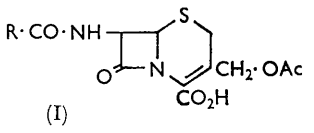
1296. *Cephalosporanic Acids. Part III.*[1] *Reactions with Pyridine—Kinetics and Mechanism*

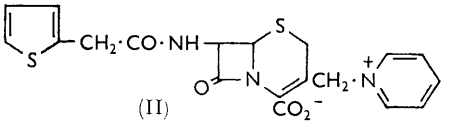
By A. B. Taylor

Displacement of the acetoxy group by pyridine and other nucleophiles proceeds by an S_N1 mechanism. No common-ion effect was detected. In aqueous solution the losses in yield of the pyridinium betaine are due chiefly and in equal measure to product decay and unselective attack of pyridine on the intermediate carbonium ion. In formamide the second cause is much less important. The reaction is supported only by protic solvents and is assisted by the carboxylate group.

When salts of cephalosporanic acids (I) undergo nucleophilic substitution by pyridine at the C-3 methylene group, yields are lower than those obtained with many other nucleophiles.[1,2] Because the pyridine derivatives showed great potential as broad spectrum antibiotics,[3] the cause of the lower yields was investigated. It was found to be partly product decay and partly non-specific attack of pyridine on a dipolar carbonium ion* intermediate. During the work, which was carried out on the sodium salt of 7-2′-thienylacetamidocephalosporanic acid (I; R = $CH_2{\cdot}\overline{C{:}CH{\cdot}CH{:}CH{\cdot}S}$), some general mechanistic features emerged, including a requirement for protic solvent and a rate enhancement by the carboxylate group.



(I)



(II)

(*a*) *Formation and Decay.*—The pyridine derivative (II) was known to decompose during its preparation from the parent acid and pyridine in aqueous solution. The kinetics of formation and decay were therefore studied to determine to what extent the latter process was responsible for the low yields.

Proton magnetic resonance (p.m.r.) spectroscopy was used to monitor the conversion of covalent into ionised acetate. An internal reference standard (t-butyl alcohol) was included in a preliminary run to establish that the total concentration of acetate remained constant, the conversion going to about 98% completion. Reactions designed to determine the kinetic order of the acetate ionisation were carried out typically at 46° on the sodium salt of the acid in 0·2M phosphate buffer, pH 7·0, the total ionic strength being adjusted to 1·0 with sodium perchlorate. Drift in pH was thus generally reduced to 0·1—0·2 units, but could be as much as 0·5 units in the absence of any nucleophile other than water. In some runs at lower pH the free acid, which is insoluble in water, was dissolved by the addition of an excess of pyridine. Concentrations of un-ionised pyridine were readily estimated, as it could be shown from a consideration of the pK_a at 46° of pyridine (5·00) [4] and of the cephalosporanic acid (2·59 in water containing 5% by volume of acetone) that hydrolysis was inappreciable at the concentrations employed. First-order rate-constants were calculated from straight-line, semi-logarithmic plots of residual covalent acetate concentration against time. They are gathered together in Table 1. They were

* The use of the term " carbonium ion " here and elsewhere is not intended to imply that the positive charge resides solely on carbon.

[1] Part II, J. D. Cocker, B. R. Cowley, J. S. G. Cox, S. Eardley, G. I. Gregory, J. K. Lazenby, A. G. Long, G. A. Somerfield, and J. C. P. Sly, *J.*, 1965, 5015.
[2] S. Eardley, J. Kennedy, A. G. Long, and G. Stocker, to be published.
[3] P. W. Muggleton, C. H. O'Callaghan, and W. K. Stevens, *Brit. Med. J.*, 1964, **2**, 1234.
[4] J. M. Essery and K. Schofield, *J.*, 1961, 3939.

found not to increase with increasing concentration of pyridine (runs 1—4). A slight decrease is evident, which may be due partly to a small medium effect and partly to about 3% conversion into a more slowly ionising tautomer (see below). Neither were they

TABLE 1

First-order rate-constants for displacement of acetoxy group in 7-2′-thienylacetamidocephalosporanate ions

Run	Nucleophile	Medium	Temp.	pH	Ionic strength	Initial [cephalosporanate]	Initial [nucleophile]	10^5k (sec.$^{-1}$)
1	None	Buffer	46°	7·1—6·6	1·0	0·120	—	2·12
2	Pyridine	,,	46	7·1—7·0	1·0	0·120	0·120	2·03
3	,,	,,	46	7·1—7·0	1·0	0·120	0·260	1·97
4	,,	,,	46	7·2—7·1	1·0	0·120	0·580	1·94
5	,,	,,	46	7·3—7·1	1·0	0·240	0·580	1·92
6	,,	,,	46	7·3—7·1	1·0	0·320	0·580	1·95
7	,,	Water	46	6·2—6·0	1·0	0·120	0·580	1·93
8	,,	Buffer	46	7·2—7·0	0·56	0·120	0·580	1·96
9	,,	Water	46	6·2—6·0	0·12	0·120	0·580	1·97
10	Azide	Buffer	46	7·1—7·0	1·0	0·120	0·120	2·28
11	,,	,,	46	7·1—7·0	1·0	0·120	0·240	2·32
12	,,	,,	46	7·1—7·0	1·0	0·300	0·300	2·11
13	Thiosulphate	Water	46	7·4—6·9	0·24	0·120	0·120	2·21
14	Azide, acetate	Buffer	46	7·2—7·0	1·0	0·120	0·120, 0·120	2·29
15	Acetate	,,	46	7·2—6·7	1·0	0·120	0·120	2·07
16	,,	Water	46	7·2—6·5	1·0	0·120	0·120	1·86
17	Azide	Buffer	39	7·1—7·0	1·0	0·120	0·120	0·952
18	,,	,,	55	7·1—7·0	1·0	0·120	0·120	6·57
19	,,	,,	65	7·2—7·1	1·0	0·120	0·120	20·1
20	Thiopicolinate	,,	46	6·7—6·5	1·0	0·120	0·120	2·29

Buffer = phosphate. pH's are at reaction temp. For pyridine, concn. is of un-ionised base.

dependent on the initial concentration of cephalosporanate ions (runs 4—6). Ambiguities associated with minor changes in pH and ionic strength were removed by showing that these were without effect on reaction rate (runs 4, 7, 8, 9). It was later shown that the yield and rate of formation of the pyridinium betaine at 46° were unaffected by larger pH changes (5·1—8·3), provided that the concentration of un-ionised pyridine was maintained. The ionisation rate remained almost unchanged when various amounts of other nucleophiles were introduced (azide and thiosulphate ions: runs 1, 10—13). These results serve to establish over-all first-order kinetics (first-order in cephalosporanate ions and zero-order in pyridine) for the conversion of covalent into ionised acetate, and thus for the formation of the carbonium ion.

Rate-constants from the integrated form of the limiting first-order equation are shown in Table 2 for ionisation in the presence of (i) pyridine, (ii) azide ions, and (iii) phosphate buffer only. The lack of a downward trend in the constants demonstrates the absence

TABLE 2

Reactions of 7-2′-thienylacetamidocephalosporanate ions in phosphate buffer at 46°

(i) *With pyridine.* $[\text{cephalosporanate}]_0 = 0{\cdot}120$; $[\text{pyridine}]_0 = 0{\cdot}580$.

t (min.)	120	235	359	446	599	732	845	1055	1328
10^2[cephalosporanate]	10·35	9·14	7·97	7·05	6·11	5·16	4·68	3·72	2·64
10^5k (sec.$^{-1}$)	2·03	1·93	1·90	1·99	1·88	1·92	1·86	1·85	1·90

(ii) *With azide ions.* $[\text{cephalosporanate}]_0 = 0{\cdot}120$; $[\text{azide}]_0 = 0{\cdot}120$.

t (min.)	96	158	275	364	484	569	678	803
10^2[cephalosporanate]	10·47	9·56	8·15	7·38	6·23	5·42	4·70	4·02
10^5k (sec.$^{-1}$)	2·35	2·38	2·34	2·23	2·26	2·32	2·31	2·27

(iii) *With buffer alone.* $[\text{cephalosporanate}]_0 = 0{\cdot}120$.

t (min.)	79	149	176	281	382	525	686	924
10^2[cephalosporanate]	10·83	9·97	9·55	8·38	7·23	6·04	4·93	3·77
10^5k (sec.$^{-1}$)	2·16	2·06	2·16	2·13	2·21	2·18	2·16	2·09

of a common-ion or mass-law effect.[5] Confirmation was provided by the failure of added acetate ions (one equivalent of sodium acetate) to reduce the rate of formation and yield of betaine in (i) and the rate of ionisation in (ii) and (iii) (Table 1, runs 10 and 14, 1 and 15). The absence of a common-ion depression in aqueous solutions probably indicates that no ionic recombination (" external ion return ") [6] occurs. (In solvents of low dielectric constant it could alternatively mean that the intermediate species is an ion-pair, possibly solvent-separated, and not a free carbonium ion.[6]) With no nucleophile present other than water, some recombination is indicated, as added acetate produced a small drop in the ionisation rate (run 16).

Rates measured at four temperatures (Table 1, runs 10, 17, 18, 19) gave an excellent Arrhenius plot, an indication against the occurrence of appreciable participation by complex reactions.[7] The activation energy (E_A) was 24·7 kcal. mole^{-1}. Other activation parameters derived for a temperature of 55·0° using transition-state theory were: entropy of activation ($\Delta S^{\ddagger}$) = −5 e.u., and enthalpy of activation ($\Delta H^{\ddagger}$) = 24·0 kcal. mole^{-1}. The

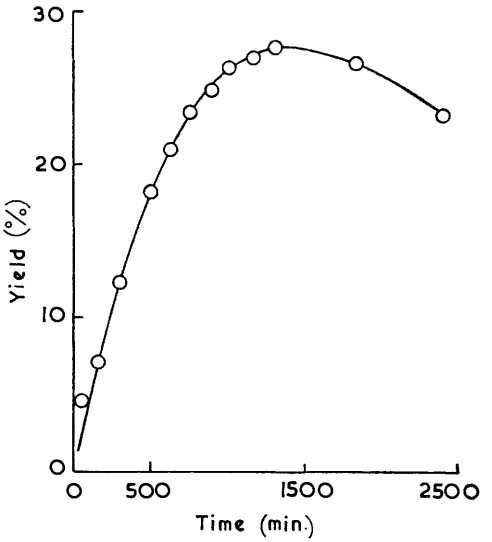


FIGURE 1. Formation of pyridinium betaine at 46°

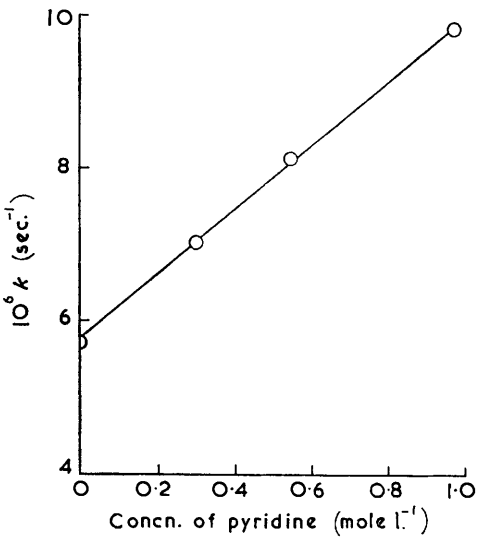


FIGURE 2. Variation in rates of decomposition of pyridinium betaine at 46° with initial concn. of un-ionised pyridine

low negative value of $\Delta S^{\ddagger}$, although partly due to the highly ordered nature of the solvent, may also be caused by an increase in vibrational and rotational freedom in the transition state. This argues against an increase in external interaction with the neighbouring carboxylate group on passing from ground to transition state [see section (*c*) (ii)].

The displacement may thus far be represented by a simple S_N1 mechanism: $TACA^- \xrightarrow{-OAc^-} TAC^{\pm} \xrightarrow{py} TACP^{\pm}$, where $TACA^-$ = cephalosporanate ion, $TAC^{\pm}$ = dipolar carbonium ion, $TACP^{\pm}$ = pyridinium betaine, and py = pyridine.

In order to investigate product decay, a spectrometric assay for betaine was devised, based on a paper-electrophoretic separation at pH 1·9. The same assay was used to determine optimum yields. A representative plot of yield as a function of time is shown in Figure 1. In a typical preparative run the cephalosporanic acid is dissolved in aqueous

[5] L. C. Bateman, M. G. Church, E. D. Hughes, C. K. Ingold, and N. A. Taher, *J.*, 1940, 979.
[6] S. Winstein, E. Clippinger, A. H. Fainberg, R. Heck, and G. C. Robinson, *J. Amer. Chem. Soc.*, 1956, **78**, 328.
[7] J. R. Hulett, *Quart. Rev.*, 1964, **18**, 227.

pyridine. No buffers are used, and the pH at the reaction temperature (46°) drops from 6·2 to 5·9. The initial concentration of the acid is 0·12M, and the concentration of unionised pyridine is 0·46M. Optimum yields are between 26 and 28% of the theoretical (isolated yields are 21—22%). The mean ionisation rate constant is $1{\cdot}96 \times 10^{-5}$ sec.$^{-1}$. For rate measurements on the decomposition of betaine, preparative reaction conditions were simulated by buffering pyridine to pH 6·1 (46°) with formic acid; in the absence of pyridine, sodium formate solution of the same pH was used. With the initial concentration of betaine held at 0·020M, and with different concentrations of pyridine, a series of first-order constants was obtained. These are plotted against the concentration of unionised pyridine in Figure 2. They are seen to represent a pseudo-unimolecular mechanism, at least for pyridine, the first-order kinetic form being attributable to (a) the partly solvolytic nature of the reaction and (b) the use of an excess of pyridine. As the concentration of pyridine is increased and (a) becomes less important, (b) becomes more so. For a concentration of 0·46M free pyridine the rate constant for decomposition is $7{\cdot}8 \times 10^{-6}$ sec.$^{-1}$. In a composite reaction of the type being considered ($A - k_1 \longrightarrow B - k_2 \longrightarrow C$, where k_1 and k_2 are first-order), the maximum yield of B (*i.e.*, betaine) is given [8] by

$$Y_{max.} = 100(k_2/k_1)^{k_2/(k_1 - k_2)} \quad (1)$$

Substituting for k_1 and k_2, the maximum yield is found to be 54%. Since the actual yield is 26—28% the decomposition of product by pyridine and water cannot be held solely responsible for the losses.

(*b*) *Side-reactions.*—Possible side-reactions are: attack of water on the carbonium ion, pyridine on the acid, water on the acid, and pyridine on the carbonium ion but not at the C-3 methylene group.

(i) *Attack of water on the carbonium ion.* If water competes effectively with pyridine for a share of the carbonium ion intermediate, *initial* rates of formation of betaine should increase with increasing pyridine concentration. (By confining attention to initial rates, the effect of concomitant decay can be discounted.) These rates, measured with the aid of the electrophoretic assay and estimated by extrapolation of slopes to zero time, were found to be notably independent of pyridine concentration when this was above about two equivalents (Table 3).

TABLE 3

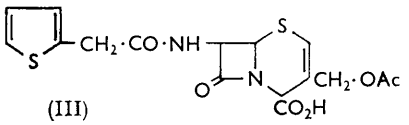
Initial rates of formation of the pyridinium betaine

Initial [free pyridine]	Initial [cephalosporanate]	Initial rates $\times 10^6$ (sec.$^{-1}$ mole l.$^{-1}$)
0·14	0·12	1·06
0·26	0·12	1·20
0·49	0·12	1·22
0·97	0·12	1·19

The test becomes increasingly insensitive as the extent of the assumed competitive reaction decreases. However, from the proportionality between d[TACP]/dt and $k_2[py]/(k_2[py] + k_3)$, sensitivity limits were assessed, and it was concluded that the proportion of this type of competition is less than 5% when five equivalents of pyridine are present. This conclusion will apply to competition from any other kinetically first-order reaction of the intermediate, such as intramolecular rearrangement and spontaneous decay. Second-order bimolecular attack by hydroxide ion is unlikely to provide serious competition at the usual reaction pH,[9] a contention supported by the finding that rates of formation and yields of betaine do not vary appreciably between pH 5·1 and 8·3.

[8] A. A. Frost and R. G. Pearson, " Kinetics and Mechanism," Wiley, New York, 1953, p. 153.
[9] O. T. Benfey, E. H. Hughes, and C. K. Ingold, *J.*, 1952, 2494.

(ii) *Attack of pyridine on the acid.* Bimolecular attack by pyridine to produce acetate ions must be ruled out on kinetic evidence [section (*a*)]. An alternative possibility is pyridine-sponsored conversion of the acid into a modified form without loss of bound acetate, the modified form then ionising at the same rate as the acid and so remaining kinetically undetected. An example would be isomerisation to the ceph-2-em acid (III), which is known to occur in neat pyridine.[10] However, the ceph-2-em acid forms a pyridine derivative that is relatively stable, and this derivative was formed in only 1·1% yield (assayed) during a standard reaction of the ceph-3-em acid with pyridine. (It was shown not to originate in the ceph-3-em pyridinium betaine.) Hence, since the yield from the ceph-2-em acid is 32% under the same conditions, the extent of isomerisation during a normal preparation is limited to about 3%. In any event the ceph-2-em acid was found to ionise five times more slowly than the ceph-3-em acid. Further objections to a pyridine-dependent side-reaction of the sort described are: no induction period is observed in the ionisation rate-plots, and increasing the pyridine concentration does not reduce initial rates of formation of betaine. (Fortuitous cancellation of two effects, produced by attack of pyridine on the acid and the carbonium ion, would not account for the fall in these initial rates when less than two equivalents of pyridine are used.) Bimolecular attack by pyridine on the acid is thus not acceptable as a side-reaction of significance.



(III)

(iii) *Remaining side-reactions.* The two remaining side-reactions are written into the scheme:

$$\text{TACA}^- \xrightarrow{k_1\ (slow)} \text{TAC}^{\pm} \xrightarrow[\text{py}]{k_2\ (fast)} \text{TACP}^{\pm} \xrightarrow[\text{H}_2\text{O, py}]{k_5\ (slow)} \text{X},$$

$$\text{TACA}^- \xrightarrow[\text{H}_2\text{O}]{k_3\ (slow)} \text{Z} \qquad \text{TAC}^{\pm} \xrightarrow[\text{py}]{k_4\ (fast)} \text{Y}$$

where X, Y, and Z are by-products. From a conventional steady-state treatment:

$$\frac{\mathrm{d}[\text{TACP}^{\pm}]}{\mathrm{d}t} = \frac{k_1k_2[\text{TACA}^-]_0\mathrm{e}^{-(k_1+k_3)t}}{k_2+k_4} - k_5[\text{TACP}^{\pm}]$$

Integration with use of the factor $\exp(k_5t)$ and application of boundary conditions ($t = 0$; $[\text{TACP}^{\pm}] = 0$) leads to:

$$[\text{TACP}^{\pm}]_t = \frac{k_1k_2[\text{TACA}^-]_0\{\mathrm{e}^{-(k_1+k_3)t} - \mathrm{e}^{-k_5t}\}}{(k_2+k_4)\,(k_5-k_1-k_3)} \qquad (2)$$

Differentiating (2) and equating to zero gives the time for optimum yield of betaine:

$$t_{\text{max.}} = \frac{\ln\{(k_1+k_3)/k_5\}}{k_1+k_3-k_5} \qquad (3)$$

From (2) and (3):

$$[\text{TACP}^{\pm}]_{\text{max.}} = \frac{k_1k_2[\text{TACA}^-]_0}{k_5\,(k_2+k_4)}\left\{\frac{k_5}{k_1+k_3}\right\}^{\frac{k_1+k_3}{k_1+k_3-k_5}} \qquad (4)$$

from which the optimum yield ($= 100[\text{TACP}^{\pm}]_{\text{max.}}/[\text{TACA}^-]_0$) may be obtained. Equation (2) may be reduced to two limiting forms, corresponding to (a) side-reaction(s) leading to Z only ($k_4 = 0$) and (b) side-reaction(s) leading to Y only ($k_3 = 0$). These will be seen to be equivalent. Hence, in terms of their effect on the formation of betaine, the two side-reactions are kinetically indistinguishable. The proportion of the total reaction of cephalosporanic acid or its carbonium ion leading to betaine formation may be calculated

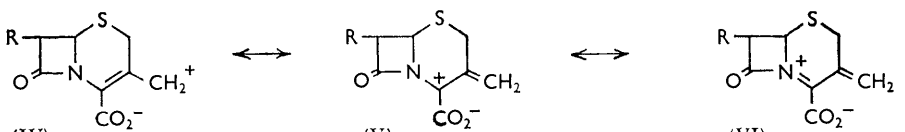
[10] S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, Abstracts of Scientific Papers, 19th International Congress of Pure and Applied Chemistry, London, 1963, p. 308; Part IV., *J.*, to be published.

from either of the two limiting forms of equation (4). Thus, putting $k_4 = 0$ and substituting $[TACP^{\pm}]_{max.}/[TACA^{-}]_0 = 0{\cdot}275$, $k_1 + k_3 = 1{\cdot}96 \times 10^{-5}$ sec.$^{-1}$ (ionisation rate), and $k_5 = 7{\cdot}8 \times 10^{-6}$ sec.$^{-1}$ (values for 46°, pH 6), k_1 is found to be $1{\cdot}0 \times 10^{-5}$ sec.$^{-1}$ and $k_1/(k_1 + k_3) = 0{\cdot}51$. Hence the maximum yield of betaine would be limited to 51% by these side-reactions (acting singly or in concert) even if the betaine did not decompose. Substitution of the appropriate values in equation (3) gives the optimum time for stopping the reaction as 1280 min. at 46°. Experimentally it was found to fall within the range 1250—1300 min. Calculation shows that, after 1280 min. at 46°, 22% of the original amount of cephalosporanic acid remains unchanged.

The two side-reactions may be distinguished non-kinetically by studying yields obtained with nucleophiles other than pyridine. Substitution proceeds at closely similar rates *via* rate-limiting ionisation (Table 1, runs 10, 13, 20), but now the yields are greatly improved.[1] When the product is protected by insolubility (nucleophile = thiopicolinate or thiobenzoate), yields of isolated product as high as 85% have been recorded.[11] It follows that " yields " of carbonium ion are probably in the region of 90% ($k_3 \simeq 0{\cdot}1\ k_1$) and the significance of bimolecular solvolytic attack on the acid substrate is correspondingly diminished.

The most important side-reaction (as opposed to product decay) in the preparation of betaine with excess pyridine (>2 equivalents) is now seen to be the attack of pyridine on the carbonium ion to give products other than the desired one. Numerous components of the reaction mixture were revealed by thin-layer chromatography, but, with the exception of carbon dioxide and the small amount of ceph-2-em derivative referred to, attempts to identify by-products were unsuccessful.

(*c*) *Mechanism.*—(i) *Nature of the carbonium ion.* Stabilisation of the transition state leading to the allylic dipolar carbonium ion (IV) is undoubtedly increased by resonance with forms resembling (V), as shown. The contribution from (VI) may be lowered because



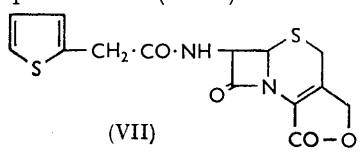
the β-lactam nitrogen atom forms part of a four-membered ring. Bond-angle constraint will shift hybridisation on nitrogen away from sp^2 in the direction of sp^3, thereby introducing some *s* character into the lone-pair orbital and so weakening the π bond. The contribution may also be lowered by sharing of conjugation with the adjacent carbonyl group, although this in turn will be reduced below that in a normal amide linkage.[12]

Further stabilisation may arise from transannular interaction with the sulphur atom of the dihydrothiazine ring. It has been suggested that this atom forms part of the chromophore responsible for the ultraviolet absorption spectrum of cephalosporin C.[12] Overlap with the electron-deficient centres should be increased by puckering of the ring,[13] as this could lead to an increase in the ratio of σ to π symmetry in the bonding orbital. It is difficult to predict to what extent it would be modified by *sp* hybridisation of sulphur valencies (to accommodate the ring valence-angle), but oxidation to sulphoxide should reduce it, as the residual lone-pair electrons will now occupy a hybrid orbital with increased *s* character, in keeping with the pyramidal geometry [14] of sulphoxides. This view receives superficial support from the observation of a five-fold reduction in acetate-ionisation rates on comparing the sulphoxide of sodium thienylacetamidocephalosporanate with its parent compound. In the presence of azide ions (one equivalent) the first-order rate-constant at 46°

[11] W. Graham, J. F. Oughton, and P. E. Sandford, unpublished results.
[12] G. C. Barrett, V. V. Kane, and G. Lowe, *J.*, 1964, 783.
[13] D. C. Hodgkin and E. N. Maslen, *Biochem. J.*, 1961, **79**, 393.
[14] C. C. Price and S. Oae, " Sulfur Bonding," Ronald Press, New York, 1962, p. 131.

is $4{\cdot}69 \times 10^{-6}$ sec.$^{-1}$; in the presence of pyridine (five equivalents) it is $4{\cdot}60 \times 10^{-6}$ sec.$^{-1}$. Unfortunately, no substitution products could be isolated, and it is possible that the sulphoxide is first rapidly converted into a more slowly ionising compound (*e.g.*, a ceph-2-em isomer or a decarboxylated form, both conversions initiated by loss of a C-2 proton and assisted by the positive charge on the sulphur atom). Some reduction in rate may also be ascribed to an adverse field effect from the sulphoxide group. In either event, the results quoted lose their proposed significance, and the role of the sulphur atom remains in doubt.

(ii) *Role of the carboxylate group.* When a solution of the sodium salt of the cephalosporanic acid (0·20M) was allowed to ionise to 80% of completion in phosphate buffer at 46° and approximately neutral pH (7·2 dropping to 6·3), the yield of γ-lactone (VII) [1,15] (estimated by means of thin-layer chromatography) was 3%. In a second experiment, one equivalent of sodium azide was added, and the yield was <0·5% (the pH fell from 7·2 to 6·8). This reluctance to undergo γ-lactonisation reflects the inferior nucleophilic powers of solvated carboxylate [16] and accords with the lack of ionic recombination noted earlier. The difference in the two yields quoted suggests that the lactone is formed *via* a free carbonium ion rather than by an intramolecular reaction.



(VII)

The carboxylate group will most probably influence the S_N1 reaction by providing additional stabilisation of the cationic centre in the transition state by charge dispersal. This could result from an inductive (+ *I*) effect or from the contribution of a zwitterionic [17] (or α-lactone [18]) structure. (Some modification of the effect will arise from a different distibution of solvation energy and entropy in the transition state.) The influence was demonstrated by measuring the acetate ionisation rate at a pH low enough to ensure that a high proportion of the electron-repelling carboxylate group was protonated to its conjugate acid. At 46° and pH 2·15 (achieved by adding an equivalent of hydrochloric acid to the sodium salt) the first-order rate-constant was $2{\cdot}84 \times 10^{-6}$ sec.$^{-1}$. After 1·5 half-lives the pH was 2·76. (A slight rise in the instantaneous " constants " was noted after one half-life.) The solvent was unbuffered aqueous dioxan (1 : 1 by vol.), the un-ionised acid being insoluble in water. From the pK_a at 46° in the same solvent (4·41) the proportion of un-ionised acid present during the kinetic run may be shown to decrease with the rising pH from 99 to 95%. With the same solvent, temperature, ionic strength, and concentration of reactants, but at pH 7·51—6·20, the ionisation rate was $7{\cdot}30 \times 10^{-6}$ sec.$^{-1}$. This value being used to correct for the presence of an average 3% residual carboxylate form, the rate for the un-ionised acid becomes $2{\cdot}7 \times 10^{-6}$ sec.$^{-1}$.

The change from carboxylate group to carboxylic acid thus appears to produce an almost threefold reduction in the rate of ionisation of the acetate group. The retarding effect is, in fact, as much as double this, because the insoluble γ-lactone was precipitated in 30% yield during the reaction at acid pH, indicating that not all of the reduced rate is that of a simple solvent-assisted ionisation. The part associated with lactone formation is presumably the result of an acid-catalysed process (*e.g.*, an intramolecular reaction or a reaction involving acyl-oxygen fission). It is considered that the accelerating effect of the carboxylate group contributes more to the rate difference than the retarding effect of the carboxylic acid group.

Attempts to carry out rate measurements on the methyl ester were frustrated by its low solubility in suitable ionising solvents.

(iii) *The ceph-2-em system.* A cursory examination of this system revealed that the sodium salt underwent ionisation of the acetate group at about one-fifth the rate of the

[15] E. Van Heyningen, *J. Medicin. Chem.*, 1965, **8**, 22.
[16] C. A. Bunton, " Nucleophilic Substitution at a Saturated Carbon Atom," Elsevier, Amsterdam, 1963, p. 75.
[17] W. A. Cowdrey, E. D. Hughes, C. K. Ingold, S. Masterman, and A. D. Scott, *J.*, 1937, 1252.
[18] S. Winstein and H. J. Lucas, *J. Amer. Chem. Soc.*, 1939, **61**, 1576; E. Grunwald and S. Winstein, *ibid.*, 1948, **70**, 841.

ceph-3-em isomer under similar conditions, the first-order constant being $4{\cdot}0 \times 10^{-6}$ sec.$^{-1}$ at 46°. With sulphur replacing nitrogen in conjugation with the double bond, the lower rate might have been explained in terms of the smaller net overlap in a $3p$–$2p\pi$ bond compared with a $2p$–$2p\pi$ bond. However, we have seen that the contribution from nitrogen is likely to be restricted stereochemically, and the rate reduction must be largely attributed to the relative remoteness of the carboxylate group from the centres of positive charge in the ceph-2-em carbonium ion.

(*d*) *Solvent Effects.*—In non-polar solvents yields of the pyridinium betaine are zero. Neither is there any detectable yield in dipolar aprotic solvents (dimethyl formamide, dimethyl sulphoxide, sulpholane, etc.), even after prolonged heating. Presumably the small degree of charge separation in the transition state lowers the singnificance of the bulk dielectric constant. Swain [19] has shown from a consideration of a large number of nucleophilic substitutions that those with an S_N1 mechanism are more susceptible to electrophilic solvation than to nucleophilic solvation, *i.e.*, the solvation of the developing anion is of paramount importance. In dipolar aprotic solvents, ion–dipole and dipole–dipole interactions are much less important for the solvation of anions than for the solvation of cations.[20] It follows that promotion of ionisation must here arise from solvation of the leaving group by hydrogen bonding. The failure of several good proton donors to support the reaction can be attributed to one of two causes: (a) they form salts with pyridine (formic and acetic acids) or (b) they rapidly destroy the product (alcohols). Formamide, which resembles water as a solvent and readily forms hydrogen bonds by proton donation,[21] does neither of these things; at 46° and with five equivalents of pyridine the optimum yield of betaine in this solvent is 22% (electrophoretic assay). The first-order rate-constants for ionisation of acetate and decay of product under these conditions are $1{\cdot}21 \times 10^{-6}$ sec.$^{-1}$ * and $1{\cdot}89 \times 10^{-6}$ sec.$^{-1}$. On using these rates in a simple formation/decay interpretation, the calculated optimum yield is found to be 29%. This is much closer to the established yield than it was in water. A treatment similar to that used for the results in water shows that side-reactions (as opposed to product decay) now account for only 24%† of cephalosporanic acid consumed. This shift in the balance of the reaction finds a ready explanation in terms of the side-reaction already proposed (misplaced attack of pyridine on the carbonium ion). Thus, in the poorer ionising solvent (formamide) the carbonium ions are more likely to be still under the influence of the departing group when the nucleophile attacks. The reduced charge delocalisation will lead to a higher proportion of product in which the nucleophile occupies the same site as the original substituent (*i.e.*, the required betaine). The effect has been noted in the solvolysis of 1- and 3-phenylallyl chlorides,[22] and a similar explanation was offered. It may be enhanced in the present instance, since pyridine will be less solvated (and thus more active) in formamide than in water.

From measurements in formamide at three temperatures, with 0·18M sodium salt and and an equivalent of azide ions, first-order rate-constants were obtained: at 46°, $2{\cdot}00 \times 10^{-6}$; at 55°, $6{\cdot}30 \times 10^{-6}$; at 65°, $2{\cdot}12 \times 10^{-5}$ sec.$^{-1}$. From these results the following activation parameters were calculated: $E_A = 26{\cdot}6$ kcal. mole^{-1}; $\Delta H^{\ddagger}_{55°} = 26{\cdot}0$ kcal. mole^{-1}; $\Delta S^{\ddagger}_{55°} = -3{\cdot}5$ e.u. Thus, the lower rate found in formamide than in water is due to an unfavourable change in $\Delta H^{\ddagger}$, partly compensated by a change in $\Delta S^{\ddagger}$. Although this may be seen as a trend in the manner of an isokinetic relationship,[23] the values of $\Delta S^{\ddagger}$

* If pyridine is replaced by azide this rate is $2{\cdot}00 \times 10^{-6}$ sec.$^{-1}$. This represents a larger increase than was found with water as solvent, the result, probably, of an observed rise to about 30% in the extent of pyridine-catalysed isomerisation to the ceph-2-em system.

† Less, if the increased isomerisation and a possible increase in bimolecular attack (due to slower ionisation) are taken into account.

[19] C. G. Swain and R. B. Mosely, *J. Amer. Chem. Soc.*, 1955, **77**, 3727; C. G. Swain, R. B. Mosely, and D. E. Bown, *ibid.*, p. 3731.
[20] A. J. Parker, *Quart. Rev.*, 1962, **16**, 163.
[21] A. J. Parker and D. Brody, *J.*, 1963, 4061.
[22] G. Valkanas, E. S. Waight, and M. Weinstock, *J.*, 1963, 4248.
[23] J. E. Leffler, *J. Org. Chem.*, 1955, **20**, 1202.

are only accurate to within about one entropy unit (the rate-constants have *ca.* 5% accuracy), and $\Delta\Delta S^{\ddagger}$ may not be significant. The small value of $\Delta\Delta S^{\ddagger}$ on changing the solvent is not unexpected when it is remembered that the substrate is ionic before it enters the polar transition state. The small values of $\Delta\Delta S^{\ddagger}$ and $\Delta\Delta H^{\ddagger}$, taken together, indicate continuity of mechanism on changing from water to formamide.[23]

Experimental

Materials.—The preparations of 7-2′-thienylacetamidocephalosporanic acid [24] and its pyridinium betaine [2] have been described. The sodium salt of the acid was recrystallised twice from aqueous n-propanol by addition of acetone, and had $[\alpha]_D^{25}$ +130 (*c* 1·0; H_2O), $\lambda_{max.}$ (H_2O) 237 mμ (ε 15,700), inflection at 262 mμ (ε 9850). The betaine was rechromatographed on " De-acidite " FF anion exchange resin in the acetate form, freeze-dried, and triturated with methanol, $[\alpha]_D^{25}$ +47 (*c* 1·0; H_2O), $\lambda_{max.}$ (H_2O) 240 mμ (ε 16,100), inflection at 255 mμ (ε 14,700). The sulphoxide was prepared by oxidation of the sodium salt with periodate.[1] It was recrystallised from ethanol–ethyl acetate. The ceph-2-em cephalosporanic acid was prepared as the pyridinium salt by treating the ceph-3-em isomer with 10% acetic anhydride in pyridine.[10] It was recrystallised twice from ethyl acetate containing 5% ethanol. Both it and the sulphoxide were characterised by infrared spectrum and mixed m. p. with authentic samples supplied by Dr. G. I. Gregory.

Pyridine was from a selected batch shown to be picoline-free by its p.m.r. spectrum, and was dried over fused potassium hydroxide and fractionated through a 24 in. column of glass helices. Formamide was used from freshly opened bottles, and was shown by Karl Fischer estimation to contain 0·10—0·13% water. Dioxan (AnalaR) was dried over molecular sieves and passed through an alumina column (Woelm, neutral, grade 1).

Formic acid, and sodium acetate, thiosulphate, and phosphates were of AnalaR quality. Sodium formate and perchlorate were prepared by neutralising the AnalaR acids with carbonate-free AnalaR sodium hydroxide. Thiopicolinic acid was supplied by Dr. B. R. Cowley. Sodium azide was recrystallised from ethanol.

Measurements.—Kinetic runs of the ionisation of acetate were begun by dissolving the sodium salt of the acid in a heat-equilibrated solution of the nucleophile in, for the most part, phosphate buffer. This was contained in 5 or 10 ml. volumetric flasks re-calibrated at the reaction temperature. After bringing the volume to the mark, the solutions were transferred to 10 ml. B.10-stoppered flasks immersed to the neck in a water-bath. The temperature was held constant to within ±0·02° by a mercury/iso-octane thermostat. For runs in formamide (except at 65°), and for those involving decomposition of the betaine, temperature-equilibration was considered unnecessary. Samples (*ca.* 0·5 ml.) were chilled in cold water and stored in solid carbon dioxide.

For ionisation rates the thawed samples were transferred to the specimen tube of a Varian Associates A60 p.m.r. spectrometer. Peaks due to covalent and ionised acetate occur, respectively, at 7·9 and 8·1 τ relative to sodium 3-(trimethylsilyl)propanesulphonate (the separation is much reduced at acid pH). A total of six integrals was recorded at two sweep-widths, and a mean value was taken. A small correction was applied to the sampling time to allow for the recording period in the spectrometer at 35°.

In the electrophoretic assay of the betaine, the thawed sample (10 μl.) was spotted on to Whatman 3MM paper and run in pH 1·9 buffer consisting of water (495 ml.), acetic acid (84 ml.), formic acid (16·7 ml.), and acetone (105 ml.) [25] for 1·5 hr. at 15 v cm.$^{-1}$. The spots were detected under a Hanovia Chromatolite ultraviolet lamp, cut out, and eluted [26] into 5 or 10 ml. volumetric flasks. The eluate extinctions were measured at 240 and 255 mμ on a Hilger Uvispek spectrometer. A linear relationship was shown to exist between extinction and concentration of betaine. The ratio of the extinctions at the two wavelengths was used as a check on purity.

A Radiometer Titrator model TTT.1., calibrated at two points against phthalate and borate buffers, was used for measuring pH and pK_a values. The pK_a values were determined by potentiometric titration of the sodium salt against hydrochloric acid. For determinations in 5% acetone solution, a non-limiting form of the Henderson equation was employed. Corrections

[24] R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Amer. Chem. Soc.*, 1962, **84**, 3401.

[25] H. Michl in " Chromatographic Reviews," Elsevier, Amsterdam, 1959, vol. 1, p. 11.

[26] C. E. Dent, *Biochem. J.*, 1947, **41**, 240.

for ionic-strength effects were applied by means of the Debye–Hückel equation. In 50% aqueous dioxan the A and B constants were calculated for 46° with the value 32 for the dielectric constant.[27] The effective ionic diameter was taken as 5×10^{-8} cm. As the ionic strengths in titration and reaction solutions were nearly the same (0·2), a closely similar correction was applied in reverse when estimating the concentration of un-ionised acid, thus minimising the error inherent in the use of the Debye–Hückel theory for relatively high salt concentrations.

Miscellaneous.—Small amounts of γ-lactone were estimated by measuring spot areas on thin-layer chromatograms. Silica containing uranyl acetate (0·02%) as fluorescing agent was used as substrate and 15% ethanol in ethyl acetate as developing solvent. The assay was calibrated against an authentic sample.

Identification of the ceph-2-em pyridine derivative by paper electrophoresis was assisted by the characteristic colour given with a potassium iodoplatinate spray. It was assayed by eluting the spots and measuring optical extinctions at 237 mμ. An authentic sample was supplied by Dr. G. I. Gregory.

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