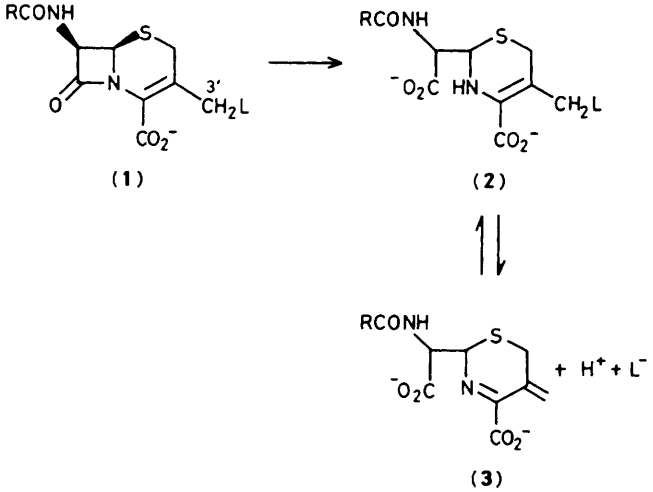
Equilibria between Enamine and α,β-Unsaturated Imine in Cephalosporin Hydrolysis

Stephen Buckwell,[a] Michael I. Page,*[a] and Jethro L. Longridge[b]

[a] *Department of Chemical and Physical Sciences, The Polytechnic, Huddersfield HD1 3DH, U.K.*
[b] *ICI Pharmaceuticals Division, Alderley Park, Macclesfield SK10 4TG, U.K.*

The normal product of hydrolysis of cephalosporins with a leaving group at C-3′ is an α,β-unsaturated imine which is demonstrated to be in equilibrium with its precursor, an enamine; the equilibrium constants for this process are reported for thiolate anions which generate a Bronsted β_{lg} of 0.71.

Cephalosporins (**1**) differ from penicillins by having a leaving group at C-3′ which is often expelled during the reactions of these antibiotics with nucleophiles that open the β-lactam ring.[1] There have been many suggestions that this process is concerted and that this mechanism is important for the lethal action of cephalosporins towards bacteria.[2] However, there have been several reports suggesting that β-lactam ring opening and fission of the C-3′–L group are separate processes and an intermediate enamine (**2**) is formed.[3] Despite this experimental evidence, theoretical arguments are still used to justify a concerted process.[4] Herein is presented evidence that there is an equilibrium between the intermediate enamine (**2**) and the product α,β-unsaturated imine (**3**).



We have synthesised a series of cephalosporins (**1**) with thiol leaving groups at C-3′ (L = SR) that have different pK_a values. The *Bacillus cereus* β-lactamase I catalysed hydrolysis of these cephalosporins produces different products. At pH 7.0, cephalosporins with good leaving groups at C-3′, *e.g.* *N*-pyridyl, give the α,β-unsaturated imine (**3**) whereas those with poor leaving groups at C-3′, *e.g.* n-butylthio, give the enamine (**2**). These two products have different u.v. spectra, notably between 260 and 270 nm ($\Delta\varepsilon \simeq 6 \times 10^3$ dm^3 mol^{-1} cm^{-1}). The addition of 10^{-2} M n-butanethiol to an aqueous solution of (**3**) (10^{-4} M) at pH 7.0 generates (**2**). This can also be observed if the α,β-unsaturated imine (**3**) is generated from cephaloridine (L = *N*-pyridyl). The addition of up to 10^{-2} M pyridine to solutions of (**3**) does not regenerate (**2**).

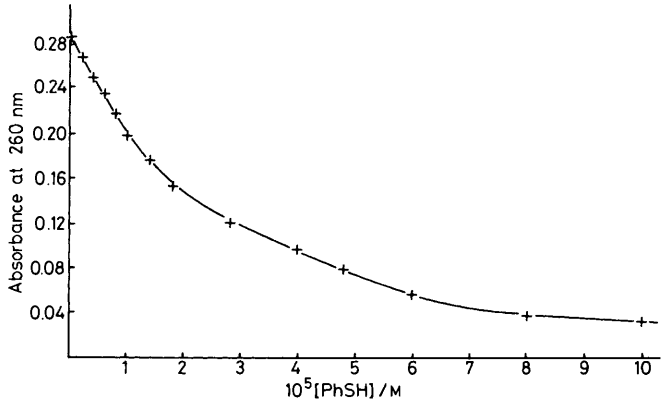


Figure 1. Absorbance at 260 nm *vs.* [PhSH] in a 10^{-4} M aqueous solution of (**3**) at pH 7.0, 30 °C.

Table 1. Equilibrium constants, K, for imine–enamine with thiols, equation (1), at 30 °C.

Thiol	pK_a	K/mol² dm⁻⁶
Bu^nSH	12.40	1.01×10^{-15}
$HO[CH_2]_2SH$	9.61	7.81×10^{-14}
PhSH	6.43	1.86×10^{-11}

On step-wise addition of a thiol to aqueous solutions of (**3**), u.v. spectra intermediate between those corresponding to (**3**) and (**2**) are observed. The equilibrium constant for the equilibrium between (**3**) and (**2**) is given by equation (1),

$$K = \frac{[(3)][RS^-][H^+]}{[(2)]} = \frac{[(3)]}{[(2)]} \quad \frac{K_a[RSH]_{total}}{1 + K_a/H^+} \qquad (1)$$

where K_a is the dissociation constant of the thiol RSH. The equilibrium constant K can be determined by measuring the change in absorbance at 260 nm as a function of thiol concentration (Figure 1). The second order rate constant for thiophenoxide ion attack on (**3**) is *ca.* 740 dm³ mol⁻¹ s⁻¹ at 30 °C.

If the pK_a of the thiol is $\gg$ pH, the ratio of imine (**3**) to enamine (**2**) is pH independent. However, if pH > pK_a of RSH the ratio decreases with decreasing pH. The reversibility of this reaction is readily demonstrated by increasing the pH of a solution of (**2**) which regenerates (**3**). The equilibrium constants for other thiols have been measured and there is a linear relationship between log K and the pK_a of the thiol which gives a Bronsted β_{lg} of 0.71. The formation of (**2**) is favoured with basic thiol anions (Table 1).

It is possible that a nucleophilic group on the enzyme, which is the killing target for cephalosporin antibacterial activity, could undergo the Michael type addition to the α,β-unsaturated imine to inactivate the enzyme irreversibly. The demonstration of a reversible equilibrium between the enamine intermediate (**2**) and the α,β-unsaturated imine (**3**) is further evidence that ring opening of the β-lactam of cephalosporins is *not concerted* with expulsion of the C-3′ leaving group.

Received, 18th March 1986; Com. 357

References

1 M. I. Page, *Acc. Chem.* Res., 1984, **17**, 144.
2 D. B. Boyd and W. H. W. Lunn, *J. Med. Chem.*, 1979, **22**, 778; H. Bundgaard, *Arch. Pharm. Chemi. Sci. Ed.*, 1975, **3**, 94; C. H. O'Callaghan, S. M. Kirby, A. Morris, R. E. Waller, and R. E. Duncombe, *J. Bacteriol.*, 1972, **110**, 988.
3 M. I. Page and P. Proctor, *J. Am. Chem. Soc.*, 1984, **106**, 3820; W. S. Faraci and R. F. Pratt, *ibid.*, 1984, **106**, 1489; D. Agathocleous, S. Buckwell, P. Proctor, and M. I. Page in 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' eds. A. G. Brown and S. M. Roberts, Royal Society of Chemistry, 1985, p. 18; E. J. J. Grabowski, A. W. Douglas, and G. B. Smith, *J. Am. Chem. Soc.*, 1985, **107**, 267.
4 D. B. Boyd, *J. Org. Chem.*, 1985, **50**, 886.