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Resolution of Amino Acids. VIII. The Preparation of the Four Optical Isomers of β-Hydroxyaspartic Acid

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The ammonolysis of *cis*-epoxy succinic acid yielded *threo*-hydroxy-DL-aspartic acid exclusively, but that of *trans*-epoxy acid produced a mixture of 68% *erythro*- and 32% *threo*-hydroxyaspartic acid. This mixture was separated into the two pure diastereomers through either a Dowex 1 column chromatography or fractional crystallization after the mixture had been changed to ammonium salt. The *threo*- and *erythro*-DL-amino acids so prepared were resolved by optically active lysine and ornithine respectively. The contribution (partial molar rotation) of the α- and β-asymmetric centers to the observed molar rotations of the optically active diastereomeric hydroxyaspartic acids in water and 5 N hydrochloric acid was calculated.

β-Hydroxyaspartic acid has been found to occur in a variety of biological materials. The presence of the amino acid has been reported in an incubation mixture of dihydroxyfumaric acid.

and glutamic acid with an enzyme,[1] and in human cerebrospinal fluid.[2] Its derivative, β-hydroxyasparagine, has been isolated from human urine.[3] β-Hydroxyaspartic acid has been known as a constituent amino acid in a peptide obtained from mushroom poison[4] or a culture solution of *Azotobacter*.[5] The earlier work of Skraup suggested that the amino acid had been found in the hydrolyzate of casein,[6] but Dakin later denied the presence of this amino acid.[7] Recently, a trace amount of the amino acid was isolated from an enzymatic digest of casein by Sallach and Kornguth,[8] but its presence was also denied by the same workers later.[9]

In view of the interest in the biological role and the natural occurrence of β-hydroxyaspartic acid, it appeared that the four optical isomers of this amino acid are needed in quantity for physicochemical determination or as starting materials for syntheses of optically active β-hydroxyasparagines. The present paper is concerned with the improved synthesis and separation of the two racemic diastereomers, and with the preparation of the four optical isomers by the resolution procedure.

Kornguth and Sallach have reported the use of a Dowex 1 column (0.8×30 cm), using 0.05 N formic acid as a developing solvent, for the quantitative determination of the racemic diastereomers of hydroxyaspartic acid.[10] We developed an improved procedure for the rapid and quantitative separation of the diastereomers using a shorter column (0.9×4 cm) of Dowex 1 and 0.5 N acetic acid as a developing solvent. Furthermore, both the diastereomers were found to be separated easily by chromatography using strong basic ion-exchange paper in a 0.5 N acetic acid solvent.

It has been known that the racemic *threo*-hydroxyaspartic acid is less soluble in water than the *erythro*-form.[7] Through the determination of the solubilities in several solvents of the free amino acids and its derivatives (Table 1), we discovered an interesting and useful fact: the ammonium salt of the *threo*-DL-amino acid is more soluble than the corresponding *erythro* isomer, whereas the free *threo*-DL-amino acid is less soluble than the *erythro* compound.

Table 1. Solubility of hydroxyaspartic acids and its salts[a]

Compound[b]	H_2O	0.5 N acetic acid	60% methanol
DL-*t*Hya	0.17	0.15	
DL-*e*Hya	2.56	2.54	
NH_4 salt of DL-*t*Hya			1.64
NH_4 salt of DL-*e*Hya			0.35
Cu salt of DL-*t*Hya	0.07		
Cu salt of DL-*e*Hya	0.17		
L-*t*Hya	1.43		
L-*e*Hya	1.08		

a) The solubilities are represented as g per 100 g of the solution at 25°C.
b) *t*Hya and *e*Hya are abbreviations of *threo*- and *erythro*-hydroxyaspartic acid, respectively.

Several methods are available for the synthesis of hydroxyaspartic acid.[11] Among them, a method *via* the ammonolysis of an epoxy-succinic acid seemed excellent for the synthesis of the amino acid in quantity. The method was first developed by Dakin,[7] and has been modified recently by Kaneko and Katsura.[12] We improved this synthetic procedure further by the following two steps. We used a mixture of barium epoxysuccinate and ammonium sulfate in aqueous ammonia instead of the mixture of free epoxysuccinic acid and aqueous ammonia which was used by Kaneko *et al.*;[12] free epoxy acid had been obtained from its barium salt with a certain loss. Furthermore, we observed that the ammonolysis of an epoxy acid was completed after 3 or 4 days at 40°C; Dakin[7] or Kaneko *et al.*[12] treated an

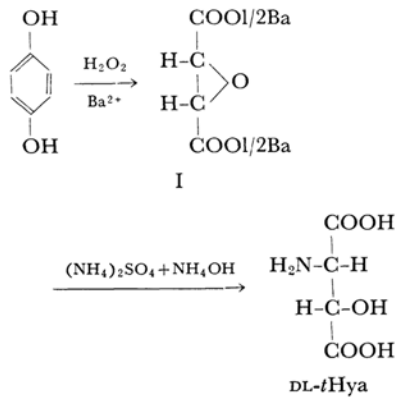

Fig. 1. Synthesis of *threo*-hydroxy-DL-aspartic acid.

1) H. J. Sallach and T. H. Peterson, *J. Biol. Chem.*, **223**, 629 (1956).
2) T. L. Perry and R. T. Jones, *J. Clin. Invest.*, **40**, 1363 (1961).
3) F. Tominaga, C. Hiwaki, T. Maekawa and H. Yoshida, *J. Biochem.*, **53**, 227 (1963).
4) T. Wieland *et al.*. *Helv. Chim. Acta*, **44**, 919 (1961); *Ann.*, **657**, 218 (1962).
5) N. F. Sarris and A. I. Virtanen, *Acta Chem. Scand.*, **11**, 1440 (1957); W.A. Bullen and J.R. LeComte, *Biochem. Biophys. Res. Commun.*, **9**, 523 (1962).
6) Z. H. Skraup, *Ber.*, **37**, 1596 (1904).
7) H. D. Dakin, *J. Biol. Chem.*, **48**, 273 (1921).
8) H. J. Sallach and M. L. Kornguth, *Biochem. Biophys. Acta*, **34**, 582 (1959).
9) M. L. Kornguth and H. J. Sallach, *Arch. Biochem. Biophys.*, **104**, 79 (1964).
10) M. L. Kornguth and H. J. Sallach, *ibid.*, **91**, 39 (1960).
11) Kornguth *et al.*[10] and Kaneko *et al.*,[12] review the synthetic methods briefly and list references.
12) T. Kaneko and H. Katsura, This Bulletin, **36**, 899 (1963).

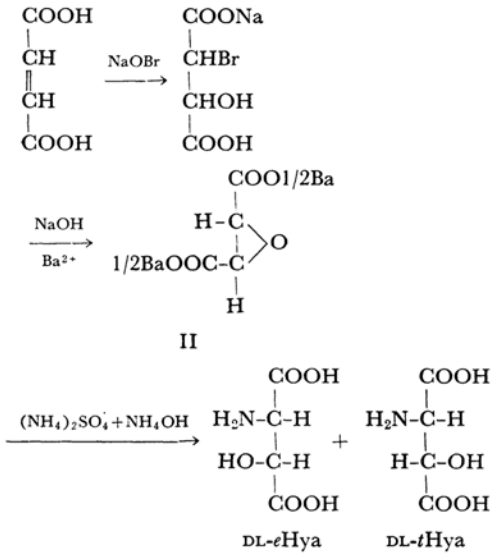

Fig. 2. Synthesis of a mixture of *erythro*- and *threo*-hydroxy-DL-aspartic acid.

epoxy acid with ammonia under pressure at 100—130°C for 10—18 hr.

The ammonolysis of a *cis*-epoxy acid for 4 days at 40°C yielded exclusively *threo*-hydroxy-DL-aspartic acid obtained in a good yield (Fig. 1). On the contrary, an analysis of the sample after the ammonolysis of a *trans*-epoxy acid showed it to be composed of 68% *erythro* and 32% *threo* form (Fig. 2).[13] It is of interest to note that, in his original synthesis, Dakin reported the formation of a compound approximately 80% *erythro* and 20% *threo*,[7] whereas Kaneko *et al.* report the exclusive formation of the *erythro* amino acid.[12] We successfully separated the mixture into the two racemic diastereomers either using a Dowex 1 column chromatography or by the fractional crystallization of the ammonium salt of the mixture.

Dakin described the resolution of *threo*-DL-amino acid with strychnine or quinine, but could not resolve *erythro*-DL-amino acid.[7] Recently, Kaneko *et al.* prepared the four optical isomers *via* optically active epoxy-succinic acid and D-glucosamine in order to determine the absolute configurations of the isomers by chemical correlation.[12] Pure L- and D-*erythro*-hydroxyaspartic acids are also isolated by two groups as natural substances.[1,4] The specific rotations reported by the above authors are summarized in Table 2. We observed previously that DL-ornithine was satisfactorily resolved by

Table 2. Summary of specific rotations of the four optical isomers of hydroxyaspartic acid

Investigators	Specific rotation, $[\alpha]_D$			
	*t*Hya		*e*Hya	
	L	D	L	D
Dakin[7]	−11.9° (H_2O)	+12.1° (H_2O)		
Sallach *et al.*[1]			+51.2° (N HCl)	
Wieland *et al.*[4]				−54.2° (N HCl)
Kaneko *et al.*[12]	−8.5° (H_2O)	+8.9° (H_2O)	+41.4° (H_2O)	
	+1.3° (N HCl)[a]	−1.2° (N HCl)	+53.0° (N HCl)	−49.2° (N HCl)
Present authors[b]	−8.5° (H_2O)	+8.6° (H_2O)	+47.0° (H_2O)	−46.8° (H_2O)
	+6.4° (5N HCl)	−6.5° (5N HCl)	+52.0° (5N HCl)	−51.8° (5N HCl)
	+2.8° (N HCl)[a]			

a) *c* 3.12 in N HCl.
b) Temperature, 20°C; *c* 1.0 in H_2O or 5N HCl.

Table 3. Contribution of the asymmetric α- and β-carbon atoms to the molar rotation of the diastereomeric hydroxyaspartic acids

Compound	$[M]_D^{20}$			$[\alpha C]_D^{20}$			$[\beta C]_D^{20}$		
	in H_2O	in 5N HCl	[M](HCl)-[M](H_2O)	in H_2O	in 5N HCl	[αC](HCl)-[αC](H_2O)	in H_2O	in 5N HCl	[βC](HCl)-[βC](H_2O)
L-*t*Hya	−12.7°	+9.5°	+22.2°	+28.7°	+43.6°	+14.9°	−41.3°	−33.9°	+7.4°
D-*t*Hya	+12.8	−9.7	−22.5	−28.5	−43.5	−15.0	+41.5	+34.0	−7.5
L-*e*Hya	+70.1	+77.6	+7.5	+28.7	+43.6	+14.9	+41.5	+34.0	−7.5
D-*e*Hya	−69.8	−77.3	−7.5	−28.5	−43.5	−15.0	−41.3	−33.9	+7.4

13) We are not certain at present if the *trans*-epoxy succinic acid barium salt used in our experiment was admixed with a small amount of the *cis*-epoxy acid barium salt or if the pure *trans*-epoxy acid yielded the epimeric hydroxyaspartic acids during the ammonolysis through partial racemization. We observed that the pure *erythro*-hydroxy-DL-aspartic acid (DL-*e*Hya) did not epimerize after the incubation of a mixture composed of DL-*e*Hya, barium sulfate, and aqueous ammonia at 40°C for 4 days.

the use of D- and L-glutamic acid.[14] Therefore, we tried optically active ornithine, lysine, and arginine for the resolution of the racemic hydroxyaspartic acid; we found that the *threo* racemate was most satisfactorily resolved with lysine, and the *erythro* racemate, with ornithine. The specific rotations of the four optical isomers so obtained are presented in Table 2. These values as determined in both water and 5 N hydrochloric acid are equal and are opposite for each diastereomeric pair within the limits of experimental error.

The determination of the molar rotations [M] of the four optical isomers made it possible to culculate the partial molar rotation of the asymmetric α- and β-carbon atoms. Lutz and Jirgensons indicated that the exhibition of a more positive optical rotation value in acid than in water appears to be a general characteristic of certain L-amino acid.[15] Greenstein *et al.* suggested further that the rotation of the diastereomeric amino acids with two asymmetric centers might be considered to be a function of the sum of the partial rotations of each center.[16] The α-asymmetric center of an amino acid of the L-configuration will make the same contribution to the total molar rotation as its diastereomeric form, but the contribution of the β-center will be of equal magnitude and opposite for the two L-diastereomers. Therefore, the partial molar rotation [αC] of the asymmetric α-carbon atom and [βC] are derived by the following equations:

$$_{\text{L-}t\text{Hya}}[\alpha\text{C}] = {}_{\text{L-}e\text{Hya}}[\alpha\text{C}] = 0.5({}_{\text{L-}t\text{Hva}}[\text{M}] + {}_{\text{L-}e\text{Hya}}[\text{M}])$$

$$_{\text{L-}t\text{Hya}}[\beta\text{C}] = -{}_{\text{L-}e\text{Hya}}[\beta\text{C}] = 0.5({}_{\text{L-}t\text{Hya}}[\text{M}] + {}_{\text{D-}e\text{Hya}}[\text{M}])$$

The sum of [αC](HCl)-[αC](H_2O) and [βC](HCl)-[βC](H_2O) would represent the observed shift in rotation from water to acid. The molar rotation data for the four optical isomers calculated are presented in Table 3.

Experimental

All the melting points are uncorrected. The optical rotations were measured on a Yanagimoto Photometric Polarimeter OR-20.

Separation Studies of Diastereomers. *On Paper Chromatography.* A slight difference in R_f values of the two diastereomers was observed; the R_f values of DL-*t*Hya and DL-*e*Hya were 0.15 and 0.17 with a *n*-butanol: acetic acid : pyridine : water (4:1:1:2, v/v) system, and 0.55 and 0.57 with a *t*-butanol : formic acid : water (75 : 15 : 10, v/v) system. Gray *et al.* also observed a similar slight difference in R_f with a *t*-amyl alcohol - acetic acid - water system.[17] Kornguth *et al.*[10] and Kaneko *et al.*[12] reported that the diastereomeric mixture could not be identified in various solvent systems.

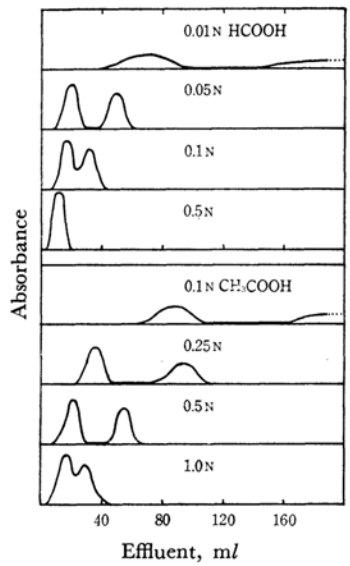

Fig. 3. The effect of concentration of developing solvent on elution pattern of a mixture of DL-*e*Hya and DL-*t*Hya. Faster peak, *e*Hya; slower peak, *t*Hya.

On Dowex 1. A column (0.9×4 cm) was filled with Dowex 1×8 (200—400 mesh), either acetate or formate form. Samples of DL-*t*Hya (7.5 mg) and DL-*e*Hya (7.5 mg) were then dissolved in 1 ml of water (total amino acid, 0.1 mmol), and the solution was applied to a column and eluted with the appropriate solvent at room temperature and at a flow rate of 14—16 ml/hr. One-milliliter fractions were collected, and 12 μl from each tube were spotted on a strip of filter paper. The strip was dried, immersed in a trough with a 0.2% ninhydrin-acetone solution for a moment, and then heated in an oven (90°C) for 3 min. The amounts of color developed were determined by an Atago AG-4 densitometer (slit 1×18 mm, 610 mμ), and the integrated areas were plotted on a graph.[18] The solvents used were 0.01—1.0 N formic acid and 0.05—4.0 N acetic acid; some of the elution patterns are shown in Fig. 3. In all the experiments we ascertained that the faster-eluting peak contains the *erythro* isomer, and the slower peak, the *threo* isomer. Among the various concentrations tested, 0.05 N formic acid and 0.5 N acetic acid separated the diastereomers most effectively. Therefore, a 0.9×4 cm Dowex 1 column with 0.5 N acetic acid was used for the quantitative analysis of a diastereomeric mixture. As may be seen in Fig. 3, the integrated area of *t*Hya is slightly larger than that of *e*Hya; its ratio was calculated to be

14) M. Kondo and N. Izumiya, Abstr. of the 17th Annual Meeting of The Chemical Society of Japan, April, 1964, p. 278.
15) O. Lutz and B. Jirgensons, *Ber.*, **65**, 784 (1932).
16) M. Winitz, S. M. Birnbaum and J. P. Greenstein, *J. Am. Chem. Soc.*, **77**, 716 (1955); L. Benoiton, M. Winitz, S. M. Birnbaum and J. P. Greenstein, *ibid.*, **79**, 6192 (1957).
17) D. O. Gray, J. Blake, D. H. Brown and L. Fowden, *J. Chromatog.*, **13**, 276 (1964).
18) There is a more detailed description in a previous communication; see H. Aoyagi, M. Ohno, N. Izumiya and B. Witkop, *J. Org. Chem.*, **29**, 1382 (1964).

100 : 97.[19]

On Ion-Exchange Paper Chromatography. For the rapid qualitative analysis of the diastereomers, a paper of Amberlite SB-II, acetate form, was successfully employed, using a 0.5 N acetic acid solvent; the R_f values of *t*Hya and *e*Hya were 0.11 and 0.25 respectively.

Solubilities of Hydroxyaspartic Acids and Its Salts. Each of the compounds was added to water (*ca.* 5 ml), and the mixture was stirred at 25°C for several hours until it reached the saturation point. The crystals which remained undissolved were then filtered off, and a part of the filtrate was evaporated to a constant weight at 80°C and 2 mmHg. The results obtained are presented in Table 1.

***threo*-Hydroxy-DL-aspartic Acid (DL-*t*Hya).** A mixture of hydroquinone (55 g) and 30% hydrogen peroxide (450 ml) was heated at 80°C until the crystals dissolved completely. To the solution 2 N potassium hydroxide (*ca.* 700 ml) was then added until pH 7 was reached. After the solution had been evapolated to 400 ml and M barium chloride (550 ml) had been added, the resultant precipitate was collected by filtration and washed with a small amount of water and acetone. Yield of barium *cis*-epoxy succinate dihydrate (I),[20] 108 g (72%).

I (87 g) was added to a solution of concentrated aqueous ammonia (870 ml) and ammonium sulfate (39 g), and the mixture was allowed to stand for 4 days at 40°C. The barium sulfate precipitated was filtered off, and the filtrate was evaporated *in vacuo* to afford the crude residue. The analysis by the Dowex 1 column revealed that this residue contains only *t*Hya, no *e*Hya. The crude residue was dissolved in water, and the solution was added to a column (3.5×50 cm) with Dowex 1 (OH^- form).[21] The column was washed with water until it was free from ammonium ions, and then eluted with 2 N acetic acid (1500 ml). The eluate was evaporated *in vacuo*, and the residue was collected by filtration with the aid of aqueous ethanol (19.8 g). The product was recrystallized by precipitation effected by the additions of 2 N hydrochloric acid (80 ml) and ethanol (40 ml) to a solution of the product in 2 N triethylamine (80 ml). Yield, 17.9 g (42%).

Found: C, 32.43; H, 4.70; N, 9.24%. Calcd for $C_4H_7O_5N$: C, 32.22; H, 4.73; N, 9.40%.

Salts of DL-*t*Hya. *Ammonium Salt.* DL-*t*Hya (0.746 g) was dissolved in aqueous ammonia, and the solution was evaporated *in vacuo*. The resulting crystals were recrystallized from water-ethanol; yield, 0.681 g (82%); mp 198—199°C (decomp.). The air-dried product did not contain the water of crystallization.

Found: C, 28.82; H, 6.21; N, 16.67%. Calcd for $C_4H_{10}O_5N_2$: C, 28.92; H, 6.07; N, 16.86%.

Cupric Salt. To a solution of the ammonium salt of DL-*t*Hya (0.166 g) in water (2 ml), there was added a solution of cupric acetate monohydrate (0.24 g) in water (8 ml). The crystals which resulted were collected by filtration, and washed with water and ethanol; yield, 0.23 g (96%); mp 233—235°C (decomp.).

Found: C, 20.15; H, 3.56; N, 5.96%. Calcd for $C_8H_{10}O_{10}N_2Cu\cdot 3H_2O$: C, 20.21; H, 3.40; N, 6.13%. The air-dried product lost 10.8% of its weight by being dried over phosphorus pentoxide at 80°C and 2 mmHg; calcd for $3H_2O$, 11.4%.

***erythro*-Hydroxy-DL-aspartic Acid (DL-*e*Hya).** *By Column Chromatography.* Maleic acid (116 g) was added to a solution of 0.5 N sodium hydroxide (4000 ml) and bromine (160 g), and the mixture was allowed to stand overnight. To the solution, 10 N sodium hydroxide (200 ml) was added, and then M barium chloride (1100 ml). The precipitate which resulted was collected; yield of barium *trans*-epoxy succinate dihydrate (II),[22] 202 g (67%).

II (98 g) was treated with a solution of aqueous ammonia (1000 ml) and ammonium sulfate (43.7 g), as has been described for the preparation of DL-*t*Hya. The filtrate from barium sulfate was shown by column chromatographic analysis to be a mixture of *e*Hya and *t*Hya in the ratio of 68 : 32. The residue obtained after evaporation was treated with the Dowex 1 column (3.5×50 cm), using 2 N acetic acid as the developing solvent. The eluate was evaporated to give the crude crystals (21 g), which were then subjected to fractional crystallization with hot water. DL-*t*Hya (4.2 g) was thus obtained as a less soluble material. The mother liquor and washings were evapolated to a small volume (*ca.* 300 ml). The solution was applied to a column (2.7×70 cm) of Dowex 1 (acetate form), the column was eluted with 0.5 N acetic acid, and 30 ml fractions were collected. The *e*Hya was found in tubes 40—87, and the *t*Hya in tubes 120—180. The tubes 120—180 were evaporated, and the residue was recrystallized from water; yield of DL-*t*Hya, 1.9 g. Total yield of DL-*t*Hya, 6.1 g (13%). The material obtained from the tubes 40—87 was recrystallized from water-ethanol; yield of *e*Hya, 11.9 g (25%). Found: C, 32.15; H, 4.83; N, 9.32%.

By Fractional Crystallization of Ammonium Salts. II (98 g) was subjected to ammonolysis as has been described above. The crude product obtained was crystallized from hot water, and the yield of DL-*t*Hya was 4.0 g. The mother liquor was treated with aqueous ammonia, and the solution was evaporated to dryness. The recrystallization of the residue from water-methanol gave ammonium DL-*e*Hya hemihydrate (III); yield, 14.9 g (28%); mp 202—204°C (decomp.). (Found: C, 27.68; H, 6.29; N, 15.71%.) The solution of III (14.8 g) in water was treated with a column of Dowex 1 (OH^- form), and the column was eluted with 2 N acetic acid. The crystals obtained by the evaporation of the eluate were recrystallized from water-ethanol; yield of *t*Hya, 11.5 g (24%). (Found: C, 32.21; H, 4.64; N, 9.29%.)

Salts of DL-*e*Hya. *Ammonium Salt.* This was prepared in the same manner as that described for the preparation of DL-*t*Hya ammonium salt. Yield of the

19) By the determination using the method described by H. Rosen (*Arch. Biochem. Biophys.*, **67**, 10 (1957)), the percentage of color yeild of DL-*t*Hya and DL-*e*Hya, based on L-leucine as 100%, were observed to be 94 and 91% respectively.

20) The synthetic procedure used for the barium salt is essentially the same as that reported by E. Weitz, H. Schobbert and H. Seibert, *Ber.*, **68**, 1163 (1935).

21) The preparative chromatographic procedure of the substance which contains DL-*t*Hya was carried out at room temperature or at 40—45°C preferably; DL-*t*Hya crystallized out in some cases in the column or from the concentrated solution at lower temperatures.

22) The synthetic procedure is essentially the same as that reported by R. Kuhn and F. Ebel, *Ber.*, **58**, 919 (1925).

air-dried product, 95%; mp 203—205°C (decomp.).

Found: C, 27.58; H, 6.29; N, 15.82%. Calcd for $C_4H_{10}O_5N_2 \cdot \frac{1}{2}H_2O$: C, 27.43; H, 6.33; N, 15.99%. The product lost 5.5% of its weight by being dried at 80°C and 2 mmHg; calcd for $\frac{1}{2}H_2O$: 5.1%.

Cupric Salt. This was prepared in the same manner as that described for DL-*t*Hya cupric salt; yield of the air-dried product, 96%; mp 224—226°C (decomp.).

Found: C, 17.88; H, 4.01; N, 5.33%. Calcd for $C_8H_{10}O_{10}N_2Cu \cdot 6H_2O$: C, 18.15; H, 4.12; N, 5.29%. Four moles of the water of crystallization were lost when the air-dried product was dried at 80°C and 2 mmHg: loss of weight, 14.3%; calcd for $4H_2O$, 14.6%.

L-Lysine *threo*-Hydroxy-L-aspartate (L-Lys·L-*t*Hya). A solution of L-lysine monohydrochloride (4.57 g, 25 mmol) in water was placed in a column (2.4×20 cm) of Dowex 50 (H^+ form), and the column was washed with water and eluted with 2 N ammonia. The eluate was then evaporated *in vacuo* to dryness. The residue was added in a mixture of DL-*t*Hya (3.73 g. 25 mmol) and water (30 m*l*), and to the solution methanol (25 m*l*) was added. It was allowed to stand overnight at room temperature. The crystals which precipitated were collected by filtration, washed with aqueous methanol, and recrystallized once from water-methanol (the mother liquor and washings were set aside for the isolation of D-*t*Hya); yield of the air-dried product, 2.97 g (76%); mp 182—183°C (decomp.); $[\alpha]_D^{20}$ −5.0° (*c* 1, H_2O), +17.2° (*c* 1, 5 N HCl).

Found: C, 38.54; H, 7.21; N, 13.46%. Calcd for $C_{10}H_{21}O_7N_3 \cdot H_2O$: C, 38.33; H, 17.40; N, 13.41%.

***threo*-Hydroxy-L-aspartic Acid.** L-Lys·L-*t*Hya·H_2O (2.82 g, 9 mmol) dissolved in water was added to a column (2.4×12 cm) of Dowex 1 (acetate form). The column was washed with 0.5 N acetic acid (50 m*l*) to remove the lysine, and then with 2 N acetic acid (200 m*l*) to elute hydroxyaspartic acid. The residue obtained by the evaporation of the eluate was recrystallized from water-ethanol; yield, 1.19 g (88%). The specific rotations are presented in Table 2. (Found: C, 31.95; H, 4.83; N, 9.32%.)

D-Lysine *threo*-Hydroxy-D-aspartate. The mother liquor and washings from L-Lys·L-*t*Hya were evaporated to a small volume, and the solution was treated with a column of Dowex 1 (acetate form), using successively 0.5 and 2 N acetic acid, as has been described for the isolation of L-*t*Hya from L-Lys·L-*t*Hya. The fractions containing hydroxyaspartic acid were evaporated to dryness, and the residue dissolved in water was neutralized with D-lysine which had been prepared from D-lysine monohydrochloride.[23] The solution was then evaporated, and the residue which remained was recrystallized from water-methanol; yield of D-Lys·D-*t*Hya·H_2O, 2.58 g (66%); mp 182—184°C (decomp.); $[\alpha]_D^{20}$ +17.0° (*c* 1, 5 N HCl). (Found: C, 38.61; H, 7.36; N, 13.62%.)

***threo*-Hydroxy-D-aspartic Acid.** D-Lys·D-*t*Hya·H_2O was treated in the same manner as has been described for the preparation of L-*t*Hya; yield, 86%. (Found: C, 31.98; H, 4.83; N, 9.25%.)

L-Ornithine *erythro*-Hydroxy-L-aspartate. DL-*e*Hya (3.73 g, 25 mmol) was dissolved in a solution of L-ornithine (25 mmol) in water (30 m*l*). After methanol (20 m*l*) had been added to the solution, it was allowed to stand overnight at room temperature. The crystals thus obtained were recrystallized from water-methanol; yield of the air-dried product (L-Orn·L-*e*Hya·H_2O), 3.14 g (84%); mp 235—236°C (decomp.); $[\alpha]_D^{18}$ +26.6° (*c* 1, H_2O), +40.2° (*c* 1, 5 N HCl). (Found: C, 35.82; H, 7.07; N, 13.88%.)

***erythro*-L-Hydroxy-L-aspartic Acid.** This was obtained from L-Orn·L-*e*Hya·H_2O (2.99 g) by the method described for the separation of L-*t*Hya; yield, 1.43 g (96%). (Found: C, 32.05; H, 4.70; N, 9.31%.)

D-Ornithine *erythro*-Hydroxy-D-aspartate. The mother liquor and washings from L-Orn·L-*e*Hya were treated with a Dowex 1 coulmn, and the portion of hydroxyaspartic acid was neutralized with D-ornithine[14] in the manner described for the preparation of L-Orn·L-*e*Hya·H_2O; yield of D-Orn·D-*e*Hya·H_2O, 2.92 g (78%); mp 233—234° (decomp.); $[\alpha]_D^{18}$ −26.2° (*c* 1, H_2O), −40.2° (*c* 1, 5 N HCl). (Found: C, 35.88; H, 7.02; N, 13.93%.)

***erythro*-Hydroxy-D-aspartic Acid.** D-Orn·D-*e*Hya·H_2O was treated as in the D-*e*Hya preparation; yield, 92%. (Found: C, 32.19; H, 4.71; N, 9.31%.)

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