On a Convenient Resolution Method for the Preparation of Isoleucine Optical Isomers

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Derivatives of the amino acids D-Ile and D-aIle for use in the synthesis of oxytocin isomers were prepared from epimeric mixtures of the derivatives by resolution with α-phenylethylamine (PEA). Epimerization of L-aIle with excess isobutyric anhydride and 4 *N* NaOH, followed by heating to 80°, led in a few minutes to an epimeric mixture of Ibu-D-Ile and Ibu-L-aIle, from which acid hydrolysis yielded the amino acids. The preparation of the (*S*)- and (*R*)-PEA salts of Z-L-Ile and Z-L-aIle made possible the knowledge of the melting points and crystallizing properties of all eight possible diastereoisomeric salts. With this knowledge, treatment of the Z derivative of the D-Ile and L-aIle epimeric mixture with (*S*)-PEA yielded (as predicted) the higher melting of the two possible diastereoisomeric salts, Z-D-Ile (*S*)-PEA. Similarly, the four diastereoisomeric salts of (*S*)- and (*R*)-PEA with Boc-L-Ile and Boc-D-aIle were made and, from these, the melting points of their four enantiomers were predicted. By the methodology used for L-aIle, L-Ile was converted to the epimeric mixture of D-aIle and L-Ile, and the latter derivatized to Boc-amino acids. Addition of (*S*)-PEA to the latter mixture gave (as predicted) the higher melting Boc-D-aIle (*S*)-PEA salt. The resolved salts were converted to the free amino acids D-Ile and D-aIle, each of which was subjected to amino acid analysis and found to be devoid of the epimer (L-aIle and L-Ile, respectively) under conditions where 0.1–0.3% of the latter could have been detected. Therefore, the routes described allow the rapid and direct preparation of useful synthetic derivatives of the unnatural amino acids D-Ile and D-aIle from which the free amino acids can also be prepared.

The need for D-isoleucine (D-Ile) and D-alloisoleucine (D-aIle) for the synthesis of D analogs of oxytocin,[1,2] as well as the erratic commercial supply of these unnatural amino acids, led us to develop practical and convenient resolution methods for certain useful derivatives of these diastereoisomers.

L-Ile has two assymetric centers so that inversion of its α carbon leads to an epimeric mixture of L-Ile and the diastereoisomer D-aIle. Therefore, epimerization of suitable derivatives of the more readily available L-Ile and L-aIle and resolution of the epimeric mixtures constitute convenient synthetic routes for the preparation of D-aIle and D-Ile, respectively. In earlier studies, the enzymatic resolution of *N*-isobutyryl (Ibu)-L-Ile (or Ibu-L-aIle) in an epimeric mixture was accomplished by formation of the anilide in the presence of papain.[3] However, this procedure was rather slow and tedious, and like most enzymatic procedures it leads first to L isomers and only after subsequent steps to D isomers. Alternative methods of resolution have been described but either involve lengthy enzymatic methods, or require special chromatographic equipment.[4]

In an extension of our earlier studies, we found that Ibu-L-Ile is epimerized cleanly and in excellent yield by treatment of its sodium salt in aqueous solution at 35–40° with excess of acetic anhydride.[5] Alternatively, treatment of the sodium salt of L-Ile with excess of isobutyric anhydride and warming leads directly to the epimeric mixture of Ibu-L-Ile and Ibu-D-aIle. In either case the rapid epimerization of the α carbon proceeds very likely through an azlactone intermediate.[5]

We decided to attempt the direct resolution of derivatives of D-Ile and of D-aIle by the general method of resolution by diastereoisomer salt formation.[6] The resolving agent selected, (*S*)- or (*R*)-α-phenylethylamine (PEA), was added to Ibu-L-Ile, to Ibu-L-aIle, and to the epimeric mixture of Ibu-L-Ile and Ibu-D-aIle in order to form the diastereoisomeric salts. However, the rates of crystallization of all salts were slow, and the derivatives obtained were low melting and poorly defined, and such attempt to resolve isobutyryl derivatives was discontinued. On the other hand, *N*-benzyloxycarbonyl Z-L-Ile as well as Z-L-aIle readily gave well-defined salts with both (*S*)- and (*R*)-PEA, so that the melting points and optical rotations for all possible diastereoisomeric salts could be either determined or predicted (Table I). In considering a model epimeric mixture of Z-L-Ile and Z-D-aIle, we predicted as well (and later verified) that (a) the addition of (*S*)-PEA, leading to diastereoisomeric salts of similar melting points, would not resolve isomers readily on account of the probably similar crystallization rates of their salts with this amine; (b) the addition of (*R*)-PEA would cause the relatively more rapid and selective crystallization of the higher melting diastereoisomer Z-L-Ile (*R*)-PEA salt, leaving the lower melting Z-D-aIle (*R*)-PEA in the mother liquor. It was also predicted that addition of (*S*)-PEA to an epimeric mixture of Z-D-Ile and Z-L-aIle would selectively yield the Z-D-Ile (*S*)-

Table I
α-Phenylethylamine (PEA) Salts of Z-Isoleucines

Z-Amino acid	(*R*)-PEA Mp, °C	(*R*)-PEA $[\alpha]^{25}D$, deg (*c* 2, EtOH)	(*S*)-PEA Mp, °C	(*S*)-PEA $[\alpha]^{25}D$, deg (*c* 2, EtOH)	Registry no. (*R*)-PEA	Registry no. (*S*)-PEA
Z-L-Ile	124–125	+10.4	134–136	+0.98	55723-44-9	55723-50-7
Z-D-aIle	108–109.5	−3.42[a]	140.5–141.5	−8.7[a]	55723-46-1	55723-51-8
Z-D-Ile	134–136	−0.98[a]	124–125	−10.4[a]	55723-47-2	55723-52-9
Z-L-aIle	140.5–141.5	+8.7	108–109.5	+3.42	55723-49-4	55723-53-0

[a] Predicted melting point and optical rotation.

Table II
α-Phenylethylamine (PEA) Salts of Boc-Isoleucines

	(R)-PEA		(S)-PEA		Registry no.	
Boc-Amino acid	Mp, °C	$[\alpha]^{25}D$, deg (c 2, EtOH)	Mp, °C	$[\alpha]^{25}D$, deg (c 2, EtOH)	(R)-PEA	(S)-PEA
Boc-L-Ile	144–145	+10.3	128–129	+4.3	55723-54-1	55723-57-4
Boc-D-aIle	142–143	−3.0	145–146	−14.2	55280-21-8	55780-91-1
Boc-D-Ile	128–129	−4.3[a]	144–145	−10.3[a]	55723-55-2	55723-58-5
Boc-L-aIle	145–146	+14.2[a]	142–143	+3.0[a]	55723-56-3	55723-59-6

[a] Predicted melting point and optical rotation.

PEA salt. Therefore, the model epimeric mixture of Ibu-L-Ile and Ibu-D-aIle was hydrolyzed with 6 *N* HCl and the epimeric amino acid mixture was isolated and subsequently acylated with the more convenient Z group. When (*R*)-PEA was added to an epimeric mixture of Z-L-Ile and Z-D-aIle, Z-L-Ile (*R*)-PEA crystallized selectively and was found to be comparable to the sample prepared directly from pure Z-L-Ile and (*R*)-PEA. As predicted, (*S*)-PEA failed to yield selective crystallization of either isomer. Consequently, L-aIle was epimerized as an Ibu derivative and the epimeric amino acids were isolated and converted to the Z derivative. When (*S*)-PEA was added to the epimeric mixture of Z-D-Ile and Z-L-aIle, the Z-D-Ile (*S*)-PEA salt crystallized selectively.

In an attempt to develop a direct resolution route for D-aIle we also studied the salts of Boc-L-Ile and Boc-D-aIle with (*S*)- and (*R*)-PEA (Table II). As predictable from inspections of Table II, addition of (*S*)-PEA to the epimeric mixture of Boc-L-Ile and Boc-D-aIle caused the selective crystallization of the higher melting Boc-D-aIle (*S*)-PEA salt, from which Boc-D-aIle was readily prepared for use in solid phase peptide syntheses.[7] The Z and Boc groups of resolved isomers were removed by hydrogenolysis and by treatment with 25% TFA–CH_2Cl_2, respectively. The free amino acids were analyzed in a Durrum automatic analyzer capable of resolving isoleucines from alloisoleucines. Both D-Ile and D-aIle emerged as sharp single peaks, devoid of visible amounts of wrong epimers (L-aIle and L-Ile, respectively), under conditions where not less than 0.3% and perhaps as much as 0.1% of contaminating epimers should have been detectable.

The methods here described allow a convenient epimerization of any optical isomer of isoleucine and the rapid resolution of the resulting epimeric mixture into a derivative of either of the two resulting diastereoisomers. The procedures developed are adaptable to the preparation of large quantities of isomers. In contrast to enzymatic resolutions our methods are particularly useful for the direct resolution of derivatives of D-Ile and D-aIle which are suitable for synthetic work or for the preparation of the free amino acids.

Experimental Section

All melting points were determined in a Thomas-Hoover melting point apparatus and are corrected. Optical rotations were measured in 1-dm tubes with a Rudolph polarimeter with a precision of ±0.01°. The (*S*)- and (*R*)-α-phenylethylamine employed were respectively *l*-(−)-α-methylbenzylamine, $[\alpha]^{20}D$ −39° (neat), and *d*-(+)-α-methylbenzylamine, $[\alpha]^{20}D$ +39° (neat), supplied by Aldrich. Amino acid analyses were determined in a Durrum automatic amino acid analyzer. The following abbreviations were used: isoleucine, Ile; alloisoleucine, aIle; DCC, dicyclohexylcarbodiimide; Ibu, isobutyryl; PEA, α-phenylethylamine; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; and DCHA, dicyclohexylamine.

Ibu-L-aIle. A solution of L-aIle (40 g, 0.30 mol) in 4 *N* NaOH (80 ml) was cooled (−10°) and isobutyryl chloride (48.5 g, 0.46 mol) and 2 *N* NaOH (80 ml) were added in several portions, maintaining the pH above 8 and the temperature of the reaction mixture below 0°. After the reaction was complete (5 min) the pH remained constant, and the reaction mixture was extracted with three 100-ml portions of $CHCl_3$. The aqueous layer was acidified with 6 *N* HCl and cooled in an ice bath. The crystalline material which precipitated was collected, washed with H_2O, and dried in vacuo over P_2O_5. Upon extraction with $CHCl_3$, the mother liquor yielded an additional crop, for a combined yield of 56.5 g (92%). A recrystallization afforded the analytical sample, mp 143–145°, $[\alpha]^{20}D$ +15.5° (*c* 4, EtOH).

Anal. Calcd for $C_{10}H_{19}NO_3$: N, 6.96. Found: N, 6.74.

Epimerization of Ibu-L-aIle. Ibu-L-aIle (56 g, 0.28 mol) was dissolved in 2 *N* NaOH (280 ml). To this solution was added H_2O (280 ml) and acetic anhydride (262 ml, 2.8 mol), and the mixture was incubated in an oven for 30 min at 50° when a clear solution resulted. The reaction mixture was cooled in ice and the crystalline mass which formed was filtered and washed three times with H_2O. The filtrate was extracted with four 50-ml portions of $CHCl_3$ and the combined extracts were washed once with H_2O, dried ($MgSO_4$), filtered, and evaporated to a residue which was combined with the crystalline product inasmuch as both crops had mp 174–176°, $[\alpha]^{20}D$ +2.9° (*c* 4, EtOH).

Ibu-L-Ile. This compound was obtained in 85% yield by the method described for Ibu-L-aIle, mp 152–153°, $[\alpha]^{19}D$ +9.62° (*c* 4, EtOH).

Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.7; H, 9.52; N, 6.96. Found: C, 59.6; H, 9.49; N, 6.85.

Epimerization of Ibu-L-Ile. Method A. Ibu-L-Ile (4 g, 0.02 mol) was dissolved in 2 *N* NaOH (20 ml). To this solution was added H_2O (20 ml) and acetic anhydride (20.4 g, 0.2 mol). After 10 min the mixture became hot (62°), and 5 min later white plates began to appear. After a few minutes the reaction mixture was cooled in ice, and the product was collected, washed with H_2O, and dried in vacuo over P_2O_5, yielding 3.4 g of product, mp 175–177°; $[\alpha]^{20}D$ −2.96° (*c* 4.5, EtOH). From the mother liquor, extraction with $CHCl_3$ yielded 0.35 g of additional material (total yield 94%), lit.[3] mp 175–176°.

Method B. L-Ile (10.5 g, 0.08 mol) dissolved in 4 *N* NaOH (80 ml) was treated with isobutyric anhydride (84 ml, 0.5 mol) with stirring. After 1 min the temperature climbed to 52° and then began to decrease. At this point 4 *N* NaOH (25 ml) was added and the temperature was raised to 80° for 10 min, when the two phases of the reaction mixture cleared up. The solution was cooled in ice and acidified to pH 2 with 20% HCl and the crystals formed were collected, washed with H_2O, and dried over P_2O_5 in vacuo, yielding 13.2 g (82%), mp 173–175°; $[\alpha]^{24}D$ −2.6° (*c* 4, EtOH).

Hydrolysis of the Epimeric Mixture of Ibu-L-aIle and Ibu-D-Ile. The above epimeric mixture of acyl amino acids (25 g, 0.225 mol) was added to 20% HCl (250 ml) and the suspension was refluxed for 3 hr. The resulting solution was evaporated in vacuo to a solid residue to which H_2O (50 ml) was added and evaporation under vacuum was repeated, the latter process being repeated several times. The residue was dissolved in H_2O (100 ml) and the pH was adjusted to 6 with concentrated ammonium hydroxide. Crystallization took place at this stage, and it was completed by the addition of EtOH (800 ml). The amino acid epimerizate was allowed to crystallize overnight in a cold room (0°). The crystals obtained were filtered and washed with H_2O, H_2O–EtOH, EtOH, and Et_2O. Drying under vacuum over P_2O_5 and KOH pellets afforded 15.4 g (94.5%), $[\alpha]^{18}D$ 0° (*c* 1, 5 *N* HCl). This product was homogeneous on silica gel TLC with *n*-BuOH–H_2O–AcOH (4:1:5) and *n*-BuOH–EtOAc–AcOH–H_2O (1:1:1:1), with Cl_2–tolidine and ninhydrin color sprays, and was indistinguishable from Ile.

Z-L-Ile. This derivative was prepared from L-Ile by the proce-

dure of Bergman[8] for the preparation of Z-amino acids. In one occasion a preparation kept in a freezer for several weeks gave prisms, mp 51–54°, $[\alpha]^{24}D$ +13° (c 2, EtOH).

Z-L-aIle. This derivative was obtained as an oil, $[\alpha]^{18}D$ +15.2° (c 2, acetone) [lit.[9] $[\alpha]^{20}D$ +16.0° (c 2, acetone)].

Salts of PEA and Z-Ile Diastereoisomers. A solution of the corresponding Z-Ile or Z-L-aIle (0.5 g, 1.9 mmol) in EtOAc (2 ml) was treated with (S)- or (R)-PEA (0.23 g, 1.9 mmol), and the solution was allowed to stand overnight. The crystals collected were washed with EtOAc–pentane and pentane and finally dried. The melting points and optical rotations of all possible diastereoisomeric salts are shown in Table I.

Resolution of an Epimeric Mixture of Z-L-Ile and Z-D-aIle. The epimeric mixture of Ibu-L-Ile and Ibu-D-aIle prepared as described earlier was hydrolyzed with 20% HCl and the freed amino acid was converted to the Z derivative (oil) by the general method of Bergman. A solution of the resulting Z-L-Ile and Z-D-aIle mixture (1.15 g, 4.3 mmol) in EtOAc (1.6 ml) was treated with (R)-PEA (0.55 ml, 4.3 mmol). Crystals began to form slowly in clusters of needles, yielding 0.50 g (60%), mp 119–121°; $[\alpha]^{19}D$ +8.42° (c 2, EtOH). Three recrystallizations of the latter material from EtOAc gave Z-L-Ile (R)-PEA, mp 124–125°, $[\alpha]^{19}D$ +10.9° (c 2, EtOH).

Anal. Calcd for $C_{22}H_{30}N_2O_4$: N, 7.25. Found: N, 7.10.

Resolution of an Epimeric Mixture of Z-L-aIle and Z-D-Ile. The epimeric mixture of amino acids was converted to the Z derivatives by the general method of Bergman. The resulting oil (43.2 g, 0.163 mol) was dissolved in EtOAc (65 ml) and (S)-PEA (22.6 ml, 0.175 ml) was added all at once. The crystals which formed overnight were collected and washed with EtOAc, yielding 19.5 g (62%), mp 122–123°. Three successive recrystallization yielded Z-D-Ile (S)-PEA salt, 14.0 g (44%), mp 124–125°; $[\alpha]^{17}D$ −10.9° (c 2, EtOH).

A sample of the latter (79 mg, 0.20 mmol) was dissolved in 80% EtOH (10 ml) and the solution was treated with BioRex 70 (H^+). The resin was filtered and to the filtrate (20 ml) was added AcOH (0.1 ml) and 5% Pd/C (100 mg), and H_2 was bubbled gently for 2 hr at room temperature. The catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. After drying overnight over P_2O_5 in vacuo the crystals were suspended in Et_2O, filtered, and dried, yielding 26 mg of the amino acid, $[\alpha]^{24}D$ −41.7° (c 1, 6 N HCl) [lit.[4] $[\alpha]^{19}D$ −41.6° (c 1, 6 N HCl)]. An amino acid analysis of this product, obtained in a Durrum automatic analyzer, showed one sharp single peak with the retention time corresponding to Ile. Although no aIle was detected, the presence of 0.3% to as low as perhaps 0.1% cannot be ruled out.

Epimeric Mixture of Boc-D-aIle and Boc-L-Ile DCHA Salts. The epimeric amino acid mixture of D-aIle and L-Ile (5.9 g, 45 mmol) was dissolved in a mixture of 2 N NaOH (45 ml) and dioxane (45 ml), and Boc-azide (10.0 g, 70 mmol) was added with stirring at room temperature while the pH of the mixture was adjusted to 10 by the occasional addition of 2 N NaOH. Excess Boc-azide was extracted with Et_2O, and the aqueous layer was carefully acidified to pH 2 wihh 20% HCl in an ice bath and extracted with EtOAc (100 ml). The organic layer was extracted and washed with H_2O, dried (Na_2SO_4), and evaporated to dryness. The residual oil was dissolved in Et_2O and treated with DCHA (9 ml). The DCHA salt was collected, yielding 14.9 g (80%), mp 133–134°; $[\alpha]^{24}D$ 0° (c 1.5, DMF).

Boc-D-aIle DCHA Salt. Boc-D-aIle was prepared from D-aIle essentially by the methods described in the preceding experiment. Because Boc-D-aIle did not crystallize readily, it was isolated as the DCHA salt in 94% yield, mp 136–137°. Recrystallization from EtOAc–hexane gave the analytical sample, mp 138–139°, $[\alpha]^{24}D$ −11.3° (c 1.5, DMF).

Anal. Calcd for $C_{23}H_{44}N_2O_4$: C, 67.0; H, 10.8; N, 6.79. Found: C, 67.1; H, 10.8; N, 6.68.

Salts of PEA with Boc-Ile Diastereoisomers. These salts were prepared essentially as described for Z-Ile diastereoisomers and their melting points and rotations are shown in Table II. In the case of the combination of Boc-L-Ile and (S)-PEA, hexane had to be added to the EtOAc solution in order to force crystallization of the salt.

Resolution of Boc-D-aIle and Boc-L-Ile. The epimeric mixture of Boc-D-aIle and Boc-L-Ile DCHA salts (8.25 g, 20 mmol) was added to EtOAc (40 ml) and 1 N H_2SO_4 (40 ml), and the mixture was shaken in a separatory funnel until the salts dissolved. The EtOAc extract was washed with H_2O, dried (Na_2SO_4), and evaporated to an oil. The latter was dissolved in EtOAc (10 ml), (S)-PEA (2.58 ml, 20 mmol) was added, and the solution was kept at room temperature for 20 hr and then at 4° for 6 hr. The crystals which formed were collected, washed with 1:1 EtOAc–hexane (15 ml) and then hexane and finally air dried, yielding 2.83 g (80%), mp 142–143.5°. Two recrystallizations from EtOAc (15 ml) gave 2.4 g (70%), mp 145–146°, $[\alpha]^{24}D$ −14.5° (c 2, EtOH). Anal. Calcd for $C_{19}H_{32}N_2O_4$: C, 64.7; H, 9.15; N, 7.95. Found: C, 64.4; H, 9.27; N, 7.88.

A sample of Boc-D-aIle (S)-PEA (0.35 g, 1 mmol) was dissolved in CH_2Cl_2 and the solution was extracted with 0.1 N H_2SO_4 and then H_2O and finally dried (Na_2SO_4). To the CH_2Cl_2 solution (about 10 ml) was added trifluoroacetic acid (3 ml) and the solution was allowed to stand at room temperature for 30 min, when the solvents were removed in a rotatory evaporator. The residue obtained was extracted with Et_2O and dissolved in H_2O and the solution was treated with Rexyn AG3-X4 (AcO^-) and filtered. The filtrate was lyophilized and the powder obtained was triturated with EtOH and Et_2O and dried, yielding 80 mg of the amino acid, $[\alpha]^{24}D$ −38.9° (c 1, 6 N HCl) [lit.[4] $[\alpha]^{19}D$ −38° (c 1, 6 N HCl)]. A sample of the product was subjected to an amino acid analysis in a Durrum automatic analyzer, which revealed only one sharp single peak with the retention time corresponding to D-aIle. A contamination with Ile of 0.3% and perhaps as low as 0.1% would have been detectable.

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Registry No.—L-Alloisoleucine, 1509-34-8; N-isobutyl-L-alloisoleucine, 55723-60-9; N-isobutyryl-D-isoleucine, 55723-61-0; N-isobutyryl-L-isoleucine, 55723-62-1; N-isobutyryl-D-alloisoleucine, 55723-63-2; L-alloisoleucine, 73-32-5; DL-isoleucine, 443-79-8; N-benzyloxycarbonyl-L-isoleucine, 3160-59-6; N-benzloxycarbonyl-L-alloisoleucine, 55723-48-3; D-isoleucine, 319-78-8; D-alloisoleucine, 1509-35-9; *tert*-butyloxycarbonyl-L-isoleucine DCHA, 55723-64-3; *tert*-butyloxycarbonyl-D-alloisoleucine DCHA, 55780-92-2.

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