Synthesis of DL-α-Aminosuberic Acid and Its Optical Resolution

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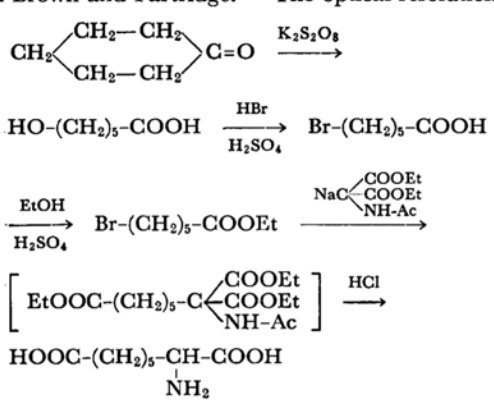
α-Aminosuberic acid is a key substance in synthesizing an analogue of deamino-oxytocin which has an ethylene linkage in place of the disulfide bond of oxytocin. DL-α-Aminosuberic acid (I) was first synthesized in 1956 by the hydrogenation of α-oximinosuberic acid, but the procedure was not practical and the optical resolution of the racemate has not yet been achieved.[1] Farkašová and Rudinger[2] derived L-α-aminosuberic acid from L-glutamic acid by the step-by-step addition of methylene groups to the side chain.

In the present study, the racemic compound I was synthesized by the acetaminomalonate procedure using ethyl ε-bromocaproate. The latter had been obtained from cyclohexanone following the procedure of Brown and Partridge.[3] The optical resolution of



the racemate was carried out by two different procedures. One route was the enzymic digestion of *N*-chloroacetyl-DL-α-aminosuberic acid (II) at pH 7.2 with Taka-acylase. The other route was the fractional crystallization of *N*-carbobenzoxy-DL-α-aminosuberic acid (III) with D-tyrosine hydrazide according to the procedure of Vogler *et al.*[4] In the former case, it took about ten days at 37°C to complete the digestion, and L-α-aminosuberic acid (IV) was obtained in about an 80% yield; the all-around efficiency was found to be lower than in the latter method. The fractional crystallization procedure afforded carbobenzoxy-L-α-aminosuberic acid (V) in a good yield, and this was immediately available for the synthesis of its peptides; the recovery of the D-form from the mother liquor was also easy in this case. The same optical rotation value was obtained for the free L-amino acids prepared by the two methods, and this value was identical with that reported previously.[2]

Experimental

Materials. Ethyl ε-bromocaproate (bp 116—120°C/14 mmHg) was synthesized following the procedure of Brown and Partridge.[3] DL-Tyrosine was prepared from L-tyrosine by racemization with acetic anhydride.[5] DL-Tyrosine was converted to the ethyl ester and then to the hydrazide as has been described in the literature;[6,7] the over-all yield from L-tyrosine was 57%, mp 171—173°C. The optical resolution of DL-tyrosine hydrazide was carried out with carbobenzoxy-L-proline as has been described by Vogler and Lanz.[4] The Takadiastase was obtained from the Sankyo Co., Ltd.*1

DL-α-Aminosuberic Acid (I). Ethyl acetaminomalonate (183 g, 0.84 mol) was added to a solution of sodium ethoxide in absolute ethanol (18.4 g sodium in 800 ml of ethanol), and the mixture was refluxed gently. Ethyl ε-bromocaproate (183 g, 0.82 mol) was then stirred slowly in over a period of 80 min; refluxing and stirring were continued for a further 7.5 hr, and then the whole mixture was kept overnight at room temperature. The precipitate formed was removed, the ethanol solution was concentrated under reduced pressure, and the residual oil was taken up in ether. The ether solution was washed with water, and the solvent was removed by evaporation. The oily residue was refluxed with conc. hydrochloric acid (1.2 *l*) for 6 hr, and then concentrated to dryness. The final residue was dissolved in water (350 ml), the pH of the solution was adjusted to 3 with aqueous ammonia, and the mixture was kept overnight in a refrigerator. The crystals which appeared were collected by filtration, washed with water and with methanol, and dried in a desiccator over sulfuric acid; crude yield, 133 g (80%). The crude product was dissolved in boiling water, and the solution was decolorized with active charcoal; on cooling compound I crystallized as the monohydrate; yeild, 127 g (77%), mp 240—241°C (decomp.). Reported, mp 233°C (decomp.).[1] For analysis, this material was dried over phosphorus pentoxide *in vacuo* for 10 hr at 90°C.

Found: C, 50.67; H, 8.04; N, 7.41%. Calcd for $C_8H_{15}O_4N$: C, 50.78; H, 7.99; N, 7.40%.

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***N*-Chloroacetyl-DL-α-aminosuberic Acid (II).** Compound II was obtained from I (151 g, 0.73 mol as the monohydrate) by a normal Schotten-Baumann reaction with chloroacetyl chloride (100 g, 0.88 mol) and 4N sodium hydroxide. The crude product was recrystallized from ethyl acetate and petroleum ether; yield, 149 g (77%), mp 116—118°C.

Found: C, 44.97; H, 6.02; N, 5.22; Cl, 13.61%. Calcd for $C_{10}H_{16}O_5NCl$: C, 45.20; H, 6.07; N, 5.27; Cl, 13.34%.

***N*-Carbobenzoxy-DL-α-aminosuberic Acid (III).** Compound I (41.5 g, 0.2 mol as monohydrate) was treated with carbobenzoxy chloride (40.8 g, 0.24 mol) and with 2N sodium hydroxide under the normal conditions of a Schotten-Baumann reaction; crude yield, 58.5 g (90%), mp 123—125°C. Recrystallization from ethyl acetate afforded needles; yield, 54.3 g (84%), mp 124—125°C.

Found: C, 59.50; H, 6.52; N, 4.31%. Calcd for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55; N, 4.33%.

Isolation of L-α-Aminosuberic Acid (IV) and of Its D-Isomer by Enzymic Digestion of II. A solution of Takadiastase (250 g) made by extraction with ice water (1.8 *l*) was added to a mixture of II (266 g, 1 mol) and cobaltous chloride (460 mg) in 4N sodium hydroxide; the pH of the solution was adjusted to 7.2 with acetic acid and with sodium hydroxide before and after the addition of the enzyme solution respectively. During the incubation at 37°C the solution was frequently adjusted to pH 7.2 with 4N sodium hydroxide. After 8 days the mixture was boiled for 10 min to precipitate proteins, which were then removed by filtration with Hyflo Supercel. The unchanged chloroacetyl derivative (mainly the D-form) was extracted with ethyl acetate at pH 1.7, and then the water layer was adjusted to pH 3 and stored overnight in a refrigerator. The crude IV was collected by filtration and recrystallized from hot water after decolorization with active charcoal; yeild, 80 g (84%), mp 243—245°C (decomp.), $[\alpha]_D^{17}$ +20.1° (*c* 1.9, 5N hydrochloric acid), $[\alpha]_D^{26}$ +19.6° (*c* 1.4, 6N hydrochloric acid). Reported, $[\alpha]_D^{25}$ +20.2° (*c* 0.1, 5N hydrochloric acid).[2)]

Found: C, 50.73; H, 8.35; N, 7.64%. Calcd for $C_8H_{15}O_4N$: C, 50.78; H, 7.99; N, 7.40%.

The ethyl acetate extract, which was rich in the D-form, was dried over sodium sulfate and concentrated *in vacuo*. The oily residue was refluxed with concentrated hydrochloric acid for 5 hr, and then the solution was concentrated to dryness. The residue was dissolved in water, the pH of the solution was adjusted to 3, and the D-α-aminosuberic acid which precipitated was collected by filtration. The crude crystals were recrystallized from hot water, as in the case of the L-form; yield, 42.5 g (45%), mp 243—245°C (decomp.), $[\alpha]_D^{15}$ −19.9° (*c* 2.1, 6N hydrochloric acid).

***N*-Carbobenzoxy-L-α-aminosuberic Acid (V).** D-Tyrosine hydrazide (29.3 g, 0.15 mol) and III (32.3 g, 0.1 mol) were dissolved in boiling methanol (600 m*l*), and a small amount of an insoluble material was filtered off. The resulting clear solution was concentrated to about 300 m*l*, and V was precipitated as the salt of D-tyrosine hydrazide by the careful addition of ethyl acetate (about 350 m*l*). When oily material appeared during the precipitation procedure, the addition of ethyl acetate was stopped and the oil was rubbed vigorously with a glass rod until it changed to crystals. After the solution had stood overnight at room temperature, the crystals were collected by filtration, washed with a mixture of methanol and ethyl acetat , and dried; yeild, 32.3 g (90%), mp 151—153°C. (The mother liquor was used later for the isolation of VI.). This material, which represented the salt of D-tyrosine hydrazide and V (2: 1 mol/mol), was recrystallized from ethanol (800 m*l*) for purification; yeild, 25.8 g (72%), mp 153—154°C, $[\alpha]_D^{24}$ −56.0° (*c* 1.8, water). A second crop of crystals was recovered from the mother liquor; weight, 2.7 g (7.4%), mp 153—154°C, $[\alpha]_D^{25}$ −56.1° (*c* 2, water).

Found: C, 57.20; H, 6.94; N, 13.66%. Calcd for $C_{34}H_{47}O_{10}N_7$: C, 57.21; H, 6.64; N, 13.73%. This salt (25.6 g, 0.036 mol) was suspended in ethyl acetate (120 m*l*), and the mixture was shaken with 4N hydrochloric acid (30 m*l*). The ethyl acetate layer was separated, washed with water, dried over sodium sulfate, and concentrated to a residue, which was then recrystallized from ethyl acetate and petroleum ether; yield of V, 10.8 g (93%), mp 119—121°C, $[\alpha]_D^{26}$ −3.3° (*c* 7.7, acetic acid), $[\alpha]_D^{25}$ −9.1° (*c* 4, dimethylformamide).

Found: C, 59.39; H, 6.56; N, 4.29%. Calcd for $C_{16}H_{21}O_6N$: C, 59.43; H, 6.55; N, 4.33%. The optical purity of this compound was confirmed after the removal of the protective group by catalytic hydrogenolysis; the compound IV thus obtained showed a mp of 246—248°C (decomp.), $[\alpha]_D^{28}$ +20.0° (*c* 2, 5N hydrochloric acid).

***N*-Carbobenzoxy-D-α-aminosuberic Acid (VI).** The mother liquor mentioned above was concentrated, and crude VI was extracted from the residue with ethyl acetate in the presence of hydrochloric acid. On the concentration of the extract, a residue of crystals was obtained; yield, 17.3 g, $[\alpha]_D^{25}$ +6.3° (*c* 4, dimethylformamide). A part of this material (3.23 g, 0.01 mol) was dissolved in boiling methanol, together with L-tyrosine hydrazide (3.90 g, 0.02 mol), and the mixture was treated as in the case of V. The yield of the salt of L-tyrosine hydrazide and VI was 4.86 g (73%); mp 153—154°C, $[\alpha]_D^{27}$ +56.1° (*c* 2, water). Compound VI was isolated from the salt as has been described previously; yield, 97%, mp 119—121°C, $[\alpha]_D^{28}$ +9.1°(*c* 4, dimethylformamide). D-α-Aminosuberic acid was obtained from VI by the removal of the protecting group; mp 246—248°C (decomp.), $[\alpha]_D^{28}$ −20.1° (*c* 2, 5N hydrochloric acid).