The Chemical Reactivity of Penicillins and Other β-Lactam Antibiotics

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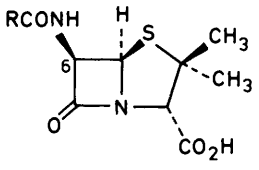
The rates of the acid catalysed hydrolysis of penicillins and cephalosporins are linear in H_0 and, unlike other amides, show no rate maximum with increasing acidity. Electron-withdrawing substituents at C-6 in penicillins decrease the rate of hydrolysis with a ρ_I of *ca.* 4 and they decrease the rate when attached to the amine leaving group. The acylamido-group at C-6 in penicillins, but not at C-7 in cephalosporins, exhibits neighbouring group participation with a rate enhancement of *ca.* 10^3. The absence of penicillenic acid formation from benzylpenicillin in acidic solution is not due to the ionisation of the carboxy-group. These observations are rationalised by a scheme involving *N*-protonation and formation of an acylium ion intermediate. The alkaline hydrolysis of penicillins proceeds 10^2 faster than a β-lactam after correction for substituent effects. There is no evidence for substantial inhibition of amide resonance in the bicyclic β-lactam antibiotics, little evidence to indicate extra strain in these systems and no evidence that expulsion of the leaving group at C-3 in cephalosporins occurs in the transition state.

THE β-lactam ring of penicillins and of the other β-lactam antibiotics is a unique feature not normally found in natural products. It is understandable therefore that chemists have assumed that the biological activity of this class of compounds is attributable to their unusual structure. Furthermore the penicillins (I) have their β-lactam fused to a thiazolidine ring resulting in a non-planar arrangement of substituents attached to nitrogen. In penicillins the nitrogen atom is 0.4 Å out of the plane defined by its substituents [1] whereas in the cephalosporins (II) it deviates by 0.3 Å.[2] The ease of nucleophilic attack upon the penicillin β-lactam carbonyl group is usually ascribed to either relief of strain upon opening the four-membered ring [3] or to a reduction in the usual amide resonance because of the non-planarity of the system.[4] Although these concepts are superficially appealing the evidence to support them is ambiguous. It has been reported that strain alone may be responsible for the enhanced reactivity,[5] particularly because of the fusion of the two rings in penicillins,[6] but, on the other hand, it is claimed that ring strain is unimportant and that inhibition of resonance stabilisation causes increased activity.[7]



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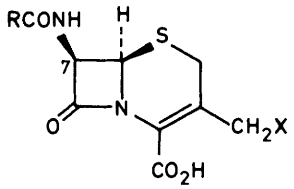


(II)

Amides are less reactive towards nucleophiles than are ketones because of the resonance stabilisation energy resulting from delocalisation of the lone pair of electrons on nitrogen over the carbonyl group. It is estimated [8] that this resonance stabilises amides by *ca.* 18 kcal mol^{-1}. If delocalisation is inhibited a reaction which involves the loss of resonance in the transition state could proceed up to 10^{13} fold faster than the analogous stabilised system. The strain energy of a four-membered ring [9] is 26—29 kcal mol^{-1} and therefore a reaction involving ring opening could occur up to 10^{20} fold faster than an analogous acyclic system. The total possible rate enhancement of a resonance inhibited β-lactam is therefore 10^{33} so if either of these effects are even slightly significant they should be easily observable.

The β-lactam of penicillins is generally more reactive towards nucleophiles than are normal amides but the exact rate enhancement is not easy to estimate. Substituent effects on both the acyl and amine portions of the β-lactam should be considered. For example, carbon–β-lactam nitrogen bond fission involves the expulsion of a better leaving group than the simple basic amine often found in amides.

This report attempts to quantify any enhanced or special chemical reactivity of the β-lactam antibiotics and to elucidate any unusual features in the mechanism of their reactions. A preliminary report of some of this work has appeared.[10]

EXPERIMENTAL

Materials.—6-Aminopenicillanic acid, 7-aminocephalosporanic acid, and the cephalosporins were the generous gifts of Glaxo Research Group. The sodium salt of nocardicin A was generously supplied by Fujisawa Pharmaceutical Co. Ltd. and that of penem (XIV) by Ciba-Geigy. Benzylpenicillin methyl ester, 6α-chloropenicillanic acid, 6α-bromopenicillanic acid, and penicillanic acid were prepared as previously described.[11] The acyclic amides were prepared from acetyl chloride and the amine and all gave satisfactory analytical data.

Kinetics.—Rate constants were obtained at 30.0 °C spectrophotometrically and computed as described previously.[11,12]

RESULTS AND DISCUSSION

Acid Hydrolysis.—If amide resonance in penicillins is significantly impaired the β-lactam nitrogen should be more basic than a normal amide nitrogen. The pK_a of *N*-protonated amides is normally *ca.* −7 to −8 [13] whereas that for resonance inhibited amide such as 1-azabicyclo[2.2.2]octan-2-one is 5.[14] Thermodynamically the most basic site for the protonation of normal amides is oxygen and the pK_a of *O*-protonated amides is 0 to

−3.[15] Whether the mechanism of the acid catalysed hydrolysis of simple amides proceeds *via* *O*- or *N*-protonation seems to have been resolved in favour of the former.[16] However, there exists the possibility that the mechanism of hydrolysis of penicillin is different.

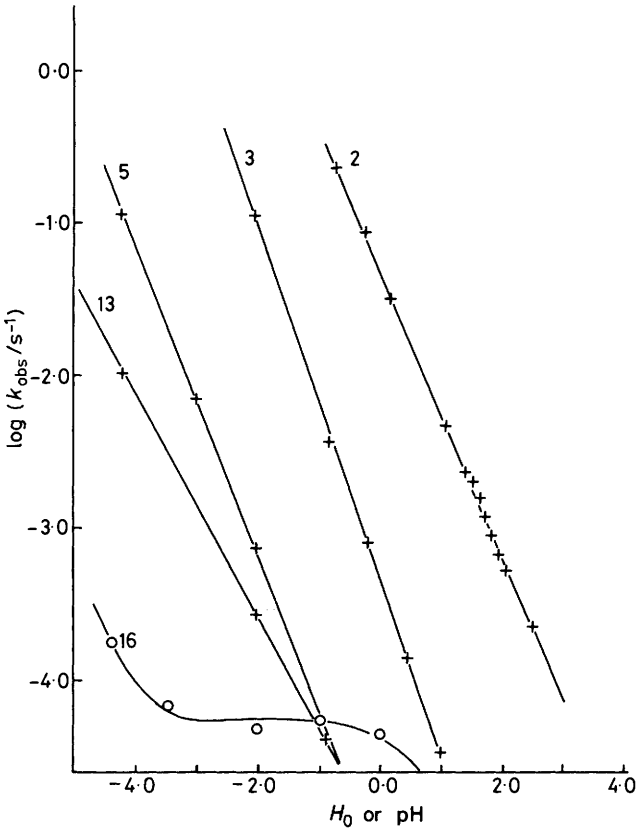


FIGURE 1 Plot of the logarithm of the observed pseudo-first-order rate constant for the decomposition of β-lactams and amides against H_0 for hydrochloric acid at 30°. The numbers refer to the compounds in Table 1

The pseudo-first-order rate constants for the hydrolysis of some β-lactam antibiotics and derivatives are shown as a function of acidity in Figure 1 and the data are given in Table 2. The rates are first-order in hydrogen-ion concentration and the second-order rate constants are given in Table 1. Shown in Figure 2 are data for a simple model amide, *N*-acetylglycine (III). Like other amides, the rate of hydrolysis of *N*-acetylglycine (III) passes through a maximum, which is attributed to complete conversion of the amide into its *O*-conjugate acid and to decreasing water activity.[17] Quite remarkably this feature is not observed for the β-lactams for which the rates of hydrolysis continue to increase up to H_0 values of −5. This is taken to indicate that the β-lactams are far less basic than normal amides for *O*-protonation and that a different mechanism is operating. A major conclusion is that the nitrogen of the bicyclic β-lactams is insufficiently basic for complete or partial conversion to the *N*-protonated amide. The pK_a for *N*-protonation must be < -5.

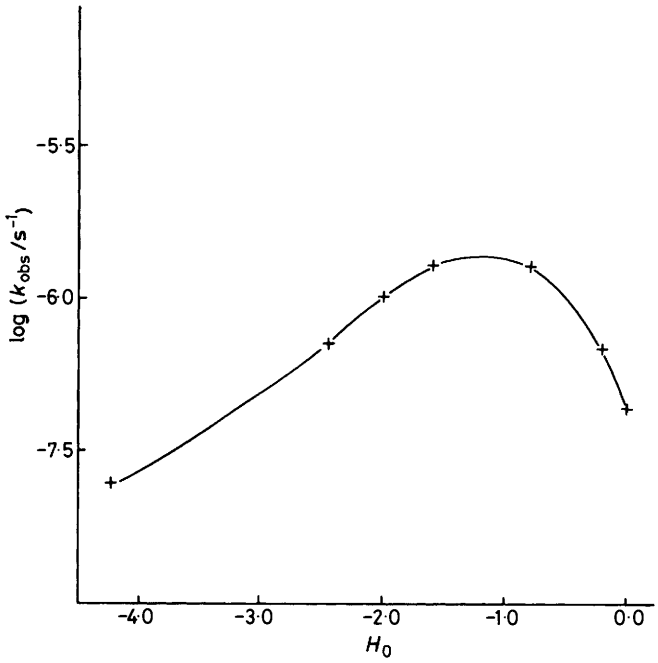


FIGURE 2 Plot of the logarithm of the observed pseudo-first-order rate constant for the hydrolysis of *N*-acetylglycine against H_0 for hydrochloric acid at 30°

There is thus *no* evidence for substantial inhibition of amide resonance in the bicyclic β-lactams. Furthermore, this behaviour is not peculiar to bicyclic β-lactams as the *monocyclic* β-lactam antibiotic, nocardicin A (IV), and 1-propylazetidinone (V) show similar reactivity and behaviour.

Amide resonance increases the *O*-basicity of amides compared with ketones and the pK_a for the *O*-conjugate acid in a significantly resonance inhibited amide would be decreased. In the extreme case such an amide could be regarded as a ketone with an electronegative *N*-substituent decreasing further the basicity of the carbonyl oxygen. The observations reported here indicate that the *O*-basicity of β-lactams is unusual. Even if *N*-protonation gives the kinetically significant species, using acid solutions of H_0 below the pK_a for the *O*-conjugate acid should mean that the rate of reaction becomes independent of H_0. We therefore conclude that the basicities of β-lactams are less than those of acyclic amides but emphasise that this is not a unique property of bicyclic β-lactams as it is also shown by monocyclic β-lactams.

The slopes of the logarithm of the pseudo-first-order rate constants against H_0 are −1 to −1.3 (Figure 1) and as water activity decreases with increasing acidity it appears that water is not involved in the transition state. We conclude that *N*-protonation takes place but that this is *not* the result of reduced amide resonance but is simply an intrinsic property of β-lactams. In fact, there has been a recent suggestion that the mechanism of acid catalysed hydrolysis of azetidin-2-one proceeds by an *A*-1 mechanism.[18]

Substituent effects in the acyl and amine portions of

TABLE 1

Second order rate constants for the degradation of β-lactam antibiotics, and derivatives, β-lactams and amides at 30 °C.

	Amide	pK_a of leaving group amine	k_{OH}/l mol^{-1} s^{-1}	k_H/l mol^{-1} s^{-1}
1	Benzylpenicillin	5.2	1.54×10^{-1}	1.35×10^{-1}
2	Benzylpenicillin methyl ester	3.2	2.49	8.20×10^{-2}
3	Penicillanic acid	7.1	7.40×10^{-3}	7.56×10^{-4}
4	Penicillanic acid methyl ester	5.1	9.38×10^{-1}	4.46×10^{-4}
5	6α-Chloropenicillanic acid	4.6	4.16×10^{-1}	1.20×10^{-5}
6	6α-Bromopenicillanic acid	4.7	6.10×10^{-1}	1.12×10^{-5}
7	6α-Bromopenicillanic acid methyl ester	2.6	7.96	
8	6β-Aminopenicillanic acid	6.4	6.31×10^{-2}	3.50×10^{-3}
9	6β-Ammoniumpenicillanic acid	3.9		1.80×10^{-4}
10	Penem XIV	5.1	5.10×10^{-1}	1.15×10^{-1}
11	Nocardicin A	7.8	1.02×10^{-4}	7.12×10^{-5}
12	Cephaloridine	2.0	6.49×10^{-1}	8.90×10^{-6}
13	Cephalothin	2.5	9.36×10^{-2}	2.54×10^{-5}
14	3-Methyl-7β-phenylacetamidoceph-3-em-4-carboxylic acid	3.7	2.90×10^{-2}	$\sim 2 \times 10^{-5}$
15	Methyl ester of 14	1.7	9.22×10^{-1}	$\sim 2 \times 10^{-5}$
16	7β-Aminocephalosporanic acid	3.7	2.65×10^{-2}	3.90×10^{-4}
17	7β-Ammoniumcephalosporanic acid	1.0		$\sim 1.0 \times 10^{-8}$
18	7α-Chlorocephalosporanic acid	1.9	1.80×10^{-1}	
19	*N*-Acetylethylamine	10.92	5.40×10^{-6}	8.0×10^{-8}
20	*N*-Acetylglycine	9.81	6.87×10^{-6}	6.3×10^{-7}
21	*N*-Acetyl-2-cyanoethylamine	8.20	5.15×10^{-6}	4.2×10^{-7}
22	*N*-Acetyl-2,2,2-trifluoroethylamine	5.81	1.31×10^{-5}	2.05×10^{-6}
23	4-Nitroacetanilide	1.05	1.63×10^{-3}	3.12×10^{-5}

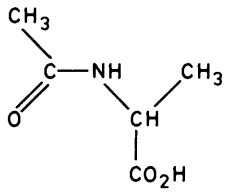
TABLE 2

Observed pseudo-first-order rate constants (s^{-1}) for the degradation of β-lactam antibiotics in acidic aqueous solution at 30.0 °C

[HCl]/ mol l^{-1}	$-H_0$	*a*	*b*	*c*	*d*	*e*		*g*
11.55	4.23				1.08×10^{-1}	2.24×10^{-1}		
9.50	3.50							
6.93	2.45			9.50×10^{-2}				
5.78	2.00				7.00×10^{-4}	1.37×10^{-3}	1.08×10^{-1}	
4.62	1.60			7.72×10^{-3}				
2.31	0.80			6.02×10^{-4}	4.06×10^{-5}	3.70×10^{-5}	3.50×10^{-3}	
2.00	0.70	0.469	2.14×10^{-1}					
1.00	0.20	0.135	8.20×10^{-2}	9.26×10^{-5}	1.20×10^{-5}	1.19×10^{-5}	7.56×10^{-4}	4.46×10^{-4}
0.71	0			7.76×10^{-5}		9.92×10^{-6}		
0.50	−0.20	6.31×10^{-2}	3.01×10^{-2}					
0.316	−0.50			4.33×10^{-5}			1.79×10^{-4}	
0.1	−1.09	9.58×10^{-3}	8.18×10^{-3}				5.91×10^{-5}	
0.04	−1.47	2.38×10^{-3}					2.35×10^{-5}	
0.01	−2.1	8.71×10^{-4}						

[HCl]/ mol l^{-1}	$-H_0$	*h*	*i*	*j*	*k*	*l*	*m*	*n*
11.55	4.23	1.05×10^{-2}	2.17×10^{-2}	3.81×10^{-2}	4.04×10^{-2}	1.77×10^{-4}	9.09×10^{-4}	1.45×10^{-2}
9.50	3.50					6.43×10^{-5}		
6.93	2.45							4.24×10^{-3}
5.78	2.00	2.65×10^{-4}	3.76×10^{-4}	7.81×10^{-4}	7.63×10^{-4}	5.00×10^{-5}	2.89×10^{-5}	
4.62	1.60							9.12×10^{-4}
2.31	0.80	2.11×10^{-5}	5.43×10^{-5}	3.00×10^{-4}		5.62×10^{-5}	1.53×10^{-5}	2.41×10^{-4}
2.00	0.70							
1.00	0.20	8.90×10^{-6}	2.54×10^{-5}			4.52×10^{-5}		7.12×10^{-5}
0.71	0							
0.50	−0.20							
0.316	−0.50							
0.1	−1.09	2.30×10^{-6}	5.48×10^{-6}			1.59×10^{-5}		
0.04	−1.47							
0.01	−2.1	1.53×10^{-6}	3.54×10^{-6}			3.90×10^{-6}		

[a] Benzylpenicillin. [b] Benzylpenicillin methyl ester. [c] 6β-Aminopenicillanic acid. [d] 6α-Bromopenicillanic acid. [e] 6α-Chloropenicillanic acid. [f] Penicillanic acid. [g] Penicillanic acid methyl ester. [h] Cephaloridine. [i] Cephalothin. [j] 3-Methyl-7β-phenylacetamidoceph-3-em-4-carboxylic acid. [k] Methyl ester of *j*. [l] 7-Aminocephalosporanic acid. [m] 7α-Chlorocephalosporanic acid. [n] Nocardicin A.



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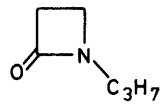

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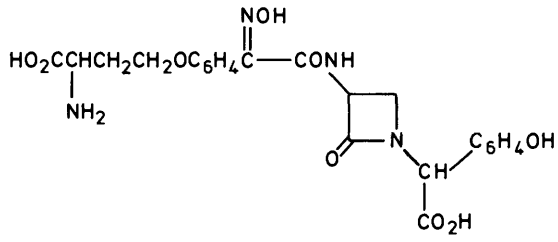

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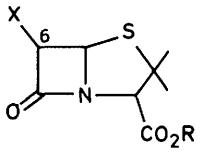

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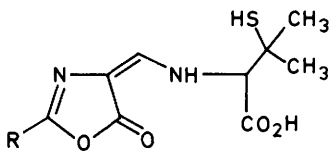


(VII)

the β-lactam shed further light on the reaction mechanism. Substitutents at C-6 in penicillins (VI) will inductively affect both the oxygen and nitrogen of the β-lactam, but their effect on C–N bond cleavage will be that predominantly of an acyl substituent. Normal penicillins rearrange to penicillenic acid (VII) in weakly acidic solution by nucleophilic attack of the acylamido side chain at C-6 on the β-lactam carbonyl group.[19-21] We therefore examined the effect of substituents which could not be involved in neighbouring group participation and in Table 1 are shown the effects of substitution on the rates of the acid-catalysed hydrolysis of 6-substituted penicillins (VI). Electron-withdrawing substituents greatly retard the rate with a Hammett ρ_I value of *ca.* 4.0—5.0 (depending upon the acidity).

The effect of acyl substituents upon the rate of acid catalysed hydrolysis of acyclic amides is small, with electron-withdrawing substituents producing either a small increase or decrease in rate.[22,23] It is therefore apparent that the β-lactams are hydrolysing by a different mechanism.

Finally, electron-withdrawing substituents in the amine portion of the β-lactam decrease the rate of acid catalysed hydrolysis and the rate of penicillenic acid formation. The Brönsted β_{lg} value is *ca.* 0.35 compared with −0.26 for acyclic anilides [24] and amides (Figure 3). Although the effects of substituents are not large they are significant and in opposite directions for β-lactams compared with other amides which again is indicative of a different mechanism.

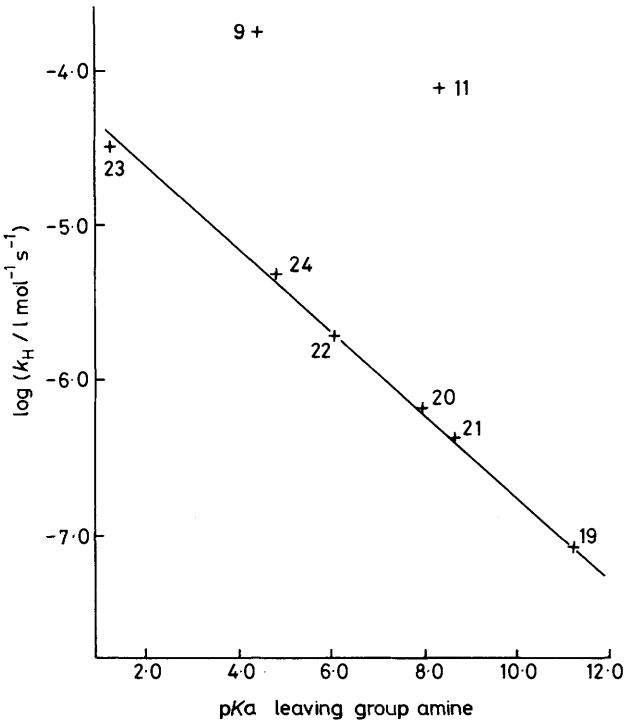
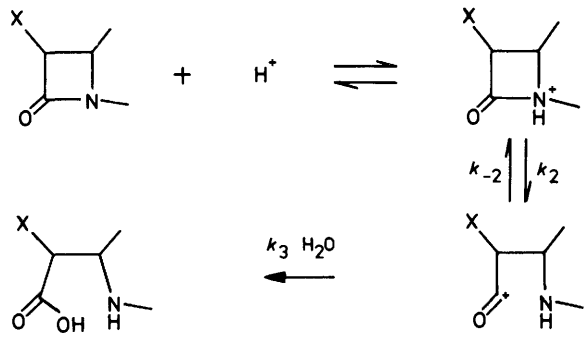


FIGURE 3 Brönsted plot of the second-order rate constants for the acid catalysed hydrolysis of β-lactams and amides against the pK_a of the leaving group amine. Numbers refer to the compounds in Table 1, 24 is acetanilide

The observations are consistent with a unimolecular mechanism for acid catalysed hydrolysis of the β-lactam ring with protonation occurring on nitrogen (Scheme 1). Such a mechanism has been suggested previously for the hydrolysis of nitroacetanilides,[25] esters,[26] and carbamates [27] in very concentrated acid (>70% w/w sulphuric acid). The rate dependence on acid concentration for the hydrolysis of nitroacetanilides shows a



SCHEME 1

maximum, followed by a minimum and subsequently increasing rate at high acid concentration.[25] There is no break in the profile for the hydrolysis of the β-lactams which indicates that there is not a change in mechanism but that Scheme 1 is operative throughout the acid region studied.

The introduction of the A-1 mechanism could result from either the normal A-2 mechanism being retarded or

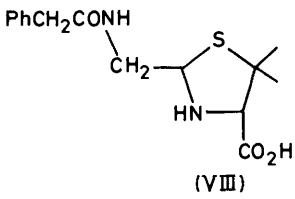
the A-1 pathway being favoured. It is difficult to rationalise the former possibility and the unimolecular mechanism could result from either more favourable N-protonation or more favourable C–N bond fission, which is more probable. There is no evidence for increased β-lactam nitrogen basicity as the pK_a for the N-protonated β-lactams is < -5.

The pK_a for N-protonation of amides is *ca.* -7 to -8 [13] and as the value of the second-order rate constant, k_H, for penicillanic acid is 7.6×10^{-4} l mol^{-1} s^{-1} a similar pK_a value for penicillanic acid would mean the rate of C–N fission (k_2 in Scheme 1) is *ca.* 10^4—10^5 s^{-1}, if k_2 is the rate-limiting step.

The rate enhancement for k_{H^+} for penicillanic acid (VI; X = H) compared with that for N-acetylglycine (III) is *ca.* 10^3 and *ca.* 10^2 compared with an amide of an amine of similar pK_a (Figure 3). As the mechanisms of their hydrolysis are different the rate enhancement for the bicyclic β-lactam proceeding by the same mechanism as that for an acyclic amide is much less than this.

There is a slight shadow cast over the neat picture for the acid-catalysed hydrolysis of β-lactams outlined in Scheme 1. Penicillins with an acylamido side chain at C-6 give penicillenic acid (VII) and other products in acidic solution.[19] This observation can be incorporated into the proposed mechanism (Scheme 1) by suggesting that the intermediate acylium ion is trapped by the intramolecular amido-group rather than by water. However, the rate of penicillin degradation is *ca.* 10^3 faster than that predicted from the σ value for RCONH. The implication is that the acylamido-group participates in the rate-limiting step, which is acceptable if k_3 is rate-limiting (Scheme 1) for hydrolysis, *i.e.* the formation of the acylium ion is reversible. However, this then reintroduces water in the transition state for hydrolysis of the penicillins lacking an acylamido-side chain, which is not indicated by the acidity dependence of the rate of reaction. An alternative explanation is that attack of the acylamido-group on the β-lactam carbonyl carbon is concerted with C–N bond fission *i.e.* the reaction does not proceed *via* the acylium ion, or that trapping of the acylium ion by the acylamido-group is more efficient than by the thiazolidine amino-group and formation of the acylium ion is the rate-limiting step.

Although several kinetic studies have been executed on the degradation of penicillins in acidic media [19–21,28,29] there is still considerable uncertainty about the details of the reaction pathway. In terms of product formation, benzylpenicillin gives benzylpenilloic acid (VIII), benzylpenicilloic acid (IX), benzylpenillic acid (X), benzylpenamaldic acid (XI), and benzylpenicillenic acid (VII), the proportion of each formed depending upon the pH.[19,30,31] There is considerable controversy about how these products are formed and their inter-relationship.[19,21,29,29] Recently it has been shown that most of the penillic acid (X) and penicilloic acid (IX) are *not* formed from penicillenic acid (VII).[21] Penicillenic acid (VII) is not formed significantly below pH 1 and is the major product only at *ca.* pH 3.[19,28] It has been



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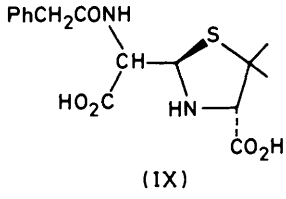

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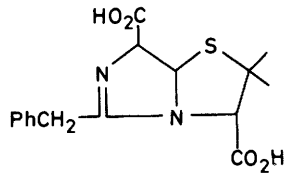

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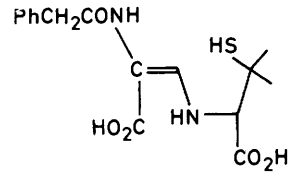


(XI)

suggested [19] that penicillenic acid is formed from the specific acid-catalysed reaction of ionised penicillin or the kinetically equivalent spontaneous rearrangement of the undissociated acid. This suggestion is based on the observation that the rate of formation of penicillenic acid reaches a plateau at pH *ca.* 2, where the overall rate constant for degradation also shows an inflection point, and was thought to correspond to ionisation of the carboxy-group.[19] However, benzylpenicillin methyl ester shows exactly the same behaviour and the rate of penicillenic acid formation follows equation (I), with k_0 3.42×10^{-4} s^{-1} and K_a $10^{-2.25}$. The ionisation of a group with pK_a 2.25 obviously cannot correspond to the carboxy-group as suggested for benzylpenicillin itself.[19]

$$k_{obs} = \frac{k_0[H^+]}{K_a + [H^+]} \quad (1)$$

It seems doubtful, therefore, that the decreased formation of penicillenic acid from penicillin at low pH is due to protonation of the ionised carboxy-group. It is of interest to note here, that because of the similarity of the rate constants for the degradation of benzylpenicillin and its methyl ester (Table 1), there is *no* evidence for neighbouring-group participation of the carboxy-group in the fission of the β-lactam ring.

The important facts to be explained are (1) the rate enhancement observed for the acid catalysed degradation of benzylpenicillin (Table I) indicates neighbouring group participation by the acylamido side chain; (2) benzylpenicillenic acid is not formed in acidic solution, pH < 1; (3) the reaction does not occur *via* the *O*-protonated β-lactam.

The intermediate in penicillenic acid formation is the protonated oxazolone-thiazolidine (XII) formed by trapping the acylium ion (Scheme 2). This can react with water to give penicilloic acid (IX), undergo intramolecular nucleophilic attack of the thiazolidine nitrogen on the carbon of the protonated oxazolone to give penillic acid (X) or eliminate across C(5)–C(6) to give penicillenic acid (VI). The pK_a of the protonated oxazolone or that of the thiazolidine nitrogen could be the kinetically important one controlling penicillenic acid formation. The protonated oxazolone probably has a pK_a of *ca.* 0 [20]

and furthermore there is no obvious chemical reason why protonation of the imine should inhibit elimination. The pK_a of the thiazolidine nitrogen is estimated [32] to be *ca.* 3. Protonation of this nitrogen would inhibit both penicillenic acid (VI) and penillic acid (X) formation. We therefore attribute the kinetically important ionisation to that of the thiazolidine nitrogen.

The dependence of the rate of hydrolysis of 6β-aminopenicillanic acid upon pH is shown in Figure 4. Three

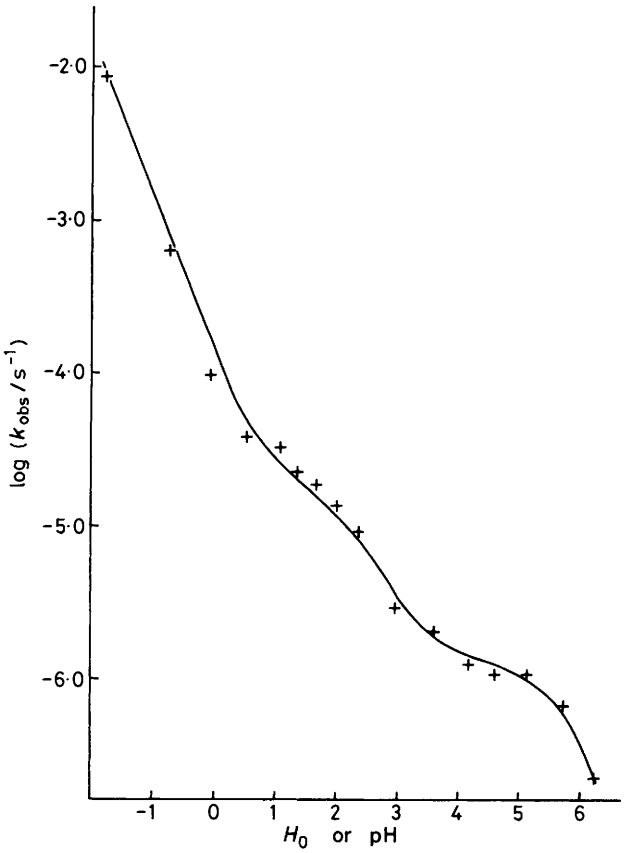


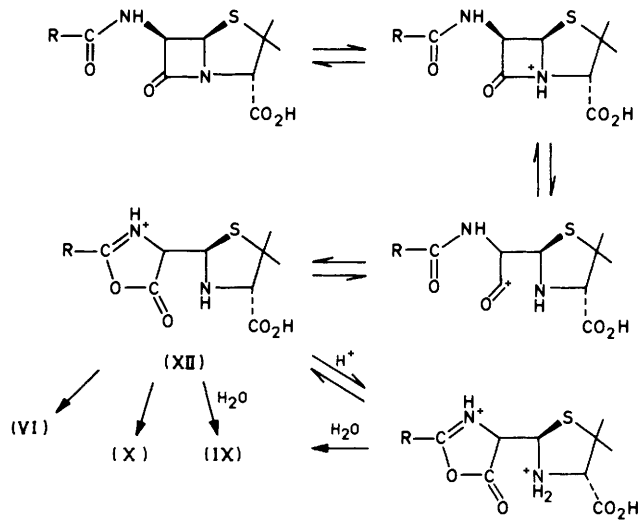
FIGURE 4 Plot of the logarithm of the observed pseudo-first-order rate constant for the hydrolysis of 6β-aminopenicillanic acid at 30° against pH or H_0. The line is theoretical generated from equation (2) using the values given in the text

terms in the rate law are required to describe the data which correspond to the acid catalysed hydrolysis of the species with a protonated amino-group and undissociated carboxy-group, k_1, the acid catalysed hydrolysis of the zwitterion, k_2, and the acid catalysed hydrolysis of the species with a free amino-group and a carboxylate anion, k_3. The line in Figure 4 is generated from equation (2)

$$k_{obs} = \frac{k_1(a_H)^3}{a_H^2 + K_1a_H + K_1K_2} + \frac{k_2K_1a_H^2}{a_H^2 + K_1a_H + K_1K_2} + \frac{k_3K_1K_2a_H}{a^2 + K_1a_H + K_1K_2} \quad (2)$$

where $a_H = 10^{-pH}$ or 10^{-H_0} and $k_1 = 1.8 \times 10^{-4}$, $k_2 = 3.5 \times 10^{-3}$, and $k_3 = 0.675$ 1 mol^{-1} s^{-1}. K_1 and K_2 are 5.0×10^{-3} and 1.78×10^{-6} 1 mol^{-1}, respectively, corresponding to pK_a values of 2.3 and 5.10 for the carboxy- and amino-groups. A 40-fold positive deviation is shown by k_1 on a Hammett plot for 6-substituted penicillins, which is rather surprising as the transition state is dipositively charged.

The acid hydrolysis of cephalosporins (II) shows similar behaviour to that of the penicillins (Figure 1). However, they are about *ca.* 10^4 fold less reactive than penicillins. Electron-withdrawing substituents at C-7 in cephalosporins (II) decrease the rate of acid hydrolysis and, as for penicillins, the Hammett ρ_I value is *ca.* 5. There is no evidence for neighbouring group participation by the 7-acylamido-group as postulated for the penicillins (Scheme 2). To us, there is no obvious reason



SCHEME 2

to explain the difference in behaviour between the cephalosporins and penicillins. Either attack of water on the acylium ion (Scheme 1) could be inhibited in penicillins relative to cephalosporins and therefore trapping of the acylium ion by the acylamido-group is more effective in penicillins or the latter process could be inhibited in cephalosorins. One obvious difference is that the dihydrothiazine of cephalosporins has a less basic nitrogen than the thiazolidine of penicillins; enamine resonance lowers the pK_a by *ca.* 2 units. This could account for the lower reactivity of 7-amino- and 7-chloro-cephalosporanic acid compared with the analogous penicillin derivatives (Table 1).

It is of interest to note that there is no evidence for the group at C-3 in cephalosporins (acetate or pyridine) affecting the rate of reaction. In fact, the 3-methyl derivative is more reactive than the cephalosporins with acetate or pyridine at C-3 (Table 1) which, contrary to other suggestions,[33] indicates that expulsion of these groups is not important in the rate-limiting step.

Alkaline Hydrolysis.—In order to assess any special reactivity of the β-lactam antibiotics, the dependence of the rate of hydrolysis of simple amides and β-lactams upon substituents must be known. Carbon–β-lactam nitrogen bond fission in penicillins (I) involves the expulsion of a better leaving group than the basic amine

of simple amides. The pK_a of the protonated amine in the thiazolidine derivative (IX) is 5.2. The alkaline hydrolysis of acyclic anilides is characterised by ^{18}O exchange with solvent occurring faster than hydrolysis [34] and a second-order dependence on hydroxide ion concentration.[34,35] Both results indicate rate-limiting breakdown of the tetrahedral intermediate. The second-order rate constants for the hydroxide catalysed hydrolysis of *N*-substituted acyclic amides were obtained by extrapolation of $k_{obs}/[OH]$ against [OH] to zero hydroxide concentration and are given in Table 1. Together with literature data [34,36] for anilides these rate constants are plotted against the pK_a of the leaving group amine in Figure 5. The Brönsted β_{lg} value is −0.07, compatible

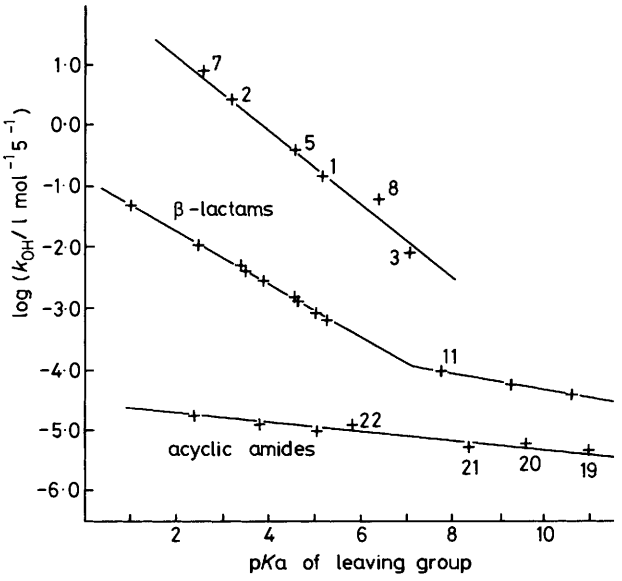


FIGURE 5 Brönsted plot of the second-order rate constants for the hydroxide ion catalysed hydrolysis of β-lactams and amides, against the pK_a of the leaving group amine. Numbers refer to the compounds in Table 1. Data for anilides are from ref. 34 and that for β-lactams of anilines from ref. 5

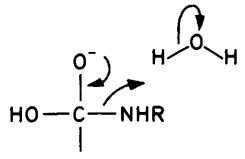
with water general acid catalysed breakdown of this intermediate.[37] The second-order rate constant for 4-nitroacetanilide shows a positive deviation of *ca.* 10-fold from the Brönsted plot (Figure 5), which has been noted previously [38] and is attributed to the rate-limiting step changing to formation of the intermediate.

The rates of alkaline hydrolysis of *N*-aryl-β-lactams exhibit mainly first-order dependence on hydroxide ion concentration.[39] Together with the second-order rate constants for the hydrolysis of other β-lactams these are plotted in Figure 5. The Brönsted β_{lg} value is −0.44, which has been interpreted in terms of rate-limiting formation of the tetrahedral intermediate.[39] However, β-lactams of the more basic amines show a positive deviation from this line and appear to exhibit a smaller dependence upon basicity of the leaving group, and may signify a change in rate-limiting step to breakdown of the intermediate.

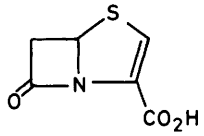
A consequence of the different dependency upon leaving group basicity (Figure 5) is that the rate enhancement of β-lactams compared with acyclic amides depends on the basicity of the leaving group amine. β-Lactams of weakly basic amines are *ca.* 5×10^2 more reactive than an acyclic amide of the same amine. However, β-lactams of basic amines are only slightly more reactive than an analogous acyclic amide.

Crystallographic [40] and spectroscopic evidence [41] show that *N*-substituted β-lactams are planar. The rate enhancement of 30—500 fold shown by β-lactams of amines of $pK_a \leqslant 6$ may be adequately rationalised by the change in co-ordination number–hybridisation of the carbonyl carbon as the tetrahedral intermediate is formed.[9,10] The magnitude is similar to the 500-fold faster rate of reduction of cyclobutanone by borohydride compared with acetone.[42]

β-Lactams of basic amines are only *ca.* 10 fold more reactive than analogous acyclic amides. If the mechanism of hydrolysis of both these systems involves a similar transition state, which is indicated by the Brönsted plot (Figure 4), then the rate limiting must involve little or no C–N bond fission. The release of strain energy accompanying the opening of the β-lactam ring could increase the rate by up to 10^{20} but obviously is not realised in the transition state. The Brönsted β_{lg} of −0.07 is indicative of a transition state in which the nitrogen behaves as if it has *ca.* 0.5 positive charge in the transition state.[37] This is incompatible with rate-limiting expulsion of the amine anion, but is consistent with the concerted mechanism (XIII) proposed for the reaction of hydroxy-amides.[37]



(XIII)



(XIV)

The monocyclic β-lactam, nocardicin (IV), hydrolyses with the expected reactivity of a monolactum but the bicyclic antibiotics are *ca.* 100 fold more reactive than β-lactams of amines of similar basicity (Figure 5). The Brönsted β_{lg} value for the bicyclic system is −0.55 which is again indicative of rate-limiting formation of the tetrahedral intermediate. Although the rate enchancement is significant it is hardly of the magnitude expected from the release of strain in opening a four-membered ring or from a system in which amide resonance is significantly inhibited. Even the penem system (XIV) shows little sign of significant strain. Monocyclic β-lactams of weakly basic amines may be as reactive as the 1-azabicyclo[3.2.0]heptan-2-ones of the more basic amines.

Electron-withdrawing substituents at C-6 increase the rate of hydroxide ion hydrolysis (Table 1) with a Hammett ρ_I value of +2.0 which is similar to the value of +2.7 reported for acyclic amides and corrected for steric effects.[22] The effect of the 6-acylamido-substituent on alkaline hydrolysis is purely inductive.

The reactivity of cephalosporins (II) is similar to that of penicillins (I). Although electron-withdrawing substituents at C-3 in cephalosporins increase the rate of hydrolysis (Table 2), the rate differences are those expected purely by an inductive effect and β_{lg} values for substitution at C-3 is −0.06 and −0.5 for substitution of C-4 which are consistent with rate-limiting attack. Contrary to a recent suggestion [33] there is no evidence for expulsion of the leaving group at C-3 occurring during the rate-limiting step.

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