

An Improved Resolution of β -Hydroxy-DL-aspartic Acid on Optically Active Resin Containing L-Lysine or L-Ornithine^{1,2)}

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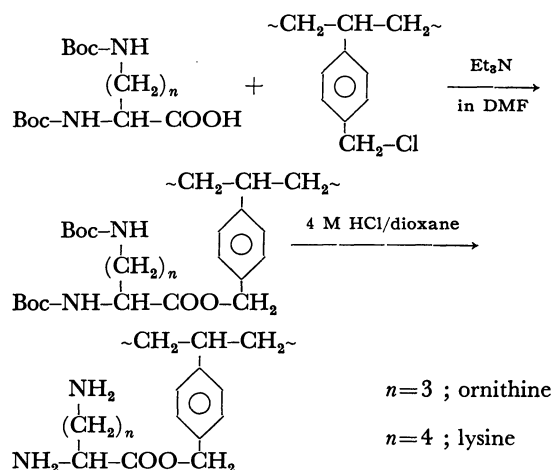
A series of experiments on column chromatography of the optically active resin containing L-lysine or L-ornithine were carried out with pyridinium acetate and ammonium acetate solvent systems as the eluents. *threo*- β -Hydroxy-DL-aspartic acid was found to be resolved on the optically active resin containing L-lysine with ammonium acetate solvent system; *erythro*- β -hydroxy-DL-aspartic acid could be resolved on that containing L-ornithine with pyridinium acetate solvent system. The *threo* or *erythro* racemate was resolved on a preparative scale, and the resolution including the complete characterization of enantiomers resolved was first accomplished.

Resolution of racemic compounds on the optically active resin has become familiar. In the initial stage, the resolution was carried out using naturally occurring adsorbent materials such as wool,³⁾ (+)-quartz,⁴⁾ and cellulose,^{5–7)} but the optical purity of the enantiomers obtained was low. The possibility of using synthetic optically active polymers as agents for the resolution was first studied by Bunnett and Marks in 1952.⁸⁾ The application of this principle to the resolution of racemic amino acids was first reported by Losse *et al.*⁹⁾ They attempted to resolve eight kinds of racemic amino acids on three types of copolymers composed by L-tyrosine and some suitable crosslinking agents. Roberts and Haigh also reported the synthesis of polymer of *S*-(*o*- and *p*-vinylbenzyl)-L-cysteine and its use in the partial resolution of DL-methionine.¹⁰⁾ Yamashita and Nakamura synthesized three types of optically active polymers by the homogeneous copolymerization of *N*-acryloylated α -amino acids with *N,N'*-dimethacryloyl-1,6-hexanediamine, and then partially resolved several kinds of DL-amino acid anilides by the use of these copolymers.¹¹⁾ Snyder *et al.* resolved DL-valine, DL-isoleucine, DL-norvaline, DL- α -aminobutyric acid, DL-alanine, and DL-proline on styrene-divinylbenzene copolymers containing (*N*-carboxymethyl-L-valine)copper(II) at 40–70% optical purity.¹²⁾ These authors suggested that the degree of resolution increased with an increase in the bulkiness of the side chain on the α -carbon of the amino acid. Davankov *et al.* reported that DL-proline was resolved at 100% optical purity using an asymmetric resin containing optically active L-proline in the Cu²⁺ form.¹³⁾ In all cases mentioned above, however, there has been no work on the resolution involving the complete characterization of enantiomers resolved.

One of the present authors has previously reported the resolution of *threo*- or *erythro*- β -hydroxy-DL-aspartic acid by the fractional crystallization of diastereomeric salt.¹⁴⁾ Although the *threo* racemate has been most satisfactorily resolved with L-lysine, and the *erythro* racemate with L-ornithine, these resolutions have required a highly skilled experimental technique. The resolutions of β -hydroxyaspartic acid were expected to become technically easier by the application of these resolutions

to column chromatography. So the authors have tried to resolve *threo*- or *erythro*- β -hydroxy-DL-aspartic acid on the optically active resin containing L-lysine or L-ornithine. In this paper, we deal with the preparations of the optically active resins and their use in the resolutions.

The optically active resins were prepared by the modification of the solid phase peptide synthesis by Merrifield.^{15,16)} *N*^a, *N'*-Bis(*t*-butoxycarbonyl)-L-lysine and chloromethylated styrene-divinylbenzene copolymer (PS-DVB) (DVB 2%, 200–400 Mesh, 1.32 mmol of Cl/g-resin) were coupled in *N,N*-dimethylformamide containing triethylamine for 72 h at 50 °C. The desired resin was afforded by the removal of the *t*-butoxycarbonyl group from the resin mentioned above by treatment with hydrogen chloride in dioxane for 90 min at room temperature. After the acidic hydrolysis



of the prepared resin, L-lysine content was determined by amino acid analysis and found to be 0.58 mmol/g-resin (correspond to 44% yield). L-Ornithylstyrene-divinylbenzene copolymer (Orn-PS-DVB) was prepared in the same manner as described for the preparation of L-lysylstyrene-divinylbenzene copolymer (Lys-PS-DVB); the L-ornithine content was 0.97 mmol/g-resin (yield 73%). The chromatography was carried out as follows: The aqueous solution of an aliquot of the racemate was applied to a column (1.2 × 10 cm) and eluted with various solvent systems at room temperature and

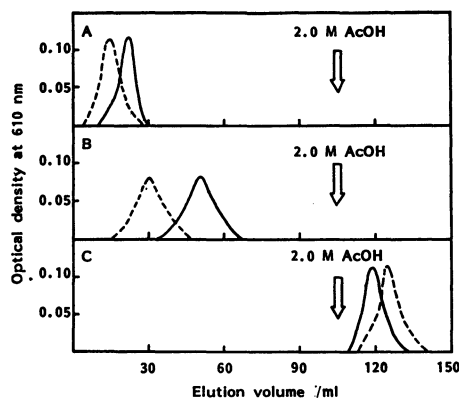


Fig. 1. Effect of pH in 0.1 M pyridinium acetate solvent system on resolution of *threo*-DL-Hyasp. Column: Lys-PS-DVB (solid line), Orn-PS-DVB (dotted line). A: pH 4.0, B: pH 6.0, C: pH 8.0.

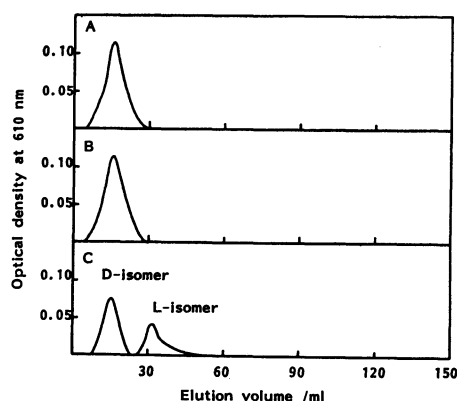


Fig. 2. Effect of pH in 0.1 M ammonium acetate solvent system on resolution of *threo*-DL-Hyasp. Column: Lys-PS-DVB. A: pH 6.0, B: pH 7.0, C: pH 8.0.

a flow rate of *ca.* 5 ml h⁻¹.

The elution patterns of *threo*- β -hydroxy-DL-aspartic acid with 0.1 M (1 M = 1 mol dm⁻³) pyridinium acetate solvent system are shown in Fig. 1. The effect of variation of pH was examined on Lys-PS-DVB and Orn-PS-DVB; in no cases was any *threo* racemate resolved. A similar experiment was run by the use of 0.1 M ammonium acetate instead of 0.1 M pyridinium acetate as an eluent. The elution patterns of the *threo* racemate are shown in Fig. 2. The *threo* racemate was successfully resolved with the column of Lys-PS-DVB using 0.1 M ammonium acetate of pH 8.0. It was confirmed that D-isomer eluted faster than L-isomer by separately eluting each enantiomer under the conditions described above. The effect of variation in concentration of ammonium acetate in the solvent system was also examined on Lys-PS-DVB; 0.1 M concentration proved to be the optimal condition for the resolution of the *threo* racemate (Fig. 3).

The resolution of *erythro*- β -hydroxy-DL-aspartic acid was carried out with column of Lys-PS-DVB and Orn-PS-DVB using 0.1 M pyridinium acetate of pH 4.0, 6.0, and 8.0. The elution patterns of the *erythro* racemate are shown in Fig. 4. The *erythro* racemate was found

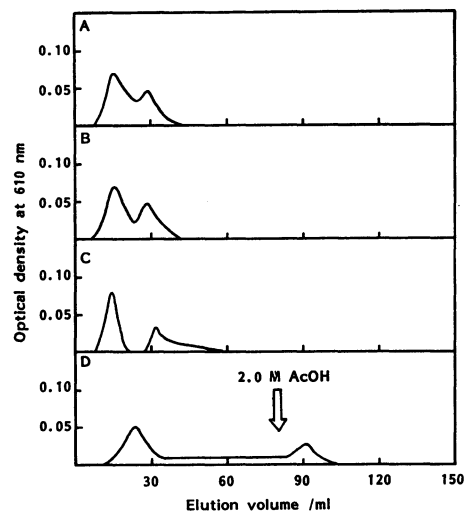


Fig. 3. Effect of concentration of ammonium acetate in solvent system (pH 8.0) on resolution of *threo*-DL-Hyasp. Column: Lys-PS-DVB. A: 0.2 M, B: 0.15 M, C: 0.1 M, D: 0.05 M.

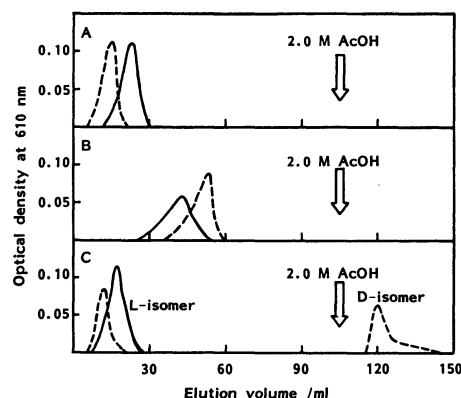


Fig. 4. Effect of pH in 0.1 M pyridinium acetate solvent system on resolution of *erythro*-DL-Hyasp. Column: Lys-PS-DVB (solid line), Orn-PS-DVB (dotted line). A: pH 4.0, B: pH 6.0, C: pH 8.0.

to be successfully resolved with the column of Orn-PS-DVB using 0.1 M pyridinium acetate of pH 8.0. The faster peak was eluted easily, as shown in Fig. 4, whereas 2.0 M acetic acid was required for the elution of the slower peak. The faster peak proved to be L-isomer, and the slower peak was D-isomer.

As an experiment on a preparative scale, we attempted to resolve the *threo* racemate with the column (1.8 $\times$ 40 cm) of Lys-PS-DVB, and the *erythro* racemate with the column of Orn-PS-DVB, under the optimal conditions which had been found previously for the resolutions on a smaller scale. Two hundred milligrams of *threo*- β -hydroxy-DL-aspartic acid was resolved, and the eluate containing each enantiomer was collected. The resolution was repeated five times, and each faster (or slower) peak was combined. We observed the presence of a small amount of lysine in the collected eluates, which seemed to be attributable to the liberation of lysine from the resin. After the removal of lysine by ion-exchange chromatography with an appropriate eluent,

TABLE 1. COMPARISON OF SPECIFIC ROTATIONS AND YIELDS OF THE FOUR OPTICAL ISOMERS OF β -HYDROXYASPARTIC ACID

Compound	Chemical procedure ¹⁴⁾		Present procedure	
	$[\alpha]_D^{20}/^\circ$	Yield/%	$[\alpha]_D^{20}/^\circ$	Yield/%
<i>threo</i> -L-Hyasp	- 8.5(H ₂ O) + 6.4(5 M HCl)	67	- 8.1(H ₂ O) + 6.2(5 M HCl)	80
<i>threo</i> -D-Hyasp	+ 8.6(H ₂ O) - 6.5(5 M HCl)	57	+ 8.3(H ₂ O) - 6.4(5 M HCl)	84
<i>erythro</i> -L-Hyasp	+ 47.0(H ₂ O) + 52.0(5 M HCl)	81	+ 47.4(H ₂ O) + 52.1(5 M HCl)	92
<i>erythro</i> -D-Hyasp	- 46.8(H ₂ O) - 51.8(5 M HCl)	72	- 47.0(H ₂ O) - 51.6(5 M HCl)	90

a) Temperature, 20 °C; *c* 1.0 in H₂O or 5 M HCl.

TABLE 2. AMINO ACID CONTENTS AND SPONTANEOUS LIBERATIONS RELATING TO THE PREPARED RESINS

Resin	Content (mmol/g-resin)	Yield ^{a)} /%	Liberated amino acid ^{b)}					
			pH 4.0 mmol/g-resin	%	pH 6.0 mmol/g-resin	%	pH 8.0 mmol/g-resin	%
Gly-PS-DVB	0.85	64	0.11	13	0.04	5	0.04	5
Ala-PS-DVB	0.66	50	0.15	23	0.12	18	0.10	15
Val-PS-DVB	0.80	61	0.01	1	<0.01	<1	<0.01	<1
Leu-PS-DVB	0.70	53	0.03	4	<0.01	<1	<0.01	<1
Ile-PS-DVB	0.65	49	<0.01	<1	<0.01	<1	<0.01	<1
Phe-PS-DVB	0.78	59	0.01	1	<0.01	<1	<0.01	<1
Tyr-PS-DVB	0.52	39	0.01	2	<0.01	<1	<0.01	<1
Pro-PS-DVB	0.91	69	0.51	56	0.12	13	0.13	14
Ser-PS-DVB	0.56	42	0.05	9	0.01	2	<0.01	<1
Thr-PS-DVB	0.69	52	0.02	3	<0.01	<1	<0.01	<1
Orn-PS-DVB	0.97	73	0.06	6	0.01	1	0.01	1
Lys-PS-DVB	0.58	44	0.07	12	0.11	19	0.04	7

a) The yields are based on Cl content (1.32 mmol/g-resin) of chloromethylated PS-DVB (DVB 2%, 200–400 Mesh).

b) The amounts of liberated amino acids below 0.01 mmol/g-resin are described as <0.01, and the percentages of liberation below 1% as <1.

the faster fraction (containing D-isomer) or the slower fraction (containing L-isomer) was concentrated *in vacuo*; optically pure *threo*- β -hydroxy-D-aspartic acid or *threo*- β -hydroxy-L-aspartic acid was obtained, in a good yield. *erythro*- β -Hydroxy-DL-aspartic acid was resolved by the elution of 500 mg of the racemate. Each enantiomer was obtained in the same manner as described for the resolution of the *threo* racemate. The specific rotations and yields of the four optical isomers so obtained are summarized in Table 1, together with those obtained by the fractional crystallization. As shown in Table 1, it was obvious that the *threo* or *erythro* racemate was completely resolved. This chromatographic procedure was far more useful than the resolution by the fractional crystallization: Such as could be obtained both a high yield and high optical purity, in a chemical procedure, were given by a facile operation.

In order to estimate the degree of the above-mentioned spontaneous degradation in PS-DVB substituted with amino acids, the authors prepared twelve kinds of resins, including Lys-PS-DVB and Orn-PS-DVB, and compared the liberations of amino acids from resins. They were prepared in the same manner as described for the preparations of Lys-PS-DVB and Orn-PS-DVB. Amino acid contents of resins are shown in Table 2. An aliquot of each resin so obtained was immersed in 0.1 M pyridinium acetate of pH 4.0, 6.0, and 8.0 for 7 d at room temperature, and the liberated amino acid in the supernatant was determined by amino acid analysis

(Table 2). Table 2 revealed the liberations of amino acids from resins, whereas amino acid benzyl esters in liquid phase were not entirely hydrolyzed under such mild conditions. The percentage of the liberated amino acid based on each amino acid content varied with the kinds of bonded amino acid. Gly-, Ala-, Pro-, and Lys-PS-DVB were unstable, while the resins containing amino acids possessing hydrophobic side chains were relatively stable. The variation of pH in the solvent also affected the liberation. The degree of liberation was greater under the acidic conditions than under the neutral and basic conditions, but only Lys-PS-DVB was unstable under the neutral conditions. These phenomena were greatly different from the commonly known propensity of hydrolysis of amino acids benzyl ester in aqueous acidic or basic solution. It seemed that an amino acid benzyl ester fixed on the stationary phase behaved in different ways from that in the liquid phase.

There have been some reports on successful resolutions of racemic amino acid derivatives by gas chromatography on optically active stationary phases,^{17,18)} but they have been used only on the analytical scale for the determination of configurations and optical purity and have restricted the variation of the racemates. For the resolutions of various kinds of racemic amino acids on a preparative scale, liquid-chromatographic resolutions have been more effective than gas-chromatographic resolutions. This report is the first on the complete resolution of a racemic amino acid on the

optically active stationary phase. It will be possible to apply the principles of this procedure to the resolutions of other racemic amino acids.

Experimental

All the melting points were uncorrected. The optical rotations were measured on a Union PM-101 Polarimeter. The quantitative analyses of amino acids were run with the high-performance liquid chromatography (HPLC) apparatus composed of; Toyo soda HLC-805, Toyo soda solvent feeder, Toyo soda sequence controller, Toyo soda reactor, Shimadzu fluorescence spectromonitor RF-500LC (for phthalaldehyde as fluorogenic reagent), Toyo soda visible spectrophotometer (for ninhydrin as color-developing reagent), and TSK pen recorder. The amounts of color developed were determined by an Atago AG-4 densitometer (slit 1×18 mm, 610 nm).

Syntheses of *N*-(*t*-Butoxycarbonyl) Amino Acid Derivatives.

All the *N*-(*t*-butoxycarbonyl) amino acid derivatives were synthesized by the ordinary manner. *N*-(*t*-Butoxycarbonyl) derivatives of lysine, ornithine, valine, isoleucine, phenylalanine, tyrosine, serine, and threonine were stored as a DCHA salt, and that of leucine as the hemihydrate. Boc-Lys(Boc)-OH·DCHA: mp 134–136 °C; $[\alpha]_D^{20} -4^\circ$ (*c* 3, AcOH). Boc-Orn(Boc)-OH·DCHA: mp 164–166 °C; $[\alpha]_D^{20} +2^\circ$ (*c* 4, AcOH). Boc-Gly-OH: mp 93–94 °C (lit.¹⁹) 94–95 °C). Boc-Ala-OH: mp 81–83 °C (lit.²⁰) 83–84 °C; $[\alpha]_D^{20} -22^\circ$ (*c* 1, AcOH) (lit.²⁰) -24° (*c* 1, AcOH)). Boc-Val-OH·DCHA: mp 138–140 °C; $[\alpha]_D^{20} -1^\circ$ (*c* 4, AcOH). Boc-Leu-OH·1/2 H₂O: mp 80–82 °C (lit.¹⁹) 78–81 °C; $[\alpha]_D^{20} -21^\circ$ (*c* 2, AcOH). Boc-Ile-OH·DCHA: mp 125–127 °C (lit.²¹) 127–128 °C; $[\alpha]_D^{20} +7^\circ$ (*c* 1.5, DMF) (lit.²¹) $+6^\circ$ (*c* 1.5, DMF)). Boc-Phe-OH·DCHA: mp 213–214 °C (lit.²²) 210–212 °C; $[\alpha]_D^{20} +30^\circ$ (*c* 1, methanol) (lit.²²) $+29.2^\circ$ (*c* 1.025, methanol at 23 °C)). Boc-Tyr-OH·DCHA: mp 208–210 °C (lit.²⁰) 205–208 °C; $[\alpha]_D^{20} +4^\circ$ (*c* 1, AcOH) (lit.²⁰) $+3.8^\circ$ (*c* 1, AcOH)). Boc-Pro-OH: mp 133–134 °C (lit.²³) 133–134 °C; $[\alpha]_D^{20} -59^\circ$ (*c* 2, AcOH) (lit.²³) -60.8° (*c* 2.04, AcOH at 25 °C)). Boc-Ser-OH·DCHA: mp 140–142 °C (lit.²⁴) 142–144 °C; $[\alpha]_D^{20} +13^\circ$ (*c* 3, methanol) (lit.²⁴) $+13.3^\circ$ (*c* 3.044, methanol at 25.5 °C)). Boc-Thr-OH·DCHA: mp 148–151 °C; $[\alpha]_D^{20} -2^\circ$ (*c* 4, AcOH). Elemental analyses of these compounds also agreed with the theoretical values.

Preparations of Optically Active Resins. *L*-Lysylstyrene-divinylbenzene Copolymer (Lys-PS-DVB): The chloromethylated PS-DVB (DVB 2%, 200–400 Mesh, 1.32 mmol of Cl/g-resin) (10.0 g) was suspended in DMF (80 ml). The suspension was degassed for 1 h *in vacuo*. The solvent was decanted and the residual resin was washed three times with DMF by decantation. Boc-Lys(Boc)-OH which was released from its DCHA salt (13.9 g, 26.4 mmol) with 1 M sulfuric acid in the usual manner was dissolved in DMF (80 ml). The solution and triethylamine (3.3 ml, 23.8 mmol) were added to the residual resin and the mixture was shaken for 72 h at 50 °C. Then the resin was filtered, washed thoroughly with DMF, acetic acid, methanol, DMF, and methanol by gradual changes of solvent composition and sucked dry. The resin was kept overnight in a vacuum desiccator: yield 12.8 g. The resulting resin was suspended in dioxane (80 ml) and the suspension was degassed for 1 h *in vacuo*. The solvent was decanted and the residual resin was washed three times with dioxane by decantation. To the residual resin, 4 M hydrogen chloride in dioxane (50 ml) was added. After shaking for 90 min at room temperature, the resin was filtered, washed with dioxane, 1 M acetic acid, water and finally methanol and

sucked dry. The resin was kept overnight in a vacuum desiccator: yield 11.0 g. Lys-PS-DVB (41 mg) weighed out precisely was introduced into a tube and hydrolyzed with a mixture of 6 M HCl (0.5 ml) and propionic acid (0.5 ml) at 110 °C in a sealed tube for 24 h. Lys-PS-DVB was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in water (1.0 ml) and the solution was diluted with water. Lysine content was determined by applying a part of the dilute solution on HPLC and found to be 0.58 mmol/g-resin. The PS-DVB substituted with ornithine, glycine, alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, proline, serine, and threonine were prepared in the same manner as described for the preparation of Lys-PS-DVB. *N*-(*t*-Butoxycarbonyl) derivatives of glycine, alanine and proline were used for the coupling directly, and Boc-Leu-OH·1/2 H₂O was used after dehydration. The amino acid content of each resin is presented in Table 2.

Resolution of threo- or erythro-DL-Hyasp on Optically Active Resin.

Resolution of threo-DL-Hyasp: Lys-PS-DVB or Orn-PS-DVB (7.5 g) was packed into a column (1.2×10 cm); each column was equilibrated by washing with an eluent. 0.1 M ammonium acetate was prepared in the following manner. Ammonium acetate (7.7 g) was dissolved in water (500 ml), and the pH of the solution was adjusted with acetic acid. The solution was made up to 1 L with water, and adjusted again to the desired pH by acetic acid or aqueous ammonia. 0.1 M Pyridinium acetate was also prepared in the same manner by using pyridine (8.1 ml) instead of ammonium acetate. The solution of threo-DL-Hyasp¹⁴ (20 mg/1 ml water) was put on the column and eluted with the appropriate solvent system at room temperature and a flow rate of *ca.* 5 ml h⁻¹. The volume of each fraction was one milliliter. When the elution volume exceeded 90 ml, 2.0 M acetic acid was used as an eluent. The color development, the determination of the developed color, and the plotting on a graph were run according to the manner by H. Okai *et al.*¹⁴ The elution patterns are shown in Fig. 1–3.

Resolution of erythro-DL-Hyasp: erythro-DL-Hyasp¹⁴ (20 mg) was treated in the same manner as has been described for the resolution of threo-DL-Hyasp. For the elution of the slower peak, 2.0 M acetic acid was used as an eluent. The elution patterns are shown in Fig. 4.

Preparative Resolution of threo-DL-Hyasp: A solution of threo-DL-Hyasp¹⁴ (0.2 g) in a suitable volume (*ca.* 10 ml) of 0.1 M ammonium acetate solvent of pH 8.0 was put on a column (1.8×40 cm) of Lys-PS-DVB (70 g, 0.63 mmol of Lys/g-resin), and eluted with the same solvent at a flow rate of *ca.* 10 ml h⁻¹. Each fraction (2 ml) was tested on a paper strip with ninhydrin, and then the ninhydrin-positive fractions were applied on paper chromatography (developing solvent, 1-butanol-acetic acid-pyridine-water (4 : 1 : 1 : 2, v/v)) to find the fractions containing hydroxyaspartic acid. The resolution of 0.2 g of the racemate was repeated five times in the above-mentioned manner. The faster eluting fractions were combined and then evaporated to dryness *in vacuo*. Most of the ammonium acetate was removed by sublimation *in vacuo* for *ca.* 1 h at 100 °C. To remove the contaminated lysine, the residue was treated with Dowex 1 (acetate form). A column (1.8×12 cm) of Dowex 1 was washed with 0.5 M acetic acid (30 ml) and then with 2.0 M acetic acid (70 ml) to elute hydroxyaspartic acid. The residue obtained by the evaporation of the eluate was crystallized from water-ethanol: yield of threo-D-Hyasp 0.42 g (84%).

Found: C, 31.99; H, 4.88; N, 9.32%. Calcd for C₄H₇O₅N: C, 32.22; H, 4.73; N, 9.40%. The slower eluting fractions were combined and then treated in the manner described above: yield of threo-L-Hyasp 0.40 g (80%).

Found: C, 31.96; H, 4.90; N, 9.28%. The specific rotations of each enantiomer are presented in Table 1.

Preparative Resolution of erythro-DL-Hyasp: A solution of erythro-DL-Hyasp¹⁴⁾ (0.5 g) in a suitable volume (ca. 20 ml) of 0.1 M pyridinium acetate of pH 8.0 was put on a column (1.8 × 40 cm) of Orn-PS-DVB (70 g, 0.91 mmol of Orn/g-resin), and eluted with the same solvent at a flow rate of ca. 10 ml h⁻¹. After the faster eluting fractions containing hydroxyaspartic acid were found in the above-mentioned manner, 2.0 M acetic acid was used as an eluent. The resolution was repeated twice, and erythro-L-Hyasp was obtained from the faster eluting fractions by the method described for the separation of threo-D-Hyasp: yield of erythro-L-Hyasp 0.46 g (92%).

Found: C, 32.02; H, 4.82; N, 9.35%. The slower eluting fractions were combined and then treated as in the separation of threo-D-Hyasp: yield of erythro-D-Hyasp 0.45 g (90%).

Found: C, 32.18; H, 4.77; N, 9.42%. The specific rotations of each enantiomer are presented in Table 1.

Spontaneous Degradation. *Determination of Liberated Amino Acid:* The prepared resin (ca. 300 mg) was weighed out precisely and introduced into three test tubes. To the tubes 6.0 ml of 0.1 M pyridinium acetate (pH 4.0, 6.0, and 8.0) were added; the tubes were then sealed and allowed to stand at room temperature. The time-course of the spontaneous degradation was followed by determining the amino acid in the supernatant at daily intervals. The supernatant (0.5 ml) was withdrawn and evaporated to dryness *in vacuo*, and an aliquot of the residue was applied on HPLC. The amounts of the amino acids in the supernatant at each time were derived by correcting the experimental values, and those at zero time were subtracted from those at each time. The amounts of the liberated amino acids after 7 d are presented in Table 2.

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- 1) This article was in preliminary form communicated at the 13th Symposium on Peptide Chemistry, Nov. 1975, Tokyo.
- 2) The abbreviations recommended by the IUPAC-IUB commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used. Amino acid symbols except glycine denote the L-configuration unless otherwise noted.

Additional abbreviations: AcOH, acetic acid; DCHA, dicyclohexylamine; DMF, *N,N*-dimethylformamide, Et₃N, triethylamine; Hyasp, β -hydroxyaspartic acid; PS-DVB, styrene-divinylbenzene copolymer.

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