Studies on the Mechanism of Action of (5*R*)-(*Z*)-6-(1-Methyl-1,2,3-triazol-4-ylmethylene)penem-3-carboxylic Acid (BRL 42715), a Potent Inhibitor of Bacterial β-Lactamase

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A novel base catalysed rearrangement of (5*R*)-(*Z*)-6-(1-methyl-1,2,3-triazol-4-ylmethylene)penem-3-carboxylic acid (BRL 42715) is reported together with its implications concerning the mechanism of inactivation of β-lactamases by this compound.

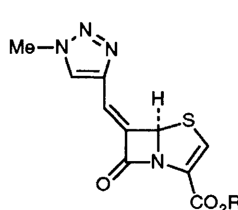
The discovery of clavulanic acid,[1] the first clinically useful β-lactamase inhibitor, clearly demonstrated the ability to protect β-lactam antibiotics from hydrolysis by β-lactamase producing organisms. The search for other potentially useful β-lactamase inhibitors has identified a number of interesting compounds of which sulbactam, 6-β-bromopenicillanic acid and tazobactam have received most attention.

Studies carried out in our own laboratories[2] highlighted the 6-(substituted methylene)penems as a novel class of β-lactamase inhibitor with activity and breadth of spectrum surpassing that of clavulanic acid, sulbactam or tazobactam. One of this series of compounds, (5*R*)-(*Z*)-6-(1-methyl-1,2,3-triazol-4-ylmethylene)penem-3-carboxylic acid (BRL 42715) **1** was identified as a particularly active inhibitor capable of protecting β-lactam antibiotics against a wide range of clinically important β-lactamases.[3] Nevertheless, SmithKline Beecham has terminated future development of BRL 42715 because of its failure to fulfil other desired technological features.

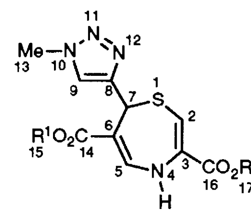
Studies on the interaction of β-lactamases with a number of β-lactamase inhibitors have been reported of which clavulanic acid, sulbactam and 6-β-bromopenicillanic acid have been examined in the greatest detail.[4] From these investigations a mechanistic model for the inactivation of β-lactamase by suicide inhibitors has emerged. Amino acid sequencing has revealed that a serine residue is conserved at the active site in all the common classes of β-lactamase except metalloenzymes.[5] Furthermore, radiolabelling studies[6] have shown that it is this serine residue that is acylated initially in the inhibition process. The acyl enzyme complex formed can then suffer three fates: (*i*) hydrolysis to regenerate the intact enzyme, (*ii*) further reactions/rearrangements to generate a transiently stabilised complex or (*iii*) further reactions/rearrangements leading to irreversible inhibition. The involvement of a serine hydroxy function has prompted many researchers to carry out parallel base catalysed hydrolysis studies on the β-lactamase inhibitor. These have served as useful probes in the understanding of the further rearrangements that lead to irreversible inhibition of β-lactamases by suicide inhibitors.

Herein we report our studies on the novel base catalysed rearrangement of BRL 42715 and its implications concerning the mechanism of inactivation of β-lactamases.

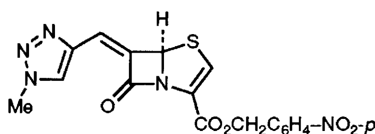
On reaction with sodium methoxide in methanol the *Z*-penem *p*-nitrobenzyl ester **2** underwent a novel rearrangement, with concomitant transesterification, to give dimethyl 4,7-dihydro-7-(1-methyl-1,2,3-triazol-4-yl)-1,4-thiazepine-3,6-dicarboxylate **3**, m.p. 162–163 °C; λ_{max} (4% $CHCl_3$–EtOH) 253 (ε_m 3037) and 370 nm (3559). Similar treatment of the *E*-penem ester **4** provided the same rearrangement product.† Confirmation of the structure of this dihydrothiazepine **3** was obtained from NMR studies. A combination of homo- and hetero-nuclear 2D NMR experiments allowed a



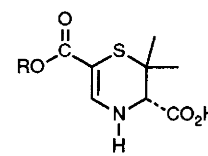
1; R = H
2; R = $CH_2C_6H_4$–NO_2-*p*
6; R = Na



3; $R^1 = R^2$ = Me
5; R^1 = Me, R^2 = Na
7; $R^1 = R^2$ = Na



4



8; R = Me / (Ser) enzyme

† This novel rearrangement has been shown to be general for a number of 6-(substituted methylene)penems.

Table 1 ^{13}C and ^{1}H Assignment (δ) and principle COLOC correlations for compound **3**[a]

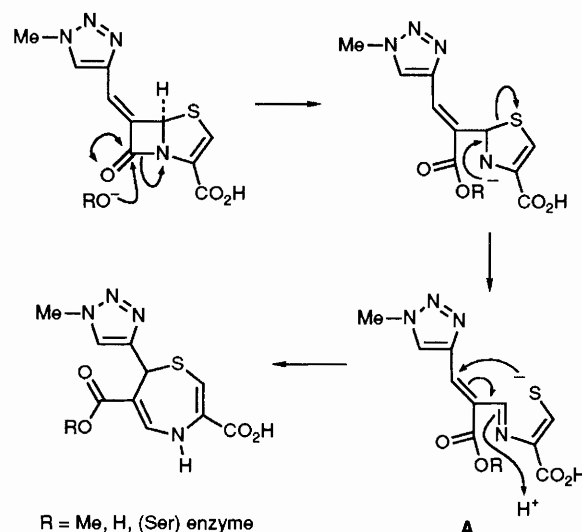
^{13}C	^{1}H	Assignment	COLOC Correlations
166.93	—	14	H(5), H(7), H(15)
163.36	—	16	H(2), H(17)
150.22	—	8	H(7), H(9)
140.07	7.71	5	H(5), H(7)
130.58	—	3	H(5)
121.90	7.22	9	H(7), H(9), Me(13)
115.88	6.76	2	H(2), H(7)
110.88	—	6	H(5), H(7)
55.34	3.81	17	Me(17)
51.93	3.71	15	Me(15)
42.15	5.93	7	H(2), H(5), H(7)
36.71	4.01	13	Me(13)

[a] ^{n}J H–H coupling constants/Hz: $^{4}J_{5,7}$ 1.0, $^{4}J_{2,7}$ 1.3, $^{4}J_{7,9}$ 0.6, $^{3}J_{5,NH}$ 8.1, $^{4}J_{2,NH}$ 1.3.

full assignment of the ^{1}H and ^{13}C spectra. Table 1 summarises these assignments and shows the connectivities established by the ^{13}C, ^{1}H COSY (correlation spectroscopy) and ^{13}C, ^{1}H COLOC (correlation by long-range coupling) experiments.[7] These data could not differentiate between the two ester methyl groups which were assigned by a NOE (nuclear Overhauser effect) experiment, strong NOEs being observed between Me(17)–H(2) and Me(15)–H(9). Hydrolysis of the diester **3** using sodium hydroxide gave the monoester **5**, λ_{max} (H_2O) 250 (ε_m 11900) and 360 nm (4500). The same monoester was obtained by rearrangement of the sodium salt **6** in the presence of sodium methoxide (1 mol. equiv.) in methanol, whereas reaction of **6** with sodium hydroxide gave the disodium salt **7**, λ_{max} (H_2O) 247 (ε_m 10150) and 355 nm (3360).

Incubation of low concentrations of BRL 42715 with a preparation[8] of the K1 β-lactamase from *Klebsiella pneumoniae* 1082E at pH 6.2 resulted in a loss of absorbance at 282–284 nm with a concomitant increase at 356 nm. An initial fast phase of penem hydrolysis was followed by a slower one which is indicative of further reaction of the acyl enzyme complex resulting in time dependent inhibition of the β-lactamase. Confirmation that this was indicative of inhibition of the enzyme responsible for penem hydrolysis was provided by the observation that at higher concentrations of the penem, the β-lactamase was completely inactivated. When hydrolysis was allowed to proceed to completion the HPLC and UV data for the product were consistent with those of the dihydrothiazepine **7**. Despite the fact that the low turnover rate (*ca.* 6×10^{-2} s^{-1} at pH 6.2) necessitated the use of large quantities of the K1 enzyme, it was possible to isolate a sample of the dihydrothiazepine **7**, following incubation and separation from the β-lactamase by gel filtration. The NMR spectrum of this sample was consistent with that of the chemically generated material with no other major products being detected. Further evidence that the rearrangement was mediated by β-lactamase was provided by pH, enzyme concentrations dependent hydrolysis rates and evidence for saturation kinetics. The profile of pH dependence was similar to that reported for benzylpenicillin[8] using this enzyme where optimum hydrolysis rates were observed at pH 6.5. Furthermore, preincubation of the enzyme preparation with potassium clavulanate virtually abolished penem hydrolysis. Other studies have shown no evidence for the degradation of BRL 42715 by bacterial isolates in general.

The proposed mechanism for the base catalysed rearrangement of BRL 42715 is shown in Scheme 1. Cleavage of the β-lactam ring by hydroxide or methoxide followed by C(5)–S bond fission provides the enethiolate **A** which undergoes an intramolecular Michael reaction to give the dihydrothiazepine. Results from our preliminary studies with the K1 enzyme lead us to propose a parallel mechanism for the



Scheme 1

inactivation of β-lactamase involving initial attack at the β-lactam by the active site serine hydroxy function. The present studies do not allow us to define, with certainty, the structure of the rearrangement product found at the active site of the inactivated enzyme. It is possible that the isolated dihydrothiazepine is formed only after hydrolytic release of an intermediate (*e.g.* **A**) from the active site of the inactivated enzyme. However, rearrangement to a dihydrothiazepine acyl enzyme is particularly attractive since this complex contains the same vinylagous urethane subunit believed to convey stability to acyl enzyme complexes following interactions with other suicide inhibitors such as clavulanic acid[9] and 6-β-halopenicillanic acids.[10] Our observations on the selective hydrolysis of the diester **3** to the monoester **5** even in the presence of an excess of sodium hydroxide attest to the resistance of vinylagous urethanes to hydrolysis. The analogy between BRL 42715 and the 6β-halopenicillanic acids is particularly noteworthy since it has been demonstrated that base catalysed and enzyme mediated hydrolysis of the latter leads to a rearrangement involving intramolecular trapping; in this case the product being the dihydrothiazine **8**.

Detailed kinetic studies involving β-lactamases and BRL 42715 will be published elsewhere.

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