

Stereospecific Synthesis of D-Isothreonine from L-Threonine¹⁾

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(Received December 23, 1977)

Synopsis. D-Isothreonine, (2*R*,3*S*)-3-amino-2-hydroxybutanoic acid (**4**), was readily prepared by the ammonolysis of optically active 2-bromo-3-hydroxybutanoic acid derived from L-threonine. The configuration of **4** was deduced from the shift of molecular rotation, Cotton effect in ORD curve of **4**, and NMR measurement of its oxazolidone derivative.

In recent years α -hydroxy β -amino carboxylic acids have been found in nature as constituent amino acids of biologically active peptides; L-isoserine²⁾ in an antibiotic, edeine, and (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoic acid³⁾ in an aminopeptidase B inhibitor, bestatin. However, the syntheses of these optically active α -hydroxy β -amino carboxylic acids are tedious. L-Isoserine was prepared by optical resolution⁴⁾ or through three steps starting from L-asparagine.⁵⁾

This paper deals with a convenient method of preparation of such optically active α -hydroxy β -amino carboxylic acids as exemplified by the first synthesis of (2*R*,3*S*)-3-amino-2-hydroxybutanoic acid (D-isothreonine)⁶⁾ (**4**) through two steps starting from L-threonine (**1**).

It is known that racemic α -halo β -hydroxy carboxylic acids are converted by the action of ammonia into racemic α -hydroxy β -amino carboxylic acids, α -amino β -hydroxy carboxylic acids, or a mixture of the two.^{7–9)} For the amination reactions Carter and Zirkle⁸⁾ and Neuberg and Mayer¹⁰⁾ proposed possible α,β -epoxy carboxylic acid intermediates. In fact, Liwschitz *et al.*¹¹⁾ prepared DL-*threo*-2-hydroxy-3-aminobutanoic acid without formation of *erythro* form by the reaction of racemic *cis*-2,3-epoxybutanoic acid with amine. The closure and opening of epoxide ring accompany an inversion of the configuration of carbon atom attacked by nucleophiles.^{12,13)} Thus we assumed that optically active α -hydroxy β -amino carboxylic acids could be stereospecifically prepared from optically active α -halo β -hydroxy carboxylic acids. In order to confirm the prediction we attempted a simple

preparation of **4** through the amination reaction of (2*S*,3*R*)-2-bromo-3-hydroxybutanoic acid (**2**) derived from **1** (Scheme 1).

Compound **2** was prepared by the action of nitrosyl bromide on **1**. This reaction is known to proceed with retention of the configuration of C $_{\alpha}$.¹⁴⁾ Treatment of **2** with 28% aqueous ammonia afforded a mixture of isothreonine and threonine (92 : 8). The mixture was separated into each component by column chromatography on Dowex 50X8 (NH $_4^+$ form).

The configuration of C $_{\alpha}$ atom of isolated isothreonine (**4**) was confirmed to be 2*R* by the negative shift in molecular rotation on acidification¹⁵⁾ and the negative Cotton effect in the region 200–240 nm.¹⁶⁾ In order to determine the configuration of C $_{\beta}$ atom of **4**, we measured the ¹H-NMR spectrum of 2-oxazolidone derivative (**6**) of **4**. The coupling constants ($J_{\alpha\beta}$) between the vicinal methine protons of the oxazolidone derivatives of α -amino β -hydroxy carboxylic acids are reported to be 5.0 $\pm$ 1.0 Hz for *threo* and 9.6 $\pm$ 0.6 Hz for *erythro* isomers in CD $_3$ OD.¹⁷⁾ Those of α -hydroxy β -amino carboxylic acids are also reported to be 4.0 Hz for *threo* and 9.0 Hz for *erythro* isomers in CD $_3$ OD.³⁾ NMR spectrum of **6** was recorded in DMSO-*d*₆ because of its insolubility in CD $_3$ OD, $J_{\alpha\beta}$ value of **6** being 5.0 Hz. $J_{\alpha\beta}$ values of reference oxazolidone derivatives of L-threonine (*threo*) and L-allothreonine (*erythro*) were 4.8 and 8.5 Hz in DMSO-*d*₆, respectively. The values in DMSO-*d*₆ are almost equal to those for *threo* and *erythro* in CD $_3$ OD, respectively. These results suggest that the configuration of **4** should be *threo* form (2*R*, 3*S*). Thus the prediction (Scheme 1) was confirmed by the first synthesis of optically active isothreonine **4**.

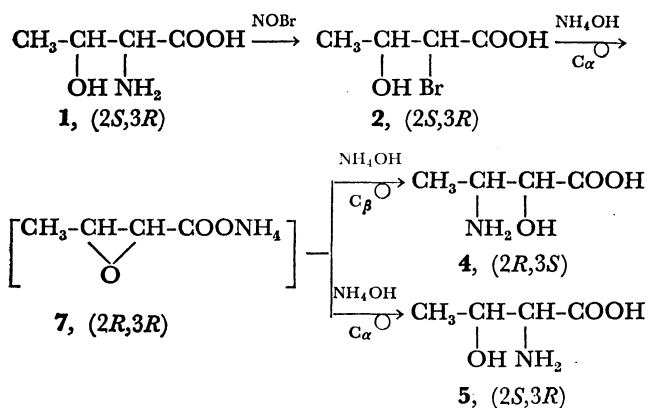
In a similar manner D-isoserine, (2*R*)-3-amino-2-hydroxypropanoic acid (**8**), was prepared from L-serine in optically pure state. On the basis of this fact and the result obtained by Liwschitz *et al.*,¹¹⁾ we assume that **4** should be optically pure as regards both C $_{\alpha}$ and C $_{\beta}$.

Experimental

The following solvent systems were used: R_f^1 , pyridine–H $_2$ O (65 : 35, v/v) for TLC and R_f^2 , cyclohexylamine–H $_2$ O–methyl ethyl ketone–*n*-BuOH (2 : 5 : 10 : 10, v/v) for paper chromatography. ¹H-NMR spectra were measured with a Hitachi R-20B spectrometer (60 MHz), using sodium 3-trimethylsilyl-1-propanesulfonate in D $_2$ O or tetramethylsilane in DMSO-*d*₆ as an internal standard.

Synthesis of D-Ith (4**).** (2*S*,3*R*)-2-Bromo-3-hydroxybutanoic Acid (**2**): This was prepared from L-Thr (5.95 g, 50 mmol), KBr (20.9 g, 175 mmol) and sodium nitrite (5.58 g, 80 mmol) in 1.25 M H $_2$ SO $_4$ (105 ml) according to the procedure of Izumiya,¹⁸⁾ yield of an oil, 8.27 g (90%); R_f^1 0.64.

Mixture (3**) of Ith and Thr:** Compound **2** (8.20 g, 45 mmol) was dissolved in 28% aqueous ammonia (82 ml) at



Scheme 1. Stereochemical reaction route.

0°C. After being left to stand for 10 d at 0°C, the solution was evaporated and the residual solid was dissolved in a small amount of water. The solution was applied on a column (2.2×20 cm) of Dowex 50X8 (H⁺ form), and the column was washed with water and eluted with 2 M NH₄OH (100 ml). The eluate was evaporated and the yellowish residue was collected; yield, 2.40 g (45%); R_f^1 0.43 (major) and 0.70 (minor); R_f^2 0.40 (major), 0.56 (minor) and 0.45 (faint). R_f s of reference compounds: L-Thr, R_f^1 0.70, R_f^2 0.56; L-aThr, R_f^1 0.70, R_f^2 0.45. The ratio of major to minor component in **3** was determined as 92 : 8 based on the chromatogram of **3** on an amino acid analyzer.

D-Ith (4): The mixture (**3**) (1.0 g) was chromatographed with Dowex 50X8 (NH₄⁺ form) under the following conditions: column, 1.8×80 cm; buffer, 0.2 M ammonium acetate in 40% MeOH at pH 3.50; flow rate, 14 ml/h. The eluate (340–580 ml) was collected and evaporated to a small volume. The solution was applied on a column (1.0×10 cm) of Dowex 50X8 (H⁺ form). The column was washed with water and eluted with 2 M NH₄OH (30 ml). The eluate was evaporated and the residue was crystallized from H₂O–EtOH; yield, 0.69 g (31% from **1**); mp 215–216°C (dec); $[\alpha]_D^{25} +23.5^\circ$ (c 2, H₂O), $+5.5^\circ$ (c 2, 5 M HCl). NMR (D₂O) δ : 4.01 (1H, d, $J=5.0$ Hz, H-2), 3.54 (1H, m, H-3), 1.31 (3H, d, $J=6.7$ Hz, CH₃).

Found: C, 40.12; H, 7.53; N, 11.67%. Calcd for C₄H₉NO₃: C, 40.33; H, 7.62; N, 11.76%.

Determination of the Configuration of 4. *Configuration of C_α:* The molecular rotation values of **4** were calculated as $+6.6^\circ$ (5 M HCl) and $+28.0^\circ$ (H₂O) based on the observed optical rotation values at D-line. Thus the shift value in the molecular rotation of **4** on acidification is -21.4° . ORD spectrum of **4** was obtained with a JASCO spectropolarimeter model ORD-CD/UV-5 in 0.5 M HCl. The value of the specific rotation at minimum absorption (220 nm) was -900° .

Configuration of C_β: According to the procedure of Futagawa *et al.*¹⁷⁾ **4** (100 mg) was converted into its 2-oxazolidone derivative (**6**) by treatment with phosgene. The obtained oil (**6**) was dissolved in DMSO-*d*₆ and the solution was directly analyzed by NMR. The coupling constant of the vicinal methine protons was 5.0 Hz. Those of the oxazolidones derived from L-Thr and L-aThr were 4.8 Hz and 8.5 Hz in DMSO-*d*₆, respectively.

Synthesis of D-Ise (8). Compound **8** was prepared from L-Ser after bromination, ammonolysis and chromatographic separation in a similar manner to that used for **4**; yield, 58% from L-Ser; mp 197–199°C (dec); $[\alpha]_D^{25} +32.0^\circ$ (c 2, H₂O), $+17.6^\circ$ (c 2, 5 M HCl). NMR (D₂O) δ : 4.24 (1H, dd, $J=7.6, 4.8$ Hz, H-1), 3.40 (1H, dd, $J=13.0, 4.8$ Hz, H-2), 3.08 (1H, dd, $J=13.0, 7.6$ Hz, H-2). R_f s on TLC

and paper chromatography of **8** were identical with those of DL-Ise prepared by the procedure of Gundermann and Holtmann.¹⁹⁾ Reported values for D-Ise;⁴⁾ mp 199–201°C (dec); $[\alpha]_D +32.4^\circ$ (c 10, H₂O).

Found: C, 34.03; H, 6.83; N, 13.21%. Calcd for C₃H₇NO₃: C, 34.28; H, 6.72; N, 13.33%.

We thank Prof. T. Yoshino and Mr. T. Shinmyozu, Kyushu University, for their ¹H-NMR measurements.

References

- 1) Presented at the 30th National Meeting of the Chemical Society of Japan, Osaka, April 1974.
- 2) G. Roncari, Z. Kurylo-Borowska, and L. C. Craig, *Biochemistry*, **5**, 2153 (1963).
- 3) H. Suda, T. Takita, T. Aoyagi, and H. Umezawa, *J. Antibiot.*, **29**, 100 (1976).
- 4) E. Fischer and W. A. Jacobs, *Chem. Ber.*, **40**, 1057 (1907).
- 5) T. Miyazawa, E. Akita, and T. Ito, *Agric. Biol. Chem.*, **40**, 1651 (1976).
- 6) Abbreviations for amino acids follow the rules of the IUPAC-IUB commission on Biochemical Nomenclature. Other abbreviations: Ise, isoserine; Ith, isothreonine, which is used for *threo*-2-hydroxy-3-aminobutanoic acid analogously to that of isoserine.
- 7) P. Melikoff, *Chem. Ber.*, **13**, 956, 1265 (1880).
- 8) H. E. Carter and C. L. Zirkle, *J. Biol. Chem.*, **178**, 709 (1949).
- 9) W. J. N. Burch, *J. Chem. Soc.*, **1930**, 310.
- 10) C. Neuberger and P. Mayer, *Biochem. Z.*, **3**, 116 (1907).
- 11) Y. Liwischitz, A. Singerman, and M. Luwisch, *Israel J. Chem.*, **1**, 441 (1963).
- 12) S. Winstein and R. B. Henderson, "Heterocyclic Compounds," John Wiley & Sons Inc., New York (1950), Vol. 1, Chap. 1.
- 13) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, **59**, 737 (1959).
- 14) P. Brewster, E. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. S. Rao, *Nature*, **166**, 179 (1950).
- 15) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acid," John Wiley & Sons, Inc., New York (1961), Vol. 1, pp. 83, 157.
- 16) F. W. Bachelor and G. A. Miana, *Can. J. Chem.*, **47**, 4089 (1969).
- 17) S. Futagawa, T. Inui, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **46**, 3308 (1973).
- 18) N. Izumiya, *Bull. Chem. Soc. Jpn.*, **26**, 53 (1953).
- 19) K.-D. Gundermann and G. Holtmann, *Chem. Ber.*, **91**, 160 (1958).