

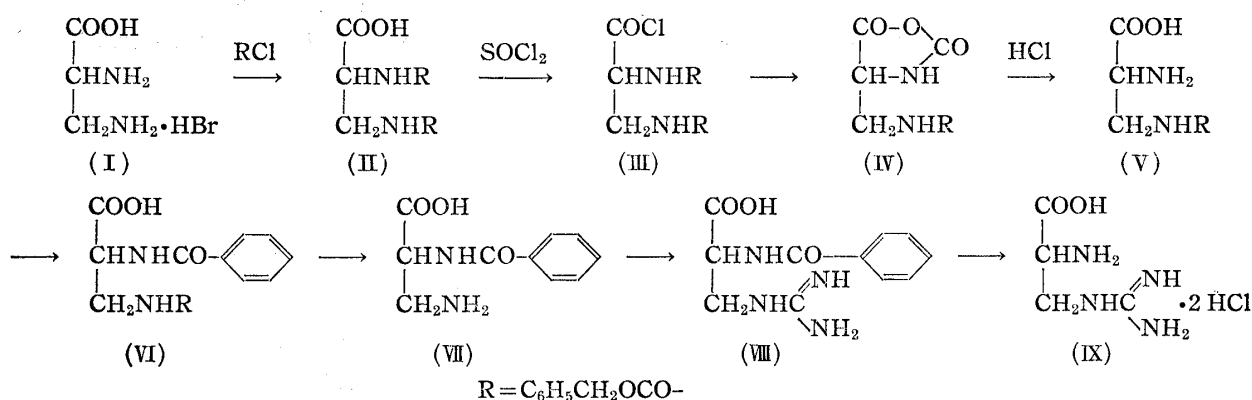
113. Seishi Takagi, Hiroaki Tsukatani, and Kyoze Hayashi :
 Syntheses of Arginine Analogs. I. Synthesis
 of DL-2-Amino-3-guanidinopropionic Acid.

(Pharmaceutical Institute, Medical Faculty, University of Kyoto*)

Some amino acids which have guanidino group have been found among the natural amino acids, such as arginine and canavanine. In an attempt to investigate their biological activity, some of the analogs of arginine were synthesized. This paper deals with the synthesis of DL-2-amino-3-guanidinopropionic acid.

Many attempts nevertheless have been made to prepare homologs of arginine, notably by Winterstein,¹⁾ Kossel,²⁾ Heckel,³⁾ and Steib.⁴⁾ The early methods simply employed the cyanamide reaction with DL-2,3-diaminopropionic acid and a mixture of mono- and diguanidino derivatives was obtained with very low yield. Greenstein⁵⁾ reported that S-methylisothiourea was reacted with lysylglutamic acid and monoguanidino derivative was obtained with the contamination of a small quantity of diguanidino derivative.

Later Steib⁴⁾ reported a new method converting amino group into guanidino group which involves the protection of amino group. This method was improved by Greenstein⁶⁾ and DL-homoarginine was successfully synthesized in a good yield. The authors applied this method for the synthesis of DL-2-amino-3-guanidinopropionic acid.



In regard to DL-2-amino-3-benzyloxycarbonylamino propionic acid (V), Kajaer⁷⁾ obtained it together with a large quantity of DL-2,3-bis(benzyloxycarbonylamino)propionic acid (II), which was produced even when equivalent of benzyl chloroformate was reacted with (I). Therefore, the authors first prepared (II), converted it into (III) using thionyl chloride, and obtained the anhydride (IV). Similar to other anhydrides of amino acid, (IV) was very sensitive to moisture, and easily lost carbon dioxide and polymerized. (VI) was prepared by Schotten-Baumann reaction of (V) and then reduced to (VII) using Pd-charcoal as a catalyst. (VII) was reacted with S-methylisothiourea sulfate to form

* Sakyo-ku, Kyoto (高木誠司, 塚谷博昭, 林 恭三).

- 1) E. Winterstein, *et al.*: *Z. Physiol. Chem.*, **59**, 141(1909).
- 2) A. Kossel, F. Weiss: *Ibid.*, **84**, 1(1913).
- 3) F. Heckel: *Monatsh.*, **29**, 779(1913).
- 4) H. Steib: *Z. physiol. Chem.*, **155**, 292(1926).
- 5) J.P. Greenstein, *et al.*: *J. Biol. Chem.*, **111**, 245(1935).
- 6) J.P. Greenstein: *J. Org. Chem.*, **2**, 480(1935).
- 7) A. Kajaer: *Acta Chem. Scand.*, **3**, 1087(1949).

(VIII) which gave positive result in Sakaguchi reaction. (VIII) was hydrolyzed by usual method and (IX) was obtained as white needles melting at 180~181°.

The authors express their deep gratitude to Prof. T. Suzuki for his kind guidance throughout the course of this work, and thanks are due to the member of the microanalytical center of this Institute for the microanalysis.

Experimental

DL-2,3-Bis(benzyloxycarbonylamino)propionic Acid (II)—It was obtained from DL-2,3-diaminopropionic acid HBr (9.3 g.), benzyl chloroformate (0.85 g./cc.) (22 cc.), and 2*N* NaOH (127 cc.). White crystals (from CHCl₃), m.p. 122~123.5°. Yield, 19.9 g.

DL-2-Amino-3-benzyloxycarbonylamino propionic Anhydride (IV)—1.01 g. of (II) was dissolved in 7 cc. of dry CHCl₃ and 0.8 cc. of SOCl₂ was added dropwise while cooling with ice. The mixture was heated on a water bath at 45~50° for 40 min. It was then concentrated *in vacuo* and SOCl₂ was removed. Further 5 cc. of dry CHCl₃ was added, heated on a water bath at 45~50° for 30 min., concentrated *in vacuo*, and SOCl₂ was completely removed. 4 cc. of dry AcOEt was added, dissolved by heating for a short time, 8 cc. of petr. ether was added, and then left standing. A slightly yellow crystals m.p. 127~128°, separated out. Yield, 0.62 g.

DL-2-Amino-3-benzyloxycarbonylamino propionic Acid (V)—1) 0.62 g. of (IV) was dissolved in 5 cc. of Me₂CO, to which was added 10 cc. of 5*N* HCl and left standing at room temperature for about 40 hr. After reaction was complete, Me₂CO was removed *in vacuo* and the residue was dissolved in 5 cc. of *N* HCl. The small quantity of the yellow, insoluble oily substance which separated was filtered off and the filtrate neutralized to pH 5.0 with dil. NH₄OH. The white crystals separating out were filtered and recrystallized from 50% EtOH, obtaining white plate crystals, m.p. 242~243°(decomp.). Yield, 0.51 g. This was identified by admixture with the authentic specimens.

2) 1.1 g. of (II) was dissolved by heating with 7 cc. of CHCl₃ and 0.8 cc. of SOCl₂ was added dropwise while cooling. After heating the mixture at 50° for 40 min., it was concentrated *in vacuo* and the residue was dissolved in 2 cc. of Me₂CO, 5 cc. of 5*N* HCl was added, and left standing at room temperature. After 40 hr., it was concentrated *in vacuo*, the residue was dissolved in 2 cc. of water, and then neutralized with dil. NH₄OH. The crystals separated were filtered. Yield, 0.55 g., m.p. 242~243°(decomp.). This was identified by admixture with the authentic specimen.

3) After 10 g. of (I) was converted into Cu salt, 103.5 cc. of 2*N* NaOH and 11.5 cc. of toluene solution of benzyl chloroformate (0.85 g./cc.) were added alternately, taking about 1 hr. for the addition, under cooling and vigorous stirring, and then the mixture was stirred for 30 min. at room temperature, green precipitate produced was filtered and washed with water. This Cu salt was suspended in water, decomposed with H₂S, heated, and CuS was filtered off. CuS was washed several times with hot water and the washing was added to the filtrate. Crystals separated on cooling was collected by filtration. Yield, 3.1 g., m.p. 242~243°(decomp.). This was identified by admixture with the authentic specimen.

4) 2.3 g. of (I) was dissolved in 25 cc. of *N* NaOH, and 6.3 cc. of ether solution containing 2.2 g. of benzyl chloroformate and 25 cc. of *N* NaOH were added alternately under stirring, cooling, and at strong alkalinity of over pH 11.0. After the reaction was completed, the residue was collected by filtration, suspended in water, and acidified with *N* HCl under cooling. The crystals separating out were filtered. Yield, 1.2 g., m.p. 121~122°. The crystals obtained were DL-2,3-bis(benzyloxycarbonylamino)propionic acid (II) which was identified by admixture with the authentic specimen. After acidifying the filtrate was extracted 3 times with 20 cc. of CHCl₃, dried over Na₂SO₄, and CHCl₃ was distilled off, further 0.5 g. of (II) was obtained. The residue from CHCl₃ extraction was concentrated *in vacuo*, HCl was removed as much as possible, the residue was dissolved in a small amount of water, and made to pH 7.0 with *N* NaOH. The crystals that separated were collected, recrystallized from 50% EtOH, and thus (V) was obtained. Yield, 0.39 g., m.p. 242~243°(decomp.). Further 0.11 g. of crystals were obtained by concentration of the filtrate. Total yield of (V) was 0.50 g. *Anal.* Calcd. for C₁₁H₁₄O₄N₂: C, 55.45; H, 5.92; N, 11.76. Found: C, 55.77; H, 6.15; N, 11.73.

DL-2-Benzamido-3-benzyloxycarbonylamino propionic Acid (VI)—4.76 g. of (V) was dissolved in 21 cc. of *N* NaOH, 25 cc. of *N* NaOH and 2.5 cc. of benzoyl chloride were added dropwise at the same time under cooling and vigorous stirring, stirring was continued for another 30 min. at room temperature. After the reaction was completed it was acidified with *N* HCl and the white precipitate changed into a white syrup. The clear layer was decanted off, the syrupy substance was extracted with benzene, and dried over Na₂SO₄. The substance obtained by distilling off benzene was extracted 4 times with 25 cc. of petr. ether to remove benzoic acid and the residue was re-

crystallized from dil. Me₂CO. Yield, 5.05 g., m.p. 150~151°. Further 1.0 g. was obtained from the recrystallizing mother liquor.

DL-2-Benzamido-3-aminopropionic Acid (VII)—5.95 g. of (VI) was dissolved in 140 cc. of 50% MeOH, 3.5 cc. of AcOH was added, 1.5 g. of Pd-charcoal (5% Pd) was added, and hydrogen gas was vigorously introduced while heating at 50° with stirring. While the reaction was progressing, water was added, because MeOH was evaporating out. Hydrogen gas was passed through for about 6 hr. After the reaction was completed, it was filtered, the filtrate was concentrated, MeOH was added, and 0.61 g. of crystals was obtained after standing. The Pd-charcoal was washed several times with hot water, the washings were concentrated *in vacuo*, and 2.41 g. of crystals were obtained. Total yield, 3.02 g., m.p. 226~227°(decomp.). *Anal.* Calcd. for C₁₀H₁₂O₃N₂: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.75; H, 6.05; N, 13.49.

DL-2-Benzamido-3-guanidinopropionic Acid (VIII)—0.83 g. of (VII) was dissolved in 3 cc. of 2*N* NaOH, to which was added 0.8 g. of S-methylisothiourea sulfate and heated on a water bath at 70° for 15 min. This was left standing in an ice box, the crystals were obtained by filtration, and then recrystallized from water. Yield, 0.91 g. White needles, m.p. 240~242°(decomp.). *Anal.* Calcd. for C₁₁H₁₄O₃N₄: C, 52.79; H, 5.64; N, 22.39. Found: C, 53.03; H 5.46; N, 22.18.

DL-2-Amino-3-guanidinopropionic Acid Dihydrochloride (IX)—0.5 g. of (VIII) and 10 cc. of 5*N* HCl were heated in an autoclave at 120~130° for 5 hr. After hydrolysis was completed, the benzoic acid that separated was removed by filtration, the filtrate was concentrated *in vacuo*, and extracted with ether. After benzoic acid was removed, it was further concentrated *in vacuo* and the syrupy substance obtained was recrystallized from water and Me₂CO. Yield, 0.2 g., m.p. 180~181°(decomp.). *Anal.* Calcd. for C₄H₁₂O₂N₄Cl₂: N, 25.58. Found: N, 25.11.

Flavianate: m.p. 256°(decomp.). *Anal.* Calcd. for C₁₄H₁₆O₁₀N₆S: C, 36.52; H, 3.50; N, 18.26. Found: C, 36.54; H, 3.29; N, 17.97.

Benzylidene-DL-2-amino-3-guanidinopropionic Acid: m.p. 171.5~172°(decomp.). *Anal.* Calcd. for C₁₁H₁₄O₂N₄·1½ H₂O: C, 50.56; H, 6.56; N, 21.45. Found: C, 50.86; H, 6.68; N, 21.44. Rf value: 0.11 (BuOH:HOAc:H₂O=4:1:5).

Summary

DL-2-Amino-3-guanidinopropionic acid was prepared in a fairly good yield from DL-2,3-diaminopropionic acid by treatment with S-methylisothiourea sulfate after protection of the amino groups in 2- and 3-positions respectively with benzoyl and benzyloxy-carbonyl group.

(Received January 8, 1959)