to react very slowly in EtOH at room temperature, since azophenine (V) scarcely dissolved even in hot EtOH containing aniline and glacial AcOH in the ratio mentioned above.

Summary

The mechanisms of formation of products obtained by oxidation of aniline with periodic acid, (I) to (VI) reported in Part X of this series, are discussed. Mechanism for the production of (I), (IV), and (VI) is considered to involve the action of hydroxyl radical. In the formation of (V) from N-phenyl-*p*-benzoquinone diimine, a radical reaction is assumed to play a part.

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36. **Seishi Takagi and Kyozo Hayashi**: Studies on the Synthesis of Amino Acids by the Schmidt Reaction. III.* Synthesis of DL-Homolysine.

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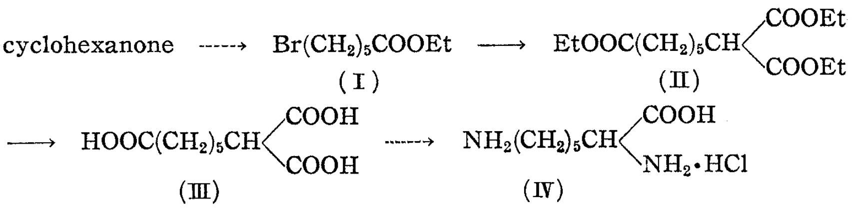
Harris, *et al.*[1] has reported that they synthesized DL-homolysine by acetamidomalonate method and obtained crystalline monohydrochloride monohydrate of (IV) melting at 176°. The authors obtained a different result from that of Harris, *et al.* in the synthesis of DL-homolysine by the following several methods and obtained it as a monohydrochloride melting at 263°.

In the first method, 1,1,6-hexanetricarboxylic acid was reacted with more than 2 moles of hydrazoic acid, in the presence of conc. sulfuric acid as the catalyst. In the second method, 7-aminoheptanoic acid was obtained from 1,8-octanedioic acid by the procedure previously described[2] and DL-2-amino-7-benzamidoheptanoic acid formed was hydrolyzed. In the third method, ethyl 2-acetamido-2-cyano-7-benzamidoheptanoate, obtained by the condensation of ethyl acetamidocyanoacetate with 1-chloro-5-benzamidopentane, was hydrolyzed.

By these methods, the objective was successfully obtained as white needles melting at 263°, instead of the substance melting at 176° reported by Harris, *et al.*

The first method was carried out by the route shown in Chart 1.



$$\text{cyclohexanone} \dashrightarrow \underset{(\mathrm{I})}{Br(CH_2)_5COOEt} \longrightarrow \underset{(\mathrm{II})}{EtOOC(CH_2)_5CH\langle^{COOEt}_{COOEt}}$$

$$\longrightarrow \underset{(\mathrm{III})}{HOOC(CH_2)_5CH\langle^{COOH}_{COOH}} \dashrightarrow \underset{(\mathrm{IV})}{NH_2(CH_2)_5CH\langle^{COOH}_{NH_2 \cdot HCl}}$$

Chart 1.

Ethyl 5-bromocaproate was synthesized from cyclohexanone by the method of Brown, *et al.*[3] and condensed with diethyl malonate to give triethyl 1,1,6-hexanetricarboxylate (II). This was hydrolyzed by the general method and 1,1,6-hexanetricarboxylic acid (III) so formed was reacted with a slight excess of 2 moles of hydrazoic acid to form (IV). From the reaction mixture, (IV) was isolated and purified as previously reported.[2] (IV) was adsorbed on Amberlite IR-120, eluted with 0.5~0.6*N* ammonia

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* Part II: This Bulletin, **7**, 99(1959).

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[1] J. I. Harris, T. S. Work: Biochem. J., **46**, 190(1950).

[2] S. Takagi, K. Hayashi: This Bulletin, **7**, 96(1959).

[3] G. B. Brown, C. W. H. Partridge: J. Am. Chem. Soc., **66**, 839(1944).

and the effluent was concentrated *in vacuo*. In this case, (IV) readily formed a carbonate and the free amino acid could not be obtained easily. Therefore, the syrupy concentrated residue was treated with dil. hydrochloric acid and ethanol, and white needles melting at 263° were obtained. This substance gave analytical data agreeing with those calculated for monohydrochloride of (IV) having the formula of $C_7H_{17}O_2N_2Cl$. Harris, *et al.* reported that (IV) was isolated as its monohydrochloride monohydrate melting at 176°, but such crystals were not obtained. The substance here obtained gave a single spot on paper chromatogram. By Schotten–Baumann's method using benzoyl chloride, the substance gave a dibenzoate having the formula of $C_{21}H_{24}O_4N_2$, m.p. 154∼155°. Therefore, it was considered that the substance must be (IV).

The second method was as shown in Chart 2.

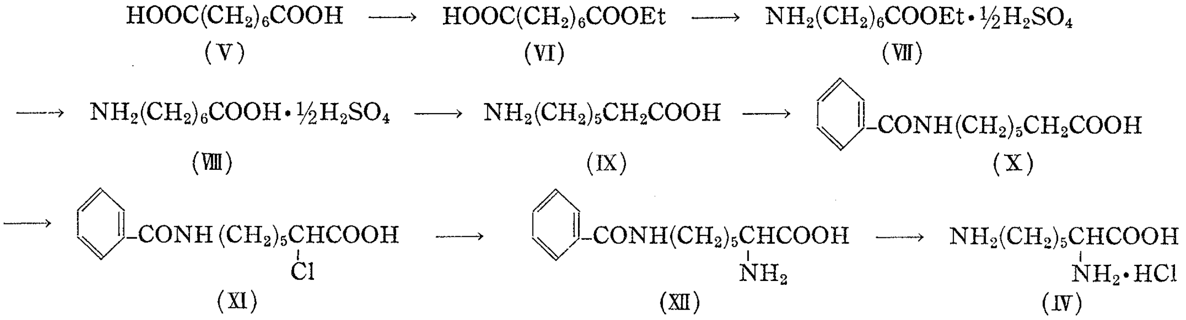

Chart 2.

7-Ethoxycarbonylheptanoic acid (VI), which was obtained by esterification of (V) with a calculated amount of ethanol saturated with hydrogen chloride gas and a slight amount of conc. sulfuric acid as a catalyst, was reacted with more than a calculated amount of hydrazoic acid as previously described, and 7-aminoheptanoic acid (IX) was obtained. From this acid, dihydrochloride of (IV) was obtained by benzoylation, chlorination, amination, and hydrolysis. The final reaction mixture was dissolved in water, the aqueous solution was passed through a column of Amberlite IR-4B, and the effluent was concentrated *in vacuo*. The residue was dissolved in a small amount of water, adjusted to pH 4.0∼5.0 with dil. hydrochloric acid, and ethanol was added to the concentrated solution. White crystals obtained were recrystallized from hydr. ethano lto crystals melting at 263°. This substance was identified by admixture with (IV) obtained from 1,1,6-hexanetricarboxylic acid.

The first and second methods afforded DL-homolysine monohydrochloride as crystals having the same melting point as mentioned above. In the third method, 1-chloro-5-benzamidopentane was condensed with acetamidocyanoacetate and hydrolyzed with hydrochloric acid. Excess of the acid was removed *in vacuo* and the residue was extracted with ethanol. The extract was evaporated, the residue was treated with phosphotungstic acid, and after the general procedure, crystals of DL-homolysine monohydrochloride was obtained. The admixture of the crystals so obtained and (IV) obtained by the former two methods showed no melting point depression.

In an attempt to obtain the same substance of m.p. 176° as that of Harris, *et al.*, the same method (Chart 3) reported by Harris and Work[1)] was followed but white needles melting at 263°, instead of 176°, were obtained.

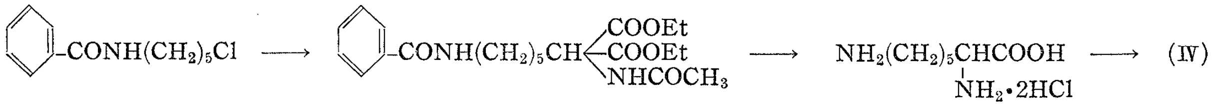

Chart 3.

The substance obtained by the above method corresponds to formula $C_7H_{17}O_2N_2Cl$ and was identified with the monohydrochloride of (IV).

The authors' thanks are due to the members of the microanalytical center of this Institute for the microanalyses.

Experimental

Ethyl 5-Bromocaproate3)—A mixture of 710 cc. of conc. H_2SO_4 in 240 cc. of water was cooled to below 10° and 500 g. of $K_2S_2O_8$ was gradually added. 500 cc. of EtOH was added to the mixture maintained at the same temperature and 90 g. of cyclohexanone dissolved in 130 cc. of EtOH was added slowly with vigorous stirring. After the addition of cyclohexanone was completed, stirring was continued for a few hrs., 2 L. of water was added to the mixture, and the diluted mixture was filtered. The filtrate was extracted with 800 cc. of ether, the extract was dried over Na_2SO_4, and ether was evaporated, leaving 90 g. of crude lactone. Without purification, 500 cc. of 48% HBr and 120 cc. of conc. H_2SO_4 were added to 90 g. of this lactone. The mixture was kept at room temperature for 2 hrs. and then heated on a water bath at 90~100° for 4 hrs. The reaction mixture was cooled and poured into 1 L. of water. The lower layer was separated, the aqueous layer was saturated with $(NH_4)_2SO_4$, and extracted with ether. The ether solution was added to the lower layer, the mixture was dried over Na_2SO_4, ether was removed, and 120 g. of crude bromo-acid was obtained. To this was added 300 cc. of dehyd. EtOH and 7.5 cc. of conc. H_2SO_4, and the mixture was heated under reflux for 8 hrs. The reaction mixture was concentrated *in vacuo* and excess of EtOH was removed. The residue was extracted with ether and the ether was removed. The residue was extracted with ether and the extract was washed with water, dried over Na_2SO_4, and fractional distillation of the residue gave a fraction of $b.p_{13}$ 115~123°. Yield, 81 g.

Triethyl 1,1,6-Hexanetricarboxylate—20 g. of Na metal was dissolved in 30 cc. of dehyd. EtOH and to this, 20 g. of diethyl malonate and 6.5 cc. of benzene were added. Under vigorous stirring, 27 g. of 5-bromocaproate was gradually added to the ethanolic solution and the mixture was heated on a water bath under reflux until the reaction mixture became neutral. EtOH was removed by distillation and some water was added to the residue to dissolve the crystalline NaBr in the residue. Oily layer was separated and the aqueous layer was extracted with ether. The ethereal solution was added to the oily layer and the mixture was dried over Na_2SO_4 and fractional distillation gave a fraction of $b.p_8$ 170~180°. Yield, 18 g.

1,1,6-Hexanetricarboxylic Acid—18 g. of triethyl 1,1,6-hexanetricarboxylate was hydrolyzed with 50% KOH according to the general method, neutralized with a calculated amount of HCl, extracted 3 times with 30 cc. of ether, and ether was evaporated. 1,1,6-Hexanetricarboxylic acid was obtained as white prisms (from a mixture of ether and petr. ether), m.p. 96~98°. Yield, 10.5 g.

Reaction of 1,1,6-Hexanetricarboxylic Acid and Hydrazoic Acid: Isolation of DL-Homolysine—A solution of 1.43 g. of 1,1,6-hexanetricarboxylic acid dissolved in 6.0 cc. of 100% H_2SO_4 was added with 10 cc. of $CHCl_3$, the mixture was heated to 50~60°, and under vigorous stirring, 17 cc. of $CHCl_3$ containing 6% of hydrazoic acid was added gradually to the mixture. After all the hydrazoic acid solution was added, the mixture was further heated for 3 hrs. under stirring. The upper $CHCl_3$ layer was separated and the lower H_2SO_4 solution was neutralized with hot saturated $Ba(OH)_2$ solution until pH 2~3. Precipitated $BaSO_4$ was removed by filtration and the filtrate was passed through a column of Amberlite IR-120. The column was washed with water until no trace of SO_4^{2-} was detected in the eluate. The adsorbed DL-homolysine was eluted with 0.3~0.5*N* NH_4OH and the effluent was concentrated to 15 cc. *in vacuo*. The residue was neutralized with dil. HCl to pH 4~5, further concentrated, and EtOH was added to the residue. Crystallized DL-homolysine was collected and recrystallized from hydr. EtOH, m.p. 263°. Yield, 0.98 g. *Anal.* Calcd. for $C_7H_{17}O_2N_2Cl$: C, 42.75; H, 8.65; N, 14.25. Found: C, 42.90; H, 8.65; N, 14.17. Rf 0.11 (BuOH : AcOH : H_2O=4 : 1 : 1).

Dibenzoyl-DL-homolysine—The Schotten-Baumann reaction was carried out with 0.5 g. of DL-homolysine monohydrochloride, 10 cc. of water, 1.6 g. of Na_2CO_3, and 0.56 g. of BzCl. White plates (from a mixture of EtOH and petr. ether), m.p. 154~155°. Yield, 0.9 g. *Anal.* Calcd. for $C_{21}H_{24}O_4N_2$: C, 68.44; H, 6.57; N, 7.61. Found: C, 68.08; H, 6.55; N, 7.59.

7-Ethoxycarbonylheptanoic Acid (VI)—34 g. of (V) was placed in a Claisen flask, 9.1 g. of dehyd. EtOH saturated with dry HCl gas and 25 cc. of butyl ester were added, and the mixture was heated in an oil bath. (V) gradually dissolved and after about 1 hr., the mixture became homogeneous. The mixture was further refluxed for 5 hrs. and the ester was isolated by a fractional distillation, as a fraction of $b.p_3$ 205°. Yield, 28 g.

7-Aminoheptanoic Acid (IX)—(VII) was prepared from 5 g. of (VI), 10 cc. of conc. H_2SO_4, 15 cc. of benzene, and 18 cc. of benzene containing 6.5% of hydrazoic acid previously described.4) Without isolating ethyl 7-aminoheptanoate, the reaction mixture was hydrolyzed and the solution was passed through a column of Amberlite IR-4B. After the effluent was concentrated *in vacuo*, EtOH was added to the residue and separated crystals were collected as white plates (from dil. acetone), m.p.

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4) S. Takagi, K. Hayashi: This Bulletin, **6**, 96(1958).

192~193°. Yield, 2.1 g. *Anal.* Calcd. for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65. Found : C, 57.70; H, 10.49; N, 9.90.

7-Benzamidoheptanoic Acid (X)—The Schotten-Baumann reaction was carried out with 2.9 g. of (IX), 3.2 g. of NaOH, 30 cc. of water, and 5.7 g. of BzCl. Reaction mixture was treated as in general procedure. White plates (from acetone-petr. ether), m.p. 82~83°. Yield, 4.2 g. *Anal.* Calcd. for $C_{14}H_{19}O_3N$: C, 67.44; H, 7.68; N, 5.62. Found : C, 67.15; H, 7.81; N, 5.71.

DL-2-Chloro-7-benzamidoheptanoic Acid—To the mixture of 2 g. (X) and 0.04 g. of iodine, 5 cc. of SO_2Cl_2 was added drop by drop under cooling. After all SO_2Cl_2 was added, the mixture was heated at 60~65° for 1.5 hrs. and then at 90~100° for 1 hr. Excess of SO_2Cl_2 was removed *in vacuo.* The viscous oily substance obtained was washed with water and twice with hot water. The substance was used in the next reaction without purifying. Yield, 2.0 g.

DL-2-Amino-7-benzamidoheptanoic Acid (XII)—A solution of 2.0 g. of (XI) dissolved in 40 cc. of 28% NH_4OH was heated in a pressurized bottle on a water bath for 7~8 hrs. After cool, crystals that separated were filtered, the filtrate was further concentrated *in vacuo,* and white plates (from hydr. EtOH), m.p. 249~250°, were obtained. Yield, 1.1 g. *Anal.* Calcd. for $C_{14}H_{20}O_3N_2$: C, 63.61; H, 7.63; N, 10.60. Found : C, 63.35; H, 7.78; N, 10.53.

Preparation of DL-Homolysine Monohydrochloride (IV) by Hydrolysis of (XII)—A mixture of 0.5 g. of (XII) and 8 cc. of 20% HCl was heated under reflux in an oil bath for 7~8 hrs. When cool, the crystallized benzoic acid was filtered off and the filtrate was concentrated *in vacuo* to remove excess of HCl as much as possible. The residue was dissolved in a small amount of water, treated with activated carbon, and concentrated *in vacuo.* The residue was dissolved in dehyd. EtOH and acetone added until the solution became slightly turbid. The mixture was kept in a refrigerator but crystalline substance was not obtained. The oily substance was dissolved in about 20 cc. of water, passed through a column of Amberlite IR-120, and treated as described above. White needles, m.p. 263°. Yield, 0.25 g. *Anal.* Calcd. for $C_7H_{16}O_2N_2{\cdot}HCl$: C, 42.74; H, 8.71; N, 14.25. Found : C, 42.69; H, 8.65; N, 14.21.

Preparation of DL-Homolysine (IV) by Acetamidocyanoacetate Method—To a solution of 0.23 g. of Na dissolved in 20 cc. of dehyd. EtOH, 1.5 g. of ethyl acetamidocyanoacetate and 2.3 g. of 1-chloro-5-benzamidopentane were added and the mixture was heated under reflux until it became neutral. EtOH was removed *in vacuo* and the oily substance formed was extracted with ether. The ethereal solution was dried over Na_2SO_4 and ether was removed by distillation. The residue was heated with 20 cc. of 20% HCl under reflux for 7~8 hrs. to effect hydrolysis. On cooling, separated benzoic acid was filtered off, the filtrate was concentrated *in vacuo,* and treated with phosphotungstic acid in the same way as that of Harris, *et al.* described below. White needles, m.p. 264°. Yield, 1.2 g.

Preparation of DL-Homolysine (IV) by the Method of Harris, *et al.*—To a solution of 1.2 g. of Na dissolved in 30 cc. of dehyd. EtOH, 16 g. of diethyl acetamidomalonate and 8.8 g. of 1-chloro-5-benzamidopentane were added and the mixture was refluxed for 18 hrs. EtOH was removed *in vacuo* and the oily substance obtained was extracted with ether. The ethereal solution was dried over Na_2SO_4, ether was removed, and the residue was hydrolyzed with 5 g. of 20% KOH at 95° for 2 hrs. The insoluble oily substance was removed and the alkaline solution was acidified with an excess of HCl whereupon an oily substance separated. The collected oily substance was heated with 6*N* HCl for 10 hrs. under reflux. Crystallized benzoic acid was removed by filtration and the filtrate was concentrated *in vacuo.* The syrupy residue was extracted with EtOH and the EtOH solution was concentrated. The residue did not crystallize, and was dissolved in *N* HCl and an excess of phosphotungstic acid was added to this. Precipitated phosphotungstate was filtered off, the filtrate was concentrated *in vacuo* to about 15 cc., and it was acidified with dil. HCl to pH 4.0~5.0. The residue was further concentrated *in vacuo* and EtOH added. (IV) was recrystallized from dil. EtOH, m.p. 263°. Yield, 1.4 g.

Summary

Harris, *et al.* synthesized DL-homolysine (IV) by the acetamidomalonate method and obtained its monohydrochloride monohydrate as crystals melting at 176°. (IV) was prepared as its monohydrochloride, m.p. 263°, by the reaction of hydrazoic acid and 1,1,6-hexanetricarboxylic acid, which was obtained from cyclohexanone, and (IV) was derived to its benzoate. Two different methods of preparation were further tried, one from 7-aminoheptanoic acid and the other from 1-chloro-5-benzamidopentane, and the same substance melting at 263° was obtained in both cases. The procedure reported by Harris, *et al.* was followed exactly but the substance of m.p. 263° was obtained instead of that melting at 176° reported by Harris, *et al.*

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