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Synthesis of Tricholomic Acid. VI.[1] Synthesis of DL-Tricholomic Acid and Its *threo*-Isomer from Diastereoisomers of β-Hydroxyglutamic Acid α-Benzyl Ester[2]

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Racemic tricholomic acid and its *threo*-isomer (I) were successfully synthesized by extending the previous method to diastereoisomers of N-carbobenzoxy-β-hydroxyglutamic acid α-benzyl ester (III), which were prepared by the selective partial esterification of the corresponding N-carbobenzoxyamino acids (III).

In the preceding paper,[1] the synthesis of DL-tricholomic acid and its *threo*-isomer (I) starting with *threo*- and *erythro*-β-hydroxyglutamic acids *via* their α-monoalkyl esters were described. Compared with the first synthetic route,[4] the route was an improvement on several points; these include, for example, the selective protection of α-carboxyl group of β-hydroxyglutamic acid and the stereochemical control on the ring closure reaction. The yield of the final product, however, was only about 12% despite our attempts at further refinements of

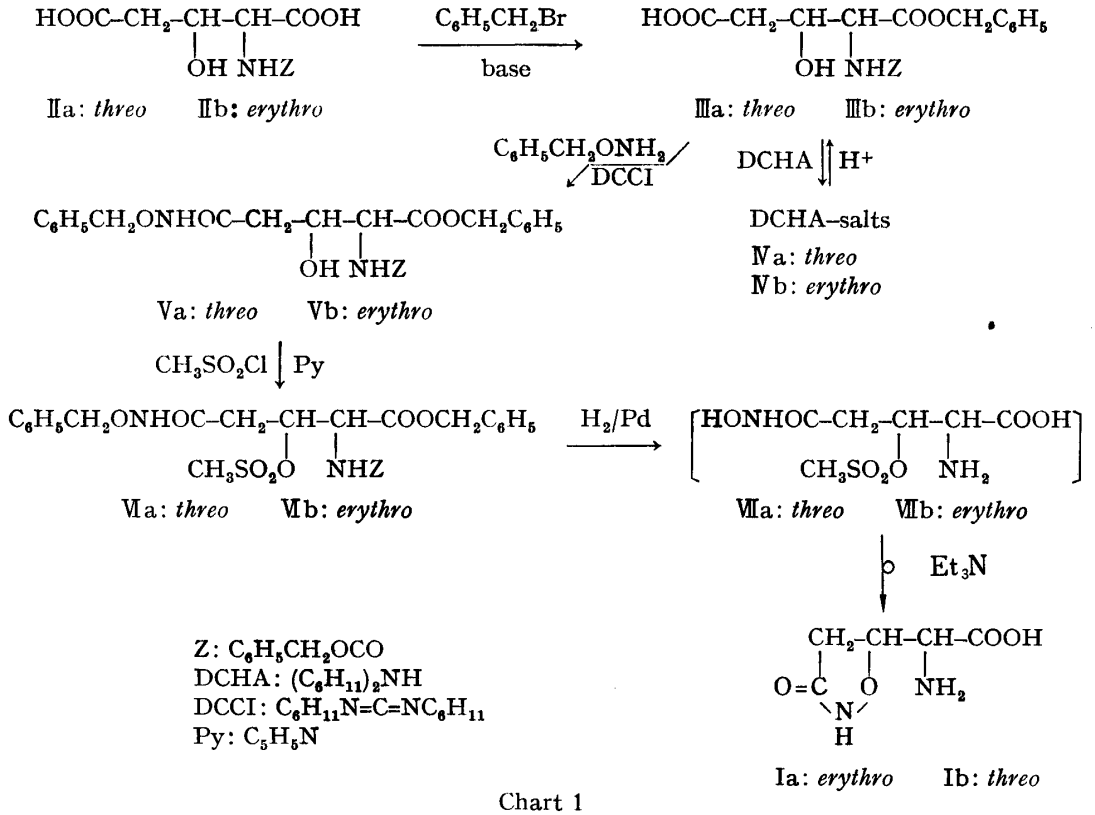

Chart 1

1) Part V: T. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **17**, 879 (1969).
2) This work was presented at Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, June 1966 and published as a preliminary communication in *Chem. Pharm. Bull.* (Tokyo), **14**, 1307 (1966).
3) Location: *Jusonishino-cho, Higashiyodogawa-ku, Osaka.*
4) H. Iwasaki, T. Kamiya, C. Hatanaka, Y. Sunada and J. Ueyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 873 (1969).

the yield. The biological interests[5] of this compound prompted us to achieve a further improvement of the yield.

The present paper describes a further modification of the previous synthesis by replacing α-alkyl esters with α-benzyl ester, which was easily convertible to a carboxylic acid by hydrogenolysis to give tricholomic acid (I) in a superior yield.

α-Benzyl ester (III) of β-hydroxyglutamic acid was obtained by the selective esterification of the corresponding N-carbobenzoxy-β-hydroxyglutamic acid (II)[6] according to the procedure reported in the synthesis of α-benzyl N-acylglutamates.[7] The α-benzyl esters (III) thus obtained were purified as dicyclohexylammonium salts (IV), and the structures were confirmed by elemental analysis, IR and NMR spectra and also by correlation with respective diastereoisomers of γ-methyl β-hydroxyglutamate (IX)[8] (Chart 2).

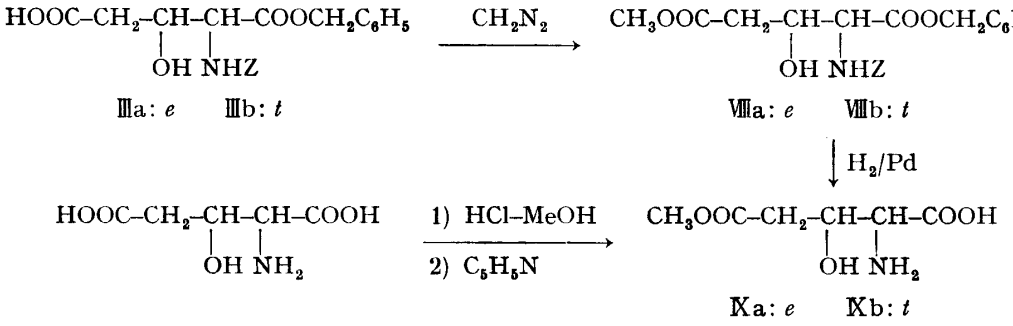

Chart 2

An attempt was made to convert N-carbobenzoxy-*threo*-β-hydroxyglutamic acid α-benzyl ester (IIIa), thus prepared, into the γ-benzyloxyamide (Va) by the treatment with O-benzylhydroxylamine in the same manner as described in the preceding paper.[1] However, it resulted in a mixture instead of the expected Va. Of two components isolated from the mixture as pure crystals by chromatography on silicagel, one was identified as the γ-benzyloxyamide (Va) and the other as an N-acylurea (XI) by elemental analysis and NMR spectra (Fig. 1) respectively. The by-product XI would have been derived from an active intermediate (X) by the acyl migration reported by Khorana.[9] This side reaction was found preventable by replacing the solvent (tetrahydrofuran) with acetonitrile according to the description of Sheehan, *et al.*[10] and both diastereoisomers of V were obtained in high yields.

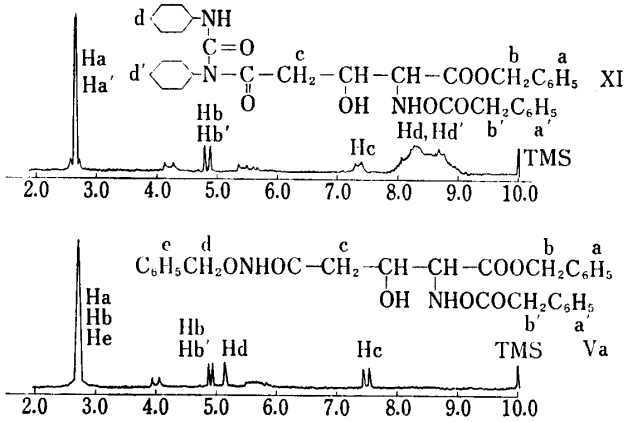

Fig. 1. Nuclear Magnetic Resonance Spectra of XI and Va in $CDCl_3$ at 60 Mc

The γ-benzyloxyamides were easily converted to the corresponding mesylates (VI) with mesylchloride in pyridine. Subsequent hydrogenolysis of VI over palladium black and cyclization of the resulting hydroxamic acid (VII) were performed in one step. The hydrolysis

5) M. Terasaki, S. Wada, E. Fujita, T. Takemoto, T. Nakajima and T. Yokobe, *Eiyo To Shokuryo*, 18, 1183 (1964).
6) T. Kaneko, R. Yoshida and H. Katura, *Nippon Kagaku Zasshi*, **75**, 942 (1954).
7) G.H.L. Nefkens and R.J.F. Nivard, *Receuil*, **83**, 199 (1964).
8) Direct synthesis of the authentic samples is described in the next paper (Part VIII).
9) H.G. Khorana, *Chem. Ind.*, **1958**, 1087.
10) J.C. Sheehan, M. Goodman and G.P. Hess, *J. Am. Chem. Soc.*, **78**, 1367 (1956).

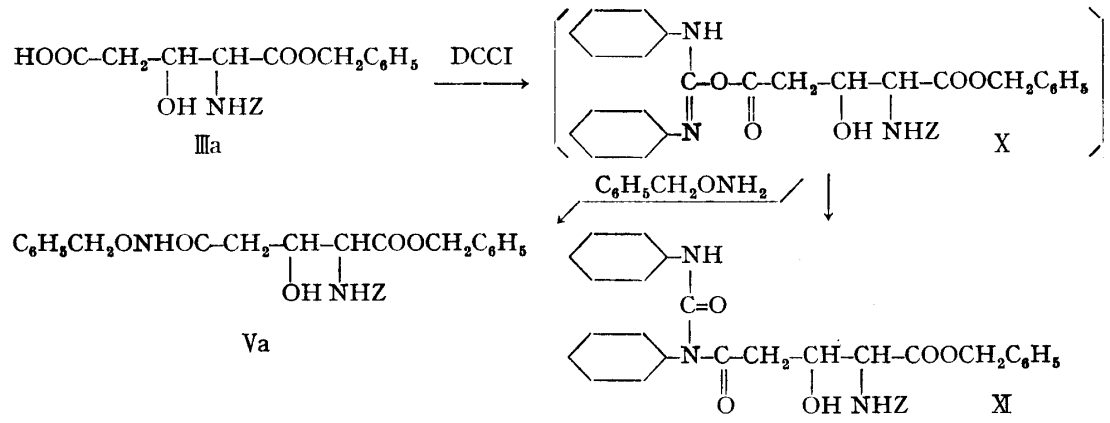

Chart 3

of the α-ester employed in the last step in the preceding work was not required in the present method, since the α-benzyl protective was removed before the cyclization was effected by the hydrogenolysis. This is the main advantage of the present method which excludes the side reactions of an α-amino ester. Thus, the final products were easily obtained in a highly purified form by evaporation of the solvent and excess triethylamine from the reaction mixture followed by recrystallization from water. Consequently, the yield of I in the last step was increased to about 80% and almost complete inversion of the configuration at the β-asymmetric carbon in the last step was observed yielding respective pure diastereoisomer of I from the corresponding mesylate.

Experimental[11]

Dicyclohexylammonium Salt (IV) of N-Carbobenzoxy-β-hydroxyglutamic Acid α-Benzyl Ester——a) To a solution of 51.6 g of IIa and 24.5 ml of triethylamine in 65 ml of dimethylformamide 20.8 ml of benzyl bromide was added, and the reaction mixture was left overnight at room temperature. Water (400 ml) was added to the reaction mixture, and an oily product separated was extracted in AcOEt, washed with H_2O and dried over Na_2SO_4. The oil (62.8 g, 93.5%) obtained by evaporation of solvent was treated with 32.4 g of dicyclohexylamine in AcOEt to yield crystals. The crystals were recrystallized from EtOH to give 68 g (69%) of IVa, mp 153—154°. *Anal.* Calcd. for $C_{32}H_{44}O_7N_2$: C, 67.58; H, 7.80; N, 4.93. Found: C, 68.03; H, 7.78; N, 4.98.

b) To a stirred solution of 3.7 g of IIb and 3 ml of dicyclohexylamine in 30 ml of dimethylformamide was added dropwise 1.8 ml of benzyl bromide at 55°, and the mixture was stirred for 1 hr. After addition of AcOEt, the precipitate of dicyclohexylammonium bromide was filtered and washed with AcOEt. The filtrate and the washing were combined and dried over Na_2SO_4. The oil (4.7 g) obtained by evaporation of the solvent was treated with dicyclohexylamine according to the procedure described above to give 4.5 g (63%) of IVb, mp 135—137°. *Anal.* Found: C, 67.56; H, 7.59; N, 4.91.

On the other hand, by the same procedure as a), IIb yielded 24.5% of IVb and 25% of di-dicyclohexylammonium salt of IIb, mp 162—163.5° (from AcOEt). *Anal.* Calcd. for $C_{37}H_{61}O_7N_3$: C, 67.34; H, 9.32; N, 6.37. Found: C, 67.08; H, 9.17; N, 5.76.

N-Carbobenzoxy-β-hydroxyglutamic Acid α-Benzyl Ester (III)——a) To a stirred suspension of 10 g of IVa in 500 ml of AcOEt was added 5 ml of 5N HCl. Dicyclohexylammonium chloride precipitated was removed by filtration and washed with AcOEt. The filtrate and the washing were combined, dried over Na_2SO_4 and evaporated to crystals. Recrystallization from AcOEt yielded 6.5 g(95%) of IIIa, mp 135—136.5°. *Anal.* Calcd. for $C_{20}H_{21}O_7N$: C, 62.01; H, 5.46; N, 3.62; Found: C, 62.17; H, 5.53; N, 3.66. NMR ($CDCl_3$) τ: 2.66 (10H, singlet); 4.84 (2H, singlet); 4.93 (2H, singlet).

b) Treatment of 2 g of IVb with 5N HCl according to the procedure described above afforded 1.2 g (88%) of IIIb, mp 87—90°. *Anal.* Found: C, 61.78; H, 5.51; N, 3.52.

N-Carbobenzoxy-β-hydroxyglutamic Acid α-Benzyl-γ-methyl Ester (VIII)——a) To a solution of IIIa in tetrahydrofuran was added a solution of diazomethane in ether until yellow color remained for several minutes. The solution was evaporated under reduced pressure and a crystalline residue was recrystallized

11) Melting points are uncorrected. Procedures and instrumentation used were the same as previously reported,[1] unless otherwise indicated.

from ether to give VIIIa (quantitative yield), mp 99°. *Anal.* Calcd. for $C_{21}H_{23}O_7N$: C, 62.83; H, 5.77; N, 3.49. Found: C, 62.98; H, 5.77; N, 3.29. NMR ($CDCl_3$) τ: 2.73 (10H, singlet); 4.87 (2H, singlet); 4.94 (2H, singlet); 6.40 (3H, singlet); 7.48 (2H, doublet).

b) Treatment of IIIb with diazomethane yield quantitatively the crystals of VIIIb. NMR ($CDCl_3$) τ: 2.70 (10H, singlet); 4.87 (2H, singlet); 4.95 (2H, singlet); 6.43 (3H, singlt); 7.36 (2H, broad.)

β-Hydroxyglutamic Acid γ-Methyl Ester (IX)——a) A solution of VIIIa in MeOH was subjected to catalytic reduction with Pd-black according to the conventional procedure. After completion of the reaction and removal of the catalyst, the filtrate was concentrated under reduced pressure to a crystalline mass. Recrystallization of the residue from H_2O–MeOH yielded IXa (quantitative yield), mp 193.5° (decomp.), which was identified with an authentic sample[8] by the mixed melting point determination and IR spectrum. *Anal.* Calcd. for $C_6H_{11}O_5N$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.90; H, 6.36; N, 7.92.

b) Hydrogenolysis of VIIIb according to the procedure described above afforded IXb, mp 182° (decomp.), which was identified with an authentic sample.[8] *Anal.* Found: C, 40.76; H, 6.31; N, 7.88.

N-Carbobenzoxy-β-hydroxyglutamic Acid α-Benzyl Ester γ-Benzyloxyamide (V)——a) According to the procedure described in the previous paper[1] 5 g of IIIa was treated with 2 g of O-benzylhydroxylamine in the presence of 2.8 g of DCCI in 100 ml of tetrahydrofuran to yield 7.5 g of crystalline residue. The residue was chromatographed on silicagel with a solvent of benzene–AcOEt (7:3). The first eluate gave 4 g of an N-acylurea (XI), mp 160—161.5° (from AcOEt). *Anal.* Calcd. for $C_{33}H_{43}O_7N_3$: C, 66.76; H, 7.30; N, 7.08. Found: C, 66.50; H, 7.42; N, 7.25. NMR spectrum: Fig. 1. Further elution afforded 3 g (47%) of Va, mp 132—133.5°. *Anal.* Calcd. for $C_{27}H_{28}O_7N_2$: C, 65.84; H, 5.73; N, 5.69. Found: C, 65.95; H, 5.62; N, 5.64. NMR spectrum: Fig. 1. By a similar treatment as described above except that 120 ml of acetonitrile was used as solvent in place of tetrahydrofuran 5 g of IIIa afforded 5.6 g (87.5%) of Va.

b) According to the procedure described in the latter example of a) 4.5 g of IIIb was treated with 1.8 g of O-benzylhydroxylamine and 2.5 g of DCCI in 50 ml of acetonitrile to give 4.8 g (84%) of Vb, mp 166—167° (from CH_2Cl_2). *Anal.* Found: C, 65.97; H, 5.86; N, 5.39.

N-Carbobenzoxy-β-mesyloxyglutamic Acid α-Benzyl Ester γ-Benzyloxyamide (VI)——According to the procedure described in the foregoing experiment,[1] Va and Vb were mesylated to yield VIa and VIb.

a) VIa: mp 117—120° (MeOH). *Anal.* Calcd. for $C_{28}H_{30}O_9N_2S$: C, 58.93; H, 5.30; N, 4.91. Found: C, 59.09; H, 5.14; N, 4.89.

b) VIb: mp 127—129° (MeOH). *Anal.* Found: C, 59.20; H, 5.33; N, 4.73.

α-Amino-3-oxo-5-isoxazolidine Acetic Acid (I)——a) To a suspension of 0.5 g of VIa in 60 ml of MeOH–H_2O (5:1) was added 50 mg of Pd-black, and the mixture was subjected to catalytic reduction at 15° for 3 hr. After completion of the reaction and removal of the catalyst by filtration, the filtrate was concentrated to 25 ml under reduced pressure below 20°. The aq. solution was stirred vigorously and kept at 0°, while 3 ml of triethylamine was added dropwise and the reaction mixture was stirred for 30 min at 0° and for additional 1 hr at room temperature. After completion of the cyclization, the mixture was concentrated under reduced pressure, and the crystalline residue was recrystallized from hot water to yield 110 mg of Ia, mp 200.5° (decomp.).[12]

b) By treating 0.5 g of VIb as described above for the *threo*-isomer, Ib (105 mg) was obtained. mp 209—210° (decomp.).[12]

12) The identity with an authentic sample[4] was confirmed by the mixed melting point determination, IR-spectroscopy, paper chromatography, paper electrophoresis and amino acid analysis.