Studies of Hydroxy Amino Acids. I. Separation of Diastereoisomers of Threonine

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It was found that DL-allothreonine forms scarcely any soluble compounds with 5-nitronaphthalene-1-sulfonic acid, α-naphthylphosphoric acid, chlorendic acid,[1] and tetrachlorophthalic acid in water. DL-Threonine formed soluble or unstable compounds in water with these acids. The synthetic threonine mixture was successfully separated into DL-threonine and DL-allothreonine.

L-Threonine is used as medicine or a nutrient-enrichment for foodstuffs. However, allothreonine which is always formed as a by-product in the synthesis of threonine has almost no practical use. Most of the synthetic methods for threonine[2] produce more DL-allothreonine than DL-threonine, except for the reaction of copper glycinate with acetaldehyde[3] and the Strecker reaction of 1-ethoxy-1,2-diacetoxypropane.[4] A few methods of separation of diastereoisomers have been reported, in which threonine was changed to its derivative, Cu-acetaldehyde-threonine complex,[5] *N*-benzoyl-DL-threonine ethyl ester,[6] or sodium DL-threoninate.[7] Some aromatic acids have been used as a specific precipitating reagent for amino acids.[8,9] However, none is found in literature for the diastereoisomers of threonine.

In the course of studies on hydroxy amino acids, it was found that DL-allothreonine forms precipitates scarcely soluble in water with 5-nitronaphthalene-1-sulfonic acid, α-naphthylphosphoric acid, chlorendic acid, and tetrachlorophthalic acid. DL-Threonine, on the contrary, formed soluble or unstable compounds in water with these reagents. This finding was successfully applied to the separation of the two diastereoisomers of threonine. When a threonine mixture was brought into contact with these reagents in water, DL-allothreonine selectively precipitated with the reagents, while DL-threonine remained almost quantitatively in the mother liquor (Table 1).

Table 1. Separation of DL-allothreonine and DL-threonine with various precipitating reagents

Reagent	Grams of aThr and Thr in 100 m*l* of aqueous solution	
	Initial solution	Mother liquor
NNS	aThr 10.0 Thr 10.0	aThr 3.8 Thr 9.8
NP	aThr 10.0 Thr 10.0	aThr 2.0 Thr 8.6
CA	aThr 3.5 Thr 7.0	aThr 1.7 Thr 6.9
TCP	aThr 10.0 Thr 10.0	aThr 0.6 Thr 9.8

Abbreviations used are as follows:
aThr, DL-Allothreonine; Thr, DL-Threonine; NNS, 5-Nitronaphthalene-1-sulfonic acid; NP, α-naphthylphosphoric acid; CA, Chlorendic acid; TCP, Tetrachlorophthalic acid.
1.2 equimolar amount of NNS, NP, and TCP, and 2.4 equimolar amount of CA were used to DL-allothreonine in the initial solution.

Elementary analyses showed that the precipitate consists of DL-allothreonine and the reagent in 1:1 molar ratio, except for the precipitate with chlorendic acid. The IR spectra of the precipitates showed the absorption band in a region 1720—1750 cm^{-1} due to the free carboxyl group as the salt of DL-allothreonine with the strong acid.

In the case of chlorendic acid, the precipitate consisted of DL-allothreonine and chlorendic acid in 1:2 molar ratio. Since its IR spectrum only slightly differed from that of the mechanical mixture of DL-allothreonine and chlorendic acid, it was assumed that the precipitate is formed by the hydrogen bonding between DL-allothreonine and chlorendic acid.

DL-Threonine formed soluble salts with α-naphthylphosphoric acid and tetrachlorophthalic acid, but did not form crystalline compounds with 5-nitronaphthalene-1-sulfonic acid and chlorendic acid in water (Table 2). The salts of DL-allothreonine with 5-nitronaphthalene-1-sulfonic acid, α-naphthylphosphoric acid and tetrachlorophthalic acid were recrystallized from aqueous methanol, whereas the salts of DL-allothreonine with chlorendic acid and of DL-threonine with α-naphthylphosphoric acid and tetrachlorophthalic acid decomposed into the two components on recrystallization from various solvent systems.

When the salt of DL-allothreonine with tetrachlorophthalic acid was gradually heated above its melting point of 117°C, the melted material solidified at 148—150°C, and the solid remelted at 203—204°C. The compound with melting point of 203—204°C was assigned to be *N*-tetrachlorophthaloyl-DL-allothreonine from its IR spectrum, with no NH absorption of the

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Table 2. Characters of precipitated compounds

Compound	Reagent	Amino acid	Component ratio	Mp °C		Elementary analysis (%)
I	NNS	aThr	1:1	224—225 (dec.)	$C_{14}H_{16}O_8N_2S$	Found C, 45.26; H, 4.18; N, 7.40; S, 8.47 Calcd C, 45.16; H, 4.33; N, 7.52; S, 8.61
	NNS	Thr	—	——	——	——
II	NP	aThr	1:1	189—190 (dec.)	$C_{14}C_{18}O_7NP$	Found C, 49.11; H, 5.25; N, 3.95; P, 8.71 Calcd C, 48.98; H, 5.29; N, 4.08; P, 9.02
III	NP	Thr	1:1	160—161 (dec.)	$C_{14}H_{18}O_7NP$	Found C, 49.14; H, 5.21; N, 4.13; P, 8.68
IV	CA	aThr	1:2	136.5—137.5	$C_{22}H_{17}O_{11}NCl_{12}H_2O$	Found C, 28.85; H, 2.09; N, 1.53; Cl, 46.13 Calcd C, 28.88; H, 2.09; N, 1.52; Cl, 46.51
	CA	Thr	—	——	——	——
V	TCP	aThr	1:1	117	$C_{12}H_{11}O_7NCl_4 2H_2O$	Found C, 31.65; H, 3.08; N, 3.06; Cl, 30.77 Calcd C, 31.39; H, 3.29; N, 3.05; Cl, 30.89
VI	TCP	Thr	1:1	129—130	$C_{12}H_{11}O_7NCl_4 H_2O$	Found C, 32.97; H, 2.87; N, 3.25; Cl, 32.36 Calcd C, 32.68; H, 2.97; N, 3.18; Cl, 32.16

compound with melting point of 117°C in the region of 1500—1600 cm^{-1}. The same phenomenon was observed with the salt of DL-threonine with tetrachlorophthalic acid. The compound with a higher melting point of 204—206°C was also assigned to be tetrachlorophthaloyl-DL-threonine from its IR spectrum.

As seen in Table 1, the most effective precipitating reagent was tetrachlorophthalic acid. Separation tests with this acid were made in more detail using the mixtures of the two diastereoisomers with various ratios and a synthetic threonine mixture obtained by the procedure described by Akabori *et al.*[3] (Table 3).

Table 3. Separation of DL-allothreonine and DL-threonine with tetrachlorophthalic acid

No.	Mixture Thr g	Mixture aThr g	TCP[a] g	Water ml	Recovery[b] Thr g(%)	Recovery[b] aThr g(%)
1	14	7	22	150	13.5(96)	5.8(84)
2	10	10	31	135	10 (100)	9.2(92)
3	15	5	16	170	14.7(98)	3.7(74)
4[c]	10.3	6.3	20	110	10 (97)	5.8(92)

a) Tetrachlorophthalic acid as hemihydrate.
b) Purity of all the amino acids not less than 95%.
c) A synthetic mixture containing 0.4 g of glycine.

The precipitate and the mother liquor were separately treated with hydrochloric acid. After removing the liberated tetrachlorophthalic acid by filtration, DL-allothreonine and DL-threonine were recovered in 74—92% and 96—100% yield, respectively, from the acidic solutions using ion-exchange column. Recrystallization gave chromatographically pure DL-allothreonine and DL-threonine.

Experimental

All melting points are uncorrected. The IR spectra were obtained in Nujol mull with a JASCO IR-S spectrometer.

Determination of DL-*Threonine and* DL-*Allothreonine.* Paper chromatographic analysis was carried out by the descending method on Toyo Roshi No. 51 paper with the solvent system, *n*-butanol-methyl ethyl ketone-28% aqueous ammonia-water (5:3:1:1 v/v),[4] and the chromatogram was stained with cadmium-ninhydrin reagent.[10] Two spots corresponding to allothreonine and threonine were individually cut off, and eluted with methanol, and the absorbances of the eluates were measured at 510 mμ.

Salt of DL-*Allothreonine with 5-Nitronaphthalene-1-sulfonic Acid (I).* A solution of 2.0 g of DL-allothreonine and 5.0 g of 5-nitronaphthalene-1-sulfonic acid in 20 ml of water was prepared and stored in a refrigerator overnight. The crystals formed were collected by filtration; yield, 3.5 g (56%). Recrystallization from aqueous methanol gave pure I as platelets. Anal. and mp (Table 2). IR: 3420, 3160 (sh), 3080, 1750, 1615 (sh), 1603, 1530, 1515 cm^{-1}. On the other hand, DL-threonine gave no crystalline salt in analytically pure state when treated with 5-nitronaphthalene-1-sulfonic acid in the same manner.

Salts of DL-*Allothreonine and* DL-*Threonine with α-Naphthylphosphoric Acid (II and III).* To a solution of 5.4 g of disodium α-naphthylphosphate in 20 ml of 2N hydrochloric acid, 2.0 g of DL-allothreonine or DL-threonine was added with stirring at room temperature. The mixture was stirred for 5 hr and kept in a refrigerator overnight. Crystalline II formed was collected by filtration; yield, 4.5 g (78%). Recrystallization from aqueous methanol gave pure II as platelets. Crystalline III was obtained from the concentrated syrupy solution; yield, 3.3 g (57%). Anal. and mp (Table 2). IR of II: 3300, 3080 (sh), 1720, 1620, 1603, 1585, 1525 cm^{-1}. IR of III: 3580, 3220, 3120, 1740, 1620, 1603 (sh), 1580 (sh), 1505 cm^{-1}.

Crystalline Compound of DL-*Allothreonine with Chlorendic Acid (IV).* To a solution of 2.0 g of DL-allothreonine in 20 ml of water, 7.8 g of chlorendic acid was added with stirring at room temperature. The suspension was stirred for 5 hr, and then allowed to stand in a refrigerator overnight. The crystalline compound formed was collected by filtration; yield, 8.2 g (89%). Anal. and mp (Table 2). IR: 3580, 3420, 3100, 1730 (sh), 1710, 1610, 1580, 1515 cm^{-1}. DL-Threonine gave no such crystalline compound with chlorendic acid.

Salts of DL-*Allothreonine and* DL-*Threonine with Tetrachlorophthalic Acid (V and VI).* A mixture of 2.0 g of DL-allothreonine or DL-threonine and 6.3 g of tetrachlorophthalic acid hemihydrate in 20 ml of water was stirred for 5 hr at room temperature, and stored in a refrigerator overnight. The crystals of V formed were collected by filtration; yield, 6.5 g (84%). Recrystallization from aqueous methanol gave pure V as platelets. The crystals of VI were obtained as

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needles from a concentrated syrupy solution; yield, 5.6 g (75%). Anal. and mp (Table 2). IR of V: 3500 (sh), 3220, 1750, 1710, 1620, 1600, 1550 (sh), 1520 (sh), 1510 cm^{-1}. IR of VI: 3460, 3200, 1735 (sh), 1705, 1595, 1525 cm^{-1}.

Separation of DL-*Allothreonine and* DL-*Threonine with Various Precipitating Reagents (Table 1).* To an aqueous solution containing DL-allothreonine and DL-threonine was added a precipitating reagent in the amount of 1.2 molar equivalent to DL-allothreonine. In the case of chlorendic acid, 2.4 molar equivalent to DL-allothreonine was used. The mixture was stirred for 5 hr and stored in a refrigerator overnight. The precipitate formed was removed by filtration. An aliquot of the filtrate was analyzed by paper chromatography and the contents of DL-allothreonine and DL-threonine in the filtrate were determined. The results are summarized in Table 1.

Separation of DL-*Allothreonine and* DL-*Threonine using Tetrachlorophthalic Acid (Table 3).* A typical run (No. 4. in Table 3) is as follows: To a solution of a synthetic threonine mixture composed of 10.3 g of DL-threonine, 6.3 g of DL-allothreonine, and 0.4 g of glycine in 110 m*l* of water,[3] was added 20 g of tetrachlorophthalic acid hemihydrate with stirring. The mixture was stirred for 5 hr at room temperature, and then kept in a refrigerator overnight. The precipitate was filtered, washed with a small amount of water, and then suspended in 100 m*l* of N hydrochloric acid. The mixture was boiled for 30 min and allowed to stand at room temperature for 3 hr. After removing tetrachlorophthalic acid liberated by filtration, the solution was passed through a column of Dowex 50 W×8 (H form). The column was washed with water and eluted with 2N ammonia. The eluate was concentrated *in vacuo* until crystallization began and a sufficient amount of methanol was added. The crystals were collected by filtration and washed with a small amount of aqueous methanol, giving 5.8 g (92% recovery) of DL-allothreonine. Recrystallized DL-allothreonine from water-methanol was chromatographically pure. The mother liquor, from which the DL-allothreonine salt was removed, was adjusted to pH 1.35 with hydrochloric acid. After removing tetrachlorophthalic acid liberated, the filtrate was treated in the same manner as described above giving 10.0 g (97% recovery) of DL-threonine. Recrystallized DL-threonine from water-methanol was chromatographically pure.

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