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On the Proposed Intermediacy of β-Hydroxyvaline- and Thiazepinone-containing Peptides in Penicillin Biosynthesis

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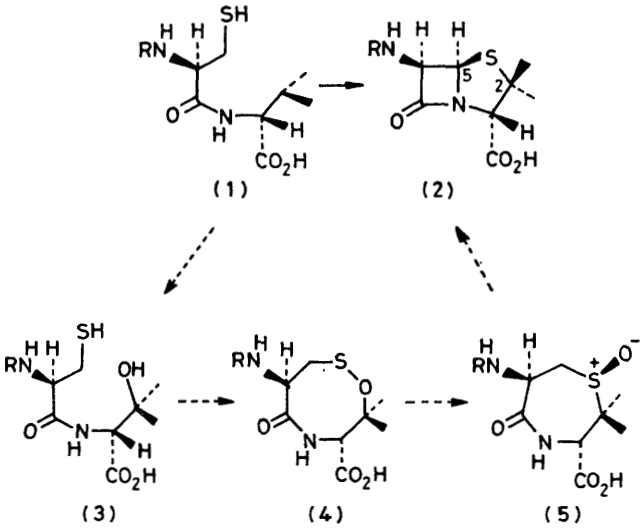
Summary The two thiazepinone-containing peptides (**5**) and (**7**), as well as the β-hydroxyvaline peptide (**3**), have been synthesised and tested as substrates for isopenicillin N synthesis in a cell-free extract from *Cephalosporium acremonium*; none of these compounds behaved as substrates, in contradiction to a recent proposal.

RECENTLY it has been proposed[1] that the established conversion[2] of the tripeptide (**1**) into isopenicillin N (**2**) might proceed by way of intermediates (**3**), (**4**), and (**5**), a process suggested to be in accord with the established stereochemical course of the conversion of (**1**) into (**2**), *i.e.* overall retention of configuration at carbons 2 and 5 in (**2**),[3] Scheme 1. We have synthesised the proposed intermediates (**3**) and (**5**) and have tested them as putative precursors in a highly active cell-free synthetase system from *Cephalosporium acremonium* for the conversion of (**1**) into (**2**).

Thus D-β-hydroxyvaline ($[\alpha]_D^{20}$ −13·3°, *c* 1, 5N-HCl), prepared and resolved by known methods,[4] was converted into its benzyl ester through esterification of its *N*-benzyloxycarbonyl derivative (Cs salt, benzyl bromide, dimethyl-



SCHEME 1. R = L-α-amino-δ-adipyl.

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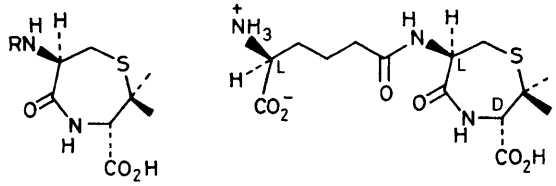
formamide) and subsequent deprotection with hydrogen bromide in acetic acid.† Reduction (Na,NH_3) restored the amino-acid with no change in optical activity. This ester was coupled (dicyclohexylcarbodi-imide, 2-hydroxybenzotriazole) with *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine to afford the dipeptide (m.p. 93 °C; 90%), which was then deprotected (40% HBr, glacial AcOH), and finally coupled with 1-benzyl-*N*-benzyloxycarbonyl-L-α-aminoadipic acid to give the tripeptide (m.p. 122—124 °C; 12%), which was deprotected (Na, NH_3) and purified with Hopkin's reagent (Hg^{3+}–H_2SO_4) to yield the tripeptide (**3**).‡

The nucleus of (**5**), *i.e.* the cyclic peptide (**6**, R=$PhCH_2O_2C$), was synthesized by a known procedure.[5] After esterification to give the benzyl ester (triethylamine, benzyl bromide; 25 °C; m.p. 126·5—127·5 °C; 86%) and deprotection (HBr, glacial AcOH, 94%) the free amine (**6**; R = H), m.p. 128·5—129 °C, was liberated ($NaHCO_3$; 96%). This substance was acylated (*N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; tetrahydrofuran; 25 °C; 89%) with 1-benzyl-*N*-benzyloxycarbonyl-L-δ-aminoadipic acid to afford the fully protected cyclic peptide which was deprotected (H_2; 10% Pd/C; MeOH, 25 °C) to give the cyclic peptide (**6**; R = L-α-amino-δ-adipyl§), m.p. 194 °C (decomp.) (67%). Oxidation (*m*-chloroperbenzoic acid; CH_2Cl_2; 98%) of the fully protected cyclic peptide gave the (*R*)-sulphoxide which was deprotected, as before, to give the peptide (**5**) (78%). The (*R*) configuration of the sulphoxide has been previously assigned and corresponds to that implicated in the proposed biosynthetic scheme.¶ The cyclic peptide (**6**) has been discussed previously as a possible intermediate in penicillin biosynthesis[6] but it was not at that time realised that the aminoadipic acid moiety was essential for penicillin biosynthesis and, furthermore, no cell-free extract with synthetic activity was then available. Consequently, the earlier experiments[6] are invalid. We therefore decided also to test (**7**) [=(**6**; R = L-α-amino-δ-adipyl)] in a cell-free system.



(**6**) (**7**)

SCHEME 2

An active cell-free extract containing all co-factors[7] was prepared from *C. acremonium* as previously described. Solutions of the substances (**3**), (**5**), and (**7**) respectively (10 μl; 40 mM) were each added to the complete system (390 μl),[7] the mixtures incubated at 27 °C (60 min; shaken at 100 cycles/min), and the reactions stopped by addition of acetone (930 μl). After centrifugation the supernatants were concentrated in a stream of air to their original volume (400 μl). All bioassays and chromatographic separations were conducted on these solutions. The controls contained either the tripeptide substrate (**1**) or no peptide, the latter showing no background level of antibiotic content. The results are presented in the Table. There is no indication of conversion of compounds (**3**), (**5**), or (**7**) into antibiotics. The incubation mixtures from (**3**), (**5**), and (**7**) were subjected to electrophoresis (paper; pH 6·5) before and after treatment with β-lactamase I (*Bacillus cereus*, 569/H/9) and showed no production of isopenicillin N from (**3**), (**5**), or (**7**), but essentially contained unchanged substrates. Furthermore, chromatography on paper (descending; Bu^nOH–HOAc–H_2O, 4:1:4) showed only unchanged starting material before or after β-lactamase treatment. When (**5**) and (**7**) (1·1 mM) were first incubated in the extract for 15 min there was no detectable inhibition of the synthesis of (**2**), after the subsequent addition of (**1**) (0·6 mM). However (**3**), under these conditions, appeared to behave as a weak inhibitor (*ca.* 9% of normal conversion).

TABLE. Production of antibiotic activity on incubation of substrate (**1**) and compounds (**3**), (**5**), and (**7**) with the extract[7] from *C. acremonium*.

Compound [a]	Conversion (%) [b]	Product
(**1**)	77	(**2**)
(**3**)	0	—
(**5**)	0	—
(**7**)	0	—

[a] Concentration *ca.* 1 mM. [b] Assay against *Staphylococcus aureus* (WCTC-6571) and *Salmonella typhi* (strain Mrs. S). Detection limit *ca.* 2% of (**2**).

We conclude that none of the proposed intermediates (**3**), (**5**), or (**7**) are acceptable as substrates for the penicillin synthetase system from *C. acremonium*.

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† It was not possible to benzylate (benzene, benzyl alcohol, toluene-*p*-sulphonic acid) this amino-acid directly.

‡ Homogeneous by t.l.c. (SiO_2, 75% aq. PrOH) and electrophoresis on paper (pH 4·5).

§ α-Amino-δ-adipyl = 5-amino-5-carboxypentanoyl.

¶ The previous study[1] showed that oxidation of the fully protected thiazepinone peptide under these conditions gave only the *R*-sulphoxide. The new data reported were in agreement with those for our compound, both in chemical shift and coupling constants.

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