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37. Kyozo Hayashi: Studies on the Synthesis of Amino Acids by the Schmidt Reaction. IV.* Synthesis of DL-Homoserine.

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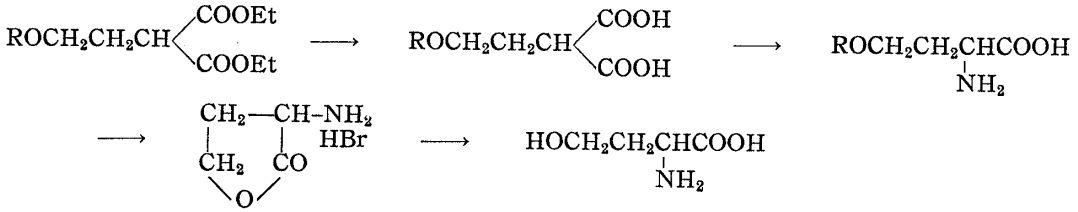
Homoserine is a natural amino acid which is a precursor in biosynthesis of methionine, and threonine, and found widely in nature.[1] Several methods for the preparation of this amino acid have been reported. For example, Fischer, *et al.*[2] prepared it by hydrolysis of DL-2-amino-4-phenoxybutyric acid with 48% hydrobromic acid and DL-2-amino-γ-butyrolactone hydrobromide formed was treated with silver carbonate and hydrogen sulfide to obtain DL-homoserine. Livak, *et al.*[3] also prepared DL-homoserine, first preparing DL-2-amino-4-hydroxybutyramide from γ-butyrolactone and treating it with lead oxide and hydrogen sulfide to obtain DL-homoserine. These methods are complicated in the isolation and there is a fear of contamination of inorganic salts in the product.

The synthesis of DL-homoserine was attempted through Schmidt reaction, starting from (2-alkoxyethyl)malonic acid or ethyl 2-(2-alkoxyethyl)acetoacetate and DL-homoserine was obtained from the latter in a good yield. The reaction process from (2-alkoxyethyl)malonic acid is shown in Chart 1.



R = CH_3–, C_2H_5–, C_3H_7–, C_4H_9–, $C_6H_5CH_2$–

Chart 1.

Ethyl (2-alkoxyethyl)malonate, obtained by Palomma's method, was hydrolyzed with potassium hydroxide giving (2-alkoxyethyl)malonic acid. This product, however, could not be crystallized and was used for the succeeding reaction in oily state. It was reacted with hydrazoic acid as a benzene solution, in the presence of sulfuric acid as a catalyst. The amino acid formed was treated with ion exchange resin for separation and purification. In the above reaction, when the alkyl was an aliphatic group, the amino acid was obtained in a low yield as shown in Table I, while in the case of aromatic group, expected amino acid was not obtained.

Table I.

Amino acid produced	Yield (%)	m.p. (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
DL-2-Amino-4-methoxybutyric acid	23	240	$C_5H_{11}O_3N$	45.10	8.33	10.52	45.28	8.26	10.78
DL-2-Amino-4-ethoxybutyric acid	29	235~238	$C_6H_{13}O_3N$	48.96	8.90	9.52	48.78	8.95	9.27
DL-2-Amino-4-propoxybutyric acid	13	250	$C_7H_{15}O_3N$	52.15	9.38	8.69	52.20	9.12	9.18
DL-2-Amino-4-butoxybutyric acid	15	235	$C_8H_{17}O_3N$	54.83	9.78	7.99	54.80	9.98	8.36
DL-2-Amino-4-phenoxybutyric acid	0								

* Part III: This Bulletin, **7**, 183(1959).

** Sakyo-ku, Kyoto (林 恭三).

1) A. I. Virtanen, J. K. Miettinen: Biochim. et Biophys. Acta, **12**, 181(1953).
2) E. Fischer, H. Blumenthal: Ber., **40**, 106(1907).
3) J. E. Livak, E. C. Britton, *et al.*: J. Am. Chem. Soc., **67**, 2218(1945).

This fact is probably due to the hindrance of an aromatic group in this reaction and this point will be described in the next report.

DL-2-Amino-4-alkoxybutyric acid thereby obtained was hydrolyzed with 48% hydrobromic acid under reflux in an oil bath. DL-Homoserine is prepared by treating DL-2-amino-γ-butyrolactone hydrobromide so formed with silver carbonate,[2] lead carbonate,[4] or lead oxide[3] followed by dehydrobromination, ring-cleavage, and liberation of excess of heavy metal salts with hydrogen sulfide. In this process, however, considerable amount of DL-homoserine is adsorbed in the precipitate of sulfide, causing a decrease in the yield. Hence, this complicated method was improved as follows: DL-2-Amino-γ-butyrolactone hydrobromide was treated with Amberlite IR-120 as described in a previous report,[5] the adsorbed amino acid was eluted with 0.3~0.5*N* ammonium hydroxide solution, and then concentrated *in vacuo*. In this process, liberation of hydrogen bromide and ring cleavage were effected simultaneously, and DL-homoserine was obtained easily. DL-Homoserine can be obtained in a good yield from DL-2-amino-4-alkoxybutyric acid but this acid cannot be prepared from derivatives of malonic acid in such a good yield.

As to synthesis from ethyl 2-(2-alkoxyethyl)acetoacetate, Schmidt has reported that the amino acid was obtained in a good yield from ethyl 2-alkoxyacetoacetate and hydrazoic acid in the presence of conc. sulfuric acid.

Thus, it was supposed that DL-homoserine would be obtained from ethyl 2-(2-alkoxyethyl)acetoacetate in a good yield by the process illustrated in Chart 2.

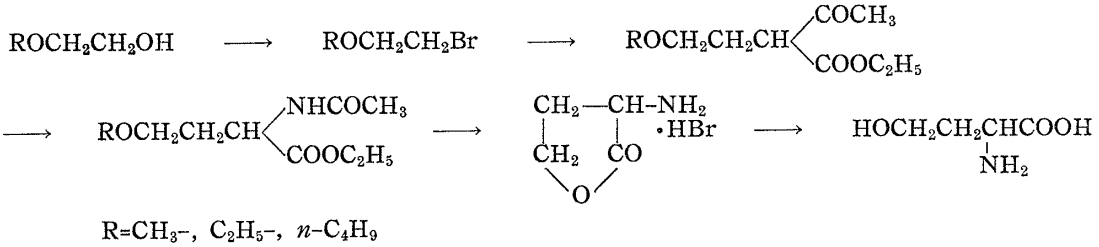


Chart 2.

The condensation of 2-alkoxyethyl bromide with ethyl acetoacetate was carried out by the general method to give ethyl 2-(2-alkoxyethyl)acetoacetate. The product was reacted with hydrazoic acid or sodium azide as a benzene or chloroform solution, in the presence, of conc. sulfuric acid, and the acid layer was diluted with water. The product, ethyl 2-acetamido-4-alkoxybutyrate, was extracted with ethyl acetoacetate, hydrolyzed with 48% hydrobromic acid by refluxing in an oil bath, and excess of the acid was removed by concentration *in vacuo*. The crystalline residue was treated with Amberlite IR-120 as previously described to give DL-homoserine.

Other method was also studied. A crude lactone hydrobromide dissolved in water was passed through a column of Amberlite IR-4B, 5~6 cc. of conc. ammonia was added to the effluent, and pure DL-homoserine was obtained by concentrating the effluent *in vacuo*. In the reaction between ethyl 2-(2-alkoxyethyl)acetoacetate and hydrazoic acid the following side reaction is considered to occur but in the present method there is no fear of a by-product contamination in the product for the following reason.

From many reports,[6] the reaction is supposed to proceed as shown in Chart 3.

4) T. Kaneko: "Chemistry of the Protein," Ed. by S. Akabori and S. Mizushima, Vol. I, 384(1954). Kyoritsu Co., Tokyo.
5) S. Takagi, K. Hayashi: This Bulletin, **7**, 96(1959).
6) M.S. Newman, H.L. Gildenhorn: J. Am. Chem. Soc., **70**, 317(1948); P.A.S. Smith: *Ibid.*, **70**, 320(1948); J.K. Sanford, F.T. Blair, *et al.*: *Ibid.*, **67**, 1941(1945); L.H. Briggs, G.C. De Ath, S.R. Eiiis: J. Chem. Soc., **1942**, 61; P.A.S. Smith, J.P. Horwitz: J. Am. Chem. Soc., **72**, 3718 (1950).

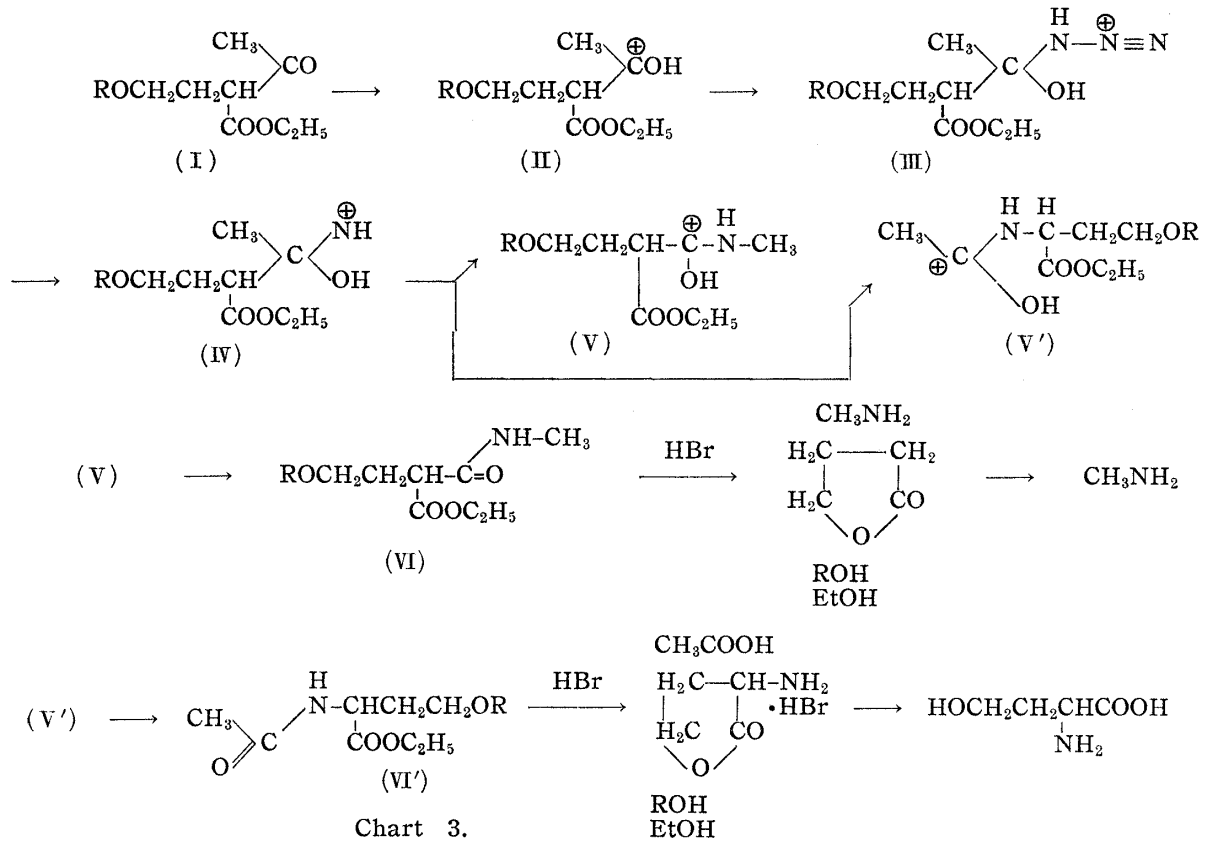


Chart 3.

In this reaction, hydrazoic acid in an active form is added after carbonium cation is formed, an addition product (III) is formed. (III) is so unstable that it is denitrogenized and dehydrated immediately to form (IV) and then undergoes intramolecular rearrangement. In this rearrangement, if the migration of methyl group occurs in (IV), (VI) is obtained from (IV) through (V) by liberating a proton. On the other hand, if the migration of 4-alkoxybutyric acid ester group occurs in (IV), (VI′) is obtained through (V′). Then, methylamine, γ-butyrolactone, and alcohol are produced on hydrolysis of (VI), while 2-amino-γ-butyrolactone hydrobromide and alcohols are formed on hydrolysis of (VI′). When these mixtures are passed through a column of Amberlite IR-120, only methylamine and 2-amino-γ-butyrolactone are adsorbed. Then, these substances are eluted with ammonia water and, when the eluate is treated as described previously, pure DL-homoserine is obtained without contamination of a by-product. The reaction of (IV)→(V)→(VI) hardly occurs, whereas the reaction of (IV)→(V′)→(VI′) progresses rapidly, and DL-homoserine is obtained from ethyl 2-(2-alkoxyethyl)acetoacetate in a good yield and high purity.

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Experimental

(2-Methoxyethyl)malonic Acid—Following Palomma's method,[7] ethyl (2-methoxyethyl)malonate (10 g.) was prepared and hydrolyzed with 40~50% KOH solution (20 cc.). The unreacted ester was extracted with ether (30 cc.). The product was neutralized with a calculated amount of 20% HCl under cooling with ice, extracted several times with ether (20 cc.) which was dried over Na_2SO_4 and ether evaporated. The oily residue failed to crystallize and was used *per se* for succeeding reaction. Yield, quantitative.

7) M. H. Palomma, A. Kenetti: Ber., **64**, 797(1931).

(2-Ethoxyethyl)malonic Acid—Obtained by the same manner as described above from ethyl (2-ethoxyethyl)malonate (20 g.) and 50% KOH solution (40 cc.). Yield, quantitative. An oily substance.

(2-Propoxyethyl)malonic Acid—Obtained by the same manner as described above from ethyl (2-propoxyethyl)malonate (10 g.) and 50% KOH solution (20 cc.). Yield, quantitative. An oily substance.

(2-Butoxyethyl)malonic Acid—Obtained by the same manner as described above from ethyl (2-butoxyethyl)malonate (10 g.) and 50% KOH solution (20 cc.). Yield, quantitative. An oily substance.

DL-2-Amino-4-methoxybutyric Acid—A mixture of (2-methoxyethyl)malonic acid (1.6 g.), conc. H_2SO_4 (5 cc.), and $CHCl_3$ or benzene (10 cc.) was heated on a water bath at 30~40° and 5 cc. of hydrazoic acid in $CHCl_3$ (9.9%) was slowly added with vigorous stirring. After the addition, the reaction was continued for further 3 hrs. Succeeding procedure was the same as in the previous reports. White amorphous powder (from hydr. acetone). Yield, 0.3 g. (cf. Table I).

DL-2-Amino-4-ethoxybutyric Acid—Obtained by the same manner as above from (2-ethoxyethyl)-malonic acid (3.6 g.), conc. H_2SO_4 (7 cc.), $CHCl_3$ or benzene (20 cc.), and hydrazoic acid in $CHCl_3$ (9.0%) (13 cc.). White plates (from hydr. acetone). Yield, 0.86 g. Easily soluble in water.

DL-2-Amino-4-propoxybutyric Acid—Obtained by the same manner as above from (2-propoxy-ethyl)malonic acid (6.6 g.), conc. H_2SO_4 (8 cc.), $CHCl_3$ or benzene (10 cc.), and hydrazoic acid in $CHCl_3$ (10.2%) (15 cc.). White powder (from hydr. acetone). Yield, 0.7 g. Easily soluble in water.

DL-2-Amino-4-butoxybutyric Acid—Obtained by the same manner as above from (2-butoxyethyl)-malonic acid (10.2 g.), conc. H_2SO_4 (16 cc.), $CHCl_3$ or benzene (20 cc.), and hydrazoic acid in $CHCl_3$ (11.5%) (23 cc.). White plates (from dil. EtOH). Yield, 1.4 g.

Preparation of DL-Homoserine from DL-2-Amino-4-alkoxybutyric Acid—i) DL-2-Amino-γ-butyro-lactone hydrobromide: A mixture of DL-2-amino-4-ethoxybutyric acid (0.75 g.) and 48% HBr (8 cc.) was refluxed in an oil bath for 2.5 hrs. and concentrated *in vacuo* to remove the excess of HBr as much as possible. The residue was dissolved in EtOH and the solution was allowed to stand with addition of petr. ether or benzene to deposit white crystals, m.p. 223°. Yield, 0.7 g.

DL-Homoserine was obtained directly from other DL-2-amino-4-alkoxybutyric acid without separation of DL-2-amino-γ-butyrolactone hydrobromide.

ii) DL-Homoserine: The purified DL-2-amino-γ-butyrolactone hydrobromide (0.7 g.) prepared by the above-mentioned procedure was dissolved in water (15 cc.) and passed through a column of Amberlite IR-120 to adsorb DL-2-amino-γ-butyrolactone. The ion exchange resin was washed until Br^- disappeared in the effluent solution and the adsorbed DL-2-amino-γ-butyrolactone was eluted with 0.3~0.5*N* NH_4OH. The eluate was concentrated *in vacuo* and EtOH was added to give white needles, m.p. 186~187°.

DL-2-Amino-γ-butyrolactone hydrobromide was dissolved in 20 cc. of water, passed through a column of Amberlite IR-4B and several cc. of conc. NH_4OH was added to the effluent giving the same product. The yield of DL-homoserine from DL-2-amino-4-alkoxybutyric acid (R=CH_3, C_2H_5, *n*-C_4H_9) was 77%, 81%, and 80%, respectively. *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.56; H, 7.70; N, 11.49.

Ethyl 2-(2-Alkoxyethyl)acetoacetate—Metallic Na (0.2*M*) was dissolved in 75 cc. of dehyd. EtOH and ethyl acetoacetate (0.2*M*) was added, refluxed on a water bath until the solution was neutralized, and the excess of EtOH was removed. The NaBr crystals formed were filtered off and the fractional distillation of the filtrate was carried out. The yield and b.p. are shown in Table II.

Table II.

R	b.p. (°C / mm. Hg)	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
CH_3	124~125/20	33	$C_9H_{16}O_4$	62.58	9.63	62.34	9.79
n-C_4H_9	145/18	51	$C_{12}H_{22}O_4$	57.43	8.63	57.36	8.57

Preparation of DL-Homoserine from Ethyl 2-(2-Alkoxyethyl)acetoacetate and Hydrazoic Acid—A benzene solution of hydrazoic acid (0.012*M*) was slowly added to a mixture of ethyl 2-(2-alkoxy-ethyl)acetoacetate (0.01*M*), benzene (10 cc.), and conc. H_2SO_4 (5~10 cc.) under vigorous stirring, the mixture was heated at 40~50° for 3 hrs., and the H_2SO_4 layer was diluted with 2~3 parts of water. This solution was extracted with AcOEt, dried over Na_2SO_4, and then AcOEt was removed. The residue without purification was refluxed with 15~20 cc. of 48% HBr in an oil bath for 3 hrs. Excess of HBr was removed as much as possible and DL-2-amino-γ-butyrolactone hydrobromide obtained was treated in the same manner as described above without purification. White needles (from hydr. acetone), m.p. 186°. The yield of DL-homoserine from ethyl 2-(2-alkoxyethyl)acetoacetate (R=CH_3, C_2H_5, *n*-C_4H_9) was 85%, 90%, and 82%, respectively.

Summary

DL-Homoserine is an amino acid which is important in the biochemical study on the metabolism of amino acids. Several methods for the synthesis of DL-homoserine have been reported, but they have many disadvantages; for example, they involve long process of reaction and extremely complicated method of separation of DL-homoserine formed. By the Schmidt reaction, DL-2-amino-4-alkoxybutyric acid and ethyl 2-acetamido-4-alkoxybutyrate were obtained respectively from (2-alkoxyethyl)malonic acid and 2-(2-alkoxyethyl)acetoacetate, and they were hydrolyzed with 48% hydrobromic acid to DL-2-amino-γ-butyrolactone hydrobromide. This was deacidified with ion exchange resin and simultaneously the ring cleavage was effected, giving DL-homoserine in a good yield and without difficulties in isolation and purification.

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38. Eiji Ochiai und Chikara Kaneko: Über eine neue Nitrierung des Chinolin-N-oxydes. (2).[1,2]

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Bei der Nitrierung von Chinolin-N-oxyd (I) mit 1 Mol Benzoylnitrat in einem indifferenten Lösungsmittel wie Chloroform oder Dioxan konnte man 3-Nitrochinolin-N-oxyd (II) mit ziemlich befriedigender Ausbeute erhalten.[1] Hierbei regenerierte man jedoch immer eine ca. 20% Menge Ausgangsmaterial und das mit der Ausbeute von 70~80% erhaltene Rohprodukt verminderte sich nach dem Reinigen bis auf 40~45% der Theorie. Diese Tatsachen weisen auf die Nebenentstehung irgendeines Polynitroderivates hin. So wurde die Reaktion mit überschüssigem Benzoylnitrat analogerweise durchgeführt. Hierbei hat sich die Menge von (II) vermindert und es wurde ein schwachgelbes nadelförmiges Kristall $C_{16}H_9O_7N_3$ (III) vom Schmp. 258° (u. Zers.) als Hauptprodukt erhalten. In der Tabelle I wurden die Ergebnisse der Nitrierung bei Anwendung von ca. 1~3 Mol Benzoylnitrat gezeigt.

Tabelle I.

Reaktionsbedingung			Reaktionsprodukte, mg (%)			
Probe	$C_6H_5CO_2NO_2$* (ccm)	Rkt.-Dauer (Tag)	(III)	(II)	(I)	gesamt (%)
1 g (I) in 20 ccm $CHCl_3$	1.5	3	195 (8)	670 (51)	230 (23)	82
	2.5	3	665 (28)	405 (31)	105 (10.5)	70
	4.5	3	1200 (51)	—	60 (6)	57

* 1 Mol Äquivalent Benzoylnitrat für 1 g Probe betrug ca. 1.5 ccm.

Es wurde weiter bestätigt, dass (III) bei analoger Nitrierung von (II) entsteht, wie in der Tabelle II gezeigt wird.

(III) stellt also in Berücksichtigung seiner Zusammensetzung höchstwahrscheinlich ein Monobenzoat eines Dinitrodihydroxychinolins oder Dinitromonohydroxychinolin-N-oxydes dar, in welchem eine Nitrogruppe auf der 3-Stellung des Chinolinkerns liegt. (III) ist gegen die säurige Hydrolyse beständig und bleibt unverändert, wenn man dasselbe mit

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1) Die vorangehende (1) Mitt. E. Ochiai, C. Kaneko: Dieses Bulletin, **5**, 56(1957).

2) Gleichzeitig CXXIX. Mitt. über die Polarisation der heterozyklischen Ringe mit aromatischem Charakter. CXXVIII. Mitt.: Yakugaku Zasshi, **78**, 1438(1958).