Retention of Valine Methyl Hydrogens in Penicillin Biosynthesis[1]

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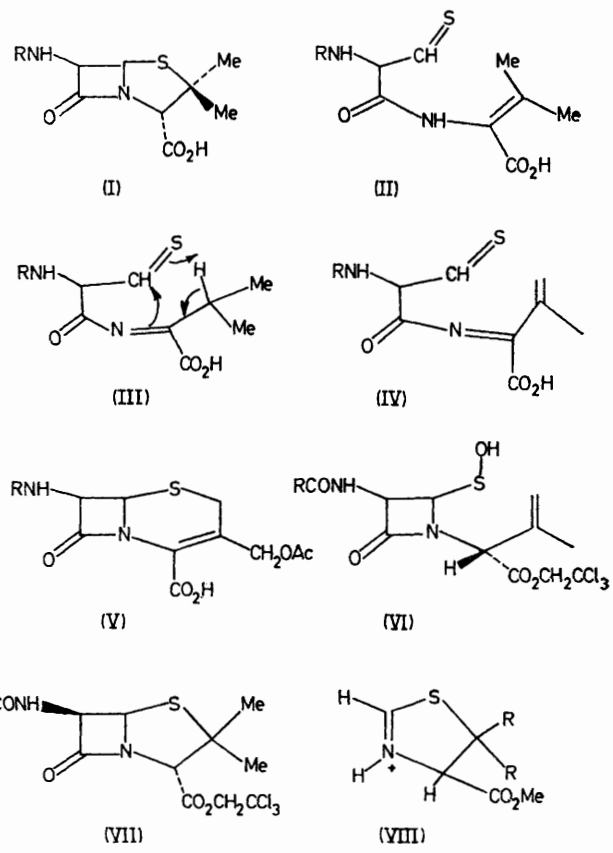
Summary [Me_2-$^2H_6$]-D,L-valine has been synthesized and incorporated into penicillin V. The mass spectrum of the penicillin V methyl ester showed the retention of all six deuterons in the biosynthetic product.

THE biosynthesis of penicillin, (I), has been suggested to occur *via* a thioaldehyde dehydrovaline intermediate, (II), derived from L-cysteinyl-L-valine (R = H) or possibly L-α-aminoadipyl-L-cysteinyl-L-valine (R = α-aminoadipyl).[2,3] A variation on this mechanism, based on the 'ene' reaction of (III) was recently suggested.[4] In addition, the structure (IV) was suggested[3] as an intermediate in the biosynthesis of cephalosporin C, (V).

It appeared to us that an intermediate such as (IV), bearing an isopropenyl group instead of an isopropylidene group as in (II), could be also considered a reasonable intermediate in the biosynthesis of penicillin. A variety of sulphenic acid derivatives have been observed[5] to cyclize forming thiazolidine rings, e.g. (VI) → (VII). To test the involvement of intermediates such as (IV) in penicillin biosynthesis, [Me_2-$^2H_6$]-valine was synthesised. [Me_2-$^2H_6$]-2-Bromopropane[6] was converted into diethyl[Me_2-$^2H_6$]-2-isopropylmalonate which was hydrolysed, decarboxylated, and brominated[7] to [Me_2-$^2H_6$]-2-bromo-isovaleric acid, which with ammonia[8] afforded [Me_2-$^2H_6$]-D,L-valine. The *N*-acetyl derivative had M^+ 165 ($C_7H_7D_6NO_3$).

[Me_2-$^2H_6$]-Valine was added to a culture of *Penicillium chrysogenum*, and the resultant phenoxymethylpenicillin (penicillin V), (I) (R = $PhOCH_2CO$), isolated as the potassium salt. The ^1H n.m.r. indicated the presence of *ca.* 10% deuterium in the methyl groups. The corresponding methyl ester of (I), prepared with diazomethane, was examined by



mass spectrometry. The most prominent fragment at *m/e* 174 [$C_7H_{12}NO_2S$, (VIIIa)] in the mass spectrum of unlabelled penicillin V methyl ester was accompanied by a new fragment (*ca.* 22%) at *m/e* 180 [$C_7H_6D_6NO_2S$, (VIIIb)]. Thus, the incorporation of [Me_2-$^2H_6$]-valine into penicillin proceeded without loss of any of the methyl hydrogens, and consequently structures such as (IV) must be excluded from consideration as intermediates in penicillin biosynthesis.

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[1] See: 'Studies on the Biosynthesis of β-Lactam Antibiotics, III'. Part I, D. J. Aberhart and L. J. Lin, *J. Amer. Chem. Soc.*, 1973, **95**, 7859; part II, submitted for publication.
[2] H. R. V. Arnstein and J. C. Crawhill, *Biochem. J.*, 1957, **67**, 180.
[3] P. A. Lemke and D. R. Brannon, 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 370.
[4] J. E. Baldwin, S. B. Haber, and J. Kitchin, *J.C.S. Chem. Comm.*, 1973, 790; see also: J. Cheney, C. J. Moores, J. A. Raleigh, A. I. Scott, and D. W. Young, *J.C.S. Chem. Comm.*, 1974, 47.
[5] R. D. G. Cooper and D. O. Spry, 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 183.
[6] V. J. Shiner, *J. Amer. Chem. Soc.*, 1952, **74**, 5285.
[7] R. Adams and R. M. Kamm 'Organic Syntheses I,' ed. A. H. Blatt, Wiley, New York, 1941, p. 250.
[8] C. S. Marvel and V. duVigneau, 'Organic Syntheses II,' 1943, p. 93.
[9] C. S. Marvel, 'Organic Syntheses III, 1955, p. 848.