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Optical Resolution of β-Hydroxynorvaline

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β-Hydroxy-DL-norvaline(HyNva) was successfully separated into its diastereoisomers by fractional crystallization from 2-propanol as *p*-toluenesulfonic acid(TsOH) salts. The two racemic diastereoisomers were resolved into four stereoisomers by Vogler's method, using diastereomers of the *N*-benzyloxycarbonyl DL-amino acid and L-tyrosine hydrazide.

β-Hydroxy-DL-norvaline (HyNva) is very useful as a threonine analogue[1] and is needed as a starting material in the synthesis of Actinomycin D analogues. However, optical resolution of the four isomers of HyNva had not been achieved. In the present study, we attempted to separate and optically resolve HyNva into its four stereoisomers.

HyNva was prepared by the condensation reaction of copper(II) glycinate with propionaldehyde, by a method originally proposed by Akabori, Okawa.[2] This method usually gives a mixture of two diastereoisomers, which is difficult to separate. Y. Ariyoshi *et al.*[3] did report separating HyNva into diastereoisomers, but the reagents used were not accessible. In the present study, we found that the *erythro-* and *threo-*forms of HyNva could be easily isolated as the TsOH salts by partial recrystallization from 2-propanol. The configurational assignment of HyNva was done by paper chromatography[4] and 60 MHz ^{1}H-NMR spectroscopy in aqueous solutions.[3] The ratio of the *erythro-* and *threo-*forms of synthetic HyNva was found to be 40:60 by HPLC analysis.[5]

Optical resolution of the two racemic compounds was attempted. First, enzymatic reaction was tried for the *threo-*form, but *N*-acetyl-HyNva(DL-*threo*) could not be resolved using aminoacylase in aqueous solution (pH=6.8). Using Vogler's method,[6] we were able to resolve the DL-*threo* (**Ia**) and DL-*erythro* (**Ib**) forms using the diastereomers of the *N*-benzyloxycarbonyl DL-amino acid (**III**) (**IV**) and L-tyrosine hydrazide. (Fig. 1).

Four stereoisomers were obtained and reduced by catalytic hydrogenation to obtain free HyNva (**Ia$_1$**) (**Ia$_2$**) (**Ib$_1$**) (**Ib$_2$**). The melting points and specific rotations of these isomers are summarized in Table 1.

The configuration of the α-carbon atom was elucidated by optical rotatory dispersion (ORD) spectroscopy in hydrochloric acid solutions (pH=1).[7] The ORD spectra of **Ia$_2$** and **Ib$_2$** exhibited a positive Cotton effect (positive absorption peaks at 225 nm), showing

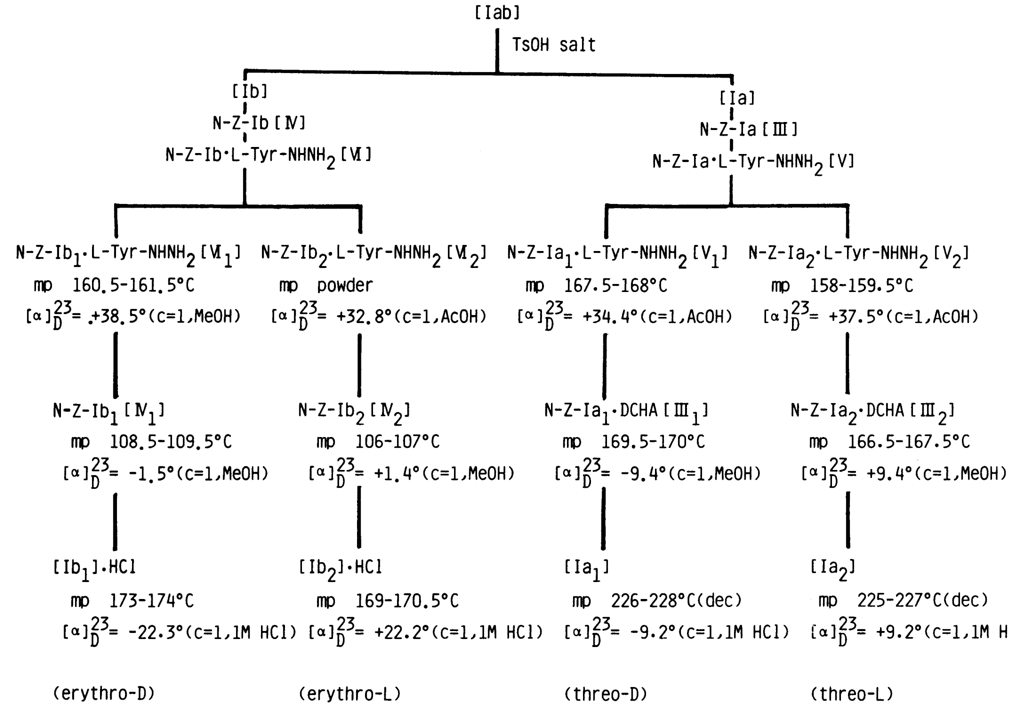

Fig. 1.

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TABLE 1. MELTING POINTS AND SPECIFIC ROTATION DATA

Form			Mp θ_m/°C	$[\alpha]_D^{23}$ °(c=1, 1M HCl)
threo	**Ia**$_1$	D	226—228(dec)	−9.2
	Ia$_2$	L	225—227(dec)	+9.2
erythro*	**Ib**$_1$	D	173—174	−22.3
	Ib$_2$	L	169—170.5	+22.2

*Hydrochloride.

these compounds to be of the L-form. **Ia**$_1$ and **Ib**$_1$ displayed a negative Cotton effect (negative absorption peak at 225 nm), which showed that they were of the D-form.

Experimental

*β-Hydroxynorvaline (**Iab**).* **Iab** was prepared by the glycine-copper method in 44% yield, mp 197—198 °C (dec) Found: C, 44.91; H, 8.33; N, 10.46%. Calcd for $C_5H_{11}O_3N$: C, 45.10; H. 8.33; N, 10.52%.

Separation of Diastereomers. A suspension of **Iab** (7.4 g, 5.6 mmol) in methanol (100 ml) was added to a methanol solution of TsOH (10.7 g, 5.6 mmol) and the solvent was removed *in vacuo*. Crystals of **Iab** TsOH salt were obtained in *ca.* 100% yield and subjected to fractional crystallization from hot 2-propanol. The first crystals of **Ib** TsOH salt (6.4 g, 35%) were removed by filtration and **Ia** TsOH salt (6.5 g, 36%) was obtained from the filtrate. Both of amino acid TsOH salts were decomposed with the pyridine–methanol solution, and recrystallization from hot water gave **Ia** [mp 215—217 °C(dec)] and **Ib** [mp 241—242 °C(dec)] in quantitative yields.

*N-Benzyloxycarbonyl-**Ia** (**III**).* **III** was prepared from **Ia** and benzyloxycarbonyl chloride by the usual method in 85% yield as a syrup.

*L-Tyrosine Hydrazide Salt of **III** (**V**).* L-Tyrosine hydrazide (26.3 g, 0.14 mol) was added to a solution of **III** (36 g, 0.14 mol) in methanol (250 ml), and the solution was warmed at 65 °C. After removal of the insoluble material, the solution was concentrated, and addition of tetrahydrofuran gave **V** in quantitative yield (62 g).

*Resolution of Racemic **III**.* A solution of **V** (62 g, 0.14 mol) in methanol (200 ml) was warmed at 65 °C. The first crop (**V**$_1$) of **V** crystallized on cooling (18.5 g, 72%). $[\alpha]_D^{23}$=+34.4° (c 1.0, AcOH), mp 167.5—168 °C. The mother solution was concentrated and the remaining crystals were dissolved in methanol (100 ml). This solution was left at room temperature for 24 h, and a second crop (**V**$_2$) of **V** was obtained (17 g, 64%). $[\alpha]_D^{23}$=+37.3° (c 1.0, AcOH), mp 158—159.5 °C. From the mother solution of **V**$_2$, racemic **V** was obtained (8.5 g). Found: **V**$_1$ C, 57.01; H, 6.60; N, 12.09%. **V**$_2$ C, 57.35; H, 6.56; N, 12.12%. Calcd for $C_{22}H_{30}N_4O_7$: C, 57.13; H, 6.54; N, 12.11%.

III$_1$ was obtained from **V**$_1$ by treatment with 3 M[††] hydrochloric acid and purified as the dicyclohexylamine (DCHA) salt. mp 169.5—170 °C, $[\alpha]_D^{23}$=−9.4° (c 1.0, MeOH). The DCHA salt of **III**$_2$ was obtained from **V**$_2$ in the same way. mp 166.5—167.5 °C, $[\alpha]_D^{23}$=+9.4° (c 1.0, MeOH). Found: **III**$_1$ C, 67.18; H, 8.92; N, 6.29% **III**$_2$ C, 66.75; H, 8.92; N, 6.40%. Calcd for $C_{25}H_{40}O_5N_2$: C, 66.90; H. 8.99; N, 6.24%.

*β-Hydroxynorvaline **Ia**$_1$, **Ia**$_2$.* The mixture of **III**$_1$, palladium black and hydrochloric acid was stirred for 3 h at room temperature under bubbling with hydrogen gas. After removal of the catalyst, the solution was neutralized and concentrated under reduced pressure, giving **Ia**$_1$. Mp 226—228 °C (dec), $[\alpha]_D^{23}$=−9.2° (c 1.0, 1 M HCl) **Ia**$_2$ was obtained by the catalytic hydrogenation described above. mp 225—227 °C (dec), $[\alpha]_D^{23}$=+9.2°(c 1.0, 1 M HCl). Found: **Ia**$_1$ C, 45.10; H, 8.33; N, 10.52% **Ia**$_2$ C, 44.93; H, 7.99; N, 10.50%. Calcd for $C_5H_{11}O_3N$: C, 44.73; H, 8.30; N, 10.66%.

*β-Hydroxynorvaline **Ib**$_1$, **Ib**$_2$.* The *erythro*-form of HyNva (**Ib** series) was obtained like the *threo*-form of HyNva (**Ia** series) and **Ib**$_1$, **Ib**$_2$ were also obtained.

[Ib$_1$]·HCl $[\alpha]_D^{23}$=−22.3° (c 1.0, 1 M HCl), mp 173—174 °C, [Ib$_2$]·HCl $[\alpha]_D^{23}$=+22.2° (c 1.0, 1 M HCl), mp 169—170.5 °C, Found: [Ib$_1$]·HCl C, 35.04; H, 6.90; N, 8.13; Cl, 21.11%, [Ib$_2$]·HCl C, 34.87; H, 6.97; N, 8.28; Cl, 19.60%. Calcd for $C_5H_{12}O_3HCl$: C, 35.41; H, 7.13; N, 8.26; Cl, 20.90%.

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[††] 1M=1 mol dm^{-3}.