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# Intra- and Inter-molecular Catalysis in the Aminolysis of Benzylpenicillin

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The rate law for the aminolysis of benzylpenicillin in water is reported. The Brønsted  $\beta$ -values for the uncatalysed, the amine-catalysed, and hydroxide-ion catalysed reactions are 1.0, 1.09, and 0.96 respectively. This indicates that in the transition states for all three pathways the amine nucleophile contains a unit positive charge which is consistent with the formation of a tetrahedral intermediate. Intramolecular general base catalysis occurs with the reaction of ethylenediamine and, despite the importance of general base catalysis in the aminolysis reaction, the effective concentration of the catalysing base is only *ca*. 1M, which is attributed to the 'loose' transition state involved in intermolecular catalysis. Intramolecular general acid catalysis occurs with the reaction of ethylenediamine monocation. This suggests that nucleophilic attack takes place from the least hindered  $\alpha$ -side.

The reaction of amines with penicillins to give penicilloyl amides [equation (1)] is of interest because the major antigenic determinant of penicillin allergy is the penicilloyl group bound by an amide linkage to  $\varepsilon$ -aminogroups of lysine residues in proteins.<sup>1</sup> The formation of the penicilloyl haptenic groups could occur by the direct aminolysis of penicillin<sup>2</sup> or by the aminolysis of



penicillenic acid (I), formed from a rearrangement of penicillin,<sup>3</sup> or by the reaction of amines with the keten (II) <sup>4</sup> formed by an elimination mechanism.

The aminolysis of penicillin is also of concern because the reaction is an amide exchange, a normally difficult process but one which occurs readily with  $\beta$ -lactams.<sup>5</sup> It is of interest to examine the susceptibility of the reactions of penicillins to catalysis. Carbon-nitrogen bond fission in amides usually requires protonation of the nitrogen to avoid expulsion of the unstable amine anion <sup>6</sup>



but in  $\beta$ -lactams this process is accompanied by a large release of strain energy and this may modify the requirements for catalysis compared with normal amides. Another important difference between carbon-nitrogen bond fission in  $\beta$ -lactams compared with that in amides is that the latter may be accompanied by a more favourable entropy change as the molecule fragments into two separate entities.<sup>7</sup>

Because of the rigidity and shape of the penicillin molecule it is a suitable substrate to study the effectiveness of intramolecular catalysis and, in particular, to elucidate any preferred direction of nucleophilic attack upon the  $\beta$ -lactam carbonyl group.<sup>8</sup> In this and the following paper <sup>9</sup> we report a kinetic study and present a detailed mechanism of the aminolysis of benzylpenicillin.<sup>10</sup>

#### EXPERIMENTAL

Materials.—Benzylpenicillin (sodium salt) and benzylpenicillenic acid were of general reagent grade and other materials of AnalaR grade. The amines were purified by crystallisation of the hydrochlorides or by distillation. Freshly boiled deionised water was used throughout and the ionic strength maintained at 0.25M with potassium chloride unless otherwise stated. The buffers were prepared by partial neutralisation of the amine or amine hydrochloride just prior to the kinetic run.

Product Analysis.—Hydrolysis of penicillin yields penicilloic acid and aminolysis gives the corresponding penicilloyl amide. Penicilloyl amide was estimated by the penamaldate analysis <sup>11</sup> and by isolation. For example, a solution of benzylpenicillin in deuterium oxide was added to a partially neutralised solution of the amine in deuterium oxide at similar concentrations to those used in the kinetic experiments. The solution was stirred for ten half-lives and then neutralised to pH 1.2 with 30% DCl. The resulting white solid was filtered off and the filtrate extracted with ether-methanol and the solvent evaporated. The combined solid products were then converted into the methyl ester with diazomethane, weighed, spectral analysis performed, recrystallised, and analysed.

Kinetics.—The reactions were initiated by the addition of 25  $\mu$ l of aqueous benzylpenicillin (sodium salt) to 2.5 cm<sup>3</sup> of the aqueous amine buffer solution, pre-incubated at  $30.0 \ ^{\circ}C \pm 0.05 \ ^{\circ}C$ , with thorough mixing. The disappearance of penicillin was followed spectrophotometrically on a Gilford 240 spectrophotometer at 235 nm. The output from the spectrophotometer was fed into a Solartron data logger equipped with a Facit tape punch, thus enabling voltages proportional to the absorbance to be punched at constant time intervals. Rate constants were calculated from the results on an I.B.M. 1130 or I.C.L. 2960 computer using a generalised least-squares method which treated the absorbances at time zero and infinity and the first-order rate constant as disposable parameters.<sup>12</sup> The slopes and intercepts of linear relationships were determined using a linear least-squares method. The pH of all solutions was checked before and after each kinetic experiment and if it had changed by more than 0.03 the experiment was rejected.

RESULTS

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The aminolysis of benzylpenicillin at 30 °C in aqueous solutions of the amine follows the rate law of equation (2)

$$\frac{\text{Rate}}{[\text{Pen}]} = k_{\text{obs}} = k_{\text{OH}}[\text{OH}^-] + k_1[\text{RNH}_2] + k_2[\text{RNH}_2]^2 + k_3[\text{RNH}_2][\text{OH}^-]$$
(2)

where  $k_{\rm obs}$  is the observed pseudo-first-order rate constant for the disappearance of penicillin and  $k_{\rm OH}$  is the secondtherefore makes a negligible contribution to the observed rate. The intercept of plots of  $k_{\rm cat}/\alpha$  against  $\alpha$  at  $\alpha = 1.0$ gives the rate constant for the term in the rate law proportional to  $[\rm RNH_2]^2$ , *i.e.*  $k_2$ . The dominant form of buffer catalysis in the aminolysis is, therefore, general base catalysis.

The intercept of plots of  $(k_{\rm obs} - k_{\rm OH})/[\rm RNH_2]_{tot}$  against  $[\rm RNH_2]_{tot}$  (Figure 2) are designated  $k_{\rm int}$  and plots of  $k_{\rm int}$  against  $\alpha$  are non-linear and show upward curvature as is illustrated in Figure 4 for methoxyethylamine. This

Summary of the rate constants for the reaction of benzylpenicillin with amines at 30 °C in water; ionic strength 0.25 M (KCl)

			k <sub>1</sub> [RNH <sub>2</sub> ]	$k_2[\mathrm{RNH}_2]^2$	$\frac{k_{3}[\text{RNH}_{2}][\text{OH}^{-}]}{l^{2} \text{ mol}^{-2} \text{ s}^{-1}}$	
	Amine	$pK_{a}$	1 mol <sup>-1</sup> s <sup>-1</sup>	l <sup>2</sup> mol <sup>-2</sup> s <sup>-1</sup>	a	b
1	Propylamine	10.79	$1.32~ imes~10^{-2}$	1.05	48.0	88.6
2	Butylamine	10.60	$1.40  imes 10^{-2}$	0.74	35.0	<b>43.5</b>
3	1,2-Diaminoethane	10.07	$8.07  imes 10^{-2}$	0.528	16.7	
4	Ethanolamine *	9.73	$5.60~ imes~10^{-3}$	$7.75 \times 10^{-2}$	11.0	12.20
5	2-Methoxyethylamine	9.66	$1.40  imes 10^{-3}$	$3.58 imes10^{-2}$	6.50	7.98
6	Taurine	9.05	$1.30 imes10^{-3}$	$3.04 imes10^{-3}$	1.41	2.13
7	2-Cyanoethylamine	8.21	$1.00  imes 10^{-5}$	$3.50  imes 10^{-4}$		0.434
8	Hydrazine <sup>e</sup>	8.18	$1.01 \times 10^{-2}$	1.42		58.7
9	1,2-Diaminoethane (monocation)	7.43	$1.36 imes10^{-3}$	$5.87 imes10^{-3}$		
10	Trifluoroethylamine	5.81	$<\!6.9 imes10^{-6}$	$< 4.1 \times 10^{-6}$		$\leq 1 \times 10^{-3}$
11	Aminoacetonitrile	5.27	$<\!5.0 imes10^{-6}$	$< 3.0  imes 10^{-6}$		$\leq$ 1 $ imes$ 10 <sup>-3</sup>

<sup>a</sup> Determined in buffer solutions of the amine. Hydroxide ion concentration taken as antilog (pH – pK<sub>w</sub>) with pK<sub>w</sub> = 13.83. <sup>b</sup> Determined in solutions of sodium hydroxide. <sup>c</sup> Terms for  $k_4[N_2H_5^+]$  and  $k_5[N_2H_4][N_2H_5^+]$  were detected with  $k_4 = 1.75 \times 10^{-3}$  1 mol s<sup>-1</sup> and  $k_5 = 0.1$  l<sup>2</sup> mol<sup>-2</sup> s<sup>-1</sup>. \*2-Aminoethanol.

order rate constant for the hydrolysis reaction. The experimental conditions for the rate measurements and the observed rate constants are given as a Supplementary Publication \* and the derived rate constants are summarised in the Table. The procedure used for determining the rate constants in the Table will be briefly described.

Typical experimental data are illustrated in Figures 1-4. The observed pseudo-first-order rate constants for monoamines such as propylamine (Figure 1) exhibit a sharp upward curvature in plots against amine concentration which is indicative of a term which is second order in amine, *i.e.* one molecule of amine acts as the nucleophile and the other as a catalyst. The individual rate constants were determined using the slopes and intercepts of plots of the observed first-order rate constants, corrected for hydrolysis, divided by the total amine buffer concentration against the total amine buffer concentration, as shown for the reaction of methoxyethylamine in Figure 2. The steep slopes and relatively small intercepts in this figure illustrate the very large contribution of the catalysed reaction to the observed rate, even at low amine concentrations. The slope of these plots,  $k_{cat}$ , is equal to  $k_2 \alpha^2$  [equation (3)], where  $\alpha$ 

$$\frac{k_{\rm obs} - k_{\rm OH}}{[\rm RNH_2]_{\rm tot}} = k_1 \alpha + k_2 \alpha^2 [\rm RNH_2]_{\rm tot} + k_3 \alpha [\rm OH^-] \quad (3)$$

is the fraction of the free base of the amine, and a plot of  $k_{\rm cat}/\alpha$  against  $\alpha$  gives a straight line as illustrated in Figure 3 for butylamine. The intercept of these plots at  $\alpha = 0$  would give the rate constant for any term in the rate law proportional to [RNH<sub>2</sub>][RNH<sub>3</sub><sup>+</sup>] and for all the amines studied, except hydrazine, it was indistinguishable from zero. The general acid-catalysed aminolysis of penicillin

indicates that the hydroxide-ion catalysed reaction makes a significant contribution to the observed rate even in buffer solutions. A plot of  $k_{\rm int}/\alpha$  against the concentration of hydroxide-ion is linear and the intercept gives the rate



FIGURE 1 Observed pseudo-first-order rate constants for the reaction of propylamine with benzylpenicillin in water at the indicated fraction of free base in the amine buffers at 30  $^{\circ}$ C, ionic strength 0.25M. The lines are calculated from the rate constants in the Table

constant  $k_1$  and the slope gives the rate constant  $k_3$ , as is illustrated for propylamine in Figure 5. For all the monoamines studied, except hydrazine, there was no term in the

<sup>\*</sup> Supplementary publication No. SUP 22636 (6 pp.); for details of the supplementary publications scheme see Notice to Authors No. 7, *J.C.S. Perkin II*, 1978, Index issue.

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rate law proportional to  $\text{RNH}_3^+$ , the concentration of the conjugate acid of the amine.

The relative importance of the terms in equation (2) depends on the basicity and the concentration of the amine



FIGURE 2 Observed second-order rate constants for the reaction of 2-methoxyethylamine with benzylpenicillin in water at the indicated fractions of free base as a function of total amine concentration at 30 °C, ionic strength 0.25M

and the pH. For strongly basic amines the amine-catalysed,  $k_2$ , and the hydroxide-ion-catalysed,  $k_3$ , terms contribute most to the observed rate, with the  $k_2$  term, of course, being more important with increasing concentration of amine. Consequently, the rate constants,  $k_1$  for the uncatalysed reactions of basic monoamines are not of high precision. For the more weakly basic amines aminolysis



FIGURE 3 The dependence of the third-order rate constants,  $k_{eat}$ , for the reaction of butylamine with benzylpenicillin in water (from the slopes of graphs like Figure 2) upon the fraction of free base,  $\alpha$ , of the buffer. The left and the right ordinate intercepts give the catalytic constants for the acidic and the basic species of the buffer

occurs mainly through the uncatalysed,  $k_1$ , and aminecatalysed,  $k_2$ , pathways because of the low concentration of hydroxide ion. In fact the hydroxide-ion catalysed term,  $k_3$ , makes a negligible contribution to the observed rate of aminolysis in buffers of amines with a  $pK_a$  of less than *ca.* 9. In order to determine the  $k_3$  values for weakly basic amines the aminolysis reactions were carried out in solutions of sodium hydroxide and the amine. The concentration of sodium hydroxide was kept low to minimise hydrolysis of penicillin and also to avoid the change in ratelimiting step which occurs at high concentrations of hydroxide ion.<sup>13</sup> Plots of the observed first-order rate constants against the concentration of amine are linear as is illustrated in Figure 6 for ethanolamine and the intercept



FIGURE 4 The dependence of the second-order rate constants,  $k_{\rm int}$ , for the reaction of 2-methoxyethylamine with benzylpenicillin in water (from the intercepts of graphs like Figure 2) upon the fraction free base of the buffer

agreed well with the calculated rate of hydrolysis. The slope of this plot when plotted against the concentration of hydroxide-ion gives a straight line of slope  $k_3$ . Where the rate constants were obtainable in both buffer solutions and sodium hydroxide the agreement was within  $\pm 10\%$ .



FIGURE 5 The dependence of the second-order rate constant,  $k_{iat\alpha}$ , for the reaction of propylamine with benzylpenicillin in water (from the intercepts of graphs like Figure 2) upon the activity of hydroxide-ion. The slope gives  $k_3$  and the intercept  $k_1$ .

#### DISCUSSION

A common method used for the elucidation of reaction mechanisms is to study the effect of substituents in the reactants upon the reaction rate. The aminolysis of penicillin lends itself particularly well to this method



FIGURE 6 The dependence of the observed pseudo-first-order rate constants for the reaction of ethanolamine with benzylpenicillin in water at 30 °C and ionic strength 0.25M upon the concentration of amine at the indicated concentration of sodium hydroxide

because it has been possible to vary *independently* the reactivity of the nucleophile and the catalyst.

(I) Intermolecular Catalysis.—(a) General base catalysis. A plot of the rate constants,  $k_2$ , for the general base-catalysed aminolysis of benzylpenicillin for a series of primary monoamines is shown in Figure 7. The slope of this line gives a Brønsted  $\beta$ -value of  $1.09 \pm 0.09$ . This means that the reaction behaves as if approximately one positive charge is developed in the transition state that is distributed between the nucleophilic and the catalysing amine molecules. A Brønsted  $\beta$ -value of



FIGURE 7 The dependence of the rate constants  $k_2$  for the reaction of amines with benzylpenicillin upon the basicity of the amine at 30 °C, ionic strength 0.25M. The data are statistically corrected. The numbers refer to the amines listed in the Table

0.68 at 60 °C has been reported for this reaction but this was based on a series of diverse amines including imidazole.<sup>14</sup> Re-plotting of the quoted data, omitting imidazole, gives a Brønsted  $\beta$ -value of 0.90  $\pm$  0.03. A Brønsted value of 0.82 at 35 °C has also been reported for this reaction, but this included data for glycine ethyl ester which is known to hydrolyse under the reaction conditions and no mechanistic conclusions were drawn.<sup>15</sup> Our observed Brønsted value of ca. unity is indicative of a transition state in which full covalent bond formation between the nitrogen of the attacking amine and the carbonyl carbon has taken place and with a proton on either the nitrogen of the nucleophilic amine of the catalytic amine molecule. The simplest mechanism that is consistent with this observation is shown in the Scheme. The first step involves nucleophilic attack of the amine to form the tetrahedral intermediate  $T^{\pm}$ , for which there is independent kinetic evidence.9,13 However, the intermediate  $T^{\pm}$  breaks down rapidly to starting materials by expulsion of the attacking amine,

 $RNH_2$ 



 $k_{-1}$ . Catalysis of the reaction occurs by proton transfer from T<sup>±</sup> to a molecule of amine to form T<sup>-</sup>,  $k_{\rm B}$ , which then breaks down to products. It is not possible to infer from the observed  $\beta$ -value whether in the ratelimiting step of the reaction the proton is located on the attacking amine, on the catalytic amine, or between the two amines.

Unlike the hydroxide-ion catalysed aminolysis <sup>13</sup> there is no kinetic evidence of a change in the rate limiting step of the reaction with increasing amine concentration up to 0.5 mol l<sup>-1</sup>. This indicates that  $k_{\rm B}[{\rm RNH_2}] < k_{-1}$ , which is not unreasonable even if  $k_{\rm B}$  were the rate constant for diffusion-controlled encounter of T<sup>±</sup> and RNH<sub>2</sub> because  $k_{-1}$  is known to be 10<sup>9</sup>—10<sup>10</sup> s<sup>-1</sup>.<sup>13</sup>

If the uncatalysed aminolysis of penicillin with monoamines occurs by proton transfer from  $T^{\pm}$  to water then general base catalysis by a second molecule of amine is *ca.* 1 000 times more efficient, when compared on the molar scale. However, the uncatalysed reaction of monoamines does not occur by water acting as a general base catalyst [section I(d)] and so the rate difference is a lower limit, and is simply the result of the greater basicity of the amino-group compared with water.

The positive deviation of hydrazine from the Brønsted plot is a well known phenomenon that is attributed to

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the  $\alpha$ -effect.<sup>16</sup> The rate enhancement is *ca.* 1 000 fold and is similar in magnitude to the deviation for hydrazine from the Brønsted plot for the uncatalysed aminolysis reaction (Figure 10). This indicates that the  $\alpha$ -effect is manifested only when hydrazine acts as a nucleophile and not as a base.

(b) Hydroxide-ion catalysis. The Brønsted plot for the rate constants,  $k_3$ , for the hydroxide-ion catalysed reaction of benzylpenicillin with a series of primary monoamines is shown in Figure 8. The  $\beta_{nuc}$  value from the slope of this line is  $0.96 \pm 0.07$  which again indicates that the reaction behaves as if a unit positive charge is developed on the attacking amine in the transition state. The assignment of charge density is unambiguous, unlike the case for the general base-catalysed reaction, and the simplest interpretation of the  $\beta_{nuc}$ -value is that the attacking amine resembles its conjugate acid, *i.e.* it is fully protonated, in the transition state. This is compatible with the simplified mechanism of the Scheme in which the bond between the attacking amine and the carbonyl carbon is fully formed.

The  $\beta_{nue}$ -value also indicates the location of the proton in the transition state, for there can be little or no proton transfer from the attacking amine to the hydroxide-ion catalyst. It is consistent with ratelimiting diffusion-controlled encounter of the tetrahedral intermediate  $T^{\pm}$  and hydroxide ion. The mechanism of this reaction is discussed in more detail in the following paper.

At high concentrations of hydroxide ion the rate of aminolysis of benzylpenicillin becomes independent of the concentration of hydroxide ion.<sup>13</sup> This is indicative of a change in the rate-limiting step of the reaction and hence of an intermediate which is probably  $T^{\pm}$  (Scheme). At high concentrations of hydroxide ion the rate of proton abstraction by the latter from  $T^{\pm}$  is faster than the rate of breakdown of the intermediate and the ratelimiting step is amine nucleophilic attack upon the  $\beta$ -lactam carbonyl group, formation of the tetrahedral intermediate.<sup>13</sup>

(c) The unimportance of general acid catalysis. Breakdown of the tetrahedral intermediate  $T^{\pm}$  could be facilitated by either proton abstraction from the attacking amine or proton donation to the  $\beta$ -lactam nitrogen. The absence in the rate law of a significant term proportional to [RNH<sub>2</sub>][RNH<sub>3</sub><sup>+</sup>] indicates that general acid catalysis is not effective compared with general base catalysis. The reason for this is apparent from an examination of the relative acidities and basicities of the species involved. The  $pK_a$  of the attacking amine in the tetrahedral intermediate,  $T^{\pm}$ , is not very different from the free amine, RNH<sub>3</sub>.<sup>17</sup> Proton transfer from  $T^{\pm}$  to RNH<sub>2</sub> is therefore a near zero free-energy change process and will occur near or at the diffusion-controlled limit.<sup>18</sup> On the other hand the  $pK_a$  of the protonated  $\beta$ -lactam, T<sup>+</sup>, is estimated <sup>17</sup> to be ca. 5.2 and proton transfer from the buffer amine, RNH<sub>3</sub>, will be thermodynamically unfavourable and occur more slowly than proton transfer from  $T^{\pm}$  to  $RNH_2$  for amines of  $pK_a > 5$ . General acid catalysis is observed when more acidic catalysts are present.<sup>8,9</sup>

A term in the rate law proportional to  $[\text{RNH}_2][\text{RNH}_3^+]$ would be expected to show a non-linear Brønsted plot. For amines of  $pK_a > 5$  proton transfer from  $\text{RNH}_3^+$  to



 $T^{\pm}$  to give  $T^{+}$  is thermodynamically unfavourable and therefore the catalyst would resemble the free base,  $RNH_2$ , in the transition state of the rate-limiting step of this reaction. The observed Brønsted  $\beta$ -value would, therefore, be *ca.* 0 reflecting the summation of a  $\beta_{nuc}$ value of *ca.* 1 for formation of the tetrahedral intermediate  $T^{\pm}$  and a  $\beta$ -value of *ca.* -1 for formation of  $T^{+}$ . For amines of  $pK_a < 5$  proton transfer from  $RNH_3^+$  to  $T^{\pm}$  to give  $T^+$  is thermodynamically favourable and therefore the catalyst would resemble the conjugate acid



FIGURE 8 The dependence of the rate constants  $k_3$  for the reaction of amines with benzylpenicillin upon the basicity of the amine at 30 °C, ionic strength 0.25M. The data are statistically corrected. The numbers refer to the amines listed in the Table

of the amine,  $\text{RNH}_3^+$ , in the transition state of the ratelimiting step of this reaction. The rate-limiting step of this process would be the diffusion-controlled encounter of T<sup>±</sup> with  $\text{RNH}_3^+$  and therefore the rate constants for the term  $[\text{RNH}_2]^2$  and  $[\text{RNH}_2][\text{RNH}_3^+]$  should be similar. The observed Brønsted β-value for the rate constants of the  $[\text{RNH}_2][\text{RNH}_3^+]$  term for amines of  $pK_a < 5$  would therefore be *ca.* 1, reflecting the summation of a  $\beta_{\text{nue}}$ -value of *ca.* 1 for the formation of the tetrahedral intermediate  $T^{\pm}$  and a  $\beta$ -value of *ca.* 0 for formation of  $T^+$ . The expected Brønsted plot for general acid-catalysed aminolysis, reflecting a  $[RNH_2]$ - $[RNH_3^+]$  term in the rate law, is therefore as shown in Figure 9. Superimposed on this hypothetical plot is the observed Brønsted plot for general base catalysis. It is not surprising therefore that for basic amines there is no term in the rate law proportional to  $[RNH_2][RNH_3^+]$ .

(d) The uncatalysed pathway  $k_1$ . A plot of the rate constants  $k_1$ , for the uncatalysed aminolysis of penicillin, against the  $pK_a$  of the amine is shown in Figure 10. The slope of this line gives a Brønsted  $\beta_{nuc}$  value of  $1.0 \pm 0.08$  which indicates that the reaction behaves as if a unit positive charge is developed on nitrogen in the transition state. The uncatalysed pathway,  $k_1$ , could represent either a purely uncatalysed reaction of amine and penicillin or solvent catalysis with water acting either as a



FIGURE 9 The hypothetical dependence of the rate constants for the general acid-catalysed aminolysis of benzylpenicillin for amine buffers upon the basicity of the amine. Superimposed on this plot is the observed Brønsted plot for general base catalysis (dashed line)

general base, removing a proton from the attacking amine, or as a general acid, donating a proton to the  $\beta$ lactam nitrogen. The  $k_1$  pathway cannot represent ratelimiting formation of the tetrahedral intermediate because the  $\beta_{nuc}$ -value for this is known to be 0.3 and the rate constants are known to be much greater than the observed  $k_1$  values.<sup>13</sup> The rate constant  $k_1$  for hydrazine divided by the concentration of water, 55M, gives a third-order rate constant with a large positive deviation from the Brønsted plot for general base-catalysed hydrazinolysis <sup>9</sup> which indicates that water is not acting as a proton acceptor. It is not possible to distinguish between uncatalysed rate-limiting breakdown of the tetrahedral intermediate, T<sup>±</sup>, (III) and water general acid-catalysed breakdown of the same intermediate (IV).

The equilibrium constant,  $K_1$ , for the formation of  $T^{\pm}$  from penicillin and propylamine is known to be  $8.86 \times 10^{-9} \, \mathrm{l} \, \mathrm{mol}^{-1.13}$  Because the rate constant for the uncatalysed reaction,  $k_1$ , is given by  $k_u K_1$  (Scheme),  $k_u$ , the rate constant for the uncatalysed or water-catalysed breakdown of the tetrahedral intermediate, is

 $k_1/K_1 = 1.5 \times 10^6 \text{ s}^{-1}$ . The rate of protonation of the  $\beta$ -lactam nitrogen of T<sup>±</sup> by water may be estimated from its  $pK_a$  of 5.2 to be ca.  $10^3 \text{ s}^{-1}$ . This means that the uncatalysed breakdown of T<sup>±</sup> cannot proceed by stepwise proton transfer from water to the  $\beta$ -lactam nitrogen. However, it could occur either by a concerted mechanism — proton transfer from water occurring synchronously



FIGURE 10 The dependence of the rate constants  $k_1$  for the reaction of amines with benzylpenicillin upon the basicity of the amine at 30 °C, ionic strength 0.25M. The data are statistically corrected. The numbers refer to the amines listed in the Table

with carbon-nitrogen bond fission—or uncatalysed expulsion of the nitrogen anion. The rate of expulsion of the imidazole anion from the tetrahedral intermediate formed in the aminolysis of acetylimidazole is  $\geq 10^6 \text{ s}^{-1.17}$ If the strain energy of the  $\beta$ -lactam ring of *ca*. 120 kJ mol<sup>-1</sup> is relieved upon ring opening then expulsion of the nitrogen as the anion should be treated as a leaving group of  $pK_a$  *ca*. 10 rather than the normal value of 30 for ordinary amines. It is conceivable therefore that



carbon-nitrogen bond fission occurs without protonation of the  $\beta$ -lactam nitrogen (III).

(II) Intramolecular Catalysis.—(a) General base catalysis. The rate constant  $k_1$  for the reaction of 1,2diaminoethane with benzylpenicillin is ca. 30-fold greater than that predicted from the Brønsted plot (Figure 9) for a monoamine of the same basicity. The rate enhancement is not attributable to a peculiarity of 1,2diaminoethane compared with monoamines because the hydroxide-ion catalysed reaction of the diamine with penicillin shows no deviation from the Brønsted plot (Figure 8). The rate enhancement is interpreted as evidence for intramolecular general base catalysis of aminolysis by the second amino-group in 1,2-diaminoethane (V), which has been suggested previously for diamines.<sup>19,20</sup> Proton transfer occurs from the aminogroup that acts as the nucleophile to the terminal amino-group acting as a general base.



Most of this rate enhancement is a result of the greater basicity of the amino-group compared with water. Very little of the rate enhancement is attributable to intramolecularity, with the catalyst being covalently linked to the nucleophile. This is evident from the 'effective molarity' of ca. 1 mol  $l^{-1}$  for the reaction which is obtained by dividing the second-order rate constant,  $k_1$ , for the reaction of 1,2-diaminoethane with penicillin by the third-order rate constant,  $k_2$ , for intermolecular catalysis of aminolysis by a second molecule of amine of similar basicity. The 'effective molarity' is the concentration of catalysing amine required to give the same rate of reaction as the diamine. Similar small ' effective molarities' have been observed for the reaction of diamines with acetylimidazole.<sup>20</sup> Intramolecular catalysis is observed because of the importance of general base catalysis in these reactions compared with uncatalysed aminolysis.

The small effective molarity of the catalysing amine group of the diamine could be caused by any one of the following: (1) An unfavourable enthalpy effect in the *intramolecular* reaction such as strain effects introduced upon ring ' closure '.<sup>7,21,22</sup> (2) An entropy effect that reflects the not-so-unfavourable entropy change accompanying the *inter*- and bi-molecular reaction that has a 'loose' transition state.<sup>7,20,22</sup> (3) An unfavourable entropy effect in the *intramolecular* reaction because of the loss of internal rotation upon ring closure. In the case of 1,2-diaminoethane this could reduce the effective molarity by a factor of *ca*. 10—100.<sup>7,21</sup>

Although there may be a significant enthalpic barrier to ring 'closure' to enable proton transfer to take place (V) it does not appear that this effect is dominant. In the similar reaction of acetylimidazole with diamines there is a remarkably small sensitivity of the rate acceleration to variations in the structure of the diamine which would not be expected if there was a large enthalpic barrier to ring 'closure'.<sup>20</sup> By analogy with the intermolecular general base-catalysed reaction the ratelimiting step in the intramolecular reaction is probably a conformational change.

It appears that the dominant contribution to the low effective molarity is the 'loose' transition state of the intermolecular reaction. The rate-limiting step of the intermolecular general base catalysed aminolysis of penicillin is the diffusion-controlled encounter of the tetrahedral intermediate,  $T^{\pm}$ , with the base <sup>9</sup> (Section I). The transition state is thus very loose and the bimolecular step  $k_{\rm B}$  in the Scheme will be associated with a small entropy change giving rise to the low effective concentration of the intramolecular reaction.<sup>7, 20, 22</sup>

Low effective molarities appear to be the norm for intramolecular general acid base-catalysed reactions  $^{7,22}$  and this suggests that very little of the enormous rate enhancements brought about by enzymes may be attributed to the fact that the general acid or base is *part* of the enzyme.<sup>23</sup>

(b) General acid catalysis. The rate constant  $k_1$  for the reaction of penicillin with the monocation of 1,2diaminoethane is ca. 100-fold greater than that predicted from the Brønsted plot (Figure 9) for a monoamine of the same basicity as  $NH_3CH_2CH_2NH_2$ . The rate enhancement is attributed to intramolecular general acid catalysis of aminolysis by the protonated amine. Breakdown of the tetrahedral intermediate,  $T^{\pm}$ , is facilitated by proton donation from the terminal protonated aminogroup to the  $\beta$ -lactam nitrogen (VI). It is not known at present whether proton transfer and carbon-nitrogen bond fission are concerted or occur by a stepwise process.

According to the theory of stereoelectronic control of Deslongchamps  $^{24}$  the breakdown of tetrahedral intermediates is facilitated by the lone pairs of the heteroatoms attached to the incipient carbonyl carbon being *anti*-periplanar to the leaving group. Application of this theory to the microscopic reverse steps predicts that the direction of nucleophilic attack on the carbonyl carbon to be such that the lone pairs on the heteroatoms will be *anti*-periplanar to the attacking group.

Penicillins have a fairly rigid structure because of the fusion of the  $\beta$ -lactam and the thiazolidine rings giving a V-shaped molecule.<sup>25</sup> The β-lactam nitrogen is consequently prevented from adopting the  $sp^2$  hybridisation found in normal amides and this is thought to reduce the conjugation between the nitrogen lone-pair and the carbonyl group.<sup>26</sup> Another consequence of the nonplanarity of the fused bicyclic ring system is that the electron density of the lone-pair of the  $\beta$ -lactam nitrogen will be concentrated heavily on the  $\alpha$ -face of the penicillin molecule (VII). According to the theory of stereoelectronic control 24 nucleophilic attack on penicillins should, therefore, take place from the  $\beta$ -side. However, this face is sterically hindered and we have previously suggested that nucleophilic attack may therefore take place from the least hindered  $\alpha$ -side.<sup>8,13</sup>

The observation of intramolecular general acid catalysis in the reaction with the monocation of 1,2-diaminoethane gives an indication of the direction of nucleophilic attack upon penicillin. In order that ready proton transfer takes place from the protonated amine to the  $\beta$ -lactam nitrogen it is essential that the tetrahedral intermediate has the geometry shown (VI). Although intramolecular general acid catalysis could conceivably take place if the amine attacked from the  $\beta$ -face (VIII) this would involve considerable non-bonded interactions and/or the proton transfer taking place through one or more water molecules. Further evidence for nucleophilic attack taking place from the  $\alpha$ -face comes from the



absence of intramolecular general base catalysis in the aminolysis of 6- $\beta$ -aminopenicillanic acid.<sup>13</sup> That the lone pair on the  $\beta$ -lactam nitrogen takes up the geometry shown with respect to the carboxy-group (VII) is supported by the observation that copper(II) ions catalyse the aminolysis of penicillin by co-ordination to the β-lactam nitrogen and the carboxy-group, thus stabilising the tetrahedral intermediate.<sup>4</sup> Thus nucleophilic attack on penicillins, at least by amines, appears to take place



from the least-hindered side in disagreement with the prediction of the theory of stereoelectronic control.<sup>24</sup>

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