Studies on the Biosynthesis of β-Lactam Antibiotics. Part I. Stereospecific Syntheses of (2*RS*,3*S*)-[4,4,4-$^2H_3$]-, (2*RS*,3*S*)-[4-^{3}H]-, (2*RS*,3*R*)-[4-^{3}H]-, and (2*RS*,3*S*)-[4-^{13}C]-Valine. Incorporation of (2*RS*,3*S*)-[4-^{13}C]-Valine into Penicillin V [1]

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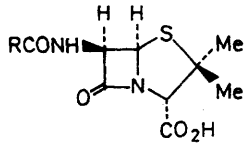
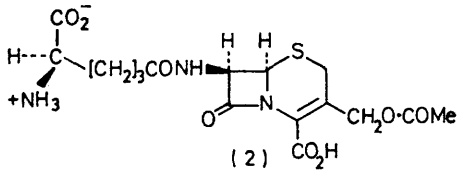
Syntheses of the title compounds are described. (−)-(2*R*,3*S*)-*trans*-2,3-Epoxybutyric acid was esterified and reduced to (2*R*,3*S*)-*trans*-2,3-epoxybutan-1-ol, which with lithium iodide-free [$^2H_3$]methyl-lithium gave (2*R*,3*S*)-3-methyl[4,4,4-$^2H_3$]butane-1,2-diol. Treatment of this with sodium metaperiodate gave [3,3,3-$^2H_3$]-isobutyraldehyde (not isolated) which was converted into the aminonitrile, and thence into (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine. N.m.r. studies on the enzymatically resolved valine and its acetate established the stereochemical homogeneity of the labelling. (2*RS*,3*S*)-[4-^{13}C]- and (2*RS*,3*S*)-[4-^{3}H]-Valine were synthesized by the same route, by using [^{13}C]methyl-lithium and [^{3}H]methyl-lithium respectively. Similarly, (2*RS*,3*R*)-[4-^{3}H]valine was synthesized from (2*S*,3*R*)-*trans*-2,3-epoxybutyric acid.

(2*RS*,3*S*)-[4-^{13}C]Valine was incorporated into phenoxymethylpenicillin, the ^{13}C n.m.r. spectrum of which showed an enhanced signal for the α-methyl group.

The biosyntheses of the β-lactam antibiotics penicillin (1) and cephalosporin C (2) have been widely investigated over the past thirty years.[2] The bicyclic systems of (1) and (2) are formed from L-valine and L-cysteine.[3,4] Some evidence suggests that the immediate precursor of



(1) a R = $PhCH_2$
b R = CO_2^-



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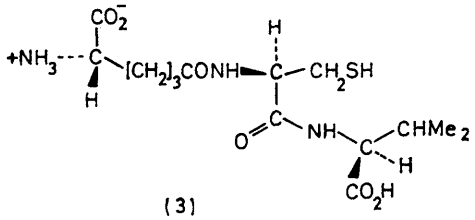
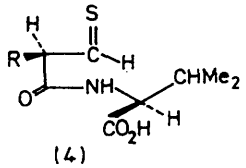


(3)

(1) and (2) is the tripeptide δ-(L-5-amino-5-carboxypentanoyl)-L-cysteinyl-L-valine (3), which has been isolated from cultures of *Penicillium chrysogenum* and *Cephalosporium acremonium*.[5] If tripeptide (3) is indeed a direct precursor of (1) and (2), it remains unclear at what stage in the biosynthesis the L-α-5-amino-5-carboxypentanoyl side-chain is exchanged for the more common side-chains of biosynthetic penicillins, *e.g.*, benzylpenicillin (1a).[6]

Numerous unsolved problems remain concerning the details of the biosynthesis of the β-lactam ring, and of the thiazolidine and dihydrothiazine rings of (1) and (2) respectively. It has been suggested that the β-lactam



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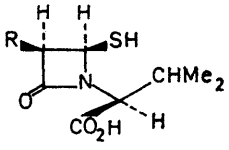


(5)

ring is formed first, yielding (5) by addition of the nitrogen atom of L-valine to a thioaldehyde (4) generated from the L-cysteine unit in (3). The retention in (1a) of at least some of the tritium from DL-[2,2′-$^3H_2$]cystine and DL-[3,3′-$^3H_2$]cystine rules out an intermediate bearing a double bond between the α and β carbons of the cysteine unit.[7] It is assumed that intermediate (5) undergoes dehydrogenation to yield a didehydrovaline unit, *e.g.* (6).† Ring formation to generate penicillin (1) occurs by addition of −SH across the double bond. Several other problems remain, including: (*a*) does the dehydrogenation leading to the didehydrovaline (6) proceed with a *cis* or *trans* elimination of hydrogens;

† An alternative didehydrovaline (7) has been ruled out by recent deuterium labelling studies in our laboratory (D. J. Aberhart, J. Y. R. Chu, N. Neuss, C. H. Nash, J. Occolowitz, L. L. Huckstep, and N. De La Higuera, *J.C.S. Chem. Comm.*, 1974, 625).

[1] Preliminary communication, D. J. Aberhart and L. J. Lin, *J. Amer. Chem. Soc.*, 1973, **95**, 7859.

[2] P. A. Lemke and D. R. Brannon, in 'Cephalosporins and Penicillins,' ed. E. H. Flynn, Academic Press, New York, 1972, p. 370.

[3] E. P. Abraham, G. G. F. Newton, and S. C. Warren, in 'Biogenesis of Antibiotic Substances,' eds. Z. Vanek and Z. Hostelak, Academic Press, New York, 1965, p. 169.

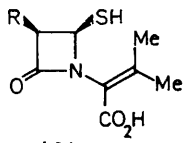
[4] (*a*) H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, 1954, **57**, 353, 360; (*b*) S. C. Warren, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, 1967, **103**, 902; (*c*) C. M. Stevens, E. Inamine, and C. W. DeLong, *J. Biol. Chem.*, 1956, **219**, 405.

[5] H. R. V. Arnstein and D. Morris, *Biochem. J.*, 1960, **76**, 357; P. B. Loder and E. P. Abraham, *ibid.*, 1971, **123**, 471.

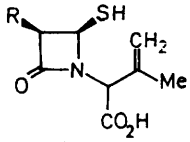
[6] G. G. F. Newton and E. P. Abraham, *Nature*, 1953, **172**, 395.

[7] H. R. V. Arnstein and J. C. Crawhill, *Biochem. J.*, 1957, **67**, 180.

(*b*) to which face of the resulting didehydrovaline (6) does the –SH of the cysteine unit add, and does the addition occur in a *cis* or *trans* manner; and (*c*) in the biosynthesis of cephalosporin C, which of the distinguishable



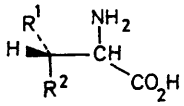
(6)



(7)

(diastereotopic) methyl groups of valine is the precursor of the $-SCH_2-$ group and which the precursor of the $-CH_2OAc$ group?

The overall stereochemical course of events (*a*) and (*b*) might be elucidated by synthesizing valine asymmetrically labelled in the isopropyl group, *e.g.* as in (8b), microbiologically converting (8) into penicillin, and determining the position of the label in the resulting penicillin. The use of ^{13}C as a tracer appeared ideal for this purpose, since the ^{13}C n.m.r. spectrum of penicillin



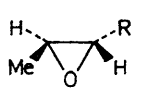
(8)

	R^1	R^2
a	CH_3	CH_3
b	$^{13}CH_3$	CH_3
c	C^2H_3	CH_3
d	C^3H_3	CH_3
e	CH_3	C^3H_3
f	CH_3	$^{13}CH_3$
g	CH_3	C^2H_3
h	CH_3	$C^1H_2{}^2H_1$

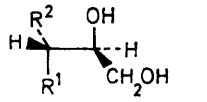
had been studied, and the signals corresponding to the α- and β-methyl groups assigned.[8] Such a labelled valine could also be used to determine the fate of the valine methyl groups in cephalosporin C biosynthesis, as its ^{13}C n.m.r. spectrum has been interpreted.[9]

We therefore undertook the synthesis of chirally-labelled [^{13}C]valine (8b). The synthesis was designed such that other chiral valines, *e.g.* (8c—g) and others, could be obtained through the appropriate choice of labelling agent and chiral starting material. Although the production of [^{13}C]valine was the ultimate goal, a trial synthesis using deuterium labelling was first attempted, and our initial objective became the synthesis of [4,4,4-$^2H_3$]valine (8c).

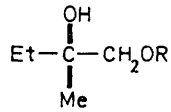
Synthesis of (2RS,3S)-[4,4,4-$^2H_3$]*Valine* (8c).—Our approach was based on the availability of optically pure (2*R*,3*S*)-*trans*-2,3-epoxybutyric acid (9a),[10] which could be converted into *trans*-2,3-epoxybutan-1-ol (9c) by reduction of the methyl ester (9b) with sodium borohydride.[11] Treatment of (9c) with methyl-lithium or a similar reagent [*e.g.* dimethyl(lithio)copper [12,13]] was expected to yield 3-methylbutane-1,2-diol (10a) which could then be converted into DL-valine. Epoxidation [14] of *trans*-crotonic acid gave racemic (9a), which was methylated (diazomethane) and reduced to (9c) by the literature procedure.[11] Treatment of this compound



(9) a R = CO_2H
b R = CO_2Me
c R = CH_2OH



(10) a $R^1 = CH_3$, $R^2 = CH_3$
b $R^1 = C^2H_3$, $R^2 = CH_3$
c $R^1 = {}^{13}CH_3$, $R^2 = CH_3$
d $R^1 = C^3H_3$, $R^2 = CH_3$
e $R^1 = CH_3$, $R^2 = C^3H_3$



(11) a R = H
b R = COPh

in ether at −70° with an ethereal solution of methyl-lithium prepared from methyl iodide and lithium metal [15] did not give the expected 3-methylbutane-1,2-diol (10a) but rather 2-methylbutane-1,2-diol (11a) as the only major product [characterized as the benzoate (11b)]. However, the desired product (10a) could be cleanly produced by treatment of (9c) with 2 equiv. of lithium iodide-free methyl-lithium, prepared by exchange of methyl iodide with n-butyl-lithium in hexane, followed by replacement of the hexane with ether.[16,*] Under these conditions, an extra equivalent of Li^+ is not available for complexation with the oxiran oxygen which could catalyse a preliminary rearrangement before attack of methyl-lithium.

We next sought to convert the diol (10a) into DL-valine, under conditions where the configurational purity of a chirally labelled diol, such as (10b), would not be lost. Intermediates bearing a carbonyl group adjacent to the isopropyl group were initially considered to be precluded from such a route. The diol (10a) was more readily prepared by epoxidation of 3-methylbut-1-ene followed by acidic hydrolysis.

One approach was *via* a circuitous route from (10a) to

* The reaction could also be carried out using commercial methyl-lithium–ether solutions, which are normally prepared from methyl chloride and lithium metal.

[8] R. A. Archer, R. D. G. Cooper, P. V. Demarco, and L. R. F. Johnson, *Chem. Comm.*, 1970, 1291.

[9] N. Neuss, C. H. Nash, P. A. Lemke, and J. B. Grutzner, *J. Amer. Chem. Soc.*, 1971, **93**, 2337.

[10] K. Harada and J. Oh-hashi, *Bull. Chem. Soc. Japan*, 1966, **39**, 2311.

[11] S. Corsano and G. Piancatelli, *Chem. Comm.*, 1971, 1106; *Gazzetta*, 1971, **101**, 204.

[12] (*a*) R. W. Herr, D. M. Wieland, and C. R. Johnson, *J. Amer. Chem. Soc.*, 1970, **92**, 3813; (*b*) R. W. Herr and C. R. Johnson, *ibid.*, p. 4978; (*c*) C. R. Johnson, R. W. Herr, and D. M. Wieland, *J. Org. Chem.*, 1973, **38**, 4263.

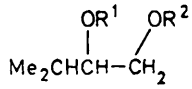
[13] B. C. Hartman, T. Livinghouse, and B. Rickborn, *J. Org. Chem.*, 1973, **38**, 4346.

[14] G. B. Payne and P. H. Williams, *J. Org. Chem.*, 1959, **24**, 54.

[15] H. Gilman, E. A. Zoellner, and W. M. Selby, *J. Amer. Chem. Soc.*, 1933, **55**, 1252. Methyl-lithium–ether solutions prepared in this manner contain an equivalent of lithium iodide.

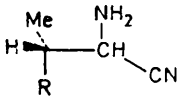
[16] T. L. Brown and M. T. Rogers, *J. Amer. Chem. Soc.*, 1957, **79**, 1859; R. West and W. Glaze, *ibid.*, 1961, **83**, 3580.

2-hydroxy-3-methylbutyric acid (13b). Thus (10a) in pyridine on treatment with trityl chloride, followed by benzoyl chloride, gave (12a) (70%). This was hydrolysed with aqueous acetic acid to the primary alcohol (12b) (95%), and chromic acid oxidation of this yielded (13a) (85%), which was then hydrolysed to (13b) (94%). In addition, treatment of (10a) in pyridine with trityl



(12) a R^1=COPh, R^2=CPh$_3$
b R^1=COPh, R^2=H
c R^1=Ts, R^2=CPh$_3$

(13) a R=COPh
b R=H



(14) a R=CH$_3$
b R=C^{2}H$_3$
c R=^{13}CH$_3$
d R=C^{3}H$_3$

chloride followed by toluene-*p*-sulphonyl chloride gave the tosylate (12c), lithium aluminium hydride reduction of which afforded isopentyl trityl ether. This latter sequence thus may be of use in the synthesis of isoprenoids (*e.g.* the side-chain of cholesterol) bearing chiral isopropyl groups. However, the large number of steps in the sequence (10a) ⟶ (13b) discouraged its use in syntheses of chiral valine.

A simple method for the conversion of (10a) into DL-valine appeared to be cleavage of the glycol with periodate to generate isobutyraldehyde and formaldehyde, and conversion of the former into valine *via* the aminonitrile (14a) followed by acid hydrolysis (Strecker method).[17] However, it was unclear whether the chirality of an asymmetrically labelled diol, *e.g.* (10b), would be retained through the periodate–Strecker sequence. Enolization of chiral isobutyraldehyde would cause a loss of configurational purity.[18] Unfortunately, it was difficult to estimate from the published data [18] the relative rates of base-catalysed enolization * of isobutyraldehyde and formation of aminonitrile. Enolization or other base-catalysed loss of configurational purity after formation of the aminonitrile was unlikely. In a preparation [19] of DL-[3,4-$^2H_2$]valine by a Strecker synthesis starting with [2,3-$^2H_2$]isobutyraldehyde, the isolated DL-valine contained *ca.* 25% less than the expected amount of deuterium, presumably as a result of some enolization of the isobutyraldehyde during the process.

However, the results below indicated that enolization might be negligible under suitably mild Strecker conditions. First glycol (10a) was treated several times with D_2O to exchange hydroxylic protons. The deuteriated analogue with sodium metaperiodate in D_2O gave a mixture of aldehydes which was distilled into a cold-trap. The thawed aqueous distillate, with $(ND_4)_2SO_4$, ND_4OD, and sodium cyanide in D_2O gave the aminonitrile (14a), the n.m.r. and mass spectra of which indicated the absence of deuterium. Similarly, [α-^{2}H]isobutyraldehyde [18] under analogous conditions but with undeuteriated reagents gave (14e) which contained one atom of deuterium. It therefore appeared the Strecker sequence could be used in a synthesis of chirally-labelled valine.

Optically pure (−)-(2*R*,3*S*)-*trans*-2,3-epoxybutyric acid (9a) was prepared by a literature procedure [10] *via* the brucine salt. In addition, the previously unknown (+)-(2*S*,3*R*)-*trans*-enantiomer (17a) was obtained by recrystallization of the racemic acid with quinine. The epoxy-acid (9a) was converted as before into (9c), which, on treatment with lithium iodide-free [$^2H_3$]methyllithium in ether, gave (2*RS*,3*S*)-3-methyl[4,4,4-$^2H_3$]-butane-1,2-diol (10b). The n.m.r. spectrum of (10b) showed a doublet for the unlabelled methyl group at δ 0·95, in contrast to the spectrum of the unlabelled diol (10a), wherein two doublets appear for the diastereotopic methyl groups at δ 0·90 and 0·95. Thus the signal at δ 0·90 can be assigned to the 3-*pro*-*S*-methyl group, and that at δ 0·95 to the 3-*pro*-*R*-methyl group. More importantly, this result proved that the epoxide cleavage (9c) ⟶ (10b) had proceeded stereospecifically, presumably in a *trans* direction.[20]

The glycol (10b) was then converted into (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine (8c) in *ca.* 25% yield *via* the aminonitrile (14b) by the periodate–Strecker sequence. The entire sequence was carried out without purification of intermediates, and the resultant valine (8c) was purified by ion-exchange on a cation exchange resin. Even the crude product was essentially pure as estimated by i.r. spectroscopy and paper chromatography, and showed only minute amounts of ninhydrin-positive impurities.

In order to estimate the configurational purity of the isopropyl group of racemic (8c), a portion was acetylated and resolved with hog kidney acylase I,[17] an enzyme which hydrolyses the *N*-acetyl groups of L-amino acids only. The resultant (2*S*,3*S*)-[4,4,4-$^2H_3$]valine (15) and (2*R*,3*S*)-*N*-acetyl[4,4,4-$^2H_3$]valine (16) were separated by a cation exchange procedure. The ^{1}H n.m.r. spectrum of (15) showed a single three-proton methyl doublet at δ 1·39, in contrast to the spectrum of racemic (8c), which showed two doublets, at δ 1·34 and 1·39 (3H total). These spectra indicated the stereochemical homogeneity of the isopropyl group in (8c), and allow assignment of the higher field doublet to the 3-*pro*-*R*-methyl and the lower field doublet to the 3-*pro*-*S*-methyl

* Typically the pH of the reaction mixture in the preparation of the aminonitrile (13a) from isobutyraldehyde is 10—11.

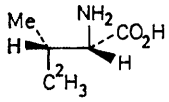
[17] J. P. Greenstein and M. Winitz, 'Chemistry of the Amino-acids,' Wiley, New York, 1961, p. 2372.

[18] For extensive studies on the catalytic dedeuteriation of [α-^{2}H]isobutyraldehyde, see J. Hine and K. W. Narducy, *J. Amer. Chem. Soc.*, 1973, **95**, 3362, and earlier papers.

[19] C. R. Kinney and R. Adams, *J. Amer. Chem. Soc.*, 1937, **59**, 897.

[20] J. G. Buchanan and H. Z. Sable, 'Stereospecific Epoxide Cleavages,' in 'Selective Organic Transformations,' vol. 2, ed. B. S. Thyagarajan, Wiley-Interscience, New York, 1972.

group of valine.* In addition, the n.m.r. spectrum of the *N*-acetyl derivative (16) was examined, for confirmation of the stereochemical homogeneity of its



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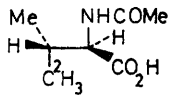


(16)

(17) a R=CO_2H
b R=CH_2OH

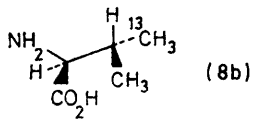
isopropyl group. Unfortunately in the 60 MHz spectrum of unlabelled DL-*N*-acetylvaline, the methyl groups were nearly magnetically equivalent, and a reliable estimate of stereochemical purity in (16) could not be made. Similarly, in the spectrum of DL-*N*-acetylvaline methyl ester, the methyl signals were superimposed. However, in the presence of $Eu(fod)_3$,[21] these signals separated and appeared at δ 1·98 and 2·42. The shifted spectrum of (16) showed a methyl signal at δ 1·98 only and no trace of absorption at 2·42, thereby confirming the stereochemical homogeneity of the labelling in this compound.

The ^{13}C-labelled chiral valine (8b) was then prepared by the established route, (−)-(2*R*,3*S*)-*trans*-2,3-epoxybutan-1-ol (9c) being treated first with 1 equiv. of unlabelled methyl-lithium followed by a second equivalent of [^{13}C]methyl-lithium. Maximum utilization of the isotopic material was thereby achieved. The resultant (2*RS*,3*S*)-3-methyl[4-^{13}C]butane-1,2-diol (10c) was then converted into (2*RS*,3*S*)-[4-^{13}C]valine (8b) as before.[22]

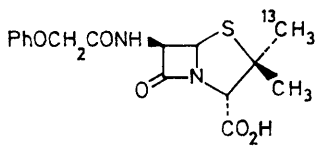
Finally, we prepared (2*RS*,3*S*)-[4-^{3}H]valine (8d) and (2*RS*,3*R*)-[4-^{3}H]valine (8e) which were required for studies on valine metabolism in mammalian systems and in micro-organisms. Compound (8d) was prepared from (−)-(2*R*,3*S*)-*trans*-2,3-epoxybutyric acid (9a) in a manner closely analogous to the synthesis of the chiral valine (8b). The enantiomer (8e) was synthesized in a similar manner starting with (+)-(2*S*,3*R*)-*trans*-2,3-epoxybutyric acid (17a) obtained by recrystallization of racemic (9a) with quinine.†

Incorporation of (2RS,3S)-[4-^{13}C]*Valine into Penicillin* V.—The incorporation of the chiral [^{13}C]valine (8b) into phenoxymethylpenicillin (18) (penicillin V) was carried out by Dr. N. Neuss and his collaborators, Lilly Research Laboratories, Indianapolis. The results were in agreement with those reported first by Neuss [22] and later by Sih.[22] The ^{13}C n.m.r. spectrum of the isolated phenoxymethylpenicillin potassium salt exhibited an enhanced signal for the α-methyl group: (8b) ⟶ (18). Thus the formation of the thiazolidine ring proceeds with an overall retention of configuration at the β carbon of L-valine.

Furthermore, assuming that the α-orientation of the carboxy-group of the penicillins is determined during the ring formation process (and is not formed by isomerization of a β-carboxy-intermediate), and assuming that a didehydrovaline intermediate such as (6) is involved in the biosynthesis, the results require that the sequence: dehydrogenation of valine, followed by addition of SH across the double bond must proceed with either (*a*) a *cis*-dehydrogenation followed by *trans*-addition, or (*b*) *trans*-dehydrogenation followed by *cis*-addition of SH. The two other possibilities (*cis*-dehydrogenation with *cis*-addition, or *trans*-dehydrogenation with *trans*-addition) are eliminated by the biosynthetic results. Clarification of the stereochemical course of the individual steps in this ring formation process will require isolation of the didehydrovaline intermediate and determination of the stereochemistry of its labelling pattern when



(8b)



(18)

biosynthesized from a chiral valine such as (8b). Alternatively, a stereospecifically labelled didehydrovaline might be synthesized, and microbiologically converted into penicillin. Such an interconversion has not yet been accomplished.

EXPERIMENTAL

I.r. spectra were taken on a Perkin-Elmer model 337 spectrometer. N.m.r. spectra were obtained on a Varian A-60 spectrometer. Mass spectra were obtained on a Varian MAT-CH-5 spectrometer at 70 eV. G.l.c. was performed using an F and M model 700 gas chromatograph, using a column of 15% SE-30 on Chromosorb W. Paper radiochromatography was carried out using Whatman no. 1 chromatography paper, with butan-1-ol–acetic acid–water (4 : 1 : 5, upper phase) as the descending mobile phase. Radiochromatogram scanning was carried out with a Nuclear Chicago Actigraph III instrument, operating in the

* A similar conclusion was reached by Professor R. K. Hill, through an independent synthesis of (2*S*,3*R*)-[4,4,4-$^{2}H_3$]valine (8g) (R. K. Hill, S. Yan, and S. M. Arfin, *J. Amer. Chem. Soc.*, 1973, **95**, 7857). We thank Professor Hill for informing us of his results prior to publication.

† These tritium-labelled chiral valines are currently being used in a study of the stereochemistry of oxidation of the valine methyl groups in their metabolism by rat liver preparations and other organisms. The results will be published elsewhere.

[21] R. E. Sievers, in 'Nuclear Magnetic Resonance Shift Reagents,' Academic Press, New York, 1973.

[22] After completion of this synthesis, two independent syntheses of ^{13}C-labelled chiral valine were reported: (2*RS*,3*R*)-[4-^{13}C]valine (8f): J. E. Baldwin, L. Löliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. De La Higuera, *J. Amer. Chem. Soc.*, 1973, **95**, 3796, 6511; (2*S*,3*S*)-[4-^{13}C]valune (8b) (2*S*-isomer only): H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1973, **95**, 6149. These authors also report the synthesis of the first monodeuteriated chiral valine (2*RS*,3*R*)-[4-$^{2}H_1$]valine (8h).

windowless mode. Assays of radiochemicals were carried out by liquid scintillation counting using a Nuclear Chicago model 701 instrument. Samples were dissolved in 10 ml of a solution of diphenyloxazole (PPO) (4 g) and *p*-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl POPOP) (100 mg) in toluene (1 l). Valine samples were dissolved in 1N-NH_4OH (0·25 ml) and diluted with 10 ml of solution of PPO (4 g), dimethyl POPOP (100 mg), and Beckman BBS-3 solubilizer (50 ml) in toluene (1 l). M.p.s were taken on a hot stage apparatus. Microanalyses were performed by Chemalytics Inc., Tempe, Arizona. [$^2H_3$]Methyl iodide was obtained from Merck, Sharpe and Dohme of Canada, [^{13}C]methyl iodide from Bio-Rad Laboratories, and [^{3}H]-methyl iodide from New England Nuclear Co.

(±)-trans-2,3-*Epoxybutan*-1-*ol* (9c).—Racemic *trans*-2,3-epoxybutyric acid [14] (9a) (10·3 g) was methylated with an excess of diazomethane in ether, and the solvent was removed *in vacuo*. The resultant methyl ester (9b) was added dropwise over 30 min to a stirred solution of sodium borohydride (3·1 g) in water (50 ml) at 20°. Stirring was continued for 1 h, then the mixture was cooled to 0°, and conc. HCl was added to pH 5, followed by NaOH to pH 7. The solution was saturated with NaCl, filtered, and extracted continuously with ether for 12 h. The extract was dried (Na_2SO_4) and evaporated to a volume of 10 ml. Benzene (300 ml) was added and the mixture was filtered and evaporated *in vacuo* to yield (9c) as an oil (4·2 g). The compound polymerized readily above room temperature and could not be distilled. The crude product had $\nu_{max.}$ (CCl_4) 3450, 1450, 1420, 1380, 1350, 1108, 1042, 950, and 870 cm^{-1}, δ ($CDCl_3$) 1·38 (3H, d, *J* 6 Hz), 2·46br (1H, s, D_2O exchangeable), 3·0 (2H, m), and 3·8 (2H, m).

2-*Methylbutane*-1,2-*diol* (11a).—A solution of methyl-lithium in ether was prepared [15] by treatment of lithium metal (29 g, 4·2 mol; cut in small pieces) in anhydrous ether (500 ml) under N_2 with methyl iodide (228 g, 1·62 mol) added over 75 min, refluxing being continued for 2 h. The resultant mixture was kept several days and the clear supernatant solution was removed. The concentration of methyl-lithium, determined by addition of a sample to water and titration with HCl, was 1·95M.

trans-2,3-Epoxybutan-1-ol (9c) (2 g, 23 mmol) in absolute ether (30 ml) under N_2 at −78° was treated with 1·95N-methyl-lithium in ether (35 ml; 68 mmol), added over 2 min. The mixture was rapidly cooled to −78° and stirred at this temperature for 30 min, then for 2 h at −20°, and 12 h at 20°. The excess of methyl-lithium was destroyed by dropwise addition of methanol, followed by water (200 ml). The pH was adjusted to 7·0, and the solution was saturated with NaCl and extracted continuously with ether for 12 h. The extract was dried (Na_2SO_4) and evaporated *in vacuo* to an oil, which was distilled at 100° and 1 mmHg in a Büchi kugelröhr apparatus, yielding crude (11a) as an oil (1·27 g), $\nu_{max.}$ (CCl_4) 3400, 1460, 1380, 1160, and 1075 cm^{-1}, δ ($CDCl_3$) 0·85 (3H, t, *J* 6 Hz), 1·09 (3H, s), 1·51 (2H, q, *J* 6 Hz), 3·42 (2H, s), and 4·0br (2H, D_2O exchangeable). This spectrum indicated minor impurities.

The foregoing product (306 mg, 3 mmol) was treated with benzoyl chloride (0·75 ml) in anhydrous pyridine for 12 h at 20°. Water (100 ml) was added and the mixture was stirred 1 h, then extracted with ether. The extract was washed (saturated Na_2CO_3, dilute HCl, H_2O, saturated NaCl), dried (Na_2SO_4), and evaporated. The major product was purified by preparative t.l.c. (20% ethyl acetate–hexane, R_F 0·4) yielding the *monobenzoate* (11b) (300 mg), distilled in a kugelröhr tube at 180° and 1·5 mmHg, $\nu_{max.}$ (CCl_4) 3620, 3500, 1725, 1270, 1110, and 708 cm^{-1}, δ ($CDCl_3$) 0·98 (3H, t, *J* 7 Hz), 1·28 (3H, s), 1·68 (2H, q, *J* 7 Hz), 3·02 (1H, s, D_2O exchangeable), 4·30 (2H, s), 7·5 (3H, m), and 8·1 (2H, m) (Found: C, 69·05; H, 7·85. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·75%).

3-*Methylbutane*-1,2-*diol* (10a).—Four 50 ml heavy-walled, narrow-necked centrifuge tubes with rubber serum caps were flushed with N_2 and charged with n-butyl-lithium–hexane (35 ml; 1·56M; 57·7 mmol per tube). The tubes were cooled in ice, and to each was added by syringe methyl iodide (3·6 ml; 1 equiv.). The mixtures were kept for 30 min at 20°, then centrifuged at low speed (*ca.* 1000 rev. min^{-1}) for 30 min. The clear supernatant was removed by syringe and discarded. Fresh hexane (30 ml) was added to each tube, the methyl-lithium resuspended by shaking, and the mixture centrifuged again. This hexane wash was repeated once and the supernatants discarded. Finally absolute ether (25 ml) was added to each tube. The methyl-lithium did not dissolve completely and was used as a suspension. An aliquot portion of the suspension, added to water and titrated with HCl, had a concentration of 1·0M.

trans-2,3-Epoxybutan-1-ol (9c) (4·0 g, 45 mmol) in tetrahydrofuran (THF) (100 ml; distilled from $LiAlH_4$) under N_2 at −20° was treated with the foregoing methyl-lithium–ether suspension (90 ml), added dropwise over 20 min. The methyl-lithium dissolved instantly upon being added to the THF solution. Gas evolution ceased after the addition of *ca.* 40 ml. Stirring was continued for 3 h at −20°, then 3 h at 20°. Methanol (5 ml) was added dropwise, then water (200 ml). The solution was acidified to pH 7, saturated with NaCl, filtered, and extracted continuously with ether for 12 h. The extract was evaporated to an oil which was distilled in a kugelröhr tube at 110° and 1 mmHg giving the diol (10a) (2·70 g). G.l.c. (15% SE-30, 115°) showed the presence * of a major product of retention time identical with that of an authentic sample of (10a), prepared by the following method.

Preparation of 3-*Methylbutane*-1,2-*diol* (10a) *from* 3-*Methylbut*-1-*ene*.—To a mixture of calcium hypochlorite (142 g) in water (240 ml) at 0° was added 3-methylbut-1-ene (100 g), followed by acetic acid (95 ml) added over 30 min with vigorous agitation and cooling. Stirring was continued for 2 h at 0°, then nitric acid (30%; 100 ml) was added to break the emulsion. The mixture was extracted with ether, and the extract was washed with K_2CO_3 solution, dried, and evaporated to an oil which was distilled to give the chlorohydrin,† b.p. 60—65° at 30 mmHg (69 g), $\nu_{max.}$ ($CHCl_3$) 3590, 1255, and 650 cm^{-1}, δ ($CDCl_3$) 0·98 (3H, d, *J* 6 Hz), 1·03 (3H, d, *J* 6 Hz), 1·3 (1H, m), 2·0br (1H, D_2O exchangeable), and 3·7 (3H, m). The chlorohydrin (66 g) was added dropwise over 30 min to a solution of KOH in water (70%; 200 ml) at 125°. The product, 1,2-*epoxy*-3-*methylbutane*, distilled out and was then redistilled, b.p. 72—75° (26 g), $\nu_{max.}$ ($CHCl_3$) 3050, 1260, 872, and 814 cm^{-1}, δ ($CDCl_3$) 0·95 (3H, d, *J* 6 Hz), 1·0 (3H, d, *J* 6 Hz), 1·30 (1H, m), and 2·60 (3H, m).

The epoxide was treated with sulphuric acid (3·5 ml) in

* Minor impurities could be detected by g.l.c.; these could not be removed by distillation or preparative g.l.c. and did not interfere with subsequent steps.

† The chlorohydrin product is probably a mixture of 2-chloro-3-methylbutan-1-ol and 1-chloro-3-methylbutan-2-ol.

water (250 ml) for 3 days at 20°. The solution was neutralized, saturated with sodium chloride, and extracted continuously with ether for 12 h. The extract was dried (Na_2SO_4), concentrated, and the resultant oil distilled to yield the *diol* (10a), b.p. 61—62° at 0·5 mmHg (14 g), $\nu_{max.}$ ($CHCl_3$), 3370, 1065, 1010, and 875 cm^{-1}, δ ($CDCl_3$) 0·90 (3H, d, *J* 6 Hz), 0·95 (3H, d, *J* 6 Hz), 1·65 (1H, m), 3·53 (3H, m), and 4·20br (2H, D_2O exchangeable) (Found: C, 57·9; H, 11·4. $C_5H_{12}O_2$ requires C, 57·65; H, 11·6%).

2-*Benzoyloxy*-3-*methylbutyl Triphenylmethyl Ether* (12a).—The diol (10a) (2·12 g, 20·4 mmol) in anhydrous pyridine (20 ml) was treated with triphenylmethyl chloride (5·6 g, 20·4 mmol; freshly recrystallized from hexane) for 24 h at 20°. To the mixture was then added benzoyl chloride (2·87 g, 20·4 mmol) and the mixture was kept at 20° for 24 h. Water was added, the product extracted with ether, and the extract washed (HCl, Na_2CO_3, saturated NaCl), dried (Na_2SO_4), and evaporated. The residue was recrystallized from methanol (6·45 g, 70%), as *prisms*, m.p. 99—100°, $\nu_{max.}$ ($CHCl_3$) 1715, 1275, 920, and 698 cm^{-1}, δ ($CDCl_3$) 0·85 (3H, d, *J* 6 Hz), 0·92 (3H, d, *J* 6 Hz), 1·18 (1H, m), 3·37 (2H, d, *J* 4 Hz), 5·23 (1H, m), and 6·95—8·25 (20H, m) (Found: C, 80·45; H, 6·85. $C_{31}H_{30}O_3,0{\cdot}5H_2O$ requires C, 80·6; H, 6·8%).

2-*Benzoyloxy*-3-*methylbutan*-1-*ol* (12b).—The ether (12a) (2 g) was treated with 75% aqueous acetic acid (10 ml) at 80° for 1 h. The mixture was cooled, and the precipitated triphenylmethanol was filtered off. The filtrate was evaporated and the residue purified by preparative t.l.c. (20% ethyl acetate–hexane, R_F 0·55) giving (12b) (880 mg) as a viscous liquid, distillable in a kugelröhr tube at 200° and 1 mmHg, $\nu_{max.}$ ($CHCl_3$) 3630, 3500, 1720, 1280, and 705 cm^{-1}, δ ($CDCl_3$) 0·98 (6H, d, *J* 6 Hz), 1·22 (1H, m), 3·86 (2H, d, *J* 5 Hz), 4·83 (1H, s, D_2O exchangeable), 5·04 (1H, m), and 7·3—8·2 (5H, m).

2-*Hydroxy*-3-*methylbutyric Acid* (13b).—The benzoate (12b) (880 mg) in acetone (4 ml) was treated with Jones reagent (3·6 ml) at 20° for 20 h. The mixture was diluted with water and extracted with ether, and the extract was dried (Na_2SO_4) and evaporated to an oil, which was distilled in a kugelröhr tube at 175° and 1 mmHg, giving (13a) (850 mg), $\nu_{max.}$ ($CHCl_3$) 3500—2500br (CO_2H), 1725, 1280, 1115, and 698 cm^{-1}, δ ($CDCl_3$) 1·13 (6H, d, *J* 6 Hz), 1·18 (1H, m), 5·20 (1H, d, *J* 5 Hz), 7·3—8·2 (5H, m), and 10·5 (1H, D_2O exchangeable).

The benzoate (13a) (240 mg) was treated with aqueous NaOH (1N; 3 ml) and ethanol (2 ml) at 60° for 24 h. The mixture was neutralized with HCl and evaporated to dryness. The solid was triturated with ether, and the ether extract evaporated. The product was sublimed at 50° and 0·5 mmHg giving (13b) (120 mg), identical with an authentic specimen.

3-*Methyl*-2-p-*tolylsulphonyloxybutan*-1-*ol* (12c).—3-Methylbutane-1,2-diol (10a) (2·12 g, 20·38 mmol) in anhydrous pyridine (20 ml) was treated with triphenylmethyl chloride (5·6 g; 1 equiv.) at 20° for 24 h. To the mixture was added toluene-*p*-sulphonyl chloride (recrystallized from hexane; 3·87 g; 1 equiv.), and reaction was continued for 36 h at 20°. Chloroform and water were added, and the $CHCl_3$ extract was washed (HCl, Na_2CO_3, H_2O), dried (Na_2SO_4), and evaporated. The residue was crystallized from ethyl acetate yielding (12c) (5·6 g), m.p. 145—146°, as *prisms*, $\nu_{max.}$ ($CHCl_3$) 1352, 1190, 1175, 908, 698, and 555 cm^{-1}, δ ($CDCl_3$) 0·70 (6H, d, *J* 6 Hz), 2·1 (1H, m), 3·20 (2H, d, *J* 5 Hz), 4·48 (1H, q, *J* 5 Hz), 7·1—7·5 (17H, m), and 7·80 (2H, m) (Found: C, 74·4; H, 6·35. $C_{31}H_{32}O_4S$ requires C, 74·4; H, 6·45%).

Tosylate (12c) (500 mg) in absolute ether (50 ml) was refluxed for 48 h with lithium aluminium hydride (250 mg). The excess of hydride was destroyed by dropwise addition of ethyl acetate, followed by water. The product was purified by chromatography on a short column of silica gel, eluting with hexane. Evaporation yielded oily isopentyl trityl ether (200 mg), identical with an authentic specimen.

Conversion of [α-^{2}H]*Isobutyraldehyde into* 2-*Amino*-3-*methyl*[3-^{2}H]*butyronitrile* (14e).—A mixture of [α-^{2}H]isobutyraldehyde [18] (3 ml), ammonium sulphate (5 g), sodium cyanide (1·8 g), ammonium hydroxide (5 ml), and water (13 ml) was stirred for 1 h at 0°, then 36 h at 20°. The mixture was diluted with water (100 ml), saturated with NaCl, and extracted continuously with ether for 12 h. The extract was dried (Na_2SO_4) and evaporated to an oil which was distilled in a kugelröhr tube at 80° and 2 mm giving (14e) (1·4 g), $\nu_{max.}$ 3420, 3345, 2230w, 2170w, 1475, and 1094 cm^{-1}, δ ($CDCl_3$) 1·05br (6H, s), 1·90br (2H, s, D_2O exchangeable), and 3·55br (1H, s), *m/e* 99 (M^+, $C_5H_9N_2D$).

2-*Amino*-3-*methylbutyronitrile* (14a).—3-Methylbutane-1,2-diol (10a) (1 g) was dissolved in D_2O (50 ml) and the solution was saturated with NaCl and extracted continuously with ether for 12 h. The extract was dried (Na_2SO_4) and redissolved in D_2O (50 ml). The solution was again saturated with NaCl and extracted with ether. The extract was evaporated to an oil which was distilled in a kugelröhr tube at 110° and 1 mmHg to yield the deuteriated diol (10a), $\nu_{max.}$ (CCl_4) 2550, 1080, and 1038 cm^{-1}.

Deuteriated (10a) (0·5 g) in D_2O (10 ml) was treated with sodium metaperiodate (1 g) at 0° for 1 h, then at 20° for 2 h. The resultant solution was distilled under vacuum, keeping the temperature <20°, and the distillate was collected in a liquid N_2 trap. The thawed distillate was treated with $(ND_4)_2SO_4$ (2 g), ND_4OD in D_2O (7M; 5 ml), and sodium cyanide (1 g) at 0° for 1 h, then at 20° for 24 h. The solution was diluted with water (50 ml), saturated with NaCl, and extracted continuously with ether. The extract was evaporated to an oil, which was distilled in a kugelröhr tube at 80° and 2 mmHg, giving the aminonitrile (14a) (0·4 g), $\nu_{max.}$ (CCl_4) 3450, 2250, 1470, and 1070 cm^{-1}, δ ($CDCl_3$) 1·05 (3H, d, *J* 6 Hz), 1·08 (3H, d, *J* 6 Hz), 2·0 (1H, m), 3·62 (1H, d, *J* 5 Hz), and 3·72br (2H, D_2O exchangeable), *m/e* 98 (M^+, $C_5H_{10}N_2$).

Resolution of (±)-trans-2,3-*Epoxybutyric Acid* (9a).—(−)-(2*R*,3*S*)-*trans*-2,3-Epoxybutyric acid was obtained by recrystallization of racemic (9a) with brucine according to a published procedure.[10] The recovered (−)-acid (9a) was more easily purified by sublimation at 60—70° and 1 mmHg.

The enantiomer, (+)-(2*S*,3*R*)-*trans*-2,3-epoxybutyric acid (17a), was obtained by the following procedure. Racemic epoxy-acid (9a) (30·2 g; sublimed) and quinine (103 g, 1 equiv.) were dissolved in acetone (400 ml). The crystals which separated after 5 days at 20° were filtered off and recrystallized twice from acetone to constant m.p. 160—163° (decomp.), $[\alpha]_D^{20}$ −135° (*c* 1·05, H_2O) (yield 28 g). This material was dissolved in water (200 ml) and cooled to 10°, then treated with 2N-NaOH (37 ml). The resultant mixture was extracted with ether (3 × 200 ml). The aqueous phase was acidified to pH 7 with HCl, and saturated with NaCl, then further acidified to pH 2·2, and extracted rapidly with ether (10 × 100 ml). The extract was dried (Na_2SO_4) and evaporated, giving crude (17a) which was

sublimed at 65° and 1 mmHg (yield 2·83 g), m.p. 62°, $[\alpha]_D^{20}$ +79° (*c* 0·6, benzene).

(2RS,3S)-[4,4,4-$^2H_3$]*Valine* (8c).—The (−)-(2*R*,3*S*)-epoxy-acid (9a) (10·3 g) in ether (30 ml) was methylated with an excess of alcohol-free diazomethane in ether. The solvent was removed under reduced pressure at 20° to yield crude ester (9b) which was reduced with sodium borohydride (3·1 g) in water (50 ml) as described above, giving the epoxy-alcohol (9c), $[\alpha]_D^{20}$ −49° (*c* 5, benzene).

Lithium iodide-free [$^2H_3$]methyl-lithium in ether was prepared from [$^2H_3$]methyl iodide (35 g) as described above for unlabelled methyl-lithium. The resultant methyl-lithium suspension was added over 75 min to (−)-(9c) (4·20 g) in THF (100 ml; distilled from $LiAlH_4$) at −20° under N_2. Stirring was continued at −20° for 3 h, then at 20° for 3 h. Methanol (5 ml) was added followed by water (200 ml). The pH was adjusted to 7·0 with HCl, and the solution was saturated with NaCl and extracted overnight with ether. The extract was evaporated to an oil, which was distilled in a kugelröhr tube at 110° and 1 mmHg to yield the glycol (10b) (2·98 g), $[\alpha]_D$ −7·6° (*c* 5, $CHCl_3$), $\nu_{max.}$ (CCl_4) 3380, 2240, 2230, 2135, 2080, 1475, 1395, 1090, 1065, and 875 cm^{-1}, δ ($CDCl_3$) 0·95 (3H, d), 1·65 (1H, m), 3·53 (3H, m), and 4·20br (2H, D_2O exchangeable) [an impurity at 1·18 (d) was visible].

The glycol (10b) (2·98 g) in water (75 ml) at 0° was treated with sodium metaperiodate (8 g) for 1 h at 0°, then 3 h at 20°. The solution was distilled under vacuum at 20°, collecting the distillate in a liquid N_2 trap. The rapidly thawed distillate was immediately treated with ammonium chloride (9 g), ammonium hydroxide (22 ml), and sodium cyanide (9 g) at 0° for 1 h, then at 20° for 24 h. Water (100 ml) was added, and the mixture was saturated with NaCl, filtered, and extracted continuously with ether for 24 h. The extract was evaporated at 30° to a volume of *ca.* 10 ml, concentrated HCl (70 ml) was added, and the mixture was refluxed for 20 h and then evaporated to dryness. The residue was applied to a 2·5 × 10 cm column of Rexyn 101(H) cation exchange resin (H^+ form), and the column was eluted with water (300 ml), followed by 1N-NH_4OH (200 ml) which eluted the DL-valine. Evaporation gave a buff powder (1·38 g) which was dissolved in a little water, treated with Norite, filtered, and evaporated. Crystallization from ethanol yielded pure (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine (8c), $\nu_{max.}$ (KBr) 3420, 3300—2400br, 1600, 1510, 1420, 1330, and 533 cm^{-1}, δ (D_2O + ND_4OD, external Me_4Si) 1·34 (d, *J* 7 Hz, Me), 1·39 (d, *J* 7 Hz, Me), 2·50 (1H, m), and 3·78 (1H, d, *J* 5 Hz).

Resolution of (2RS,3S)-[4,4,4-$^2H_3$]*Valine* (8c).—(2*RS*,3*S*)-[4,4,4-$^2H_3$]Valine (530 mg) was treated with glacial acetic acid (20 ml) and acetic anhydride (10 ml) at reflux for 30 min. Water (100 ml) was added and the solution evaporated to dryness. The product was dissolved in water (30 ml) and the pH was adjusted to 7·2 with dilute NH_4OH. Hog kidney acylase I (15 mg) was added and the solution was incubated at 37° for 44 h. Water (25 ml) was added and the solution was filtered, then applied to a 2·5 × 10 cm column of Rexyn 101 (H^+) cation exchange resin and eluted with water (350 ml). The eluate was freeze-dried, yielding (2*R*,3*S*)-*N*-acetyl[4,4,4-$^2H_3$]valine (16) (303 mg), $[\alpha]_D^{20}$ −7° (*c* 2, HOAc). A portion of (16) was methylated with diazomethane in ether; the n.m.r. spectrum of the methyl ester is discussed in the text.

The ion exchange column was then eluted with 1M-NH_4OH (200 ml) and the eluate freeze-dried, yielding (2*S*,3*S*)-[4,4,4-$^2H_3$]valine (15) (170 mg), $[\alpha]_D$ +70° (*c* 1, HOAc), δ (D_2O + ND_2OD, external Me_4Si) 1·39 (3H, d, *J* 7 Hz), 2·50 (1H, m), and 3·78 (1H, d, *J* 5 Hz).

(2RS,3S)-[4-^{13}C]*Valine* (8b).—[^{13}C]Methyl-lithium (lithium-iodide free, in ether) was prepared from [^{13}C]methyl iodide (5·0 g; 60 atom % ^{13}C) according to the above procedure. A solution of (−)-(2*R*,3*S*)-*trans*-2,3-epoxybutan-1-ol (9c) (2·20 g, 25 mmol) in anhydrous THF (50 ml) at −20° under N_2 was treated dropwise with unlabelled methyl-lithium in ether (12 ml; 2·1M; 1 equiv.) added over 15 min. Gas production ceased abruptly, and then the [^{13}C]methyl-lithium suspension was added dropwise over 1 h. Reaction continued for 3 h at −20° and 3 h at 20°. After work-up as above, the diol (10c) (867 mg) was obtained, $\nu_{max.}$ 3400, 1420, 1070, and 830 cm^{-1}, δ_H ($CDCl_3$) 0·90 (3H, m, J^1H-^1H 6, $J^{13}C-^1H$ 128 Hz), 0·95 (3H, d, *J* 6 Hz), 1·6 (1H, m), and 3·6 (3H, m).

This compound was then converted by the periodate–Strecker sequence as described above into (2*RS*,3*S*)-[4-^{13}C]valine (8b) (225 mg), $\nu_{max.}$ (KBr) 3500—2500br, 1600, 1510, 1425, 1330 cm^{-1}, δ_H (D_2O + ND_4OD, external Me_4Si) 1·34 (3H, m, J^1H-^1H 7, $J^{13}C-^1H$ 126 Hz), 2·50 (1H, m), and 3·7 (1H, m).

(2RS,3S)-[4-^{3}H]*Valine* (8d) *and* (2RS,3R)-[4-^{3}H]*Valine* (8e).—[^{3}H]Methyl-lithium (lithium-iodide free, in ether) was prepared from [^{3}H]methyl iodide (5·0 g; 25·0 mCi; prepared by dilution of higher specific activity material with inactive carrier) as described above. (−)-(2*R*,3*S*)-*trans*-2,3-Epoxybutan-1-ol (9c) (2·1 g) was treated as described above with 1 equiv. of unlabelled methyl-lithium, followed by [^{3}H]methyl-lithium yielding the glycol (10d) (989 mg; 6·9 mCi), which *via* the periodate–Strecker method as described previously was converted into (2*RS*,3*S*)-[4-^{3}H]-valine (8d) (364 mg; 2·25 mCi). A paper radiochromatogram of the product indicated at least 98% of the radioactivity associated with DL-valine.

In an identical manner, (+)-(2*S*,3*R*)-*trans*-2,3-epoxybutan-1-ol (17b), obtained *via* methylation and reduction of (17a) as described above, was converted into the glycol (10e) (1·18 g; 6·4 mCi), which *via* the periodate–Strecker sequence afforded (2*RS*,3*R*)-[4-^{3}H]valine (8e) (612 mg; 4·3 mCi), radiochemically pure as shown by radiochromatogram scanning.

Incorporation of (2RS,3S)-[4-^{13}C]*Valine* (8b) *into Penicillin* V.—(2*RS*,3*S*)-[4-^{13}C]Valine (8b) (50 mg) was incubated with *Penicillium chrysogenum* as described elsewhere,[22,23] and the resultant penicillin V potassium salt isolated. The ^{13}C n.m.r. spectrum of this material was identical with that published [8] for the unlabelled penicillin V potassium salt, except that the signal at 26 p.p.m. for the α-methyl group showed a *ca.* 2% enhanced intensity.

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