine shows a linear dependence upon hydroxide ion concentration is that under the conditions studied $k_{-1} \geqslant k_2[OH^-]$. The very rapid rate of breakdown could give rise to a change in the mechanism to one of the pre-association type.

Another possibility is that the aminolysis reaction proceeds by an elimination–addition type mechanism.[23] An *E*1cB mechanism would involve proton abstraction at C-6 to give an intermediate carbanion which then collapsed to a keten followed by reaction with the amine.[23] Changes in the rate-limiting step of reactions proceeding through *E*1cB mechanisms have been previously observed.[24] However, the hydroxide ion catalysed hydrolysis of benzylpenicillin in D_2O shows no incorporation of deuterium at C-6.[23] It seems unlikely, therefore, that an *E*1cB mechanism could explain the present observations.

In view of the similar values of the rate constants, k_1, for the formation of the tetrahedral intermediates from 6-aminopenicillanic acid and benzylpenicillin (Table) it appears that there is no evidence for intramolecular general base catalysis, in this step, by the neighbouring amino group in 6-β-aminopenicillanic acid. The differences in the rate constants may be adequately rationalised by electronic substituent effects. Of course, if amine attack does occur preferentially from the least hindered side to give the tetrahedral intermediate (V) then the neighbouring amino group is *anti* to the attacking amine and so not suitably placed to act as a catalyst.

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* Note these are obtained directly from plots of the logarithm of the rate constants against the pK_a of the conjugate acid of the amine *i.e.*, $-\log K_a$.

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