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Synthesis of Tricholomic Acid. VIII.[1] Synthesis of Diastereoisomers of Isotricholomic Acid from β-Hydroxyglutamic Acid γ-Esters[2]

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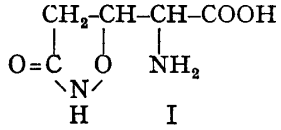
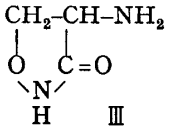
Two diastereoisomers of isotricholomic acid (II), structural isomers of tricholomic acid (*erythro*-L-I) were synthesized from the corresponding diastereoisomers of β-hydroxyglutamic acid γ-ester (V) by the method for the synthesis of I. During the synthesis of II, the diastereoisomers of I were obtained by a novel intramolecular rearrangement.

In the previous papers of this series[1,4] the stereochemically controlled synthesis of tricholomic acid (*erythro*-L-I), its three optically active isomers and two racemates derived from the corresponding β-hydroxyglutamic acid α-esters have been reported. In this paper attempts were made to synthesize the amino acid IIa,b[5] (isotricholomic acid), structural isomers of I and homologs of cycloserine (III), from β-hydroxyglutamic acid γ-esters (V) by the same reaction route as that for the synthesis of I. Some interesting results observed during the synthesis are also described.



I



III

The half-esters (V)[6] were prepared from the two diastereoisomers of β-hydroxyglutamic acid (IV) according to the procedures used for the synthesis of glutamic acid γ-esters: two types of selective partial esterification with methanolic hydrochloric acid[7] and with benzyl alcohol and sulfuric acid[8] were utilized.

Conversion of V to II which included carbobenzoxylation of V, condensation of VI with O-benzylhydroxylamine, mesylation of VII, hydrogenolysis of VIII, cyclization of IX, and saponification of X, and purification of the final products by ion-exchange chromatography was carried out by the procedures described previously,[4,9] and the details in each reaction are shown in the experimental part. The yields of the last step are summarized in Table I.

1) Part VII: T. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **17**, 890 (1969).
2) This work was presented at Meeting of Kinki Branch, Pharmaceutical Society of Japan, Osaka, Dec. 1966.
3) Location: *Jusonishino-cho, Higashiyodogawa-ku, Osaka.*
4) T. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **17**, 879, 886 (1969).
5) Recently the synthesis of diastereoisomers of II by other route was reported by R. M. Khomutov, E.S. Severin and G.K. Kovaleva, *Dokl. Akad. Nauk. SSSR*, **161**, 1227 (1965).
6) After the present study was completed (Patent application; August 4, 1965), synthesis of γ-methyl *erythro*-β-hydroxy-DL-glutamate (Va) was reported at Meeting of Tokai Branch, Pharmaceutical Society of Japan, Gifu, November 1965 by I. Ito, K. Tanabe and H. Hattori.
7) T. Kaneko and M. Izumi, "Chemistry of Proteins," Vol. I, ed. by S. Mizushima and S. Akabori, Kyoritsu Shupan Co., Tokyo, 1954, p. 404.
8) T. Hayakawa, H. Nishi, J. Noguchi, K. Ikeda, T. Yamashita and J. Isemura, *Nippon Kagaku Zasshi*, **82**, 601 (1961).
9) H. Iwasaki, T. Kamiya, O. Oka and J. Ueyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 866 (1969).

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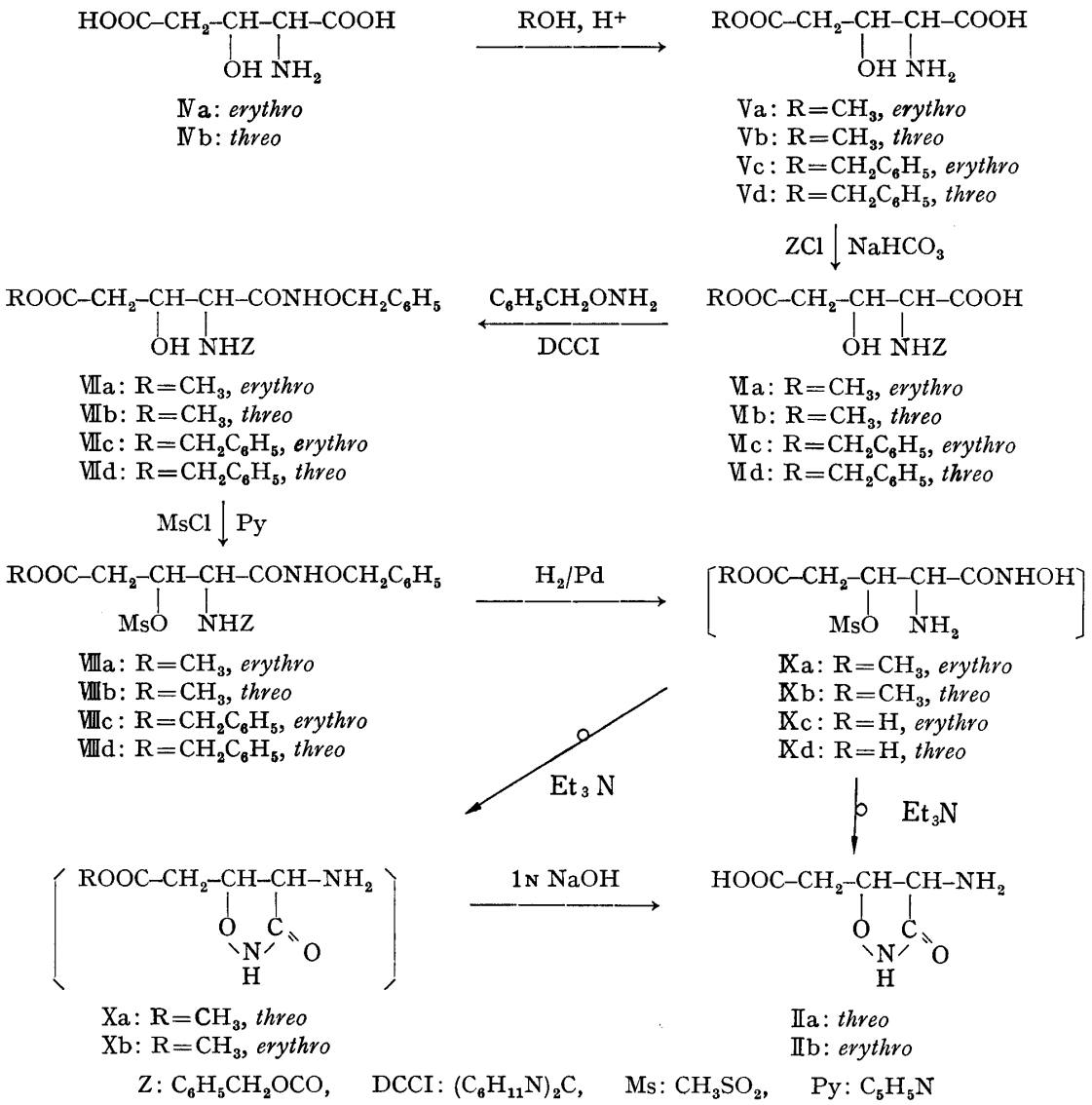

Chart 1

Table I. Yield of Final Products from Various Mesylates

Mesylate	Product (%) Ia	Ib	IIa	IIb
VIIIa	0.5	0.5	*a*)	*a*)
VIIIb	3	12	*a*)	*a*)
VIIIc	—	—	60	—
VIIId	—	—	—	60

a) These products were observed by paper electrophoresis, but not isolated.

As shown in the table, both the *erythro*- and *threo*-isomers of DL-tricholomic acid (I) were unexpectedly obtained from the corresponding diastereoisomer of mesylate having γ-methyl ester (VIII). Their structures were confirmed by comparison of melting points and infrared (IR) spectra (Fig. 1) with the authentic samples[9] and also by catalytic hydrogenolysis to the diastereoisomers of β-hydroxyglutamine (XI). Although the expected isotricholomic acid (II) was detectable in the reaction mixture by paper electrophoresis (PE), all attempts to isolate II by ion-exchange chromatography were unsuccessful due to contamination of other

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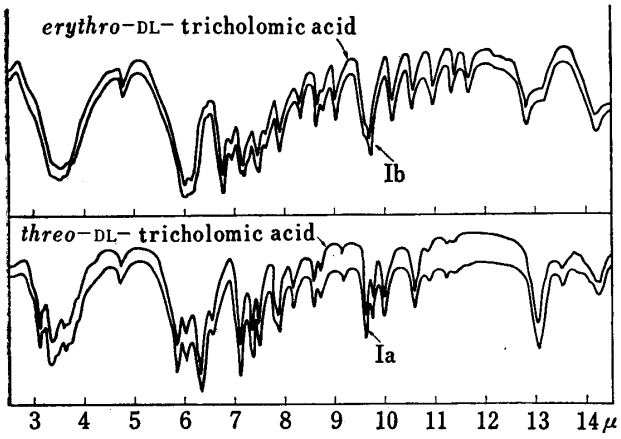

Fig. 1. Infrared Absorption Spectra of Ia and Ib (in KBr)

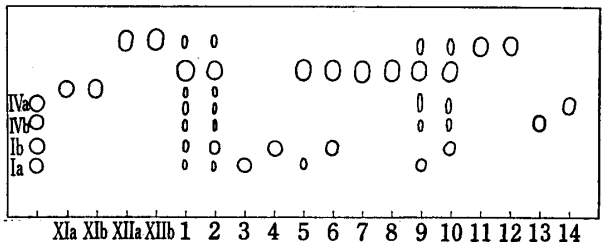

Fig. 2. Chromatograms of Paper Electrophoresis (10% AcOH, 700 V, 1.5 hr)

1: raw product from VIIIa
2: raw product from VIIIb
3: Ia obtained from VIIIa and VIIIb.
4: Ib obtained from VIIIa and VIIIb.
5: Mixture obtained by separation of 1.
6: Mixture obtained by separation of 2.
7: product from VIIIc
8: product from VIIId
9: resultant of recrystallization of 7
10: resultant of recrystallization of 8
11: hydrogenation product of 7
12: hydrogenation product of 8
13: hydrolysate of 11
14: hydrolysate of 12

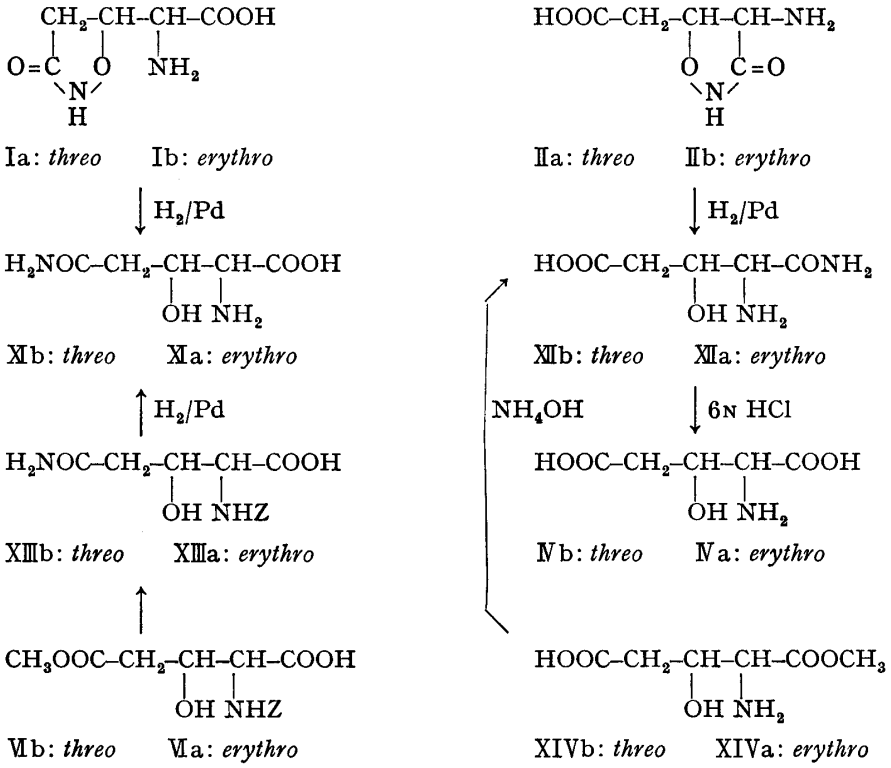

Chart 2

compounds which were identified as Ia and Ib (Fig. 2). On the other hand, the *erythro*- or *threo*-mesylate having γ-benzyl ester (VIII) afforded IIa or IIb as white powder respectively in satisfactory yield. The structures were ascertained by hydrogenolytic conversion to β-hydroxyisoglutamines (XII) and also by acid hydrolysis to diastereoisomers of β-hydroxyglutamic acid (IV). The authentic β-hydroxyglutamines were prepared according to the procedure employed in the synthesis of glutamine[10] as shown in Chart 2. Crystallization

10) E. Abderhalden and H. Nienburg, *Z. Physiol. Chem.*, **219**, 155 (1933); A.F. Beecham, *J. Am. Chem. Soc.*, **76**, 4615 (1954).

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of IIa from water, however, gave several ninhydrin positive substances when detected by PE, and one of them was identified as Ia by PE and amino acid analysis. Same observation was obtained in crystallization of IIb. It is interesting to note that the retention of configuration was observed during the conversion of II to I, and thus, *erythro*- or *threo*-I was formed from the corresponding *erythro*- or *threo*-II.

A possible interpretation on the formation of I from IX may be that an intramolecular rearrangement occurs in the cyclization of IX in a similar manner to the "$\alpha \rightarrow \gamma$-transpeptidation" of glutamylpeptides[11] as shown in Chart 3. This mechanism is consistent with the observed yields of I from VIIIa and VIIIb since the preferred conformation of IXb derived from VIIIb is more favorable for the formation of the oxyimide ring (XVb) than IXa from VIIIa.

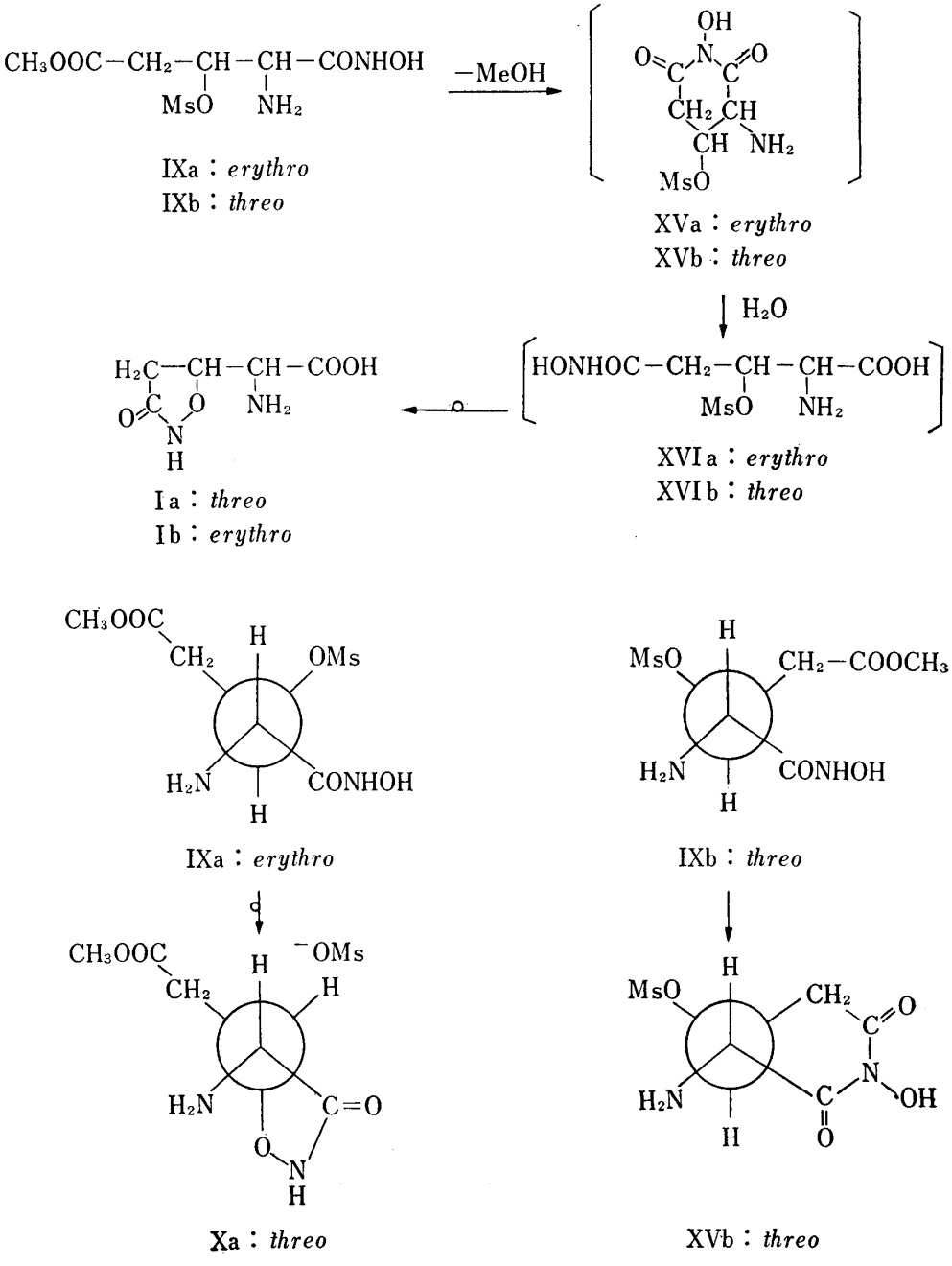

Chart 3

11) E. Schröder and K. Lübke, "The Peptides," Vol. 2, Academic Press, New York, N.Y., 1965, p. 188.

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Experimental[12]

β-Hydroxyglutamic Acid γ-Ester (V)——a) Dry hydrogen chloride was passed in a suspension of 15 g of IVa in 80 ml of MeOH until complete solution was effected. After cooling, crystals separated were collected by filtration and washed with MeOH. Recrystallization from MeOH–ether yielded 15.2 g (94.5%) of Va·HCl, mp 190.5° (decomp.). *Anal.* Calcd. for $C_6H_{12}O_5NCl$: C, 33.73; H, 5.66; N, 6.56; Cl, 16.60. Found: C, 34.02; H, 5.65; N, 6.64; Cl, 16.75. To a solution of 7 g of the hydrochloride in 50 ml of MeOH was added 2.7 ml of dry pyridine. After cooling, crystals separated were collected and recrystallized from H_2O–EtOH to give 5.4 g (93%) of Va, mp 182° (decomp.). *Anal.* Calcd. for $C_6H_{11}O_5N$: C, 40.68; H, 6.26; N, 7.91. Found: C, 40.71; H, 6.25; N, 8.04.

b) Treatment of IVb with methanolic hydrochloric acid gave a 87.5% yield of Vb·HCl, mp 153—158° (decomp.). *Anal.* Found: C, 33.91; H, 5.73; N, 6.84; Cl, 17.00. Treatment of the hydrochloride with pyridine yielded also a 93% of Vb, mp 176—177° (decomp.). *Anal.* Found: C, 40.58; H, 6.23; N, 7.67.

c) To a suspension of 5.4 g of IVa in 4 g of benzylalcohol was added 5.4 g of 60% H_2SO_4 and the mixture was stirred for 2 hr at 70°. To the mixture was added an aqueous solution of 2 mole equivalent of $NaHCO_3$,

Table II $R^1OOC-CH_2-\underset{R^2O}{CH}-\underset{NHZ}{CH}-COR^3$

	R^1	R^2	R^3	Configuration	mp (°C)	Recryst. solvent
VIa	CH_3	H	OH	*erythro*	116 —117	$CHCl_3$–CCl_4–ether
VIb	CH_3	H	OH	*threo*	108.5—109.5	$CHCl_3$–CCl_4–ether
VIc[a]	$C_6H_5CH_2$	H	OH	*erythro*	125 —126	AcOEt
VId[b]	$C_6H_5CH_2$	H	OH	*threo*	110 —111.5	AcOEt
VIIa	CH_3	H	$NHOCH_2C_6H_5$	*erythro*	130 —131	MeOH
VIIb	CH_3	H	$NHOCH_2C_6H_5$	*threo*	114 —115	MeOH
VIIc	$C_6H_5CH_2$	H	$NHOCH_2C_6H_5$	*erythro*	161 —162	CH_2Cl_2
VIId	$C_6H_5CH_2$	H	$NHOCH_2C_6H_5$	*threo*	127 —130	CH_2Cl_2–ether
VIIIa	CH_3	Ms	$NHOCH_2C_6H_5$	*erythro*	132 —133.5	MeOH
VIIIb	CH_3	Ms	$NHOCH_2C_6H_5$	*threo*	110 —111	MeOH
VIIIc	$C_6H_5CH_2$	Ms	$NHOCH_2C_6H_5$	*erythro*	120 —121	MeOH
VIIId	$C_6H_5CH_2$	Ms	$NHOCH_2C_6H_5$	*threo*	113 —114	MeOH

	Yield (%)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VIa	90	$C_{14}H_{17}O_7N$	54.01	5.51	4.50	53.59	5.53	4.63
VIb	64.5	$C_{14}H_{17}O_7N$	54.01	5.51	4.50	53.99	5.43	4.80
VIc[a]	95	$C_{20}H_{21}O_7N$	62.01	5.46	3.62	62.16	5.30	3.61
VId[b]	77	$C_{20}H_{21}O_7N$	62.01	5.46	3.62	62.76	5.46	3.76
VIIa	96	$C_{21}H_{24}O_7N_2$	60.57	5.81	6.73	60.36	5.74	6.62
VIIb	80	$C_{21}H_{24}O_7N_2$	60.57	5.81	6.73	60.83	5.75	6.75
VIIc	87.5	$C_{27}H_{28}O_7N_2$	65.84	5.73	5.69	65.92	5.95	5.86
VIId	65.5	$C_{27}H_{28}O_7N_2$	65.84	5.73	5.69	65.33	5.67	5.87
VIIIa	85	$C_{22}H_{26}O_9N_2S$	53.43	5.30	5.67	53.52	5.27	5.63
VIIIb	88	$C_{22}H_{26}O_9N_2S$	53.43	5.30	5.67	53.44	5.36	5.52
VIIIc	92.5	$C_{28}H_{30}O_9N_2S$	58.93	5.30	4.91	58.88	5.18	4.88
VIIId	90.5	$C_{28}H_{30}O_9N_2S$	58.93	5.30	4.91	59.27	5.20	4.65

a) An oily product obtained by carbobenzoxylation of Vc was crystallized after purification as the dicyclohexylammonium salt. VIc-DCHA (overall yield of two steps: 47%), mp 143—145° (AcOEt)
Anal. Calcd. for $C_{34}H_{44}O_7N_2$: C, 67.58; H, 7.80; N, 4.93 Found: C, 67.67; H, 7.64; N, 5.23
b) VId-DCHA (40%), mp 153—154° (AcOEt). *Anal.* Found: C, 67.35; H, 7.73; N, 4.92.

12) Melting points are uncorrected. Procedures and instrumentation used were the same as previously reported,[4,9] unless otherwise indicated.

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and the solid separated was crystallized from aqueous MeOH to yield 2.3 g (30%) of Vc, mp 202—203° (decomp.). *Anal.* Calcd. for $C_{12}H_{15}O_5N$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.03; H, 5.89; N, 5.53.

d) Treatment of IVb with benzylalcohol and 70% H_2SO_4 as described above yielded a 60% yield of Vd, mp 180° (decomp.). *Anal.* Found: C, 57.17; H, 5.98; N, 5.29.

N-Carbobenzoxy-β-hydroxyglutamic Acid γ-Ester (VI, see Table II)[13]

N-Carbobenzoxy-β-hydroxyglutamic Acid α-Benzyloxyamide γ-Esteyr (VII, see Table II)[13]

N-Carbobenzoxy-β-mesyloxyglutamic Acid α-Benzyloxyamide γ-Ester (VIII, see Table II)[13]

N-Carbobenzoxy-β-hydroxyglutamine (XIII)——a) Compound VIa (1 g) was dissolved in 5 ml of 28% NH_3 and the solution was left to tand overnight at room temperature. After the reaction had completed, the excess NH_3 was removed under reduced pressure and the solution was stored in a refrigerator after adjusting its pH to 3 with 10% HCl. Crystals separated were recrystallized from H_2O to give 0.85 g (94%) of XIIIa, mp 164.5° (decomp.). *Anal.* Calcd. for $C_{13}H_{16}O_6N_2$: C, 52.70; H, 5.44; N, 9.46. Found: C, 52.81; H, 5.51; N, 9.43.

b) Treatment of VIb with 28% NH_3 described as above yielded a 89% yield of XIIIb.

β-Hydroxyglutamine (XI)——a) Catalytic hydrogenation of 0.5 g of XIIIa according to the usual procedure (with Pd-black, in 100 ml of H_2O, for 3 hr) and recrystallization of the product from H_2O–EtOH afforded 0.25 g (91%) of XIa, mp 178° (decomp.). *Anal.* Calcd. for $C_5H_{10}O_4N$: C, 37.03; H, 6.22; N, 17.28. Found: C, 36.85; H, 6.23; N, 16.87. PE (Fig. 2).

b) Catalytic hydrogenation of XIIIb described as above yielded a 93% yield of XIb, mp 195° (decomp.). *Anal.* Found: C, 36.86; H, 6.24; N, 17.05.

β-Hydroxyisoglutamine (XII)——a) α-Methylester XVa (1 g) was dissolved in 2 ml of 28% NH_4OH and the solution was left to stand overnight at room temperature. After the reaction had completed, the reaction mixture was concentrated to dryness under reduced pressure and the residue was crystallized from H_2O to yield 0.77 g (87.5%) of XIIa, mp 223—224° (decomp.). *Anal.* Found: C, 36.91; H, 6.33; N, 17.09.

b) Treatment of XVb with 28% NH_4OH described as above yielded a 76% yield of XIIb, mp 211—212° (decomp.). *Anal.* Found: C, 37.21; H, 6.32; N, 17.14.

D-L-Tricholomic Acid and Its *threo*-Isomer (I) from VIII aand VIIIb (See Table I)——a) MesylateVIIIa was subjected to hydrogenolysis, cyclization and saponification according to the procedures previously reported.[4] The reaction mixture obtained was subjected to the separation by ion-exchange chromatography on Amberlite IR-120, Dowex-1 and Dowex-50 to yield Ia and Ib. The structure of each diastereoisomer of I was ascertained by elemental analysis, amino acid analysis, PE, PC and IR-spectroscopy.

b) According to the procedures described above the diastereoisomers of I were obtained from VIIIb as shown in Table I.

Isotricholomic Acid (II) from VIIIc or VIIId (See Table I)——a) Mesylate VIIIc (1 g) was subjected to hydrogenolysis and cyclization according to the procedures previously reported.[7] The reaction mixture was evaporated to dryness under reduced pressure at low temperature, and the residue was taken up in a small amount of H_2O. To the solution was added a large amount of EtOH and cooled with ice-salt. The white precipitate was isolated by filtration, washed with EtOH, and dried in a desicator containing P_2O_5 in the cold. The product IIa, obtained as white powder in a 75% yield (210 mg), was homogeneous on PE, and decomposed to a black gum at about 95—100° on measurement of its mp. On crystallization from H_2O–EtOH, IIa decomposed partially as shown in Fig. 2. Catalytic hydrogenation of IIa with Pd-black yielded XIIa mainly and the product afforded XIVa mainly by acid hydrolysis with 6N HCl (Fig. 2).

b) The same treatment of VIIId as in the case of VIIIc afforded 20 mg of IIb, which was a white powder and exhibited almost analogous behavior as IIa on crystallization and mp measurement. By catalytic hydrogenation IIb yielded mainly XIIb, which afforded XIVb by acid hydrolysis.

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13) The procedure described in the previous paper[4] was satisfactorily adopted for this preparation.

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