sequently takes place with *retention* of configuration, paralleling the result deduced earlier[1] for the analogous step in isoleucine biosynthesis.

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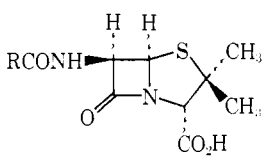
Studies on the Biosynthesis of β-Lactam Antibiotics. I. Synthesis of (2*RS*,3*S*)-[4,4,4-$^2H_3$]Valine

Sir:

The biosynthesis of the β-lactam antibiotics penicillin (**1**) and cephalosporin C (**2**) has been under investiga-



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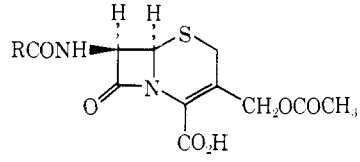
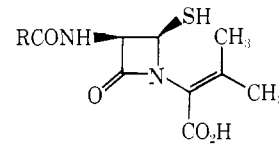


2

tion for many years.[1] The ring systems of **1** and **2** have been shown to be formed from L-valine and L-cysteine,[2] possibly *via* the intermediacy of the tripeptide 5-(L-2-aminoadipoyl)cysteinylvaline.[1,2a] In addition it is frequently assumed[1] that ring formation occurs *via* a dehydrovaline intermediate, *e.g.*, **3**. However, little experimental evidence is available in support of this theory, and the detailed mechanism of the biosynthesis of **1** and **2** remains a mystery and a challenge.

In an effort to shed some light on these biosynthetic processes, we began a study of the fate of the diastereotopic methyls of L-valine in the course of their incorporation into **1** and **2**. For this purpose a synthesis of chirally labeled CD_3-valine (**4**) was undertaken. The present communication describes the accomplishment of this synthesis in a six-step sequence starting from *trans*-(2*R*,3*S*)-(−)-2,3-epoxybutyric acid (**5a**) of established absolute configuration.[3-5]

Methylation of **5a** with diazomethane gave the



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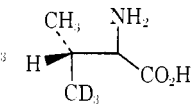

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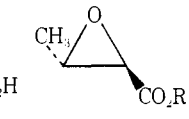
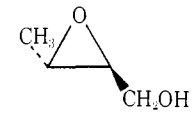
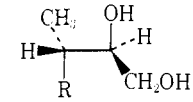


5a, R = H
b, R = CH_3

methyl ester **5b**, bp 150°. Reduction with sodium borohydride[6] in water at 20° for 75 min, followed by adjustment of the pH to 7.0 and continuous extraction, gave *trans*-(2*S*,3*S*)-2,3-epoxy-1-butanol (**6**), 50%, $[\alpha]_D$ −49° (*c* 5, benzene). Treatment of **6** in THF at −20° with a solution of [$^2H_3$]methyllithium[7] in ether gave (2*R*,3*S*)-[4,4,4-$^2H_3$]-3-methyl-1,2-butanediol (**7a**), 50%,



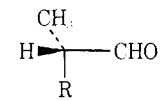
6



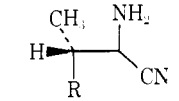
7a, R = CD_3
b, R = CH_3

purified by Kugelrohr distillation at 110° (1 mm), $[\alpha]_D$ −7.6° (*c* 5, $CHCl_3$), identical in vpc retention[8] with an authentic sample (**7b**). This was prepared by reduction of D,L-α-hydroxyisovaleric acid methyl ester with lithium aluminum hydride, and on a larger scale by conversion of 3-methyl-1-butene with calcium hypochlorite and acetic acid into 2-chloro-3-methyl-1-butanol, 38%, bp 60–65° (30 mm),[9] thence with 70% KOH at 125° into 3-methyl-1,2-epoxybutane, 58%, bp 70–74°, which with aqueous acid at reflux gave glycol **7b**, ^1H nmr ($CDCl_3$) δ 0.90 (3 H, d, *J* = 6 Hz), 0.95 (3 H, d, *J* = 6 Hz), 1.65 (1 H, m), 3.53 (3 H, m), 4.20 (2 H, OH). This is to be compared with the ^1H nmr of **7a**, identical with that of **7b** except that only a single methyl doublet appeared at 0.95 ppm indicating that the epoxide opening reaction **6** → **7a** had proceeded in a clean stereospecific, trans manner.[10]

We next sought a method of converting **7a** into (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine (**4**). The method of choice appeared to be cleavage of the glycol with periodate generating chiral isobutyraldehyde (**8a**), which could then be converted to **4** *via* the aminonitrile (**9a**) fol-



8a, R = CD_3
b, R = CH_3



9a, R = CD_3
b, R = CH_3

lowed by acid hydrolysis (Strecker method).[11] At the

(1) P. A. Lemke and D. R. Brannon in "Penicillins and Cephalosporins," E. H. Flynn, Ed., Academic Press, New York, N. Y., 1972, p 370.

(2) (a) E. P. Abraham, G. G. F. Newton, and S. C. Warren in "Biogenesis of Antibiotic Substances," Z. Vanek and Z. Hostelak, Ed., Academic Press, New York, N. Y., 1965, p 169; (b) H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, **57**, 353, 360 (1954); (c) S. C. Warren, G. G. F. Newton, and E. P. Abraham, *Biochem J.*, **103**, 902 (1967); (d) C. M. Stevens, E. Inamine, and C. W. DeLong, *J. Biol. Chem.*, **219**, 405 (1956).

(3) Studies on the synthesis of the analogous ^{13}C-labeled compound and the microbiological studies will be reported in subsequent communications.

(4) A report by Baldwin and coworkers of the synthesis of (2*RS*,3*S*)-[4-^{13}C]valine recently appeared in this journal: J. E. Baldwin, J. Löliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. DeLa Higuera, *J. Amer. Chem. Soc.*, **95**, 3796 (1973).

(5) K. Harada and J. Oh-hashi, *Bull. Chem. Soc. Jap.*, **39**, 2311 (1966).

(6) S. Corsano and G. Piancatelli, *Chem. Commun.*, 1106 (1971); *Gazz. Chim. Ital.*, **101**, 204 (1971).

(7) For success in this ring-opening reaction, it was essential to use lithium iodide free methyllithium prepared by exchange with *n*-butyllithium in hexane, followed by replacement of hexane with absolute ether; see T. L. Brown and M. T. Rogers, *J. Amer. Chem. Soc.*, **79**, 1859 (1957); R. West and W. Glaze, *ibid.*, **83**, 3580 (1961). Use of methyllithium–ether solutions prepared from methyl iodide and lithium metal in ether led to the formation of complex mixtures of products.

(8) 15% SE-30, 115°, retention 6 min; vpc indicated the presence of minor impurities which could not be removed and did not interfere with subsequent steps.

(9) See: C. E. Wilson and H. J. Lucas, *J. Amer. Chem. Soc.*, **58**, 2396 (1936). Satisfactory analytical data have been obtained for new compounds.

(10) A stereospecific cis epoxide opening would also be consistent with this result, but is considered unlikely.

(11) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Wiley, New York, N. Y., 1961, p 2372.

outset, however, it was unclear whether the chirality of **8a** would be retained throughout the Strecker sequence. Enolization of isobutyraldehyde would cause a loss of configurational purity.[12] However, the results of two experiments indicated that enolization might be negligible under suitably mild Strecker conditions. First, glycol **7b** was treated several times with D_2O to exchange hydroxylic hydrogens. The deuterated analog with sodium metaperiodate in D_2O at 0°, then 20°, gave a mixture of aldehydes which was distilled at 20° into a cold trap. The thawed aqueous distillate, with $(ND_4)_2SO_4$, ND_4OD, and sodium cyanide in D_2O at 0°, then 20°, gave aminonitrile **9b**, purified by Kugelrohr distillation at 70° (0.5 mm). The nmr and mass spectra of this compound indicated the absence of deuterium. Similarly, [α-^{2}H]isobutyraldehyde[12] under analogous conditions but with undeuterated reagents in H_2O gave **9b** which contained one atom of deuterium, as did the D,L-valine *N*-acetate obtained after acid hydrolysis and acetylation.

Hence, glycol **7a**, with sodium metaperiodate in water at 0° for 1 hr, then 20° for 3 hr, gave a mixture containing (2*S*)-[3,3,3-$^2H_3$]isobutyraldehyde (**8a**) which was distilled nearly to dryness at 20° *in vacuo*. The distillate trapped in liquid N_2 was thawed and immediately treated with ammonium chloride, ammonium hydroxide, and sodium cyanide, at 0° for 1 hr and then for 19 hr at 20°. The crude[13] (2*RS*,3*S*)-[4,4,4-$^2H_3$]-2-amino-3-methylbutyronitrile (**9a**) isolated by continuous ether extraction was hydrolyzed with concentrated HCl at reflux for 24 hr. Evaporation gave a crude residue from which D,L-[$^2H_3$]valine (**4**) was isolated (20%) by ion-exchange chromatography on Rexyn 101 (H) cation exchange resin. Only negligible traces of glycine were obtained. Crystallization from ethanol gave pure (2*RS*,3*S*)-[4,4,4-$^2H_3$]valine (**4**): ^{1}H nmr (D_2O + ND_4OD, external TMS) δ 1.34 (d, CH_3, J = 7 Hz), 1.39 (d, CH_3, J = 7 Hz), 2.50 (1 H, m), 3.78 (1 H, d, J = 5 Hz). A portion was acetylated[11] and resolved with kidney acylase I,[11] the resultant (2*S*,3*S*)-[4,4,4-$^2H_3$]valine (**10**) ([α]D +70° (*c* 1, HoAc)) and (2*R*,3*S*)-[4,4,4-$^2H_3$]valine *N*-acetate (**11**) ([α]D −7.0 (*c* 2, HoAc)) being separated by ion exchange on Rexyn 101 (H). The ^{1}H nmr of **10** showed a single 3 H methyl doublet at 1.39 ppm indicating the stereochemical homogeneity of the isopropyl group. The acetate **11** was converted to the methyl ester: ^{1}H nmr ($CDCl_3$ with Eu(fod)$_3$, 0.30 mol) showed a single doublet, 1.98 ppm, J = 7 Hz, whereas the nmr of unlabeled (2*RS*)-valine *N*-acetate methyl ester showed doublets at 1.98 and 2.42 ppm, again indicating the stereochemical purity of **4**, estimated to be close to 100%.

Acknowledgment. We are indebted to the National Institutes of Health for Grant GM 18872 in support of this work.

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

(13) For preparative purposes, much higher yields of valine are obtained if the aminonitrile is not purified before hydrolysis.

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Effect of Solvent on Neighboring Aryl Group Participation. Is k_Δ Enhanced?[1]

Sir:

The β-phenethyl tosylate/ethyl tosylate solvolysis rate ratio varies dramatically from 0.24 to 1770 (7500-fold!) in going from ethanol to trifluoroacetic acid (Table I, column 4). While these results indicate a solvent-induced shift of mechanism from aryl unassisted to aryl assisted,[2] the factors responsible for this extreme variation, although often discussed,[2-7] have not been quantitatively elucidated.

Solvolyses of primary and most secondary substrates are now established to proceed not by free carbocations but by strongly assisted transition states and cationoid intermediates.[2,6,8-10] Simple substrates solvolyze by the k_s (solvent assisted) route, but competition between two discrete pathways, k_s and k_Δ (or Fk_Δ,[11] anchimerically assisted), is possible when a neighboring group is present. Thus, eq 1 summarizes

$$\frac{k_t(\beta\text{-PhEtOTs})}{k_t(\text{EtOTs})} = \frac{Fk_\Delta + k_s(\beta\text{-PhEtOTs})}{k_s(\text{EtOTs})} \qquad (1)$$

the β-PhEtOTs/EtOTs rate ratio. Changes in this ratio in going from one solvent to another could be caused by either of two effects (or their combination[3a]):[12] (1) by a decrease in $k_s(\beta\text{-PhEtOTs})$ and k_s(EtOTs relative to Fk_Δ as solvent nucleophilicity is decreased, and/or (2) by an enhancement of the relative magnitude of Fk_Δ as solvent ionizing power is increased. For these primary substrates, the k_s route is a simple displacement reaction which is extremely sensitive to solvent nucleophilicity, while k_Δ is an ionization process and could be more sensitive than k_s to ionizing power. The enhancement of k_Δ might be due (a) merely to a difference in response of the two dissimilar[6] pathways to solvent ionizing power ($m_\Delta > m_s$) or (b) to an increase with increased solvent ionizing power of the kinetically significant magnitude of aryl bridging in the transition state (TS_Δ) leading to the phenonium intermediate.[4,7]

Our findings indicate that a combination of effects 1 and 2a is operative and that 2b is not important.

To determine if the magnitude of assistance provided by aryl bridging in the transition state (TS_Δ) is enhanced by solvent ionizing power (effect 2b), we probed

(1) A preliminary account of this work was presented at the 7th Annual Middle Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 15, 1972.

(2) For a recent review, see C. J. Lancelot, D. J. Cram, and P. v. R. Schleyer in "Carbonium Ions," Vol. III, G. A. Olah and P. v. R. Schleyer, Ed., Interscience, New York, N. Y., 1972, Chapter 27, p 1347.

(3) (a) S. Winstein and H. Marshall, *J. Amer. Chem. Soc.*, **74**, 1120 (1952); (b) S. Winstein, C. R. Lindegren, H. Marshall, and L. L. Ingraham, *ibid.*, **75**, 147 (1953).

(4) H. C. Brown, R. Bernheimer, C. J. Kim, and S. E. Scheppele, *ibid.*, **89**, 370 (1967).

(5) J. E. Nordlander and W. G. Deadman, *ibid.*, **90**, 1590 (1968).

(6) A. Diaz, I. Lazdins, and S. Winstein, *ibid.*, **90**, 6546 (1968).

(7) J. A. Thompson and D. J. Cram, *ibid.*, **91**, 1778 (1969).

(8) Citations to recent work at Princeton regarding this point are listed in ref 9, footnote 11. For a review see D. J. Raber and J. M. Harris, *J. Chem. Educ.*, **49**, 60 (1972).

(9) T. W. Bentley, F. L. Schadt, and P. v. R. Schleyer, *J. Amer. Chem. Soc.*, **94**, 992 (1972).

(10) A. F. Diaz and S. Winstein, *ibid.*, **91**, 4300 (1969).

(11) F is the fraction of intimate phenonium ion pair which does not return.

(12) More reactive leaving groups also have an effect on the competition between k_s and k_Δ.[2] See J. L. Coke, F. E. McFarlane, M. C. Mourning, and M. G. Jones, *J. Amer. Chem. Soc.*, **91**, 1154 (1969); R. J. Jablonski and E. I. Snyder, *ibid.*, **91**, 4445 (1969).