Buffer Catalysis in the Hydrazinolysis of Benzylpenicillin

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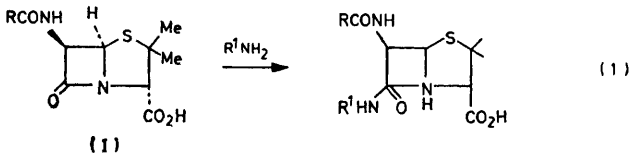
The Brønsted plot for general base catalysis of the reaction of hydrazine with benzylpenicillin in water is non-linear with limiting slopes of $\beta < 0.2$ and $\beta > 0.8$ for strong and weak bases, respectively. It is concluded that there is a change in the rate-limiting step of the reaction with changing basicity of the catalyst and it is suggested that the mechanism proceeds by a step-wise mechanism of proton transfer. For strongly basic catalysts the rate-limiting step is thought to be the diffusion-controlled encounter of the pre-formed tetrahedral intermediate and the catalysing base, whereas for weakly basic catalysts it is the diffusion apart of the deprotonated tetrahedral intermediate and the conjugate acid of the catalyst or the breakdown of the deprotonated tetrahedral intermediate in the presence of the conjugate acid of the catalyst. The α-effect for hydrazine is manifested by an increase in the equilibrium constant for formation of the tetrahedral intermediate of 350-fold, which is reflected by an increase of 15-fold in the value of k_1 and a decrease of 22-fold in the value of k_{-1}.

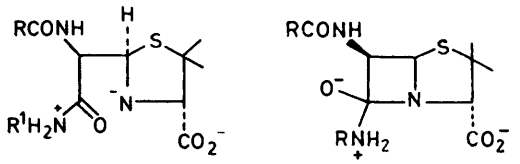
THE aminolysis of penicillin (I) is a substitution reaction in which an acyl group is transferred from one amino-group to another [equation (1)]. This reaction requires at least two proton transfers, proton removal from the attacking amine and proton addition to the leaving amino-group. These proton transfers are facilitated by buffers [1] and the kinetic importance of such catalysis is usually related to the observation that ' catalysis occurs where it is most needed '.[2] Buffer catalysis is needed in the aminolysis of penicillin because covalent bond formation and fission between heavy atoms is accompanied by large changes in the acidity and basicity of the reacting groups.[3] If a proton is not removed from



(1)

(I)

the attacking amine at some stage during the reaction the acidity of the NH group would change by *ca.* 40 pK units, from a pK_a of *ca.* 30 in the reactant to *ca.* −10 in the hypothetical *N*-protonated amide (II). Similarly bond fission of the β-lactam ring causes a change of *ca.* 35 pK units in the basicity of the leaving amino-group. These large changes in pK can give rise to unstable intermediates such as (II) and (III) and buffer catalysis is observed [1] because it can increase the rate of the reaction by either trapping such unstable intermediates or by stabilising or by-passing the transition states leading to their formation.[3]



(II) (III)

It is generally accepted that acyl-transfer reactions involve the intermediate formation of tetrahedral addition compounds,[4] such as (III), *i.e.* bond formation to the attacking group occurs before bond fission to the leaving group. The bond-breaking process may occur after, during, or even before [5] the rate-limiting step. Further complications in the elucidation of the detailed mechanism of acyl-transfer reactions arise from the problem of the timing of the proton transfer steps, *i.e.* are they concerted with, or separate processes from, covalent bond changes between heavy atoms? We have reported evidence for the formation of a tetrahedral intermediate during the aminolysis of benzylpenicillin based on the observation of a change in the rate-limiting step of the reaction.[6] In the preceding paper we reported Brønsted β-values for the general base-catalysed aminolysis of benzylpenicillin by variation of the structure of the nucleophile with a constant base, hydroxide ion.[1] Herein we describe the dependence of the rate upon the structure of the general base with a constant nucleophile, hydrazine, and propose a detailed mechanism of the aminolysis of penicillin.

EXPERIMENTAL

The materials and methods used in the experiments were similar to those described previously.[1] Unless otherwise stated the reactions were carried out in aqueous solutions at 30.0 °C and at ionic strength 0.25M, maintained with potassium chloride.

The hydrazinolysis of benzylpenicillin was usually carried out with 0.005—0.2M hydrazine in the presence of increasing concentrations of buffer solutions prepared from the catalyst being examined. For weakly basic catalysts the catalytic rate constants were determined from experiments carried out either in hydrazine buffers at a fixed concentration and pH with addition of varying concentrations of the sodium salt of the catalyst, or in buffer solutions of the catalyst with a fixed concentration of added hydrazine monocation.

The intercepts of the plots of rate constants against buffer concentration were found to agree closely with those calculated from the sum of the various hydrolysis and hydrazinolysis terms expected under the conditions of the experiment.[1] In acetate buffers and solutions of hydrochloric acid the reaction was accompanied by an *increase* in absorbance at 235 nm, unlike the decrease observed under all other conditions. The product was not identified under these acidic conditions. Except for the experiments in hydrochloric acid, benzylpenicillin was present predominantly with the carboxy-group ionised (p$K_\alpha = 2.7$).[7] Where catalytic terms could not be detected upper limits were

obtained by assuming that a 20% rate increase would not have been detected and assigning all this increase to the rate constant for general base catalysis.

RESULTS

The experimental conditions and rate constants are given in Supplementary Table 1 * and examples of the experimental results are shown in Figures 1—3. k_{obs} Is the observed pseudo-first-order rate constant and k_{cat} is the slope of a plot of k_{obs} against the total buffer concentration of each buffer or catalyst; these are corrected for terms first and second order in buffer concentration when these are significant. In Figure 1 is shown catalysis of hydrazinolysis by propylamine buffers at different pH values; the dashed lines show the reaction (aminolysis and hydrolysis) in the absence of hydrazine. Plots of k_{cat} against the fraction of free base in the buffer yield the rate constants for general base and any general acid catalysis of hydrazinolysis. Typical plots are shown in Figure 2. Except for

Summary of the rate constants for the general base-catalysed reaction of hydrazine with benzylpenicillin in water at 30 °C and ionic strength 0.25M (KCl)

	Base	pK_a [a]	k_3 (B, N_2H_4, Pen–CO_2^-) / l^2 mol^{-1} s^{-1}
1	Hydroxide-ion	15.74	58.7
2	Trifluoroethoxide ion	12.27	6.00
3	Quinuclidine	11.46	4.64
4	Propylamine	10.79	5.17
5	Quinuclidinol	10.17	2.28
6	2-Methoxyethylamine	9.66	1.51
7	Hexafluoroisopropoxide ion	9.28	1.54
8	Taurine	9.05	0.50
9	2-Cyanoethylamine	8.21	0.54
10	Hydrazine	8.18	1.42
11	Cacodylate	6.08	$<3.0 \times 10^{-2}$
12	Trifluoroethylamine	5.81	1.94×10^{-2}
13	Acetate	4.76	$<1.0 \times 10^{-2}$
14	Water	−1.74	1.82×10^{-4}

[a] The pK_a of the conjugate acid was determined from measurements of the pH of partially neutralised solutions or from titration curves under the conditions of the kinetic experiment.

experiments conducted in acetic acid–acetate buffers the intercept for general acid catalysis was indistinguishable from zero. For the less basic catalysts the rate constant for general base catalysis was also obtained from experiments in hydrazine buffers (Figure 3).

The rate law for the reaction of benzylpenicillin with hydrazine is given in equation (2) where k_B represents all the kinetic terms proportional to the catalyst in the absence

$$\frac{\text{Rate}}{[\text{Pen}]} = k_{obs} = k_o[OH^-] + k_B[B] + k_1[N_2H_4] + k_2[N_2H_5^+] + k_3[N_2H_4][B] + k_4[N_2H_4][BH^+] \quad (2)$$

of hydrazine. In experiments in dilute acid, a small additional term $k_5[N_2H_5^+][\text{Pen } CO_2H]$ was found, the value of k_5 being 1.9×10^{-3} l mol^{-1} s^{-1}. This term is kinetically equivalent to $k_5'[N_2H_4][H^+][\text{Pen}{-}CO_2H]$ with k_5' equal to 3.0×10^5 l^2 mol^{-2} s^{-1}. The product of this reaction was not determined.

* Supplementary publication No. SUP 22637 (5 pp.); for details of the supplementary publications scheme see Notice to Authors No. 7, *J.C.S. Perkin II*, 1979, Index issue.

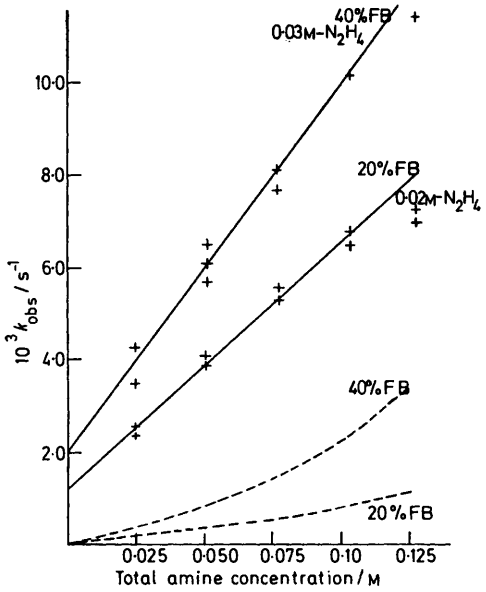


FIGURE 1 Observed pseudo-first-order rate constants for the reaction of hydrazine with benzylpenicillin (solid lines) as a function of propylamine buffer concentration at the indicated fractions of free base and at the given concentration of hydrazine in water at 30 °C, ionic strength 0.25M. The dashed lines show the rate constants in the same buffers but in the absence of hydrazine

DISCUSSION

Hydrazine shows an enhanced nucleophilic reactivity towards penicillin compared with amines of similar basicity [1] and this is attributed to the so-called α-effect.[8] This has allowed a study of the effect of varying the basicity of the catalyst with a constant nucleophile even in the presence of strongly basic catalysts. For example, catalysis of the reaction of hydrazine with benzyl-

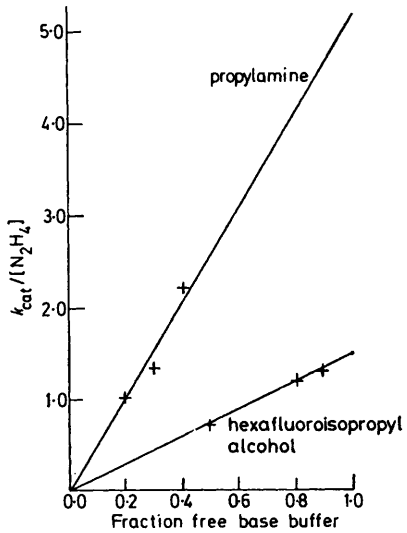


FIGURE 2 Dependence of the observed rate constants for catalysis of the reaction of hydrazine with benzylpenicillin by propylamine and by hexafluoroisopropyl alcohol buffers upon the fraction of free base of the buffer. The left and right ordinate intercepts give the catalytic constants for the acidic and basic species of the buffer

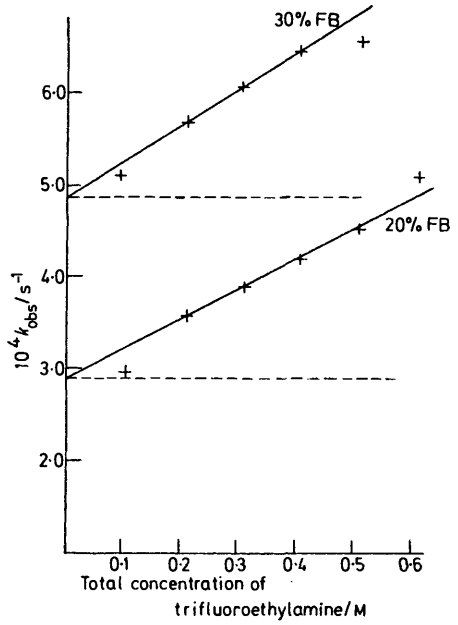


FIGURE 3 Observed pseudo-first-order rate constants for the reaction of hydrazine (0.05M total) with benzylpenicillin as a function of added trifluoroethylamine concentration at 30 °C, ionic strength 0.25M. The dashed lines show the rate constants expected if there were no reaction of hydrazine, trifluoroethylamine and benzylpenicillin

penicillin occurs even with the strongly basic amine propylamine. This reaction cannot represent nucleophilic catalysis by the amine as the product penicilloyl amide is stable to hydrazine under the reaction conditions.

The dependence of the rate constants, k_3, for the general base-catalysed hydrazinolysis of benzylpenicillin upon the basicity of the catalyst is shown in Figure 4. This Brønsted plot cannot be described by a single straight line nor by a series of straight lines for the different

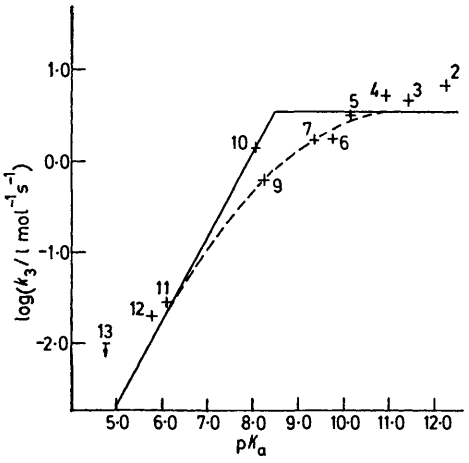


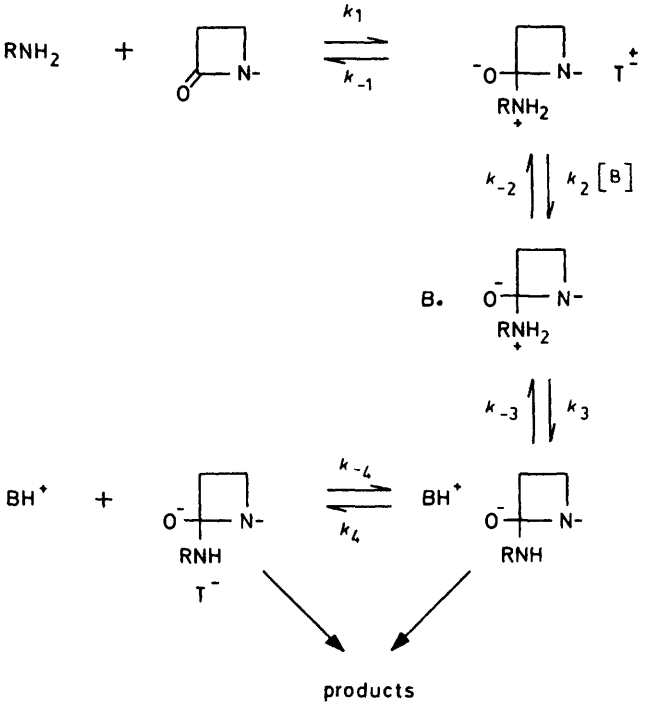
FIGURE 4 Brønsted plot for the general base-catalysed hydrazinolysis of benzylpenicillin at 30 °C. Upper limits for the rate constants when no base catalysis could be detected are indicated by arrows. The numbers refer to the bases listed in the Table. The solid line is drawn for guidance and represents a simple proton transfer that is controlled by the rate of diffusion together or apart of the acidic and basic species, and the dashed line shows the curvature actually observed for a simple proton-transfer reaction [12]

classes of catalysts that were examined. For strongly basic catalysts there is little dependence of the rate constants upon basicity and the Brønsted β-value is $\leqslant 0.2$. However, catalysis by weak bases shows a much stronger dependence upon the basicity of the catalyst, $\beta \geqslant 0.8$. A curved or non-linear Brønsted plot is required to describe the behaviour of both oxygen and nitrogen bases.

The large sensitivity of the rate constants to base strength for weakly basic catalysts indicates that the catalyst resembles its conjugate acid in the transition state, *i.e.*, there is a large amount of, or complete, proton transfer to the catalyst in the transition state. For strongly basic catalysts the small sensitivity of the rate constants upon base strength suggests that the catalyst resembles its free unprotonated basic form in the transition state.

Non-linear Brønsted plots may reflect a gradual change in transition-state structure with the reactivity of the reactants as predicted by the Hammond–Bell–Polanyi–Leffler [9] postulates and the Marcus theory.[10] This would then be consistent with a concerted reaction in which the proton in the transition state is either in flight and at a potential energy maximum or in a potential energy minimum but not associated closely with either the proton donor or acceptor.[11] There are several reasons why we think that the observed non-linear Brønsted plot is inconsistent with a concerted proton transfer mechanism but is consistent with a stepwise reaction in which there is a change in the rate-limiting step with changing basicity of the catalyst. The change in slope of Figure 4 is much sharper than is expected or observed for reactions reflecting a gradual change in transition-state structure.[10] Furthermore, there is a non-linear dependence of the rate of aminolysis of penicillin upon the concentration of hydroxide-ion which has been interpreted in terms of a change in rate-limiting step and consequently indicates the formation of an intermediate in the reaction which is probably the tetrahedral intermediate (III).[6] The simplest mechanism compatible with the observations involves the formation of an unstable dipolar tetrahedral addition intermediate, $T^{\pm}$, which rapidly reverts to the starting materials by expulsion of the attacking amine, k_{-1}. Reaction only proceeds if the intermediate is trapped by an encounter with a base that results in proton transfer to form the anionic intermediate, T^-, which rapidly breaks down to products (see Scheme). Proton transfer between electronegative atoms is thought to occur by a stepwise process involving the diffusion-controlled encounter of the proton donor and acceptor, followed by proton transfer itself and then diffusion apart.[12] Proton transfer itself, k_3, is not usually rate-limiting. The application of these suggestions to the mechanism of aminolysis of penicillin provides an explanation for the non-linear Brønsted plot, Figure 4. When the tetrahedral intermediate, $T^{\pm}$, is a stronger acid than the conjugate acid of the basic catalyst proton transfer is thermodynamically favourable. The rate-limiting step

will therefore be the diffusion-controlled encounter of $T^{\pm}$ and the catalyst, k_2, and the observed rate will be independent of the basicity of the catalyst.[3] However, for weakly basic catalysts proton transfer is thermodynamically unfavourable and the rate-limiting step changes to the diffusion apart of the deprotonated intermediate, T^-, and the protonated catalyst, k_4. This kinetic scheme explains the large dependence of the rate upon the basicity of the catalyst for weakly basic catalysts and its insensitivity for strongly basic catalysts.



SCHEME

In the intermediate region of curvature the proton-transfer process itself, k_3, or associated processes involving the solvent, become partially rate-limiting.[12,13]

There are several aspects of the mechanism that deserve further consideration.

In order that the k_2 step of the Scheme be rate-limiting it is necessary that $k_{-1} > k_2[B]$. Since k_2 is the rate constant for the diffusion-controlled encounter of $T^{\pm}$ and B it is presumably *ca.* 10^9 l mol^{-1} s^{-1} and k_{-1} must therefore be $>1 \times 10^8$ s^{-1} because the buffer plots (Figure 1) were linear up to at least 0.1M-free base. There is independent evidence that this is the case. The equilibrium constant for the formation and the rate constants for the formation and breakdown of the tetrahedral intermediate $T^{\pm}$ have been determined from the change in rate-limiting step that is observed for the hydroxide-ion-catalysed hydrazinolysis of benzylpenicillin.[6,*] The rate constant k_{-1} for the breakdown of $T^{\pm}$ is 7.6×10^8 s^{-1} which makes it compatible with the proposed mechanism. The equilibrium constant K for the formation of $T^{\pm}$ is 1.4×10^{-8} l mol^{-1} and k_1 is 10.7 l mol^{-1} s^{-1}. It is interesting to note that the α-effect is manifested by an increase in the equilibrium constant of 350-fold which is reflected by an *increase* of 15-fold in the value of k_1 and a *decrease* of 22-fold in the value of k_{-1}, compared with an amine of similar basicity to hydrazine.[6,*]

The equilibrium constant of 1.4×10^{-8} l mol^{-1} for the formation of $T^{\pm}$ from benzylpenicillin and hydrazine may be used to calculate the maximum value of the rate constant for general base catalysis as $k_{obs.} = k_2K$ for strongly basic catalysts. Assuming a value of 10^9 l mol^{-1} s^{-1} for k_2 the maximum value of k_{obs} would be 14 l mol^{-1} s^{-1} in good agreement with the value of ' levelling off ' in Figure 4.

A stepwise mechanism of catalysis for the aminolysis of penicillin is in accord with the rule proposed by Jencks that concerted catalysis can occur only when the reactive site undergoes a large change in pK in the course of the reaction so that an unfavourable proton transfer to the catalyst becomes favourable, *i.e.*, when the basicity of the catalyst is between that of the reactive site in the starting material and product.[14] The pK_a of the intermediate $T^{\pm}$ is estimated [15] to be *ca.* 8.5 which is not very different from those of the conjugate acids of many of the catalysts used and so a stepwise mechanism of catalysis is expected.

The estimated pK_a of $T^{\pm}$ agrees well with the observed break in the Brønsted plot of Figure 4. Treatment of the proton transfer from $T^{\pm}$ to the catalysing base as a simple acid-base reaction [12] predicts that for catalysts which have conjugate acids of p$K_a > 8.5$ proton transfer will be thermodynamically favourable and hence the rate-limiting step will be simply diffusion-controlled encounter of $T^{\pm}$ and the catalyst, whereas those of p$K_a <$ 8.5 proton transfer will be thermodynamically unfavourable and hence the rate-limiting step will be the diffusion apart of the conjugate acid of the catalyst and the deprotonated intermediate, T^-.

The deprotonated intermediate $T^{\pm}$ is expected to breakdown very rapidly. The rate of breakdown of $T^{\pm}$ to products is, estimated as previously described,[1] *ca.* 7×10^5 s^{-1}. There is an extra large driving force for fission of the carbon-β-lactam nitrogen in T^- that arises from the non-bonded pair of electrons on the hydrazine nitrogen atom and the incipient resonance of the amide product, amounting to *ca.* 70 kJ mol^{-1} of stabilisation in the fully formed amide.[16] Breakdown of T^- to products may therefore proceed at a rate which is faster than the diffusion apart of T^- and the conjugate acid of the base catalyst, presumably *ca.* 10^{10}—10^{11} s^{-1}.[17] Catalysis by weak bases, therefore, may occur by breakdown of T^- in the presence of the conjugate acid of the

* N. P. Gensmantel, unpublished observations. No α-effect has been observed for the attack of hydrazine upon phenyl-isocyanate where $\beta_{nuc} = 0.3$, which is attributed to the small amount of bond formation (A. F. Hegarty, C. N. Hegarty, and F. L. Scott, *J.C.S. Perkin II*, 1975, 1166). The reaction of hydrazine with methyl formate exhibits an enhanced rate of expulsion as well as attack, *i.e.* the α-effect is manifest to a greater extent in the rate constant than in the equilibrium constant for adduct formation (G. M. Blackburn and W. P. Jencks, *J. Amer. Chem. Soc.*, 1968, **90**, 2638).

catalyst as a ' spectator ' [18] or, if T^- has too short a lifetime to exist as an intermediate, through a ' concerted ' process.[15] Although, in principle, it is possible to distinguish between these and other subtle variations of the mechanism [15] it is dependent upon the pK_a of $T^{\pm}$ and estimates of this are too uncertain to make a definitive statement about the mechanism of catalysis by weak bases.

The mechanism of general-acid base catalysis in acyl-transfer reactions depends critically upon the life-time of the intermediates involved.[3,14,19] The aminolysis of penicillin is subject to general base catalysis because the initially formed tetrahedral intermediate, $T^{\pm}$, breaks down rapidly to reactants and at a rate which is faster than proton abstraction by the solvent water. Because of the low acidity of $T^{\pm}$, proton donation to water (see Scheme, $k_2[B]$ with $B = H_2O$) is a relatively slow process (*ca.* $1—10^4$ s^{-1} for amines of pK_a 10—6, respectively) compared with the rate of breakdown of $T^{\pm}$ to reactants, k_{-1}. Catalysis occurs because the intermediate is *unstable* [3] if $T^{\pm}$ was more stable proton transfer would occur to water, and if $k_{-1} < k_2[H_2O]$ no catalysis would be observed. Diffusion-controlled deprotonation of $T^{\pm}$ upon encounter with a basic buffer species provides a favourable route to the product by ' trapping ' the intermediate [3] and therefore the observed rate is increased by the addition of such species.

It is interesting to note that the mechanism of aminolysis of penicillin is not of the pre-association type or one that involves hydrogen-bonding between the catalyst and the attacking amine.[3] When the rate of breakdown of the intermediate $T^{\pm}$ to reactants is faster than the rate of separation of the intermediate and catalyst the reaction must proceed through a pre-association of all three reactants.[3,18,19] However, the observation of a change in rate-limiting step with increasing concentration of catalyst shows that this mechanism does not occur even though the rates of expulsion of the attacking amine from $T^{\pm}$ approach 10^{10} s^{-1} for the less basic amines (*e.g.* 2-cyanoethylamine, pK_a 8.2).[6] For very weakly basic amines it is likely that there is a change in mechanism because, for example, the value of k^{-1} for trifluoroethylamine (pK_a 5.8) is predicted to be *ca.* 5×10^{11} s^{-1}.[6]

In summary, two changes in the rate-limiting step in the base-catalysed aminolysis of penicillin have been observed. With *decreasing concentration* of hydroxide ion the rate-limiting step changes from the formation of the tetrahedral intermediate, k_1, to its diffusion-controlled encounter with a base, k_2 (see Scheme).[6] With *decreasing basicity* of the catalyst the rate-limiting step changes from k_2 to a subsequent step (see Scheme).

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