[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD., AND THE LABORATORY OF BIOCHEMISTRY, FACULTY OF SCIENCE, KYUSHU UNIVERSITY, FUKUOKA, JAPAN]

Stereochemistry of the Conversion of Allylglycine to γ-Hydroxyornithine and 4-Hydroxyproline

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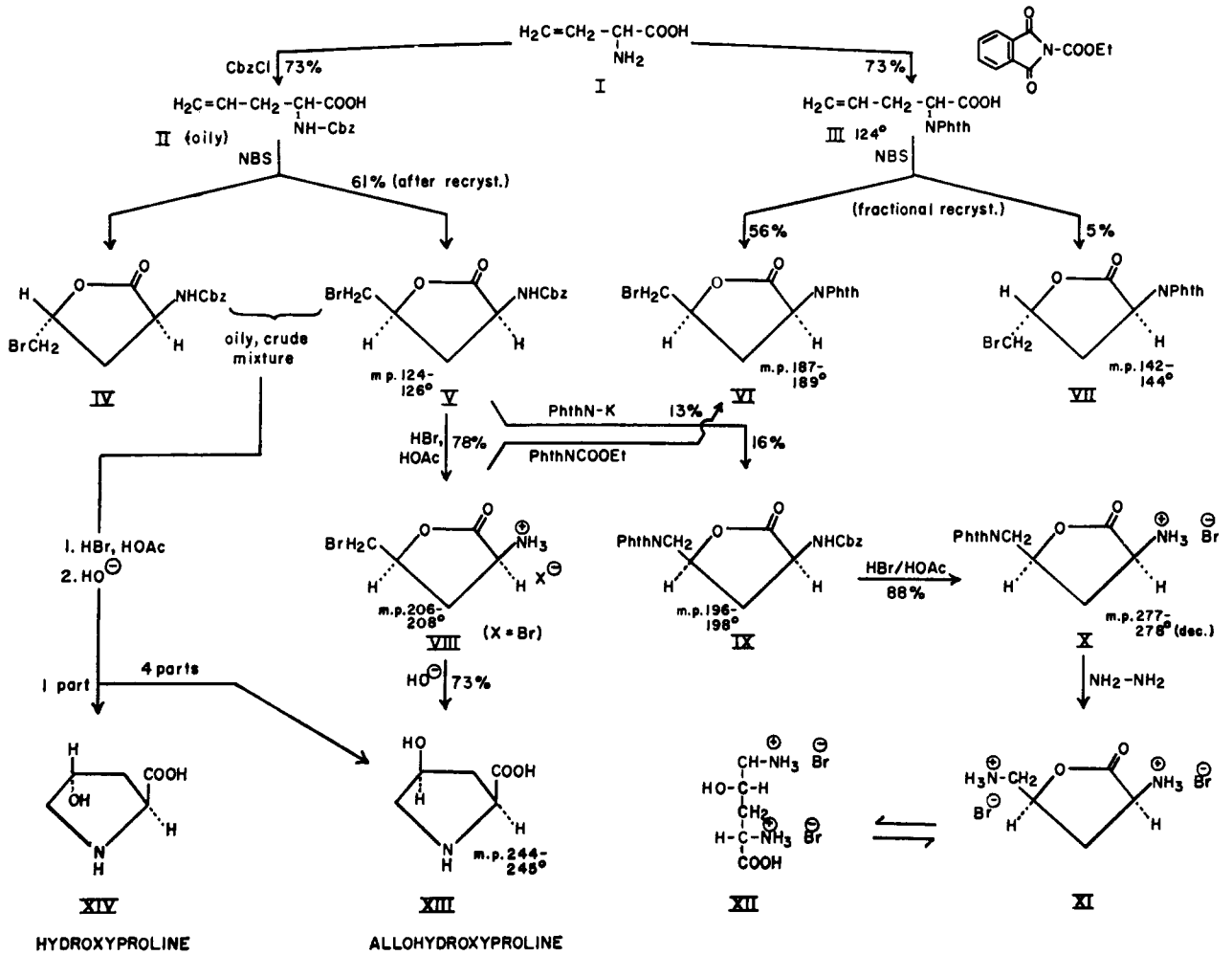
The reaction of carbobenzyloxy-DL-2-amino-4-pentenoic acid (carbobenzyloxyallylglycine, II) with N-bromosuccinimide leads to a crystalline bromolactone V whose *cis* configuration was proved by conversion to allohydroxy-DL-proline XIII *via* VIII. A mixture of 77% allohydroxy-DL-proline (XIII) and 23% hydroxy-DL-proline was obtained when the crude mixture of bromolactones IV and V was subjected to the same transformations. The *cis*-bromoaminolactone VIII was converted to the phthalimido derivative VI which was also obtained as the major product in the reaction of NBS with phthaloyl-DL-2-amino-4-pentenoic acid (III) together with the isomeric *trans*-lactone VII. The *cis*-bromolactone V was converted to the phthalimido derivative IX which, after decarbobenzoxylation (X) and hydrazinolysis gave sterically homogeneous *threo*-γ-hydroxy-DL-ornithine (XI ⇄ XII). A mechanism for the preferential formation of *cis*-lactones is discussed. The method provides a practical, stereoselective synthesis of allohydroxyproline.

N-Bromosuccinimide (NBS) cleaves peptides and amides derived from "allylglycine" (DL-2-amino-4-pentenoic acid, I) with the formation of crystalline bromolactones.[2] The subject of this paper is the establishment of the configuration and stereochemistry of these lactones.

Decarbobenzoxylation (HBr in acetic acid) of V gave the salt of the aminolactone VIII which underwent base-catalyzed intramolecular cyclization to yield, after recrystallization, pure allohydroxy-DL-proline (XIII), as assayed by chromatography, electrophoresis and ion exchange chromatography.[3] The unrecrystallized

STERIC RELATIONS AND INTERCONVERSIONS OF THE BROMOLACTONES DERIVED FROM DL-2-AMINO-4-PENTENOIC ACID



The yield of bromolactone V from the reaction of N-carbobenzyloxyallylglycine (II) with NBS was in excess of 60%. This lactone, m.p. 124–126°, is sterically homogeneous and is a *cis*-disubstituted γ-lactone. The following transformations prove these two points.

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(2) N. Izumiya, J. E. Francis, A. V. Robertson and B. Witkop, *J. Am. Chem. Soc.*, **84**, 1702 (1962).

allohydroxyproline contained 6% hydroxy-DL-proline (XIV), which presumably was formed as a result of base-catalyzed epimerization in the cyclization step VIII → XIII, at the center next to the lactone carbonyl.

When this same sequence of reactions was carried out with the oily reaction mixture of lactones IV and V, there was obtained a mixture of 23% of hydroxy-DL-

(3) K. A. Piez, *J. Biol. Chem.*, **207**, 77 (1954).

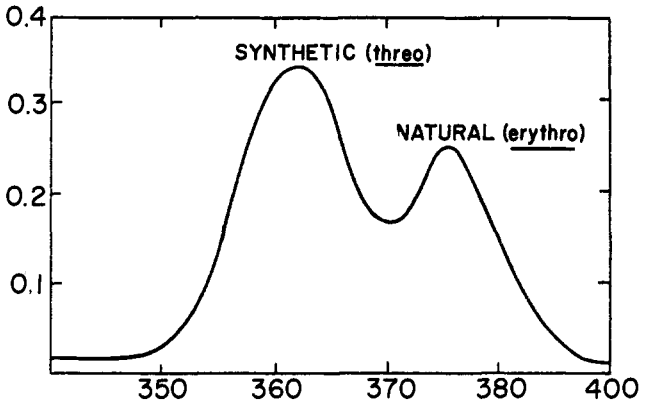
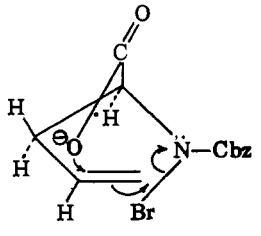
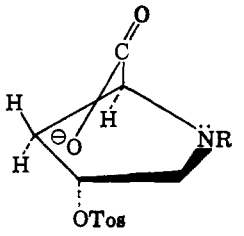


Fig. 1.—Elution pattern of *threo*- and *erythro*-γ-hydroxyornithine from ion exchange chromatography (Dowex 50W-X8).

proline (XIV) and 77% of allohydroxy-DL-proline (XIII). Allowing for minor epimerization (see above) these values should be corrected to 18% *vs.* 82%, *i.e.*, 1 part of XIV *vs.* 4 parts of XIII. There is then a noticeable steric control[3a] operative in the bromolactonization favoring formation of the *cis*-disubstituted bromolactone V. This might be rationalized in terms of an intermediate N-bromo derivative XV in which the intramolecular transfer of bromine from nitrogen



XV



XVa

to the terminal carbon of the double bond would be followed by participation of the carboxylate anion, possibly by a (diaxial) concerted process. The reaction is analogous to the intramolecular displacement (XVa) of N-acyltosyloxyprolines leading to allohydroxyproline lactones.[4] This intramolecular transfer of bromine,[5] if exclusively operative, would lead to pure *cis*-lactone V.

In order to test this hypothesis, the N-phthaloyl derivative III, in which there is no free NH and consequently no potential N–Br intermediate, was prepared by the elegant method of Nefkens.[6] The action of NBS again led to a mixture from which in this case the diastereoisomeric lactones were both isolatable by fractional crystallization. The *cis*-lactone VI was again the major product. Its configuration was proved by phthalation of the *cis*-aminolactone VIII with N-carbethoxyphthalimide.

The ratio of *cis*- *vs.* *trans*-lactone based on the yields of crystalline material, 56% of VI and 5% of VII, should of course be supplemented by direct analysis of the final mixture of hydroxyprolines. Since it was not possible to remove the phthaloyl blocking group by hydrazinolysis and to effect simultaneous ring closure, it is difficult to assess the effects of a possible N–Br intermediate on the ratio of *cis*- and *trans*-lactones, IV + V and VI + VII.

(3a) *Cf.* G. Berti, *Tetrahedron*, **4**, 393 (1958).

(4) A. A. Patchett and B. Witkop, *J. Am. Chem. Soc.*, **79**, 185 (1957); *cf.* A. V. Robertson, E. Katz and B. Witkop, *J. Org. Chem.*, **27**, 2676 (1962).

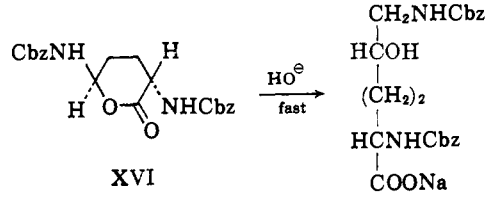
(5) Observations on the formation of N-bromo derivatives in the reaction of NBS with acylated amino acids have been made by L. A. Cohen and G. Schmir in this laboratory.

(6) G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. trav. chim.*, **79**, 688 (1960).

Care was taken to ensure the absence of epimerization of the *cis*-lactone VIII in the course of the base-catalyzed phthaloylation. A test case was investigated, namely, N-phthaloyl-L leucine ethyl ester, which was prepared from L-leucine ethyl ester hydrochloride in chloroform by the action of N-carboethoxyphthalimide in the presence of excess triethylamine. Neither in the phthaloylation step nor in the subsequent saponification of the ester was there any racemization. The final product, N-phthalamyl-L-leucine, had the same m.p. (139°) and rotation ($[\alpha]^{23}D$ −48.0°), as a sample prepared from phthaloyl-L-leucine[6] by the controlled opening of the phthalimido ring.

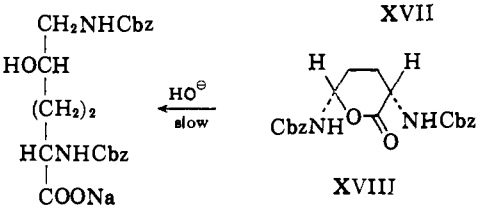
There are only two procedures known by which pure or nearly pure allohydroxyproline may be prepared. One is the sodium borohydride reduction of N-carbobenzyloxy-4-keto-L- or -D-proline[4] or the reduction with PtO_2 in methanol of 4-keto-DL-proline.[7] The second synthesis uses the intramolecular cyclization of 2-bromo-5-amino-4-valerolactone (prepared from epichlorhydrin and diethyl malonate) which Traube believed to lead to pure allohydroxy-DL-proline.[8] Exactly where steric control is exercised in the latter sequence of reactions is not clear, especially since closely related recent syntheses of hydroxyproline lead to mixtures of *equal* amounts of diastereoisomers.[9-11] By chromatographic criteria, the synthesis of allohydroxyproline *via* II → V → VIII → XIII is the first and only stereospecific approach to this amino acid in which all intermediates are obtained in sterically pure form by simple crystallization.[12]

Another proof for the *cis* configuration of the bromolactone V was its conversion to pure *threo*-γ-hydroxy-DL-ornithine (XII) (and its lactone XI) *via* IX and X, in analogy to similar transformations that led from histidine to γ-hydroxyornithine.[13] The replacement of the bromine in the *cis*-lactone V by potassium, or less well by silver, phthalimide proceeded in a yield of only 16%. The steric homogeneity of *threo*-γ-hydroxy-DL-ornithine (XII), which was obtained on a non-preparative scale, was rigidly ascertained by paper as well as ion exchange column chromatography.[13] Individual elution patterns of *threo*-γ-hydroxy-DL-ornithine as well as of *erythro*-γ-hydroxy-L-ornithine (obtained *via* γ-hydroxy-L-arginine from sea cucum-



XVI

XVII



XIX

XVIII

(7) R. Kuhn and G. Osswald, *Chem. Ber.*, **89**, 1423 (1956).

(8) W. Traube, R. Johow and W. Tepohl, *ibid.*, **56**, 1861 (1923).

(9) *Cf.* R. Gaudry and C. Godin, *J. Am. Chem. Soc.*, **76**, 139 (1954).

(10) J. Capková-Jirku, J. Kostir and M. Vondracek, *Chem. Listy.*, **44**, 19 (1950).

(11) T. Wieland and U. Wintermeyer, *Chem. Ber.*, **90**, 1721 (1957).

(12) By starting from L-allylglycine [S. Black and N. G. Wright, *J. Biol. Chem.*, **213**, 39 (1955)] allohydroxy-L-proline as well as hydroxy-L-proline, *e.g.*, by the procedure of Neuberger [*J. Chem. Soc.*, 429 (1945)], will become easily available.

(13) B. Witkop and T. Beiler, *J. Am. Chem. Soc.*, **78**, 2882 (1956).

bers[14]) gave single sharp peaks which were clearly separated when a mixture of the two diastereoisomers was run simultaneously (Fig. 1). One of us (N. I.) has established the fact that opening of the lactone XVI of natural[15] or *erythro*-N,N-dicarbobenzyloxy-δ-hydroxylysine (XVII) by base is much faster than that of the *cis*-disubstituted lactone XVIII of the *threo* isomer XIX and an elegant separation of the diastereoisomers has been based on this rate phenomenon.[16] Such a difference has so far not been observed with the lactones derived from γ-hydroxyornithine, presumably because of the inherently greater stability and the more planar character of γ-lactones.

Experimental

Carbobenzyloxy-DL-2-amino-4-pentenoic Acid (II).—DL-Allylglycine (I, 9.21 g., 0.08 mole) was dissolved in 2.0 *N* sodium hydroxide (40 ml.), and to this solution was added carbobenzoxy chloride (16.6 ml.) and 2.0 *N* sodium hydroxide (80 ml.) with stirring and ice-cooling in the course of 30 minutes. Stirring was continued overnight at room temperature.[17] The solution was acidified with 6.0 *N* HCl (40 ml.) and extracted two times with ethyl acetate. The organic solution was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. There was obtained 14.6 g. (73%) of an almost colorless oil, which did not solidify even after standing for 2 months.

***cis*-2-Carbobenzyloxyamino-4-hydroxy-5-bromopentanoic Acid Lactone (V).**—To a solution of 11.86 g. (0.0476 mole) of carbobenzyloxyallylglycine (II) in acetonitrile (130 ml.) and water (65 ml.) was added a solution of 4.45 g. (slightly more than 0.0476 mole) of N-bromosuccinimide (NBS) in acetonitrile (130 ml.) and water (65 ml.). The reaction mixture was allowed to stand for 4 hours in the refrigerator and evaporated to dryness *in vacuo*. The crystalline residue was dissolved in ethyl acetate (400 ml.), and the solution was washed with 0.048 *M* $NaHCO_3$ and then water, and dried over Na_2SO_4. The filtrate was evaporated *in vacuo*. The crystalline residue was collected and washed with a mixture of ethyl acetate–ether–petroleum ether. There was obtained 10.61 g. (69%) of crystalline material, part of which was used directly for the conversion to a mixture of 23% hydroxy- and 77% allohydroxy-DL-proline (see below), m.p. 96–117°. The major portion was recrystallized from ethyl acetate (60 ml.)–ether (60 ml.)–petroleum ether (180 ml.). There was obtained 9.58 g. (61%) of colorless crystals (V), m.p. 124–126°.

Anal. Calcd. for $C_{13}H_{14}NO_4Br$ (328.16): C, 47.58; H, 4.30; N, 4.27; Br, 24.35. Found: C, 48.07; H, 4.66; N, 4.57; Br, 23.72.

2-Amino-4-hydroxy-5-bromopentanoic Acid Lactone Hydrochloride (VIII).—A solution of 1.312 g. (4 mmoles) of the *cis*-bromolactone V in methanol (50 ml.) and 1 *N* HCl (4.2 ml.) was hydrogenated with 10% palladium-on-charcoal (0.4 g.) for 3 hours until no more CO_2 was evolved. The filtrate was evaporated *in vacuo*. The oily crystals were washed with acetone to yield 0.477 g. of crystalline material, m.p. 205–214°, which was dissolved in hot methanol (15 ml.), then concentrated to a small volume to which was added ethanol (5 ml.) and acetone (10 ml.). After several hours at room temperature the fine crystals (0.426 g., 46%) were collected; m.p. 211–212° dec.

Anal. Calcd. for $C_5H_9NO_2BrCl$ (230.50): C, 26.06; H, 3.94; N, 6.08; Cl, 15.38 (ionic form). Found: C, 26.32; H, 4.10; N, 5.72; Cl, 15.61 (ionic form).

2-Amino-4-hydroxy-5-bromopentanoic Acid Lactone Hydrobromide (VIII).—To 3.28 g. (10 mmoles) of the bromolactone V was added a saturated solution of HBr in acetic acid (15 ml.) at room temperature. After occasional shaking for 1 hour, ether (130 ml.) and petroleum ether (30 ml.) were added. The oil which initially separated solidified on scratching. The crystalline mass was collected and washed with ether (2.686 g.). It was recrystallized in the same manner as the hydrochloride. There was obtained 2.13 g. (78%) of colorless crystals, m.p. 206–208° dec.

Anal. Calcd. for $C_5H_9NO_2Br_2$ (274.96): C, 21.84; H, 3.30; N, 5.10; Br, 29.07 (ionic form). Found: C, 21.84; H, 3.49; N, 4.87; Br, 29.35 (ionic form).

Base-Catalyzed Conversion of *cis*-Bromolactone VIII to Allohydroxy-DL-proline. A. Autotitration Studies.—The recrystallized *cis*-bromolactone VII (about 10 μmoles) was dissolved in 0.1 *N* KCl (3 ml.) and titrated with 0.11 *N* NaOH in a self-recording autotitration apparatus. The final *p*H was set at 9.6. The results are summarized.

Sample	*cis*-Bromolactone VIII mg.	μmoles	Temp., °C.	Time for complete cyclization, min.	Total consumption of base (0.11 *N* NaOH), ml.
1	2.745	10.00	30	70	13.0
2	2.739	9.96	40	30	13.1
3	2.741	9.97	50	10	13.5

B. Analysis of the Cyclization Product by Electrophoresis.—Each of the three solutions (no. 1–3) after treatment with 0.11 *N* NaOH was passed through a column of Dowex 50W-X8 (200–400 mesh), H^+ form. The column was washed with water and the product was eluted with 2 *N* NH_4OH. The eluate was evaporated, the residue was dissolved in water, and the solution was assayed by high voltage electrophoresis for the presence of hydroxy-DL-proline and allohydroxy-DL-proline.[18] The following conditions were maintained: 1300 v., 40 ma., *p*H 1.9 buffer (150 ml. of acetic acid, 50 ml. of formic acid diluted to 1 l. with water), length of time 2.5 hours. By this method it was shown that the cyclization samples 1–3 contained practically pure allohydroxy-DL-proline. The accuracy of this method is limited and would not allow the detection of a few per cent of the diastereoisomer.

C. Assay by Ion Exchange Column Chromatography (Stein and Moore Technique). 1. Assay of Hydroxyprolines from the Crude Mixture of Bromolactones IV and V.—The crude mixture of oily carbobenzyloxybromolactones IV and V (0.164 g., 0.5 mmole) was decarbobenzoxylated with HBr–CH_3CO_2H (0.8 ml.) in the usual fashion. After evaporation to an oily residue and after washing with ether, intramolecular cyclization of the bromoaminolactone mixture was effected by addition of 0.5 *N* NaOH at 50–55° until *p*H 9.5 was reached. The solution was poured onto a column of Dowex 50W and the product was eluted with 2 *N* aqueous ammonia. The ammonia solution was evaporated *in vacuo* and the residue was dissolved in water (6 ml.). Of this solution 0.2 ml. was used for chromatographic analysis which showed the presence of 23% 4 hydroxy-DL-proline and of 77% allohydroxy-DL-proline.

2. Assay of Hydroxyprolines from the Crude Cyclization Mixture Obtained from Pure Crystalline *cis*-Bromolactone IV.—An aqueous solution containing 1 mg. of unrecrystallized material from the intramolecular cyclization of pure *cis*-bromoaminolactone VIII was assayed by ion exchange technique which showed a ratio of 94% allohydroxyproline *vs.* 6% hydroxyproline.

3. Assay of Recrystallized Allohydroxyproline (XIII) from Pure *cis*-Lactone VIII.—A solution of 1 mg. of recrystallized XIII in citrate buffer *p*H 3.20 (1 ml.) was passed through a column of Dowex 50W-X8 (0.9 × 50 cm.) maintained at 37°, and eluted (4 ml./hr.) with the same buffer. The effluent was collected in 1-ml. fractions and each fraction was analyzed by ninhydrin colorimetry. The solution pattern of the product was a single peak, while a mixture of XIII and commercial hydroxy-L-proline (1 mg.) was easily resolved into two peaks.

D. Preparation of Pure Allohydroxy-DL-proline (XIII).—Recrystallized bromoaminolactone VIII (0.825 g. or 3 mmoles of hydrobromide, m.p. 206–208°) was dissolved in water (10 ml.) at 50–55° and 0.5 *N* NaOH was added until the *p*H, which was followed in an autotitrator ("*p*H-Stat"), reached a final value of 9.5–9.6. The temperature was maintained at 50–55° for 15 minutes, adjusting to *p*H 9.5–9.6 by additions of 0.5 *N* NaOH. Total volume of 0.5 *N* NaOH used was 17.8 ml. The solution was passed through a column (1.5 × 8 cm.) of Dowex 50W-X8 (200–400 mesh), H^+ form, washed with water and eluted with 2 *N* ammonia (70 ml.). The ammonia solution was evaporated *in vacuo*, and the residual solid (0.376 g., 96%) was collected and washed with ethanol. The unrecrystallized allohydroxyproline (containing 6% hydroxyproline as assayed under C-2) was dissolved in hot water, treated with charcoal, evaporated to a small volume, and ethanol was added. The yield of colorless crystalline allohydroxyproline was 0.286 g. (73%), m.p. 244–245° dec. The literature reports m.p. 238° dec. The product gave a single ninhydrin spot in several solvent systems indistinguishable from that given by commercial allohydroxy-D-proline used as control. The ion exchange analysis (C-1) showed this fraction to be 100% pure allohydroxyproline.

Anal. Calcd. for $C_5H_9NO_3$ (131.13): C, 45.79; H, 6.92; N, 10.68. Found: C, 46.11; H, 6.82; N, 10.51.

α-Carbobenzyloxy-δ-phthalyl-*threo*-γ-hydroxy-DL-ornithine Lactone (IX).—A suspension of the pure *cis*-bromolactone V (4.60 g., 14 mmoles) and potassium phthalimide (2.60 g., 14 mmoles) in dimethylformamide (1.2 ml.) was heated to 100–105° and stirred for 4 hours. The reaction product was poured into an excess of water and separated as an oil which was washed with

(14) Y. Fujita, *Bull. Chem. Soc. Japan*, **33**, 1379 (1960).

(15) *Cf.* B. Witkop, *Experientia*, **12**, 372 (1956).

(16) N. Izumiya, Y. Fujita and M. Ohno, *Bull. Chem. Soc. Japan*, in press.

(17) When the time of stirring at room temperature was reduced to 2 hours the yield decreased to 49%.

(18) E. Gross and U. Wintermeyer, unpublished; *cf.* Th. Wieland and G. Pfleiderer, *Angew. Chem.*, **69**, 200 (1957).

water. The amount of $Br^{\ominus}$ in the combined washings was 87% of theory as determined by precipitation as AgBr from the solution acidified with 1.0 *N* HNO_3. The oily reaction product was left for several days in contact with a small amount of acetone and ether. The crystals which had formed were separated from a viscous oily portion by washing with a mixture of ethyl acetate–ether (2:5) and then with ether. There was obtained 1.527 g. of a crystalline material, m.p. 165–190°. This product could not be recrystallized from the usual solvents or from a combination such as dimethylformamide–ether. The following procedure gave the analytical sample. The crude product was heated with ethanol (45 ml.) which dissolved all the colored impurities and left the colorless crystals in suspension. After cooling to room temperature, the crystals (0.835 g., 16%) were collected and washed with ethanol, acetone and ether; m.p. 196–198°.

Anal. Calcd. for $C_{21}H_{18}N_2O_6$ (394.37): C, 63.95; H, 4.60; N, 7.10. Found: C, 64.51; H, 4.81; N, 6.90.

When the bromolactone V was heated with silver phthalimide (free of water of crystallization) in dimethylformamide as above only 55% of the expected $Br^{\ominus}$ was found in the precipitated AgBr. The insolubility of silver phthalimide limits its usefulness as a partner in this condensation.

δ-Phthaloyl-γ-hydroxy-DL-ornithine Lactone Hydrobromide (X).—The phthaloylcarbobenzyloxy lactone IX (m.p. 196–198°, 0.438 g., 1.29 mmoles) was decarbobenzoxylated with HBr in acetic acid (2.5 ml.) at room temperature. After occasional shaking for 1 hour, ether (25 ml.) was added. The crystals (0.438 g., 100%) which separated immediately were collected and washed with a mixture of acetone–ether (1:2). For recrystallization the product was dissolved in hot methanol (40 ml.) and the solution was concentrated to 2–3 ml. Hot ethanol (8 ml.), acetone (8 ml.) and ether (4 ml.) were added. The recrystallized material was collected and washed with acetone and ether. There was obtained 0.385 g. (88%) of colorless crystals, m.p. 277–278° dec.

Anal. Calcd. for $C_{13}H_{13}N_2O_4Br$ (341.16): C, 45.72; H, 3.84; N, 8.21; Br, 23.39 (ionic). Found: C, 45.66; H, 3.99; N, 7.95; Br, 23.08.

Studies on the Action of Base on δ-Phthaloylhydroxyornithine Lactone X. A. Action of Dilute Alkali.—Compound X (3.40 mg., 10 μmoles) in water (3 ml.) was titrated with 0.11 *N* NaOH in a self-recording titrator (*p*H-Stat). The final *p*H was set at 9.6 and the temperature was adjusted to 40°. In the course of 50 min., 30 μmoles of 0.11 *N* NaOH was added. After this addition the *p*H stayed constant at 9.6. The consumption of 3 equivalents of base may be due to opening of the lactone as well as phthalimido ring. The resulting product when tested in paper chromatography gave only one spot in the following solvent systems: pyridine–AcOH–*n*-BuOH–H_2O (20:6:30:24), R_f 0.04; HCO_2H–*n*-BuOH–H_2O (25:75:10), R_f 0.095.

B. The Action of Triethylamine.—To a very small amount of the lactone hydrobromide X dissolved in water was added a slight excess of triethylamine. The solution was left overnight at room temperature, evaporated to dryness, redissolved in water and analyzed by chromatography in the solvent systems: pyridine–AcOH–*n*-BuOH–H_2O (20:6:30:24), R_f 0.28; HCO_2H–*n*-BuOH–H_2O (25:75:10), R_f 0.12. This material may be the ω-phthaloylhydroxyornithine.

However, in another experiment even triethylamine seemed to be capable of opening the phthalimido ring and of producing the same slow-moving material obtainable with alkali as under A. The lactone X (17.1 mg., 0.05 mmole) was dissolved in water (4 ml.) and triethylamine (0.28 ml., 2 mmoles) and was stored overnight. The analysis of the product by chromatography in the solvent system pyridine–AcOH–*n*-BuOH–H_2O (25:75:10) gave an R_f value of 0.10.

***threo*-γ-Hydroxy-DL-ornithine (XII ⇄ XIII).**—The lactone X was dephthalated by the method of King, *et al.*[19] To the lactone X (0.1 g., 0.29 mmole) dissolved in 0.5 *M* Na_2CO_3 (1 ml.) and water (0.5 ml.) was added an aqueous solution (0.32 ml.) of 0.5 *M* hydrazine. After 48 hours at room temperature, the solution was passed through a column of Dowex 50W, H^+ form, 2 × 9 cm. The column was washed with water and then with 70% methanol in order to remove some crystalline phthalylhydrazine which had separated in the column. The column was eluted with 0.5 *N* ammonia (40 ml.), and the ammonia solution was evaporated *in vacuo*. The residue, dissolved in a small volume of water, was passed through a column of Dowex 50W, NH_4^+ form, and the column was washed with water and eluted with 0.5 *N* ammonia (30 ml.). The ammonia solution was evaporated *in vacuo*. The amorphous residue was too small in amount to permit further fractionation and crystallization and, therefore, was assayed for purity and steric homogeneity both by paper, as well as column, chromatography. The results are shown below.

The more accurate assay for γ-hydroxyornithine by ion exchange chromatography was carried out in the following way. About 1 mg. of the amorphous residue from the dephthalation of X was dissolved in 0.1 *M* phosphate buffer *p*H 7.8 (1 ml.) and poured onto a column (0.9 × 100 cm.) of Dowex 50W-X8. Elution in the usual way gave a single sharp peak, distinctly different from the peak of *erythro*-γ-hydroxy-L-ornithine which was run separately and in admixture under identical conditions, indicative of pure *threo*-γ-hydroxyornithine.

(19) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

TABLE I

R_f VALUES OF *threo*- AND *erythro*-γ-HYDROXYORNITHINES FROM DIFFERENT SOURCES

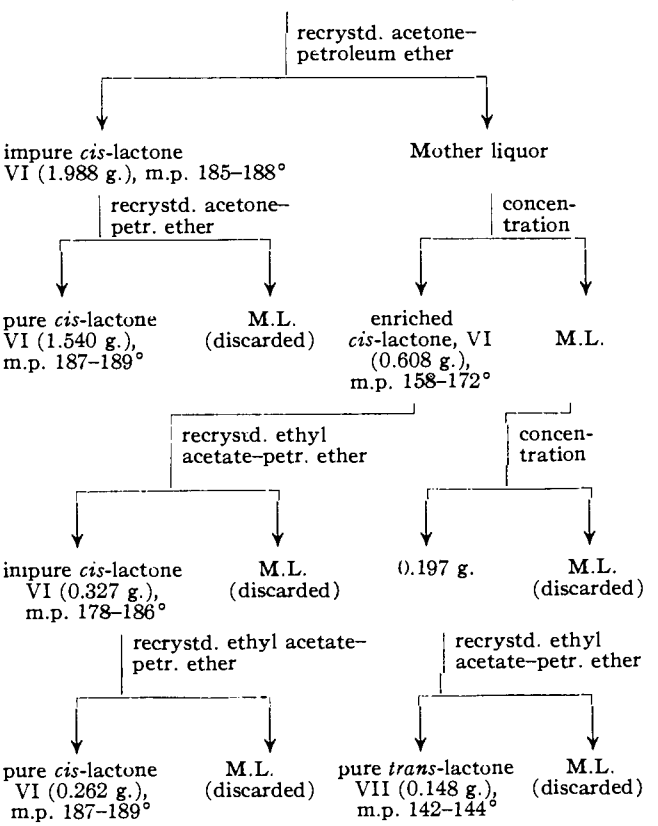
Solvent systems	Material from dephthalation of lactone X	*erythro*-γ-Hydroxy-L-ornithine (from natural sources)[14]	Mixture of diastereoisomeric γ-hydroxyornithines (obtd. by syn.)[13]
n-BuOH–pyridine–AcOH–H_2O (4:1:1:2)	0.15 (0.09 faint)	0.15	0.15
sec-BuOH–pyridine–H_2O–diethylamine (20:28:13:0.8)	0.37	0.50	(0.37) (0.50)

Phthalyl-DL-allylglycine (III). A. Method of Nefkens.—To a solution of allylglycine (11.5 g., 0.1 mole) dissolved in water (15 ml.) and Na_2CO_3 (1.06 g., 0.1 mole) was added N-carboethoxyphthalimide (22.5 g., 0.104 mole). Stirring was continued for 0.5 hour. A small amount of insoluble product was filtered off and 6.0 *N* HCl (3.7 ml.) was added. The oil which separated solidified after 1 hour and it was collected (19.4 g., m.p. 119–122°). Recrystallization from ethyl acetate–petroleum ether yielded 17.8 g. (73%) of colorless crystals, m.p. 124°.

Anal. Calcd. for $C_{13}H_{11}NO_4$ (245.22): C, 63.67; H, 4.52; N, 5.71. Found: C, 63.87; H, 4.42; N, 5.71.

CHART I

SEPARATION OF THE *cis*- AND *trans*-PHTHALOYLBROMOLACTONES VI AND VII BY FRACTIONAL CRYSTALLIZATION



B. Thermal Phthalation Method.—A mixture of allylglycine (3.45 g., 0.03 mole) and phthalic anhydride (4.44 g.) was heated in a bath (160°) for 20 minutes with stirring. The cooled reaction product was digested with ethanol (12 ml.) and water (30 ml.). The initially oily reaction product became crystalline after standing overnight in a refrigerator. It was collected and recrystallized from ethyl acetate (15 ml.)–petroleum ether (40 ml.) to yield 4.167 g. (57%) of slightly grayish crystals, m.p. 1.18–120°, which gave a correct analysis.

2-Phthaloylamino-4-hydroxy-5-bromopentanoic Acid Lactone. A. *cis*-Lactone VI, M.p. 187–189°.—To phthalylallylglycine (III, 2.45 g., 0.01 mole) in acetonitrile (30 ml.) and water (15 ml.) was added NBS (1.87 g., 0.0105 mole) in acetonitrile (30 ml.) and water (15 ml.). After 4 hours in the refrigerator the solution was evaporated. Crystallization started during evaporation. The crystals were collected and washed with water and petroleum ether to yield 2.880 g. (89%) of a mixture of diastereoisomers, m.p. 165–180°. Repeated fractional crystallization in the manner indicated in Chart I gave two products. The higher melting isomer was obtained as the major product, *viz.* 1.802 g. (56%), m.p. 187–189°.

Anal. Calcd. for $C_{13}H_{10}NO_4Br$ (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.55; H, 3.22; N, 4.00.

B. ***cis*-Lactone VI, M.p. 187–189°, by Phthalation of *cis*-Bromoamino-lactone VIII.**—To a solution of the lactone VIII (0.138 g., 0.5 mmole) and N-carboethoxyphthalimide (0.11 g., 0.5 mmole), dissolved in dimethylformamide (0.7 ml.), was added tributylamine (0.5 mmole) diluted with dimethylformamide under ice-cooling. The reaction mixture was left for 0.5 hour under ice-cooling and 0.5 hour at room temperature. When water (5 ml.) was added crystals began to separate after some minutes. The crystals were collected and washed with water to yield 80 mg. of colorless crystals, m.p. 80–160°. After recrystallizations from ethyl acetate–petroleum ether there was obtained 21 mg. (13%) of the pure *cis*-lactone VI, m.p. 186–189°, undepressed on admixture with a sample of the same lactone VI prepared by route A.

Anal. Calcd. for $C_{13}H_{10}NO_4Br$ (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.50; H, 3.39; N, 4.39.

C. ***trans*-Lactone VII, M.p. 142–144°.**—By fractional crystallization from acetone–petroleum ether or from ethyl acetate–petroleum ether in the manner indicated in Chart I it was possible to obtain 0.148 g. (5%) of the *trans*-lactone VII, m.p. 142–144°.

Anal. Calcd. for $C_{13}H_{10}NO_4Br$ (324.13): C, 48.18; H, 3.11; N, 4.32. Found: C, 48.49; H, 3.29; N, 4.06.

Several attempts to convert the *cis*-lactone VI into allohydroxyproline failed. In one experiment there was added to a suspension of the *cis*-lactone VI (0.162 g., 0.5 mmole) in boiling ethanol (15 ml.) a solution of hydrazine (0.5 mmole) in ethanol. This resulted in a clear solution. Refluxing was continued for 1 hour. The solution was then evaporated to dryness, 0.1 *N* HCl (1 ml.) was added to the residue, and the mixture was warmed for 10 minutes. The solution was filtered and the filtrate (*ca.* 15 ml.) was treated with 1.0 *N* NaOH at 50° with the final *p*H being set at 9.6 (*p*H-Stat). Subsequent assay by paper chromatography and electrophoresis failed to demonstrate the presence of hydroxyproline.

In another experiment, at room temperature, there was added to a suspension of the *cis*-lactone VI (0.081 g., 0.25 mmole) in dioxane (1 ml.) and water (0.3 ml.) a mixture of aqueous soda (0.163 mmole) and hydrazine (0.375 mmole) under stirring. The solution became clear immediately. After 2 days at room temperature the solution was treated with 1.0 *N* NaOH in the *p*H-Stat in the same manner described above. The assay for hydroxyproline showed a very faint ninhydrin-positive spot on paper electrophoresis identical in position with a control spot of allohydroxyproline.

N-Phthalamyl-L-leucine. A. From Phthaloyl-L-leucine.—To phthaloyl-L-leucine[6] (0.261 g., 1 mmole), dissolved in methanol (3 ml.), was added 2.0 *N* NaOH (1.1 ml.). The solution was kept at 45° for 1.5 hours, evaporated and treated with 1.0 *N* HCl (2.2 ml.). After standing in a refrigerator for several hours the crystals were collected and washed with a small volume of cold water. Recrystallization from ethyl acetate–ether–petroleum ether yielded 0.128 g. (46%) of colorless crystals, m.p. 139–140°, $[\alpha]^{23}D$ −48.5° (*c* 2, ethanol).

Anal. Calcd. for $C_{14}H_{17}NO_5$ (279.28): C, 60.20; H, 6.14; N, 5.02. Found: C, 60.14; H, 6.20; N, 4.99.

B. **From Ethyl Phthaloyl-L-leucinate.**—To a solution of L-leucine ethyl ester hydrochloride (0.978 g., 5 mmoles) in a mixture of chloroform (20 ml.) and triethylamine (0.7 ml.) was added N-carboethoxyphthalimide (1.096 g., 5 mmoles) under ice-cooling. After 5 hours the solution was washed with water, dilute hydrochloric acid and water, and dried over Na_2SO_4. The oily ethyl phthaloyl-L-leucinate (1.75 g.) failed to crystallize, even on prolonged standing. It was therefore saponified as described above. After recrystallization there was obtained 0.49 g. (35%) of colorless crystals, m.p. 138–139°, $[\alpha]^{23}D$ −48.0° (*c* 2, ethanol), identical in all respects with the material obtained under A.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ST. JOHN'S UNIVERSITY, NEW YORK, N. Y.]

Glutamic and Aspartic Anhydrides. Rearrangement of N-Carboxyglutamic 1,5-Anhydride to the Leuchs' Anhydride and Conversion of the Latter to Pyroglutamic Acid

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New rearrangements of glutamic acid derivatives are described. N-Carboxy-L-glutamic-1,5-anhydride (II), an intermediate in the hydrogenolysis of carbobenzoxy-L-glutamic anhydride (I), rearranges immediately to N-carboxy-L-glutamic anhydride (Leuchs' anhydride, III). Compound III is also obtained from N-carboxy-L-glutamic anhydride γ-benzyl ester (IV) by catalytic hydrogenation. Diazomethane treatment of III gave the known N-carboxy-L-glutamic anhydride γ-methyl ester. The Leuchs' anhydride III is unstable and slowly rearranges to L-pyroglutamic acid, probably through anhydride V. Unlike other N-carboxy anhydrides III yielded only a small amount of polypeptide in polymerization experiments; the main product was pyroglutamic acid. Compound I gave glutamic acid hydrobromide and acetic anhydride with hydrogen bromide–acetic acid, indicating that glutamic anhydride hydrobromide (VII) is unstable in contrast to aspartic anhydride hydrobromide. The latter, however, is very reactive because of the inductive effect of the protonated amino group and is readily opened with alcohols almost exclusively in the α-position.

The free anhydride of aspartic acid has been used in the synthesis of α,β-poly-L-aspartic acid.[1] An attempted synthesis of glutamic-1,5-anhydride for the preparation of α,γ-polyglutamic acid has also been reported[2]; however, the catalytic hydrogenation of carbobenzoxy-L-glutamic anhydride yields pyroglutamic acid instead of the expected anhydride. Further study of aminodicarboxylic acid anhydrides and related compounds reported here led to the recognition of interesting new rearrangements of glutamic acid derivatives and to a convenient synthesis of aspartic acid α-esters and of isoasparagine.

When N-carbobenzoxy-L-glutamic 1,5-anhydride[3] (I) undergoes catalytic hydrogenation in absolute dioxane or ether, one mole of hydrogen is consumed without carbon dioxide evolution, and crystalline N-carboxy-L-glutamic anhydride (Leuchs' anhydride, III) may be isolated from the reaction mixture in 85% yield. Care was taken to exclude moisture in all procedures. Peaks at 5.50 and 5.66 μ in the infrared spectrum of N-carbobenzoxy-L-glutamic anhydride disappeared during the reduction, and absorption maxima at 5.37 and 5.57 μ characteristic of Leuchs' anhydrides[4] appeared.

Crystalline N-carboxy-L-glutamic anhydride (III) was also obtained when N-carboxy-L-glutamic an-

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