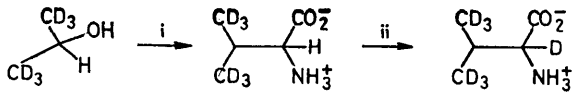
Penicillin Biosynthesis: Conversion of Deuteriated (L-α-Amino-δ-adipyl)-L-cysteinyl-D-valine† into Isopenicillin N by a Cell-free Extract of *Cephalosporium acremonium*

By JACK E. BALDWIN,* MANKIL JUNG, and JOHN J. USHER
(*Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford* OX1 3QY)
and SIR EDWARD P. ABRAHAM,* JOYCE A. HUDDLESTON, and ROBERT L. WHITE
(*Sir William Dunn School of Pathology, Oxford University, South Parks Road, Oxford* OX1 3RE)

Summary The tripeptide (L-α-amino-δ-adipyl)-L-cysteinyl-D-[2-^{2}H, Me_2-$^2H_6$]valine was converted into isopenicillin N by a cell-free extract of *Cephalosporium acremonium*; complete retention of all deuterons was confirmed by ^{2}H n.m.r. spectroscopy.

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

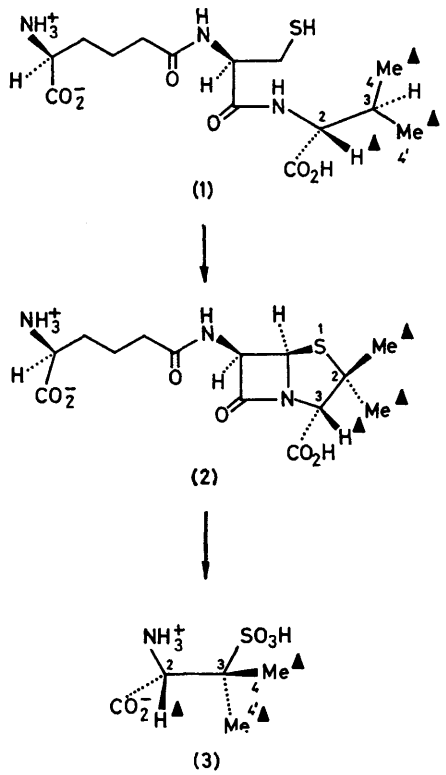
RECENTLY the incorporation of methyl-deuteriated valines into penicillin, with retention of the six valine deuterons, was shown by mass spectrometry.[1,2] Also, Abraham *et al.* demonstrated the retention of at least part of the valine α-proton during biosynthesis of isopenicillin N from (L-α-amino-δ-adipyl)-L-cysteinyl-D-[2-^{3}H]valine (LLD-ACV).[3] It was of interest to confirm these results directly by ^{2}H n.m.r. spectroscopy, with a tripeptide deuteriated at the α- and methyl positions, using the more efficient cell-free system recently obtained.[4]



SCHEME 1. i, PBr_3 then $HCONHCH(CO_2Et)_2$, hydrolysis. ii, Acetylation, exchange D_2O, resolution with hog acylase.

Thus, deuteriated valine was synthesized, as in Scheme 1, from [$^2H_6$]propan-2-ol[5] and then converted into the corresponding tripeptide (L-α-amino-δ-adipyl)-L-cysteinyl-D-[2-^{2}H,Me_2-$^2H_6$]valine (**1**) by known methods.[6] The deuterium abundance in the tripeptide (**1**) was 63 atom % ^{2}H at C-2 and 99·5 atom % ^{2}H at C-4,4′ as determined by ^{1}H and ^{2}H n.m.r. spectroscopy. This represents a ratio of 0·63 : 6·00 deuterons between C-2 and C-4,4′. Incubation of this peptide at 27 °C for 60 min with an extract of *Cephalosporium acremonium*[4] gave, after removal of protein with acetone, a mixture of isopenicillin N (**2**) and some unconverted tripeptide (**1**) [ratio (**1**) : (**2**) = 1·04 : 1] (Scheme 2). The ratio of deuterons at C-2 in the tripeptide and at C-3 of isopenicillin N to the methyl deuterons (in the valine and penicillin methyl groups) was 0·63 : 6·00. Confirmation of the retention of deuterium was obtained by oxidation of the mixture (1·5% performic acid, 5 h) to the penicillaminic acid (**3**), which was separated by preparative electrophoresis at pH 1·8. The ^{2}H n.m.r. spectrum of (**3**) showed a ratio of deuterons at C-2 to C-4,4′ of 0·64 : 6·00.

In another experiment, the unlabelled LLD-ACV peptide was incubated with the cell-free extract in tritiated water. Conversion of the so-formed isopenicillin N into penicillaminic acid, purified as before, gave a product containing no tritium (<0·2% exchange of one proton).



SCHEME 2. ▲ = ^{2}H.

In conclusion, these experiments confirm that none of the hydrogen atoms at C-2 and C-4,4′ in the valine moiety of the tripeptide (**1**) are lost during bioconversion into isopenicillin N.

We thank the S.R.C. and N.R.D.C. for financial support, N.S.E.R.C. of Canada for a Postdoctoral Fellowship to R. L. W., and Lady E. Richards for assistance.

(*Received*, 22*nd December* 1980; *Com.* 1359.)

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