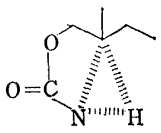
61°,[7] $[\alpha]_D^{12}$ −6.9° (c=2.06, $C_2H_5OH$). *Anal.* Calcd. for $C_8H_{13}O_3N$ : C, 56.12; H, 7.65; N, 8.18. Found : C, 56.11; H, 7.71; N, 8.22. IR $\nu_{max}^{KBr}$ $cm^{-1}$ : 1769 (2-oxazolidinone >C=O), 1706 (amide >C=O).

On the other hand, *S*(+)-2-methylbutyl azidoformate (*S*(+)-II), $\alpha_D^{11}$ +4.59° (*l* ldm., neat) was prepared from commercially available *S*(−)-2-methyl-1-butanol (*S*(−)-I) ($\alpha_D^{10}$ −4.18° (*l* ldm., neat), 87% optically pure) in 64% yield according to the method of Smolinsky, *et al.*[1] The thermal decomposition of *S*(+)-II in diphenyl ether at 190~210°[8] followed by the purification by chromatography on alumina, subsequent distillation under the reduced pressure and column chromatography on silicic acid afforded (+)-III, $[\alpha]_D^8$ +2.0° (c=11.1, $C_2H_5OH$), infrared spectrum of which was superimposable with that of DL-III in neat. This oxazolidinone ((+)-III) was submitted to N-acetylation under the similar procedure discribed above to yield (−)-VII, white crystals, m.p. 72.5~74°,[9] $[\alpha]_D^8$ −10.8° (c=1.83, $C_2H_5OH$). *Anal.* Calcd. for $C_8H_{13}O_3N$ : C, 56.12; H, 7.65; N, 8.18. Found : C, 56.18; H, 7.61; N, 8.02. Infrared spectrum (in $CHCl_3$) of this compound was identical with that of *R*(−)-VII prepared from *R*(−)-isovaline.

In our reaction, that is, thermal decomposition of azidoformate II in solution phase, it is concluded clearly that the nitrene insertion reaction proceeded with retention of configuration, and the extent of retention percent of this reaction resulted in nearly 100% retention based on the calculation of optical purity of the starting materials, I and V. It is also shown that the reaction in vapour phase decomposition reported by Smolinsky, *et al.*[1] also took place with retention of configuration. These facts suggest that the intermediate of nitrene insertion reaction of this type may be shown as VIII, although other reaction mechanism cannot be definitely ruled out. Since our finding showed that this reaction proceeds with full retention of configuration, this type of reactions would have a high potentiality to utilize for the synthesis from optically active $\overset{*}{\text{C}}$-H bond to $\overset{*}{\text{C}}$-N bond. The scope and detailed mechanism of this reaction are under investigation.



VIII

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7) DL-VII has m.p. 49.5~51.5°. The IR spectrum of DL-VII in solid state is essentially superimposable with that of (−)-VII.
8) G. Smolinsky : J. Am. Chem. Soc., 83, 2489 (1961).
9) The mixed melting point of this sample with *R*(−)-VII obtained from *R*(−)-V was shown to be 61~69°.

[Chem. Pharm. Bull. 13(6) 753~758 (1965)]

*UDC 547.466.1.07:581.19:582.287.238*

## Synthesis of Tricholomic Acid, a Flycidal Amino Acid. I.

In 1964 Takemoto, *et al.*[1] isolated a flycidal constituent "tricholomic acid" from *Tricholoma muscarium* KAWAMURA, an edible mushroom in the northern part of Japan.

1) T. Takemoto, T. Nakajima : J. Pharm. Soc. Japan, 84, 1183 (1964).

They found that it is a new amino acid with very good taste and assumed its structure as *erythro*-α-amino-3-oxo-5-isoxazolidineacetic acid (I).[2] Further studies attracted our attention with an interesting result that its delicious taste is much stronger than L-monosodium glutamate and synergistic with the flavor of inosinic acid and guanylic acid.[3]

This communication is concerned with the synthesis of tricholomic acid (I) and its *threo* isomer (II), of which the former has a good taste and flycidal property, while the latter almost none of them.

As the starting materials, *erythro*-diethyl(or dimethyl)DL-3-hydroxyglutamate(IVa, b)[4] and *threo*--diethyl DL-3-hydroxyglutamate (VI)[4] were synthesized by the procedures of Izumi, *et al.*[5] and Akabori, *et al.*[6] IVa, b were also obtained by alcoholysis of *erythro*-methyl 3-hydroxy-5-oxo-2-pyrrolidinecarboxylate (V)*1 which was newly isolated from the reduction product of diethyl 2-(phenylazo)-3-oxopentanedioate (XIX). Chlorination of IVa, b and VI with phosphorus pentachloride in chloroform gave *threo*-diethyl (dimethyl)

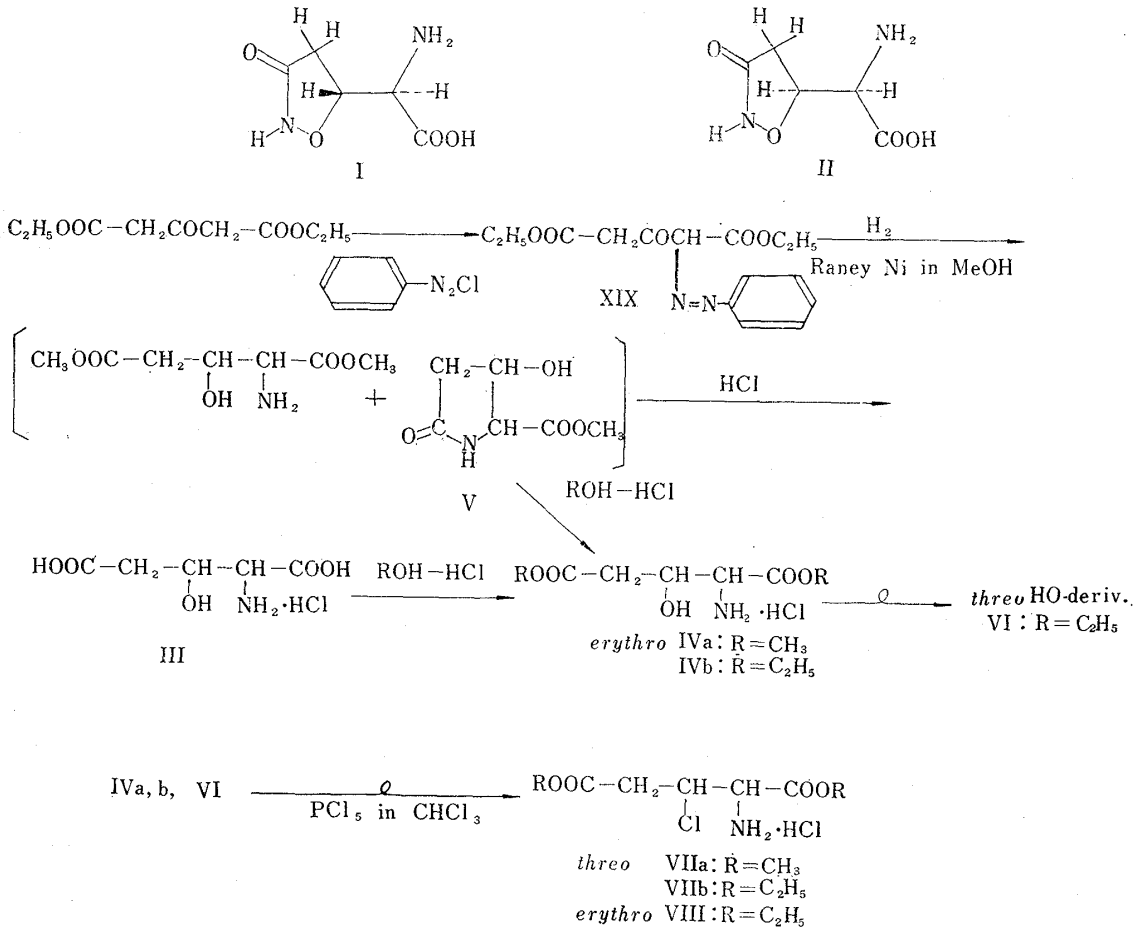


Chart 1.

*1 Izumi and Konishi described the reduction product as ethyl ester without isolation, but the ethoxyl group is actually ester-interchanged with methoxyl group of methanol, the solvent in the reduction reaction.

2) T. Takemoto, T. Nakajima : J. Pharm. Soc. Japan, **84**, 1230 (1964).
3) T. Takemoto, T. Nakajima, T. Yokobe, E. Fujita, S. Wada, M. Terasaki : personal communication and patent application.
4) T. Kaneko, Y. Yoshida, H. Katsura : J. Chem. Soc. Japan, **80**, 316 (1959).
5) Y. Izumi, S. Konishi : *Ibid.*, **74**, 960 (1953).
6) S. Akabori, T. Kaneko, S. Sakurai, Y. Izumi : J. Chem. Soc. Japan, **75**, 942 (1954).

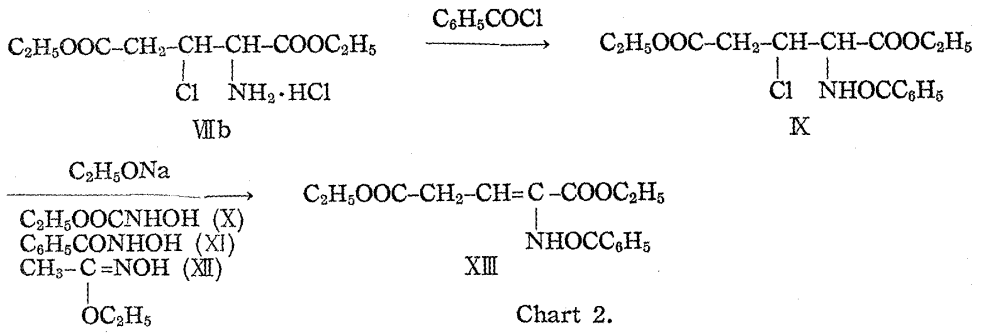


Chart 2.

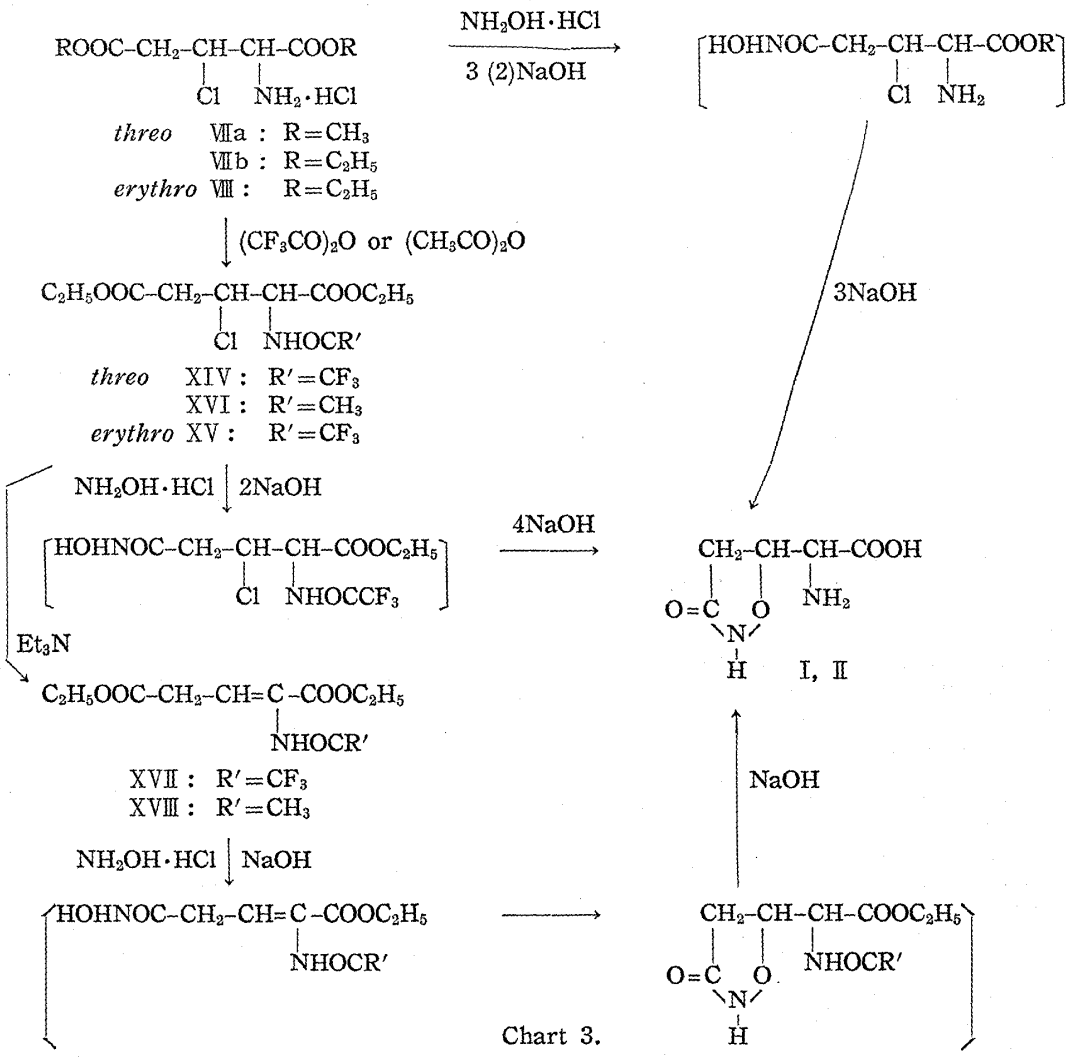


Chart 3.

DL-3-chloroglutamate (VIIa, b) and *erythro*-diethyl DL-3-chloroglutamate (VIII), respectively with steric inversion as in case of 3-chloro-derivatives from threonine analogues.[7]

Any attempt to substitute the chlorine of N-benzoyl-3-chloroglutamate (IX) wit RNO-residue by the reaction of ethyl hydroxycarbamate (X), N-benzoylhydroxamic ac (XI) or ethyl hydroximinoacetate (XII) in alkaline media failed and a yellow oil was obtain in all cases. This compound was proved to be diethyl 2-benzamido-2-pentenedioa (XIII) from nuclear magnetic resonance and infrared spectra, and also obtained by t treatment of IX with sodium alcoholate.

7) Pl. A. Plattner, A. Boller, H. Frick, A. Fuerst, B. Hegedues, H. Kirchensteiner, St. Majr R. Schlaepfer, H. Spiegelberg : Helv. Chim. Acta, **40**, 1531 (1957).

Table I. Ratios of Racemic Tricholomic Acid and its *Threo* Isomer in the Reaction Products by Various Methods

| Synthetic method | Starting material | Ratios (%) | | | |
|---|---|---|---|---|---|
| | | Paper electrophoresis method | | A. A. Analyzer analysis of derived HO-Glu | |
| | | *rac* trichlomic acid | *threo* isomer | *erythro* | *threo* |
| One step method | *threo* (Ⅶa) | 72.3 | 27.7 | 85 | 15 |
| | *threo* (Ⅶb) | 76.7 | 23.3 | 83 | 17 |
| | *threo* (Ⅶa)[a] | 50.0 | 50.0 | 54 | 46 |
| | *erythro* (Ⅷ) | 31.7 | 68.3 | 23 | 77 |
| Through N-trifluoroacetyl derivatives | *threo* (Ⅶb) | 23.3 | 76.7 | 16 | 84 |
| | *erythro* (Ⅷ) | 17.3 | 82.7 | 12 | 88 |
| Through 2-amino-2-pentenedioate | XVII | 23.3 | 76.7 | 16 | 84 |
| | XVIII | 58.7 | 41.3 | 65 | 35 |

a) 5 moles alkali in total was used, while other experiment by one step method were carried out with 6 moles. Ratios were calculated from (1) optical densities at 575 mμ of the eluate of purple bands on the paper developed by ninhydrin reagent after paper electrophoresis in 10% acetic acid and (2) quantitative analysis of *erythro*- and *threo*-hydroxyglutamic acids obtained from the mixture of I and II by reduction and hydrolysis.[2]

Table II. Physico-chemical Properties of New Compound

| No. | Compounds | m.p. (°C) | Formula | Analysis (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calcd. | | | Found | | |
| | | | | C | H | N | C | H | N |
| I | *Erythro*-DL-α-amino-3-oxo-5-isoxazolidineacetic acid | 195～198 (decomp.) | $C_5H_8O_4N_2$ | 37.50 | 5.04 | 17.50 | 37.21 | 5.20 | 17.34 |
| II | *Threo*-DL-α-amino-3-oxo-5-isoxazolidineacetic acid | 213～214 (decomp.) | 〃 | 37.50 | 5.04 | 17.50 | 37.43 | 5.26 | 17.27 |
| Ⅳa | *Erythro*-dimethyl DL-3-hydroxyglutamate hydrochloride | 150～151 (decomp.) | $C_7H_{14}O_5NCl$ | 36.93 | 6.20 | (Cl) 15.58 | 36.75 | 6.06 | (Cl) 15.64 |
| V | DL-*Erytho*-methyl 3-hydroxy-5-oxo-2-pyrrolidinecarboxylate | 150.0～151.5 | $C_6H_9O_4N$ | 45.28 | 5.70 | 8.80 | 45.29 | 5.69 | 8.85 |
| Ⅶa | *Threo*-dimethyl DL-3-chloroglutamate hydrochloride | 142～144 (decomp.) | $C_7H_{13}O_4NCl_2$ | 34.16 | 5.32 | 5.69 | 34.36 | 5.46 | 5.63 |
| Ⅶb | *Threo*-diethyl DL-3-chloroglutamate hydrochloride | 112～113 | $C_9H_{17}O_4NCl_2$ | 39.43 | 6.25 | 5.11 | 39.40 | 6.16 | 5.10 |
| Ⅷ | *Erythro*-diethyl DL-3-chloroglutamate hydrochloride | oily crys. —80 | 〃 | 39.43 | 6.25 | 5.11 | 38.40 | 6.16 | 5.10 |
| Ⅸ | *Threo*-diethyl DL-N-benzoyl-3-chloroglutamate | 59～60 | $C_{16}H_{20}O_5NCl$ | 56.22 | 5.90 | 4.10 | 56.38 | 5.76 | 4.02 |
| XIII | Diethyl 2-benzamido-2-pentenedioate | $b.p_{0.1}$ 200 | $C_{16}H_{19}O_5N$ | 62.94 | 6.27 | 4.59 | 62.84 | 6.00 | 4.72 |
| XIV | *Threo*-diethyl DL-N-trifluoracetyl-3-chloroglutamate | $b.p_{0.04}$ 123～124 | $C_{11}H_{15}O_5NClF_3$ | 39.59 | 4.53 | 4.20 | 39.62 | 4.71 | 4.07 |
| XV | *Erythro*-diethyl DL-N-trifluoroacetyl-3-chloroglutmate | $b.p_{0.07}$ 134～137 | 〃 | 39.59 | 4.53 | 4.20 | 39.78 | 4.83 | 4.13 |
| XVI | *Threo*-diethyl DL-N-acetyl-3-chloroglutamate | $b.p_{0.04}$ 157 | $C_{11}H_{18}O_5NCl$ | 47.23 | 6.49 | 5.01 | 47.07 | 6.46 | 5.18 |
| XVIII | Diethyl 2-acetamido-2-pentenedioate | 75～76 | $C_{11}H_{17}O_5N$ | 54.31 | 7.04 | 5.76 | 54.53 | 7.07 | 5.74 |

As shown in Chart 3, procedures from VIIa, b or VIII through γ-hydroxamic acids as intermediates succeeded to synthesize I and its stereoisomer (II). Table I shows the ratios of I and II obtained by these methods.

a) One step method: 3-Chloroglutamate (VIIa, b) and VIII, in aqueous alcohol were treated with one equivalent of hydroxylamine hydrochloride and 3 moles of alkali at −5∼0° for two hours, then added with more 3 moles of alkali and stirred at room temperature for 4 hours for cyclization and hydrolysis. Reaction mixture was purified by ion-exchanger chromatography. From *threo*-3-chloroglutamate (VIIa, b), I and small amount of II were obtained, while *erythro* 3-chloro derivative (VIII) gave *threo* isomer (II) as major product. However, when 5 moles of alkali in total were used, the ratio of I to II was unexpectedly 1:1.

b) From N-trifluoroacetyl derivatives: In order to avoid some complicated side reactions, and because isoxazolidinone nucleus is more stable to alkali than acid, amino groups of VIIb and VIII were protected with trifluoroacetyl residue which is easily removed with alkali. Treatment of XIV and XV with one equivalent of hydroxylamine hydrochloride and two moles of alkali at −5∼0° for 2 hours, followed by standing with more 3 moles of alkali at room temperature overnight, gave *threo* isomer II as major product. These facts suggest that both XIV and XV cyclized through the same intermediate XVII.

c) From 2-amino-2-pentenedioate: *Threo*-N-acyl-3-chloro compounds XIV and XVI were easily converted to dehydro derivatives (XVII) and (XVIII) by treating with triethylamine at room temperature. XVII was not isolated, but XVIII was isolated and its structure was confirmed from nuclear magnetic resonance spectrum. The dehydro derivative (XVII) was treated with hydroxylamine and sodium hydroxide under the conditions described in (b) to yield the mixture of I and II in the same ratio as (b). N-Acetyl dehydro compound (XVIII) was treated with hydroxylamine and alkali, then followed by cyclization and hydrolysis under the same condition as in cycloserine.[8] Ratio of I:II was 59:41.

To isolate (I) or (II) from the mixture of them, the following methods were used independently or in combination: (1) recrystallization from water, (2) Dowex 1×8 ($CH_3COO^-$ form) column chromatography with 0.5*N* acetic acid, (3) Dowex 50 W×8 (pyridine form) column chromatography with 0.1*M* formic acid-pyridine buffer (pH 3.1) containing methanol, (4) precipitation method of I as copper salt. Racemic tricholomic acid (I): colorless plate, m.p. 195∼198°(decomp.), its *threo* isomer: colorless plate, m.p. 213∼214° (decomp.).

Paper electrophoresis with 10% acetic acid, paper chromatography (solvent system: butanol-acetic acid-water=120:30:50, methanol-pyridine-water=160:8:40) and amino acid analysis by an autoanalyzer (buffer pH 5.28) of synthesized DL-I agreed with those of natural tricholomic acid, while *threo* isomer (II) was distinguished from the latter, except by paper chromatography. Infrared spectrum of DL-I was essentially identical with that of tricholomic acid, but II differed remarkably from the latter.

Structures of I and II was confirmed as follows: catalytic reduction[2] of DL-I or II gave *erythro*- or *threo*-3-hydroxyglutamine, which was converted to *erythro* or *threo*-3-hydroxyglutamic acid by hydrolysis. These derivatives were identical with the authentic samples.

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8) W. F. Runge, T. Haute: U. S. Pat., 2,815,348 (Dec. 3, 1957), 2,794,022 (May 28, 1957).

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