  Bull. Chem. Soc. Jpn., 53, 2605—2609 (1980)

Synthesis of *erythro*- and *threo*-γ-Hydroxy-L-ornithines

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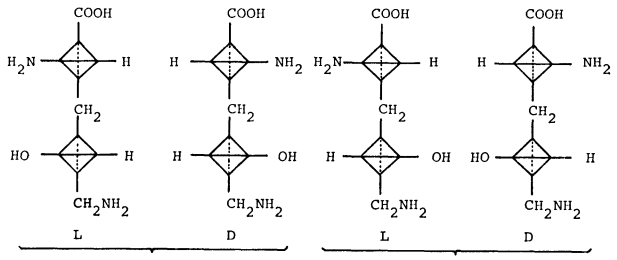
(Received January 25, 1980)

γ-Hydroxy-DL-ornithine was obtained from an alkaline hydrolysate of 2,5-diamino-4-pentanolide synthesized according to the method of Talbot *et al.*[1] The amino acid preparation was found to be a racemic mixture composed of 34% *erythro* and 66% *threo* isomers by a new chromatographic assay. This assay is based on the conversion of the basic amino acid to a neutral amino acid by acetylation of the δ-amino group and the analysis of the resulting N^{δ}-acetyl derivative with an automatic amino acid analyzer by the ordinary method. Two diastereoisomers of γ-hydroxy-DL-ornithine were separated by column chromatography on Dowex 50 and isolated as their crystalline hydrochlorides. Guanidination of the separated racemic diastereoisomers gave the corresponding diastereoisomeric γ-hydroxyarginines, hydrolysis of which with arginase yielded *erythro*- and *threo*-γ-hydroxy-L-ornithines.

γ-Hydroxyornithine is a diamino monocarboxylic acid which, by virtue of its two asymmetric centers, may exist as four optically active stereoisomers or two racemic diastereoisomers (Scheme 1). Since this amino acid was first synthesized in 1916 by Hammarsten,[2] several synthetic attempts, leading to racemic mixtures in varying proportions of the two possible diastereoisomers of 2,5-diamino-4-pentanolide (**2**), have been studied by several researchers.[3-5] Although the synthesis of the two diastereoisomers of this amino acid from L-histidine and the establishment of the stereochemistry of γ-carbon, to which the secondary hydroxyl group is attached, have been achieved in 1956 by Witkop and Beiler,[5] none of the four optically active isomers has been characterized. Several years later an optically active γ-hydroxyornithine was isolated from an enzymatic hydrolysate of the natural γ-hydroxyarginine and the overall configuration of the amino acid was determined to be *erythro*-γ-hydroxy-L-ornithine by Fujita.[7,8] The occurrence of this amino acid in a plant was also reported.[9] However, none of the optically active isomers, other than the *erythro* L-isomer, has hitherto been characterized, nor has any description for the separation of the two diastereomeric racemates appeared. This paper is primarily concerned with the preparative separation of these diastereomeric racemates, and the resolution of each racemic pair to yield *erythro* and *threo* isomers of γ-hydroxy-L-ornithine.

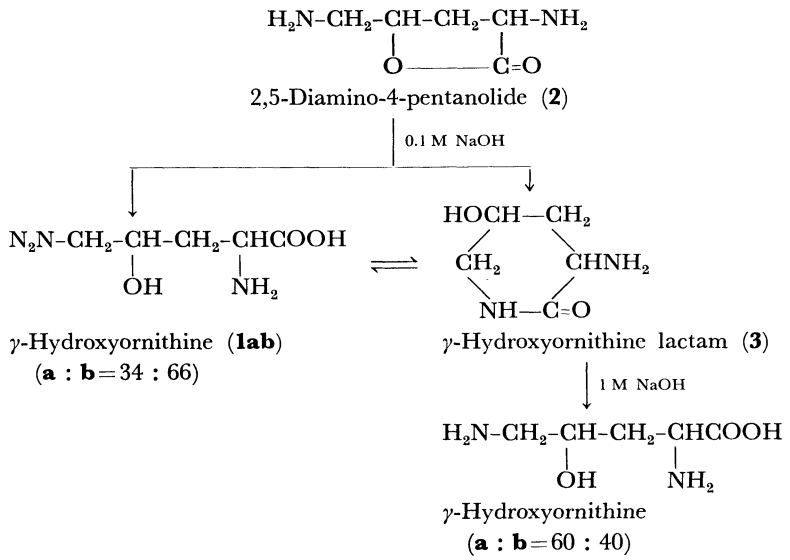


erythro-γ-hydroxyornithine (**1a**) *threo*-γ-hydroxyornithine (**1b**)

Scheme 1. Fischer projections of four stereoisomers of γ-hydroxyornithine.

Results and Discussion

*Preparation of γ-Hydroxy-DL-ornithine (**1ab**).* Compound **2** was prepared according to the directions of Talbot *et al.*[1] The preparation started from diethyl allylmalonate and involved treatment with sulfuryl



Scheme 2. Alkaline hydrolysis of 2,5-diamino-4-pentanolide.

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chloride, followed by hydrolysis and distillation to yield 2,5-dichloro-4-pentanolide. Condensation of this with potassium phthalimide gave 2,5-diphthalimido-4-pentanolide. Talbot *et al.* reported that the latter compound, on hydrolysis by a mixture of glacial acetic acid and concentrated hydrochloric acid, yielded γ-hydroxy-DL-ornithine, which was isolated as a crystalline dihydrochloride monohydrate of the corresponding **2** in a good yield (95%), mp 239 °C. But acid hydrolysis of the diphthalimido derivative in the present work gave a crude diamino lactone hydrochloride as a gummy residue. This crystallized on repeated trituration with ethanol but the crystalline product had an indistinct melting point. Additional recrystallization was impossible, and the lactone was unstable. The crude lactone hydrochloride was subjected to alkaline hydrolysis (Scheme 2). Paper chromatographic analysis of the hydrolysate in 1-butanol–acetic acid–pyridine–water system revealed many spots upon spraying with ninhydrin. Therefore, the hydrolysate neutralized with acid was freed from contaminated acidic and neutral amino acids by passage down a column of Dowex 50 (NH_4^+ form). The basic amino acid were eluted with aqueous ammonia. Further purification was performed by ion exchange chromatography on a column of Dowex 50 equilibrated with pyridinium acetate buffer of pH 4.8. The component that appeared as the first major peak was confirmed to be **1ab** by the chromatographic and electrophoretic behavior on paper. From the fractions comprising the peak, a mixture of the two racemic diastereoisomers of γ-hydroxyornithine was isolated as its hydrochloride (**1ab**·HCl) (see Experimental section). The isomeric composition of the sample of **1ab**·HCl was estimated to be 66% *threo* and 34% *erythro* forms by the assay with an amino acid analyzer, as described below. The next major peak was found to be principally composed of a derivative of γ-hydroxyornithine. From the fractions comprising the peak, a product that gave a characteristic brown-gray color with ninhydrin on paper was isolated as the crystalline hydrochloride. The compound was identified as γ-hydroxyornithine lactam (3-amino-5-hydroxy-2-piperidone) (**3**) by elementary analysis. The characteristic amide bond (lactam) of **3** was well defined by the band at 1670 cm^{-1} in the IR spectrum. Another γ-hydroxyornithine preparation was also recovered from **3** hydrochloride by treatment with alkali. This preparation was composed of 40% *threo* and 60% *erythro* isomers. If γ-hydroxyornithine hydrochloride was treated with warm aqueous ammonia or the free base with refluxing water, a fraction of the amino acid was converted into the lactam. In the same manner, ornithine lactam (3-amino-2-piperidone) was formed from ornithine hydrochloride. Fischer and Zemplen[10] have reported that piperidone was formed from 5-aminovaleric ester and 3-amino-2-piperidone from ornithine ester as a result of spontaneous cyclization. Taking into account the isomeric compositions of the two γ-hydroxyornithine preparations, we can assert that *erythro* isomer is liable to cyclize more easily than *threo* isomer.

*Preparative Separation of Two Diastereoisomers of γ-Hydroxyornithine (**1ab**).* The successful separation of the diastereoisomers of hydroxylysine on a column (3.4×115 cm) of Dowex 50 (Na^+ form) was reported by Hamilton and Anderson.[11] For the separation of the two racemic diastereoisomers of **1ab**, we also tried this technique, but only partial separation was obtained. Upon passage through a long column (3.8×275 cm) of Dowex 50, a good separation of the diastereoisomers into two peaks resulted. A typical elution pattern is shown in Fig. 1. The component of the first major peak was confirmed to be **1b** and that of the second peak to be **1a** by the assay with an amino acid analyzer. From the combined peak fractions, **1a** and **1b** were isolated as their crystalline hydrochlorides, respectively. Their stereoisomeric purity was ascertained by the assay with an amino acid analyzer.

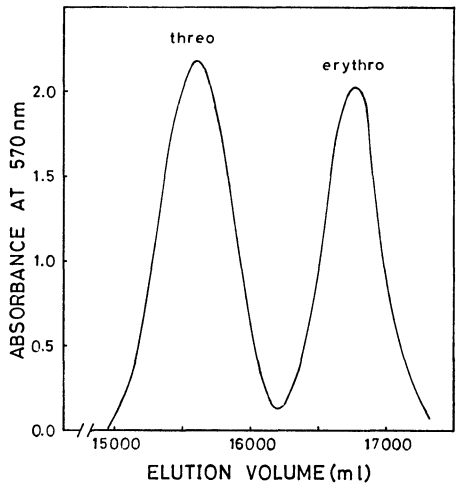

Fig. 1. Elution pattern of γ-hydroxyornithine diastereoisomers on a column (3.8×275 cm) of Dowex 50.

Assay with an Automatic Amino Acid Analyzer. The analytical separation of the diastereoisomers of γ-hydroxyornithine on a Dowex column (0.9×100 cm) by the method of Hirs *et al.*[12] as modified by Piez[13] was reported by Witkop and Beiler.[6] However, the procedure involves many time-consuming operations. A new attempt was made to assay the diastereoisomeric composition of γ-hydroxyornithine with an automatic amino acid analyzer after transformation of this basic amino acid into a neutral amino acid by acetylation of the δ-amino group. As a standard we used "natural" *erythro*-γ-hydroxy-L-ornithine, obtainable by enzymatic hydrolysis of natural γ-hydroxy-L-arginine with arginase. The elution profiles of some N^δ-acetyl derivatives of γ-hydroxyornithine are shown in Fig. 2. The separation of the two isomers was not complete, but was sufficient to allow us to estimate their compositions. The elution times were 40 min for the *threo* and 45 min for the *erythro* isomers. This is a suitable method for routine analysis of the stereochemical purity and of the composition of the diastereoisomers of γ-hydroxyornithine preparations.

Epimerization of erythro-*γ-Hydroxy-L-ornithine by Treatment with Acid and Alkali.* Hamilton and An-

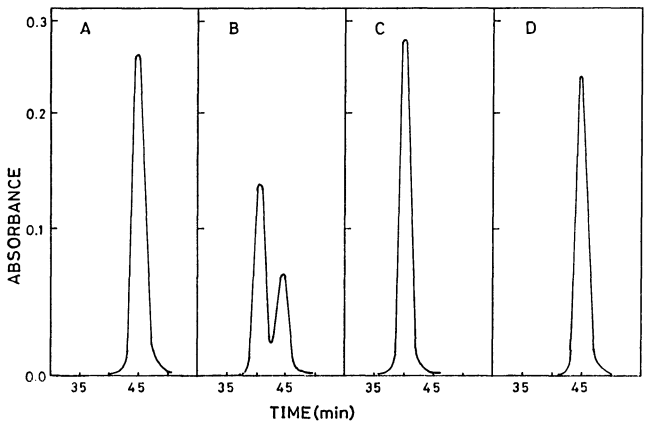

Fig. 2. Elution pattern of N^{δ}-acetyl-γ-hydroxyornithine on a long column (0.8×50 cm) of an amino acid analyzer. A: N^{δ}-Acetyl derivative of γ-hydroxyornithine derived from natural γ-hydroxyarginine, B: N^{δ}-Acetyl derivative of diastereoisomeric γ-hydroxy-DL-ornithine (**lab**), C and D: N^{δ}-Acetyl derivatives of *threo* L- and *erythro* L-isomers obtained in this study.

Table 1. Epimerization of *erythro*-γ-hydroxyornithine on refluxing in acid and alkali

Conditions time (h)	6 M HCl			0.5 M $Ba(OH)_2$	
	0	24	72	0	2
erythro Isomer, %	100	90	78	100	80
threo Isomer, %	0	10	22	0	20

derson[11] have reported that refluxing solutions of δ-hydroxy-L-lysine with 6 M (1 M=1 mol dm^{-3}) hydrochloric acid for 72 h led to epimerization at the α-carbon; a nearly equal mixture of δ-hydroxy-L-lysine and allo-δ-hydroxy-D-lysine resulted. A "natural" γ-hydroxyornithine preparation was refluxed with 6 M hydrochloric acid or 0.5 M barium hydroxide and the extent of epimerization was determined by the assay with an amino acid analyzer. The results are presented in Table 1. Epimerization of *erythro*-γ-hydroxy-L-ornithine was shown to take place more slowly in boiling 6 M hydrochloric acid than that of δ-hydroxy-L-lysine. On the other hand, after refluxing of natural γ-hydroxyarginine in 0.5 M barium hydroxide for 2 h, the content of the diastereoisomers of the resulting γ-hydroxyornithine was found to be 20% *threo* and 80% *erythro* isomers.

Preparation of γ-Hydroxy-DL-arginine. Compounds **1a** and **1b** obtained above were guanidinated with 1-amidino-3,5-dimethylpyrazole nitrate to give γ-hydroxyarginines in the usual manner.[14] After column chromatography of the reaction mixture, γ-hydroxyarginine and unchanged γ-hydroxyornithine were isolated as their hydrochlorides. Assays with an amino acid analyzer of each isomer of the unchanged γ-hydroxyornithine showed that no racemization occurred under the conditions of guanidination.

Hydrolysis of γ-Hydroxy-DL-arginine with Arginase. The optical resolution of racemic γ-hydroxyarginine into γ-hydroxy-L-ornithine and γ-hydroxy-D-arginine was performed by the L-directed hydrolytic action of beef liver arginase. Thus *threo*-γ-hydroxy-L-ornithine and *threo*-γ-hydroxy-D-arginine were obtained from the hydrolysate of *threo*-γ-hydroxy-DL-arginine, and *erythro*-γ-hydroxy-L-ornithine and *erythro*-γ-hydroxy-D-arginine from *erythro*-γ-hydroxy-DL-arginine, respectively. Two optically active isomers of γ-hydroxy-L-ornithine were isolated in crystalline state as their hydrochlorides. The *erythro* isomer obtained here was found to be optically identical with "natural" *erythro*-γ-hydroxy-L-ornithine obtained from natural γ-hydroxy-L-arginine. Rivard and Carter[15] have reported that L- and D-ornithines were obtained from L- and D-arginines, respectively, by hydrolysis with barium hydroxide and careful crystallization with ethanol. Refluxing natural γ-hydroxy-L-arginine with 0.5 M barium hydroxide for 2 h led to complete conversion to γ-hydroxyornithine, but partial epimerization in the product occurred as described above. Therefore, no attempt was made to prepare the isomers of γ-hydroxy-D-ornithine.

Experimental

All melting points are uncorrected. The 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 done 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). The spots were made visible with ninhydrin and Sakaguchi reagents.

Preparation of 2,5-Diphthalimido-4-pentanolide. This compound was prepared from 2,5-dichloro-4-pentanolide (52 g, 30.8 mmol) and potassium phthalimide (122 g, 67.8 mmol) according to the method reported by Talbot *et al.*[1] Yield of crude product, 108 g (27.7 mmol). Recrystallization from a large volume of glacial acetic acid gave crystals, mp 261—263 °C (lit,[1] 263 °C).

Found: C, 64.14; H, 3.71; N, 7.07%. Calcd for $C_{21}H_{14}O_6N_2$: C, 64.62; H, 3.61; N, 7.18%.

*Preparation of γ-Hydroxyornithine (**1ab**) and Its Lactam (**3**).* The crude 2,5-diphthalimido-4-pentanolide (10 g, 25.6 mmol) was hydrolyzed by a mixture of glacial acetic acid (50 ml) and concentrated hydrochloric acid (100 ml) according to the literature.[1] The filtrate was evaporated to dryness *in vacuo* after the phthalic acid had been removed from the cold solution. The residue was dissolved in water and the solution was decolorized with Norit, concentrated to a small volume, and crystallized by repeated trituration with ethanol. The crystalline product was hygroscopic and had an indistinct melting point. The product (2.1 g) was dissolved in 0.1 M sodium hydroxide (30 ml); the solution was refluxed for 3 h and subsequently neutralized with dilute hydrochloric acid. The solution was washed onto a column (2×15 cm) of Dowex 50 (H^+ form); the column was washed with water and then eluted with 2 M aqueous ammonia (200 ml). The eluate was evaporated to dryness *in vacuo*. The residue taken up in a small volume of water was applied to a column (2×15 cm) of Dowex 50 (NH_4^+ form). The column was washed with water and then eluted with 2 M aqueous ammonia (200 ml), and the eluate was evaporated to dryness. The residue dissolved in a small volume of 0.3 M pyridinium acetate buffer, pH 4.8, was placed on a Dowex 50 column (3×54 cm) equilibrated with the same buffer. The column

was developed with the same buffer at a flow rate of about 10 ml/h and the effluent was collected in 5 ml fractions. The position and shape of the resulting peaks were established by ninhydrin spot test on paper of 10 μl samples taken from alternate fractions. Two major peaks appeared on the chromatogram. The component of the first major peak was proved to be **lab** and that of the second to be **3**, by means of paper chromatography and electrophoresis.

*γ-Hydroxy-*DL*-ornithine Hydrochloride (**1ab**·HCl)*: The first major peak fractions were pooled and evaporated to dryness *in vacuo*. The residue dissolved in water was passed through a column (2×10 cm) of Dowex 50 (H^+ form) and the column was washed with water. The amino acid was eluted with 2 M aqueous ammonia (100 ml) and the evaporated eluate was dissolved in water (5 ml). The solution was adjusted to pH 5.0 with 1 M hydrochloric acid, decolorized with charcoal, and concentrated to a syrup. The syrup dissolved in a small volume of 50% ethanol was subjected to crystallization in the cold with the addition of absolute ethanol; yield, 806 mg (4.4 mmol); mp 180—181 °C; *erythro*:*threo*=34:66.

Found: C, 32.77; H, 7.29; N, 15.04%. Calcd for $C_5H_{12}O_3N_2$·HCl: C, 32.53; H, 7.10; N, 15.17%.

*γ-Hydroxy-*DL*-ornithine Lactam Hydrochloride (**3**·HCl)*: The next major peak fractions were pooled and evaporated to dryness. The residue was dissolved in 2 M hydrochloric acid and the solution treated with charcoal, filtered, and evaporated to a syrup. Ethanol was added to the syrup and triturated in the cold to yield crystals. Recrystallization from water–ethanol gave 268 mg (1.6 mmol) of pure **3**·HCl; mp 217—218 °C (dec). This compound gave the characteristic brown-gray color on paper with ninhydrin reagent. IR (KBr): 3200, 1670, and 1580 cm^{-1} (lactam).

Found: C, 35.96; H, 6.66; N, 16.80%. Calcd for $C_5H_{10}O_2N_2$·HCl: C, 36.01; H, 6.60; N, 16.81%.

The combined mother liquors of the crystallization of **3**·HCl were evaporated to dryness. The residue dissolved in 1 M sodium hydroxide (30 ml) was refluxed for 3 h. After neutralization with 1 M hydrochloric acid, the solution was passed through a column (2×11 cm) of Dowex 50 (H^+ form) and the column was washed with water. The amino acid was eluted with 2 M aqueous ammonia (150 ml) and the eluate was evaporated to dryness. From the residue, racemic γ-hydroxyornithine was crystallized as its hydrochloride as described for **lab**·HCl. Yield, 486 mg (2.6 mmol); mp 168—169 °C; *erythro*:*threo*=60:40.

*Separation of Two Diastereoisomers of γ-Hydroxy-*DL*-ornithine (**1ab**).* A preparation of **lab**·HCl (600 mg) was dissolved in a small volume of water and the solution was applied to a column (3.8×275 cm) of Dowex 50 equilibrated with 0.2 M acetate buffer, pH 3.4. The column was developed at room temperature with 0.1 M phosphate buffer, pH 7.8, at a flow rate of 20 ml/h. The first 15 liters of effluent were discarded and the next 2.5 liters were collected in 20 ml fractions. The position and shape of the resulting peaks were established by the colorimetric ninhydrin analysis[16] of 0.2 ml aliquots taken from each fraction. Figure 1 shows a typical chromatogram. The component and the degree of overlap were confirmed by the assay with an amino acid analyzer of 1—2 ml aliquots of the peak fractions. Fractions comprising the fore and after peaks were found to be composed of **1b** and **1a**, respectively, and fractions that overlapped were discarded.

threo-*γ-Hydroxy-*DL*-ornithine Hydrochloride (**1b**·HCl)*: The fractions of 15100—15940 ml were collected and adjusted to pH 5.0 with 2 M hydrochloric acid. The solution was passed through a column (3.8×20 cm) of Dowex 50 (H^+ form) and the column was washed with 50% ethanol. The amino acid was eluted with 2 M aqueous ammonia (900 ml) and the eluate was treated as described for **lab**·HCl; yield of crystals, 300 mg (76%), mp 193—194 °C (dec). The stereochemical purity of the compound was ascertained by the assay with an amino acid analyzer, as shown in Fig. 2.

Found: C, 32.05; H, 7.15; N, 15.15%. Calcd for $C_5H_{12}O_3N_2$·HCl: C, 32.53; H, 7.10; N, 15.17%.

erythro-*γ-Hydroxy-*DL*-ornithine Hydrochloride (**1a**·HCl)*: The fractions of 16460—17120 ml were treated as described for **lb**·HCl; yield of crystals, 153 mg (75%), mp 175—175.5 °C (dec).

Found: C, 32.44; H, 7.04; N, 15.15%. Calcd for $C_5H_{12}O_3N_2$·HCl: C, 32.53; H, 7.10; N, 15.17%.

Preparation of N^δ*-Acetyl-γ-hydroxy-*L*-ornithine.* *erythro*-γ-Hydroxy-L-ornithine hydrochloride (92.3 mg, 0.5 mmol) obtained from natural γ-hydroxy-L-arginine hydrochloride was dissolved in a small volume of water; the solution was brought to 90 °C and copper(II) carbonate was added. Excess copper carbonate was filtered off and the filtrate chilled. With stirring in an ice–water bath, 2 M lithium hydroxide (0.5 ml, 1 mmol) and acetic anhydride (0.1 ml, 1 mmol) were added alternately in portions. After standing for 30 min at room temperature, the solution was acidified with glacial acetic acid and poured onto a column (1×3 cm) of Dowex 50 (H^+ form). After washing with water, the N^δ-acetyl derivative was eluted with 2.5 M pyridine (20 ml) and the eluate was evaporated to dryness *in vacuo*. The residue was triturated in ethanol, filtered off, and washed with ethanol and ether. The product was recrystallized from water–ethanol; yield 49.2 mg (52%); mp 211 °C (dec).

Found: C, 44.02; H, 7.38; N, 14.39%. Calcd for $C_7H_{14}O_4N_2$: C, 44.21; H, 7.37; N, 14.73%.

Amino Acid Analysis of N^δ*-Acetyl-γ-Hydroxyornithines.* A small amount of γ-hydroxyornithine preparation (1—2 mg) was transformed into its N^δ-acetyl derivative, as described above, on a small scale. Without crystallization, the product was dissolved in 0.2 M sodium citrate buffer (5 ml) of pH 3.25 (the first buffer). A sample of the solution was applied to a long column (0.8×50 cm) and the column was eluted with the first buffer in the usual way.

Epimerization of erythro-*γ-Hydroxy-*L*-ornithine with Strong Acid.* *erythro*-γ-Hydroxy-L-ornithine hydrochloride (9.3 mg) was refluxed in 6 M hydrochloric acid (10 ml). Two milliliter aliquots of the reaction mixture were taken out and evaporated to dryness. The product was converted to N^δ-acetyl derivative as described above and subjected to amino acid analysis. The results of the samples refluxed for 24 and 72 h are presented in Table 1.

Degradation of erythro-*γ-Hydroxy-*L*-arginine with Weak Alkali.* Natural γ-hydroxyarginine hydrochloride (12 mg) was refluxed in 0.5 M barium hydroxide (2 ml) for 2 h. The reaction mixture was no longer positive to a Sakaguchi reaction. Then it was neutralized with 0.25 M sulfuric acid and centrifuged. The concentrated supernatant was treated with copper(II) carbonate. This was followed by acetylation with acetic anhydride and alkali, as described above. N^δ-Acetyl derivative of the possible reaction product (γ-hydroxyornithine) was subjected to amino acid analysis. The result is presented in Table 1.

Preparation of γ-Hydroxyarginine from γ-Hydroxyornithine. Guanidination of γ-hydroxyornithine with 1-amidino-3,5-dimethylpyrazol nitrate and chromatographic separation of the product and unchanged amino acid on a Dowex 50 column were carried out essentially according to the literature.[14]

erythro-*γ-Hydroxy*-DL-*arginine Hydrochloride*: Compound **1a**·HCl (203 mg, 1.1 mmol) was guanidinated with 1-amidino-3,5-dimethylpyrazol nitrate (443 mg, 2.2 mmol) and the reaction mixture subjected to chromatography on a Dowex 50 column (2×16 cm). From the afterpeak fractions, the product was crystallized as its hydrochloride. The yield of hygroscopic crystals was 160 mg (64%). The homogeneity of this compound was confirmed by ninhydrin and Sakaguchi reagents by paper electrophoresis and chromatography. A small amount of **1a**·HCl was recovered from the forepeak fractions.

threo-*γ-Hydroxy*-DL-*arginine Hydrochloride*: Guanidination of **1b**·HCl (277 mg, 1.5 mmol) with the guanidinating reagent (604 mg, 3 mmol), followed by column chromatography, gave hygroscopic crystals; yield 140 mg (41%).

Optical Resolution of Racemic γ-Hydroxyarginine by Arginase. The partially purified arginase preparation was obtained as reported by Schmike.[17] Racemic γ-hydroxyarginine hydrochloride (120—140 mg) and the arginase (50 mg) were dissolved in 0.025 M Tris-Cl buffer of pH 9.6 (5 ml) containing 0.01 M manganese chloride, and incubated for 24 h at 37 °C. The progress of the hydrolysis of the susceptible L-antipode was followed by amino acid analysis as usual. To reach complete hydrolysis, arginase (25 mg) were added to the reaction mixture and it was incubated for a further 24 h. From the mixture deproteinized with 0.5 M perchloric acid, the products were recovered by chromatographic separation on a Dowex 50 column (2×16 cm) essentially according to the literature.[14]

erythro-*γ-Hydroxy*-L-*ornithine Hydrochloride.* *erythro-γ*-Hydroxy-DL-arginine hydrochloride (140 mg) was treated with arginase and the deproteinized solution was subjected to chromatographic separation. From the forepeak fractions, *erythro-γ*-hydroxy-L-ornithine was crystallized as its hydrochloride; yield 32 mg (56%); mp 175—176 °C (dec); $[\alpha]_D^{20}$ +10.5° (c 2, H_2O), lit,[8] $[\alpha]_D^{20}$ +10.6° (*c* 5, H_2O).

Found: C, 32.39; H, 7.06; N, 14.81%. Calcd for $C_5H_{12}O_3N_2 \cdot HCl$: C, 32.53; H, 7.10; N, 15.17%.

From the afterpeak fractions, *erythro-γ*-hydroxy-D-arginine was crystallized as its hydrochloride; yield of hygroscopic crystals, 42 mg (60%).

threo-*γ-Hydroxy*-L-*ornithine Hydrochloride*: *threo-γ*-Hydroxy-DL-arginine hydrochloride (120 mg) was treated as described for *erythro* racemate. The yield of *threo-γ*-hydroxy-L-ornithine hydrochloride was 48 mg (98%); mp 200—201 °C (dec); $[\alpha]_D^{20}$ −7.0° (*c* 2, H_2O).

Found: C, 32.53; H, 7.09; N, 14.99%. Calcd for $C_5H_{12}O_3N_2 \cdot HCl$: C, 32.53; H, 7.10; N, 15.17%.

The yield of hygroscopic crystals of *threo-γ*-hydroxy-D-arginine hydrochloride was 51 mg (85%).

References

1) G. Talbot, R. Gandry, and L. Berlinguet, *Can. J. Chem.*, **34**, 911 (1956).
2) E. Hammarsten, *Compt. Rend. Trav. Lab. Carlsberg*, **11**, 223 (1916).
3) W. Traube, R. Tohow, and W. Tepohl, *Ber.*, **66**, 1861 (1923).
4) W. Langenbeck and R. Hutschenreuter, *Ber.*, **162**, 305 (1929).
5) A. N. Dey, *J. Chem. Soc.*, **1937**, 1166.
6) B. Witkop and T. Beiler, *J. Am. Chem. Soc.*, **78**, 2282 (1956).
7) Y. Fujita, *Bull. Chem. Soc. Jpn.*, **32**, 439 (1959).
8) Y. Fujita, *Bull. Chem. Soc. Jpn.*, **33**, 1379 (1960).
9) E. Bell and A. S. L. Tirmanna, *Nature (London)*, **197**, 901 (1963).
10) E. Fischer and G. Zemplen, *Ber.*, **42**, 1022, 4878 (1909).
11) P. B. Hamilton and R. A. Anderson, *J. Biol. Chem.*, **213**, 249 (1955).
12) C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Biol. Chem.*, **195**, 669 (1952).
13) K. A. Piez, *J. Biol. Chem.*, **207**, 77 (1954).
14) S. Ohyama, K. Mizusaki, H. Tsunematsu, and S. Makisumi, *J. Biochem. (Tokyo)*, **86**, 11 (1979).
15) D. E. Rivard and M. E. Carter, *J. Am. Chem. Soc.*, **77**, 1260 (1955).
16) E. N. Yemm and E. C. Cooking, *Analyst (London)*, **80**, 209 (1955).
17) R. T. Schimke, *J. Biol. Chem.*, **239**, 3808 (1964).