*Oxazolidone Derivatives of Hydroxyamino Acids. III. Inversion of the erythro- to the threo-Form of Threonine via Oxazolidone Ester**

By Takeo KANEKO and Toshishige INUI

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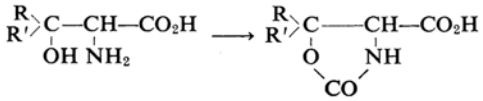
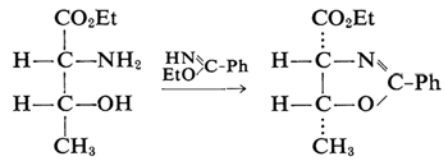
In a previous paper of this series[1], it was shown that α-amino-β-hydroxylic acids (I) in an alkaline solution cyclised to oxazolidone derivatives (II) under the action of phosgene. During this reaction the configuration at both



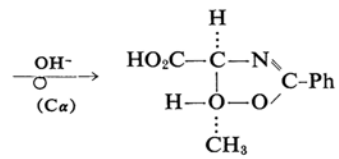
(I) (II)

the asymmetric centers remains unchanged, and DL-*trans*- and DL-*cis*-5-methyl-2-oxo-oxazolidine-4-carboxylic acids (II; R=H, R′=CH_3) are easily obtained from DL-threonine and DL-allothreonine respectively.

Oxazoline derivatives, as is well known, are important intermediates for the configurational transformation of allothreonine into threonine, and this conversion has been achieved by epimerization at either the α- or the β-carbon position. According to the first method, the less stable *cis*-oxazoline-ester (III) derived from allothreonine ester is transformed into the more stable trans-configuration IV with epimerization at the α-carbon atom and with

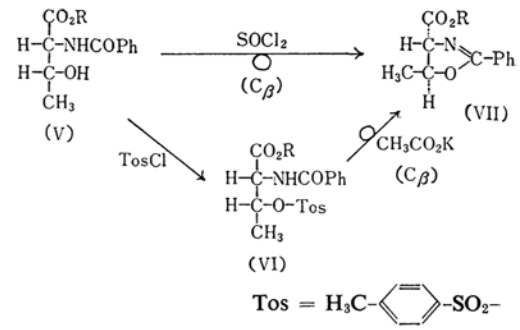


(III)



(IV)

the simultaneous hydrolysis of the ester group[2,3] under the action of aqueous alkali. According to the second method, on the other hand, *N*-benzoyl-allothreonine ester (V) or its *O*-tosyl derivative (VI) is converted into the *trans*-oxazoline ester (VII), with the inversion



(V) (VI) (VII)

* This work was partly presented at the 11th and 13th Annual Meetings of the Chemical Society of Japan, Tokyo, April, 1958, and April, 1960.

1) T. Kaneko and T. Inui, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 1075 (1961).

2) D. F. Elliott, *Nature*, **162**, 657 (1948); *J. Chem. Soc.*, **1949**, 589.

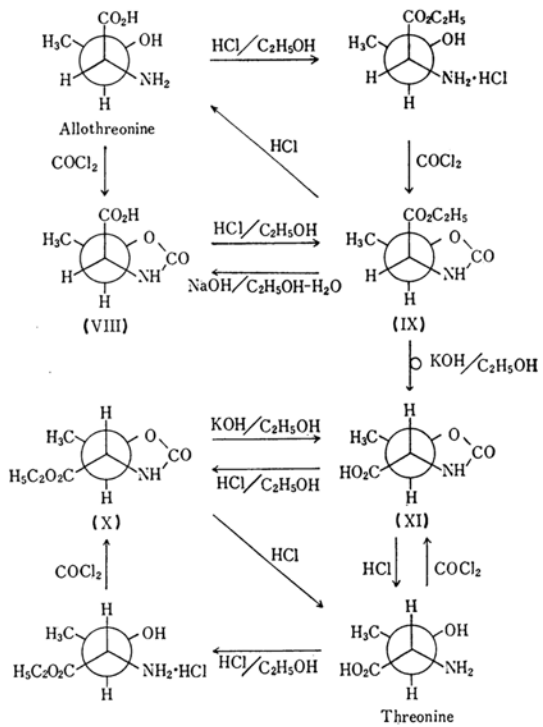
3) D. F. Elliott, *J. Chem. Soc.*, **1950**, 62.

of the configuration at the β-carbon atom, by the action of thionyl chloride[4,5] or potassium acetate[5] respectively. In both cases, the resulting *trans*-oxazoline derivatives (IV and VII) give threonine on acid hydrolysis.

These facts suggest that the *cis*-oxazolidone derivative derived from allothreonine by the present authors may be similarly converted into the stable trans-configuration, because of the expected instability of the cis-configuration of oxazolidone ester. In the present paper, it is reported that *cis*-oxazolidone-carboxylic ester converts into *trans*-carboxylic acid on alkaline hydrolysis.

Upon treatment with hydrogen chloride in absolute alcohol, DL-*cis*-5-methyl-2-oxo-oxazolidine-4-carboxylic acid (VIII)[1] derived from DL-allothreonine yielded DL-*cis*-4-ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (IX), b. p. 126~128 °C/10^{-3} mmHg. without any detectable change in the configuration. The configuration assigned was confirmed by isolating pure DL-allothreonine from the acid-hydrolyzate of this oxazolidone-carboxylic ester (IX). The same ester IX was also obtained by the reaction of DL-allothreonine ethyl ester hydrochloride with phosgene in aqueous potassium carbonate. In a similar way, DL-threonine ethyl ester hydrochloride gave DL-*trans*-4-ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (X), b. p. 115~118°C/10^{-3} mmHg. The configuration of this ester X was also assigned by hydrolyzing it with hydrochloric acid and by isolating the resulting pure DL-threonine.

When DL-*cis*-ester IX was heated with one equivalent of alcoholic aqueous sodium hydroxide according to the method employed by Elliott[3], only the hydrolysis of the ester group took place, without any inversion; on the other hand, if the same ester IX was treated with a slightly excessive amount of 0.4 N alcoholic potassium hydroxide, both the conversion and the hydrolysis of ester took place. The resulting product possessed an infrared spectrum similar to that of DL-*trans*-5-methyl-2-oxo-oxazolidine-4-carboxylic acid (XI) derived from DL-threonine, but its melting point (120~122°C) was somewhat lower than that of XI (127~128°C). On the other hand, DL-*trans*-ester X gave DL-*trans*-carboxylic acid XI with the retention of configurations under the same conditions, and the infrared spectrum of the product was identical with that of an authentic sample. Therefore, it may be supposed that the less stable DL-*cis*-ester IX converted to the more stable DL-*trans*-form XI but not from

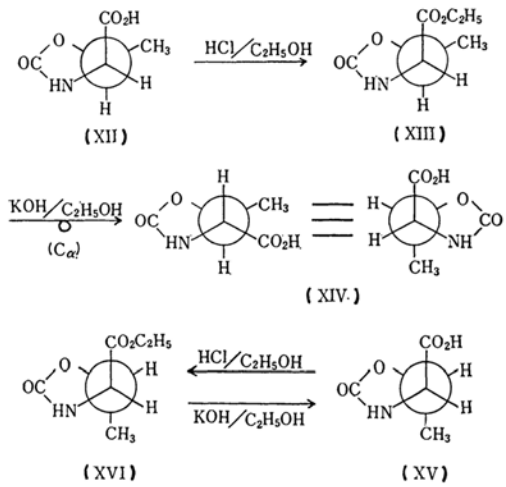


trans to cis, under the conditions used. The compound obtained from DL-*cis*-ester IX seemed to be impure DL-*trans*-carboxylic acid XI contaminated with DL-*cis*-carboxylic acid VIII, as the hydrolysis of this compound with hydrochloric acid gave DL-threonine, together with a small quantity of DL-allothreonine, both of which were identified by paper chromatographic analysis[6]. Recently, chromatographic separation and determination of a synthetic mixture of *N*-2,4-dinitrophenylated threonine and allothreonine were carried out by Seki using a column of carboxylic type resin, Amberlite IRC-50[7]. By the use of this method, the ratio of DL-threonine to DL-allothreonine in the acid-hydrolyzate was estimated to be 6.1. From these results, it was presumed that the conversion was due to epimerization at the asymmetric center corresponding to the α-carbon atom of the original amino acid molecules.

In order to prove this presumption exactly, this conversion should be applied to optically active material. Thus, the conversion at the α-carbon atom should result in the conversion of L-*cis*-ester to D-*trans*-carboxylic acid. To obtain L(−)-*cis*-ester XIII, the DL-*cis*-carboxylic acid VIII was resolved by means of brucine[8], and the resulting L(−)-*cis*-carboxylic acid (XII)

4) K. Pfister, C. A. Robinson, A. C. Shabica and M. Tishler, *J. Am. Chem. Soc.*, **70**, 2297 (1948); **71**, 1101 (1949).
5) J. Attenburrow and D. F. Elliott, *J. Chem. Soc.*, **1948**, 310.
6) K. N. F. Shaw and S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).
7) T. Seki, *J. Biochem. Japan*, **47**, 253 (1960).
8) T. Inui and T. Kaneko, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 1078 (1961).

was treated with hydrogen chloride in absolute alcohol. Hydrolysis of this ester XIII with hydrochloric acid yielded pure L(+)-allothreonine, confirming the configuration assigned. Upon the treatment of this L(−)-*cis*-ester XIII with alcoholic potassium hydroxide, an optically active acid XIV, m. p. 131~133.5°C, $[\alpha]_D$ −37.7°, was obtained. Hydrolysis with hydrochloric acid yielded a mixture of D(+)-threonine and L(+)-allothreonine. Chromatographic analysis of this mixture as the dinitrophenyl derivative[7] showed that the ratio of D(+)-threonine to L(+)-allothreonine was identical with that of the optically inactive case mentioned already. As a comparison, L(+)-*trans*-carboxylic acid XV was obtained by the re-



(XII) (XIII) (XIV) (XVI) (XV)

action of L(−)-threonine with phosgene in the presence of potassium hydroxide[8]. The infrared spectrum of the compound XIV was similar to that of L(+)-*trans*-carboxylic acid XV. As a result, it was proved that this levorotatry acid XIV was D(−)-*trans*-carboxylic acid contaminated with L(−)-*cis*-carboxylic acid XII. The L(+)-*trans*-ester XVI derived from L(+)-*trans*-carboxylic acid XV gave pure L(−)-threonine upon hydrolysis with hydrochloric acid. Moreover, the treatment with alcoholic potassium hydroxide resulted only in the hydrolysis of ester group, yielding L(+)-*trans*-carboxylic acid XV without any configurational change.

From the results of this investigation, it was concluded that this conversion of the cis- to trans-form was the result of epimerization at the asymmetric center corresponding to the α-carbon atom in the original amino acid molecules.

** All melting points and boiling points are uncorrected. Infrared spectra were recorded with a Hitachi EPI-2 type infrared spectrophotometer, and refractive indices were determined with a Bausch and Lomb precision refractometer.

Experimental**

DL-*cis*-Ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (IX). *Method A. By Esterification of DL-cis-5-Methyl-2-oxo-oxazolidine-4-carboxylic Acid (VIII).*—a) A suspension of DL-*cis*-carboxylic acid[1] VIII (8.0 g., 0.055 mol.) in 100 ml. of absolute alcohol was saturated with hydrogen chloride by passing the dry gas as a vigorous stream at 0°C. The solution, after being left standing overnight at room temperature, was evaporated to dryness under reduced pressure. The residue was dissolved in water and extracted with four 60 ml. portions of ethyl acetate. The combined extract was dried over sodium sulfate, and the solvent was removed by distillation. The residue, on distillation under reduced pressure, yielded 8.0 g. (84%) of DL-*cis*-ester IX, b.p., 126~128°C/10^{-3} mmHg, n_D^{20} 1.46376.

IR, 3300 (amide), 1760 (cyclic amide and ester) cm^{-1} (liquid film).

Found: C, 48.41; H, 6.39; N, 7.87. Calcd. for $C_7H_{11}O_4N$: C, 48.55; H, 6.40; N, 8.09%.

b) A suspension of the above DL-*cis*-carboxylic acid[1] VIII (3.6 g., 0.025 mol.) in 80 ml. of ether was treated with an excess of diazoethane dissolved in ether. After the evaporation of the solvent and the reagent, the residue was distilled under reduced pressure. Yield, 2.7 g. (63%); b. p., 138~140°C/10^{-2} mmHg. The infrared spectrum was identical with that of the ester obtained by a).

Method B. By Cyclization of DL-Allothreonine Ethyl Ester Hydrochloride.—Crude DL-allothreonine ethyl ester hydrochloride[2] obtained from DL-allothreonine (7.2 g., 0.06 mol.) was dissolved in 200 ml. of water, and the solution was covered with 105 ml. of toluene. To the mixture, after it was cooled to 3°C, 1 N potassium hydroxide (70 ml., 0.07 mol.) and 26.0 g. (0.18 mol.) of anhydrous potassium carbonate were added, and then a solution of 14.6 g. (0.147 mol.) of phosgene in toluene (45 ml.) was stirred in drop by drop over a 40 min. period. After being stirred for an additional hour, the aqueous layer separated from the toluene layer was acidified with concentrated hydrochloric acid and extracted with four 60 ml. portions of ethyl acetate. The combined extract was dried over sodium sulfate, the solvent was removed by distillation, and the residue was distilled under reduced pressure. Yield, 4.1 g. (39% for DL-allothreonine); b. p., 122~124°C/10^{-3} mmHg., n_D^{20} 1.46420. All properties of this sample were identical with those of the product obtained by the above method A.

The aqueous layer remaining after the extraction with ethyl acetate was evaporated under reduced pressure, and the residue was extracted with four 50 ml. portions of ethyl acetate while hot. The extract was concentrated under reduced pressure. On scratching of the side of the vessel, DL-*cis*-carboxylic acid VIII crystallized out. Yield, 2.0 g. (23% for DL-allothreonine); m. p., 193~195°C (decomp.). All properties of this sample were identical with those of an authentic sample[1].

DL-Allothreonine.—A solution of 0.99 g. (0.0057 mol.) of DL-*cis*-ester IX in 20 ml. of 1:1 hydrochloric acid was refluxed for 8 hr. After being cooled at room temperature, the reaction mixture was extracted with ethyl acetate, and the aqueous

solution was evaporated to dryness under reduced pressure. The residue was dissolved in 17 ml. of 94% alcohol, and pyridine (3 ml.) was added. DL-Allothreonine crystallized out. Yield, 0.5 g. (86%); m. p., 241~242°C (decomp.). On recrystallization from water and alcohol, its melting point rose to 246~247°C (decomp.). All properties of this sample were identical with those of an authentic sample.

DL-*cis*-5-Methyl-2-oxo-oxazolidne-4-carboxylic Acid (VIII). — *By Hydrolysis of DL-cis-Ester IX with Aqueous Alcoholic Sodium Hydroxide.* — To a solution of 0.74 g. (0.00428 mol.) of DL-*cis*-ester IX in 40 ml. of 94% alcohol, 3.9 ml. (0.00426 mol.) of 1.09 N sodium hydroxide was added. The mixture was heated under reflux for 20 min. After being cooled, the solvent was evaporated in vacuo, and the residue was dissolved in water. The aqueous solution was extracted with ethyl acetate, acidified with concentrated hydrochloric acid, and then evaporated in vacuo to dryness. The residue was extracted with three 40 ml. portions of ethyl acetate. When the combined extract was evaporated in vacuo, DL-*cis*-carboxylic acid VIII crystallized out. Yield, 0.42 g. (68%); m. p., 193~195°C (decomp.). The melting point could be raised to 196~197°C (decomp.) by recrystallization. All properties of this sample were identical with those of an authentic sample[1].

DL-*trans*-4-Ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (X).—According to the same method as used in the synthesis of DL-*cis*-ester IX by method B, DL-threonine ethyl ester hydrochloride[2] obtained from DL-threonine (4.8 g., 0.04 mol.) was cyclized to DL-*trans*-ester X with phosgene (11.8 g., 0.119 mol.) in the presence of potassium carbonate. Yield, 3.4 g. (49%); b. p., 115~118°C/10^{-3} mmHg., n_D^{20} 1.46105.

IR, 3300 (amide), 1765 (cyclic amide and ester) cm^{-1} (liquid film).

Found: C, 48.17; H, 6.32; N, 7.81. Calcd. for $C_7H_{11}O_4N$: C, 48.55; H, 6.40; N, 8.09%.

DL-*trans*-Carboxylic acid XI could not be obtained from the remaining aqueous solution.

Treatment of DL-*trans*-carboxylic acid XI with hydrogen chloride in absolute alcohol also yielded the same ester X.

DL-*trans*-5-Methyl-2-oxo-oxazolidine-4-carboxylic Acid (XI).—*A) By Hydrolysis of DL-cis-Ester IX with Alcoholic-Potassium Hydroxide.*—To a solution of 1.16 g. (0.00670 mol.) of DL-*cis*-ester IX in 7.5 ml. of absolute alcohol, 7.5 ml. (0.00677 mol.) of 0.89 N alcoholic potassium hydroxide was added, and the mixture was refluxed for 15 min. After the mixture had been cooled, 15 ml. of water was added, and the solution was acidified with concentrated hydrochloric acid. The aqueous solution was evaporated to dryness under reduced pressure. The residue was extracted with 3 times with 30 ml. of ethyl acetate. The combined extract was evaporated in vacuo to a syrup, which was crystallized on scratching of the side of the vessel. Yield, 0.57 g. (77%); m. p., 119~121.8°C. The melting point could be raised to 121~123°C by recrystallization. Its infrared spectrum was similar to that of DL-*trans*-carboxylic acid XI[1], m. p. 127~128°C.

B) By Hydrolysis of DL-trans-Ester X.—In a similar way, DL-*trans*-ester X (1.72 g, 0.010 mol.) was hydrolyzed with 0.40 N alcoholic potassium hydroxide (25 ml., 0.010 mol.). Yield, 1.07 g. (74%); m. p., 125~126.5°C. The melting point could be raised to 126.5~127.5°C by recrystallization, and it was undepressed on admixture with a pure authentic sample[1]. All properties of both compounds were identical.

DL-Threonine.—*A) By Hydrolysis of DL-trans-Carboxylic Acid XI Derived from DL-cis-Ester IX.*—The substance XI (6.06 g., 0.035 mol.) was refluxed with 120 ml. of 1 : 1 hydrochloric acid. From the hydrolyzate, DL-threonine containing a small quantity of DL-allothreonine was obtained by the usual method. Yield, 3.71 g. (73%); m. p., 230.5~231.5°C (decomp.). Its infrared spectrum was similar to that of pure DL-threonine. The proportion of DL-threonine (R_f 0.16) to DL-allothreonine (R_f 0.12) present was determined by paper chromatography using the upper layer from a mixture of *n*-butanol-acetone-concentrated aqueous ammonia-water (8 : 1 : 1 : 6 by volume)[6] as the developer. The ratio of DL-threonine to DL-allothreonine was about 5. Chromatographic analysis of the dinitrophenylated sample[7] showed that the ratio was 6.1. On recrystallization from water and alcohol, its melting point rose to 231~232°C (decomp.). Chromatographic analysis of this sample showed that the ratio was 10.1.

B) By Hydrolysis of DL-trans-Ester X.—One gram (0.0058 mol.) of DL-*trans*-ester X was hydrolyzed with hydrochloric acid (1 : 1; 16 ml.) in the usual way. Yield, 0.46 g. (67%); m. p., 232.5.~233.5°C (decomp.). The melting point could be raised to 236~237°C (decomp.) by recrystallization. All properties of this sample were identical with those of an authentic sample.

L(−)-*cis*-Ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (XIII).—Four grams (0.0376 mol.) of L(−)-*cis*-carboxylic acid XII[8], m. p., 169.5~170.5°C, $[\alpha]_D$ −19.2±0.5°C (H_2O), were esterified with hydrogen chloride in absolute alcohol. Yield, 4.1g. (86%); m. p., 71~73°C. After recrystallization from ethyl acetate and petroleum ether, it melted at 72~73°C. $[\alpha]_D^{27}$ −3.6°C (*c* 3.29, 99% EtOH).

IR, 3290 (amide), 1760, 1750, 1730 (cyclic amide and ester) cm^{-1} (Nujol).

Found: C, 48.75; H, 6.39; N, 8.16. Calcd. for $C_7H_{11}O_4N$: C, 48.55; H, 6.40; N, 8.09%.

L(+)-Allothreonine.—L(−)-*cis*-Ester XIII (0.79 g., 0.00456 mol.) was hydrolyzed with hydrochloric acid (1 : 1; 20 ml.) in the usual way. Yield, 0.47g. (89%); m. p., 273.5~275°C (decomp.), $[\alpha]_D^{29}$ +9.1° (*c* 2.97, H_2O). All properties of this sample were identical with those of an authentic sample[2].

L(+)-*trans*-4-Ethoxycarbonyl-5-methyl-2-oxo-oxazolidine (XVI).—Four and a half grams (0.031 mol.) of L(+)-*trans*-carboxylic acid XV[8], m. p., 139.5~140.5°C, $[\alpha]_D$ +41.2±0.5° (H_2O), were esterified with hydrogen chloride in absolute alcohol. Yield, 4.0 g. (75%); b. p., 123~125°C/10^{-3} mmHg; m. p., 63~65°C. On recrystallization from small amounts of ethyl acetate and petroleum ether, its melting point rose to 64~66°C. $[\alpha]_D^{28}$ +37.4° (*c* 3.50, 99% EtOH).

IR, 3260 (amide), 1750, 1715 (cyclic amide and ester) cm^{-1} (Nujol).

Found: C, 48.53; H, 6.12; N, 7.98. Calcd. for $C_7H_{11}O_4N$: C, 48.55; H, 6.40; H, 8.09%.

L(−)-Threonine.—In the usual way, 0.84 g. (0.00485 mol.) of L(+)-*trans*-ester XVI was hydrolyzed with 20 ml. of hydrochloric acid (1 : 1). Yield, 0.51 g. (89%); m. p., 261~262°C (decomp.), $[\alpha]_D^{28}$ −28.4° (*c* 2.81, H_2O). All properties of this sample were identical with those of an authentic sample[5].

L(+)-*trans*-5-Methyl-2-oxo-oxazolidine-4-carboxylic Acid (XV).—To a solution of 1.14 g. (0.00658 mol.) of L(+)-*trans*-ester XVI in 8.0 ml. of absolute alcohol, 7.6 ml. (0.00661 mol.) of 0.87 N alcoholic potassium hydroxide was added. After the mixture bad been lett standing for 1.5 hr. at room temperature, 20 ml. of water was added. The solution was acidified with concentrated hydrochloric acid and evaporated in vacuo to dryness. The residue was extracted 3 times with 40 ml. of hot ethyl acetate. When the extract was evaporated under reduced pressure, L(+)-*trans*-carboxylic acid XV crystallized out. Yield, 0.8 g. (85%); m. p., 139.8~140.2°C, $[\alpha]_D^{29.8}$ +41.8°(*c* 2.70, H_2O). All properties of this sample were identical with those of an authentic sample[8].

D(−)-*trans*-5-Methyl-2-oxo-oxazolidine-4-carboxylic Acid (XIV).—*Conversion of L(−)-cis-Ester XIII.*—In a similar way, 1.57 g. (0.00907 mol.) of L(−)-*cis*-ester XIII were hydrolyzed with 21.2 ml. (0.00915 mol.) of 0.43 N alcoholic potassium hydroxide. Yield, 1.10 g. (84%); m. p., 131~133.5 °C, $[\alpha]_D^{27}$ −37.7° (*c* 2.71, H_2O). Its infrared spectrum was similar to that of L(+)-*trans*-carboxylic acid XV.

D(+)-Threonine.—In the usual way, 0.54 g. (0.00454 mol.) of D(−)-*trans*-carboxylic acid XIV obtained from L(−)-*cis*-ester XIII was hydrolyzed with 20 ml. of hydrochloric acid (1 : 1). Yield, 0.37 g. (83%); m. p., 251~253.5°C (decomp.), $[\alpha]_D^{29}$ +24.8° (*c* 2.82, H_2O). Chromatographic analysis of the dinitrophenylated sample[7] showed that the ratio of D(+)-threonine to L(+)-allothreonine was 5.7.

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Department of Chemistry
Faculty of Science
Osaka University
Nakanoshima, Osaka