Studies of Hydroxy Amino Acids. II.[1] The Separation of Diastereoisomers of Hydroxy Amino Acids

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It has been found that *erythro*-β-hydroxy-DL-norvaline, *erythro*-β-hydroxy-DL-norleucine, and *threo*-β-hydroxy-DL-leucine form scarcely-soluble compounds in water with α-naphthylphosphoric acid, tetrachlorophthalic acid, and chlorendic acid, respectively. On the other hand, the compounds of their diastereoisomers with the reagents were soluble in water. On the basis of these facts, β-hydroxy-DL-norvaline, β-hydroxy-DL-norleucine, and β-hydroxy-DL-leucine were successfully separated into their diastereoisomers. The configurational assignment of α-amino-β-hydroxy acids without a branched side chain on the γ-carbon atom was established by means of NMR spectroscopy.

α-Amino-β-hydroxy acids are easily obtained by the condensation of copper glycinate with aldehydes or ketones, the method of which was originally proposed by Akabori *et al.*[2] and developed by Mix[3] and by Otani and Winitz.[4] However, this method usually gives a mixture of two diastereoisomeric racemates. Furthermore, the separation of the diastereoisomers is generally so difficult and complicated that only a few methods of doing so have been known. In these methods, β-hydroxy-DL-norvaline and β-hydroxy-DL-norleucine were changed to their copper chelates[3] and β-hydroxy-DL-leucine was converted to its sodium salt.[5]

In the previous paper, the present authors reported the separation of DL-threonine.[1] The separation and the configurational assignment of the diastereoisomers of β-hydroxy-DL-norvaline, β-hydroxy-DL-norleucine, and β-hydroxy-DL-leucine will be described in this paper. *erythro*-β-Hydroxy-DL-norvaline, *erythro*-β-hydroxy-DL-norleucine, and *threo*-β-hydroxy-DL-leucine formed scarcely-soluble compounds in water with α-naphthylphosphoric acid, tetrachlorophthalic acid, and chlorendic acid respectively. The elementary analyses showed that the compound consisted of the amino acid and the reagent in a 1:1 molar ratio, except for the compound with chlorendic acid, of which we failed to obtain an analytical sample. The IR spectra of all the compounds showed the absorption band in a region of 1715—1720 cm^{-1} due to the free carboxyl group as the salt of the amino acids with the strong acids. On the contrary, the compounds of their diastereoisomers with the reagents were soluble in water. These characteristics were successfully used in the separation of the two diastereoisomers of β-hydroxy-DL-norvaline, β-hydroxy-DL-norleucine, and β-hydroxy-DL-leucine.

The precipitates and the mother liquors were individually treated with hydrochloric acid. After removing the liberated reagents by filtration, the *erythro*- and *threo*-forms of the amino acids were recovered from the acidic solutions with an ion-exchange column. The results are summarized in Table 1. The *threo*-forms of β-hydroxy-DL-norvaline and β-hydroxy-DL-leucine also formed comparatively insoluble compounds in water with trimesic acid. These characteristics were also successfully used in the separation of the *threo*-forms of these amino acids, but the separation of the *erythro*-forms failed because of contamination with the *threo*-forms.

TABLE 1. SEPARATION OF THE DIASTEREOISOMERS

Amino acid	Ratio of diastereoisomers		Reagent	Recovery of pure isomers %	
	erythro	*threo*		*erythro*	*threo*
HyNva	53	47	NP	68	64
HyNle	44	56	TCP	71	66
HyLeu	25	75	CA	68	75

Abbreviations used are as follows:
HyNva, β-Hydroxy-DL-norvaline; HyNle, β-Hydroxy-DL-norleucine; HyLeu, β-Hydroxy-DL-leucine; NP, α-Naphthylphosphoric acid; TCP, Tetrachlorophthalic aicd; CA, Chlorendic acid.
To *erythro*-forms of HyNva and HyNle, and *threo*-form of HyLeu, 1.2 equimolar amount of the reagents was used.

α-Amino-β-hydroxy acids with long side chain are difficult to separate into their diastereoisomers by paper or thin-layer chromatography, and a convenient method of determining the configuration has not been found in the literature. The NMR spectra of the diastereoisomers of the amino acids obtained in the investigation were observed at 60 MHz in aqueous solutions, with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference.

In an alkaline solution, the chemical shifts of α-CH protons of the *erythro*- and *threo*-forms without a branched side chain on the γ-carbon atom were found in the region of δ 3.30—3.32 ppm and δ 3.13—3.16 ppm respectively (Table 2). However, no such regularity was observed in a neutral or an acidic solution for any of them (Table 2). From these results, it is possible to distinguish the diastereoisomers of α-amino-β-hydroxy acid without a branched side chain on the γ-carbon atom by means of NMR spectroscopy in an alkaline solution. Thus, the ratio of the *erythro*- and *threo*-forms of the synthetic DL-α-amino-β-hydroxy pelargonic acid could be determined to be 30:70 on the basis of the NMR spectrum, though the amino acid could not be clearly separated into its diastereoisomers by paper or thin-layer chromatography.

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Table 2. Chemical shifts (δ ppm) of α-CH in aqueous solutions

Amino acid	Configuration	Solution		
		alkaline	neutral	acidic
DL-α-Amino-β-hydroxybutyric acid	*erythro*	3.30	3.83	3.92
	threo	3.16	3.58	3.72
β-Hydroxy-DL-norvaline	*erythro*	3.32	3.82	4.22
	threo	3.15	3.62	4–4.2
β-Hydroxy-DL-norleucine	*erythro*	3.32	3.82	3.95
	threo	3.13	3.62	3.85
β-Hydroxy-DL-leucine	*erythro*	3.33	3.90	4.33
	threo	3.32	3.82	4.08
DL-α-Amino-β-hydroxypelargonic acid[a)]	*erythro*	3.29	—	—
	threo	3.13	—	—

a) The compound is insoluble in a neutral or an acidic solution.

Experimental

The melting points are uncorrected. The NMR spectra were obtained with a Varian A-60 or T-60 spectrometer at 60 MHz in aqueous solutions, and chemical shifts are given from sodium 2,2-dimethyl-2-silapentane-5-sulfonate, used as the internal reference. Paper chromatography was carried out by the descending method on Toyo Roshi No. 51 paper with the solvent system of *n*-butanol - methyl ethyl ketone - 28% aqueous ammonia - water (5:3:1:1 v/v). The determination of the diastereoisomers was described in the previous paper.[1] The IR spectra were recorded in Nujol mull with a JASCO IR-S spectrometer.

Preparation of Starting Materials. β-Hydroxy-DL-norvaline, β-hydroxy-DL-norleucine, and DL-α-amino-β-hydroxypelargonic acid were synthesized by the method described by Mix.[3] β-Hydroxy-DL-leucine was obtained according to the method described by Ikutani *et al.*[5] DL-allothreonine and DL-threonine were on hand from the previous investigation.[1]

Salt of Erythro-*β-Hydroxy*-DL-*norvaline with α-Naphthylphosphoric Acid (I).* A solution of 1.3 g of *erythro*-β-hydroxy-DL-norvaline and 3.2 g of disodium α-naphthylphosphate in a mixture of 25 m*l* of water and 1 m*l* of concentrated hydrochloric acid was stored overnight in a refrigerator. The crystals thus formed were collected by filtration; yield, 1.2 g (32%). The compound was recrystallized from aqueous methanol to give **I** as fine crystals; mp 172.5—173.5°C (decomp.). IR: 3360, 3180, 1720, 1595, 1515 cm^{-1}. Found: C, 47.81; H, 6.03; N, 3.74; P, 8.20%. Calcd for $C_{15}H_{20}O_7NP \cdot H_2O$: C, 48.00; H, 5.91; N, 3.73; P, 8.25%.

Salt of Erythro-*β-Hydroxy*-DL-*norleucine with Tetrachlorophthalic Acid (II).* Into a solution of 1.47 g of *erythro*-β-hydroxy-DL-norleucine in 30 m*l* of water, 3.1 g of tetrachlorophthalic acid hemihydrate was dissolved with shaking at room temperature. The crystals precipitated immediately were dissolved by the addition of methanol. The resulting solution was stored overnight in a refrigerator to give **II** as needles; yield, 3.0 g (64%); mp 129.5—130°C (decomp.). IR: 3340, 3200, 1715, 1630, 1535 cm^{-1}. Found: C, 35.85; H, 3.74; N, 2.92; Cl, 30.03%. Calcd for $C_{14}H_{15}O_7NCl_4 \cdot H_2O$: C, 35.84; H, 3.65; N, 3.00; Cl, 30.23%. *threo*-β-Hydroxy-DL-leucine failed to give such a crystalline compound with chlorendic acid in an analytically pure state.

Separation of Diastereoisomers of β-Hydroxy-DL-*norvaline.* A solution of 6.4 g of disodium α-naphthylphosphate and 5.0 g of β-hydroxy-DL-norvaline (*erythro*: *threo*=53: 47) in 55 m*l* of water containing 4 m*l* of concentrated hydrochloric acid was stirred for 2 hr at room temperature, and then stored overnight in a refrigerator. The crystals thus formed were collected by filtration. The IR spectrum of the compound was identical with that of **I**. The crystals were dissolved in a mixture of 50 m*l* of water and 8 m*l* of concentrated hydrochloric acid by warming. The resulting solution was poured onto a column of Dowex 50 W×8 (in the H-form). After washing with water, the amino acid was eluted with 2N ammonium hydroxide (If α-naphthylphosphoric acid crystallized out from the acidic solution, it was filtered off and the filtrate was treated as above). The eluate was filled up to 500 m*l* with water, and 5 μ*l* of it was subjected to quantitative analysis. The solution contained 2.4 g of the *erythro*-form and 0.3 g of the *threo*-form. It was concentrated *in vacuo* to dryness. The residue was crystallized from water - ethanol to give *erythro*-β-hydroxy-DL-norvaline as plates; yield, 1.8 g (68%); mp 247–248°C (decomp.). lit,[3] 245—246°C (decomp.). The compound was confirmed to be a pure *erythro*-form by paper chromatography. (Found: C, 45.38; H, 8.46; N, 10.46%).

The mother liquor excluding the *erythro*-form was poured onto a column of Dowex 50 W×8 (in the H-form) and treated as has been described above. The eluate contained 2.1 g of the *threo*-form and 0.2 g of the *erythro*-form. The eluate was concentrated *in vacuo* and crystallized from water. The *threo*-form was obtained as plates; yield, 1.5 g (64%); mp 218—219°C (decomp.). lit,[3] 217—218°C (decomp.). (Found: C, 45.15; H, 8.44; N, 10.33%). The crystals were confirmed to be pure *threo*-β-hydroxy-DL-norvaline by paper chromatography.

Separation of Diastereoisomers of β-Hydroxy-DL-*norleucine.* A solution of 18.5 g of β-hydroxy-DL-norleucine (*erythro*: *threo*= 44: 56) and 20.8 g of tetrachlorophthalic acid hemihydrate in 185 m*l* of water was stirred for 3 hr at room temperature and then stored overnight in a refrigerator. The crystals (26 g) thus formed were collected by filtration. The IR spectrum of the compound was identical with that of **II**. The crystals were suspended in a mixture of 130 m*l* of water with 10 m*l* of concentrated hydrochloric acid and then boiled under reflux for 30 min. The reaction mixture was stored overnight in a refrigerator. The tetrachlorophthalic acid (17.2 g) thus liberated was filtered off. The filtrate was poured onto a column of Dowex 50 W×8 (in the H-form) and treated in the manner used in the separation of β-hydroxy-DL-norvaline to give the *erythro*-form as plates. Yield, 5.8 g (71%); mp 253—254°C (decomp.). lit,[3] 248—250°C (decomp.). (Found: C, 49.19; H, 8.96; N, 9.54%).

The filtrate (pH 2.70) from the scarcely-soluble compound was acidified to pH 1.20 with concentrated hydrochloric acid and kept for 2 hr at room temperature. The tetrachlorophthalic acid (2.5 g) thus liberated was filtered off. The

filtrate was treated in the manner used in the separation of the *erythro*-form to give the *threo*-form as plates. Yield, 6.8 g (66%); mp 232—233°C (decomp.). lit,[3] 224—226°C (decomp.). (Found: C, 48.90; H, 8.89; N, 9.64%).

*Separation of Diastereoisomers of β-Hydroxy-*DL*-leucine.* A mixture of 10 g of β-hydroxy-DL-leucine (*erythro*: *threo*=25: 75) and 23.8 g of chlorendic acid in 100 m*l* of water was stirred for 2.5 hr at room temperature and then kept overnight in a refrigerator. The scarcely-soluble compound (30.9 g) thus formed was collected by filtration and dissolved in a mixture of 150 m*l* of water and 10 m*l* of concentrated hydrochloric acid by heating. The resulting suspension, containing an oily substance formed during the heating, was stirred at room temperature for 30 min. In the course of stirring, the oily substance turned to crystals, which were then filtered off. The crystals were identified as chlorendic acid by studying the IR spectrum. The filtrate was treated in the manner used in the separation of β-hydroxynorvaline to obtain the *threo*-form as plates. Yield, 5.6 g (75%), mp 232—233°C (decomp.). Recrystallization from water - ethanol raised the melting point to 239—240°C (decomp.). lit, 240—241°C (decomp.),[5] 239—240°C (decomp.).[6] The IR spectrum of the compound was identical with that of the *threo*-form.[7] (Found: C, 48.95; H, 8.96; N, 9.58%).

The filtrate (pH 3.20) from the addition compound was acidified to pH 1.20 with concentrated hydrochloric acid. After standing for 1 hr at room temperature, the chlorendic acid thus liberated was filtered off. The filtrate was treated in the manner used in the separation of the *threo*-form to afford the *erythro*-form as plates. Yield, 1.7 g (68%); mp 241—242°C (decomp.). Recrystallization from water raised the melting point to 249—250°C (decomp.). lit, 255—256°C (decomp.),[5] 253—255°C (decomp.).[6] The compound was obtained as hemihydrate. The IR spectrum of the compound was almost identical with that of the *erythro*-form except for the band due to the water of crystallization.[7] Found: C, 46.27; H, 9.13; N, 9.11%. Calcd for $C_6H_{13}O_3N \cdot 1/2H_2O$: C, 46.14; H, 9.04; N, 8.97%.

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