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Synthesis of Tricholomic Acid. III.[1] Synthesis of DL-*erythro*-α-Amino-3-oxo-5-isoxazolidineacetic Acid (DL-Tricholomic Acid)[2] and Its Optical Resolution

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DL-*erythro*-α-Amino-3-oxo-5-isoxazolidineacetic acid (I) was synthesized from dialkyl β-hydroxyglutamate. Optical resolution afforded L- and D-isomers, of which the former was proved to be identical with tricholomic acid, a flycidal and tasty constituent of *Tricholoma muscarium* KAWAMURA. The D-isomer lacked the biological activities.

Tricholomic acid is a novel amino acid isolated by Takemoto, *et al.*[4a] as a flycidal and tasty constituent of a mushroom, *Tricholoma muscarium* KAWAMURA (Haetori-shimeji in Japanese) and its chemical structure was established by them[4b] as L-*erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid[5] (L-*erythro*-I). The amino acid kills the fly when the insects lick the compound; moreover, the compound was a tasty substance for mankind.[4a] In fact,

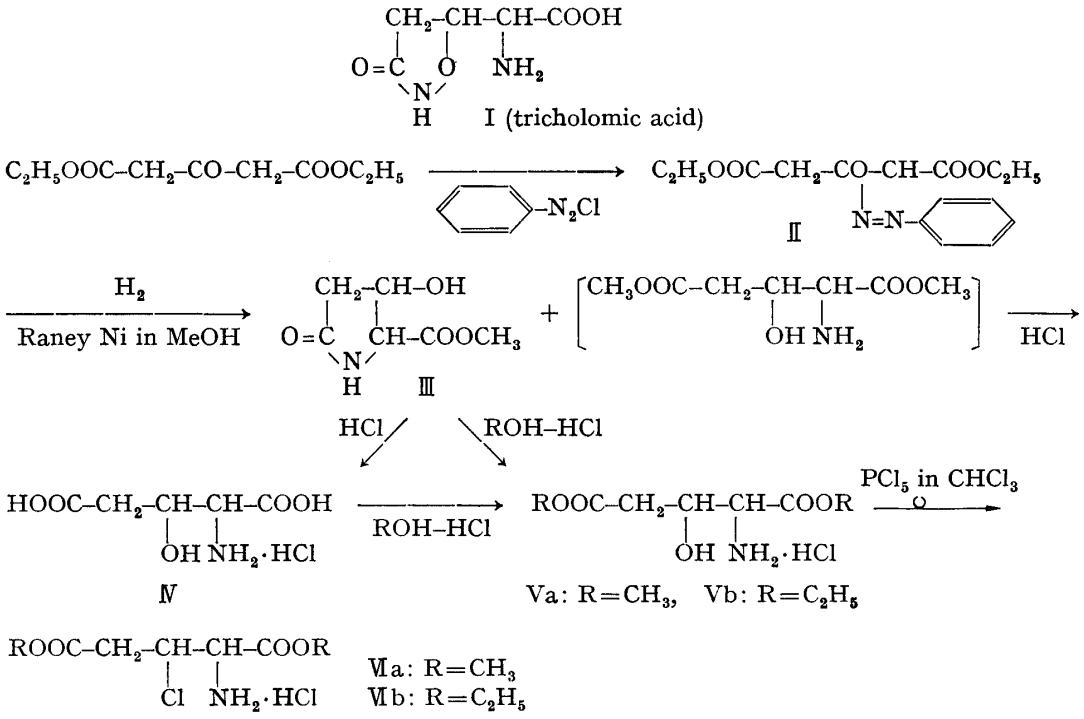

Chart 1

1) Part II: T. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **14**, 1307 (1966).
2) Presented at Meeting of Kinki Branch, Pharmaceutical Society of Japan, May 1965.
3) Location: *Juso, Higashiyodogawa-ku, Osaka.*
4) *a*) T. Takemoto and T. Nakajima, *Yakugaku Zasshi*, **84**, 1183 (1964); *b*) *ibid.*, **84**, 1230 (1964).
5) All amino acids and their derivatives have optical isomers (L, D and DL). Unless described particularly, individual compounds are DL form in this series of paper.

Terasaki, *et al.*[6] reported that the taste of this new amino acid was in the same order or stronger than that of sodium inosinate or sodium guanylate, and showed a potentiation with these 5′-ribonucleotides.

This paper deals with the synthesis of DL-tricholomic acid, DL-*erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid, and its optical resolution into L- and D-isomers, of which the former was found to be identical with natural tricholomic acid.

First of all, the starting materials, dimethyl and diethyl *erythro*-β-hydroxyglutamate (*erythro*-Va,b), and diethyl *threo*-β-hydroxyglutamate (*threo*-Vb) were synthesized by the procedures of Izumi, *et al.*[7a] and Akabori, *et al.*[7b]

The catalytic reduction of diethyl 2-(phenylazo)-3-oxo-pentanedioate (II) afforded methyl *erythro*-3-hydroxy-5-oxo-2-pyrrolidinecarboxylate (III), which had not been reported by Izumi, *et al.* From the ethyl ester (II) was obtained almost exclusively the methyl ester (III) owing to the ester exchange with methanol during the reaction.

The chlorination of the hydroxyl group of *erythro*-Va,b and *threo*-Vb was effected by usual manner using phosphorus pentachloride in acetyl chloride in poor yields; the yields were increased thereafter by using chloroform as solvent. Thus, *erythro*-Va,b and *threo*-Vb yielded dimethyl and diethyl *threo*-β-chloroglutamate[7b] (*threo*-VIa,b), and diethyl *erythro*-β-chloroglutamate (*erythro*-VIb), respectively.[8]

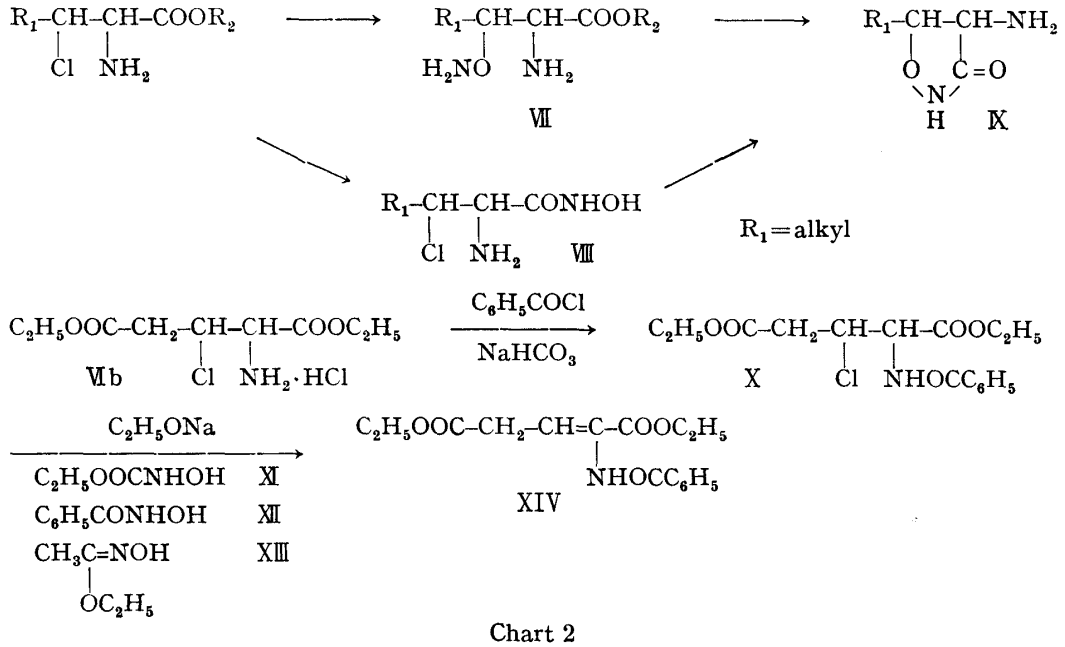

Chart 2

To date two different routes have been known for the syntheses of cycloserine analogues (IX) which have the same nucleus as that of tricholomic acid; *i.e.*, i) the cyclization of β-aminooxy derivatives (VII)[9] and ii) the cyclization of β-chlorohydroxamic acids (VIII).[8]

In order to study the reaction route by the former method *threo*-VIb was converted into diethyl *threo*-N-benzoyl-β-chloroglutamate (X) by the Schotten-Baumann reaction. Attempts to substitute the chlorine of X with a RNO– residue by the reaction with ethyl hydroxy-

6) M. Terasaki, E. Fujita, S. Wada, T. Takemoto, T. Nakajima and T. Yokobe, *J. Japan. Soc. Food Nutr.*, **18**, 172, 222 (1965).

7) *a*) Y. Izumi and S. Konishi, *J. Chem. Soc. Japan*, **74**, 960 (1953); T. Kaneko, Y. Yoshida and H. Katsura, *ibid.*, **80**, 316 (1959); *b*) S. Akabori, T. Kaneko, S. Sakurai and Y. Izumi, *ibid.*, **75**, 942 (1954).

8) Pl. A. Plattner, A. Boller, H. Frick, A. Fürst, B. Hegedüs, H. Kirchensteiner, St. Majnoni, R. Schläpfer and H. Speigelberg, *Helv. Chim. Acta*, **40**, 1531 (1957).

9) F. Winternitz, M. Mousseren and R. Lachazette, *C.A.* **54**, 1334*i* (1960); S. Iwasaki, M. Kondo and Y. Miyaji, Japan. Patent Sho-35-7476 (1960).

carbamate (XI), N-benzoylhydroxamic acid (XII) or ethyl hydroximinoacetate (XIII) have thus far met with failure; instead, a yellow oil, bp 200° (0.1 mmHg), was obtained in all cases. This compound was proved to be diethyl 2-benzamido-2-pentenedioate (XIV) by elemental analysis, NMR, and infrared spectra. This implies that the compound (X) is more susceptible to dehydrochlorination rather than the substitution of chlorine under these reaction conditions. In fact, when X was treated with an equivalent amount of sodium ethylate in ethanol the production of XIV proceeded even at room temperature.

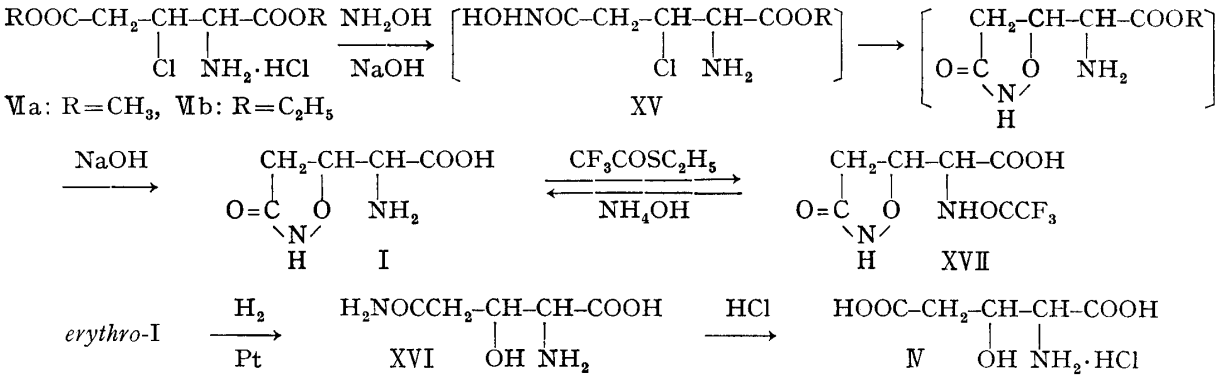

Chart 3

Owing to serious drawbacks in the first synthetic route as described above, the efforts were then directed towards the second route *via* hydroxamic acids (VIII). In the hydroxamation and cyclization of VIa,b, the side reactions such as the formation of pyrrolidone or diketopiperazine derivatives are presumed to occur. For prohibiting these reactions, the amino group of VIa,b should be protected by any means. On the other hand, the isoxazolidone nucleus contained in tricholomic acid is susceptible to hydrolysis to open the ring, and especially so to acid; *e.g.* decomposition occurred even at room temperature in dilute hydrochloric acid. Therefore, the removal of an amino-protecting group by hydrolysis after cyclization possibly cause a considerable degradation of the isoxazolidone ring formed. On the contrary to these anticipations, somewhat surprisingly, when VIa,b was subjected to the reaction with hydroxylamine and sodium hydroxide in aqueous solution without protecting the amino group the formation of γ-hydroxamic acid (XV), the cyclization and saponification of the α-ester group had proceeded all in one step to give *erythro*-I as shown in Chart 3. It was desirable to carry out the reaction at low temperature to minimize the side reactions as in the syntheses of cycloserine analogues.[8,10] The conversion yields of VIa,b into hydroxamic acid (XV) with hydroxylamine hydrochloride in the presence of alkali were investigated under a variety of conditions using two to three moles of alkali at various reaction temperatures between −10 and 10° with water, methanol or their mixture as solvent without isolating the reaction product.

Table I. Ratios of DL-Tricholomic Acid and Its *threo*-Isomer in the Reaction Products

Starting material	Ratios (%)	
	DL-Tricholomic acid	*threo*-Isomer
threo-VIa	72.3	27.7
threo-VIb	76.7	23.3
erythro-VIb	31.7	68.3

10) R.M. Khomutov, M. Ya. Karpeiskii, C. Chi-pin and N.K. Kochetkov, *J. Gen. Chem. USSR* (Eng. Transl.), **30**, 3030 (1960).

The cyclization and saponification then followed under the fixed condition with stirring at room temperature for three hours with four moles of alkali, and the ratio and the yield of *erythro*-I and *threo*-I in the reaction products were measured by means of the amino acid analysis and paper electrophoresis. The results are summarized as follows: (1) the lower reaction temperatures suppress the side reactions to a greater extent, (2) the ratio of *erythro*-I and *threo*-I varies delicately with the reaction conditions, but generally *erythro*-I from *threo*-VIa,b and *threo*-I[11] from *erythro*-VIb were obtained as shown in Table I.

For the isolation of I the reaction mixture was percolated through a column of Amberlite IR-120B (H type). The column was then eluted with 3% ammonia and the eluate was again submitted to column chromatography (Dowex 1, acetate type) using 0.5N acetic acid containing methanol[12] as developer. The ninhydrin-positive fractions were examined by paper electrophoresis with 10% acetic acid as solvent, whereby the fractions containing *erythro*-I and *threo*-I were recognized distinctively from each other. The fractions containing *erythro*-I were collected and evaporated *in vacuo* to give crystalline *erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid (DL-tricholomic acid) (DL-*erythro*-I), colorless plates, mp 195—198° (decomp.). The yield after recrystallization from water was 1.7% (3.03% in the reaction mixture before isolation) from *threo*-VIa.

The synthetic DL-*erythro*-I could not be distinguished from natural tricholomic acid (L-*erythro*-I) by the paper electrophoresis, paper chromatography, amino acid analysis by the autoanalyzer and NMR spectra. Their infrared spectra (KBr disk) closely resembled to each other.

For confirmation of the chemical structure, synthetic DL-*erythro*-I and natural tricholomic acid (L-*erythro*-I) were both reduced catalytically to *erythro*-β-hydroxyglutamine (XVI), which was hydrolyzed to *erythro*-β-hydroxyglutamic acid (*erythro*-IV) in the manner described by Takemoto, *et al.*[4b] Therefore, the synthetic and the natural compounds were determined to have the same *erythro* configuration at the 5-position.

DL-*erythro*-I was found to have a taste potency about one half of that of natural tricholomic acid and showed the flycydal activity. Optical resolution was cairred out to determine which of L- or D-isomer of *erythro*-I was biologically active. Takemoto, *et al.*[4b] assigned the L-configuration to tricholomic acid. Further on the basis of the measurement of the optical rotatory dispersion curve[13] and Lutz–Jirgensons' rule,[14] the configuration of the carbon at the α–position of tricholomic acid was confirmed to be L form. For optical resolution, a protection of the amino or carboxyl group is considered a prerequisite, however, the isoxazolidone nucleus in DL-*erythro*-I easily opens by acid hydrolysis or hydrogenation as described before. Therefore, the amino group of DL-*erythro*-I was protected with a trifluoroacetyl residue which could be removed by treatment with aqueous ammonia at room temperature. Trifluoroacetylation was carried out using the technique of Schallenberg, *et al.*[15] with ethyl thioltrifluoroacetate and alkali. The N-trifluoroacetyl derivative (XVII) thus obtained was converted into a quinine salt (XVIII). The product was submitted to fractional crystallization from ethanol to separate slightly-soluble crystals from more-soluble ones, and the both were treated with aqueous ammonia at room temperature to remove the quinine and trifluoroacetyl residue. From the former crystals were obtained D-*erythro*-I and from the latter L-*erythro*-I. The physico-chemical properties of L-*erythro*-I thus obtained were in complete accord with those of natural tricholomic acid. The flycidal and flavour enhancing activities

11) See next paper of this series.
12) H. Aoyagi, H. Okai, M. Bessho and N. Izumiya, *J. Chem. Soc. Japan*, **85**, 651 (1964).
13) J.C. Craig and S.K. Roy, *Tetrahedron*, **1965**, 391; I.P. Dirkx and F.L.J. Sixma, *Rec. Trav. Chim.*, **83** 522 (1964); W. Gaffield, *Chem. Ind.* (London), **1964**, 1460.
14) O. Lutz and B. Jirgensons, *Chem. Ber.*, **63**, 448 (1930); **64**, 1221 (1931); **65**, 784 (1932).
15) E.E. Schallenberg and M.C. Calvin, *J. Am. Chem. Soc.*, **77**, 2779 (1955).

of synthetic tricholomic acid were in the same degree as those of natural compound, while the antipode, D-*erythro*-I, was found to have almost none of such properties.

Experimental

Methyl *erythro*-3-Hydroxy-5-oxo-2-pyrrolidinecarboxylate (III)——A solution of 500 g of diethyl 2-(phenylazo)-3-oxo-pentanedioate (II) in 2.5 liters of MeOH was reduced by the procedure of Izumi, *et al.*[7a] After removal of the catalyst by filtration, the reaction mixture was concentrated *in vacuo* to yield 96.7 g of III. Recrystallization from MeOH yielded colorless plates, mp 150.5—151.5°. *Anal.* Calcd. for $C_6H_9O_4N$ (methyl *erythro*-3-hydroxy-5-oxo-2-pyrrolidinecarboxylate): C, 45.28; H, 5.70; N, 8.80. Found: C, 45.29; H, 5.69; N, 8.85. Ninhydrin reaction: negative. Mass spectrum *m/e*: 159 (M^+) (Calcd. mol. wt. 159.14). NMR (in D_2O) τ: 7.5 (2H, multiplet), 6.2 (3H, singlet), 5.7 (1H, doublet), 5.4 (1H, multiplet).

***erythro*- and *threo*-β-Hydroxyglutamic Acid Hydrochloride (*erythro*- and *threo*-IV)**——(1) The filtrate from the crystalline III was evaporated *in vacuo* and the residue was hydrolyzed with 6N HCl. The hydrolyzate was worked up by the procedure of Izumi, *et al.*[7a] to yield *erythro*-β-hydroxyglutamic acid hydrochloride (*erythro*-IV) as the first crop and *threo*-IV as the third crop. The second crop was a mixture of the both isomers. The IR spectra of free amino acids (IV) obtained by neutralization with pyridine were identical with those in the literature.[7b] Furthermore, the amino acid analysis and the paper electrophoresis clearly indicated their stereochemical homogeneities. (2) III was hydrolyzed with 6N HCl and from the hydrolyzate was obtained *erythro*-IV hydrochloride in a quantitative yield. The configuration of III, therefore, was proved to be *erythro* (*trans*). (3) The second crop of crystal, a mixture of *erythro* and *threo*-IV, was subjected to Dowex column chromatography by a technique similar to that of Izumiya, *et al.*[12] in the separation of threonine from *allo*-threonine. One gram of the mixture was put on the top of column (4 × 120 cm) of Dowex 50W × 8 (200—400 mesh, pyridine salt type) and run with a 0.2 M pyridine–acetate buffer containing MeOH (20%) (pH 4). The fraction between 700 and 950 ml of the effluent yielded 300 mg of *threo*-IV and the fraction between 1000 and 1300 ml gave 500 mg of *erythro*-IV after evaporation *in vacuo*.

Dimethyl *erythro*-β-Hydroxyglutamate Hydrochloride (erythro-Va)——A suspension of 30 g of III in MeOH was saturated with dry HCl and boiled for 3 hr. The reaction mixture was kept in a refrigerator overnight to yield a massive crystal. Recrystallization from MeOH–ether gave 36 g (84%) of *erythro*-Va, colorless needles, mp 150—151° (decomp.). *Anal.* Calcd. for $C_7H_{14}O_5NCl$ (dimethyl *erythro*-β-hydroxyglutamate hydrochloride): C, 36.93; H, 6.20; Cl, 15.58. Found: C, 36.75; H, 6.06; Cl, 15.64.

Diethyl erythro-β-Hydroxyglutamate Hydrochloride (*erythro*-Vb)——III was submitted to ethanolysis in the same manner as described in the synthesis of Va to give DL-*erythro*-Vb. Yield, 81.5%.

Dimethyl *threo*-β-Chloroglutamate Hydrochloride (*threo*-VIa)——To a suspension of 16.6 g (80 mmoles) of finely powdered PCl_5 in 110 ml of anhydrous $CHCl_3$ was added portionwise 12.9 g (57 mmoles) of *erythro*-Va hydrochloride under stirring and ice-cooling at 5—10° over a period of about 40 min. Stirring was continued for 5 hr at 5°, then the solution was left standing at room temperature for 20 hr. The reaction mixture was treated under ice-cooling with 5 ml of MeOH to decompose the excess PCl_5 and evaporated *in vacuo*. The residual oil was washed with ether and crystallized from EtOH–ether to give 11.1 g (79%) of *threo*-VIa, mp 151—152° (decomp.). *Anal.* Calcd. for $C_7H_{13}O_4NCl_2$ (dimethyl *threo*-β-chloroglutamate hydrochloride): C, 34.16; H, 5.32; N, 5.69. Found: C, 34.36; H, 5.46; N, 5.63.

Diethyl β-Chloroglutamate Hydrochloride (VIb)——i) *threo*-VIb: *erythro*-Vb hydrochloride was treated in the same manner as described in dimethyl ester (Va). Recrystallization from EtOH–ether afforded *threo*-VIb in 75% yield, mp 112—113° (decomp.). *Anal.* Calcd. for $C_9H_{17}O_4NCl_2$ diethyl *threo*-β-chloroglutamate hydrochloride): C, 39.43; H, 6.25; N, 5.11. Found: C, 39.40; H, 6.16; N, 5.10.

ii) *erythro*-VIb: *threo*-Vb hydrochloride was treated in the same manner as described in *erythro*-Vb. Recrystallization from $CHCl_3$–ether gave colorless needles, *erythro*-VIb in 45% yield, mp 80—81°. *Anal.* Cacld. for $C_9H_{17}O_4NCl_2$ (diethyl *erythro*-β-chloroglutamate hydrochloride): C, 39.43; H, 6.25; N, 5.11. Found: C, 38.40; H, 6.29; N, 5.12.

Diethyl *threo*-N-Benzoyl-β-chloroglutamate (X)——*threo*-VIb (2.7 g, 10 mmoles) and 16.8 g (200 mmoles) of $NaHCO_3$ were dissolved in 170 ml of H_2O. To the solution was added dropwise 2.8 g (200 mmoles) of benzoyl chloride with stirring under ice-cooling. After 6 hr stirring under ice-cooling, the solution was extracted with benzene. The extract was washed with a saturated solution of $NaHCO_3$ and H_2O in succession, and dried over anhydrous Na_2SO_4. Benzene was distilled off under reduced pressure and the residues was crystallized from petroleum benzine. The crystals were washed with petroleum ether and recrystallized from a mixture of benzene and petroleum ether to give 2.6 g (76%) of X, colorless plates, mp 59—60°. *Anal.* Calcd. for $C_{16}H_{20}O_5NCl$ (diethyl *threo*-N-benzoyl-β-chloroglutamate): C, 56.22; H, 5.90; N, 4.10; Cl, 10.37. Found: C, 56.38; H, 5.76; N, 4.02; Cl, 10.55.

Diethyl 2-Benzamido-2-pentenedioate (XIV)——To a solution of 70 mg (3 mmoles) of Na in 10 ml of EtOH was added a solution of 1 g (2.9 mmoles) of X in 10 ml of EtOH. After being stirred at room temperature for 30 min, the solution was filtered to remove NaCl precipitated. The filtrate was evaporated *in*

vacuo and the residue was shaken with 20 ml of EtOAc and 5 ml of H_2O. The EtOAc layer was separated and washed several times with H_2O. After being dried over anhydrous $MgSO_4$, the EtOAc solution was evaporated *in vacuo* to afford 670 mg of an yellow oil. Qualitative analysis of halogen: negative. Distillation of this oil gave a pale yellow oil, bp 200° (0.1 mmHg). *Anal.* Calcd. for $C_{16}H_{19}O_5N$ (diethyl 2-benzamido-2-pentenedioate): C, 62.94; H, 6.27; N, 4.59. Found: C, 62.84; H, 6.13; N, 4.72. NMR (in CCl_4) τ: 3.2 (1H triplet, -CH=C-), 6.7 (2H doublet, $J=7$ cps, OC-CH_2-C=C).

DL-*erythro*-α-Amino-3-oxo-5-isoxazolidineacetic Acid (DL-Tricholomic Acid) (DL-*erythro*-I)——$NH_2OH \cdot HCl$ (11.1 g, 160 mmoles) was added portionwise to 80 ml of 10N NaOH with stirring under cooling with ice-NaCl. To this solution was added 39.8 g (160 mmoles) of *threo*-VIa all at one time, while the temperature being kept below −5°. The mixture was stirred for 2 hr at −8——5° in the atmosphere of N_2, followed by stirring at room temperature for 3 hr. The reaction mixture was diluted to 500 ml with H_2O and percolated through a column of Amberlite IRC-50 (H type) (100 ml) to remove the excess NaOH. The resin was washed with 200 ml of H_2O, the effluent and the washing were combined, and, the solution was passed through a column of Amberlite IR-120B (H type) (700 ml). The column was washed with each 700 ml of H_2O, 70% MeOH and H_2O, successively. Amino acids were eluted with 1500 ml of 3% aq. NH_3. The eluate was submitted to the quantitative analysis of *erythro*- and *threo*-I by an autoanalyzer with 150 cm column and the buffer of pH 5.28. The yield of the former was 3.03%, while that of the latter was no more than 0.6%. The residue obtained by evaporation of the eluate was dissolved in 30 ml of MeOH-AcOH-H_2O (200:58:1742, v/v) and was chromatographed on a column of 6.0×100 cm packed with Dowex 1×8 (200—400 mesh, acetate type) run with the MeOH·AcOH·H_2O mixture. The effluent was collected in a 15 ml fraction, while the elution being conducted at a rate of 3—4 ml/min. The ninhydrin-positive fractions were submitted to paper electrophoresis (Toyo No. 51, 10% AcOH, 28 V/cm, 2.5 hr) to recognize the fractions containing DL-*erythro*-I in comparison with natural tricholomic acid. The fractions were evaporated *in vacuo* to give DL-*erythro*-I, which was crystallized from H_2O after decolouring with active carbon. Yield: 443 mg (1.7%), colorless platelets, mp 195—198° (decomp.). *Anal.* Calcd. for $C_5H_8O_4N_2$ (DL-*erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid): C, 37.50; H, 5.04; N, 17.50. Found: C, 37.21; H, 5.20.; N, 17.34. Paper electrophoresis (Toyo No. 51, 10% AcOH, 28 V/cm, 2.5 hr): −8 cm, Paper chromatography (*n*-BuOH-AcOH-H_2O 120:30:50, v/v): *Rf* 0.5; (MeOH-pyridine-H_2O 160:8:40, v/v): *Rf* 0.4. Amino acid autoanalysis (Beckman-Spinco Model 120B, pH 5.28 citrate buffer, 150 cm column): 135min, NMR (in NaOD-D_2O): Fig. 1.

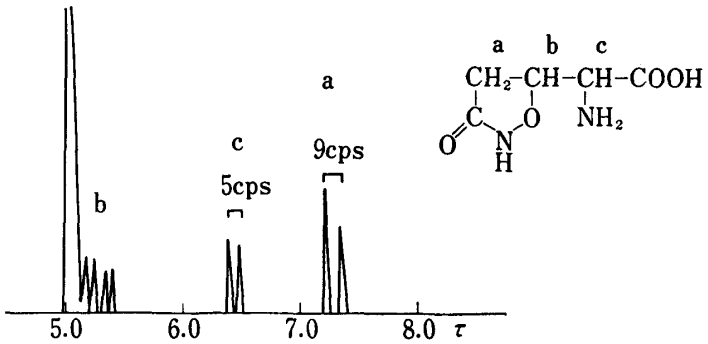

Fig. 1. NMR Spectrum of DL-Tricholomic Acid (in D_2O+NaOD)

a: 7.25 doublet
b: 5.2 overlapping with H_2O-signal
c: 6.4 doublet
$J_{a,b}$: 9 cps
$J_{b,c}$: 5 cps

DL-*erythro*-α-Trifluoroacetamido-3-oxo-5-isoxazolidineacetic Acid (XVII)——DL-*erythro*-I (800 mg, 5 mmoles) was dissolved in 10 ml (10 mmoles) of 1N NaOH and stirred vigorously with 1.26 g (8 mmoles) of ethyl thioltrifluoroacetate for 8 hr at room temperature. The reaction mixture, after being acidified with dil. HCl and saturated with NaCl, was extracted repeatedly with EtOAc. The EtOAc solution was dried over $MgSO_4$ and evaporated *in vacuo*. The oily residue was crystallized from EtOAc-petroleum ether to give 877 mg (69%) of XVII, colorless needles, mp 186° (decomp.). *Anal.* Calcd. for $C_7H_7O_5N_2F_3$ (DL-*erythro*-α-trifluoroacetamido-3-oxo-5-isoxazolidineacetic acid): C, 32.82; H, 2.75; N, 10.94. Found: C, 32.71; H, 2.85; N, 10.67.

L- and D-*erythro*-α-Amino-3-oxo-5-isoxazolidineacetic Acid (L- and D-*erythro*-I)——To solution of 590 mg of XVII and 1.5 g of quinine in 10 ml of MeOH was added H_2O until the solution had become opaque, and the mixture was left standing in a refrigerator overnight to give 1.4 g of quinine salt (XVIII). *Anal.* Calcd. for $C_{47}H_{59}O_{11}N_6F_3$ (DL-*erythro*-α-trifluoroacetamido-3-oxo-5-isoxazolidineacetic acid with 2 moles of quinine and 2 moles of H_2O): C, 59.99; H, 6.32; N, 8.93. Found: C, 60.25; H, 6.40; N, 8.55. XVIII was subjected to the fractional crystallization from ethanol to separate slightly-soluble crystal (crystal-1) from more-soluble ones (crystal-2). Crystal-1: 532 mg, $[\alpha]_D^{24}$ −158° ($c=0.5$, $l=1$, MeOH), crystal-2: 468 mg, −108° ($c=0.5$, $l=1$, MeOH). Each of both crystals was dissolved in 5 ml of MeOH and treated with a mixture of 20 ml of $CHCl_3$ and 10 ml of 14% aq. NH_3. The mixture was shaken well and the $CHCl_3$ layer was extracted three times with 14% aq. NH_3 (30 ml). The combined 14% NH_3 solution was washed twice with $CHCl_3$ and left standing for 5 hr at room temperature. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in 1 ml of H_2O, then left standing in a refrigerator after acidifying with AcOH

to give crystals. From the crystal-1 was obtained D-tricholomic acid (D-*erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid), 51 mg colorless plates, mp 202—204° (decomp.), $[\alpha]_D^{22}$ −104° ($c=0.2$, $l=1$, H_2O), while from the crystal-2 was obtained L-tricholomic acid (L-*erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid), 60 mg, colorless plates, mp 201—202° (decomp.) with no depression on admixture with tricholomic acid, $[\alpha]_D^{22}$ +105.5° ($c=0.2$, $l=1$, H_2O),[16] $[\alpha]_D^{21}$ +185.5° ($c=0.2$, $l=1$, 0.1N HCl), +34.5° ($c=0.2$, $l=1$, 0.1N NaOH). ORD ($c=1.94$ mg/2 ml in 0.1N HCl): positive Cotton effect. 3-Hydroxyglutamic acid was obtained by reduction and hydrolysis by the procedure of Takemoto, *et al.*,[4b] $[\alpha]_D^{22}$ +7.7° ($c=0.3$, $l=1$, H_2O), ref. 7a: L-*erythro*-β-hydroxyglutamic acid, $[\alpha]_D$ +8.4° ($c=2.01$, $l=1$, H_2O). L-Tricholomic acid takes two crystalline forms with completely different IR spectra; *i.e.*, the one from H_2O showed mp 201—202° (decomp.), IR: Fig. 2. while the other obtained from 30% AcOH melted at 207° (decomp.) (IR: Fig. 3). These IR spectra were in complete accord with those of natural tricholomic acid.

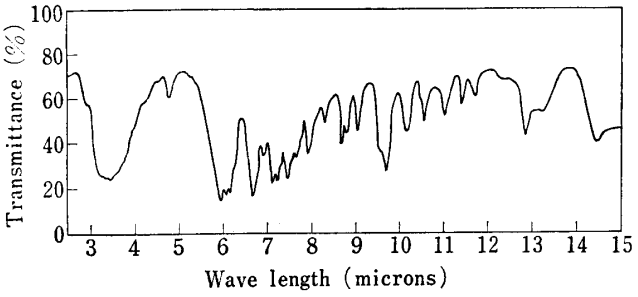

Fig. 2. IR Spectrum of Tricholomic Acid crystallized from H_2O (KBr)

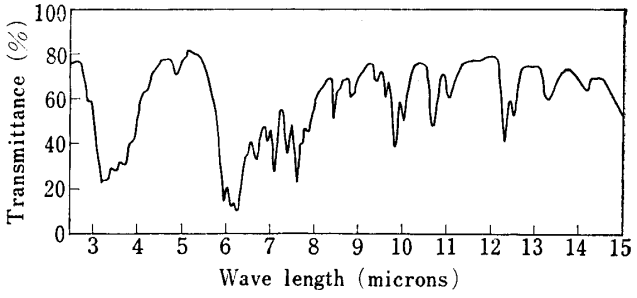

Fig. 3. IR Spectrum of Tricholomic Acid crystallized from dil. AcOH (KBr)

16) Although Takemoto, *et al.*[4b] reported $[\alpha]_D$ +80.0° ($c=0.2$, $l=1$, H_2O) for natural tricholomic acid, the specimen provided by Takemoto showed $[\alpha]_D^{21}$ +106° ($c=0.2$, $l=1$, H_2O), when measured by Perkin-Elmer Model 141.