Synthesis and Biological Activity of δ-(L-α-Aminoadipoyl)-L-cysteinyl-*N*-hydroxy-D-valine:† a Proposed Intermediate in the Biosynthesis of the Penicillins

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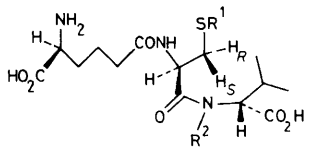
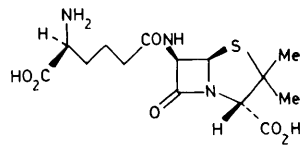
δ-(L-α-Aminoadipoyl)-L-cysteinyl-*N*-hydroxy-D-valine (**3a**) has been prepared from the appropriately protected amino acids; (**3a**) was not converted into isopenicillin N (**2**) using a cell-free system from *Cephalosporium acremonium* but inhibited the formation of (**2**) from δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (**1**) by this system.

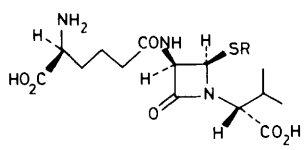
While it is now generally accepted that the cyclisation of the tripeptide, δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (**1**) to isopenicillin N (**2**) is the final step in the series of reactions common to pencillin and cephalosporin biosynthesis,[1] the mechanisms by which the β-lactam and thiazolidine rings of the penam nucleus are elaborated *in vivo* remain unexplained. On the basis of *in vitro* analogy several mechanisms for the formation of the β-lactam ring have been proposed.[2,3] One such possibility involves enzymic hydroxylation at the nitrogen of the D-valine residue of (**1**) to generate a hydroxamic acid (**3a**) followed by abstraction of the cysteinyl 3-*pro-S* proton and ring closure with elimination of the *N*-hydroxy group or of the acyl group of an *N*-acyl derivative (**3b**) to afford an enzyme bound β-lactam derivative (**4**, R = enzyme).[2c,3] This route appears attractive since a number of microbial peptide hydroxamic acids have been isolated[4] and the feasibility of such a pathway has been demonstrated by a model chemical reaction[3] (Scheme 1). While recent results[5] have shown that the oxygen atoms of the α-aminoadipoyl residue of (**1**) are retained in the enzymic conversion of (**1**) into (**2**), precluding intermediacy of a thiazolinesulphone or a thiazoline species, the retention of the δ-carbonyl oxygen does



(1) $R^1 = R^2 = H$
(3)a; $R^1 = H$, $R^2 = OH$
b; R^1 = enzyme, R^2 = Oacyl or OH

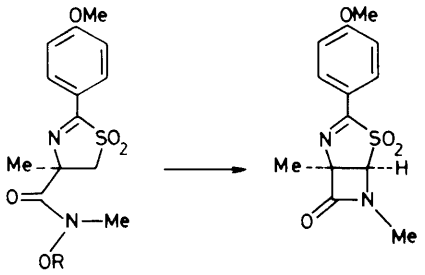


(2)



(4)

† δ-(L-α-aminoadipoyl) = 5-(5*S*)-amino-5-carboxypentanoyl.



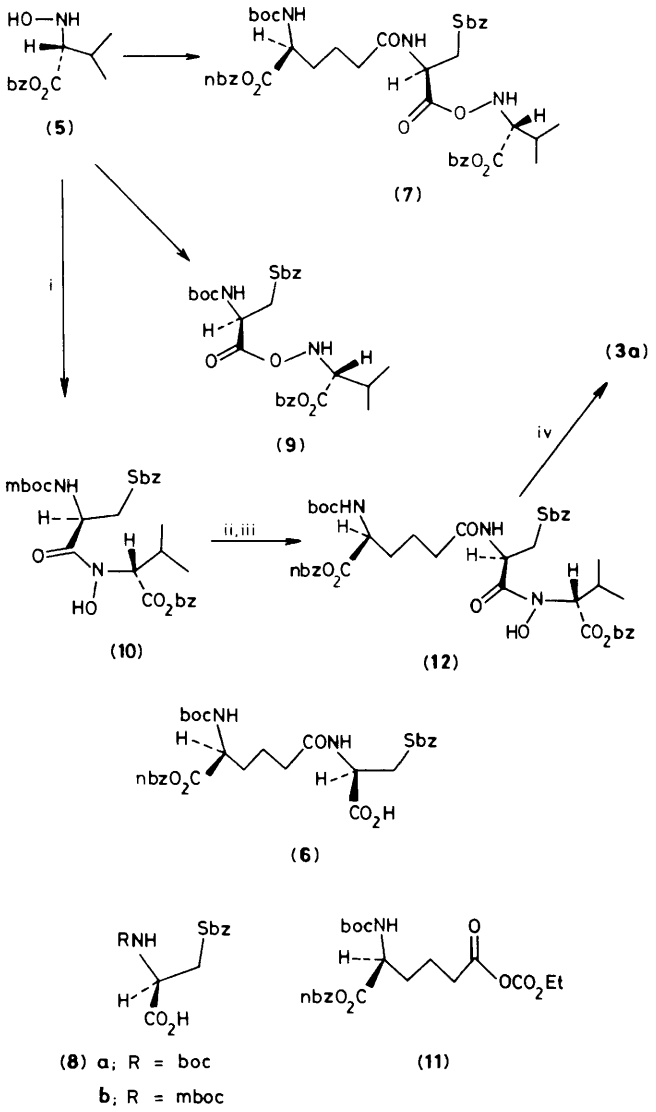
Scheme 1. R = 4-$NO_2C_6H_4SO_2$–.

not obviate the possible role of a linear *N*-hydroxy derivative such as (**3a**) as an intermediate. To test this hypothesis it was necessary to prepare the *N*-hydroxytripeptide (**3a**) and to evaluate it as a substrate.

Attempts to prepare a protected derivative of (**3a**) directly by acylation of (**5**)[6] with the protected dipeptide (**6**)[7] afforded only the *O*-acyl derivative (**7**). Similarly, acylation of (**5**) with *N*-benzyloxycarbonyl-*S*-benzyl-D-cysteine (**8a**) under a variety of peptide coupling conditions yielded the *O*-acyl derivative (**9**) as the major product. Selective *N*-acylation of (**5**) with *N*-(4-methoxybenzyloxycarbonyl)-*S*-benzyl-D-cysteine (**8b**) to give (**10**) and subsequent elaboration to the desired *N*-hydroxytripeptide (**3a**) were carried out as shown in Scheme 2.‡

The *N*-hydroxytripeptide (**3a**), gave a positive colour reaction with ferric chloride in solution and exhibited an ion at *m/z* 378 [378.1327, (*M* − 1)$^-$, $C_{14}H_{24}N_3O_7S$ requires 378.1329] in its negative ion mass spectrum (fast atom bombardment). The ^{13}C n.m.r. spectrum (75 MHz, D_2O) exhibited resonances at δ 19.57, 19.65 (val C-4, 4'), 21.73 (aaa C-4), 25.54 (cys C-3), 28.61 (val C-3), 30.55, 35.54 (aaa C-3, 5), 53.13, 55.15 (cys C-2, aaa C-2), and 66.61 p.p.m. (val C-2). The observation of the *N*-hydroxy-D-valine C-2 resonance at higher frequency than the chemical shift of the valine C-2 in the spectrum of (**1**) (δ 59.77 p.p.m.)[7] appears diagnostic of the hydroxamic acid structure. In the spectrum of *N*-hydroxy-D-valine benzyl ester (**5**) in D_2O the α carbon resonance appears at δ 72.81 p.p.m. while in the spectrum of (**12**) in $CDCl_3$ the corresponding carbon resonates at δ 63.10 p.p.m.

The *N*-hydroxytripeptide (**3a**) was administered to a partially purified enzyme system derived from homogenised cells of *C. acremonium* CW-19[1b] under conditions in which (**1**) was efficiently converted into (**2**).§ The resultant incubation mixture was assayed for isopenicillin N production using a hole-plate assay with *Staphococcus aureus*.[8] No significant antibiotic activity was detected and the *N*-hydroxytripeptide could be recovered unchanged from the incubation mixture suggesting that the compound is not directly involved as a free intermediate in the enzymatic conversion of (**1**) into (**2**).



Scheme 2. *Reagents*: i, (**8b**)–dicyclohexylcarbodi-imide (1 mol. equiv.)–dimethylformamide; ii, HCl–$MeNO_2$; iii, (**11**)–*N*-methylmorpholine–CH_2Cl_2; iv, Na–NH_3. Protecting groups, boc = benzyloxycarbonyl; mboc = 4-methoxybenzyloxycarbonyl; bz = benzyl; nbz = 4-nitrobenzyl.

However, addition of the *N*-hydroxytripeptide, at a concentration of 50 μM, to the crude enzyme system was found to completely inhibit formation of isopenicillin N from (**1**).

Peptide hydroxamic acids and *N*-acyl-*N*-hydroxypeptides are known to be powerful active site specific inhibitors of a number of zinc containing metallopeptidases.[9] While even relatively simple hydroxamic acids inhibit *Aeromonas* aminopeptidase, hydroxamic acids corresponding to L-amino acid amides which act as substrates for the metalloenzyme have been shown to be potent inhibitors.[10] It may be significant that acetohydroxamic acid also inhibits conversion of (**1**) into (**2**) by the *C. acremonium* enzyme system but only at concentrations higher than those required for inhibition by the *N*-hydroxytripeptide.¶

‡ Satisfactory elemental analyses and concordant spectroscopic data were obtained for compounds described in Scheme 2.

§ Incubations were carried out at 25 °C on a gyrorotatory shaker at 210 r.p.m. in 50 mM 3-(*N*-morpholino)propanesulphonic acid buffer, pH 7.2, containing 1.3 mM $FeSO_4$ and 2.5 mM dithiothreitol with a protein concentration of 5.5 mg/ml and substrate concentrations of 0.15—3.0 mM. For 0.3 mM (**1**) conversions were typically in the range 60—80% in 1 h. The limit of detection of the assay was 20 μg (**2**)/ml.

¶ Acetohydroxamic acid at 1 mM and (**3a**) at 40 μM were required for 50% inhibition of the conversion of (**1**) into (**2**).

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