[Chem. Pharm. Bull. 24(2) 326—329 (1976)]

UDC 547.466.45.04 : 547.234.04

Syntheses of Ornithine Decarboxylase Inhibitors: D- and DL-α-Hydrazinoornithine

TADAHIRO SAWAYAMA, HIROAKI KINUGASA and HARUKI NISHIMURA

Research Laboratory, Dainippon Pharmaceutical Co., Ltd.[1]

(Received May 7, 1975)

The absolute configuration of optically active α-hydrazinoornithine which was prepared by the reaction of hydrazine with the α-bromo acid derived from L-ornithine was proved to be the D-configuration. In addition, racemic α-hydrazinoornithine was first prepared by two different methods.

Polyamines are found to be ubiquitous in nature. Recent studies[2] show that polyamines may participate in the biosynthesis of protein and ribonucleic acid (RNA), and may be associated with cell growth and rapidly growing tumors. In polyamine biosynthesis, decarboxylation of L-ornithine is the rate-determining step. Therefore, inhibitors[3] of ornithine decarboxylase (ODC) became important in relation to studies on the role of polyamines.

Johansson, Alto and Skinner[4] have recently patented so-called α-hydrazinoornithine (5-amino-2-hydrazinovaleric acid, hereinafter, HVA) which was derived from L-ornithine. Harik and Snyder[5] reported that HVA donated by them was a potent and relatively selective inhibitor of ODC and that 70% of the compound was in the L-form on the basis of the optical rotation data ($[\alpha]_D^{21}$ +6.56°, c=1, in H_2O). However, there was no description on the preparation of the L-isomer and no detail on the assignment of the L configuration in both literatures[4,5] mentioned above, in spite of that the configuration was of importance and interest in connection with the biological activity.

This note will describe the absolute configuration of optically active HVA derived from L-ornithine and, in addition, the preparation of racemic HVA.

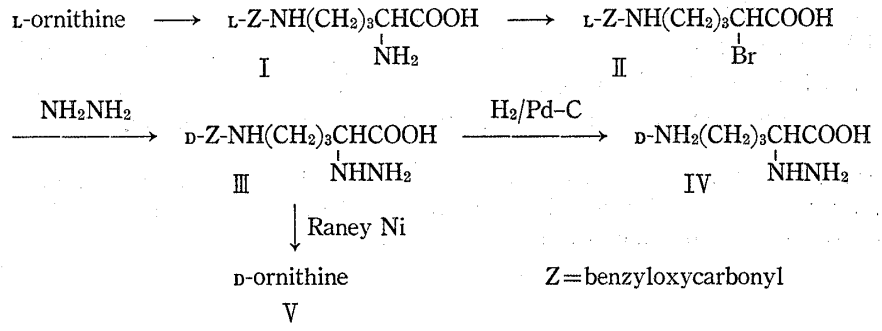

Chart 1

Optically active HVA (IV) was prepared according to the method of Johansson, *et al.*[4] with some modifications (see Chart 1). The oxalate of IV obtained showed $[\alpha]_D^{26}$ +6.8° (c=2, in H_2O). The optical rotatory dispersion curve for IV indicates that the rotation becomes

1) Location: *33-94, Enokicho, Suita City, Osaka.*
2) Y. Takeda, *Igaku no Ayumi*, **91**, 609 (1974) and references cited therein.
3) W.A. Skinner and J.G. Johansson, *J. Med. Chem.*, **15**, 427 (1972); M.M. Abdel-Monem, N.E. Newton and C.E. Weeks, *ibid.*, **17**, 447 (1973).
4) J.G. Johansson, P. Alto and W.A. Skinner, U.S. Patent 3754027 (1973)[*C. A.*, **79**, 137504*t* (1973)].
5) S.I. Harik and S.H. Snyder, *Biochim. Biophys. Acta.*, **327**, 501 (1973).

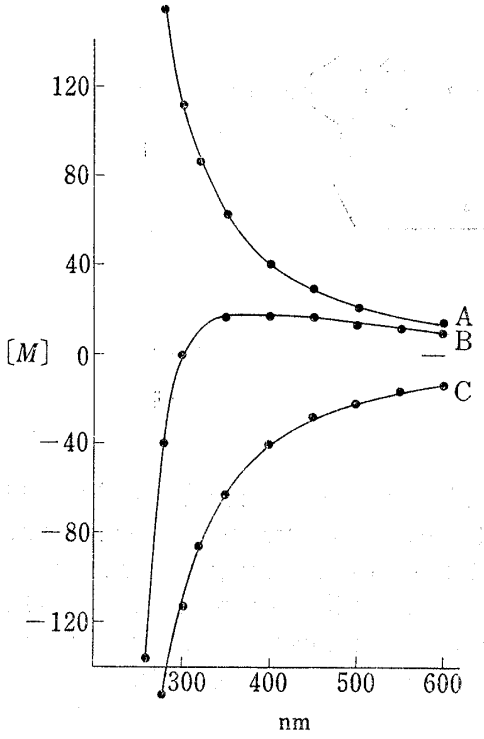

Fig. 1. Optical Rotatory Dispersion Curves

A, L-ornithine HCl; B, D-HVA (IV) oxalate; C, D-ornithine (V) HCl; Measurements were made at 26° on 2% solutions of A, B and C in water.

strongly negative in the 250—300 nm against that of L-ornithine (see Fig. 1). This suggests that IV is the D-isomer. In confirmation of this suggestion, 5-benzyloxycarbonylamino-2-hydrazinovaleric acid(III), the precursor of IV, was reduced[6] to the corresponding ornithine in water by Raney nickel. On the basis of the optical rotation data shown in Table I, *ca.* 97.5% of the ornithine (V) obtained proved to have the D configuration (5% racemate). Consequently, the absolute configuration of IV was established to be the D configuration because of no inversion of the configuration during the course of both reductions (III→V and III→IV). In general, it is known that the conversion of an optically active α-amino acid to the corresponding α-halo acid proceeds with retention of configuration,[6,7] and a hydrazino displacement reaction[8] on an α-halo acid was shown to proceed by an S_N2 mechanism.[6] Therefore, in this preparation of IV, the overall sequence L-ornithine→(L-α-bromo acid)→D-HVA is considered to have occurred.

As Table I shows, IV indicates a positive shift in rotation on going from alkaline to acidic solution. This is an interesting finding which suggests that the Lutz-Jirgensons rule[9] on α-amino acids does not apply to α-hydrazino acids.

TABLE I. Optical Rotation

	$[\alpha]_D^{26}$ (c=2)						
	1N HCl	H_2O	pH 7.0 phosphate	0.05N NaOH	0.1N NaOH	0.2N NaOH	1N NaOH
D-HVA (IV) oxalate	+10.7	+ 6.80	+4.4	+1.9[a]	−2.0[a]	−4.7[a]	−3.7[a]
L-Ornithine HCl	+22.7	+11.5					
D-Ornithine (V) HCl		−11.0					

a) c=1.0

In expectation of having more biological activity than the D-isomer, racemic HVA (VI) was obtained *via* two independent syntheses (see Chart 2). The first synthesis of VI was carried out by the racemization of IV; when refluxed in 1.4N LiOH under argon,[10] IV provided VI in 32% yield. The overall yield from L-ornithine to VI was just a few. In the second synthesis, 2-bromo-5-phthalimidovaleric acid (VII), the known compound which was easily derived from δ-valerolactam, was utilized as the precursor of VI. When treated with an excess

6) For references to reduction of α-hydrazino acids to the corresponding α-amino acids see H. Niedrich and R. Grupe, *J. Prakt. Chem.*, **27**, 108 (1965); M. Sletzinger, R.A. Firestone, D.F. Reinhold and C.S. Roony, *J. Med. Chem.*, **11**, 261 (1968).
7) N. Izumiya, *Nippon Kagaku Zasshi*, **72**, 149 (1951).
8) A. Darapsky, *J. Prakt. Chem.*, **99**, 179 (1919).
9) O. Lutz and B. Jirgensons, *Chem. Ber.*, **63**, 448 (1930); *idem*, *ibid.*, **64**, 1221 (1931).
10) α-Hydrazinoornithine in a solution decomposes in the presence of air.

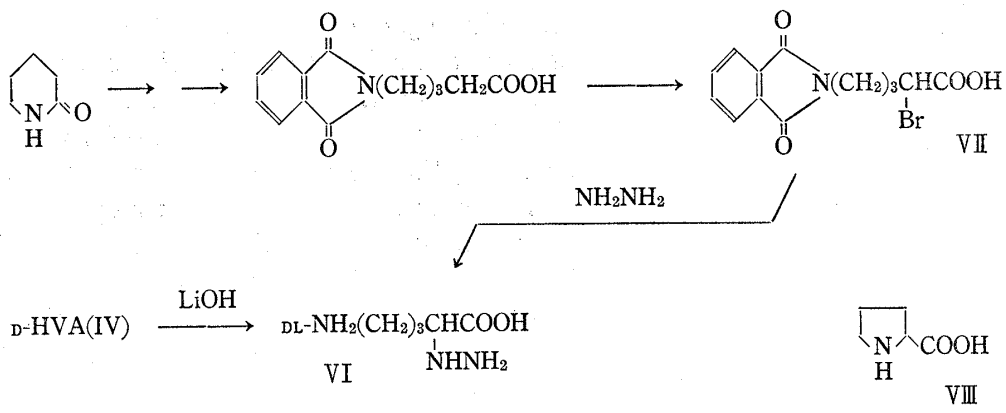

Chart 2

of hydrazine in N,N-dimethylformamide at 60° for 1 hr, VII gave VI, though not good yield (28%). This hydrazine displacement reaction was found to be accompanied with the complete removal of phthalimido group, affording the target (VI). Such a low yield might be due to the rapid removal of phthalimido group, followed by intramolecular cyclization to proline (VIII). However, attempts to detect proline by means of thin-layer chromatography using cellulose, were not successful because of disturbance of color reaction due to an excess of hydrazine.

Experimental[11]

D-5-Benzyloxycarbonylamino-2-hydrazinovaleric Acid (III)——To a stirred solution of N^{δ}-benzyloxycarbonyl-L-ornithine (I) (60 g) and KBr (94 g) in 2.5 N H_2SO_4 (460 ml) was added $NaNO_2$ (26 g) in portions for 3 hr on an ice-cooled water bath, and the mixture was stirred for another 1 hr. The pale yellow oil which formed was separated by decantation, washed with cold water and dissolved in $CHCl_3$. The solution was dried on Na_2SO_4 and concentrated to dryness *in vacuo* below 50°. The residual oil was a crude of 5-benzyloxycarbonylamino-2-bromovaleric acid (II) and dissolved in a cooled 30% aqueous solution (200 ml) of hydrazine with stirring. The resulting solution was allowed to stand at room temperature for 2 days. The solution was concentrated to dryness *in vacuo*. The residual oil was dissolved in 10% HCl and the solution adjusted to about pH 6 with dil. NaOH. The free hydrazino acid that precipitated was collected by filtration, washed with EtOH and dissolved in 10% HCl. The solution was concentrated to dryness *in vacuo* below 50°. The residual white crystals were recrystallized from a mixture of EtOH and ether, giving white leaflets (12.3 g) of III hydrochloride, mp 120—140°, in 17.2% yield. *Anal.* Calcd. for $C_{13}H_{19}O_4N_3 \cdot HCl$: C, 49.14; H, 6.34; N, 13.22; Cl, 11.16. Found: C, 48.91; H, 6.31; N, 13.22; Cl, 11.34. The free α-hydrazino acid was recrystallized from water, showing mp 190—193° (lit.[4] mp 161—164°). *Anal.* Calcd. for $C_{13}H_{19}O_4N_3 \cdot 1/2H_2O$: C, 53.78; H, 6.94; N, 14.47. Found: C, 54.16; H, 6.95; N, 14.63.

D-5-Amino-2-hydrazinovaleric Acid (IV)——Hydrogen was passed through a stirred solution of III·HCl (7.0 g) in 50% H_2O-EtOH (100 ml) in the presence of 5% palladium carbon (0.5 g) for 7 hr in a hood. The catalysts were filtered off and the filtrate was applied to a column of Dowex 50 W×8 (H-form, 50 ml). The column was well washed with water until the washings was neutral and then elution was carried out with 5% NH_4OH (250 ml). The resulting eluate was concentrated to dryness *in vacuo* below 50°. The residual oil was dissolved in an aqueous solution of oxalic acid (3.2 g). To the resulting solution was added EtOH. The precipitates were collected by filtration and recrystallized from a mixture of H_2O and EtOH, giving white leaflets (3.6 g) of IV oxalate, mp 173—173.5° (decomp.), in 69% yield. *Anal.* Calcd. for $C_5H_{13}O_2N_3 \cdot C_2H_2O_4$: C, 35.44; H, 6.37; N, 17.72. Found: C, 35.22; H, 6.39; N, 17.77. The hydrochloride of IV melted at 180—182° (decomp.) (*lit.*[4] mp 163—166°). $[\alpha]_D^{26}$ +8.17 (c=1, in H_2O). *Anal.* Calcd. for $C_5H_{13}O_2N_3 \cdot HCl$: C, 32.70; H, 7.68; N, 22.88; Cl, 19.31. Found: C, 32.80; H, 7.48; N, 22.93; Cl, 19.25.

DL-5-Amino-2-hydrazinovaleric Acid (VI) from IV——A solution of IV oxalate (0.3 g) in 1.4 N LiOH (15 ml) was refluxed under argon for 15 hr until the solution showed no rotation. The lithium oxalate that precipitated was filtered off and the filtrate applied to a column of Dowex 50 W×8 (H-form, 25 ml). The column was well washed with water and elution was carried out with 5% NH_4OH (150 ml). The eluate was concentrated to dryness *in vacuo* below 50°. The residue was dissolved in an aqueous solution (3 ml) of oxalic acid (0.22 g). To the resulting solution was added EtOH. The precipitates were collected by filtra-

11) All melting points were determined on a Yanagimoto micro melting apparatus and uncorrected. Optical rotatory dispersion (ORD) curves were taken with Jasco ORD/UV-5. Rotations were measured with Rex photoelectric polarimeter NEP-2.

tion and repeatedly recrystallized from a mixture of H_2O and EtOH, giving white crystals (97 mg) of VI oxalate, mp 161—162° (decomp.), in 32% yield. *Anal.* Calcd. for $C_5H_{13}O_2N_3 \cdot C_2H_2O_4$: C, 35.44; H, 6.37; N, 17.72. Found: C, 35.15; H, 6.40; N, 17.72.

Reduction of III with Raney Nickel——A solution of III·HCl (3.5 g) in water (1 liter) was adjusted to about pH 8 with dil. NaOH. To this solution was added Raney nickel (30 g) and the mixture was stirred at room temperature for 2 hr. Raney nickel was filtered off and the filtrate concentrated to about 40 ml at 60°. The resulting solution was applied to a column of Dowex 50 W×8 (H-form, 50 ml). The column was well washed with water and elution was carried out with 5% NH_4OH (300 ml). The effluent was concentrated to dryness *in vacuo*, affording a residual oil which was dissolved in a small amount of water. The solution was adjusted to pH 6 with dil. HCl. On addition of EtOH, white crystals were deposited. These crystals were collected by filtration and recrystallized from a mixture of H_2O and EtOH, giving white leaflets (0.53 g) of D-ornithine (V) hydrochloride, mp 239—240° (lit.[7] mp 234—235°), in 28.6% yield. *Anal.* Calcd. for $C_5H_{12}O_2N_2 \cdot HCl \cdot 1/4H_2O$: C, 34.67; H, 7.86; N, 16.18; Cl, 20.48. Found: C, 34.60; H, 8.07; N, 16.02; Cl, 20.61.

DL-5-Amino-2-hydrazinovaleric Acid (VI) from VII——To a solution of 2-bromo-5-phthalimidovaleric acid[12] (VII) (6.5 g) in dimethyl formamide (DMF) (100 ml) was added 100% hydrazine hydrate (4.95 g). The solution was allowed to stand at 60° on a water bath for 1 hr and concentrated to about 20 ml *in vacuo* below 50°. To this solution was added an aqueous solution of oxalic acid (5.0 g) and the mixture was stirred for a while. The precipitates composed of mainly 1,4-dioxophthalazine and hydrazine oxalate, were filtered off and the filtrate was applied to a column (2.5ϕ×10 cm) of Dowex 50 W×8 (H-form). The column was well washed with water and then elution was carried out with 5% NH_4OH. The eluate (200 ml) was concentrated to dryness *in vacuo*. The oily residue was dissolved in a small volume of water. To the aqueous solution was added a solution of oxalic acid (5.0 g) in a small volume of water, in order to remove the remaining hydrazine as the oxalate. The precipitates were filtered off and the filtrate was concentrated to dryness *in vacuo*. To the residue was added a small volume (*ca.* 4 ml) of water and the mixture was stirred for a while. Insoluble materials were filtered off and the filtrate was concentrated to dryness *in vacuo*. The residual solid was repeatedly recrystallized from a mixture of H_2O and EtOH, affording white crystals (1.3 g) of VI oxalate which were identified with those prepared from IV by mixed melting point and by comparison of infrared spectra.

Acknowledgement The authors are grateful to Prof. Y. Takeda and Dr. H. Inoue, Department of Biochemistry, Osaka University, Dental School, for their valuable discussion and to Drs. H. Takamatsu and S. Minami for their encouragement throughout this work.

12) R. Guadry and L. Berlinguet, *Can. J. Research*, **28B**, 245 (1950).