Direct ^{1}H N.M.R. Observation of the Cell-free Conversion of δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine and δ-(L-α-Aminoadipyl)-L-cysteinyl-D-(−)-isoleucine into Penicillins

By GULAM BAHADUR, JACK E. BALDWIN,* LESLIE D. FIELD, EEVA-M. M. LEHTONEN, JOHN J. USHER, and CARLOS A. VALLEJO

(*Dyson Perrins Laboratory, South Parks Road, Oxford* OX1 3QY)

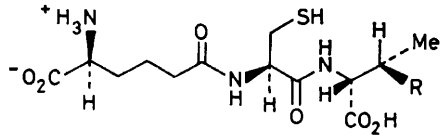
and EDWARD P. ABRAHAM* and ROBERT L. WHITE

(*Sir William Dunn School of Pathology, South Parks Road, Oxford* OX1 3RE)

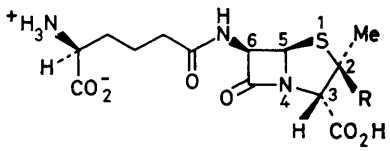
Summary The cell-free conversion of δ-(L-aminoadipyl)-L-cysteinyl-D-(−)-isoleucine (**1b**) into a penicillin (**2b**) was observed directly by ^{1}H n.m.r. spectroscopy and the thiazolidine ring formation was shown to occur with retention of stereochemistry at C-3 of the isoleucinyl residue by ^{1}H nuclear Overhauser enhancement studies of the product penicillin in the deproteinated incubation mixture.

RECENTLY, the conversion of the ^{13}C-enriched tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine (LLD-ACV, **1a**) into isopenicillin N (**2a**) was observed directly by ^{13}C n.m.r. spectroscopy in cell-free extracts of *Cephalosporium acremonium*.[1] We now report the observation of the same conversion by ^{1}H n.m.r. spectroscopy, and of the conversion of the D-(−)-isoleucine tripeptide (**1b**) into a biologically active β-lactam antibiotic (**2b**), the structure of which was determined by ^{1}H nuclear Overhauser enhancement (n.O.e.) studies performed directly on the deproteinated incubation mixture.

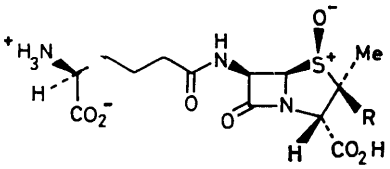
When LLD-ACV (**1a**) was incubated with a cell-free extract of *C. acremonium*[2] at 10 °C in the probe of an n.m.r. spectrometer, the intensities of the two doublets at δ 0·99 and 1·03† (due to the diastereotopic methyl protons of the substrate) decreased in intensity with time. As the incubation progressed, signals characteristic of isopenicillin N



(1a) R = Me (LLD-ACV)
(1b) R = Et (LLD-ACI)



(2a) R = Me
(2b) R = Et



(3a) R = Me
(3b) R = Et

† ^{1}H N.m.r. spectra were recorded on a Bruker WH 300 n.m.r. spectrometer at 300 MHz. Chemical shifts are given in p.p.m. with acetic acid (δ 2·030 p.p.m.) as internal reference.

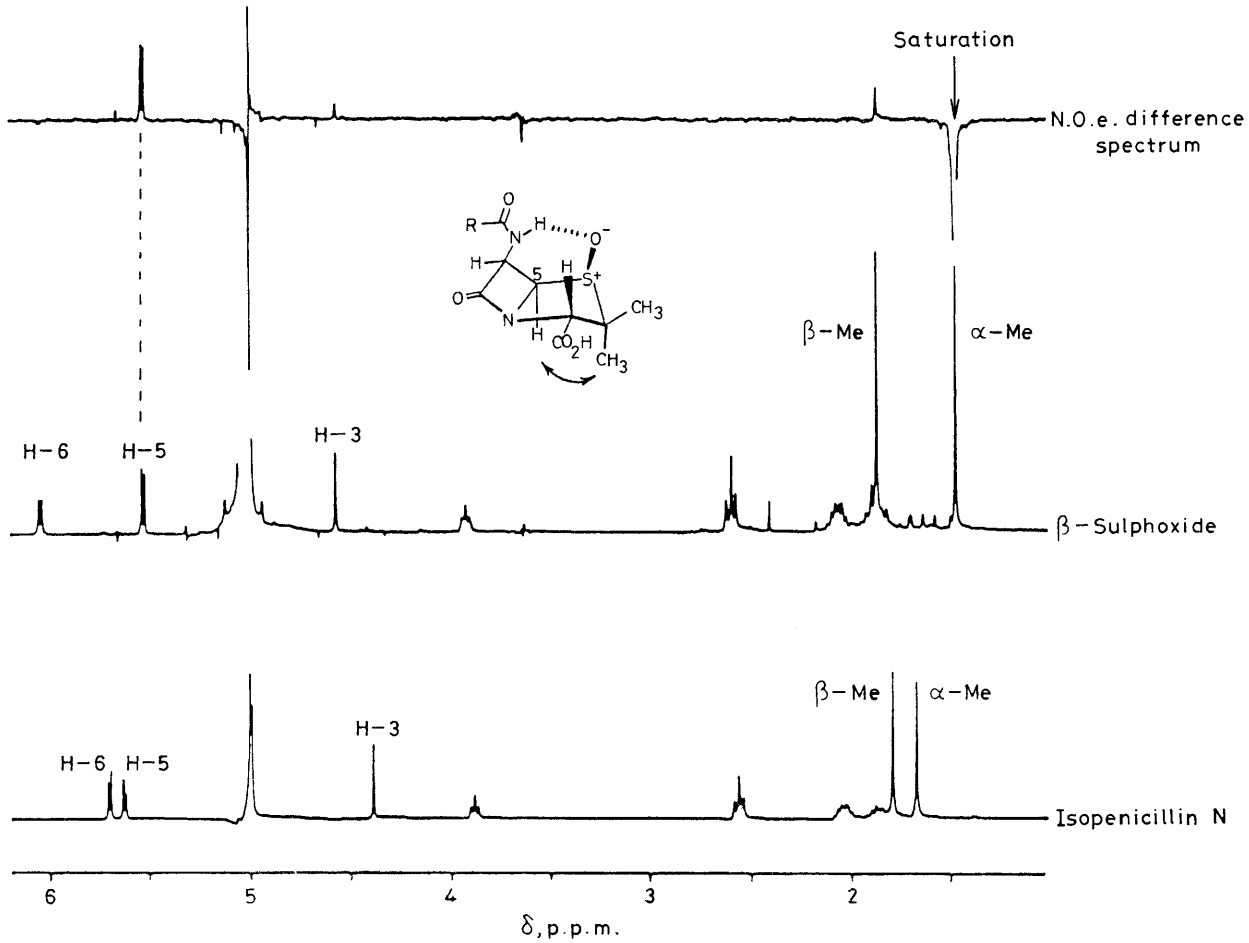


FIGURE 1. (a) ^{1}H N.m.r. spectrum of isopenicillin N in D_2O. (b) ^{1}H N.m.r. spectrum of isopenicillin N β-sulphoxide in D_2O. (c) ^{1}H N.O.e. difference spectrum[7] of isopenicillin N β-sulphoxide with saturation at δ 1·38 p.p.m.

(**2a**)‡ appeared at δ 1·62 (s, α-Me), 1·74 (s, β-Me), 4·35 (s, H-3), 5·57 (d, *J* 4·4 Hz, H-6), and 5·65 (d, *J* 4·4 Hz, H-5). When δ-(L-α-aminoadipyl)-L-cysteinyl-D-(−)-isoleucine (LLD-ACI, **1b**) was incubated in the same manner, the characteristic resonances of the β-lactam ring protons (H-5 and H-6) were observed as well as a new methyl singlet at δ 1·58 (Figure 1, Table). Deproteination and treatment of the resultant solution with penicillinase[4] removed these signals from the spectrum of the reaction mixture.

Aqueous periodate oxidation of penicillin derivatives yields the β-sulphoxides stereospecifically.[5] Cooper *et al.*[6] have shown that in the β-sulphoxide of phenoxymethylpenicillin methyl ester, the conformation of the oxidised thiazolidine ring is such that an n.O.e. (*ca.* 17%) can be observed between the 2-α-methyl groups and H-5; *i.e.* across the α-face of the molecule. Periodate oxidation of an authentic sample of isopenicillin N[3] gave the β-sulphoxide and saturation of the high field (α-) methyl resonance produced a nuclear Overhauser enhancement (18%) of the H-5 proton as indicated in the n.O.e. difference spectrum[7] (Figure 1).

TABLE. ^{1}H N.m.r. chemical shift data (δ values) for isopenicillin N (**2a**), isopenicillin N β-sulphoxide (**3a**), and their homologues (**2b**) and (**3b**).

	H-5	H-6	H-3	α-Me	β-Me
(**2a**)	5·58	5·66	4·35	1·62	1·74
(**2b**)	5·50	5·63	4·30	1·57	—
(**3a**)	5·43	5·96	4·53	1·38	1·78
(**3b**)	5·41	5·98	4·46	1·35	—

In situ periodate oxidation of the deproteinated reaction mixture resulting from the incubation of LLD-ACI (**1b**) gave a sulphoxide with a methyl singlet at δ 1·35 (Table, Figure 2). Saturation of this resonance resulted in an n.O.e. (*ca.* 12%) of the high-field β-lactam proton (H-5) and hence led to assignment of the (3*S*)-configuration to (**2b**) and (**3b**). This observation highlights the potential of n.O.e. difference spectroscopy in the determination of the three-dimensional structures of small molecules in complex mixtures.

A number of workers have shown that the biosynthesis of penicillins proceeds with retention of configuration at C-3 of the valine precursor.[8] The results of our ^{1}H n.O.e. studies show that the configuration of isoleucine at C-3 is also retained in the biosynthesis of (**2b**) from (**1b**).[8]

‡ By comparison with an authentic sample of isopenicillin N (ref. 3).

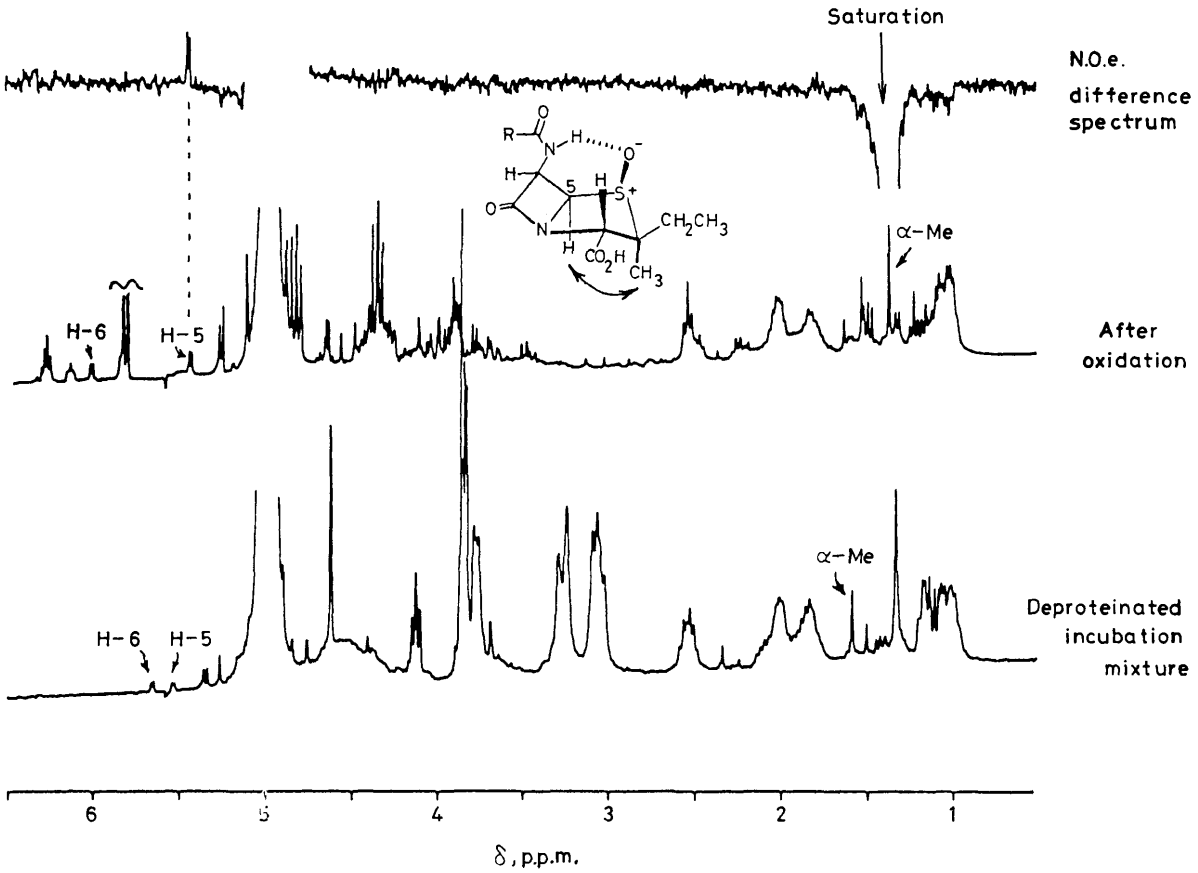


FIGURE 2. ^{1}H N.m.r. spectra of cell-free incubation mixture and LLD(−)-ACI. (a) After incubation at 10 °C for 2 h and precipitation of protein with acetone, lyophilisation of the supernatant, and redissolution in D_2O. (b) After *in situ* oxidation with $NaIO_4$ (1 h, 20 °C). (c) ^{1}H N.O.e. difference spectrum of (b) with saturation at δ 1·35 p.p.m. As well as the peptide (initial concentration 4·8 mM) the incubation mixtures also contained dithiothreitol (4·8 mM), L-ascorbic acid (4·8 mM), $FeSO_4$ (0·06 mM), and bovine liver catalase (895 units per ml) in an ammonium hydrogen carbonate buffer at pH 7·6 (37 mM)

We thank Lady Richards for her assistance and interest in the n.m.r. aspects of the work, the S.R.C. for a grant for the Bruker WH 300 spectrometer, and the S.R.C. and N.R.D.C. for financial support. One of us (R.L.W.) is indebted to the N.S.E.R.C. of Canada for a Post-doctoral Fellowship.

(*Received*, 17*th June* 1981; *Com.* 712.)

[1] J. E. Baldwin, B. L. Johnson, J. J. Usher, E. P. Abraham, J. A. Huddleston, and R. L. White, *J. Chem. Soc., Chem. Commun.*, 1980, 1271.

[2] E. P. Abraham, J. A. Huddleston, G. S. Jayatilake, J. O'Sullivan, and R. L. White, in 'Recent Advances in the Chemistry of β-Lactam Antibiotics' (2nd International Symposium), ed. G. I. Gregory, Special Publication, The Royal Society of Chemistry, 1981, pp. 125—134.

[3] J. E. Baldwin and C. A. Vallejo, unpublished results.

[4] β-Lactamase I from *Bacillus cereus* 569/H/9; R. B. Davies, E. P. Abraham, and J. Melling, *Biochem. J.*, 1974, **143**, 115.

[5] P. G. Sammes, *Chem. Rev.*, 1976, **76**, 113.

[6] R. D. G. Cooper, P. V. Demarco, J. C. Cheng, and N. D. Jones, *J. Am. Chem. Soc.*, 1969, **91**, 1408; R. D. G. Cooper, P. V. Demarco, C. F. Murphy, and L. A. Spangle, *J. Chem. Soc. C*, 1970, 340.

[7] N.O.e. difference spectra were obtained directly from the undegassed reaction mixtures using a modification of the procedure described by G. E. Chapman, B. D. Abercrombie, P. D. Levy, and E. M. Bradbury, *J. Magn. Reson.*, 1978, **31**, 459.

[8] N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *J. Am. Chem. Soc.*, 1973, **95**, 3797 (correction on p. 6511); H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, p. 6149; D. J. Aberhart and L. J. Lin, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2320; H. Kluender, F. C. Huang, A. Fritzberg, H. Schroes, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Am. Chem. Soc.*, 1974, **96**, 4054; F. C. Huang, J. A. Chan, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, 1975, **97**, 3858; J. E. Baldwin and T. S. Wan, *Tetrahedron*, 1981, **37**, 1589; G. Bahadur, J. E. Baldwin, J. J. Usher, E. P. Abraham, G. Jayatilake, and R. L. White, *J. Am. Chem. Soc.*, submitted for publication.