Enzymatic Ring Expansion of Penicillins to Cephalosporins: Side Chain Specificity

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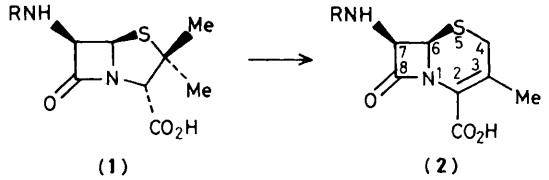
Structural variants on the acylamino-side chains of penicillins as substrates for the ring expansion enzyme from *Cephalosporium acremonium* CO728 show that a six carbon chain terminating in a carboxy group permits efficient conversion into cephems with the exception of δ-(L-α-aminoadipoyl) [5-(5*S*)-amino-5-carboxypentanoyl].

The biosynthesis of cephalosporins involves a ring expansion of penicillin N (**1**, R = **a**) to deacetoxycephalosporin C (DAOC) (**2**, R = **a**).[1] The δ-(α-aminoadipoyl) side chain of (**1**) is D-configured, deriving from the L-side chain of isopenicillin N,[2] the first formed penicillin.[3] We have purified the ring expansion activity from *Cephalosporium acremonium* CO728[4] and used it to assess the side chain specificity of this important enzymatic step. Thus a series of penicillins were exposed to this purified activity and their conversions into cephems monitored by ^{1}H n.m.r. spectroscopy, bioassay, and steady-state initial rate measurements based on the generation of increased u.v. absorption at $\lambda_{max.}$ 260 nm, characteristic of the dihydrothiazine moiety of cephalosporins.[5] In those cases where the efficiency of the conversion permitted we isolated and characterised the so-formed cephalosporins. The results are shown in Table 1. In the case of (**1**, R = **b**) the product, active against *Escherichia coli* both in the presence and absence of β-lactamase 1 (from *Bacillus cereus*), was purified by h.p.l.c. [reverse phase ODS column, acetonitrile : water (1 : 40)] to give the cephem (**2**, R = **b**), δ_H (D_2O, 500 MHz)† 1.77 (3H, s, 3-Me), 3.03, 3.35 (2H, ABq, *J* 18 Hz, 4-H), 3.53, 3.58 (2H, ABq, *J* 15 Hz, $COCH_2$), 4.88 (1H, d, *J* 4.5 Hz, β-lactam-H), 5.36 (1H, d, *J* 4.5 Hz, β-lactam-H), and 7.23—7.35 (4H, 2 × m, ArH), *m/z* (fast atom bombardment) 360 (MH^+, 45%), which was identical to a synthetically prepared sample.‡

Similarly with (**1**, R = **c**) the product was purified by h.p.l.c. (reverse phase ODS column, 7.5 mM NH_4HCO_3) to yield the cephem (**2**, R = **c**), δ_H (D_2O, 500 MHz)† 1.46—1.53 (4H, m, $[CH_2]_2CH_2CO$), 1.79 (3H, s, 3-Me), 2.06—2.10 (2H, m, CH_2CO), 2.22—2.30 (2H, m, CH_2CO), 3.12, 3.47 (2H, ABq, *J* 13 Hz, 4-H), 5.44 (1H, d, *J* 4.5 Hz, β-lactam-H), and 5.96 (1H, d, *J* 4.5 Hz, β-lactam-H), which was identical to an authentic sample. The dimethyl ester of (**2**, R = **c**) obtained (diazomethane) from the enzymatic reaction gave *m/z* (NH_3 desorption chemical ionisation) 388 (MNH_4^+, 32%) and 371 (MH^+, 34%), identical to an authentic sample.§ This result is in contrast to that of Kupka *et al.*[6] who reported, using a protoplast lysate from *C. acremonium* CW-19, containing ring expansion activity, that 'carboxy n-butyl penicillin' [which we assume to be (**1**, R = **c**)] did not give cephalosporin products (by bioassay or h.p.l.c.).

It is of some interest that the *m*-carboxyphenylacetyl side chain of (**1**, R = **b**) provides a 'rigid' version of the adipoyl side chain of (**1**, R = **a**) and (**1**, R = **c**), *cf.* Figure 1.

Of the other penicillins (**1**, R = **d**—**k**) we tested as substrates for the ring expansion activity only the γ-(D-glutamyl) penicillin (**1**, R = **d**) gave a low conversion into a cephem product detectable by ^{1}H n.m.r. spectroscopy and

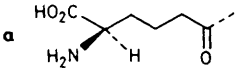
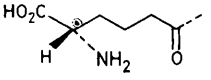
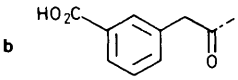
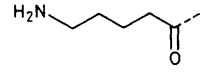
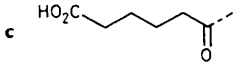
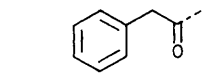
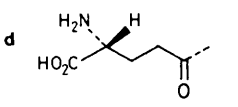
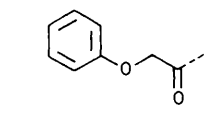
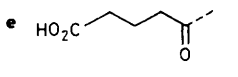


† Referenced to sodium [2,2,3,3-$^2H_4$]-3-trimethylsilylpropanoate (TSP) = 0.00 p.p.m.

‡ Prepared by hydrogenation (Pd/C, H_2, 20 °C) of the bis-*p*-nitrobenzyl ester of (**2**, R = **b**).

§ Prepared by hydrogenation (Pd/C, H_2, 20 °C) of the bis-*p*-nitrobenzyl ester of *N*-adipoyl-7-aminodeacetoxycephalosporin C.

Table 1. Side chain specificity of the ring expansion of penicillins to cephalosporins.[a]

Conversion				No conversion	
R	Initial velocity / μ mol min^{-1} x10^4	% (rel. to **1**, R = **a**)		R	
a 	3.88	100	**f** 		
b 	1.93	50	**g** 		
c 	1.56	40	**h** 		
d 	0.54	14	**j** 		
e 	0.13	3	**k** H		

[a] Conditions: [substrate] = 1 mM; [enzyme] = 4 μM; cofactors $FeSO_4$, O_2, α-ketoglutarate, ascorbate, dithiothreitol; buffer: Tris·HCl, pH 7.5; temp. 30 °C.

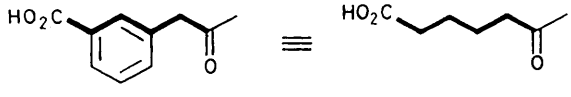


Figure 1

antibacterial activity in the presence of β-lactamase 1. However, using the more sensitive spectrophotometric assay, based on the observation of the 260 nm chromophore of the products (**2**) we found that both the γ-(D-glutamyl) penicillin (**1**, R = **d**) and the glutaryl penicillin (**1**, R = **e**) were poor substrates (see Table 1).

In conclusion these studies indicate that a six carbon-*N*-acyl side chain, terminating in a carboxy group, permits reasonable penicillin into cephem conversion by the ring expansion enzyme. Although we have found a broadly similar requirement for the isopenicillin N synthetase enzyme,[7,8] the ring expansion enzyme differs in its inability to process isopenicillin N (**1**, R = **f**) bearing the δ-(L-α-aminoadipoyl) side chain.[9]

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