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New Method for the Separation of Diastereomeric Mixtures of β-Methylnorleucine and β-Methylleucine

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β-Methylnorleucine and β-methylleucine were synthesized, separated into diastereomers and optically resolved. The configurational assignments of these isomers and the determinations of the optically active isomers were achieved by means of NMR spectroscopy and the enzymatic method respectively.

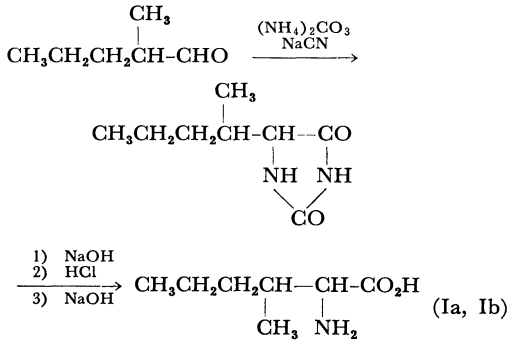
The present authors previously discovered the asymmetric hydrogenation catalyst, which is obtained by the modification of the Raney nickel catalyst with optically active α-amino or α-hydroxy acid, and found an experimental rule on the relation between the asymmetric activity of the catalyst and the structures of the modifying reagent.[1]

The syntheses, the separations in to diastereomers, and the optical resolutions of β-methylnorleucine and β-methylleucine were performed in order to study the effect of the β-asymmetric center of the α-amino acid on the asymmetric activity of the modified Raney nickel catalyst.

β-Methylnorleucine and β-methylleucine have β-asymmetric carbons in addition to α-asymmetric carbons and each has two diastereomers.

β-Methylnorleucine and β-methylleucine are not naturally occurring amino acids. For β-methylnorleucine, no descriptions of the synthesis, the diastereomers, or the optically active isomers have yet been published.

β-Methylleucine has been synthesized as the intermediate of the *N*,β-dimethylleucine, which is a component of antibiotic Ethamycin, and its four optically active isomers have been obtained by Kotake *et al.* and one of the present authors.[2]



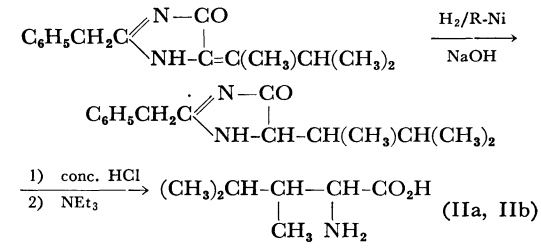
Scheme 1

In the present work, β-methylnorleucine was synthesized starting from α-methylvaleraldehyde by the general method developed by Bucherer and Steiner; it is described in Scheme 1.

The fine crystalline hydantoin derivative, obtained from the reaction of α-methylvaleraldehyde, ammonium carbonate, and sodium cyanide, was hydrolyzed with alkali in the autoclave; subsequent acidification with concentrated hydrochloric acid afforded an excellent yield of β-methylnorleucine hydrochloride.

β-Methylleucine was synthesized by a modification of the procedure developed by Kotake *et al.* The modification was based on catalytic hydrogenation with a Raney nickel catalyst of the α-methylisobutylidene derivative of the 2-benzyl-4-(5*H*)-imidazolone in a sodium hydroxide solution and successive treatment with concentrated hydrochloric acid.

By this method, β-methylleucine, which is a mixture of threo and erythro isomers in the molar ratio of 2 : 1, was directly obtained in a 87% yield (based on the α-methylisobutylidene derivative). (Scheme 2)



Scheme 2

Diastereomeric mixtures of β-methylnorleucine and β-methylleucine were crystallized from the concentrated hydrochloric acid, and the threo isomers (Ia and IIa) were obtained as the less soluble hydrochloride salts.

1) Part XII of the series: Y. Izumi, S. Tatsumi and M. Imaida, This Bulletin, **42**, 2373 (1969).

2) H. Kotake, T. Saito and K. Ōkubo, *ibid.*, **42**, 1367 (1969).

The erythro isomers (Ib and IIb) of these amino acids were purified as the less soluble copper salts in a hot 1% aqueous solution of cupric sulfate.

The extraction of the copper salt of the threo isomer with a hot 1% aqueous solution of cupric sulfate is a new, quite convenient method for the purification of the erythro isomers of these amino acids.

The relative configurational assignments of these amino acids were performed by the acylase resolution and the NMR spectroscopy. The stereochemically pure racemates, Ia, Ib, IIa, and IIb, were each *N*-acetylated and then resolved with acylase by the ordinary method to afford L-amino acids.

Recently Ōtsuka *et al.* introduced the use of NMR spectroscopy to elucidate the relative configurations at the α- aud β-carbon atoms of *N*,β-dimethylleucine diastereomers, which have $\rangle\underset{\alpha}{CH}-\underset{\beta}{CH}\langle$ structures, compared with the similar known compounds, the *N*-methylisoleucine diastereomers.[3] They proved that the threo form has a smaller coupling constant, $J_{\alpha,\beta}$.

Since the Karplud equation could be applied to β-methylnorleucine, (Ia and Ib) and β-methylleucine (IIa and IIb), although these amino acids were not *N*-methylated, we extended that theory, in the present work, to these amino acids. The observed coupling constants obtained from the doublet signals of the α-protons of the L-isoleucine, L-alloisoleucine, β-methyl-L-norleucine (Ia-L), β-methyl-L-norleucine (Ib-L), β-methyl-L-leucine (IIa-L), and β-methyl-L-leucine (IIb-L) are shown in Table 1.

Table 1. $J_{\alpha,\beta}$ Values obtained from the doublet signal of the α-proton

	$J_{\alpha,\beta}$ (Hz)
L-Alloisoleucine (*threo*)	3.6±0.1
L-Isoleucine (*erythro*)	4.1±0.1
β-Methyl-L-norleucine (Ia-L)	3.5±0.1
β-Methyl-L-norleucine (Ib-L)	4.0±0.1
β-Methyl-L-leucine (IIa-L)	3.5±0.1
β-Methyl-L-leucine (IIb-L)	5.2±0.1

As could be expected, L-alloisoleucine (*threo*) has a smaller coupling constant, $J_{\alpha,\beta}$, than L-isoleucine (*erythro*). For β-methylnorleucine and β-methylleucine, Ia and IIa have smaller coupling constants than Ib andIIb. From these results, Ia and IIa are said to be of the threo form, and Ib and IIb of the erythro form.

Additionally, this result coincides with the experimental rule that, in the asymmetric hydrogenation of methyl acetoacetate to methyl 3-hyoroxybutyrate, a Raney nickel catalyst modified with the optically active threo form has a stronger asymmetric activity than the optically active erythro form.*1

Experimental

Preparation of α-Methylvaleraldehyde (2-Methylpentanal). Two hundred and fifty-five grams of 2-methyl-2-pentenal were hydrogenated with a catalyst prepared from 4 g of $PdCl_3$ by Willstätter's method, at 90 atm and at 70°C. The hydrogenation product was distilled fractionally to yield 200 g (77.4%) of α-methylvaleraldehyde; bp 110—120°C.

Preparation of 5-(1-Methylbutyl)-hydantoin. A solution consisting of 125 g of sodium cyanide, 550 g of commercial ammonium carbonate, 1500 m*l* of methanol, and 1500 m*l* of water was placed in a 5-*l* four-necked, round-bottomed flask fitted with a mechanical stirrer, a dropping funnel, a reflux condenser, and a thermometer; the flask was then kept at 40°C in a water bath. Through the dropping funnel we stirred in 106 g of α-methylvaleraldehyde, drop by drop, where upon a vigorous reaction took place. After this vigorous reaction had ceased, the reaction mixture was stirred continuously at 55°C for 6 hr. When the resulting solution was concentrated to about one-half volume under reduced pressure, it became a sludge with crystals. The residual solution was boiled with 800 m*l* of concentrated hydrochloric acid for 1 hr in a draft chamber and then kept in a refrigerator. The precipitated crystals were collected, washed with water, and dried in air. The yield was 180 g (100%) of a practically pure material; mp 122°C.

Found: C, 56.25; H, 8.54; N, 16.55%. Calcd for $C_8H_{14}O_2N_2$: C, 56.45; H, 8.29; N, 16.46%.

Preparation of β-Methylnorleucine (Ia, Ib). In an autoclave, 170 g of 5-(1-methylbutyl)-hydantoin was hydrolyzed with 800 m*l* of aqueous sodium hydroxide containing 380 g of sodium hydroxide at 140°C for 5 hr. After removing the ammonia by boiling, the reaction mixture was neutralized with hydrochloric acid and treated with charcoal. Then, the solution was sufficiently acidified with hydrochloric acid and dried *in vacuo*, and the residue was extracted with 500 m*l* of methanol. The extract was dried *in vacuo*, and the residue was taken up with 300 m*l* of water and adjusted to pH 6.0 with aqueous sodium hydroxide to afford 136 g of β-methylnorleucine; another 9 g of the product were obtained from the mother liquid. Total yield, 145 g (100%).

A part of the product was recrystallized from 10% ethanol for the elemental analysis.

Separation and Purification of β-Methylnorleucine. One hundred and seventeen grams of the crude β-methylnorleucine (Ia+Ib) were recrystallized twice from 400 m*l* of concentrated hydrochloric acid; crystalline hydrochloride salt was collected and thoroughly washed with concentrated hydrochloric acid. Yield, 57 g (Ia·HCl).

The mother liquor obtained from the recrystallization was combined and concentrated to dryness under reduced pressure, and the residue was recrystallized twice from concentrated hydrochloric acid; another 17 g of β-

3) J. Shoji, K. Tori and H. Ōtsuka, *J. Org. Chem.*, **30**, 2772 (1965).

*1 These results will be reported fully in the near future in this Bulletin.

methylleucine hydrochloride were thus recovered. Yield, 74 g (50.8%).

The mother liquor and washings obtained by the above treatment were combined and evaporated to dryness, and the residue was taken up with 50 ml of water. The solution was neutralized to pH 6.5 with 20% sodium hydroxide to afford 32 g of crude β-methylnorleucine (Ib).

β-Methylnorleucine cupper salt was instantly precipitated from a solution containing 23 g of the crude β-methylnorleucine (Ib) and 6.5 g of sodium hydroxide in 300 ml of water, by the addition of 20 g of cupric sulfate. After heating the sluggish mixture, with occasional stirring, in a boiling-water bath for 1 hr, the precipitated cupper salt of β-methylnorleucine was collected from the hot, sluggish mixture and washed with methanol. The crude cupper salt was treated with 600 ml of a 1% cupric sulfate solution during heating on the water bath for 20 min with occasional stirring. An insoluble cupper salt was then collected from the hot mixture. This treatment with 1% cupric sulfate solution was repeated twice. The pure cupper salt obtained in this manner was dissolved in 10% hydrochloric acid and treated with hydrogen sulfide. After the cupric sulfide was filtered off, the filtrate was evaporated to dryness *in vacuo* to afford stereochemically pure β-methylnorleucine hydrochloride (Ib·HCl). The residue was recrystallized from hot acetone. Yield, 15 g (10.3%).

One portion of the hydrochloride salt was dissolved in a small amount of water and neutralized to pH 6.0 with aqueous hydroxide. The amino acid (Ib) thus precipitated was collected and washed with water.

Preparation of 2-Benzyl-5-(α-methylisobutylidene)-4-imidazolone. This material was prepared

Table 2. Derivatives of β-methylnorleucine and β-methylleucine (Formula I and II)

Compound	Mp (°C)	$[\alpha]_D$	Anal. (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
DL-Ia+Ib	275	—	57.90	10.41	9.65	57.32	11.07	9.61
DL-Ia	275	—	57.90	10.41	9.65	57.47	10.92	10.07
Acetyl-DL-Ia	151	—	57.73	9.15	7.48	57.74	9.35	7.76
DL-Ia·HCl	219	—	46.28	8.88	7.71	46.16	8.93	8.15
L-Ia	280	+46.7° (*c* 1.50, 6 N HCl)	57.90	10.41	9.65	57.50	10.48	9.38
Acetyl-D-Ia	151	−34.8° (*c* 2.07, EtOH)	57.73	9.15	7.48	57.00	9.59	7.87
D-Ia·HCl	253	−35.7° (*c* 35.7, 6N HCl)	46.28	8.88	7.71	46.09	9.16	7.71
D-Ia	280	−45.3° (*c* 1.50, 6N HCl)	57.90	10.41	9.65	57.59	10.59	9.36
DL-Ib	275	—	57.90	10.41	9.65	57.39	10.87	10.18
Acetyl-DL-Ib	131	—	57.73	9.15	7.48	57.80	9.60	7.53
DL-Ib·HCl	187	—	46.28	8.88	7.71	45.73	9.35	8.25
L-Ib	280	30.4 (*c* 1.51, 6N HCl)	57.90	10.41	9.65	57.42	10.56	9.44
Acetyl-D-Ib	146	−17.5° (*c* 2.12, EtOH)	57.73	9.15	7.48	56.57	9.41	7.01
D-Ib·HCl	203	−25.4° (*c* 2.44, 6N HCl)	46.28	8.88	7.71	45.72	9.11	7.56
D-Ib	280	−32.2° (*c* 1.55, 6N HCl)	57.90	10.41	9.65	57.32	10.54	9.37
DL-IIa+IIb	270		57.90	10.41	9.65	57.46	10.58	9.46
DL-IIa	270		57.90	10.41	9.65	57.21	10.71	9.45
Acetyl-DL-IIa	157—158		57.73	9.15	7.48	57.42	9.40	7.59
L-IIa	270	+40.6° (*c* 1.0, 6N HCl)	57.90	10.41	9.65	57.81	10.87	9.71
Acetyl-D-IIa	185—186	− 2.3° (*c* 8.3, EtOH)	57.73	9.15	7.48	57.31	9.54	7.48
D-IIa	270	−39.9° (*c* 1.2, 6N HCl)	57.90	10.41	9.65	57.64	10.74	9.63
DL-IIb	270		57.90	10.41	9.65	57.17	10.80	9.48
Acetyl-DL-IIb	169—170		57.73	9.15	7.48	57.42	9.55	7.47
L-IIb	270	+38.6° (*c* 1.2, 6N HCl)	57.90	10.41	9.65	57.59	10.84	9.62
Acetyl-D-IIb	199—200	− 2.8° (*c* 6.9, EtOH)	57.73	9.15	7.48	57.47	9.57	7.48
D-IIb	270	−39.4° (*c* 1.1, 6N HCl)	57.90	10.41	9.65	56.99	10.88	9.31

from 2-benzyl-5(*H*)-imidazolone and methylisopropylketone according to the procedure of Kotake *et al.* The crude product was recrystallized from ether-hexane for elemental analysis.

Found: C, 74.33; H, 7.49; N, 11.64%. Calcd for $C_{15}H_{20}ON_2$: C, 74.35; H, 7.49; N, 11.56%. Mp 134—136°C.

Preparation of β-Methylleucine (IIa, IIb). One hundred and ninety grams of an imidazolone derivative were dissolved in a solution of 80 g of sodium hydroxide in 100 m*l* of methanol and 220 m*l* of water; this was followed by hydrogenation in the presence of a Raney nickel catalysts from 25 g of the alloy at 100°C and at an initial pressure of 90 kg/cm².

After the removal of the catalyst by filtration, the filtrate was concentrated to about 200 m*l* *in vacuo* and then boiled with 100 m*l* of concentrated hydrochloric acid for 10 hr. After cooling, the phenyl acetic acid thus precipitated was removed by filtration; the filtrate was washed with ether and evaporated *in vacuo* to a syrup, and the residue was taken up in water and again evaporated. The residue was dissolved in 400 m*l* of ethanol, and the insoluble material was removed by filtration. Into the cooled ethanol solution, triethylamine was added cautiously with gentle stirring; then the resultant crystals were collected, washed with a large amount of ethanol, and dried in air. A small amount of the crude product was then recrystallized from water. Yield, 102 g (80%).

Found: C, 10.68; H, 57.46; N, 9.46%. Calcd for $C_7H_{15}O_2N$: C, 10.41; H, 57.90; N, 9.65%.

Separation and Purification of β-Methylleucine. The crude β-methylleucine (IIa+IIb), 102 g was recrystallized twice from 200 m*l* of concentrated hydrochloric acid. The crystalline hydrochloride salt (IIa·HCl) was collected by filtration and washed thoroughly with concentrated hydrochloric acid. Yield, 85 g (67%).

One portion of the hydrochloride salt was dissolved in ethanol and neutralized with triethylamine to afford an amino acid (IIa).

The mother liquor and washings were combined and adjusted to pH 6.8 with an aqueous hydroxide solution, and then 30 g of cupric sulfate were added to the solution. The resultant blue-purple solution was gently heated on the water bath, allowed to cool, and then carefully neutralized with 20 g of sodium bicarbonate. The crystalline cupper salt of the amino acid was collected by vacuum filtration and washed repeatedly with water and then with methanol. The crude cupper salt was suspended in a 1% solution of cupric sulfate, and the mixture was slightly heated. Insoluble crystals were collected by filtration under reduced pressure and then washed with methanol. This treatment was repeated twice, and then the crystals were dried in air. Yield, 56 g.

The pure copper salt obtained in this manner was dissolved in 10% hydrochloric acid and treated with hydrogen sulfide. The cupric sulfide thus precipitated was removed. The filtrate and washings were combined and evaporated *in vacuo* to afford stereochemically-pure β-methylleucine hydrochloride (IIb·HCl). Yield, 27 g (21.2%).

Resolution of the Amino Acid (Ia, Ib, IIa, IIb). IIa·HCl was acetylated with 2 N sodium hydroxide and acetic anhydride by the ordinary method. Fifty-six grams of the acetyl derivative of IIa were dissolved in 1.5 *l* of water, and the pH was adjusted to 6.8 with 2 N sodium hydroxide. To this solution, 3 g of prozyme acylase were then added, after which the mixture was allowed to stand for 50 hr at 42°C. After 50 hr, the pH of the solution was again adjusted to 6.8 with 2 N NaOH, and the hydrolysis was continued. Then, the solution was evaporated to 100 m*l* *in vacuo*; the crystals of the L-amino acid were subsequently collected by filtration and washed with a small amount of water. The crude L-amino acid was purified by recrystallization from the water by treatment with charcoal. Yield, 12 g (55.3%). $[\alpha]_D^{33}$ +40.6 (*c* 1, 6N HCl).

The mother liquor was acidified with 6 N hydrochloric acid. The *N*-acetyl-D-amino acid thus precipitated was collected and washed with dilute hydrochloric acid and then water. Yield, 24 g (85.7%). $[\alpha]_D^{25}$ −2.3 (*c* 8.3 EtOH).

One portion of the *N*-acetyl-D-amino acid was refluxed with 10% hydrochloric acid; the solution was then neutralized with triethylamine to afford, quantitatively, D-amino acid. $[\alpha]_D^{25}$ −39.9 (*c* 1.2, 6 N HCl).

The other amino acids, Ia, Ib, and IIb were resolved by a procedure similar to that used for IIa.

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