Retention of Configuration at C-3 of (2*S*,3*S*)-[4-^{13}C]Valine in the Biosynthesis of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine, the Acyclic Precursor of the Penicillins

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(2*S*,3*S*)-[4-^{13}C]Valine has been incorporated into the title compound (**1**) by a β-lactam negative mutant of *Cephalosporium acremonium;* isolation [as the corresponding sulphonic acid (**1a**)] and subsequent hydrolysis afforded (2*R*,3*S*)-[4-^{13}C]valine, showing retention of the chirality at the 3-position.

A considerable weight of evidence now exists for the role of δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (ACV, **1**) as the acyclic precursor of isopenicillin N (**2**).[1–5] The nature of the mechanisms resulting in formation of the β-lactam and thiazoline rings of the penam nucleus *in vivo* are as yet unexplained, although several *in vitro* models have been proposed.[6–9] In this regard the stereochemical fate of the prochiral methyl groups of ACV during cyclisation to the penam structure is of considerable interest. Several independent studies[10–13] have shown that chiral valines are incorporated into penicillins with *overall* retention of configuration at C-2 (derived from C-3 of valine), but this result *in itself* does not distinguish between a route involving retention of the valine C-3 configuration at each step or one in which this position is inverted in the formation of ACV and reinverted in a subsequent ring closure. The recent demonstration[13] that [3-^{3}H]-valines are incorporated into ACV with no net loss of tritium suggests that no inversion at C-3 occurs during ACV biosynthesis yet does not preclude the intermediacy of an enzyme-bound dehydrovaline species in which the abstracted

C-3 hydrogen is subsequently reinserted on the opposite face of the molecule. Specific intramolecular transfer of hydrogen without exchange with solvent has been observed with isomerase[14] and racemase[15] enzymes.

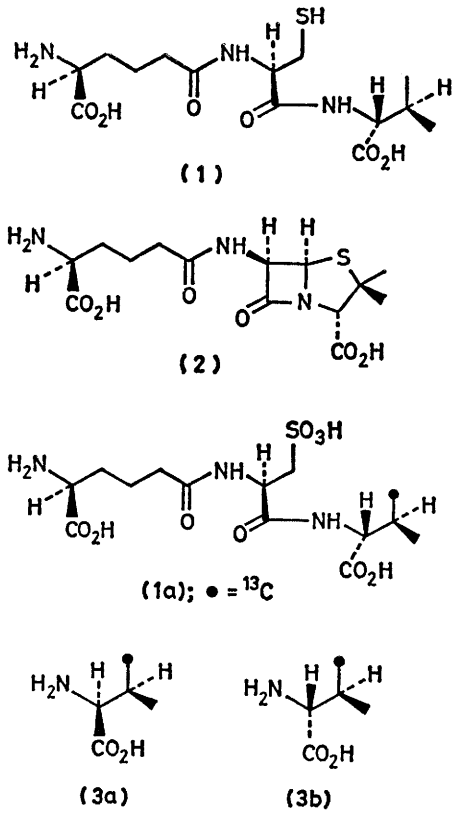
In order to provide an unambiguous answer to this question we have examined the stereochemistry of the valine residue in ACV derived biosynthetically from valine asymmetrically labelled in the *pro-S* methyl group with ^{13}C.

(2*RS*, 3*S*)-[4-^{13}C]valine was prepared by a modification of the route of Aberhart and Lin[12,16] and resolved by treatment of the corresponding *N*-acetyl derivative with hog kidney acylase[17] to afford the diastereomeric (2*S*,3*S*)- and (2*R*,3*S*)-[4-^{13}C]valines, (**3a**) and (**3b**) respectively. The ^{13}C n.m.r. spectrum (D_2O—H_2O, 1:2, pH 10) of (**3a**) showed a single resonance at 16·4 p.p.m. while that of (**3b**) showed a peak at 18·0 p.p.m.

Incorporation of labelled valine into ACV was achieved using a β-lactam negative mutant of *Cephalosporium acremonium*, N-2.[18] Thus (2*S*,3*S*)-[4-^{13}C]valine (128 μmol, 85 atom % ^{13}C) together with (2*S*)-[*U*-^{14}C]valine (5 μCi, 0·5 μmol) was administered over a period of 36 h to a three-day old culture which was harvested after a total of seven days growth. Following chromatography on charcoal—keiselguhr and ion exchange on 50wx2 resin, labelled ACV could be readily identified by comparison with a synthetic sample by paper electrophoresis (3000 V/m, pH 3·5). The crude isolate was oxidised with performic acid and the resultant mixture of sulphonic acids fractionated by ion exchange on 50wx2 resin and paper electrophoresis to afford ACV sulphonic acid (**1a**) {9·9 mg; 1·01 × 10^4 d.p.m./mg, 1·0% radio-chemical yield based on (2*S*)-[*U*-^{14}C]-valine}.

The ^{13}C n.m.r. spectrum of the labelled (**1a**), shown in Figure 1, shows an enhanced signal at 18·0 p.p.m. (2·0 ± 0·2 × natural abundance) corresponding to one of the methyl groups of the valine residue. Hydrolysis (5M-HCl, 95 °C, 30 h) of the labelled tripeptide and separation of the amino acids by paper electrophoresis afforded (2*R*) valine [>98% (2*R*) as shown by treatment with D-amino acid oxidase[19]]. The ^{13}C n.m.r. spectrum of the isolated valine showed significant enhancement (2·0 × natural abundance) of the intensity of the 3-*pro-S*-methyl resonance at 18·0 p.p.m. and no significant enhancement of the 3-*pro-R*-methyl signal at 16·4 p.p.m. The sample is therefore (2*R*,3*S*)-[4-^{13}C]valine.

This result clearly demonstrates that the inversion of configuration at C-2 of (2*S*)-valine during formation of ACV (**1**) is not accompanied by inversion at C-3. Hence the biosyn-



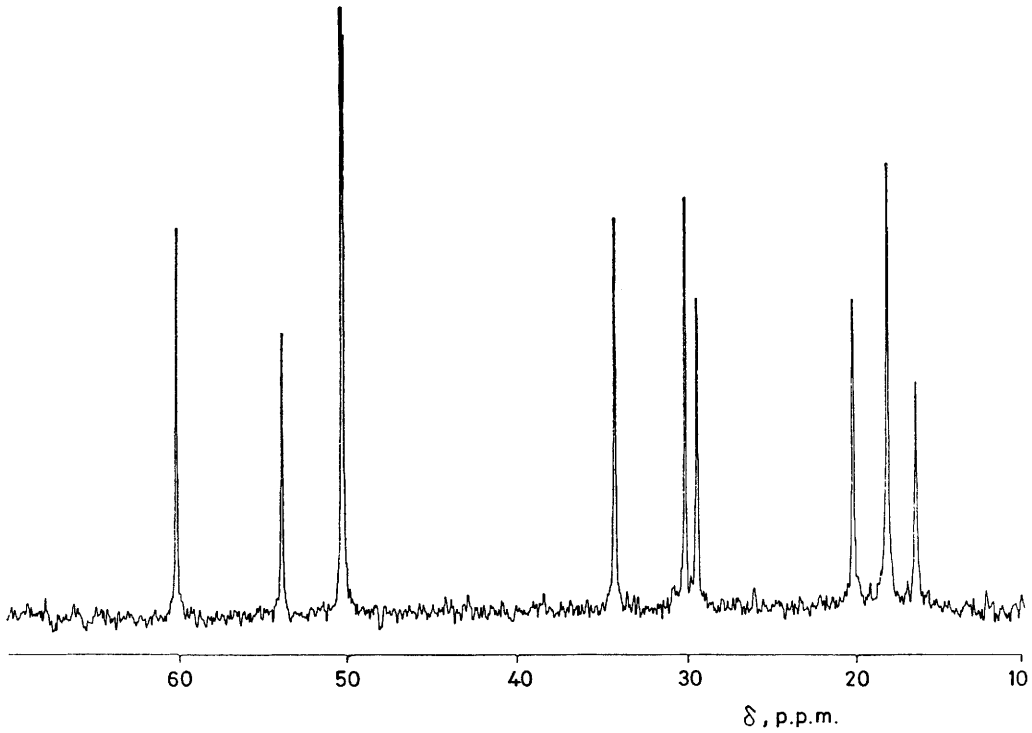


Figure 1. Fully proton decoupled 75 MHz ^{13}C n.m.r. spectrum of labelled (**1a**). The spectrum was the result of 130 000 free induction decays (repetition time 0·44 s; approximate pulse angle 66°). Chemical shifts are expressed relative to external C_6H_6 at 128·0 p.p.m.

thesis of isopenicillin N (**2**) from (**1**) must proceed with overall retention of configuration at this position. While this does not preclude the hypothesis[9] that a 3-hydroxyvaline tripeptide might be a later intermediate, it does demand that introduction of a hydroxy-group would proceed with inversion, since subsequent formation of the S–C (1) bond of the penam nucleus would most likely be an S_N2 process. Although hydroxylation generally proceeds with retention of configuration, examples of hydroxylation with inversion have been reported.[20]† An alternative hypothesis[13] of a radical cyclisation remains valid, although it has yet to be demonstrated experimentally that this would proceed with retention of configuration.

We thank the Takeda Chemical Industries for providing us with the N-2 mutant and the National Institutes of Health for support.

Received, 1st October 1981; Com. 1152

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