Stereochemistry of valine and isoleucine biosynthesis. III. Enzymic discrimination between diastereotopic enol faces in the dehydrase step of vline biosynthesis

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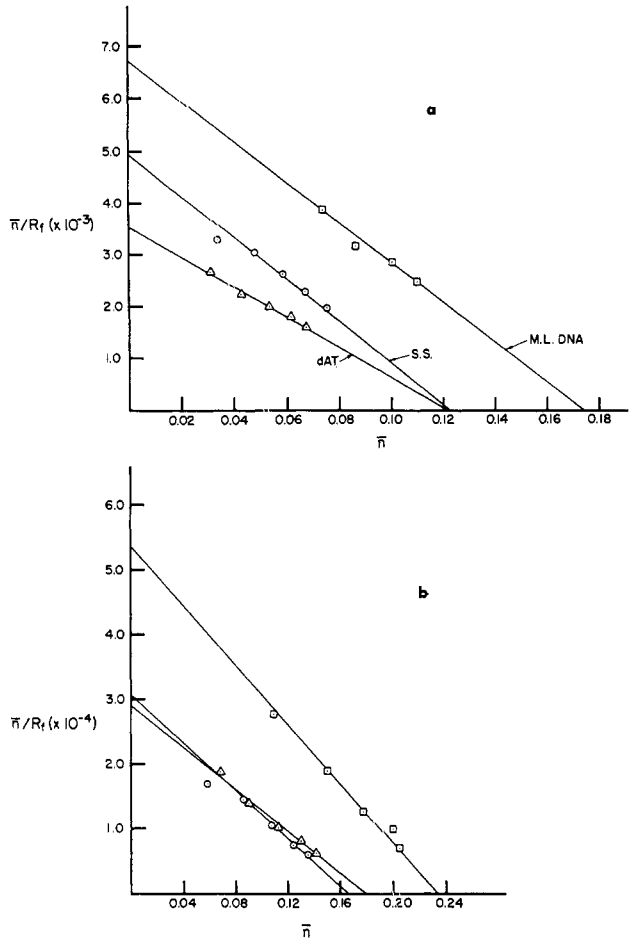


Figure 8. The Scatchard plots of the binding studies data obtained by equilibrium dialysis technique for interactions of cations **1** (a) and **6** (b) with poly(dA-T)-poly(dA-T) (Δ) salmon sperm DNA (○) and *Micrococcus luteus* DNA (□) (see Table III).

separate by distances greater than 6.8 Å in order to accommodate a bulkier intercalating cation, *e.g.*, **6**. In the latter case a separation distance of at least 7.6 Å is required. (2) The effect of nucleic acid base composition on the apparent binding constant and the maximum number of binding sites was studied for cations **1** and **6** (Table III). The results show the following order of increasing affinity of **1** to the nucleic acids, poly(dA-T)-poly(dA-T) (100% A-T) < *M. luteus* DNA (28% A-T) < salmon sperm DNA (58% A-T), and the following order of increasing affinity of **6** to the nucleic acids, poly(dA-T)-poly(dA-T) < salmon sperm DNA < *M. luteus* DNA. In addition, it is noted that G-C rich DNA, *i.e.*, *M. luteus* DNA, shows a higher maximum number of strong binding sites for **1** and **6** than the other nucleic acids. Steric hindrance to intercalation between A-T sites is one possible explanation for the above observation. For example, the separation distance required for intercalation of **6** between base pairs composed of A-T sites may be as high as 8.4 Å if the CH_3 group of thymine is in an eclipsed conformation with respect to a CH_3 substituent of the intercalating cation, **6**. Such effects are illustrated in Figure 9.

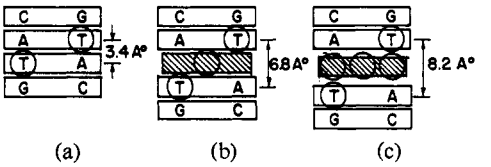


Figure 9. Schematic illustrations of the complexes of I to DNA showing the possible separation distances between base pairs required to accommodate unsubstituted (b) and methyl-substituted *N*-methylphenanthrolinium cation (c).

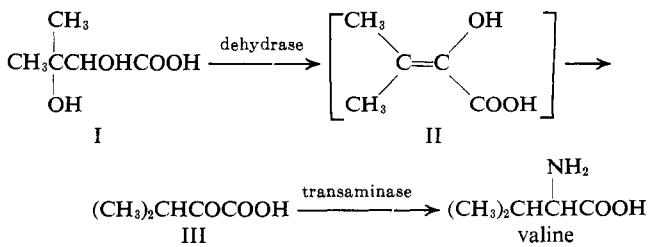
In summary, systematic studies of the interaction specificities of methyl-substituted *N*-methylphenanthrolinium cations, I, with nucleic acids of various base compositions have been carried out. In all cases, a common mode of binding is observed, *i.e.*, intercalation between base pairs of DNA. Selective interactions of I with DNA are noted as a function of the position and number of methyl substituents on the *N*-methylphenanthrolinium ring. For example, the more highly substituted systems exhibit (i) higher affinity, (ii) greater stabilization of the helix, and (iii) higher viscosity upon binding to DNA. Moreover, selective binding to G-C sites (and/or a combined G-C/A-T site) by the more highly substituted aromatic cations is observed. These as well as other effects are discussed and the results can be accounted for in terms of reasonable structural models.

Communications to the Editor

Enzymatic Discrimination between Diastereotopic Enol Faces in the Dehydrase Step of Valine Biosynthesis[1]

Sir:

The penultimate step of valine biosynthesis in bacteria[2] is the dehydration of (−)-α,β-dihydroxyisovalerate (I) to α-ketoisovalerate (III), catalyzed by an α,β-dihydroxy acid dehydrase. Arfin has shown conclusively by labeling experiments[3] that dehydration must proceed *via* an enol intermediate (II).



(1) Stereochemistry of Valine and Isoleucine Biosynthesis. III. For paper II in this series, see R. K. Hill and S. Yan, *Bioorg. Chem.*, 1, 446 (1971).

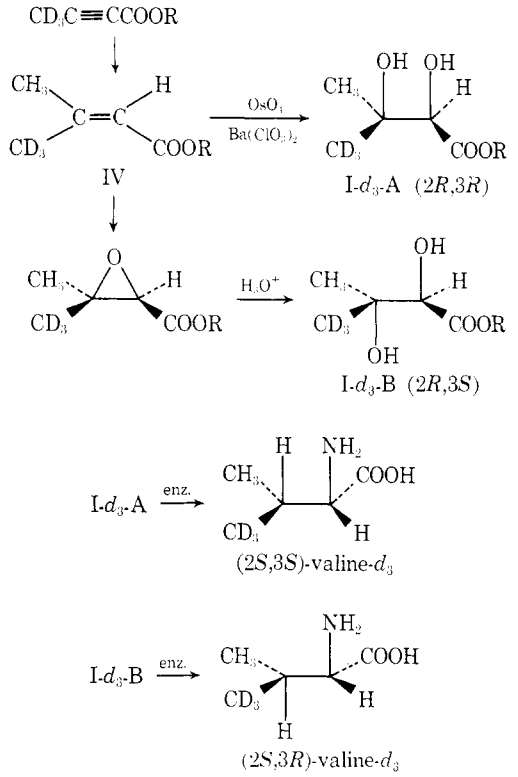
(2) A. Meister, "Biochemistry of the Amino Acids," 2nd ed, Vol. 2, Academic Press, New York, N. Y., pp 729–739.

(3) S. M. Arfin, *J. Biol. Chem.*, **244**, 2250 (1969).

Protonation of enol II generates a prochiral center at C-3 in III. By analogy with the stereospecific enzymatic protonation of phosphoenolpyruvate[4] and of isopentenyl pyrophosphate,[5] as well as with other enzymatic reactions[6] which generate a prochiral carbon by stereospecific addition to one of the diastereotopic faces of a system $a_2C{=}Cxy$, it might be anticipated that the protonation step would be stereospecific. We present evidence with labeled substrates that enzymatic discrimination between the diastereotopic faces of II is essentially absolute, another striking example of enzymatic recognition of diastereotopic groupings.[7]

The diastereotopic methyls of I give rise to two methyl singlets, δ 1.20 and 1.28, in the nmr spectrum of its methyl ester. Samples of I stereospecifically labeled with deuterium in one of the methyls were prepared as outlined in Scheme I and their stereochemical purity

Scheme I. Synthesis of Diastereomeric Samples of I-d_3



was analyzed by nmr. Addition of lithium dimethylcuprate[8] to ethyl tetrolate-d_3 in THF at $-70°$ gave IV, which showed only the methyl doublet at δ 1.86 for the methyl trans to COOR and no signal at δ 2.14 for methyl cis to COOR. Cis hydroxylation with OsO_4–$Ba(ClO_3)_2$ gave the (2*R*,3*R*;2*S*,3*S*)-I-d_3-A (methyl singlet at δ 1.28, no detectable signal at δ 1.20), while epoxidation of IV with *m*-chloroperbenzoic acid followed by aqueous acid hydrolysis gave predominantly the 2*R*,3*S*;2*S*,3*R* isomer, I-d_3-B (singlets at δ 1.20 and 1.28 in a 78:22 ratio).

These samples of I-d_3 were saponified and the acids used as substrates for enzymatic dehydration. Since it has been shown that only the *R* enantiomer serves as a substrate,[1,9] the racemic deuterated samples could be used without resolution; the biologically active enantiomers are depicted in Scheme I. A partially purified dehydrase from *Escherichia coli* was used; to avoid having to analyze the optically labile III, a transaminase was added to the incubation mixtures[10] to convert III to valine.

The nmr spectra of the valine-d_3 samples isolated from these biosynthetic experiments revealed the stereospecificity of their formation. The diastereotopic methyls of L-valine appear in the nmr spectrum (taken in aqueous alkali) as two doublets, δ 1.38 and 1.45. L-Valine biosynthesized from I-d_3-A shows only the doublet at δ 1.45, with no more than 5% contamination by the 1.38 signal, while L-valine biosynthesized from I-d_3-B shows conversely a predominant doublet at δ 1.38. Enzymatic protonation of enol II accordingly takes place with a minimum of 95% stereospecificity.

The absolute stereochemical sense of the protonation step could be elucidated by an absolute assignment of the two valine methyl signals to the (*pro*-*R*)- and (*pro*-*S*)-methyls. This has been accomplished by stereospecific synthesis of (2*S*,3*R*)- and (2*R*,3*R*)-valine-d_3 beginning with (*S*)-(+)-2-propanol-d_3.[11] Displacement of the benzenesulfonate by diethyl sodiomalonate led to (3*R*)-(−)-diethyl isopropyl-d_3-malonate, which was converted to (2*RS*,3*R*)-valine-d_3 by a published procedure.[12] Kinetic resolution[13] of the *N*-acetyl derivative with hog kidney acylase I gave (2*S*,3*R*)-(+)-valine-d_3 (nmr doublet at δ 1.38) and (2*R*,3*R*)-(−)-*N*-acetylvaline-d_3 (nmr doublet at δ 1.43).

These results show that the (*pro*-*S*)-methyl of L-valine gives the nmr signal at δ 1.38, and the (*pro*-*R*)-methyl at 1.45.[14] Accordingly, the enzymatic dehydration specifically converts (2*R*,3*R*)-I-d_3 to (2*S*,3*S*)-valine-d_3 and (2*R*,3*S*)-I-d_3 to (2*S*,3*R*)-valine-d_3. The overall replacement of the C-3 hydroxyl in dihydroxy isovalerate by hydrogen during valine biosynthesis con-

(4) I. A. Rose, *J. Biol. Chem.*, **245**, 6052 (1970); M. Cohn, J. E. Pearson, E. L. O'Connell, and I. A. Rose, *J. Amer. Chem. Soc.*, **92**, 4095 (1970).

(5) K. Clifford, J. W. Cornforth, R. Mallaby, and G. T. Phillips, *Chem. Commun.*, 1599 (1971); J. W. Cornforth, *Chem. Soc. Rev.*, **2**, 1 (1973).

(6) (a) I. A. Rose, E. L. O'Connell, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, T. G. Cooper, and M. Benziman, *J. Biol. Chem.*, **244**, 6130 (1969); (b) M. G. Kienle, R. K. Varma, L. J. Mulheirn, B. Yagen, and E. Caspi, *J. Amer. Chem. Soc.*, **95**, 1996 (1973).

(7) R. Bentley, "Molecular Asymmetry in Biology," Academic Press, New York, N. Y., 1969; W. L. Alworth, "Stereochemistry and its Application in Biochemistry," Wiley-Interscience, New York, N. Y., 1972.

(8) E. J. Corey and J. A. Katzenellenbogen, *J. Amer. Chem Soc.*, **91**, 1851 (1969); J. B. Siddall, M. Biskup, and J. H. Fried, *ibid.*, **91**, 1853 (1969). We thank Professor Katzenellenbogen for his kindness in furnishing experimental details.

(9) (a) J. Sjölander, K. Folkers, E. A. Adelberg, and E. Tatum, *J. Amer. Chem. Soc.*, **76**, 1085 (1954); (b) B. E. Nielsen, P. K. Larsen, and J. Lemmich, *Acta Chem. Scand.*, **23**, 967 (1967).

(10) A typical 8.0-ml incubation mixture contained, besides the two enzymes, 150 mg of I-d_3, 750 μmol of Tris-HCl (pH 7.8), 10 mmol of sodium glutamate, 100 μmol of pyridoxal phosphate (neutralized), 40 μmol of $FeSO_4$, and 200 μmol of cysteine (neutralized). After 15-hr incubation, the reaction was stopped by heating at 100° for 3 min and centrifuged to remove denatured protein, and valine was isolated from the supernatant by paper chromatography.

(11) K. Mislow, R. E. O'Brien, and H. Schaefer, *J. Amer. Chem. Soc.*, **84**, 1940 (1962).

(12) C. S. Marvel and V. Du Vigneaud, "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1943, p 93; C. S. Marvel, *ibid.*, Collect. Vol. III, 1955, p 848.

(13) C. G. Baker and H. A. Sober, *J. Amer. Chem. Soc.*, **75**, 4058 (1953).

(14) An independent synthesis of (2*S*,3*S*)-valine-d_3 (D. J. Aberhart and L. J. Lin, *J. Amer. Chem. Soc.*, **95**, 7859 (1973)) confirms the assignment of the upfield and downfield methyl signals. We thank Professor Aberhart for notifying us of his results before publication. For recent syntheses of valine stereospecifically labeled with C-13 in the methyl groups, see (a) J. E. Baldwin, J. Löliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. De La Higuera, *ibid.*, **95**, 3796 (1973); (b) H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, **95**, 6149 (1973).

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

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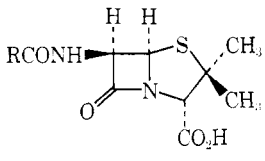
Studies on the Biosynthesis of β-Lactam Antibiotics. I. Synthesis of (2*RS*,3*S*)-[4,4,4-$^2H_3$]Valine

Sir:

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



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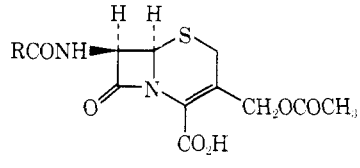
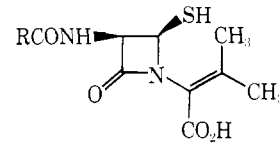


2

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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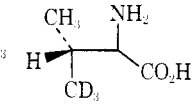

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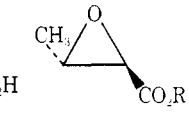
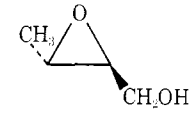


5a, R = H
b, R = CH_3

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%,



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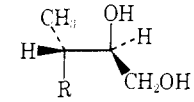
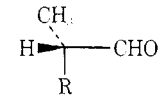


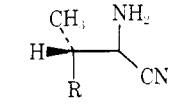
7a, R = CD_3
b, R = CH_3

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.