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Synthesis of Tricholomic Acid. V.[1] Synthesis of DL-Tricholomic Acid and Its *threo*-Isomer from Diastereoisomers of β-Hydroxyglutamic Acid through α-Mono Esters[2]

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Racemic tricholomic acid and its *threo*-isomer (I) were prepared by a new route starting from the diastereoisomers of β-hydroxyglutamic acid (II) through α-mono esters (XI) and γ-benzyloxyamides (XIII).

In the preceding paper,[1] the synthesis of DL-tricholomic acid and its *threo*-isomer (I) from β-hydroxyglutamic acid (II) *via* dialkyl β-chloroglutamate (III) and γ-hydroxamic acid (IV) was reported. Despite our efforts at further refinements of the reaction conditions, the result was not satisfactory from the view point of yield and stereospecificity. In this paper, first the discussion is given in regard to possible side reactions in the previous synthesis and then the synthesis of I by a new route is described.

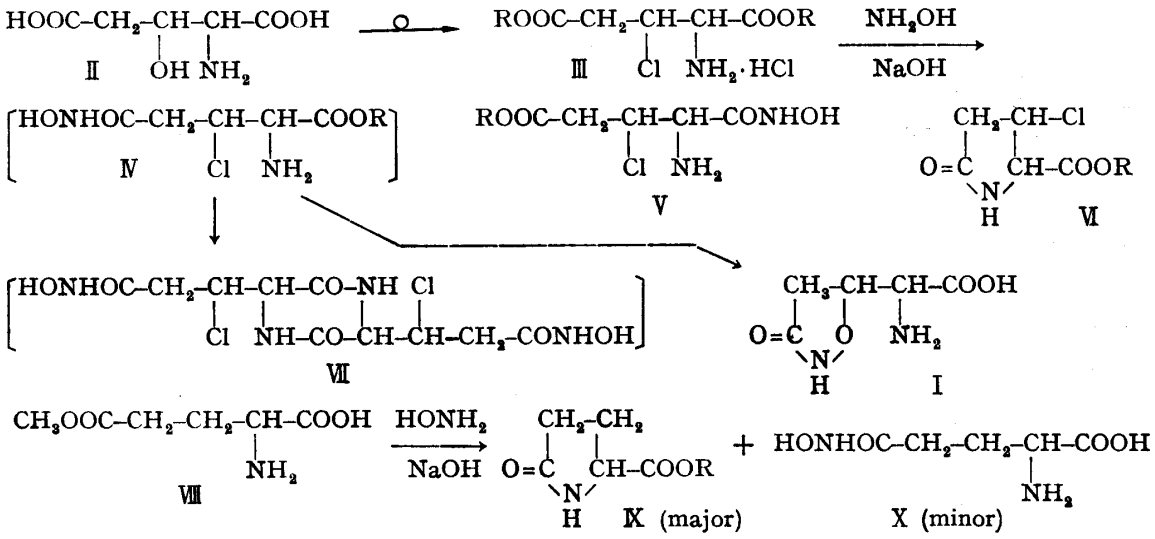

Chart 1

Reaction of γ-methyl glutamate (VIII) with hydroxylamine under the same conditions as those previously employed in the transformation (III→IV) gave pyroglutamic acid (IX) in a high yield (86%), while the yield of the γ-hydroxamic acid (X) was less than 14%. This model reaction suggests that the formation of the pyrrolidone (VI) by an intramolecular cyclization would have predominated, thus leading to a principal side reaction in the previous synthesis. Moreover, III offers other possibilities to produce other by-products such as an α-hydroxamic acid (V) and a diketopiperazine (VII).

1) Part IV: H. Iwasaki, T. Kamiya, C. Hatanaka, Y. Sunada and J. Ueyanagi, *Chem. Pharm. Bull.* (Tokyo), **17**, 873 (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.*

In order to avoid the formation of these by-products a new synthetic route was designed, as shown in Chart 2.

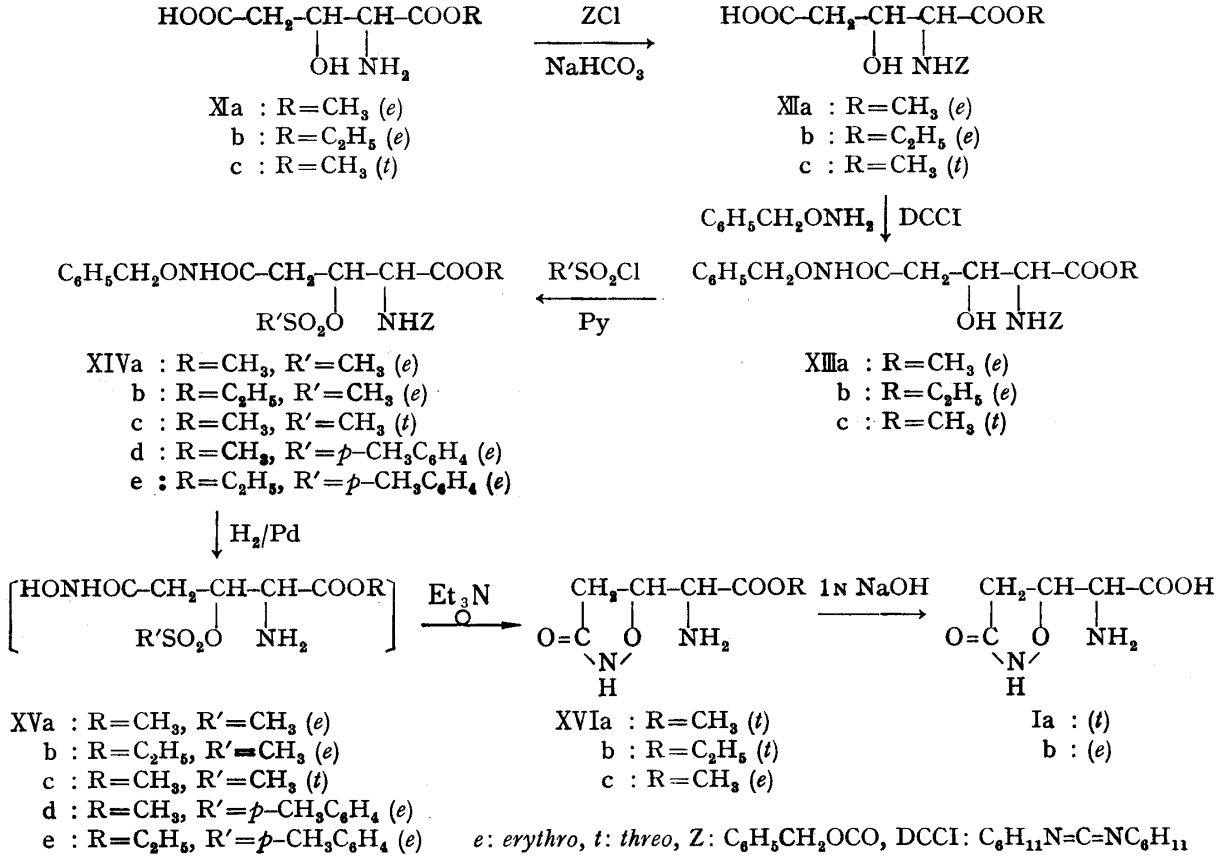

Chart 2

Preparation of the α-halfesters (XI) was done in accordance with the method for the synthesis of glutamic acid α-esters.[4] Dialkyl β-hydroxyglutamates (XVII) were treated with tritylchloride and triethylamine to give N-tritylamino acid diesters (XVIII), which were partially hydrolyzed with 1N sodium hydroxide on an equivalent molar basis. The yield of the partial hydrolysis was high due to steric effect of the bulky trityl group, and the removal of the trityl group was effected easily by warming with 50% acetic acid.

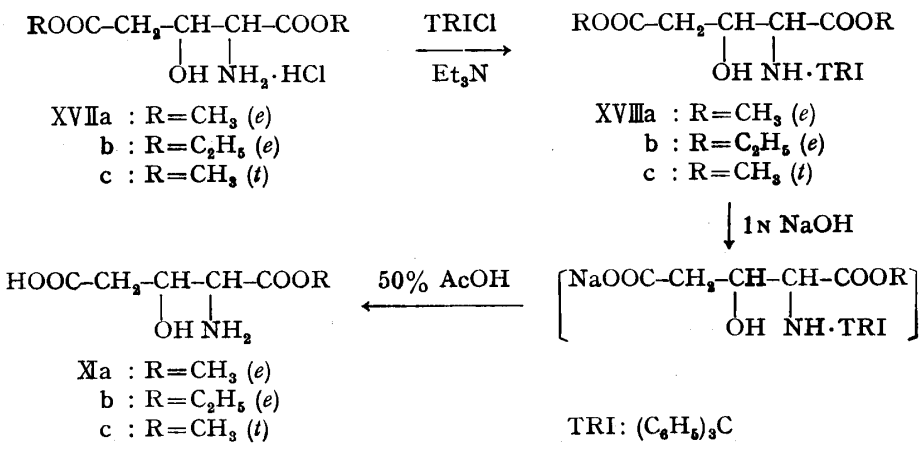

Chart 3

4) G. Amiard, R. Heymes and L. Velluz, *Bull. Soc. Chim. France*, **1956**, 97.

The structure of XI was confirmed by elemental analysis, paper chromatography (PPC)[5] and more exactly by nuclear magnetic resonance (NMR) studies described later.

These α-mono esters were converted to the N-carbobenzoxy compounds (XII) by the procedure used for the synthesis of N-carbobenzoxythreonine.[6] Condensation of XII and O-benzylhydroxylamine by the action of N,N'-dicyclohexylcarbodiimide (DCCI)[7] gave γ-benzyloxyamides (XIII). Of the two diastereoisomers of XIII, the *erythro*-isomers were easily obtained as crystals, while the *threo*-isomer was obtained as an oily substance which was successfully crystallized by purification with silicagel.

All the γ-benzyloxyamides (XIIIa—c) were then converted to the mesylates (XIVa—c) or the tosylates (XIVd, e) by the usual procedure, and their structures were confirmed by the NMR spectra (Fig. 1).

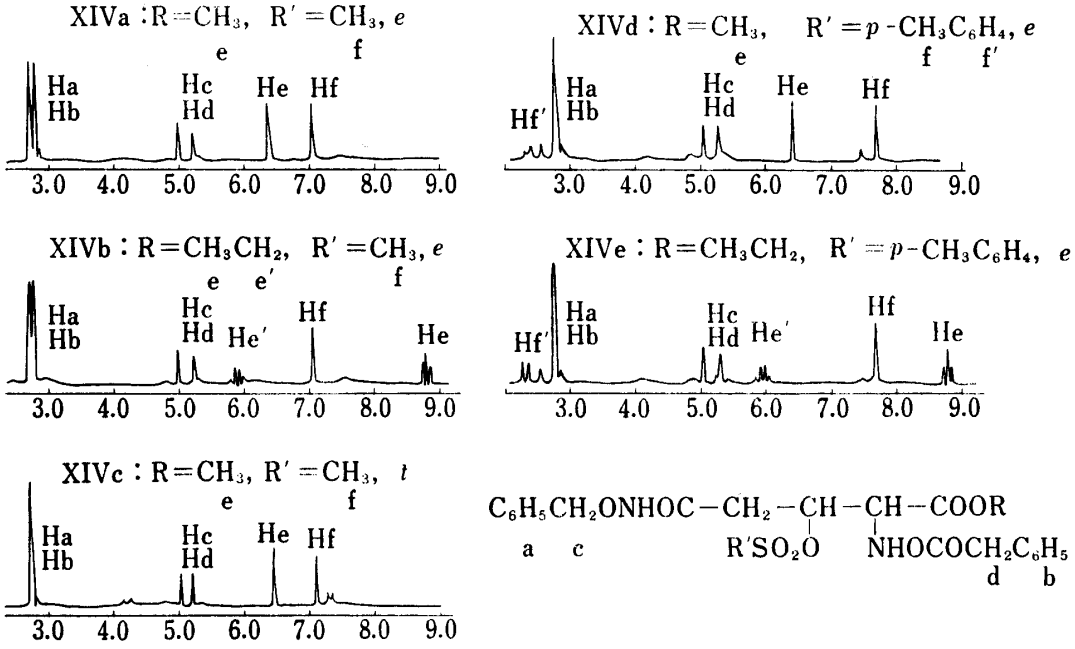

Fig. 1. Nuclear Magnetic Resonance Spectra of XIV in $CDCl_3$ at 100 Mc

The catalytic hydrogenolysis of XIVe by the method of Plattner, *et al.*[7] (palladium black in ethanol, in the presence of one mole equivalent of hydrobromic acid at room temperature), somewhat surprisingly, led to a diester (XIX), the structure of which was identified by the elemental analysis, infrared (IR) spectrum and color reactions.[8] In the absence of hydro-

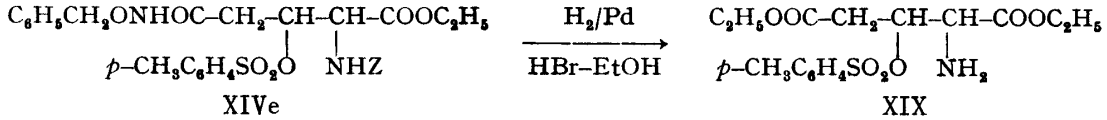

Chart 4

bromic acid, however, the catalytic hydrogenolysis (palladium black in aqueous methanol at 10—15°) gave XVe in a high yield. Each of five benzyloxyamides (XIVa—e) was subjected to this modified hydrogenolysis, and to cyclization with triethylamine, followed by hydrolysis

5) XIa, b, c are located between II and XVII on the chromatogram.
6) J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N.Y., 1961, p. 893.
7) Pl. A. Plattner, A. Boller, H. Frick, A. Fürst, B. Hegedüs, H. Kirchensteiner, St. Majnoni, R. Schläpfer and H. Spiegelberg, *Helv. Chim. Acta*, 40, 1531 (1957).
8) Ninhydrin reaction is positive, while $FeCl_3$ reaction is negative.

of the α-ester without isolation of intermediates (XV, XVI) to give α-amino-3-oxo-5-isoxazolidineacetic acid (I).

Isolation and identification of I were carried out by the procedures described in the preceding paper[1,9] and by ion-exchange chromatography on Dowex-50,[10] and the racemic diastereomers (Ia, b) of α-amino-3-oxo-5-isoxazolidineacetic acid were obtained from all sulfonates (XIVa—e) in about 10% yields without any noticeable effect of the substituents and the configuration of XIV (Table I). It was of interest to note that the cyclization of

Table I. Yield of Ia and Ib from XIV

XIV	Yield (%) Ia (*threo*)	Ib (*erythro*)
a (*erythro*, R: CH_3, R′: CH_3)	12.5	1.7
b (*erythro*, R: C_2H_5, R′: CH_3)	12.7	1.8
c (*threo*, R: CH_3, R′: CH_3)	2.1	12.8
d (*erythro*, R: CH_3, R′: *p*-$CH_3C_6H_4$)	12.1	1.5
e (*erythro*, R: C_2H_5, R′: *p*-$CH_3C_6H_4$)	12.2	1.6

the hydroxamic acids (XV) had brought for the inversion of the configuration at β-asymmetric carbon yielding *erythro*-I from *threo*-XIV and *threo*-I from *erythro*-XIV. The overall yield in the last three steps was not improved by the kind of sulfonate under various reaction conditions.

NMR Spectra of the Mono Esters (XIa, b, c)

The NMR spectra of XIa, b, c provide a clear proof for their structures. For example, the ester signal of XIa appeared as a singlet, while that of the corresponding dimethyl ester (XVIIa) as a doublet, and the chemical shift of the singlet was almost equal to that of the lower-field peak of the doublet as shown in Fig. 2. The same relationship was observed in the ethyl esters (XVIIb—XIb) and the *threo*-esters (XVIIc—XIc) (Table II). Since the singlet was assigned to the α-esters (XI), the higher field signal of the doublet observed with the diesters should be assigned to the γ-esters. Thus, the NMR spectra provided a useful criterion not only for the identification of α- and γ-esters of β-hydroxyglutamic acid but also for the purity test. Chemical shifts of various esters of α-aminodicarboxylic acids are listed in Table II.

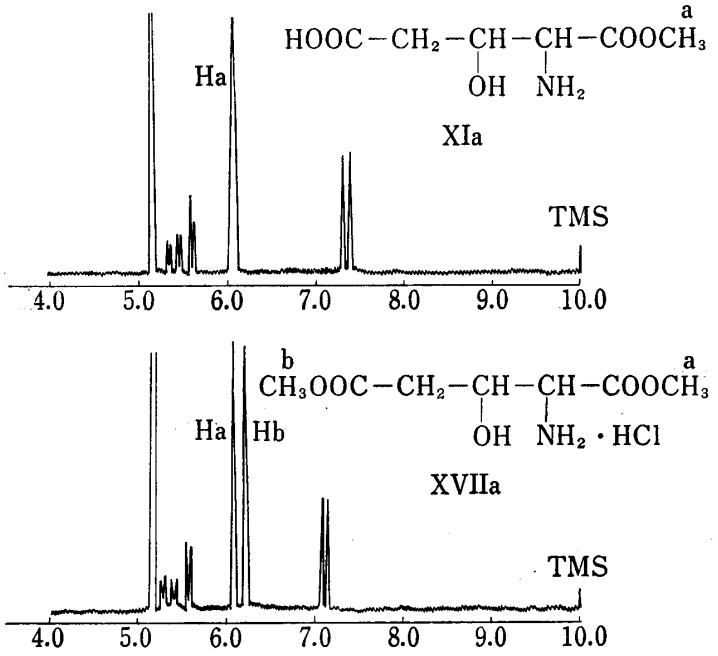

Fig. 2. Nuclear Magnetic Resonance Spectra of XIa and XVIIa in D_2O at 60 Mc

9) Isolation and separation by ion-exchange chromatography on IR-120 and Dowex-1, and identification by PPC, paper electrophoresis (PE) and IR and NMR spectroscopies.

10) This procedure was useful for the separation of diastereomers of I.

Table II. Chemical Shifts of Various Esters of α-Aminodicarboxylic Acids in D_2O

Substance		α-Ester	γ-Ester (β-Ester)
$HOOC-CH_2-\underset{OH}{CH}-\underset{NH_2}{CH}-COOCH_3$	(*e*)[a]	6·08	—
$CH_3OOC-CH_2-\underset{OH}{CH}-\underset{NH_2\cdot HCl}{CH}-COOCH_3$	(*e*)	6.06	6.20
$HOOC-CH_2-\underset{OH}{CH}-\underset{NH_2}{CH}-COOC_2H_5$	(*e*)	8.62[b]	—
$C_2H_5OOC-CH_2-\underset{OH}{CH}-\underset{NH_2\cdot HCl}{CH}-COOC_2H_5$	(*e*)	8.63[b]	8.70[b]
$HOOC-CH_2-\underset{NH_2}{\underset{OH}{CH}}-CH-COOCH_3$	(*t*)[a]	6.06	—
$CH_3OOC-CH_2-\underset{NH_2\cdot HCl}{\underset{OH}{CH}}-CH-COOCH_3$	(*t*)	6.03	6.17
$CH_3OOC-CH_2-CH_2-\underset{NH_2}{CH}-COOH$		—	6.25
$CH_3OOC-CH_2-CH_2-\underset{NH_2\cdot HCl}{CH}-COOCH_3$		6.11	6.24
$CH_3OOC-CH_2-\underset{NH_2}{CH}-COOH$		—	6.20
$CH_3OOC-CH_2-\underset{NH_2}{CH}-COOCH_3$		6.12	6.21

a) *e* and *t* designate *erythro* and *threo*, respectively b) chemical shift of methyl proton

Experimental[11]

Reaction of γ-Methyl L-Glutamate (VIII) with Hydroxylamine——A solution of 110 mg of hydroxylamine hydrochloride and 190 mg of NaOH in 2 ml of H_2O was added dropwise under stirring at −5° to a solution of 300 mg of VIII in 5 ml of EtOH. The mixture was stirred at −5°—room temperature for 3 hr. The solution was diluted with H_2O and passed through a column of Amberlite IR-120 (H^+-form). The effluent and washings were combined and evaporated to give crystals, 168 mg (86%): mp 161—162° (EtOH–H_2O). IR spectrum of the crystal was identical with that of an authentic IX, mp 162—163°.[12] Ninhydrin positive part of 5% NH_4OH eluate was evaporated to a white solid (30 mg), which was identified as a mixture of X and L-glutamic acid by PPC.

General Preparation of N-Trityl β-Hydroxyglutamic Acid Dialkyl Ester (XVIII, see Table III)——To a suspension of 0.05 mole of XVII in 120 ml of dry $CHCl_3$ was added 11 g (0.11 mole) of triethylamine and 0.05 mole of tritylchloride, and the mixture was stirred for 5 hr at room temperature. Chloroform was added and the solution was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to dryness. The residue was recrystallized from MeOH to yield XVIII.

General Preparation of β-Hydroxyglutamic Acid α-Ester (XI, see Table III)——To a hot solution of 0.02 mole of XIII in 60 ml of MeOH was added one drop of phenolphthalaine solution and 20 ml of 1N NaOH. The red colored solution was refluxed for several minutes until the color had faded and evaporated *in vacuo*. The residue was treated with 40 ml of 50% AcOH for 20 min at about 50° and white precipitates that separated were filtered, washed with H_2O. The filtrate and the washing were combined and concentrated to a crystalline residue, which was recrystallized from H_2O–EtOH to yield XI.

General Preparation of N-Carbobenzoxy-β-hydroxyglutamic Acid α-Alkyl Ester (XII, see Table III)——To a solution of 0.01 mole of XI and 2 g of $NaHCO_3$ in 30 ml of H_2O was added 2 ml of carbobenzoxychloride at 0°, and the mixture was stirred for 4 hr at 0°—room temperature. The solution was washed with ether,

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

12) M. Bergmann and L. Zervas, *Z. Physiol. Chem.*, **221**, 51 (1933).

TABLE III. $R'OOC-CH_2-\underset{\underset{OH}{|}}{CH}-\underset{\underset{NH\cdot X}{|}}{CH}-COOR$

	R	R′	X	Configuration	mp (°C)	Yield (%)	IR (cm^{-1})	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
XVIIIa	CH_3	CH_3	TRI[a]	*erythro*	140.5—141	94	1730, 1747	$C_{26}H_{27}O_5N$	72.03	6.28	3.23	71.97	6.14	3.41
XVIIIb	C_2H_5	C_2H_5	TRI	*erythro*	98 —99	84.5	1730, 1743	$C_{28}H_{31}O_5N$	72.86	6.77	3.04	72.58	6.57	3.25
XVIIIc	CH_3	CH_3	TRI	*threo*	146.5	89	1726, 1745	$C_{26}H_{27}O_5N$	72.03	6.28	3.23	71.98	6.25	3.27
XIa	CH_3	H	H	*erythro*	181 (d.)[a]	93.5	1748	$C_6H_{11}O_5N$	40.68	6.26	7.91	40.87	6.35	7.78
XIb	C_2H_5	H	H	*erythro*	165.5—166(d.)	96	1743	$C_7H_{13}O_5N$	43.97	6.85	7.32	44.06	6.76	7.34
XIc[b]	CH_3	H	H	*threo*	163 (d.)	72	1745	$C_{11}H_{11}O_5N$	40.68	6.26	7.91	40.58	6.35	7.93
XIIa	CH_3	H	Z[a]	*erythro*	120.5	90	1726	$C_{14}H_{17}O_7N$	54.01	5.51	4.50	54.23	5.47	4.50
XIIb	C_2H_5	H	Z	*erythro*	108 —110	88	1723	$C_{15}H_{19}O_7N$	55.38	5.89	4.31	55.61	5.78	4.13
XIIc	CH_3	H	Z	*threo*	oil	90	—	$C_{14}H_{17}O_7N$	54.01	5.51	4.50	—	—	—

a) TRI: $(C_6H_5)_3C$, Z: $C_6H_5CH_2OCO$, d.: decomp.
b) A crude product contaminated with β-hydroxyglutamic acid was isolated, however, the pure diester was obtained by chromatography on IRC-50 (0.5N acetate).

TABLE IV. $C_6H_5CH_2ONHOC-CH_2-\underset{\underset{R'O}{|}}{CH}-\underset{\underset{NHOCOCH_2C_6H_5}{|}}{CH}-COOR$

	R	R′	Configuration	mp (°C)	Yield (%)	τ-value (100 Mc, $CDCl_3$) $O\cdot CH_2-C_6H_5$, R, R′	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
XIIIa	CH_3	H	*erythro*	87—89	91.5		$C_{21}H_{24}O_7N_2$	60.57	5.81	6.73	60.45	6.06	7.04
XIIIb	C_2H_5	H	*erythro*	106	83		$C_{22}H_{26}O_7N_2$	61.38	6.09	6.51	61.57	6.35	6.18
XIIIc[a]	CH_3	H	*threo*	110—112	36	5.04, 5.23, 6.45, —	$C_{21}H_{24}O_7N_2$	60.57	5.81	6.73	60.80	5.75	6.77
XIVa	CH_3	Ms[b]	*erythro*	139—140	85	5.00, 5.25, 6.33, 7.07	$C_{22}H_{26}O_9N_2S$	53.43	5.30	5.69	53.23	5.16	5.57
XIVb	C_2H_5	Ms	*erythro*	106—110	83	4.99, 5.23, 8.78[c], 7.07	$C_{23}H_{28}O_9N_2S$	54.32	5.52	5.51	54.07	5.52	5.50
XIVc	CH_3	Ms	*threo*	108	84	5.02, 5.21, 6.43, 7.10	$C_{22}H_{26}O_9N_2S$	53.43	5.30	5.69	53.38	5.26	5.58
XIVd	CH_3	Ts[b]	*erythro*	120—121.5	65	5.04, 5.30, 6.41, 7.71[d]	$C_{28}H_{30}O_9N_2S$	58.93	5.30	4.91	59.05	5.24	4.74
XIVe	C_2H_5	Ts	*erythro*	94	80	5.03, 5.30, 8.82[c], 7.70[d]	$C_{29}H_{32}O_9N_2S$	59.57	5.52	4.79	59.43	5.31	4.58

a) Oily raw product was purified by chromatography on silica gel (benzene–EtOH; 8:2) and recrystallized from benzene. b) Ms: CH_3SO_2- Ts: p-$CH_3C_6H_4SO_2-$
c) chemical shift of methyl protons; triplet d) chemical shift of methyl protons; singlet

acidified to pH 3 with 6N HCl and extracted with ether. The extract, dried over Na_2SO_4, was condensed to a white crystalline residue, which was recrystallized from $CHCl_3$–CCl_4–ether to yield XII.

General Preparation of N-Carbobenzoxy-β-hydroxyglutamic Acid α-Alkyl Ester γ-Benzyloxyamide (XIII, see Table IV)——A solution of 0.03 mole of DCCI in 25 ml of tetrahydrofuran was slowly added to a stirred solution of 0.03 mole of XII and 0.033 mole of O-benzylhydroxylamine in 100 ml of tetrahydrofuran. The mixture was stirred for 6 hr at room temperature and the precipitated dicyclohexylurea was removed by filtration. Evaporation of the filtrate under reduced pressure yielded a crystalline residue. The residue was recrystallized from ether to yield XIII.

General Preparation of N-Carbobenzoxy-β-mesyloxy- (or β-tosyloxy-) glutamic Acid α-Alkyl Ester γ-Benzyloxyamide (XIV, see Table IV)——To a solution of 0.01 mole of XIII in 20 ml of dry pyridine was added 0.01 mole of mesylchloride (or tosylchloride) and the solution was allowed to stand at 5° overnight. The solution was treated with 200 g of ice–water and extracted with three 100 ml portions of CH_2Cl_2. The extract was washed with H_2O, dried over Na_2SO_4, and condensed to a heavy syrup under reduced pressure. The residue was crystallized from MeOH to yield XIV.

Catalytic Hydrogenolysis of XIVe by the Conventional Procedure[7]——To a solution of 552 mg of XIVe in 20 ml of EtOH was added 0.18 ml of 47% HBr and 30 mg of palladium black, and the reaction mixture was subjected to catalytic reduction at room temperature for 16 hr according to the conventional procedure.[7] The reaction mixture was filtered and the filtrate was condensed to 8 ml under reduced pressure. One half of the concentrate was left standing in a refrigerator overnight to yield 100 mg of XIX, mp 133—134°. *Anal.* Calcd. for $C_{16}H_{23}O_7NS$: C, 51.46; H, 6.21; O, 29.99; N, 3.75. Found: C, 51.32; H, 5.65; O, 29.96; N, 4.23. The other half of the concentrate was subjected to cyclization, hydrolysis and isolation according to the procedure described in the next experiment.

General Preparation of α-Amino-3-oxo-5-isoxazolidineacetic Acid (I, see Table I)——To a solution of 2 g of XIV in 160 ml of MeOH–H_2O (5:1) was added 150 mg of Pd-black, and the reaction mixture was subjected to catalytic reduction at 15° for 3 hr. After the reaction had completed, the catalyst was removed by filtration and the solution was concentrated under reduced pressure. The aqueous solution thus obtained was treated with 10 ml of Et_3N at 0° and stirred for 3 hr, during which the reaction temperature raised to 18°. The solution was stirred for further 2 hr at room temperature after addition of 10 ml of 1N NaOH. Excess Et_3N was removed by evaporation under reduced pressure at low temperature and the resulting solution was passed through a column (3×15 cm) of ion–exchanger (Amberlite IR–120, H-type). After being washed with H_2O, the column was eluted with 0.5N NH_4OH. The eluate was concentrated under reduced pressure keeping the temperature below 50°. The residue (*ca.* 200 mg) was chromatographed on a column (4×120 cm) of Dowex-1 ion–exchanger (200—400 mesh) with 0.5N AcOH as solvent in an automated fraction collector. Each fraction (15 ml) was examined by ninhydrin reaction. Ninhydrin positive by–products were collected in fractions 10 to 16, and a diastereomeric mixture of I in fractions 18 to 26. The fractions 18 to 26 were combined and condensed to a crystalline residue under reduced pressure.

Diastereoisomers of I in the mixture were separated by a column (4.5×130 cm) of Dowex–50 ion–exchanger (200—400 mesh) with the eluent of 0.1 mole pyridine–acetate buffer (pH 3.1). The identity of each diastereoisomer with authentic sample[1] was established by the mixed melting point determination, IR–spectroscopy, PPC, PE and the automatic amino acid analysis.