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A new simple preparation of D-alloisoleucine suitable for large-scale manufacture

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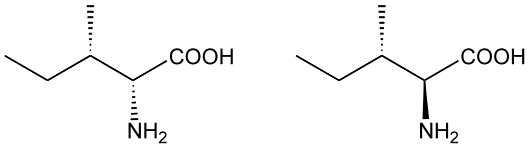
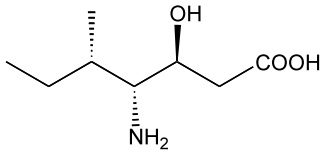
Abstract—D-Alloisoleucine (D-aIle) was obtained by the resolution of the epimer mixture of L-isoleucine (L-Ile) and D-aIle, which was formed by epimerization of L-Ile, with a resolving agent such as (2*S*,3*S*)-dibenzoyltartaric acid ((2*S*,3*S*)-DBTA) or (2*S*,3*S*)-di-4-toluoyl tartaric acid ((2*S*,3*S*)-DTTA).

1. Introduction

D-Alloisoleucine (D-aIle) is a well-known stereoisomer of L-isoleucine (L-Ile) and seems to have a potentially wide usage as a component of unnatural peptides or medicines. It is a precursor of (3*S*,4*R*,5*S*)-isostatine (Ist), which is the characteristic component of cyclic peptide didemnins isolated from a Caribbean tunicate[1,2] and known to have a potent antitumor activity as well as a potential immunosuppressive activity. A patent[3] describes the syntheses of drugs for cardiovascular disease starting from D-aIle.





(3*S*, 4*R*, 5*S*)-Ist

Unfortunately, there was no efficient method to obtain this unnatural amino acid in large quantities. As a consequence this amino acid is very expensive and only available in sub-gram units on the market.[†]

To obtain this unnatural amino acid, much effort has been expended and several methods have been proposed.[4] Although the epimer mixture of L-Ile and D-aIle is known to be obtained by epimerization of L-Ile[5–8] or by synthesis from (*S*)-2-methylbutanol (optically active isoamylalcohol),[4] no efficient method to separate the epimer mixture has been reported. The epimer mixture has to be protected by an acetyl[9] or benzyloxycarbonyl[10,11] group and separated as the salts with optically active amines. Inversion of 2-amino group of L-Ile is also devised by Schmidt et al.,[12] but this method requires five steps and is very laborious. Several stereoselective syntheses of D-aIle being also described, all remain of laboratory use.[2,13]

We report here a new simple method for the preparation of D-aIle. It starts from L-Ile, which is manufactured in an industrial scale and inexpensive, and comprises a very efficient resolution method of the epimer mixture with a resolving agent and the improved procedure for epimerizing L-Ile, which is applicable for other amino acids. Final liberation of the amino acid from the resulting complex or salt is very simple and the enantiomerically pure amino acid is obtained in a high yield.

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[†] D-Alloisoleucine is available only as reagents in 100 mg to 1 g packages.

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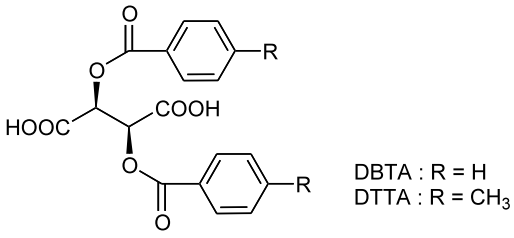
2. Results and discussion

Various methods for the isomerization (racemization or epimerization) of amino acids have been proposed and well documented.[8] Of these, the method found by Chibata et al.,[6,14] in which amino acids are heated with a catalytic amount of salicylaldehyde in glacial acetic acid, is most favourable for Ile, because no protection of the amino group is required. However, this method imposes a severe difficulty of evaporating a large amount of glacial acetic acid from the reaction mass to isolate racemized amino acids. We have found that the isomerization of amino acids can be performed in aromatic solvents and the isomerized acid separates as a solid and is easily recovered in a high recovery after cooling the reaction mass. Thus, L-Ile is refluxed in toluene with 4 equiv. of glacial acetic acid and a catalytic amount of salicylaldehyde. Epimerization is completed within about 3 h. After cooling the reaction mixture, the precipitated epimer mixture is isolated by filtration to give a highly pure product in ca. 90% yield. The mother liquor containing acetic acid and salicylaldehyde can be reused in next batches by supplementing some loss of acid and aldehyde. This isomerization procedure in an aromatic solvent is generally applicable for other amino acids very efficiently (Table 1). The degree of isomerization is defined by the following equation:

$$\text{Degree of isomerization } (\%) = \{(P_0 - P_I)/P_0\} \times 100$$

where P_0 is the enantiomeric or diastereomeric purity of the amino acid before the isomerization, and P_I, is that after the isomerization.

The epimer mixture has been found to be efficiently separated as the salt or complex with a resolving agent such as (2*S*,3*S*)-dibenzoyltartaric acid ((2*S*,3*S*)-DBTA) or (2*S*,3*S*)-di-4-toluoyl tartaric acid ((2*S*,3*S*)-DTTA). The most favorable separation is achieved when 0.5 equiv. of the resolving agent and hydrochloric acid are mixed with the epimer mixture as shown in Table 2. L-Ile in the mother liquor is also recovered and recycled to the epimerization step. From elemental analysis, and the ^{1}H NMR spectrum, D-aIle is estimated to form a less soluble 1:1 complex with (2*S*,3*S*)-DBTA (anhydrous) or with (2*S*,3*S*)-DTTA (mono- or dihydrate).



The complex or salt formed from D-Ile and tartaric acid derivative (2*S*,3*S*)-DBTA or (2*S*,3*S*)-DTTA easily decomposes in alcoholic solvents to give diastereomerically pure D-aIle as a solid by filtration. Better results are obtained by decomposing the complex or salt in polar solvents such as lower alcohols or acetic acid ester containing 5–10% of water. These facts suggest that D-Ile is interacted with (2*S*,3*S*)-DBTA or (2*S*,3*S*)-DTTA mainly by weak bonds such as a hydrogen bond or a van der Waals force; it seems appropriate to regard the solid as a complex, but not a salt.[15]

Table 1. Racemization of L-amino acids

Entry	Amino acid	Time (h)	Degree of racemization (%)	Yield (%)
1	Alanine	3	97	92
2	Valine	2	48	90
3	Leucine	2	98	88
4	Isoleucine	3	100[a]	90
5	Phenylalanine	3	87	82
6	Tryptophan	2	82	87
7	Methionine	2	86	86
8	Serine	2	81	92
9	Aminobutanoic acid	2	96	85

Amino acid 5.0 g; $PhCH_3$ 25 ml; AcOH 4.0 equiv.; salicylaldehyde 0.2 equiv.
[a] Epimer mixture of L-isoleucine and D-alloisoleucine.

Table 2. Diastereomeric resolution of epimer mixture

Entry	Resolving agent (mol equiv.)	HCl (equiv.)	Yield (%)	DP[a] (% de)	E[b] (%)
1	(2*S*,3*S*)-DBTA/0.50	None	42	96	81
2	(2*S*,3*S*)-DBTA/0.50	0.50	44	96	85
3	(2*S*,3*S*)-DBTA/0.50	0.50	45	95	86
4	(2*S*,3*S*)-DTTA/0.50	0.50	44[c]	95	84

Solvent: entries 1, 2 and 4, H_2O; entry 3, H_2O/MeOH = 5/1.
DBTA, dibenzoyltartaric acid; DTTA, di-4-toluoyltartaric acid.
[a] Diastereomeric purity.
[b] Resolution efficiency (E, %) = yield (%)×diastereomeric purity (%)/100.
[c] Monohydrate.

The complex having a diastereomeric purity higher than 95% de gives diastereomerically pure D-aIle (100% de) in a yield of up to 90%. The resolving agent in the mother liquor can be recovered and reused. This simple and efficient process could be successfully scaled up with no trouble to give multi-kilograms of highly pure D-aIle in our pilot plant.[16]

3. Experimental

3.1. General

^{1}H NMR spectra were measured on a JEOL GSX 270 spectrometer. IR spectra were measured on a Jasco IR 700 spectrometer. Enantiomeric or diastereomeric purities of amino acids were determined by HPLC analysis with a Jasco UV-975 detector (wavelength 254 nm) under the following conditions. Column: Sumichiral OA-5000 4.6 mm I.D.×150 mm for isoleucine, alanine, valine, leucine, phenylalanine, methionine, serine, and 2-aminobutanoic, Daicel Chiralpak MA (+) 4.6 mm I.D.×50 mm for tryptophan. Mobile phase: 2 mmol $CuSO_4$+isopropnol (IPA) (98:2) for isoleucine, 2 mmol $CuSO_4$ for alanine, valine, serine, and 2-aminobutanoic acid, 2 mmol $CuSO_4$+IPA (90:10) for phenylalanine, 2 mmol $CuSO_4$+methanol (85:15) for tryptophan.

3.2. Epimerization of L-Ile

A suspension of L-Ile (60 g, 0.457 mol), glacial acetic acid (109.8 g, 1.83 mol) and salicylaldehyde (11.2 g, 91.4 mol) in toluene (300 ml) was heated under reflux for 3 h and then cooled to room temperature. The precipitated crystals were filtered, rinsed with toluene and dried to give the epimeric mixture of L-Ile and D-aIle (54.0 g, yield 90%) as a white crystalline powder.

As for other amino acids, the typical epimerization procedure is as follows: a suspension of L-amino acid (5 g), glacial acetic acid (4 mol equiv.) and salicylaldehyde (0.2 mol equiv.) in toluene (25 ml) was heated under reflux for 2 h. Subsequent treatment was similarly carried out for L-Ile. The results of the racemization of the various amino acids are shown in Table 2.

3.3. Separation of epimer mixture of L-Ile and D-aIle by (2*S*,3*S*)-DBTA

To a suspension of the epimer mixture of L-Ile and D-aIle (5.0 g, 38.1 mmol) in water (90 ml) was added (2*S*,3*S*)-DBTA monohydrate (7.19 g, 19.1 mmol) under stirring, and the mixture was heated at 70°C for 1 h. The slurry formed by reaction of the amino acids with the DBTA was then cooled to 25°C. The precipitated crystals were filtered, rinsed with water and dried to give a 1:1 complex of D-aIle and (2*S*,3*S*)-DBTA (7.77 g, yield (based on the epimer mixture) 41.7%) as a white crystalline powder of 95.6% de (Table 2, entry 1); mp 175.5–176.5°C; water (Karl–Fisher) found: 0.08%; Elemental analysis found: C, 59.0; H, 5.6; N, 3.1%. Calcd for ($C_{24}H_{27}NO_{10}$): C, 58.9; H, 5.6; N, 2.9%; IR (KBr): 3156, 2972, 2942, 2882, 1733 (s), 1692 (s), 1601 (m), 1528 (m), 1320, 1266 (s), 1118 (s), 719 (s) cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.97 (3H, t, J=4.0 Hz, D-aIle-5 CH_3), 1.00 (3H, t, J=4.0 Hz, CH_3), 1.24–1.40, 1.43–1.59 (1H×2, each m, CH_2), 2.01–2.14 (1H, m, D-aIle-3 CH), 3.71 (1H, d, J=3.5 Hz, D-aIle-2 CH), 4.90 (7.5H, s, CD_3OH), 5.94 (2H, s, DBTA CH×2), 7.50 (4H, t, Bz-3,5×2), 7.64 (2H, tt, Bz-4×2), 8.11 (4H, dd, Bz-2,6×2).

Other experimental results are summarized in Table 2 (entries 2 and 3).

3.4. Preparation of D-aIle

To a suspension of the epimer mixture of L-Ile and D-aIle (50.0 g, 381 mmol) in water (490 ml) was added 35% hydrochloric acid (7.94 g, 76.2 mmol) under stirring. A suspension was added dropwise to a mixture of (2*S*,3*S*)-DBTA monohydrate (71.7 g, 190.5 mmol) in methanol (100 mL) under stirring. A further 11.9 g (114 mmol) 35% hydrochloric acid was added to the slurry, and the mixture was heated at 70°C for 1 h. The slurry formed by reaction of the amino acids and the DBTA was then cooled to 25°C. The precipitated crystals were filtered, rinsed with water and dried to give a 1:1 complex of D-aIle and (2*S*,3*S*)-DBTA (84.7 g, yield (based on the epimer mixture) 45.5%) as a white crystalline powder of 94.8% de. This complex (80.0 g, 163 mmol), obtained by the above procedures, was placed in a mixture of 2-propanol (720 ml) and water (80 ml). The mixture was heated under reflux for 1 h and, after cooling, the solid was filtered from the mixture at 25°C. The solid was rinsed three times with 2-propanol and dried to give D-aIle (19.0 g, yield (based on the complex) 89.0%) as a white crystalline powder of 99.9% de.

3.5. Separation of epimer mixture of L-Ile and D-aIle by (2*S*,3*S*)-DTTA

To a suspension of the epimer mixture of L-Ile and D-aIle (5.0 g, 38.1 mmol) in water (90 ml) was added (2*S*,3*S*)-DTTA monohydrate (7.72 g, 19.1 mmol) under stirring, and the mixture was heated at 70°C for 1 h. The slurry formed by reaction of the amino acids with the DTTA was then cooled to 25°C. The precipitated crystals were filtered, rinsed with water and dried to give a complex of D-aIle:(2*S*,3*S*)-DTTA:water in a ratio of 1:1:1–2 (9.2 g, yield (based on the epimer mixture) 44.3%). The diastereomeric purity of this complex was 94.8% de (Table 2, entry 4); mp 157–161°C; water (Karl–Fischer) found: 5.64% (dihydrate calcd 6.51%); Elemental analysis found: C, 58.9; H, 6.1; N, 2.8%. Calcd for ($C_{26}H_{31}NO_{10}\cdot H_2O$): C, 58.3; H, 6.2; N, 2.6%; ($C_{26}H_{31}NO_{10}\cdot 2H_2O$): C, 56.4; H, 6.4; N, 2.5%; IR (KBr): 3526, 2966, 2924, 1717 (s), 1609 (s), 1546 (m), 1259 (s), 1176, 1123, 1108 (s), 755 (s) cm^{-1}; ^{1}H NMR (CD_3OD): δ 0.97 (3H, t, J=4.0 Hz, D-aIle-5 CH_3), 1.00 (3H, t, J=4.0 Hz, CH_3), 1.26–1.40, 1.43–1.58 (1H×2, each m, CH_2), 2.03–2.13 (1H, m, D-aIle-3 CH), 2.42 (6H, s, toluoyl CH_3), 3.70 (1H, d, J=3.5 Hz, D-aIle-2 CH), 4.89 (11H, s, CD_3OH), 5.91 (2H, s, DTTA CH×2), 7.31 (4H, d, toluoyl 3,5×2), 8.03 (4H, d, toluoyl 2,6×2).

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