Reaction Rates and Thermodynamic Equilibration of Tetrahedral Intermediates: the Alcoholysis of Cephalosporins

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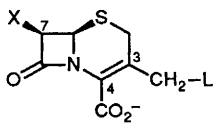
The rate of reaction of cephalosporins with alkoxide ions shows general acid-catalysed inhibition, which is attributed to the trapping of the anionic tetrahedral intermediate (T^-) by proton donors to give the thermodynamically more stable neutral intermediate T^0 at a rate which is faster than breakdown of T^-.

The alcoholysis of cephalosporins **1** is an interesting reaction as a model for the interaction of β-lactam antibiotics with the serine transpeptidase and β-lactamase enzymes.[1] However, the reaction of alcohols with cephalosporins shows such very unusual kinetic behaviour that it is of relevance to the mechanism of acyl transfer reactions[2] in general. A major step forward in the understanding of nucleophilic substitution at acyl centres was the realisation that the bond making to the nucleophile and bond breaking to the leaving group generally occurred in separate stages, *i.e.* an unstable tetrahedral intermediate is usually formed in a low steady-state concentration.[3] Since then tetrahedral intermediates stable enough to be identified spectroscopically have been studied.[4] A second important development was that the tetrahedral intermediate is not necessarily at equilibrium with respect to proton transfer and that this step rather than bond making or breaking between heavy atoms may be rate-limiting.[5] A corrollary of this suggestion is that some reaction rates may be as fast as they are because the tetrahedral intermediate is not at equilibrium with respect to its protonation states. Thermodynamically more stable states may be kinetically less reactive and if they accumulate, a slower reaction rate may result. We report herein an example of such inhibition.

Alcohols react with cephalosporins **1** in aqueous solution to open the β-lactam and form the ester **2**. Reaction rates were studied using the alcohol as both buffer and nucleophile and under such conditions the observed pseudo-first-order rate constants are first order in alcohol concentration (Fig. 1). However, there is a dramatic decrease in the second-order rate constants for the alkoxide ion k_{RO^-} as the pH of the solution is lowered, such that they fall several hundredfold over a narrow pH range (Fig. 2). This observation is not an artefact of the buffer system used because some cephalosporins show 'normal' behaviour, *i.e.* the second-order rate constant (k_{RO^-}) is pH independent, whereas others show 'abnormal' dependence upon pH in the same buffer solutions. At the high pH range of the alcohol buffer the second-order rate constant (k_{RO^-}) becomes pH independent and the same value of the rate constant is obtained using fully dissociated alcohols in solutions of sodium hydroxide (Fig. 2). The 'abnormal' pH dependence of k_{RO^-} is not a consequence of the substituents attached to the cephalosporin skeleton since it is observed with cephalosporins lacking a side chain amido



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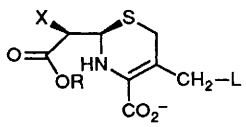

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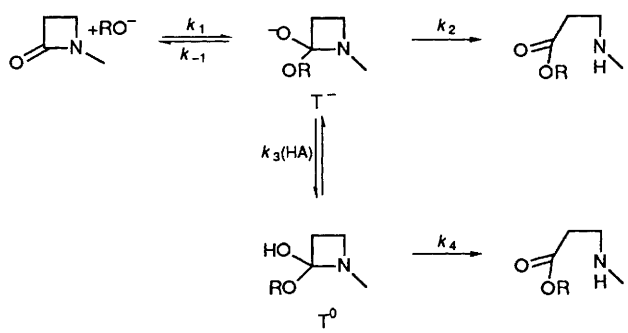

Scheme 1

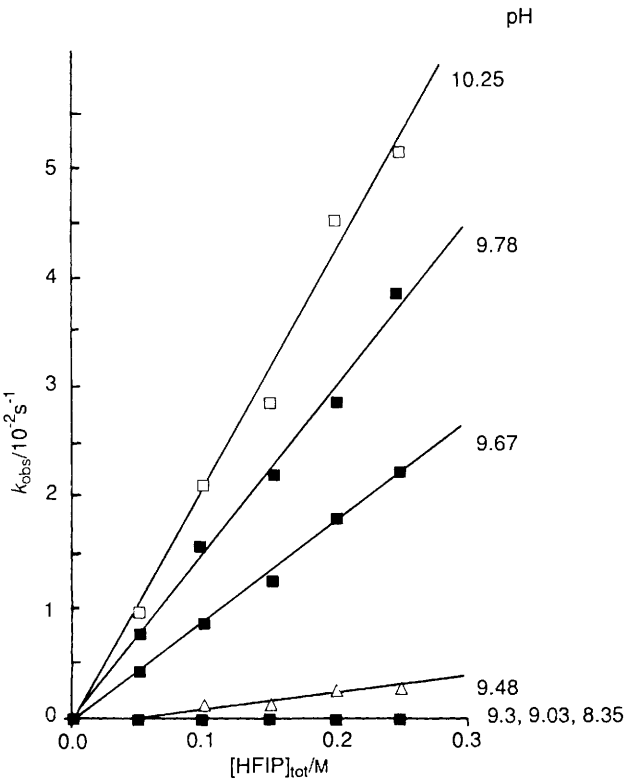

Fig. 1 The observed pseudo-first-order rate constant for the alcoholysis of cephaloridine in water at 30 °C, I = 1.0 M (KCl) as a function of total hexafluoropropan-2-ol concentration at the indicated pH

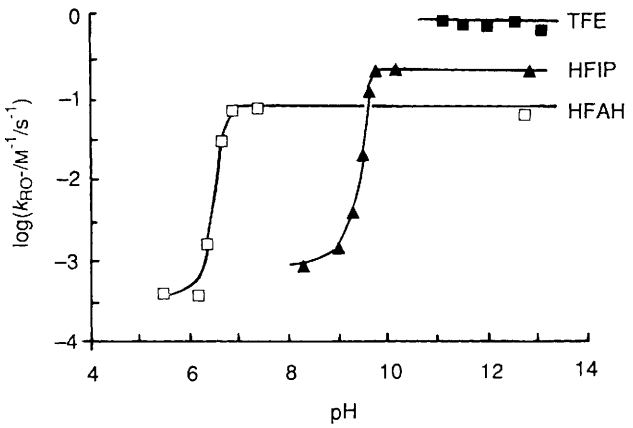

Fig. 2 The second-order rate constant for alkoxide ions reacting with cephaloridine in water at 30 °C I = 1.0 M (KCl) as a function of pH; TFE = trifluoroethanol, HFIP = hexafluoropropan-2-ol and HFAH = hexafluoroacetone hydrate

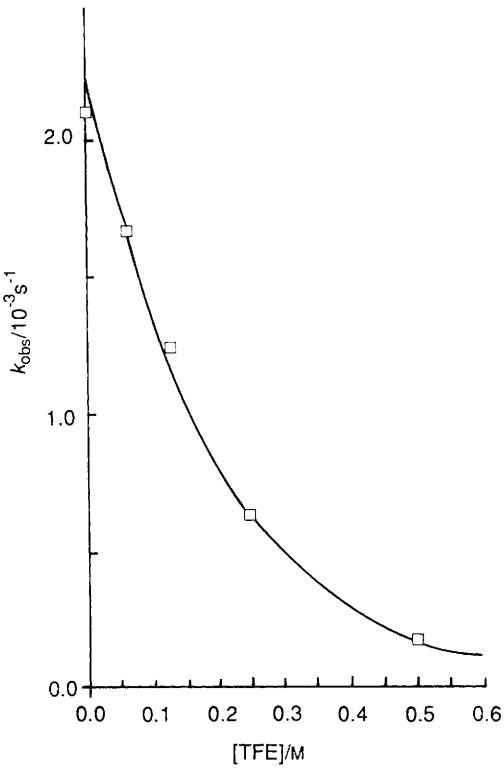

Fig. 3 The effect of increasing trifluoroethanol concentration on the observed pseudo-first-order rate constant for the alcoholysis of the 3-methyl cephalosporin (**1**, L = CH_3) in 0.2 M hexafluoropropan-2-ol buffer pH 10.25 at 30 °C, I = 1.0 M (KCl)

group at C-7, *i.e.* **1**: X = NH_2, with cephalosporins lacking a leaving group at C-3 *i.e.* **1**: L = H, with cephalosporins having an ester function at C-4 rather than a carboxylate and also with Δ2-cephalosporins. It is, therefore, concluded that the observation is an intrinsic property of the alcoholysis reaction.

In the lower pH range of the buffer system the concentration of undissociated alcohol increases and it was assumed that there may be a general acid-catalysed inhibition of the reaction. This was confirmed by adding general acids such as higher pK_a alcohols or ammonium salts to the alcohol buffer system. At the higher pH range where 'normal' behaviour is observed adding these general acids causes the onset of inhibition, whereas in the lower pH region, in which the slower rate is observed, adding these additional proton donors has no effect (Fig. 3).

We can think of no explanation other than that the anionic tetrahedral intermediate T^- formed by nucleophilic attack of the alkoxide ion on the β-lactam is trapped by proton donors to form the thermodynamically more stable but less reactive neutral intermediate T^0 (Scheme 1). Even at pH below the pK_a of T^0 the intermediate T^- cannot be protonated if the rate constant for this step is lower than the breakdown of T^- to reactants and products. The half-life of anionic tetrahedral intermediates[6] is commonly of the order of 10^{-8} to 10^{-11} s and the longer the lifetime the more likely is the intermediate to be protonated by acids at the diffusion controlled limit, k_3[HA] (Scheme 1). Tetrahedral intermediates of carbonyl carbon in four-membered rings are expected to be relatively more stable than their acyclic analogues.[1,7] The opening of four-membered rings is not the facile process expected from the release of strain energy.[1,8] It is, therefore, not surprising that the tetrahedral intermediate, T^-, formed from the alkoxide-ion attack on cephalosporins has a sufficiently large lifetime to allow protonation to occur.

In the high pH region of the buffers the rate of breakdown of T^- is faster than protonation, $(k_{-1} + k_2) > k_3$[HA]. Under these conditions the second-order rate constants for alkoxide-ion attack, k_{RO^-}, generate Brønsted β_{nuc} values of 0.2 to 0.3; consistent with rate-limiting formation of the tetrahedral intermediate. At lower pH, or in the presence of additional proton donors, protonation of T^- to T^0 becomes competitive with breakdown of T^- and k_3[HA] > $(k_{-1} + k_2)$. Under these conditions the Brønsted β_{nuc} values are 0.8 to 1.0 and are

consistent with the rate-limiting breakdown of the intermediate.

There is some 270 MHz NMR evidence that the tetrahedral intermediate actually accumulates. In trifluoroethanol buffers at low pH, but not at high pH, the 3-methyl cephalosporin, **1** (L = H, X = $PhCH_2CONH$), shows high field shifts of the β-lactam 6-H and 7-H from an AB 'quartet' centred at δ 5.06 and 5.56 ppm with $J_{6,7}$ 4.3 Hz to two different AB 'quartets' with different intensities centred at δ 4.52 and 4.62 ($J_{6,7}$ 9.85 Hz) and 4.55 and 4.65 δ ($J_{6,7}$ 7.88 Hz). There are two new methyl resonances at δ 1.88 and 1.92 and the H-2 protons also move upfield from δ 3.24 and 3.61, $J_{\alpha,\beta}$ 17.2 Hz, to a doublet at δ 3.10 but a pair of doublets at δ 3.38 and 3.45, $J_{\alpha,\beta}$ 16.8 Hz. These spectral changes are consistent with the formation of the two stereoisomers of the tetrahedral intermediate T^0 formed in a ratio of *ca.* 60:40. Only β protons of H-2 are affected by the stereochemistry at the tetrahedral intermediate.

It has been previously suggested that although electrophilic catalysis at the carbonyl oxygen may enhance nucleophilic attack at carbon, complete neutralisation of the incipient charge on oxygen is inhibitory for expulsion of the leaving group from the carbonyl centre.[9] This is illustrated by the present example where the decomposition of the neutral tetrahedral intermediate T^0 is several hundredfold slower than T^-.

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