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L-*THREO*- AND L-*ERYTHRO*-2-AMINO-3-HYDROXYHEX-4-YNOIC ACIDS NEW AMINO ACIDS FROM *TRICHOLOMOPSIS RUTILANS**

YUKIO NIIMURA and SHIN-ICHI HATANAKA

Department of Biology, College of General Education, University of Tokyo, Komaba, Meguro-ku, Tokyo

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Abstract—2-Amino-3-hydroxyhex-4-ynoic acid, reported previously from *Tricholomopsis rutilans*, was shown to be a mixture of its *threo*- and *erythro*-forms They were separated from each other and characterized by elementary analysis, optical rotation, TLC, IR, NMR spectra, catalytic hydrogenation, and by chemical synthesis Their configurations were determined by the comparison of their hydrogenation products with known *threo*- and *erythro*-2-amino-3-hydroxyhexanoic acids

INTRODUCTION

PREVIOUSLY we reported the isolation and characterization of L-2-aminohex-4-ynoic acid from fruit b of *Tricholomopsis rutilans* (Fr) Sing [1] This fungus contains another amino acid, dered to be 2-amino-3-hydroxyhex-4-ynoic acid whose stereochemistry was not known [2]

Natural 2-amino-3-hydroxyhex-4-ynoic acid proved, by further investigation, to be composed of L-*threo*- and L-*erythro*-forms Occurrence of two diastereomeric amino acids in one species is already known Thus, 2(*S*), 3(*S*), 4(*R*)- and 2(*S*), 3(*R*), 4(*R*)-β-hydroxy-γ-methylglutamic acids were recently reported from the seeds of *Gymnocladus dioicus* (L) Koch [3,4]

The present paper describes the separation and characterization of the natural L-*threo*- and L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acids and their synthesis

RESULTS AND DISCUSSION

Since 2-amino-3-hydroxyhex-4-ynoic acid has two centres of asymmetry, four stereoisomers are possible The NMR spectrum, determined in D_2O, showed two pairs of doublets, both assigned to the proton of α-carbon suggesting that natural acid was a mixture of *threo*- and *erythro*-forms TLC on cellulose developed with *n*-BuOH–MeCOEt–NH_4OH–H_2O[5] gave two well-distinguished brown spots with ninhydrin, fast-moving *A*

* Part VI in the series "Biochemical Studies on Nitrogen Compounds of Fungi" For Part V see HATANAKA, S-I, NIIMURA, Y and TANIGUCHI, K (1973) *Z Naturforsch* in press

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and slow-moving *B* This system has been used to separate *threo-* and *erythro*-forms of several α-amino-β-hydroxyacids [5-7]

We then separated the mixture preparatively into *A* and *B* by cellulose column chromatography with the same solvent c Colorimetric determination[8] of the eluates from the column gave the ratio of *A B*, 2 3 Elementary analysis of the recrystallized samples both correspond to $C_6H_9NO_3$ and from the values of optical rotation, measured in a neutral and an acidic solution both belong to L-series of amino acids The presence of an acetylene-bond in each was ascertained by quantitative total and partial catalytic hydrogenation with Adams' Pt- and Lindlar's Pd-catalysts,[9] respectively The hydrogenation products of *A* and *B* with the former catalyst both correspond to β-hydroxynorleucine[6] by TLC c

Threo-forms of several known α-amino-β-hydroxyacids move faster than their *erythro*-forms on PC with the solvent used[5-7] and the order of R_f-values are reversed in second solvent *iso*PrOH-HOAc-H_2O [10] The chromatographic behaviour of separated *A* and *B* in these solvents suggested that the former is of *threo-* and the latter *erythro*-configuration The R_{Leu} values are shown in Table 1

TABLE 1 R_{Leu}-VALUES OF THE NEW AMINO ACIDS ON CELLULOSE TLC

	Solvent	
	c	*d*
A (*threo*)	0 73	0 53
B (*erythro*)	0 51	0 64

Solvent c *n*-BuOH–MeCOEt–NH_4OH(28%)–H_2O (15 9 4 2) solvent *d* isoPrOH–HOAc–H_2O (14 1 5)

Though their IR spectra showed considerable differences, a weak absorption of a triple bond was determined in both samples at 2230 cm^{-1} The NMR spectra of both compounds were very similar but not identical Signals of their methyl groups, with the same long range coupling constant (J 2 Hz) were observed at 8 13τ (*A*) and 8 16τ (*B*) Two doublets at 6 13τ (J 3 5 Hz) in *A* and at 6 05τ (J 4 0 Hz) in *B* could be assigned to an α-methyne group From the Karplus relationship,[11] a larger coupling constant $J_{2,3}$ is expected in *anti-* or *erythro-* form than in *gauche-* or *threo*-form Thus the values of $J_{2,3}$ of *threo-* and *erythro*-β-hydroxyphenylserines were reported to be 3 7 and 4 6 Hz, respectively [12] The chemical shifts of α-methyne groups of *threo*-α-amino-β-hydroxyacids are generally at higher field than those of the corresponding *erythro*-compounds For example, the values 5 62τ and 5 34τ have been reported for threonine and *allo*-threonine respectively [12] From all these facts it could be shown, that *A* is L-*threo-* and *B* L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid

The chemical synthesis of 2-amino-3-hydroxyhex-4-ynoic acid was carried out by the condensation of tetrolaldehyde and copper glycinate The product was separated into the

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fast- and slow-moving compounds, as described for the natural amino acids The ratio of *A B* formed was 2 3, as in the natural amino acids

All the four natural and synthetic amino acids gave the same fragmentation patterns in MS The highest mass-ions in each, *m/e* 125, correspond to their dehydrated forms The chemical shifts in NMR spectra of synthetic *A* and *B* were identical with those of natural *A* and *B*, respectively Comparison by TLC in three solvents also confirmed the identity

Synthesis and separation of diastereomers, followed by their stereochemical characterization of β-hydroxynorleucine were reported by Mix [6] It enabled us, therefore, to compare the hydrogenated forms of synthetic *A* and *B* with known DL-*threo*- and DL-*erythro*-β-hydroxynorleucines For the separation of diastereomers, trimesic acid[13,14] was applied as a selective precipitant for its *threo*-form (see Experimental) The IR spectra of the *threo*-form and hydrogenated synthetic *A*, and *erythro*-form and hydrogenated synthetic *B* were superimposable, respectively, which support conclusively the above consideration about the stereochemical nature of the natural 2-amino-3-hydroxyhex-4-ynoic acid

Two related fungal species were surveyed for the possible occurrence of these amino acids by 2-D PC While the extract of *Tricholomopsis decora* (Fr) Sing showed a brown spot in the same position as the original mixture, *T platyphylla* (Fr) Sing did not

EXPERIMENTAL

General M ps were uncorrected IR were measured in KBr discs and NMR recorded in D_2O with DSS as an internal standard Evaporation of solvents was carried out with a rotary evaporator *in vacuo* below 40°

Chromatography Solvents used were *n*-BuOH–HOAc–H_2O (63 10 27) (*a*), PhOH–H_2O (in presence of NH_3 vapour) (25 9) (*b*), *n*-BuOH–MeCOEt–NH_4OH(28%)–H_2O(15 9 4 2)[5] (*c*) and *iso*PrOH–HOAc–H_2O (14 1 5)[10] (*d*) "Avicel SF" (cellulose plates and powder were used for TLC and column chromatography, respectively

Isolation and separation of diastereomers of natural 2-amino-3-hydroxyhex-4-ynoic acid The fruit bodies of *Tricholomopsis rutilans* (Fr) Sing * (2 kg), stored deep-frozen, were extracted with EtOH (20 l) and filtered The filtrate was passed through a column of Amberlite IR-120 (H^+) (300 ml) After the resin was washed thoroughly with EtOH and H_2O, the amino acids were eluted with 2 N NH_4OH (3 l) The ammonia eluate was evaporated *in vacuo* to *ca* 20 ml The concentrate was then applied to a column of Dowex 1 × 4 (Me COO^-) (4 5 × 90 cm) and eluted with 0 025 N HOAc The relevant fractions were combined, concentrated, and treated with a cellulose column (3 × 80 cm) with solvent *a*, to give pure fraction

In order to separate the diastereomers, the fraction was applied again to a cellulose column (3 × 75 cm) and developed with solvent *c* After repeated chromatography with the same solvent, fast-moving *A* and slow-moving *B* were separated completely Each fraction was extracted with H_2O and the aqueous layers concentrated The decolourized concentrates were treated with EtOH and Et_2O, yielding 55 mg *A* and 32 mg *B* *A* L-*threo*-2-amino-3-hydroxyhex-4-ynoic acid m p 155–161° (decomp) (Found C, 50 20, H, 6 27, N, 9 82 $C_6H_9NO_3$ requires C, 50 35, H, 6 34, N, 9 79%) $[\alpha]_D^{28°}$ −75° (*c* 1, H_2O), −72° (*c* 0 5, 3 N HCl) NMR τ 8 13 (*d*, *J* 2 Hz, –Me), 6 13 (*d*, *J* 3 5 Hz, –CH(ND_3^+)–), 5 04 (*m*, –CH(OD)–) *B* L-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid m p 140–154° (decomp) (Found C, 50 60, H, 6 04, N, 9 89%) $[\alpha]_D^{28}$ −24° (*c* 1 15 H_2O), +7 2° (*c* 0 58 3 N HCl) NMR τ 8 16 (*d* *J* 2 Hz Me) 6 05 (*d*, *J* 4 Hz CH(NH_3^+)) 4 98 (*m* CH(OD))

Synthesis and separation of diastereomers of DL-*2-amino-3-hydroxyhex-4-ynoic acid* Copper glycinate[15] (1 6 g) and tetrolaldehyde [16] (2 5 g) were added to 1 N NaOH (50 ml) and the mixture was stirred at room temp under N_2 for 4 hr After the removal of cupric ion with H_2S, the filtrate was passed through Amberlite IR-120 (H^+) (100 ml), and the amino acids were eluted with NH_3 (2 N, 1 l) Cellulose column chromatography (5 × 75 cm) with solvent *c* gave two fractions They were separately extracted with H_2O and the extracts evaporated *in vacuo* to give crude crystals after the addition of EtOH The fast-moving *A*(S) and slow-moving *B*(S) yielded 623 and 478 mg respectively Total yield 55% from copper glycinate *A*(S) DL-*threo*-2-amino-3-hydroxyhex-4-ynoic acid m p 187–192 (decomp) (Found C 50 34, H, 6 09, N, 9 94 $C_6H_9NO_3$ requires C 50 35, H, 6 34, N 9 79%)

* Voucher specimens were deposited in the Department of Biology, College of General Education, University of Tokyo

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NMR τ 8·13 (*d*, *J* 2 Hz), 6·13 (*d*, *J* 3·5 Hz) 5·04 (*m*) *B*(S) DL-*erythro*-2-amino-3-hydroxyhex-4-ynoic acid m.p. 154–162° (decomp.) (Found: C, 50·21; H, 6·36; N, 9·98%). NMR τ 8·16 (*d*, *J* 2 Hz), 6·05 (*d*, *J* 4 Hz) 4·97 (*m*).

Separation of diastereomers of β-hydroxynorleucine. A mixture of diastereomers of β-hydroxynorleucine was prepared by the condensation of copper glycinate and *n*-butylaldehyde according to Mix.[6] The product (1 g) and trimesic acid[13,14] (1·43 g) were added to H_2O (5·4 ml) and the mixture was warmed at 60° for 10 min, followed by stirring at 30° for 12 hr. After the filtration, the precipitate was dissolved in hot H_2O and the crystallization with EtOH (×2) gave DL-*threo*-β-hydroxynorleucine (163 mg) m.p. 215–226° (decomp.) (Lit.[6] 224–226°) (Found: C, 49·19; H, 9·04; N, 9·64. Calc. for $C_6H_{13}NO_3$: C, 48·92; H, 8·90; N, 9·52%). The filtrate was evaporated *in vacuo* and DL-*erythro*-β-hydroxynorleucine was crystallized from EtOH (×2) (70 mg) m.p. 226–232° (decomp.) (Lit.[6] 248–250°) (Found: C, 48·92; H, 8·90; N, 9·43%).

Hydrogenation and analysis of the products. A few mg of pure sample were hydrogenated at room temp. and pressure with stirring magnetically. Adams Pt catalyst (a few mg) suspended in H_2O (2·5 ml) and Lindlar's Pd catalyst[9] (a few mg) in 40% EtOH (2·5 ml) with a trace of freshly distilled quinoline were used for the total and partial catalytic hydrogenation respectively. Equivalent mol H_2 absorbed were as follows: total hydrogenation L-*threo*- 2·00, L-*erythro*-, 2·07, DL-*threo*- 2·02, DL-*erythro*- 2·00; partial hydrogenation L-*threo*- 1·03, L-*erythro*- 1·04, DL-*threo*- 0·98, DL-*erythro*- 0·99. Total hydrogenation products of DL-*threo*- and DL-*erythro*-2-amino-3-hydroxyhex-4-ynoic acids, the catalyst being removed, were crystallized from H_2O containing EtOH (×2). The former DL-*threo*-β-hydroxynorleucine m.p. 213–225° (decomp.) (Found: C, 48·78; H, 8·81; N, 9·67. Calc. for $C_6H_{13}NO_3$: C, 48·92; H, 8·90; N, 9·52%). The latter DL-*erythro*-β-hydroxynorleucine m.p. 226–230° (decomp.) (Found: C, 49·19; H, 8·98; N, 9·30%).

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