A Tetrahedral Intermediate in the Aminolysis of Benzylpenicillin

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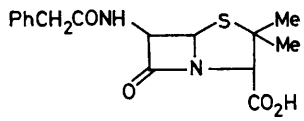
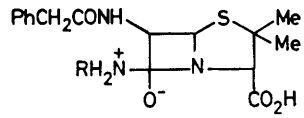
Summary There is a non-linear dependence of the rate of aminolysis of benzylpenicillin upon hydroxide ion concentration which is interpreted in terms of formation of a tetrahedral intermediate; the rate of breakdown of the intermediate into reactants is *ca.* 10^9 s^{-1}.

THE aminolysis of penicillin (I) is of interest because the principal antigenic determinant of penicillin allergy is the penicilloyl group bound by an amide linkage to amino groups on proteins.[1] This amide exchange reaction occurs readily and the susceptibility of penicillins to attack by nucleophiles has been attributed to strain in the β-lactam ring[2] and to the nonplanarity of the system which inhibits the usual amide resonance.[3]

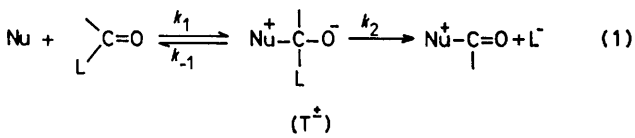


(I)



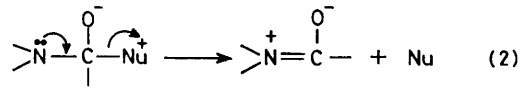
(II)

Nucleophilic substitution at the carbonyl centre is often presumed to proceed through the formation of a tetrahedral addition intermediate ($T^{\pm}$) [equation (1), Nu = nucleophile], in which the bond to the attacking group is made before the bond to the leaving group L is broken.[4] The rate-limiting step of these reactions is determined by the relative rates of partitioning of the tetrahedral intermediate to reactants and products, *i.e.*, the relative values of k_{-1} and k_2.



($T^{\pm}$) (1)

The β-lactam of penicillins is an unusual amide. Carbonyl carbon–β-lactam nitrogen bond fission is accompanied by the relief of a large amount of strain energy[2] which facilitates this process which in normal amides requires protonation of the nitrogen atom to prevent amine anion expulsion.[4,5] Carbonyl carbon–nucleophile bond fission to expel the attacking nucleophile may be expected to be a relatively slower process than that found in normal tetrahedral intermediates. There are two reasons for this: (i) 3-co-ordinate approximately sp^2 hybridised centres in four-membered rings are more strained than 4-co-ordinate approximately sp^3 hybridised centres[6] and (ii) a significant driving force for the collapse of tetrahedral intermediates is the conjugation of the amide nitrogen lone-pair with the incipient carbonyl group[7] [equation (2)]. This resonance interaction is severely reduced in penicillins.[3] It may therefore be possible to detect tetrahedral intermediates in the reactions of penicillins. We report here kinetic evidence for such an intermediate.



(2)

The rate law for the aminolysis of the sodium salt of benzylpenicillin in water at 30·0 °C is given in equation (3), where k_{obs} is the observed pseudo-first-order rate constant and k_0 is the first-order rate constant for the hydrolysis reaction.[8] The individual rate constants are normally determined using the amine as both buffer and reactant.[8,9]

$$k_{obs}=k_0+k_1[RNH_2]+k_2[RNH_2]^2+k_3[RNH_2][OH^-] \quad (3)$$

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 this pathway.

With glycine and methoxyethylamine in sodium hydroxide solutions, at constant ionic strength (I = 1 mol l^{-1}, KCl) there is a non-linear dependence of the apparent second-order rate constant, $(k_{obs} - k_0)/[RNH_2]$, upon the concentration of hydroxide ion (Figure). At low concentrations of hydroxide ion the rate is first-order in hydroxide ion

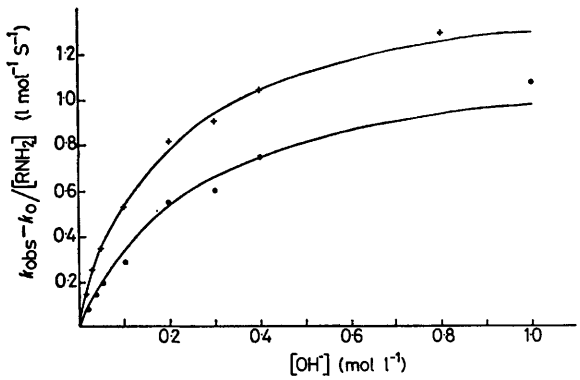
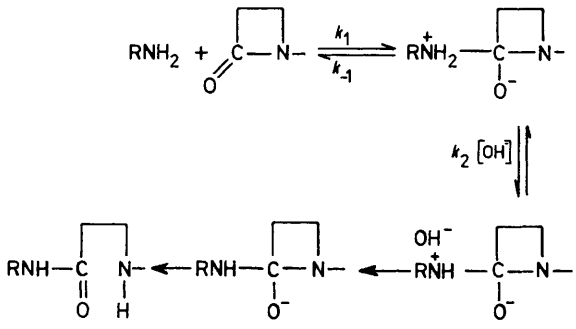


FIGURE. Plot of the apparent second-order rate constant $(k_{obs} - k_0)/[RNH_2]$ as a function of hydroxide ion concentration for the reaction of benzylpenicillin with glycine (●) and methoxyethylamine (+), at 30·0 °C (I = 1·0) (KCl). The lines are calculated from the constants given in the text.

and the slope of this line gives k_3 which agrees well with the value determined at lower pH in buffer solutions.[8,9] At high concentrations of hydroxide ion the rate becomes independent of the concentration of hydroxide ion.

The break indicates, to us, that the reaction proceeds through at least two steps and an intermediate, which is probably the tetrahedral addition intermediate (II). Other possible interpretations of such breaks[10] are not relevant in this case. The proposed mechanism involves formation of a tetrahedral intermediate followed by diffusion of hydroxide ion into the same solvent cage as the intermediate. Rapid proton transfer is followed by rapid collapse to products (Scheme). The apparent first-order



SCHEME

rate constant for such a scheme is given in equation (4). At low concentrations of hydroxide ion $k_{-1} > k_2[OH^-]$ and the rate constant is dependent upon the hydroxide ion

$$k_{obs} - k_0 = \frac{k_1 k_2[RNH_2][OH^-]}{k_{-1} + k_2[OH^-]} \quad (4)$$

concentration and k_2, the diffusion-controlled step, is rate-limiting. Support for this hypothesis comes from the Brønsted β-value of 1·1 for the dependence of k_3 upon amine basicity.[8,9] This indicates that the reaction behaves as if approximately a unit positive charge has been developed in the transition state which is consistent with rate-limiting diffusion of the tetrahedral intermediate and hydroxide ion together. At high concentrations of hydroxide ion $k_2[OH^-] > k_{-1}$ and the rate constant becomes independent of hydroxide ion concentration and k_1, the rate of formation of the tetrahedral intermediate, is rate-limiting. The rates of aminolysis of benzylpenicillin catalysed by bases other than hydroxide ion do not show a non-linear dependence upon the concentration of the base, [B], because $k_2[B] < k_{-1}$ up to 0·2 mol l^{-1} base.[8,9]

From equation (4), a plot of the reciprocal of the apparent second-order rate constant $(k_{obs} - k_0)/[RNH_2]$ against $1/[OH^-]$ gives a straight line (not shown) of intercept $1/k_1$ and of slope $k_{-1}/(k_1k_2)$. The values of k_1, the rate of formation of the tetrahedral intermediate, are 1·24 ± 0·1 and 1·54 ± 0·1 l mol^{-1} s^{-1} for glycine and methoxyethylamine, respectively. The diffusion-controlled step, k_2, has a value[11] of *ca.* 10^{10} l mol^{-1} s^{-1} and hence, from the slopes and intercepts, the values of k_{-1}, the rate of breakdown of the tetrahedral intermediate to reactants, are *ca.* $2{\cdot}7 \times 10^9$ and $1{\cdot}9 \times 10^9$ s^{-1} for glycine and methoxyethylamine, respectively. Because these rate constants are expected to be larger for other systems, this provides some justification for the postulated rapid breakdown of normal tetrahedral intermediates.[5,7]

The equilibrium constants for the formation of the tetrahedral intermediates from benzylpenicillin and glycine and methoxyethylamine are $4{\cdot}5 \times 10^{-10}$ and $7{\cdot}9 \times 10^{-10}$ l mol^{-1}, respectively. The similar values are to be expected in view of the similar pK_a's of the amines (*ca.* 9·7).[7]

We thank the S.R.C. for a grant and Kirklees M.C. (N.P.G.) for support.

(*Received, 16th January* 1978; *Com.* 036.)

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