Optical Resolution of DL-Valine, DL-Leucine, and DL-Isoleucine by Formation of Adduct with L-Phenylalanine

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An alkaline solution containing L-phenylalanine (L-Phe) and one of DL-valine, DL-leucine, and DL-isoleucine is allowed to selectively form a precipitate composed of L-Phe and the aliphatic D-amino acid by adjusting the pH of initial solution to around 5.5 with hydrochloric acid. ^{1}H NMR spectra in deuterium oxide, elemental analyses, and infrared spectra of the precipitates indicate that they are adducts of the aliphatic D-amino acid and L-Phe in the molar ratio of 1:1. The free aliphatic D-amino acids with high optical purity (84—100%) may be recovered from the adducts in 49—63% yield. An attempt was made to obtain both the aliphatic D- and L-amino acids by using an aqueous solution containig the aliphatic DL-amino acid and L-Phe as an initial solution. The aliphatic L-amino acids obtained had optical purity of 47—85%.

A number of studies have been reported on optical resolution of DL-amino acids, and most of them have employed the preferential crystallization procedure.[1-8] Although the advantages of this procedure have been well recognized,[9,10] it should be noted that a racemate crystallizes as a conglomerate from a racemic solution and not as a racemic compound. Most of free DL-amino acids form racemic compounds and have no properties suitable for this procedure. Therefore, many of optical resolutions by this procedure have been carried out in the form of salts[3,6] or their derivatives such as *N*-acyl compounds.[1,2,4,5,7,8] Free optically active amino acids are recovered by hydrolysis of resolved derivatives, and the process may result in lowering of yield and partial racemization.

The diastereomeric procedure has also been employed for the optical resolution of DL-amino acids,[11-15] which does not require the racemate to be a conglomerate. However, since free DL-amino acids scarcely form salts with ordinary optically active organic acids or bases, their derivatives, such as *N*-acyl compounds and esters,[11-15] have been resolved by this procedure. Therefore, this procedure may have a disadvantage similar to that of the preferential crystallization procedure. In order to overcome this disadvantage, the free D- or L-amino acid should selectively form a salt or adduct with an optically active compound, and the free optically active amino acid should be easy to be recovered from the resolved salt or adduct.

Since it is possible to regard a DL-amino acids as an adduct composed of a pair of enantiomers, one enantiomer may form an adduct with an optically active amino acid. This paper describes an attempt to resolve aliphatic DL-amino acids, DL-valine (abbreviated as DL-Val), DL-leucine (DL-Leu), and DL-isoleucine (DL-Ile), *via* formation of adduct with L-phenylalanine (L-Phe). An alkaline solution containing the aliphatic DL-amino acid and L-Phe selectively gave a crystalline adduct of the aliphatic D-amino acid with L-Phe when the pH of the initial solution was adjusted to around 5.5 with hydrochloric acid. The free aliphatic D-amino acids were easy to be recovered from the adducts since active carbon is capable of adsorbing L-Phe and scarcely Val, Leu, or Ile.[16] The optical resolution of DL-Val, DL-Leu, and DL-Ile has been achieved with these ideas, and D-Val, D-Leu, and D-Ile with high optical purity (84—100%) were obtained in 49—63% yield. It was attemted to obtain the aliphatic D- and L-amino acids by using an aqueous solution as an initial solution, and the L-amino acids with optical purity of 47—85% were also obtained.

Experimental

Amino Acids. DL-Val and DL-Ile were purchased from Nakarai Chemicals, Ltd., and DL-Leu and L-Phe from Wako Pure Chemicals Ind. These amino acids were recrystallized from water. The specific rotation of the recrystallized L-Phe was $[\alpha]_D^{20}$ −4.5° (*c* 1.0, 5 mol dm^{-3} HCl) ($[\alpha]_D^{20}$ −4.5° (*c* 1.0, 5 mol dm^{-3} HCl)).[17]

Optical Resolution of DL-Valine. Procedure I: Equimolar amounts (0.01 mol) of L-Phe and DL-Val were dissolved in 20 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide and 10 cm^3 of water. The pH was adjusted to around 5.5 with 1 mol dm^{-3} hydrochloric acid. After adding 80 cm^3 ethanol, the solution was stirred for 90 min at room temperature. The precipitate (I-**1**) formed was collected by filtration. The filtrate was concentrated to 20 cm^3 under reduced pressure at around 40 °C, and the precipitate (I-**2**) deposited on cooling the solution at 0 °C for 45 min was filtered off.

Procedure II: A solution containing equimolar amounts (0.01 mol) of L-Phe and DL-Val in 150 cm^3 of water was concentrated to 40 cm^3 under reduced pressure at around 40 °C. The precipitate (II-**1**) formed was collected by filtration. After adding 20 cm^3 of ethanol to the filtrate, the solution was allowed to stand overnight at 0 °C. The precipitate (II-**2**) formed was collected by filtration. To the filtrate was added 160 cm^3 of ethanol and the solution was concentrated to 30 cm^3. The precipitate (II-**3**) deposited after cooling overnight at 0 °C was collected by filtration. The filtrate was evaporated to dryness under reduced pressure at around 40 °C, and the residue (II-**4**) was obtained.

Optical Resolution of DL-Leucine. Procedure I: Equimolar amounts (0.01 mol) of L-Phe and DL-Leu were dissolved in 20 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide and 40 cm^3 of water. The pH was adjusted to around 5.5 with 1 mol dm^{-3} hydrochloric acid. The solution was concentrated to 30 cm^3, and the precipitate (I-**1**) formed was collected by filtration. After adding 20 cm^3 of ethanol to the filtrate, the solution was concentrated to 20 cm^3. The precipitate (I-**2**) formed was filtered off.

Procedure II: Equimolar amounts (0.01 mol) of L-Phe and DL-Leu were added to 200 cm^3 of water and dissolved at around 60 °C. The solution was concentrated to 100 cm^3, and the precipitate (II-**1**) deposited after cooling overnight at 0 °C was collected by filtration. The filtrate was concentrated to 30 cm^3, and the precipitate (II-**2**) formed was collected by filtration. After adding 20 cm^3 of ethanol to the filtrate, the

solution was allowed to stand overnight at 0 °C, and the precipitate (II-3) formed was collected by filtration. The filtrate was evaporated to dryness under reduced pressure, and the residue (II-4) was obtained.

Optical Resolution of DL-*Isoleucine.* *Procedure I*: Equimolar amounts (0.01 mol) of L-Phe and DL-Ile were dissolved in 20 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide and 30 cm^3 of water. The pH was adjusted to around 5.5 with 1 mol dm^{-3} hydrochloric acid. The solution was concentrated to 20 cm^3, and the preciptitate (I-1) formed was collected by filtration. After adding 150 cm^3 of ethanol to the filtrate, the solution was stirred for 60 min at room temperature, and the precipitate (I-2) formed was filtered off.

Procedure II: A solution containing equimolar amounts (0.01 mol) of L-Phe and DL-Ile in 200 cm^3 of water was concentrated to 50 cm^3. The solution was allowed to stand overnight at 0 °C, and the precipitate (II-1) formed was collected by filtration. After adding 100 cm^3 of ethanol to the filtrate, the solution was stirred for 60 min at room temperature, and the precipitate (II-2) formed was collected by filtration. The filtrate was concentrated to 25 cm^3 and the solution was allowed to stand overnight at 0 °C. The precipitate (II-3) formed was collected by filtration. The filtrate was evaporated to dryness under reduced pressure, and the residue (II-4) was obtained.

Preparation of Free Aliphatic Amino Acids. The precipitates obtained in the above procedures were dissolved in a minimum amount of water. To the aqueous solution was added active carbon, and the mixture was shaken for 3 h at room temperature. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure at around 40 °C. The ^1H NMR spectrum of the residue was measured without recrystallization, and it was confirmed that the residue was the free aliphatic amino acid and was free from L-Phe. The optical purities of the free aliphatic amino acids obtained were determined on the basis of the specific rotations of the corresponding L-amino acids.[17]

Preparation of Adducts. L-Phe (0.005 mol) and 0.01 mol of DL-Val, DL-Leu or DL-Ile were dissolved in 15 cm^3 of 1 mol dm^{-3} aqueous sodium hydroxide. Only to the alkaline solution containing L-Phe and DL-Leu was added 30 cm^3 of water. The pH of the alkaline solutions was adjusted to around 5.5 with 1 mol dm^{-3} hydrochloric acid at 0, 25, or 50 °C. From the solution containing L-Phe and DL-Leu, a precipitate appeared after a stirring for several minutes. From the solution containing L-Phe and DL-Val or DL-Ile, a precipitate was deposited by adding an appropriate amount of ethanol. After stirring for 5—120 min at the respective temperatures, the precipitates were filtered off and the ^1H NMR spectra were measured without recrystallization. The precipitates were treated with active carbon in a similar manner as above.

The adduct composed of L-Phe and D-Val with optical purity of 100%. Found: C, 59.34; H, 7.93; N, 9.95. Calcd for $C_{14}H_{22}N_2O_4$: C, 59.56; H, 7.85; N, 9.92. $[\alpha]_D^{20}$ −14.6 ° (*c* 0.50, 5 mol dm^{-3} HCl).

The adduct composed of L-Phe and D-Leu with optical purity of 100%. Found: C, 60.50; H, 8.19; N, 9.45. Calcd for $C_{15}H_{24}N_2O_4$: C, 60.79; H, 8.16; N, 9.45. $[\alpha]_D^{20}$ −9.5 ° (*c* 0.50, 5 mol dm^{-3} HCl).

The adduct composed of L-Phe and D-Ile with optical purity of 96.8%. Found: C, 60.49; H, 8.23; N, 9.44. Calcd for $C_{15}H_{24}N_2O_4$: C, 60.79; H, 8.16; N, 9.45. $[\alpha]_D^{20}$ −19.8 ° (*c* 0.50, 5 mol dm^{-3} HCl).

Measurements. Specific rotations were measured with a Union Giken high sensitivity PM-101 digital polarimeter in 5 mol dm^{-3} hydrochloric acid by using a 0.5 dm path length quartz cell. ^1H NMR spectra were recorded on a JEOL JNM-PMX 60 NMR spectrometer in deuterium oxide. Infrared spectra were obtained in the range 4000—400 cm^{-1} with a JASCO A-102 infrared spectrophotometer by the KBr disk method.

Results and Discussion

Formation of Adducts. The adducts of Val, Leu, and Ile with L-Phe were prepared under several conditions as given in Table 1. The adducts were determined by elemental analyses and ^1H NMR spectra in deuterium oxide and found to be composed of equimolar amounts of L-Phe and the aliphatic amino acid. Aqueous solutions of each adduct were treated with active carbon to remove L-Phe by adsorption.[16] The recovered free aliphatic amino acids were of D-configuration, and those obtained under the prescribed conditions had optical purity of about 100%. The results indicate that the alkaline solution containing the aliphatic DL-amino acid and L-Phe selectively give

Table 1. Results of Preparation of Adducts with L-Phenylalanine

Aliphatic DL-amino acids[a]	Conditions			Yield of adduct	Free aliphatic amino acids		
	Temperature	Stirring time	Amount of added ethanol		Yield	Specific rotation[b]	Optical purity
	°C	min	cm^3	g	g(%[c])	°	%
DL-Val	0	5	10	0.385	0.154(26.3)	−28.8	100
	0	30	0	0.124	0.017(2.9)	−17.4	60.4
	0	30	50	0.951	0.367(62.6)	−21.6	75.1
	25	30	50	0.519	0.207(35.3)	−28.8	100
	50	120	150	0.352	0.139(23.7)	−28.8	100
DL-Leu	0	5	0	0.363	0.157(23.9)	−16.0	100
	0	30	0	0.779	0.324(49.4)	−9.6	60.2
	25	30	0	0.552	0.240(36.6)	−6.0	37.3
	50	120	0	0.332	0.142(21.6)	−4.8	30.1
DL-Ile	0	30	10	0.909	0.331(50.5)	−32.9	81.2
	25	5	30	0.351	0.125(19.1)	−39.2	96.8
	25	10	30	0.370	0.160(24.4)	−35.8	88.4
	25	30	30	0.524	0.222(33.8)	−35.7	88.1
	50	30	250	0.312	0.115(17.5)	−24.3	60.1

a) The amount of aliphatic DL-amino acids consumed by reaction was 0.01 mol and that of L-Phe was 0.005 mol. b) $[\alpha]_D^{20}$ (*c* 1.00, 5 mol dm^{-3} HCl). c) Yield(%)=(Yield(g)×100)/(1/2) (Reacted amount of DL-amino acid).

a crystalline adduct of the aliphatic D-amino acid with L-Phe.

Infrared Spectra. Infrared spectra of the adducts of the aliphatic D-amino acids with L-Phe were measured and were compared with those of the free DL- and L-amino acids. The spectral patterns are similar to those of the free DL-amino acids, different from those of the free L-amino acids. The bands due to groups COO^- and NH_3^+ were assigned as such since it is considered that the groups are most strongly influenced by formation of the adducts. The band at about 2950 cm^{-1} was assigned to the NH streching, the band at about 1625 cm^{-1} (shoulder) to the antisymmetric NH deformation, and the band at about 1500 cm^{-1} to the symmetric NH deformation. The antisymmetric CO band (ν_{CO}(as)) appears at about 1590 cm^{-1}, and the symmetric band at 1410 cm^{-1}. A most significant difference in the spectra is noticed between the ν_{CO}(as)'s of the adducts and L-Phe. The ν_{CO}(as) of L-Phe appears at 1558 cm^{-1}, whereas the spectra of the adducts have no bands in the range 1570—1530 cm^{-1}. The ν_{CO}(as)'s of the adducts are observed at wave number positions similarly to that of DL-Phe (1585 cm^{-1}). These results suggest that the interaction between the aliphatic D-amino acid and L-Phe in the adduct is analogous to that between a pair of the enantiomers in the DL-amino acid, and that these adducts are not mere mixtues of the aliphatic D-amino acid and L-Phe.

Optical Resolutions. The optical resolution of DL-Val, DL-Leu, and DL-Ile was achieved at room temperature by using two kinds of procedures (I and II). The results are given in Tables 2, 3, and 4. The precipitate numbers in these tables have been defined in the experimental section.

In procedure I, an alkaline solution containing equimolar amounts of L-Phe and the aliphatic DL-amino acid was used as an initial solution. The ^1H NMR spectra of precipitates I-1 and I-2 indicated that these precipitates are composed of equimolar amounts of L-Phe and the aliphatic amino acid. The Val, Leu, and Ile from the precipitates are of D-configuration, and the aliphatic D-amino acids with optical purity of 84—100% were obtained in 49—63% yield. After these precipitates were filtered off, sodium chloride was

Table 2. Optical resolutions of DL-valine

Procedure	Precipitate No.	Precipitate Yield / g	Free valine Yield / g(%[a])	Free valine Configuration	Free valine Specific rotation[b] / °	Free valine Optical purity / %
I	I-1	0.871	0.289(49.4)	D	−28.8	100
	I-2	0.689	0.125(21.3)	D	−5.5	19.1
II	II-2	1.029	0.300(51.2)	D	−28.3	98.3
	II-3	0.667	0.360(61.5)	L	+11.4	39.6
	II-4	0.349	0.204(34.8)	L	+24.6	85.4

a) Yield(%)=(Yield(g)×100)/0.586(g). b) $[\alpha]_D^{20}$ (*c* 1.00, 5 mol dm^{-3} HCl).

Table 3. Optical resolutions of DL-leucine

Procedure	Precipitate No.	Precipitate Yield / g	Free leucine Yield / g(%[a])	Free leucine Configuration	Free leucine Specific rotation[b] / °	Free leucine Optical purity / %
I	I-1	1.283	0.327(49.9)	D	−16.0	100
	I-2	0.375	0.065(9.9)	D	−16.0	100
II	II-1	0.378	0.149(22.7)	D	−16.0	100
	II-2	0.672	0.244(37.2)	D	−15.3	95.6
	II-3	0.199	0.045(6.9)	D	−10.7	66.9
	II-4	1.316	0.345(52.6)	L	+7.6	47.5

a) Yield(%)=(Yield(g)×100)/0.656(g). b) $[\alpha]_D^{20}$ (*c* 1.00, 5 mol dm^{-3} HCl).

Table 4. Optical resolutions of DL-isoleucine

Procedure	Precipitate No.	Precipitate Yield / g	Free isoleucine Yield / g(%[a])	Free isoleucine Configuration	Free isoleucine Specific rotation[b] / °	Free isoleucine Optical purity / %
I	I-1	0.810	0.369(56.3)	D	−34.3	84.7
	I-2	0.220	0.046(7.0)	D	−36.2	89.6
II	II-1	0.757	0.230(35.1)	D	−33.1	81.7
	II-2	0.265	0.083(12.7)	D	−37.8	93.3
	II-3	0.899	0.244(37.2)	D	−4.7	11.6
	II-4	0.673	0.276(42.1)	L	+27.6	68.1

a) Yield(%)=(Yield(g)×100)/0.656(g). b) $[\alpha]_D^{20}$ (*c* 1.00, 5 mol dm^{-3} HCl).

also deposited with the mixture of the amino acids by further concentrating the filtrate or adding ethanol. Since it was tedious to remove sodium chloride, no attempt was made to obtain the aliphatic L-amino acids.

In procedure II, an aqueous solution containing only equimolar amounts of L-Phe and the aliphatic DL-amino acid was used as an initial solution, and it was attempted to obtain both the aliphatic D- and L-amino acids.

In the optical resolution of DL-Val by this procedure, the ^{1}H NMR spectrum and specific rotation indicated that precipitate II-**1** is L-Phe ($[\alpha]_D^{20}$ −4.4° (*c* 0.50, 5 mol dm^{-3} HCl)); L-Phe is allowed to crystallize first since the solubility of DL-Val is much higher than that of L-Phe. Precipitate II-**2** is composed of equimolar amounts of L-Phe and Val. The Val from this precipitate is of D-configuration, and was recovered in 51.2% yield with optical purity of 98.3%. Precipitates II-**3** and II-**4** are composed of Val and L-Phe in the molar ratio of around 1.8:1. L-Val was recovered from precipitate II-**4**, and was obtained in 34.8% yield, with optical purity of 85.4%.

In the optical resolution of DL-Leu by procedure II, precipitates II-**1** and II-**2** are composed of equimolar amounts of L-Phe and Leu. The D-Leu from these precipitates had over 95% optical purity, and was obtained in 60% total yield. L-Leu with optical purity of 47.5% was obtained from precipitate II-**4** in 52.6% yield. Precipitate II-**4** is composed of Leu and L-Phe in the molar ratio of 0.8:1.

In the optical resolution of DL-Ile by procedure II, precipitate II-**1** deposited from the initial solution is composed of Ile and L-Phe in the molar ratio of about 0.6:1, and precipitate II-**2** in 1:1. However, the D-Ile from these precipitates had high optical purity (81.7 and 93.3%), and the total yield was 47.8%. The molar amount of Ile contained in precipitates II-**3** and II-**4** was about 1.8 times that of L-Phe. The L-Ile from precipitate II-**4** had an optical purity of 68.1% and was obtained in 42.1% yield.

The above results indicate that it is possible to resolve DL-Val, DL-Leu, and DL-Ile *via* formation of adducts with L-Phe.

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