

## Studies on Hydroxy Amino Acids. II.\*<sup>1</sup> The Optical Resolution of $\alpha$ -Amino- $\beta$ -hydroxy- $\gamma$ -benzyloxybutyric Acid\*\*<sup>2</sup>

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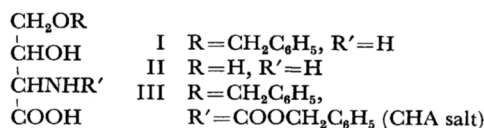
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The optical resolution of  $\alpha$ -amino- $\beta$ -hydroxy- $\gamma$ -benzyloxybutyric acid ( $I_{ab}$ ) into the four stereoisomers was investigated with success as a study related to the synthesis of homoserine analogues. The synthesis and the stereochemistry of  $\alpha$ -amino- $\beta$ , $\gamma$ -dihydroxybutyric acid were first investigated by Niemann<sup>1)</sup> and by Hamel<sup>2,3)</sup> in order to clarify the structure of Sphingosine; they succeeded in synthesizing three isomers of this amino acid, the D-erythro, L-threo, and D-threo forms, from D-mannitol. However, another isomer, the L-erythro form, could not be obtained.

The present authors prepared  $\alpha$ -amino- $\beta$ -hydroxy- $\gamma$ -benzyloxybutyric acid ( $I_{ab}$ ) by the condensation reaction of copper glycinate<sup>4)</sup> with benzyloxyacetaldehyde,<sup>5)</sup> while diastereoisomeric DL-erythro and DL-threo forms were isolated by the partial crystallization from *s*-butyl alcohol. We studied the optical resolution of both of the racemic modifications; we thus confirmed that the DL-erythro form ( $I_a$ ) could be resolved by the use of Takadiastase, but the threo form ( $I_b$ ) could not, though the latter could be resolved by Vogler's method<sup>6)</sup>

using the diastereoisomeric salt formation of the *N*-acyl-DL-threo amino acid (III) with L-tyrosine hydrazide (Fig. 1).



The four stereoisomers obtained were reduced by catalytic hydrogenation to give free  $\alpha$ -amino- $\beta$ , $\gamma$ -dihydroxybutyric acids (II);  $\gamma$ -lactone derivatives (IV) of these isomers were also obtained by treatment with dry hydrogen chloride in methanol. The melting points and specific rotations of these isomers are summarized in Tables 1 and 2.

### Experimental

$\alpha$ -Amino- $\beta$ -hydroxy- $\gamma$ -benzyloxybutyric acid ( $I_{ab}$ ) was prepared by the glycine-copper method in a 14% yield; mp 205—207°C.

Found: C, 58.43; H, 6.63; N, 6.07%. Calcd for C<sub>11</sub>H<sub>15</sub>O<sub>4</sub>N: C, 58.65; H, 6.71; N, 6.22%.

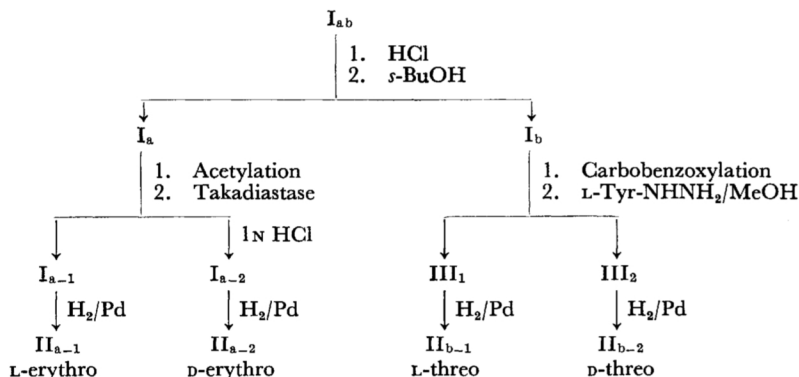


Fig. 1.

\*<sup>1</sup> Part I: K. Okawa, S. Sakai and T. Kinutani, This Bulletin, **41**, 1353 (1968).

\*\*<sup>2</sup> Cf. Original Paper: K. Okawa, K. Hori, K. Hirose and Y. Nakagawa, *Nippon Kagaku Zasshi* (J. Chem. Soc. Japan, Pure Chem. Sect.), **89**, 998 (1968).

1) C. Niemann and P. Nichols, *J. Biol. Chem.*, **143**, 191 (1942).

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TABLE 1. MELTING POINTS AND SPECIFIC ROTATIONS OF THE FOUR ISOMERS OF  $\alpha$ -AMINO- $\beta,\gamma$ -DIHYDROXYBUTYRIC ACID (II)

Author	Mp ( $^{\circ}$ C)	II <sub>a-1</sub> 194—195 [ $\alpha$ ] <sub>D</sub> <sup>25</sup> ( $^{\circ}$ )	II <sub>a-2</sub> 194 +11.3	II <sub>b-1</sub> 214 -13.5	II <sub>b-2</sub> 214—215 +13.6
		L-erythro	D-erythro	L-threo	D-threo
Hamel	Mp ( $^{\circ}$ C) [ $\alpha$ ] <sub>D</sub> <sup>25</sup> ( $^{\circ}$ )	— —	193—194 +15.3	214—215 -13.6	214—215 +13.1
Niemann	Mp ( $^{\circ}$ C) [ $\alpha$ ] <sub>D</sub> <sup>24</sup> ( $^{\circ}$ )	— —	192—194 +16.0	215 -13.7	—

TABLE 2. MELTING POINTS AND SPECIFIC ROTATIONS OF THE FOUR ISOMERS OF  $\alpha$ -AMINO- $\beta$ -HYDROXY- $\gamma$ -BUTYRORACTONE HYDROCHLORIDE (IV)

Author	Mp ( $^{\circ}$ C)	IV <sub>a-1</sub> 176 +55.6	IV <sub>a-2</sub> 176.5—177 -56.6	IV <sub>b-1</sub> 173—174 -26.2	IV <sub>b-2</sub> 175—175.5 +26.0
		L-erythro	D-erythro	L-threo	D-threo
Hamel	Mp ( $^{\circ}$ C) [ $\alpha$ ] <sub>D</sub> <sup>25</sup> ( $^{\circ}$ )	174—175 +50.4	174—175 -51.2	Oil	—

**Separation of Diastereomers.** A suspended solution of I<sub>ab</sub> hydrochloride (29 g) in *s*-butyl alcohol (300 ml) was stirred for 2 hr at 50°C. The undissolved crystals, I<sub>a</sub> hydrochloride (19 g), were then filtered off. From the mother liquor, I<sub>b</sub> hydrochloride (10 g) was obtained. Both of the amino acid hydrochlorides were decomposed with a pyridine-methanol solution; subsequent recrystallization from hot water gave I<sub>a</sub> (11 g; mp 202—203°C), and I<sub>b</sub> (6 g; mp 195—196°C).

**Resolution of Racemic I<sub>a</sub>.** *N*-Acetyl-I<sub>a</sub> was prepared in an 88% yield by the usual Schotten-Baumann method, mp 151—153°C, as CHA<sup>3</sup> salt. The salt obtained (7.33 g, 0.02 mol) was dissolved in 2N sodium hydroxide, and the liberated CHA was extracted with ether. After the pH of the aqueous layer had been adjusted to 6.8, the solution was incubated with Takadiastase at 37°C for 4 days. The precipitate was then filtered off, and the filtrate was concentrated under reduced pressure until crystals appeared. The crystals of I<sub>a-1</sub> were obtained in a 67% yield and were recrystallized from hot water; mp 194—195°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +21.9° (c 5.7, 1N HCl).

Found: C, 58.62; H, 6.75; N, 6.20%. Calcd for C<sub>11</sub>H<sub>16</sub>O<sub>4</sub>N: C, 58.65; H, 6.71; N, 6.22%.

The mother liquor of I<sub>a-1</sub> was acidified to pH 2.0 and was extracted with ethyl acetate. *N*-Acetyl-I<sub>a-2</sub> was obtained from the concentrated extract as CHA salt in an 84% yield. Recrystallization from methanol-ethyl acetate gave pure crystals; mp 145—146°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -12.5° (c 2.5, EtOH). The partial hydrolysis of the CHA salt of *N*-acetyl-I<sub>a-2</sub> with 1N hydrochloric acid gave free amino acid I<sub>a-2</sub> in a 82% yield; mp 194°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -21.9° (c 5.5, 1N HCl).

Found: C, 58.78; H, 6.67; N, 6.25%. Calcd for C<sub>11</sub>H<sub>16</sub>O<sub>4</sub>N: C, 58.65; H, 6.71; N, 6.22%.

***N*-Carbobenzoxy-I<sub>b</sub> (III).** *N*-Carbobenzoxy-I<sub>b</sub> was prepared from I<sub>b</sub> and carbobenzoxychloride by the usual method; it was obtained as CHA salt in a 79%

yield; mp 158—159°C.

**L-Tyrosine Hydrazide Salt of III.** The free acid (III) was obtained from CHA salt (13.7 g, 0.03 mol) by the use of 3N hydrochloric acid and by ethyl acetate extraction. L-Tyrosine hydrazide (5.86 g, 0.03 mol) was added to a solution of III in methanol (50 ml), and the solution was warmed at 65°C. After the removal of insolubles, the solution was concentrated; the subsequent addition of ethanol gave L-tyrosine hydrazide salt of III in a quantitative yield (16.6 g), [ $\alpha$ ]<sub>D</sub><sup>25</sup> +36° (c 1.0, water).

Four recrystallizations of this salt (5.96 g) from methanol gave a small amount (310 mg) of optically pure L-tyrosine hydrazide salt (V<sub>1</sub>), mp 158.5—159°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +30.6° (c 1.0, water). V<sub>1</sub> was used as the seed of the following partial crystallization.

**Resolution of Racemic III.** A solution of L-tyrosine hydrazide salt of III in methanol (50 ml) was similarly prepared from the CHA salt of III (6.88 g, 0.015 mol). After the insolubles had been filtered off, a small amount of V<sub>1</sub> was seeded to the filtrate; then the mixture was stirred for 1.5 hr and kept at 25°C for 22.5 hr. The precipitated first crop (V<sub>1</sub>) was then collected (2.12 g); mp 158.5°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +30.5° (c 1.0, water). The mother liquor was concentrated, and the resulting crystals were dissolved in methanol (50 ml). After the solution had been stirred at 25°C for 4 hr and then kept at 25—17°C for 20 hr, the second crop (V<sub>2</sub>) was obtained (2.66 g, 64%); mp 163—165°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +39.3° (c 1.0, water). From the filtrate of V<sub>2</sub>, an additional crop of V<sub>1</sub> was obtained; the total yield of V<sub>1</sub> was 73%.

III<sub>1</sub> was obtained from V<sub>1</sub> by treatment with 3N hydrochloric acid; it was purified as CHA salt; mp 138.0°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +10.9° (c 2.0, EtOH).

Found: C, 65.61; H, 7.60; N, 6.18%. Calcd for C<sub>25</sub>H<sub>34</sub>O<sub>8</sub>N<sub>2</sub>: C, 65.48; H, 7.47; N, 6.11%.

The CHA salt of III<sub>2</sub> was obtained from V<sub>2</sub> in the same way; mp 137—138°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> -10.3° (c 2.0, EtOH). Found: C, 65.62; H, 7.57; N, 6.14%.

**$\alpha$ -Amino- $\beta,\gamma$ -dihydroxybutyric Acids (II).** A

<sup>3</sup> CHA=cyclohexylamine

mixture of  $I_{a-1}$  (675 mg, 3 mmol), water (10 ml), palladium charcoal (160 mg), and *N* hydrochloric acid (3 ml) was stirred for 5 hr at room temperature under the bubbling of hydrogen gas. After the removal of the catalyst, the solution was neutralized and subsequently concentrated under reduced pressure. The resulting crystals were recrystallized twice from water-methanol to give  $II_{a-1}$  (305 mg, 75%); mp 194–195°C;  $[\alpha]_D^{25} -11.3^\circ$  (*c* 7.2, water).

Found: C, 35.56; H, 6.77; N, 10.30%. Calcd for  $C_4H_8O_4N$ : C, 35.55; H, 6.71; N, 10.37%.

$II_{a-2}$ ,  $II_{b-1}$ , and  $II_{b-2}$  were obtained from  $I_{a-2}$ ,  $III_1$ , and  $III_2$  by the catalytic hydrogenation described above.

$II_{a-2}$ : mp 194°C;  $[\alpha]_D^{25} +11.3^\circ$  (*c* 7.0, water).

Found: C, 35.99; H, 6.91; N, 10.63%.

$II_{b-1}$ : mp 214°C;  $[\alpha]_D^{25} -13.5^\circ$  (*c* 2.0, water).

Found: C, 35.32; H, 6.72; N, 10.21%.

$II_{b-2}$ : mp 214–215°C;  $[\alpha]_D^{25} +13.6^\circ$  (*c* 4.8, water).

Found: C, 35.61; H, 6.74; N, 10.98%.

**$\alpha$ -Amino- $\beta$ -hydroxy- $\gamma$ -butyrolactone Hydrochlorides (IV).**  $IV_{a-1}$ ,  $IV_{a-2}$ ,  $IV_{b-1}$ , and  $IV_{b-2}$  were prepared from  $II_{a-1}$ ,  $II_{a-2}$ ,  $II_{b-1}$ , and  $II_{b-2}$  respectively in the way described by Hamel and Painter.<sup>2)</sup>

$IV_{a-1}$ : mp 176°C;  $[\alpha]_D^{25} +55.6^\circ$  (*c* 1.5, water).

Found: C, 31.31; H, 5.24; N, 9.58; Cl, 22.73%.

Calcd for  $C_4H_8O_3NCl$ : C, 31.28; H, 5.25; N, 9.12; Cl, 23.09%.

$IV_{a-2}$ : mp 176.5–177°C;  $[\alpha]_D^{25} -56.6^\circ$  (*c* 1.5, water).

Found: C, 31.23; H, 5.51; N, 9.18; Cl, 23.05%.

$IV_{b-1}$ : mp 173–174°C;  $[\alpha]_D^{25} -26.2^\circ$  (*c* 1.0, water).

Found: C, 31.24; H, 5.26; N, 9.13; Cl, 23.23%.

$IV_{b-2}$ : mp 175–175.5°C;  $[\alpha]_D^{25} +26.0^\circ$  (*c* 1.0, water).

Found: C, 31.39; H, 5.27; N, 9.13; Cl, 23.15%.