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Stereospecific Synthesis of D-Isothreonine from L-Threonine[1)]

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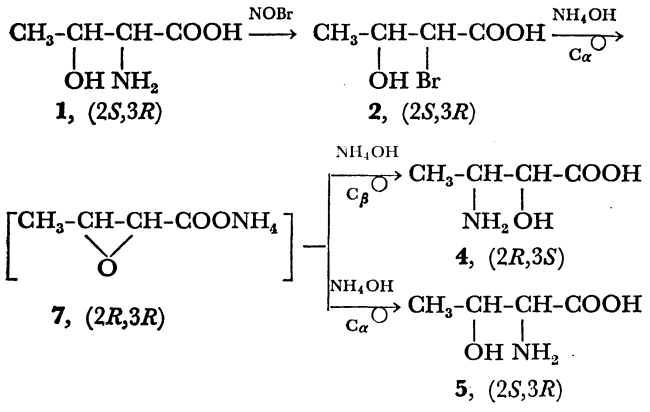
Synopsis. D-Isothreonine, (2*R*,3*S*)-3-amino-2-hydroxybutanoic acid (**4**), was readily prepared by the ammonolysis of optically active 2-bromo-3-hydroxybutanoic acid derived from L-threonine. The configuration of **4** was deduced from the shift of molecular rotation, Cotton effect in ORD curve of **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-isoserine[2)] in an antibiotic, edeine, and (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoic acid[3)] 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 resolution[4)] 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 Zirkle[8)] and Neuberg and Mayer[10)] 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).



Scheme 1. Stereochemical reaction route.

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_α.[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_α atom of isolated isothreonine (**4**) was confirmed to be 2*R* by the negative shift in molecular rotation on acidification[15)] and the negative Cotton effect in the region 200—240 nm.[16)] In order to determine the configuration of C_β atom of **4**, we measured the ^{1}H-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±1.0 Hz for *threo* and 9.6±0.6 Hz for *erythro* isomers in CD_3OD.[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_3OD.[3)] NMR spectrum of **6** was recorded in DMSO-d_6 because of its insolubility in CD_3OD, $J_{\alpha\beta}$ 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_3OD, 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_α and C_β.

Experimental

The following solvent systems were used: R_f^1, pyridine–H_2O (65 : 35, v/v) for TLC and R_f^2, cyclohexylamine–H_2O–methyl ethyl ketone–*n*-BuOH (2 : 5 : 10 : 10, v/v) for paper chromatography. ^{1}H-NMR spectra were measured with a Hitachi R-20B spectrometer (60 MHz), using sodium 3-trimethylsilyl-1-propanesulfonate in D_2O or tetramethylsilane in DMSO-d_6 as an internal standard.

Synthesis of D-*Ith* (**4**). *(2S,3R)-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_2SO_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

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 eluated with 2 M NH_4OH (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_fs 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 (NH_4^+ 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 eluated with 2 M NH_4OH (30 ml). The eluate was evaporated and the residue was crystallized from H_2O–EtOH; yield, 0.69 g (31% from **1**); mp 215—216 °C (dec); $[\alpha]_D^{20}$ +23.5° (*c* 2, H_2O), +5.5° (*c* 2, 5 M HCl). NMR (D_2O) 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, CH_3).

Found: C, 40.12; H, 7.53; N, 11.67%. Calcd for $C_4H_9NO_3$: 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° (5 M HCl) and +28.0° (H_2O) 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); $[\alpha]_D^{20}$ +32.0° (*c* 2, H_2O), +17.6° (*c* 2, 5 M HCl). NMR(D_2O) 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_fs 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° (*c* 10, H_2O).

Found: C, 34.03; H, 6.83; N, 13.21%. Calcd for $C_3H_7NO_3$: C, 34.28; H, 6.72; N, 13.33%.

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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.
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