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Note

Preparation of Optically Active Allothreonine by Separating from a Diastereoisomeric Mixture with Threonine

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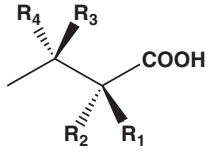
A simple procedure is described to obtain D- and L-allothreonine (D- and L-aThr). A mixture of *N*-acetyl-D-allothreonine (Ac-D-aThr) and *N*-acetyl-L-threonine (Ac-L-Thr) was converted to a mixture of their ammonium salts and then treated with ethanol to precipitate ammonium *N*-acetyl-L-threoninate (Ac-L-Thr·NH_3) as the less-soluble diastereoisomeric salt. After separating Ac-L-Thr·NH_3 by filtration, Ac-D-aThr obtained from the filtrate was hydrolyzed in hydrochloric acid to give D-aThr of 80% *de*, recrystallized from water to give D-aThr of >99% *de*. L-aThr was obtained from a mixture of the ammonium salts of Ac-L-aThr and Ac-D-Thr in a similar manner.

Key words: allothreonine; threonine; *N*-acetylated derivative; separation of diastereoisomeric ammonium salts

L- and D-Allothreonines ((2*S*,3*S*)- and (2*R*,3*R*)-2-amino-3-hydroxybutanoic acids; L- and D-aThr) are non-proteinogenic α-amino acids and diastereoisomers of L-threonine ((2*S*,3*R*)-2-amino-3-hydroxybutanoic acid; L-Thr), a normal constituent of proteins, and are difficult to obtain in large quantities. D-aThr has been identified as a constituent of the *Hafnia alvei* strain PCM 1206 lipopolysaccharide.[1] L-aThr is an important constituent of such cyclic peptides as coibamide A[2] and lysobactin,[3] displaying very strong antibacterial activity against methicillin-resistant *Staphylococcus aureus*. In addition, D- and L-aThr serve as useful chiral building blocks in asymmetric syntheses.[4] D- and L-aThr have been obtained by an asymmetric synthesis,[5] optical resolution of DL-aThr,[6–8] enzymatic resolution of *N*-acylated and *O*-methylated DL-aThr,[9] and selective enzymatic hydrolysis of a mixture of *N*-acetylated and *O*-benzylated L-Thr and D-aThr.[10] Of these methods, optical resolution by separating the diastereoisomers by crystallization seemed to be a simple and efficient procedure. However, such optical resolution would require an optically active compound such as a resolving agent in separating the diastereoisomeric salts,[6] seed crystals in preferential crystallization,[7] and an optically active cosolute in replacing crystallization.[8] We have already reported the preparation of aThr by replacing crystallization with alanine as a cosolute.[8] We report in the present work a procedure to obtain D- and L-aThr from diastereoisomeric mixtures of Thr and aThr, which were given by epimerizing Thr, by a simpler and more efficient procedure without employing such a chiral factor as alanine.



2-Amino-3-hydroxybutanoic acids

	R_1	R_2	R_3	R_4
L-Thr	NH_2	H	OH	H
L-aThr	NH_2	H	H	OH
D-Thr	H	NH_2	H	OH
D-aThr	H	NH_2	OH	H

Optically active α-amino acids undergo racemization or epimerization at the C-2 position with carbonyl compounds as a catalyst in carboxylic acid.[11] L-Thr was subjected to epimerization by stirring in acetic acid at 90 °C for 4 h, using salicylaldehyde as the catalyst, to give a diastereoisomeric mixture of L-Thr and D-aThr in a molar ratio of 1:0.7;[8] the molar ratio of L-Thr and D-aThr in the mixture was determined by the intensity ratio of the methine proton signals at the C-2 positions in the ^{1}H-NMR spectrum of the mixture, the signals for L-Thr and D-aThr respectively appearing at 3.57 and 3.83 ppm. The epimerization of D-Thr also gave a mixture of D-Thr and L-aThr in a molar ratio of 1:0.7. As L-Thr is more soluble than D-aThr in water,[8,12] we first attempted to separate aThr from the diastereoisomeric mixtures by recrystallizing from water. However, separation seemed to be difficult even by repeated recrystallization.[8,13]

We next attempted to separate aThr by transforming the mixture into a simple derivative. Optical resolution by separating diastereoisomers generally gives the enantiomer with higher optical purity from the less-soluble diastereoisomer. *N*-Acetyl-L-allothreonine (Ac-L-aThr) has been reported to have a higher melting point (mp 153–154 °C)[9] than *N*-acetyl-L-threonine (Ac-L-Thr; mp 118–119 °C).[10] Since the solubility and melting point are correlated to some extent, the levels of solubility of Ac-D- and -L-aThr were expected to be lower than those of Ac-D- and -L-Thr. A mixture (50.0 mmol) containing 3.51 g of L-Thr and 2.45 g of D-aThr, which had been obtained by epimerizing L-Thr with salicylaldehyde in acetic acid, was allowed to react with acetic anhydride in acetic acid to give an *N*-acetylated mixture as an oily substance, but attempts

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Abbreviations: aThr, allothreonine; Thr, threonine; Ac-D-aThr, *N*-acetyl-D-allothreonine; Ac-L-Thr, *N*-acetyl-L-threonine; Ac-D-aThr·NH_3, ammonium *N*-acetyl-D-allothreoninate; Ac-L-Thr·NH_3, ammonium *N*-acetyl-L-threoninate

to crystallize the *N*-acetylated mixture were unsuccessful.

N-Acetylated amino acids tend to form good crystalline ammonium salts; for example, the ammonium salts of *N*-acetyl-D-alloisoleucine and *N*-acetyl-L-isoleucine can be separated from their mixture by recrystallization.[14,15] The networks of hydrogen bonds with the ammonium ions in the crystals of these ammonium salts determine the rigidity of the crystal packing, so that a difference in the side chain conformation of the amino acids would greatly affect their solubility. An ammoniacal aqueous solution of a mixture of Ac-L-Thr and Ac-D-aThr was evaporated to dryness *in vacuo* to give a mixture of the ammonium salts of Ac-L-Thr and Ac-D-aThr (Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3) as a viscous residue, this giving a white solid after adding 2-propanol. The ^{1}H-NMR spectrum of the white solid exhibited the presence of only Ac-L-Thr, indicating that all of Ac-D-aThr·NH_3 remained in the 2-propanol solution. The crystals obtained by recrystallizing the solid from ethanol were determined by an X-ray crystal structure analysis to be Ac-L-Thr·NH_3, whose crystal structure had already been reported.[16] Ac-L-Thr·NH_3 was recovered from the suspension in 2-propanol in a yield of 63.4%, this being calculated on the basis of the amount of L-Thr (3.51 g) in the starting mixture (5.96 g, 50.0 mmol). The gathered Ac-L-Thr·NH_3 gave optically pure Ac-L-Thr, exhibiting $[\alpha]_D^{20}$ +12.4 (*c* 4.84, CH_3OH) (lit.[17] $[\alpha]_D^{20}$ +12 (*c* 4.7, CH_3OH)), after adding HCl aq., suggesting that L-Thr had not been racemized in the procedure for obtaining Ac-D-aThr·NH_3. After collecting Ac-L-Thr·NH_3 by filtration, Ac-D-aThr·NH_3 was allowed to precipitate by cooling the filtrate in a yield of 41.4%, this being calculated on the basis of the amount of D-aThr (2.45 g) in the same starting mixture. The filtrate was calculated to contain approximately a 1:1 mixture of Ac-L-Thr·NH_3 (1.92 g) and Ac-D-aThr·NH_3 (2.15 g). It seemed to be difficult to obtain further Ac-D-aThr·NH_3 from the 2-propanol filtrate by recrystallization, because the respective solubility of Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3 was 0.074 g and 0.174 g in 100 cm^3 of 2-propanol at 25 °C.

The respective solubility of Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3 in 100 cm^3 of ethanol at 20 °C was 0.240 g and 1.474 g, indicating that Ac-D-aThr·NH_3 was about 6 times more soluble than Ac-L-Thr·NH_3. These results implied that Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3 might be more efficiently separated by using ethanol rather than 2-propanol as the solvent. After adding ethanol to the viscous mixture of Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3, precipitated Ac-L-Thr·NH_3 was collected by filtration in a yield of 72.7%. On the other hand, the filtrate was evaporated *in vacuo* to give a mixture of Ac-L-Thr·NH_3 and Ac-D-aThr·NH_3 as an oily residue. After hydrolyzing the mixture in hydrochloric acid and then evaporating to dryness *in vacuo*, an ethanol solution of the residue was treated with triethylamine to precipitate D-aThr of 80% *de* in a yield of 1.92 g; the diastereoisomeric excess of D-aThr was determined by the intensity ratio of the methine proton signals at the C-2 positions of aThr and Thr in the ^{1}H-NMR spectrum. This crude D-aThr was recrystallized from water-ethanol to give diastereoisomerically pure D-aThr in a yield of 49.0% (1.20 g). On the other hand, a mixture of D-Thr and L-aThr (50.0 mmol) was treated in a manner similar to give L-aThr of >99% *de* in a yield of 48.2% (1.18 g). D-aThr and L-aThr of >99% *de* were determined from the ^{1}H-NMR spectra.

The structure of Ac-L-Thr·NH_3 (Fig. 1), which had already been reported,[16] was investigated in detail concerning the intermolecular hydrogen bonds (Table 1). The O(1) atom of the carboxylate group of the Ac-L-Thr ion interacted with the hydroxyl group of another Ac-L-Thr ion (H(3)···O(1) = 1.88 Å) and an ammonium ion (H(12)···O(1) = 1.89 Å) more strongly than the O(2) atom, which interacted with two other ammonium ions (1.94 and 1.93 Å). This is consistent with the electronic imbalance inferred from the difference in bond length between C(1)–O(1) (1.2626(12) Å) and C(1)–O(2) (1.2517(14) Å) of the carboxylate group. The fourth N–H bond of the ammonium ion interacted strongly with the C=O moiety of an acetyl group (H(11)···O(4) = 1.87 Å), indicating that this C=O moiety was more polarized in comparison with the carboxyl group. It seems to have made Ac-L-Thr·NH_3 less soluble that an Ac-L-Thr ion interacted with the other Ac-L-Thr ions with two strong hydrogen bonds and with ammonium ions with

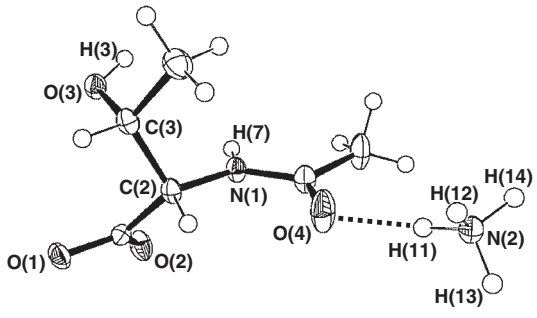

Fig. 1. ORTEP View of Ac-L-Thr·NH_3.

Table 1. Distances (Å) and Angles (°) for Hydrogen Bonds of Ac-L-Thr·NH_3

D–H···A	D···A	D–H	H···A	D–H···A
O(3)–H(3)···O(1)[a]	2.6719(12)	0.821(16)	1.881(16)	161.4(16)
N(1)–H(7)···O(3)[a]	2.8912(12)	0.895(13)	1.998(13)	175.5(14)
N(2)–H(11)···O(4)	2.7711(13)	0.919(14)	1.869(14)	166.7(12)
N(2)–H(12)···O(1)[b]	2.8382(14)	0.957(17)	1.892(17)	169.4(14)
N(2)–H(12)···O(3)[c]	3.2496(14)	0.957(17)	2.752(15)	113.1(11)
N(2)–H(13)···O(1)[d]	3.3113(14)	0.914(14)	2.560(15)	139.7(12)
N(2)–H(13)···O(2)[d]	2.8265(14)	0.914(14)	1.940(14)	162.8(13)
N(2)–H(14)···O(2)[e]	2.8339(14)	0.919(18)	1.926(18)	169.0(16)

Symmetry operations: a) $-1/2+x, 1/2-y, 1-z$; b) $2-x, 1/2+y, 1/2-z$; c) $3/2-x, 1-y, -1/2+z$; d) $3/2+x, -y, -1/2+z$; e) $1-x, 1/2+y, 1/2-z$

some hydrogen bonds involving the carboxylate groups and the amide C=O group.

The method reported in this paper is simpler and more efficient than that already reported and might be suitable for producing aThr on an industrial scale because of the easy preparation which does not require another chiral reagent.

Experimental

General. Specific rotation data were collected at 589 nm and 20 °C with a Horiba Seisakusho SEPA-300 auto-polarimeter equipped with a quartz cell with a 10.0-cm path length. ^{1}H-NMR spectra were recorded by a Jeol JNM-AL400 FT NMR system in deuterium oxide with sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) as an internal standard. Chemical shift values are reported in δ units downfield from DSS. L- and D-Thr were purchased from Wako Pure Chemical Industries.

Epimerization of L- and D-threonine. L-Thr (23.8 g, 200 mmol) was dissolved in 400 cm^3 of acetic acid at 90 °C. After adding salicylaldehyde (2.44 g, 20.0 mmol) to the solution, the mixture was stirred for 2 h at 90 °C. The mixture was then concentrated *in vacuo* at 60 °C to give a mixture of L-Thr and D-aThr as the diastereoisomeric residue. After adding 400 cm^3 of methanol to this residue and then stirring for 0.5 h at 40 °C, the mixture was collected by filtration, washed thoroughly with methanol, and dried. The molar ratio of L-Thr and D-aThr in the mixture was determined by the intensity ratio of the methine proton signals at the C-2 positions in the ^{1}H-NMR spectrum of the mixture. Epimerization of D-Thr (23.8 g, 200 mmol) was carried out in a manner similar to that for L-Thr. The mixtures were composed of Thr and aThr in a molar ratio of 1:0.70 with a yield of 16.4 g and $[\alpha]_D^{20}$ −22.1 (*c* 1.00, 1 mol dm^{-3} HCl) for the mixture of L-Thr and D-aThr, and with a yield of 11.4 g and $[\alpha]_D^{20}$ +22.0 (*c* 1.00, 1 mol dm^{-3} HCl) for the mixture of D-Thr and L-aThr.

Preparation of ammonium N-*acetyl-L-threonine and ammonium* N-*acetyl-D-allothreonine.* The diastereoisomeric mixture, which was composed of L-Thr and D-aThr in a molar ratio of 1:0.7, (5.96 g, 50.0 mmol) was dissolved in 50 cm^3 of acetic acid. After adding acetic anhydride (6 cm^3, 63.5 mmol) dropwise to the solution and stirring for 0.5 h at 90 °C, the mixture was evaporated *in vacuo*. To a solution of the residue in 50 cm^3 of water was added concentrated aqueous ammonia. After evaporating the mixture *in vacuo*, 50 cm^3 of 2-propanol was added to the residue. The precipitated Ac-L-Thr•NH$_3$ was collected by filtration, washed with a small amount of cold 2-propanol, and dried. The filtrate was solidified by stirring for 15 min in an ice bath, and then 25 cm^3 of 2-propanol was added to the resulting solid. After the solid had been finely broken up in an ultrasonic cleaner while cooling, Ac-D-aThr•NH$_3$ was collected by filtration, washed with a small amount of cold 2-propanol, and dried.

Ac-L-Thr•NH$_3$. Yield, 3.32 g; mp 152–154 °C (decomp.); $[\alpha]_D^{20}$ +28.4 (*c* 1.00, methanol). ^{1}H-NMR (400 MHz, D$_2$O, DSS) δ: 4.27–4.20 (1H, m, 3-C*H*), 4.14 (1H, d, 2-C*H*), 2.07 (3H, s, –NHCOC*H*$_3$), 1.17 (3H, d, 4-C*H*$_3$). *Anal*. Calcd. for $C_6H_{14}N_2O_4$: C, 40.44; H, 7.92; N, 15.72%. Found: C, 40.38; H, 7.63; N, 15.58%.

Ac-D-aThr•NH$_3$. Yield, 1.52 g; mp 151–153 °C (decomp.); $[\alpha]_D^{20}$ −16.2 (*c* 1.00, methanol). ^{1}H-NMR (400 MHz, D$_2$O, DSS) δ: 4.39 (1H, d, 2-C*H*), 4.28–4.18 (1H, m, 3-C*H*), 2.06 (3H, s, –NHCOC*H*$_3$), 1.14 (3H, d, 4-C*H*$_3$). *Anal*. Calcd. for $C_6H_{14}N_2O_4$: C, 40.44; H, 7.92; N, 15.72%. Found: C, 40.52; H, 7.69; N, 15.56%.

Preparation of D- and L-allothreonine. A mixture of L-Thr and D-aThr (5.96 g, 50.0 mmol) was acetylated, and then concentrated aqueous ammonia added to give a mixture of Ac-L-Thr•NH$_3$ and Ac-D-aThr•NH$_3$, in a manner similar to that just described. To the mixture was added 50 cm^3 of ethanol. The precipitated Ac-L-Thr•NH$_3$ was collected by filtration, washed with a small amount of cold ethanol, and dried. After evaporating the filtrate *in vacuo* and then refluxing a solution of the residue in 65 cm^3 of 5 mol dm^{-3} hydrochloric acid for 2 h, the solution was evaporated *in vacuo*. To the residue was added 50 cm^3 of ethanol. After removing ammonium chloride by filtration, the filtrate was adjusted to pH 6 with triethylamine. The precipitated D-aThr of 80% *de* was collected by filtration, washed with ethanol, and dried, yielding 1.92 g. After adding 40 cm^3 of ethanol to a solution of the crude D-aThr in 25 cm^3 of water and then stirring for 30 min at room temperature, D-aThr of >99% *de* was collected by filtration, washed with methanol, and dried. L-aThr of >99% *de* was prepared from the mixture (50 mmol) of L-Thr and D-aThr in a manner similar to that for D-aThr.

D-aThr. Yield, 1.20 g; $[\alpha]_D^{20}$ −31.3 (*c* 1.00, 5 mol dm^{-3} HCl). ^{1}H-NMR (400 MHz, D$_2$O, DSS) δ: 4.39–4.32 (1H, m, 3-C*H*), 3.83 (1H, d, 2-C*H*), 1.19 (3H, d, 4-C*H*$_3$).

L-aThr. Yield, 1.18 g; $[\alpha]_D^{20}$ +31.8 (*c* 1.00, 5 mol dm^{-3} HCl) (ref.[18] $[\alpha]_D^{20}$ +31.7 (5 mol dm^{-3} HCl)). The ^{1}H-NMR spectrum was virtually identical to that of D-aThr.

Solubility. Ac-L-Thr•NH$_3$ (1.843 g) or Ac-D-aThr•NH$_3$ (2.563 g) was dissolved in 25 cm^3 of 2-propanol at 50 °C. After vigorously stirring the solution for 24 h at 25 °C, the precipitated Ac-L-Thr•NH$_3$ or Ac-D-aThr•NH$_3$ was rapidly collected by filtration and thoroughly dried. The solubility at 25 °C was calculated on the basis of the weight of Ac-L-Thr•NH$_3$ or Ac-D-aThr•NH$_3$. The solubility of Ac-L-Thr•NH$_3$ and Ac-D-aThr•NH$_3$ in ethanol at 20 °C was measured in a manner similar to that just described.

X-Ray crystallography. The X-ray experiment for Ac-L-Thr•NH$_3$ was carried out with a Rigaku RAXIS imaging plate area detector with graphite-monochromated MoKα radiation (λ = 0.71070 Å). The crystal was mounted on a nylon loop at −150 °C. To determine the cell constant and orientation matrix, three oscillation photographs were taken for each frame with an oscillation angle of 3° and exposure time of 30 s. Intensity data were collected from the oscillation photographs, and the reflection data were corrected for Lorentz and polarization effects. The structure was solved by the direct method[19] and expanded by the Fourier technique.[20] Non-hydrogen atoms were refined anisotropically by a full-matrix least-squares calculation. Hydrogen atoms were found from the difference Fourier map and isotropically refined. All calculations were performed by using the CrystalStructure[21] and Crystals[22] crystallographic software packages. Crystallographic data: $C_6H_{14}O_4N_2$, M = 178.19, orthorhombic, $P2_12_12_1$, a = 7.0136(7) Å, b = 8.0869(8) Å, c = 16.7882(15) Å, V = 952.20(16) Å^3, Z = 4, D_{calc} = 1.243 g cm^{-3}, 2176 unique reflections. Refinement with all 9335 reflection converged at final R = 0.0323 and wR_2 = 0.0595; CCDC 779488.

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