The Imidazole-catalysed Isomerisation of Benzylpenicillin

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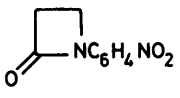
The isomerisation of benzylpenicillin to benzylpenicillenic acid is catalysed by imidazole, *N*-methylimidazole, and 2-methylimidazole. For each catalyst there is a different rate equation. The dominant reaction path is general-acid assisted reaction of benzylpenicillin with imidazole. A mechanism is proposed in which, after reaction with the imidazole at the carbonyl group of the β-lactam ring, there is protonation of the β-lactam nitrogen and ring-opening. The mechanism proposed parallels that suggested by Morris and Page for the aminolysis of penicillins.

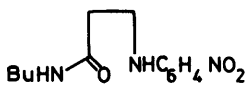
AMIDES are generally resistant to aminolysis.[1] However, cyclic amides do react and the rate of reaction depends upon the ring size. Blackburn and Plackett [2] report that the aminolysis of β-lactams occurs *ca.* 10^3 times faster than the corresponding γ-lactam. The rate equation for the aminolysis of a β-lactam has the form (1). For the reaction of 1-*p*-nitrophenylazetidin-2-one (I)

$$k_{obs} = k'[\text{Amine}] + k''[\text{Amine}]^2 + k'''[^-\text{OH}][\text{Amine}] \quad (1)$$

with n-butylamine the product is (II), and the value of k', k'', and k''' are such that at moderate concentrations of butylamine and at high pH assisted aminolyses are the predominant reactions. It is a general phenomenon



(I)



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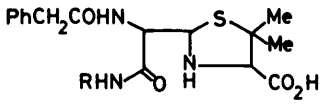
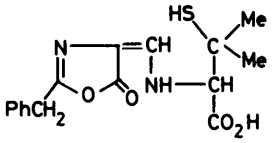


(III)

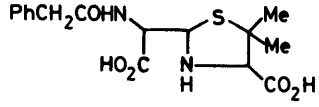
that assisted terms occur in rate equations where there is a poor leaving group and with β-lactams the leaving group (*viz.* an amide anion) is particularly poor.

The β-lactam ring of a penicillin is much more susceptible to aminolysis than is a monocyclic β-lactam. This matter has been considered in detail by Morris and Page.[3] The reason for this enhanced reactivity is not entirely clear for the leaving group is essentially unchanged in a penicillin, but the fused thiazolidine ring does confer upon the β-lactam ring of penicillin so much reactivity [4] that a bicyclic structure for penicillin was questioned for some time. The product of reaction of benzylpenicillin with a primary amine is a benzylpenicilloylamine (III), a product analogous to that obtained from a monocyclic β-lactam. Also, the rate equation has the same form as (1). The greatest enhancement occurs in term k'' and general base-assisted aminolysis is the most important pathway in the aminolysis of benzylpenicillin.

The Brønsted β factor for the k'' term is about unity and so the reaction of imidazole (pK_a 7.0) with benzylpenicillin should be very slow. However, experiment shows that imidazole is a particularly good reactant in effecting ring opening of penicillins. The first report of this appears to be the work of Grant *et al.*[5] They found that, although benzylpenicillin is fairly stable in an imidazole buffer, its antibiotic activity is destroyed if the solution is frozen. The strange effect was explained [6] by concentration of the reactants in pools of water between crystals of ice. It was not shown, in this work, that the loss of antibiotic activity was due to opening of the β-lactam ring. However, Bundgaard [7] proved that



(IV)



(V)

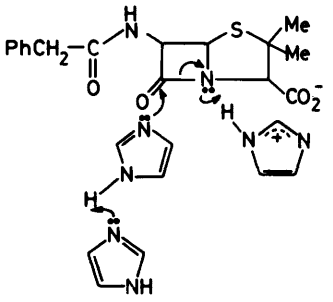
imidazole catalyses the isomerisation of benzylpencillin to benzylpenicillenic acid (IV). There is no evidence that this reaction is catalysed by other amines. A further study by Bundgaard [8] showed that the same reaction occurs with other penicillins.

Benzylpenicillenic acid is not stable in solution and readily changes into benzylpenicilloic acid (V).[9] However, benzylpenicillenic acid can be stabilised in solution by addition of mercury(II) chloride.[10] It has an intense u.v. spectrum and this permitted Bundgaard and Ilver [11] to develop a spectrophotometric method for the determination of penicillins. The concentration is obtained from the absorbance at 325—345 nm due to penicillenic acid mercuric mercaptide, formed from the penicillin in quantitative yield by addition of 1.2M-imidazole and 10^{-3}M-mercury chloride solution at pH 6.8. This method allows detection of penicillins at concentrations as low as 10^{-6} g ml^{-1}.

Bundgaard [7] reported that the rate of imidazole-catalysed isomerisation of benzylpenicillin is given by equation (2). Although the product of reaction has

$$k_{obs} = k'[\text{Im}][\text{ImH}^+] + k''[\text{Im}]^2 \quad (2)$$

been changed, the reaction is characterised by the same assisted pathways found for the aminolysis of penicillins by primary amines. The mechanistic interpretation of Bundgaard's results is of some interest. He proposed the mechanism shown in Scheme 1, with formation of



SCHEME 1

benzylpenicilloylimidazole as the first step in the isomerisation. This, we feel, is an unsatisfactory scheme. Tsuji *et al.*[12] proposed a more satisfactory mechanism of parallel pathways, each corresponding to a term in the rate equation. In view of the importance of the penicillin assay, and the relevance of the aminolysis of penicillin in understanding penicillin allergies,[13] we decided to make a further kinetic study in an effort to elucidate the mechanism fully.

EXPERIMENTAL

Materials—Specially purified imidazole from B.D.H. Ltd. was used. Other chemicals were commercial samples which were used without further purification. Buffers were prepared from imidazole and HCl and the ionic strength adjusted to 0.5M by addition of KCl. The buffers were made 10^{-4}M in $HgCl_2$. Deuteriated imidazole was prepared by crystallisation of imidazole from D_2O.

Kinetics—The conversion of benzylpenicillin to benzylpenicillenic acid was followed by observation of changes in the absorbance at 325 nm on addition of benzylpenicillin to an imidazole buffer thermostatted at 37°. A Pye–Unicam SP8-100 spectrophotometer was used. Rate constants were calculated by the method of Swinbourne.[14] The initial concentration of benzylpenicillin was *ca.* 5×10^{-5}M.

RESULTS

It is known from the work of Bundgaard [7] that conversion of benzylpenicillin to benzylpencillenic acid, by reaction with imidazole, is quantitative. We examined this for reaction with *N*-methylimidazole and 2-methylimidazole by observation of the maximum absorbance attained on addition of a known amount of benzylpenicillin to the imidazole buffer. The extinction coefficient at 325 nm for benzylpenicillenic acid is 2.63×10^4. Conversion was found to be almost quantitative ($97 \pm 0.5\%$) for *N*-methylimidazole, but only $53 \pm 1\%$ of the expected benzylpenicillenic acid was produced with 2-methylimidazole.

As described previously, mercury(II) chloride is added to prevent further reaction of the benzylpenicillenic acid. For our kinetic study it was important to know that addition of the mercury salt did not affect the rate of reaction. This was examined by a determination of the first-order rate constant k_{obs} for the reaction of benzylpenicillin in an imidazole buffer as a function of mercury(II) chloride concentration. The results are displayed in Table 1

TABLE 1

Variation of k_{obs} with $[HgCl_2]$ for the isomerisation of benzylpenicillin at 37 °C

$10^4[HgCl_2]$/M	Maximum absorbance at 325 nm	10^4k_{obs}/s^{-1}
10	1.26	1.8
8	1.33	1.7
6	1.30	1.7
4	1.29	1.7
0.1	1.25	2.4
0.08	1.08	2.4
0.04	0.74	2.8

$[Imidazole]_{tot}$ 0.30M. $[Benzylpenicillin]_0$ 0.53×10^{-4}M. pH 7.00.

and indicate that the concentration of mercury(II) ions does not affect the value of k_{obs}, provided the concentration is high enough to complex all the benzylpencillenic acid formed. If this condition is not met, the theoretical maximum optical density is not achieved and the observed rate constants are higher than they should be. This apparent increase in rate is an artefact, arising from the degradation of uncomplexed penicillenic acid, a process which gives rise to further spectral changes.

TABLE 2

Kinetic data for the isomerisation of benzylpenicillin in imidazole buffer at 37 °C

pH	10^2[Im]/M *	10^5k_{obs}/s^{-1}
6.66	9.8	12.5
6.66	7.8	7.6
6.66	5.9	4.4
6.66	3.9	1.6
6.84	12.7	14.0
6.84	10.2	9.4
6.84	7.6	5.5
6.84	5.1	2.4
7.07	16.7	16.5
7.07	13.3	10.0
7.07	10.0	6.3
7.07	6.7	2.6
7.26	19.6	16.0
7.26	15.7	11.0
7.26	11.8	6.4
7.26	7.8	2.3

* Concentration of unprotonated imidazole.

The variation of k_{obs} with changes in the concentration of free imidazole is shown in Table 2. A plot of k_{obs} against concentrations of unprotonated imidazole [Im] at each pH considered is curved and this suggests that complex terms occur in the rate equation, as in (1). However, plots of $k_{obs}/[Im]$ against [Im] are rectilinear (Figure 1) and all the curves pass through the origin. The slopes are pH-dependent and this suggests that the rate equation may be (3), which is what Bundgaard [7] proposed. As the inter-

$$k_{obs} = k_1[Im] + k_2[Im][ImH^+] + k_3[Im]^2 \qquad (3)$$

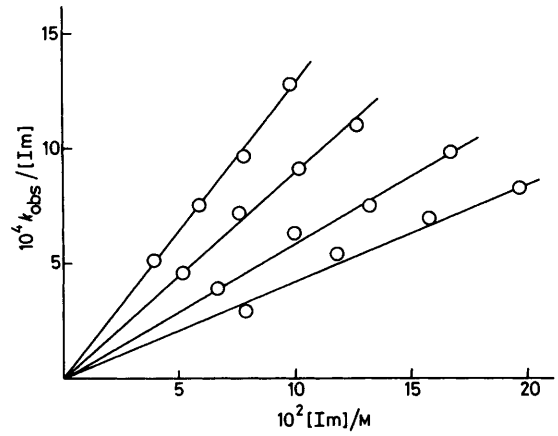


FIGURE 1 Plot of $10^4 k_{obs}/[Im]$ against concentration of unprotonated imidazole for the isomerisation of benzylpenicillin

cepts in Figure 1 are all zero k_1 must be zero. We may rewrite (3) as (4) and this means that the slope of each

$$k_{obs} = k_2[Im]^2[H^+]/K_a + k_3[Im]^2 \qquad (4)$$

curve in Figure 1 is $(k_2[H^+]/K_a + k_3)$. A plot of slope against $[H^+]/K_a$ is shown in Figure 2. The slope and intercept give the values of k_2 and k_3, 5.7×10^{-3} and 1.4×10^{-3}

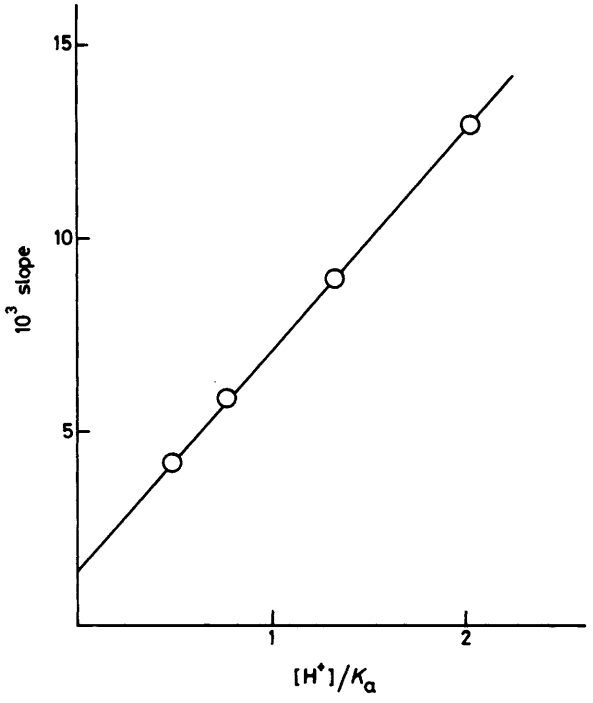


FIGURE 2 Graphical analysis of rate data for the isomerisation of benzylpenicillin

$l^2\ mol^{-2}\ s^{-1}$, respectively. These values are in reasonable agreement with those of Bundgaard [7] (6.3×10^{-3} and $2.0 \times 10^{-3}\ l^2\ mol^{-2}\ s^{-1}$) and those of Tsuji *et al.*[12] (6.2×10^{-3} and $1.6 \times 10^{-3}\ l^2\ mol^{-2}\ s^{-1}$). However, the latter workers quote a value for k_1 ($9.7 \times 10^{-6}\ l\ mol^{-1}\ s^{-1}$) but we obtained no evidence for this term. In view of its size in relation to k_2 and k_3 it is doubtful if it is experimentally detectable.

The isomerisation of benzylimidazole in a buffer made from *N*-methylimidazole was examined in the same way and the results are displayed in Table 3. Mathematical treatment of the results as described above gives both k_1 and k_3 as zero and k_2 as $2.3 \times 10^{-3}\ l^2\ mol^{-2}\ s^{-1}$. This is in good agreement with the value obtained by Tsuji *et al.*[12]

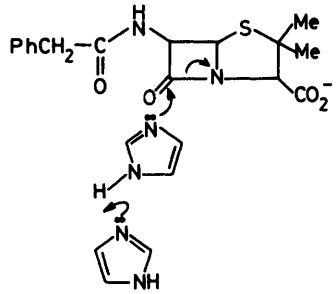
TABLE 3

Kinetic data for the isomerisation of benzylpenicillin in *N*-methylimidazole buffer at 37 °C

pH	10^2[*N*-MeIm]/M *	$10^5 k_{obs}/s^{-1}$
6.80	12.5	7.0
6.80	10.0	4.5
6.80	8.0	3.15
6.80	6.4	1.75
7.03	17.2	7.85
7.03	13.8	5.05
7.03	11.0	3.5
7.03	8.8	1.85
7.18	20.4	7.7
7.18	16.3	5.0
7.18	13.0	3.5
7.18	10.4	2.0
7.41	31.4	9.5
7.41	25.1	7.0
7.41	20.1	5.0
7.41	12.8	1.9

* Concentration of unprotonated *N*-methylimidazole.

($2.2 \times 10^{-3}\ l^2\ mol^{-2}\ s^{-1}$) but, again, these workers reported a k_1 term ($9.7 \times 10^{-6}\ l\ mol^{-1}\ s^{-1}$) which we could not detect. The absence of a k_3 term with *N*-methylimidazole could mean that this term is due to assistance of attack of imidazole of benzylpenicillin by a second molecule of imidazole (Scheme 2), as suggested by Tsuji *et al.*[12] The mechanism cannot operate when one nitrogen of imidazole ring does not carry a replacable hydrogen and so it is consistent that the k_3 term disappears with *N*-methylimidazole. However, the problem with this mechanism is that it is difficult to know how to accommodate it in parallel with the k_2 term. Here the role of protonated imidazole is simple to envisage; it protonates the leaving group and turns it from a particularly poor one into one that is reasonably good. To avoid the termolecular collision proposed by Tsuji *et al.*,[12] let alone the tetramolecular one proposed by Bundgaard,[7] we suggest that the reaction occurs in two steps. The proton transfer must be slow for $[ImH^+]$ to appear in the rate equation as it does with term k_2. However, if for the k_3 term the mechanism is attack by a hydrogen-bonded dimer of imidazole this step must be rate-determining step for there is no protonated species in this term of the rate equation. For k_2, reaction with imidazole is not rate-determining and so reaction of a stronger nucleophile cannot be. This interpretation of k_3 is, therefore, unsatisfactory.



SCHEME 2

We then examined the reactivity of the more basic compound 2-methylimidazole (pK_a 7.89). The results are displayed in Table 4. A plot of concentration of unprotonated 2-methylimidazole against k_{obs} is linear and not pH-dependent (Figure 3). Thus for 2-methylimidazole the rate equation contains only the k_1 term and the value is

TABLE 4

Kinetic data for the isomerisation of benzylpenicillin in 2-methylimidazole buffer at 37 °C

pH	10^2[2-MeIm]/M *	$10^6k_{obs}/s^{-1}$
8.01	15.8	19.5
8.01	12.6	14.7
8.01	9.5	11.3
8.01	6.3	8.7
7.75	11.8	14.2
7.75	9.4	10.3
7.75	7.1	7.7
7.74	4.7	5.3
7.48	7.8	8.3
7.48	6.2	6.8
7.48	4.7	5.0
7.48	3.1	3.3

* Concentration of unprotonated 2-methylimidazole.

1.2×10^{-4} l mol^{-1} s^{-1}. This is very different from the value obtained by Tsuji *et al.*[12] (0.092×10^{-4} l mol^{-1} s^{-1}) but they detected k_2 and k_3 terms, although they could not calculate the values with any degree of certainty. Our experimental data are unambiguous and there is no evidence for either routes k_2 or k_3.

The three imidazoles examined all show different behaviour, none has all three pathways characterised by k_1, k_2, and k_3, and only one where the unassisted pathway is significant is 2-methylimidazole.

All the rate constants mentioned so far were determined again with deuterium oxide as the solvent. The results of the work are summarised in Table 5 and are not particularly illuminating. The k_1 and k_2 terms show substantial kinetic solvent isotope effects of *ca.* 2 but that for k_3 is smaller at 1.4. Tsuji *et al.*[12] report a similar change in k_1 for catalysis by 2,4-dimethylimidazole.

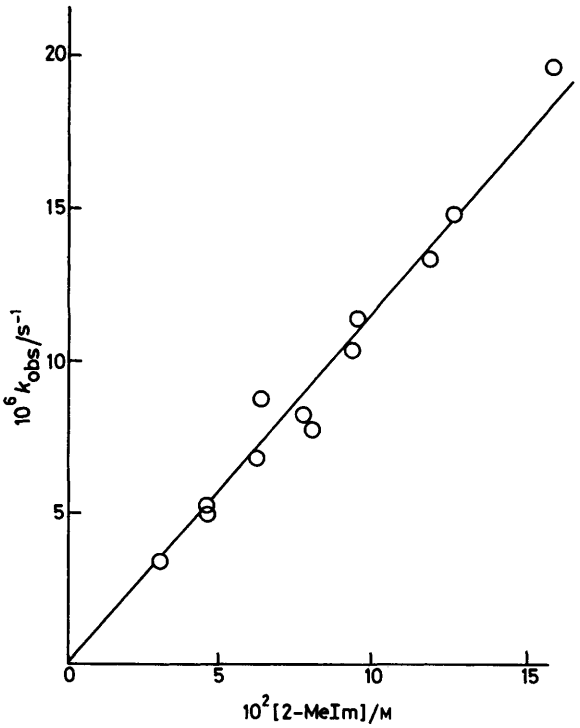


FIGURE 3 Plot of 10^6k_{obs} against concentration of unprotonated 2-methylimidazole for the isomerisation of benzylpenicillin

TABLE 5

Solvent kinetic isotope effects for the isomerisation of benzylpenicillin catalysed by various imidazoles at 37 °C

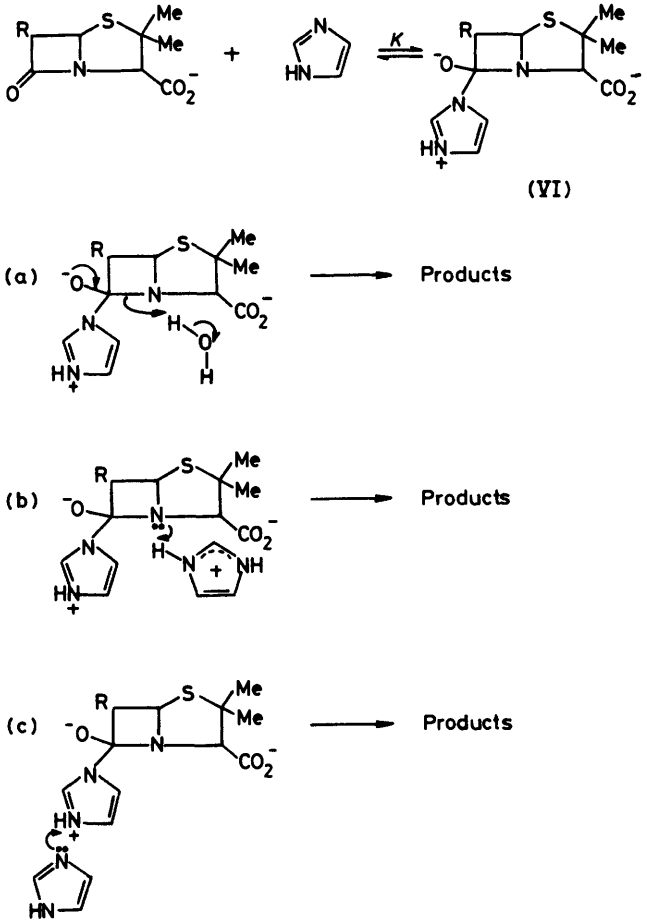
	10^4k_1/ l mol^{-1} s^{-1}	10^3k_2/ l^2 mol^{-2} s^{-1}	10^3k_3/ l^2 mol^{-2} s^{-1}
Imidazole (H_2O)		5.7	1.4
Imidazole (D_2O)		2.9	1.0
N-Methylimidazole (H_2O)		2.3	
N-Methylimidazole (D_2O)		1.1	
2-Methylimidazole (H_2O)	1.2		
2-Methylimidazole (D_2O)	0.62		

DISCUSSION

We seek a mechanism of reaction for the imidazole-catalysed step in the isomerisation of benzylpenicillin which avoids the difficulty of the rate-determining process mentioned above and which is also unified. This, we think, has been achieved in Scheme 3. The initial reaction is attack by unprotonated imidazole on the carbonyl group of the β-lactam ring to give a dipolar tetrahedral intermediate (VI). For this to react further on the path towards formation of penicillenic acid there must be fission of the carbon–nitrogen bond. The conditions under which this can occur during the aminolysis of benzylpenicillin have been discussed in detail by Morris and Page[3] and we can apply their considerations to the particular case of reaction with imidazole. There are, however, important differences. In the aminolysis and hydrazinolysis of benzylpenicillin general acid catalysis is unimportant but this, as the figures in Table 5 show, is not the case for reaction with imidazole. Also, with amines the isolable product of reaction is a penicilloyl amide but reaction with imidazole, although initially similar, results in formation of benzylpenicillenic acid.

Route (a) of Scheme 3 is the uncatalysed pathway. Our view is that *N*-protonation is necessary for opening of the β-lactam ring but Morris and Page[3] have argued convincingly that the uncatalysed pathway for aminolysis cannot involve a slow proton transfer from water to the β-lactam nitrogen. The alternative of proton transfer from water occurring synchronously with carbon–nitrogen bond fission is the most likely pathway. Uncatalysed expulsion of the amide anion is, we think, unlikely although Morris and Page[3] believe it possible because of the large strain energy which is released on ring-opening. However, Woodward *et al.*[15] have shown that the chemical reactivity of fused β-lactam rings is not directly related to the strain energy. The uncatalysed pathway was detected for only 2-methylimidazole but, if our view is correct, it should occur in all cases. The required manipulation of the data makes the evaluation of k_1, if it is small, difficult.

Page *et al.*[16] detected general acid catalysis in penicillin aminolysis only with more acidic amines and our results are consistent with this observation. 2-Methylimidazole is more basic than the other two and no general acid catalysis was detected. We envisage that k_2 arises from slow transfer of a proton from protonated imidazole to the β-lactam nitrogen as a prelude to ring-opening [route (b)].



SCHEME 3

The general base-catalysed term k_3 is, in some ways, the most difficult to understand, although it is the predominant term in most penicillin aminolyses.[3] The mechanism is not one of pre-association between two molecules of imidazole,[17] although absence of the term with *N*-methylimidazole is consistent with this possibility. As proposed by Morris and Page,[3] the simplest mechanism compatible with the results is removal of a proton from the dipolar intermediate (VI) and rapid collapse of the anionic intermediate to give products [route (c)]. Probably, with a base as weak as imidazole, the slow process is diffusion a part of the deprotonated intermediate and protonated imidazole. Clearly route (c) cannot apply to *N*-methylimidazole.

The solvent isotope effects are difficult to interpret and it is not claimed that they are diagnostic. However, they may be partially rationised in terms of the proposed mechanism. In route (a) water is involved in the rate-determining step and k_1 $(H_2O)/k_1$ (D_2O) is greater than unity, which is as expected. In route (b) the slow step is transfer of a proton or deuteron and again k_2 $(H_2O)/k_2$ (D_2O) is greater than unity.[18] If, in route (c), diffusion is the slow process then a smaller isotope k_3 $(H_2O)/k_3$ (D_2O) is not unreasonable.

The absence of base catalysed terms in the reaction of 2-methylimidazole is not easy to understand. It is a stronger base than imidazole but a weaker nucleophile. It is almost ten times less effective as a nucleophile in its reactions with 4-nitrophenyl acetate, although it is about ten times more basic.[19] The reason for the low nucleophilicity may well be steric. In this study we noted that only 53% of the product of the 2-methylimidazole catalysed reaction is benzylpenicillenic acid. This does not affect our kinetic study as in following the appearance of benzylpenicillenic acid we obtain the rate equation for consumption of benzylpenicillin by all routes.[20] In view of the increased basicity it may act as a general base catalyst and effect hydrolysis of benzylpenicillin to benzylpenicilloic acid (V).

In this paper we have considered only the imidazole-catalysed ring-opening. The subsequent processes giving rise to benzylpenicillenic acid will be discussed in a future publication where we will describe our work on some acid-catalysed reactions of penicillin.

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