Synthesis of Four Stereoisomers of γ-Hydroxyarginine *via* the Corresponding Isomers of γ-Hydroxyornithine

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Diastereomeric γ-hydroxy-L- and D-ornithines were prepared by reduction of the corresponding γ-oxoornithines synthesized from L- and D-histidines with sodium borohydride. The isomeric composition of the product obtained from L-histidine was estimated to be 68% *erythro* and 32% *threo* isomers and that of the product from D-histidine 65% *erythro* and 35% *threo* isomers by automatic amino acid analysis after conversion of the basic amino acids into neutral N^{δ}-acetyl derivatives. The diastereoisomers of each γ-hydroxyornithine were separated by chromatography on a Dowex 50 column, four optically active isomers being isolated as their hydrochlorides in crystalline state. Guanidination of these isomers with 1-amidino-3,5-dimethylpyrazole nitrate (ADPN) gave the corresponding optically active isomers of γ-hydroxyarginine which were isolated as crystalline hydrochlorides. *Erythro*-γ-hydroxy-L-arginine hydrochloride shows a specific rotation which agrees very closely with that of natural product of γ-hydroxyarginine.

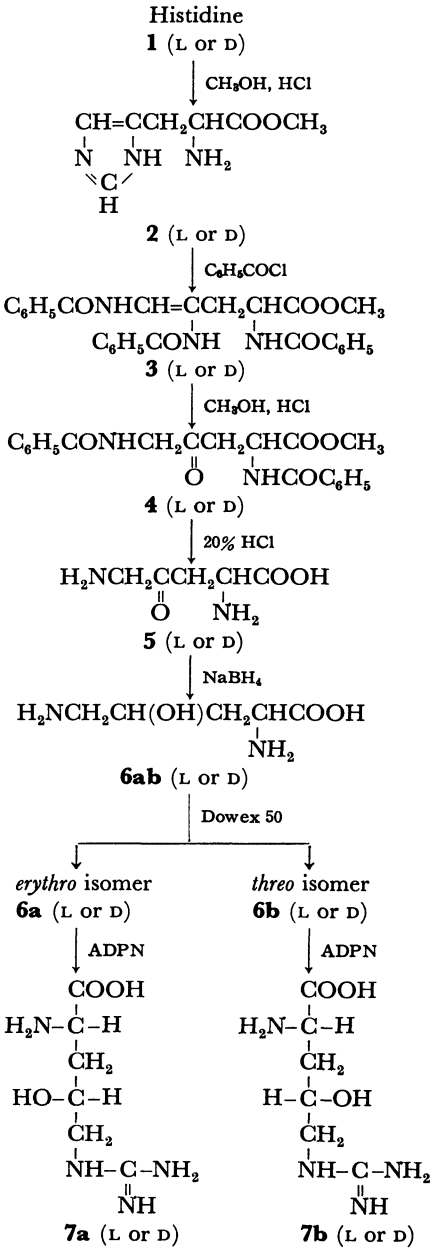
γ-Hydroxyarginine was first isolated from the sea cucumber *Polycheira rufescens* by Fujita[1] and subsequently from the sea anemone *Anthopleura japonica* Verrill by Makisumi.[2] In plants, this amino acid has been detected in the seeds of many species of *Vicia*[3] and isolated as its lactone from the seeds of *Vicia sativa* by Bell and Tirimanna.[4] Although little is known concerning the significance of the presence of this amino acid in animals and plants, the compound is known to participate in several enzymatic reactions.[1,2,5-7] An assumption that this amino acid exists as an intermediate in the metabolic pathway of L-arginine to 2-nitroimidazole in *Streptomyces eurocidicus* has been proposed by Nakane *et al.*[8]

γ-Hydroxyarginine contains two asymmetric carbon atoms (α and γ) and should thus exist theoretically in four stereoisomeric forms. The whole configuration of the natural γ-hydroxyarginine was confirmed to be *erythro*-γ-hydroxy-L-arginine by Fujita.[9] Synthetic attempts leading to an epimeric mixture of γ-hydroxyarginine have been reported.[1,10] However, none of the optically active isomers, other than the natural product, has hitherto been isolated and characterized.

In the present study the four stereoisomers of γ-hydroxyarginine were prepared by guanidination of the corresponding isomers of γ-hydroxyornithine synthesized from optically active histidines (Scheme 1).

Results and Discussion

γ-Oxoornithine (**5**) was synthesized from optically active histidine by the method of Heath *et al.*[12] Histidine (**1**) was first converted into the methyl ester (**2**) dihydrochloride by the conventional method and then transformed into methyl 2,4,5-tribenzamido-4-pentenoate (**3**) by Bamberger cleavage.[13] α,δ-Dibenzoyl-γ-oxoornithine methyl ester (**4**) was formed on heating the tribenzoyl compound (**3**) in methanolic hydrogen chloride. Acid hydrolysis of the ester **4** with 20% hydrochloric acid afforded the keto amino acid (**5**) dihydrochloride as oily residues. Owing to the instability of the acid hydrochloride, the oily product was used for further reduction without crystallization. Treatment of the aqueous solution of **5** dihydrochloride with sodium



Scheme 1. Synthesis of stereoisomers of γ-hydroxyornithine (**6**) and γ-hydroxyarginine (**7**).

borohydride gave diastereoisomeric γ-hydroxyornithine (**6ab**), which was isolated as a crystalline hydrochloride. The diastereoisomeric composition of **6ab** thus obtained was estimated by amino acid analysis of its N^{δ}-acetylated derivative.[11] The amino acid L-**6ab** was composed of 68% *erythro* and 32% *threo*, the D-enantiomorph (D-**6ab**) of 65% *erythro* and 35% *threo* isomers.

Witkop and Beiler[14] pointed out the significance of their failure to esterify or reduce with sodium borohydride γ-oxo-L-ornithine and its derivatives. However, the keto amino acid **5** was hydrogenated smoothly to the hydroxy amino acid **6ab** with the same reducing agent in the present study. With regard to the racemization of α-carbon atom of **5**, no racemization took place during the course of synthesis of mercaptohistidine *via* L-**5** from L-histidine.[12,15,16] The optical purity of L-**5** obtained in this study was ascertained by the conversion of γ-hydroxy-L-ornithine (L-**6ab**) prepared from the compound L-**5** into γ-hydroxy-L-arginine and the digestion of the resulting arginine derivative with the L-directed arginase. The approach described abov seems to be useful for the synthesis of γ-hydroxyornithine which retains the configuration of α-carbon atom, starting from readily available histidine through simple and relatively high-yield operations.

Diastereoisomeric γ-hydroxyornithines (L-**6ab** and D-**6ab**) were separated into the two diastereoisomers by chromatography using a column (3.8×275 cm) of Dowex 50 (Na^+ form).[11] The separated diastereoisomers of γ-hydroxyornithine were isolated as their crystalline hydrochlorides, their stereochemical purities being ascertained by assay using an amino acid analyzer.[11] The specific rotations of natural γ-hydroxyornithine obtained enzymatically from natural γ-hydroxyarginine and the four stereoisomers obtained are given in Table 1. The value for *erythro* L-isomer attained by synthesis is close to that of natural γ-hydroxyornithine. This suggests that surprisingly little racemization or epimerization occurred during the series of reactions and, consequently, the synthetic isomers of γ-hydroxy-L-ornithine were almost optically pure.

The four stereoisomers of γ-hydroxyornithine were separately guanidinated with 1-amidino-3,5-dimethylpyrazole nitrate (ADPN) and the products, the corresponding γ-hydroxyarginines, were isolated as their hydrochlorides.[11] The data of specific rotation for natural and synthetic preparation of γ-hydroxyarginine measured in alkaline medium are given in Table 1. The value for *erythro* L-isomer agrees very closely with that for natural γ-hydroxyarginine. The specific rotations of D-antipodes are expected to be equal but opposite to those of L-antipodes. The value for *erythro* D-isomer is also in good agreement with the value expected. It is evident that the synthetic *erythro* L- and D-isomers are optically pure. On the other hand, *threo* isomers seem to be optically pure.

Table 1. Specific rotations of γ-hydroxyornithine and γ-hydroxyarginine hydrochlorides

Isomer		γ-Hdyroxyornithine hydrochloride $[\alpha]_D^{20}$ (*c* 2, H_2O)	γ-Hydroxyarginine hydrochloride $[\alpha]_D^{20}$ (*c* 1, 0.5 M NaOH)
Natural			
erythro	L	+10.5°[a]	− 8.5°
Synthetic			
erythro	L	+ 9.4°[b]	− 8.4°
	D	− 9.8°	+ 8.5°
threo	L	− 6.3°[c]	+11.0°
	D	+ 6.4°	−11.0°

a) Ref. 9, $[\alpha]_D^{20}$ +10.6° (*c* 5, H_2O). b) Ref. 11, $[\alpha]_D^{20}$ +10.5° (*c* 2, H_2O). c) Ref. 11, $[\alpha]_D^{20}$ −7.0° (*c* 2, H_2O).

Two different values measured in 5 M (1 M=1 mol dm^{-3}) hydrochloric acid were reported with regard to the specific rotation of natural preparations of γ-hydroxyarginine. We also measured the specific rotation of natural γ-hydroxyarginine in 5 M hydrochloric acid, but obtained no such values as reported.[1,2] We found that the solution had at first $[\alpha]_D$ +17.6° which dropped to +7.5° in 10 min, to −5.6° in 3 h, and then remained constant. Partial transformation of γ-hydroxyarginine into a compound more basic than the original amino acid was ascertained by paper electrophoresis. The mutarotation may be ascribed to the formation of the lactone[4] of γ-hydroxyarginine. The above-cited authors[1,2] overlooked this mutarotative nature of γ-hydroxyarginine.

Experimental

All melting points are uncorrected. Amino acid analyses were carried out using a JEOL-6AS amino acid analyzer. The optical rotations were measured with a Union high sensitivity polarimeter PM-71. Paper chromatography was carried out by the ascending technique on Toyo Roshi No. 52 paper with 1-butanol–acetic acid–pyridine–water(4 : 1 : 1 : 2,v/v). Paper electrophoresis was carried out at 300 V for 2.5 h on Toyo Roshi No. 52 paper in a buffer solution of pyridine–acetic acid–water (20 : 10 : 970, v/v). Spots were made visible with ninhydrin and Sakaguchi reagents.

Methyl 2,4,5-Tribenzamido-4-pentenoate (3). Compound **3** was prepared from the ester **2**·2HCl by Bamberger cleavage[13] according to the procedure of Heath *et al.*[12] The product was recrystallized from Methyl Cellosolve. The yield of L-**3** from L-**2**·2HCl (24.1 g, 0.1 mol) was 34.3 g (73%); mp 215—216 °C.

Compound D-**3** was obtained from D-**2**·2HCl (14.5 g, 60 mmol); yield, 21.8 g (77%); mp 214—215 °C.

Found (L-**3**): C, 68.59; H, 5.33; N, 9.00%. Found (D-**3**): C, 68.54; H, 5.28; N, 8.70%. Calcd for $C_{27}H_{25}O_5N_3$: C, 68.73; H, 5.34; N, 8.91%.

α,δ-Dibenzoyl-γ-oxoornithine Methyl Ester (4). Compound **4** was obtained from the parent tribenzoyl compound **3** by boiling for 30 min with 10% methanolic hydrogen chloride.[12] Recrystallization of the crude product was performed from hot ethanol. The yield of L-**4** from L-**3** (22 g, 47 mmol) was 7.1 g (42%); mp 168—170 °C.

The yield of D-**4** from D-**3** (21.2 g, 45 mmol) was 6.5 g (40%); mp 168—170 °C.

Found (L-**4**): C, 65.01; H, 5.45; N, 7.55%. Found (D-**4**): C, 65.11; H, 5.43; N, 7.50%. Calcd for $C_{20}H_{20}O_5N_2$: C, 65.20; H, 5.47; N, 7.61%.

γ-Oxoornithine (5) Dihydrochloride. Compound **5**·2HCl was prepared from the dibenzoyl ester **4** (5.5 g, 15 mmol) by

refluxing with 20% hydrochloric acid (54 ml) for 7 h. The oily product could not be crystallized by treatment with ethanol. Paper electrophoretic analysis showed that it was almost pure. Owing to the instability of the acid dihydrochloride the oily product was used directly.

*Diastereoisomeric γ-Hydroxyornithine (**6ab**) Hydrochloride.* γ-Oxoornithine (**5**) dihydrochloride obtained as above was dissolved in water (30 ml) and cooled in an ice–water bath. To the solution was added 2 equivalents of sodium borohydride (0.57 g, 15 mmol) in small portions with continuous stirring over a period of 30 min. The cold mixture was stirred for 2 h and neutralized with 2 M hydrochloric acid. The progress of the reaction was checked by paper electrophoresis. The solution containing γ-hydroxyornithine was applied to a column (3.6×9 cm) of Dowex 50 (H^+ form). After the column had been washed with water and 2.5 M pyridine (300 ml), the amino acid was eluted with 2 M aqueous ammonia (350 ml) and the pooled eluate was evaporated to dryness *in vacuo*. The residue, taken up in water, was adjusted to pH 5 with 1 M hydrochloric acid, decolorized with charcoal and concentrated to a syrup *in vacuo*. The syrup was dissolved in a small amount of 50% ethanol, γ-hydroxyornithine being crystallized as its hydrochloride with the addition of absolute ethanol. Yield of L-**6ab**·HCl, 2.5 g (90%); mp 167—168 °C (dec). Yield of D-**6ab**·HCl, 2.4 g (88%); mp 169 °C (dec).

Found (L-**6ab**·HCl): C, 32.21; H, 7.01; N, 14.83%. Found (D-**6ab**·HCl): C, 31.92; H, 7.16; 14.93%. Calcd for $C_5H_{12}O_3N_2$·HCl: C, 32.53; H, 7.10; N, 15.17%.

*Separation of the Diastereoisomers of γ-Hydroxyornithine (**6ab**).* A diastereoisomeric compound **6ab**·HCl (600 mg) was separated into its two diastereoisomers by chromatography using a column (3.8×275 cm) of Dowex 50 (Na^+ form).[11] Two ninhydrin positive peaks were obtained. The component of the forepeak was confirmed to be *threo* isomer and that of the afterpeak *erythro* isomer by the assay using an amino acid analyzer.

erythro-*γ-Hydroxy*-L-*ornithine* (L-*6a*) *Hydrochloride.* The diastereoisomers of γ-hydroxy-L-ornithine were separated chromatographically as described above. The combined eluate containing *erythro* L-isomer was treated as described for L-**6ab**. The *erythro* isomer was obtained as its hydrochloride. It was recrystallized from water–ethanol; yield, 272 mg (67%); mp 176—177 °C (dec); $[\alpha]_D^{20}$ +9.4° (*c* 2, H_2O). [lit,[9] $[\alpha]_D^{20}$ +10.6° (*c* 5, H_2O), lit,[11] $[\alpha]_D^{20}$ +10.5° (*c* 2, H_2O)].

threo-*γ-Hydroxy*-L-*ornithine* (L-*6b*) *Hydrochloride.* From the pooled forepeak fractions of the eluate, the *threo* isomer was obtained as its hydrochloride. It was recrystallized from water–ethanol; yield, 146 mg (76%); mp 193—194 °C (dec); $[\alpha]_D^{20}$ −6.3° (*c* 2, H_2O). [lit,[11] $[\alpha]_D^{20}$ −7.0° (*c* 2, H_2O)].

erythro-*γ-Hydroxy*-D-*ornithine* (D-*6a*) *Hydrochloride.* The diastereoisomers of γ-hydroxy-D-ornithine were separated as described above. From the combined afterpeak fractions of the eluate, the *erythro* isomer was obtained as its hydrochloride. It was recrystallized from water–ethanol; yield, 260 mg (67%); mp 174—175 °C (dec); $[\alpha]_D^{20}$ −9.8° (*c* 2, H_2O).

threo-*γ-Hydroxy*-D-*ornithine* (D-*6b*) *Hydrochloride.* From the pooled forepeak fractions of the eluate, the *threo* isomer was obtained as its hydrochloride. It was recrystallized from water–ethanol; yield, 132 mg (63%); mp 189—190 °C (dec); $[\alpha]_D^{20}$ +6.4° (*c* 2, H_2O).

Found (L-**6a**·HCl): C, 32.05; H, 7.12; N, 14.81%. Found (L-**6b**·HCl): C, 32.44; H, 7.12; N, 14.91%. Found (D-**6a**·HCl): C, 31.97; H, 7.11; N, 14.87%. Found (D-**6b**·HCl): C, 32.32; H, 7.10; N, 14.71%. Calcd for $C_5H_{12}O_3N_2$·HCl: C, 32.53; H, 7.10; N, 15.17%.

Guanidination of Four Stereoisomers of γ-Hydroxyornithine. Each isomer of γ-hydroxyornithine (184.5 mg) was converted into the corresponding γ-hydroxyarginine by treatment with 1-amidino-3,5-dimethylpyrazole nitrate (ADPN), the unchanged material and the product being separated by chromatography using a column (1.6×16 cm) of Dowex 50 (pyridinium form).[11]

erythro-*γ-Hydroxy*-L-*arginine* (L-*7a*) *Hydrochloride.* Compound L-**6a**·HCl was guanidinated with ADPN and the reaction mixture subjected to column chromatography, as described above. From the pooled afterpeak fractions of the eluate, *erythro* L-isomer (L-**7a**) was crystallized as its hydrochloride. It was recrystallized from water–ethanol; yield, 95 mg (42%); mp 188—189 °C (dec); $[\alpha]_D^{20}$ −8.4° (*c* 1, 0.5 M NaOH).

erythro-*γ-Hydroxy*-D-*arginine* (D-*7a*) *Hydrochloride.* Compound D-**7a**·HCl was obtained from D-**6a**·HCl in the same manner as described above. It was recrystallized from water–ethanol; yield, 115 mg (51%); mp 188—189 °C (dec); $[\alpha]_D^{20}$ +8.5° (*c* 1, 0.5 M NaOH).

threo-*γ-Hydroxy*-L-*arginine* (L-*7b*) *Hydrochloride.* Compound L-**6b**·HCl was treated in the same manner as above. *threo* L-Isomer L-**7b** was obtained as a hygroscopic crystalline hydrochloride; yield, 90 mg (40%); $[\alpha]_D^{20}$ +11.0° (*c* 1, 0.5 M NaOH).

threo-*γ-Hydroxy*-D-*arginine* (D-*7b*) *Hydrochloride.* Compound D-**7b**·HCl was obtained from D-**6b**·HCl in the same manner as described above. It was obtained as a hygroscopic crystalline hydrochloride; yield, 105 mg (46%); $[\alpha]_D^{20}$ −11.0° (*c* 1, 0.5 M NaOH).

Found (L-**7a**·HCl): C, 31.76; H, 6.75; N, 24.65%. Found (D-**7a**·HCl): C, 31.86; H. 6.69; N, 24.77%. Found (L-**7b**·HCl): C, 31.70; H, 6.72; N, 24.20%. Found (D-**7b**·HCl): C, 31.39; H, 6.85; N, 24.40%. Calcd for $C_6H_{14}O_3N_4$·HCl: C, 31.79; H, 6.67; N, 24.72%.

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