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Syntheses of Arginine Analogs. II.[1] Synthesis of L-2-Amino-4-guanidinobutyric Acid.

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The synthesis of DL-2-amino-3-guanidinopropionic acid as a structural analog of arginine had been described.[1] This paper deals with the synthesis of L-2-amino-4-guanidinobutyric acid.

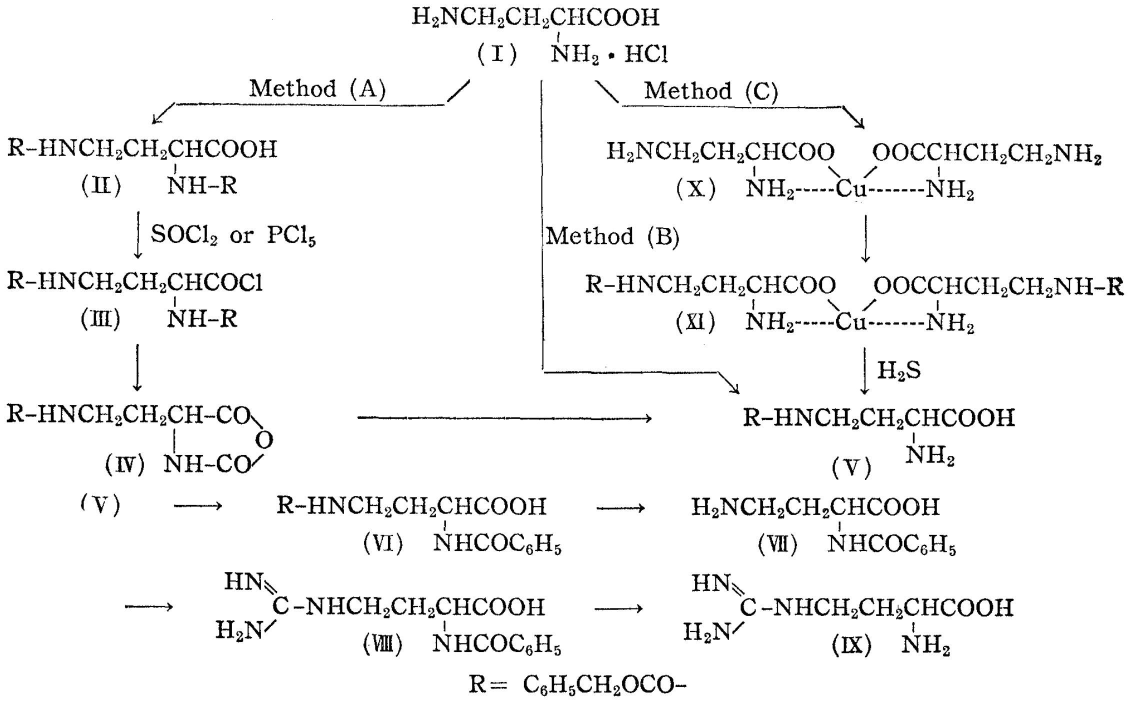

Chart 1.

Process of the Synthesis of L-2-Amino-4-guanidinobutyric Acid Dihydrochloride

The starting meterial (I) was prepared by Adamson's method and a good yield was obtained by improvement of reaction conditions. In the preparation of (V), the following three methods were tried. Method (A) was principally the same as the synthesis of DL-2-amino-3-benzyloxycarbonylaminopropionic acid from DL-2,3-diaminopropionic acid but the yield was very low. Method (B) consists of the reaction of benzyl chloroformate, treating reaction mixture with $CHCl_3$ and separation of insoluble L-2-amino-4-benzyloxycarbonylaminobutyric acid. The yield of (V) was only 6.8%. In method (C), after amino group at 2-position was protected by converting (I) into Cu-salt with basic cupric carbonate, 4-amino group was benzyloxycarbonylated and (V) was obtained by treating with H_2S. (V) was obtained with the maximum yield of 36.8% after examination of various reaction conditions. (VI) was obtained by benzoylation of 2-amino group of (V) in Schotten-Baumann's reaction, and (VII) was obtained in a good yield after catalytic reduction of (VI) with Pd-charcoal. Guanidination of (VII) with S-methylisothiourea sulfate

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[1] S. Takagi, *et al.* : This Bulletin, **7**, 616(1959).
[2] D. W. Adamson : J. Chem. Soc., **1939**, 1564.

Table I. Experimental Conditions in Guanidination of (VII) with S-Methylisothiourea Sulfate

Expt. No.	(VII) (g.)	S-Methylisothiourea sulfate (g.)	2*N* NaOH (cc.)	Yield (g.)	Yield (%)
1	0.96	0.55	4.0	0.42	36.5
2	0.96	0.83	3.0	1.09	96.3
3	0.96	1.10	3.0	1.00	88.0
4	3.50	3.20	12.0	3.87	94.2

was studied in various conditions and (VIII) was obtained as shown in Table I.

After removing benzoic acid formed on the hydrolysis of (VIII) with conc. HCl under reflux in a pressurized bottle, the reaction mixture was concentrated *in vacuo* and (IX) was purified by recrystallization from acetone and water, or ethanol and water. Dihydrochloride of (IX) was strongly hygroscopic and could not be obtained in crystalline form. The flavianate was obtained as needles of m.p. 260° and the benzylidene compound as white crystals of m.p. 214°.

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Experimental

L-2,4-Diaminobutyric Acid Monohydrochloride (I)—120 g. of L-glutamic acid was dissolved in 210 cc. of 100% H_2SO_4 and 470 cc. of 9.2% solution of hydrazoic acid in $CHCl_3$ was dropped during 5 hr. keeping the temperature at 43~46°. After completion of the reaction, the H_2SO_4 layer was poured into 750 g. of ice water and neutralized to pH 3~4 with hot saturated $Ba(OH)_2$ solution. $BaSO_4$ that separated out was removed by centrifugation and hot saturated aqueous solution of 350 g. of picric acid was added to this clear solution which was allowed to stand over night. Precipitated crystals were recovered by filtration and recrystallized from water. Yield, 265.5 g. m.p. 182~183°. The obtained crystals were dissolved in hot water (2500 cc.) and conc. HCl (1000 cc.) added. After cooling and removal of picric acid the filtrate was extracted with ether and evaporated to dryness on a water bath. Without purification, the dihydrochloride obtained was dissolved in 1500 cc. of boiling 95% EtOH and a solution of 40 g. of pyridine in 60 cc. of hot 95% EtOH was added. The separated crystals were collected and recrystallized from hydr. EtOH. Yield, 66.5 g. m.p. 233.5° (decomp.).

L-2,4-Bis(benzyloxycarbonylamino)butyric Acid (II)—1.5 g. of (I) was dissolved in 20 cc. of H_2O and 0.6 g. of MgO was added under stirring and cooling, 4 cc. of solution of benzyl chloroformate (0.85 g./cc.) was added, diluted with 4 cc. of Et_2O. After completion of the reaction, the solution was acidified to pH 2.0 with HCl to give white resinous substance. After extraction with $CHCl_3$, the extract was dried with Na_2SO_4, $CHCl_3$ was removed by evaporation, and the residue was kept for a long time in a desiccator to give a white powder; yield was 1.6 g. m.p. 112~114°.

L-2-Amino-4-benzyloxycarbonylaminobutyric Acid (V)—1.25 g. of (II) was dissolved in 1.5 cc. of hot $CHCl_3$, and 0.7 g. of PCl_5 was added to it under cooling. After keeping at 40° for 30 min., the reaction mixture was concentrated *in vacuo* to remove $CHCl_3$, the residue was dissolved in Me_2CO, 5*N* HCl was added, and left to stand for 18 hr. After removing HCl by vacuum evaporation, residue was dissolved in 5 cc. of H_2O and then neutrallized with NH_4OH to pH 7.0. The crystals formed were collected. Yield was 0.07 g. m.p. 235°. *Anal.* Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.13; H, 6.39; N, 11.17. Found: C, 57.22; H, 6.52; N, 11.27.

Method (C)—10 g. of (I) was converted into Cu salt and 104.0 cc. of 2*N* NaOH and 14.4 cc. of benzyl chloroformate solution in toluene (0.85 g./cc.), were added alternatively, 1/6 portion each and under vigorous stirring during about 1 hr. After it was restored to room temperature, stirring was continued for 30 min. and light blue precipitate produced was filtered and washed with water. The Cu salt thus obtained was suspended in 250 cc. of H_2O, treated with H_2S, heated, and CuS was removed by filtration. The residue was washed several times with hot water and the filtrate together with the washings were cooled to give crystals of (V), which were collected by filtration; yield was 3.0 g. Crystals obtained were found by admixture to be respectively identical with the ones obtained by methods (A) and (B).

Method (B)—1.08 g. (I) was dissolved in 10 cc. of *N* NaOH, and 1.4 cc. of benzyl chloroformate and 11.0 cc. of *N* NaOH were added alternatively, under stirring and cooling, so as to keep the solution constantly basic at pH above 11.0. After completion of the reaction, the solution was made acid to pH 2.0, the by-product, bis(benzyloxycarbonyl) compound, was extracted with $CHCl_3$, and L-2,4-bis(benzyloxycarbonyl)aminobutyric acid was obtained, yield was 1.25 g. Remaining solution after extraction with $CHCl_3$ was neutralized to pH 7.0 again, precipitating white crystals (V); yield was 0.12 g. m.p. 235°.

L-2-Benzamido-4-benzyloxycarbonylaminobutyric Acid (VI)—1.0 g. of (V) was dissolved in 4 cc. of *N* NaOH, and 4.5 cc. of *N* NaOH and 0.5 cc. of benzoyl chloride were added dropwise alternately under stirring and cooling for 30 min. After completion of the reaction, the solution was made acid to pH 3~4 with *N* HCl to give white gel, which was separated by filtration. It was recrystallized from aqueous Me_2CO; yield was 1.33 g. m.p. 143~143.5°. $[\alpha]_D^{15}$ +5.37(Me_2CO). *Anal.* Calcd. for $C_{19}H_{20}O_5N_2$: C, 64.03; H, 5.65; N, 7.86. Found : C, 64.02; H, 5.63; N, 7.68.

L-2-Benzamido-4-aminobutyric Acid (VI)—6.8 g. of (VI) was dissolved in 180 cc. of MeOH, 3.8 cc. of AcOH and 2.5 g. of 5% Pd-C were added, and H_2 gas was vigorously introduced for 6 hr. under heating at 50°. After the reaction completed, the solution was boiled, filtered with heating, the filtrate was concentrated *in vacuo*, and white crystals were obtained. Recrystallized from 30% Me_2CO, yield was 2.5 g. m.p. 213°. $[\alpha]_D^{15}$ +15.1(H_2O). *Anal.* Calcd. for $C_{11}H_{14}O_3N_2 \cdot H_2O$: C, 54.98; H, 6.71; N, 11.65. Found : C, 54.76; H, 6.84; N, 11.90.

L-2-Benzamido-4-guanidinobutyric Acid (VIII)—(Ⅷ) was dissolved in 2*N* NaOH and after addition of S-methylisothiourea sulfate, it was heated at 70° for 15 min. Crystals were formed on cooling and recrystallized from H_2O. m.p. 247~248°. $[\alpha]_D^{17}$ −32.48(*N* HCl). Reaction conditions were examined and results are shown in Table I. *Anal.* Calcd. for $C_{12}H_{16}O_3N_4 \cdot H_2O$: C, 51.05; H, 6.45; N, 19.85. Found : C, 51.17; H, 6.45; N, 19.49.

L-2-Amino-4-guanidinobutyric Acid Dihydrochloride (IX)—0.5 g. of (Ⅷ) was dissolved in 10 cc. of conc. HCl and was hydrolyzed by heating to 120~130° for 5 hr. in a pressurized bottle. Precipitated benzoic acid was filtered off when cool. Filtrate was concentrated *in vacuo* and residual benzoic acid was further removed. Water was added again and concentration was continued repeatedly to remove the excess of HCl. A syrupy substance thus obtained was purified from Me_2CO and water, and was left in a desiccator. It was strongly hygroscopic. *Anal.* Calcd. for $C_5H_{14}O_2N_4Cl_2$: N, 24.03. Found : N, 24.21. $[\alpha]_D^{15}$ +36.0(2*N* HCl). Rf : 0.12 (BuOH : AcOH : H_2O = 4 : 1 : 5).

Flavianate : m.p. 260°(decomp.). *Anal.* Calcd. for $C_{15}H_{18}O_{10}N_6S$: C, 37.97; H, 3.82. Found : C, 37.96; H, 4.06.

Benzylidene compound : m.p. 214~215°(decomp.). *Anal.* Calcd. for $C_{12}H_{16}O_2N_4$: C, 58.06; H, 6.45; N, 22.58. Found : C, 57.67; H, 6.61; N, 22.10.

Summary

L-2-Amino-4-guanidinobutyric acid was prepared in a fairly good yield from L-2,4-diaminobutyric acid by treatment with S-methylisothiourea sulfate after converting the acid to its copper salt and protecting the amino groups in 2- and 4-positions respectively with benzoyl and benzyloxycarbonyl group.

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