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179. Shiro Terashima, Kazuo Achiwa, and Shun-ichi Yamada : Studies on Optically Active Amino Acids. Ⅵ.*1 Studies on α-Alkyl-α-amino Acids. II.*2 Resolution of Some α-Methyl-α-amino Acids through *l*-Menthyl Ester.*4

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Studies on optically active α-methyl-α-amino acids are relatively limited as compared with those on naturally occurring optically active α-amino acids. A few of them were resolved by chemical, physical and biochemical methods. N-Formylisovaline,[1,2] N-formyl-α-methylphenylglycine,[3] N-acetyl-α-methylphenylalanine,[4] N-acetyl-α-methyltyrosine,[4] N-acetyl-α-methyl-β-(2-naphthyl)alanine,[4] N-acetyl-α-methyl-β-(3,4-diacetoxyphenyl)alanine,[5,6] N-acetyl-α-methyl-β-(3,4-dimethoxyphenyl)-alanine[5~7] were all amino acids of this series resolved by chemical methods employing optically active bases such as brucine, quinine, cinchonidine and (+) or (−)-α-phenethylamine until now. As to the biochemical resolution, Greenstein, *et al.* reported that N-chloroacetyl derivatives of DL-isovaline,[8,9] DL-α-methylvaline,[9] DL-α-methylnorvaline[9] and DL-α-methylserine[10] were resolved into their optical enantiomorphs by asymmetric hydrolysis of hog renal acylase. It should be noted, however, that a presence of methyl substituent causes a remarkable diminution of the hydrolytic rate, compared with the rate of substrates containing a hydrogen substituent on the asymmetric α-carbon atom of amino acids.[8,9] On the other hand, (−) isovaline,[11] and (−)-α-amino-α-methylvaleric acid[12] were isolated from yeast fermentations with DL-isovaline and DL-α-amino-α-methylvaleric acid respectively and (−)-α-methylserine was also isolated from a culture medium of *Pseudomonas* MS with DL-α-methylserine as a substrate by E. M. Wilson, *et al.*[13] However, both enantiomorphs in these cases could not be obtained, since one of them was assimilated during the culture. Recently, DL-α-methyl-β-(3,4-dihydroxyphenyl)alanine (DL-α-methyl-DOPA), one enantiomorph to which recently considerable attentions have been paid from the biochemical studies, was found to be resolved by preferential crystallization.[14]

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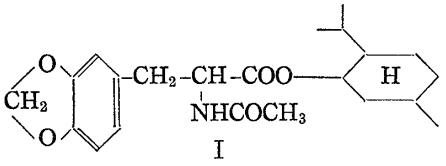
*1 Part Ⅴ : This Bulletin, **13**, 995 (1965).
*2 Part Ⅰ : This Bulletin, **13**, 1001 (1965).
*3 Hongo, Tokyo (寺島孜郎, 阿知波一雄, 山田俊一).
*4 This work was presented at the 84th Annual Meeting of Pharmaceutical Society of Japan, April, 1964, Tokyo.

1) E. Fischer, R. von Grävenitz : Ann., **406**, 1 (1914).
2) S. Akabori, T. Ikenaka, K. Matsuki : Nippon Kagaku Zasshi, **73**, 112 (1952).
3) A. McKenzie, G. W. Clough : J. Chem. Soc., **101**, 390 (1912).
4) H. R. Almond, Jr., D. T. Manning, C. Niemann : Biochem., **1**, 243 (1962).
5) E. W. Tristram, J. ten Broeke, D. F. Reinhold, M. Sletzinger, D. E. Williams : J. Org. Chem., **29**, 2053 (1964).
6) Merck & Co., Inc. South African Patent, 61/950 (1962).
7) Merck & Co., Inc. Brit. Patent, 936, 074 (C. A,. **60**, 661h (1964)).
8) C. G. Baker, Shou-Cheng J. Fu, S. M. Birnbaum, H. A. Sober, J. P. Greenstein : J. Am. Chem. Soc., **74**, 4701 (1952).
9) Shou-Cheng J. Fu, S. M. Birnbaum : J. Am. Chem. Soc., **75**, 918 (1953).
10) J. P. Greenstein, M. Winitz : "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York, London, 1961, vol. 1, p. 748 and vol. 3, p. 2573.
11) F. Ehrlich, A. Wendel : Biochem. Z., 8, 438 (1908).
12) K. Kurono : *Ibid.*, **134**, 424 (1922).
13) E. M. Wilson, E. E. Snell : J. Biol. Chem., **237**, 3180 (1962).
14) Merck & Co., Inc. Belg. Patent, 620, 113 (C. A., **59**, 8871e (1963)).

Chemical resolution method using optically active bases is always tedious and sometimes unsuccessful. Biochemical methods take much time in the case of amino acids containing methyl substituents on the α-asymmetric atom, and in some cases only one enanthiomorph was obtained and the yield was generally poor. Moreover, preferential crystallization method is not suitable for laboratory process.

In our laboratory, various kinds of optically active α-methyl-α-amino acids being requested for the study of the establishment of their absolute configurations,[15] it was considered desirable to explore generalized resolution methods of racemic α-methyl-α-amino-acids. In the previous paper,[16] one of the authors reported that a diastereomeric mixture of N-acetyl-β-(3,4-methylenedioxyphenyl)alanine *l*-menthyl ester (I) was easily separated into two diastereomers by the fractional recrystallization. The authors tried to apply this finding to the resolution of DL-2-acetamido-2-methyl-4-benzyloxybutyric acid (IIIa), since all the attempts to resolve with various optically active bases in a variety of solvents were thoroughly unsuccessful.



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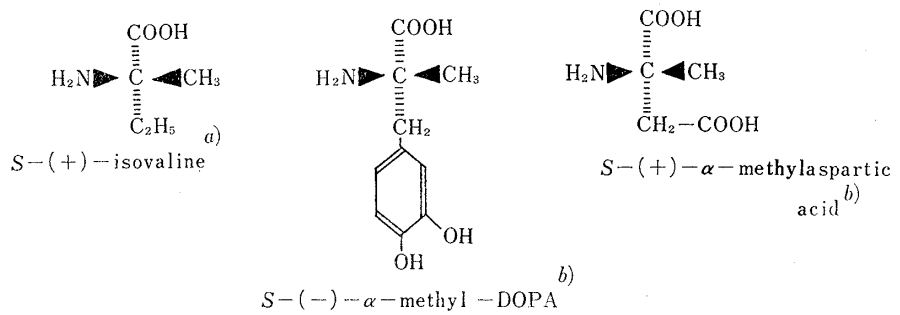
DL-2-Acetamido-2-methyl-4-benzyloxybutyric acid (DL-IIIa) derived from DL-2-amino-2-methyl-4-benzyloxybutyric acid (DL-IIa)[17~19] was converted to DL-4-(2-benzyloxyethyl)-2,4-dimethyl-2-oxazolin-5-one (DL-IVa)[20] in a good yield by the reflux in acetic anhydride.

Reaction of DL-IVa with sodium l-menthoxide in anhyd. benzene afforded a mixture of two diastereoisomers (Va and VIa) which was separated into two sorts of crystals Va, m.p. 103~104°, $[\alpha]_D^{25}$ −51.7° (CH_3OH) in 52% yield and VIa, m.p. 72.5~73.5°, $[\alpha]_D^{28}$ −29.2° (CH_3OH) in 12% yield by the fractional recrystallization from hexane. Selective hydrolysis of Va and VIa with nine equivalents of potassium hydroxide in 50% aqueous alcohol for 5 hours' reflux gave (−) and (+)-N-acetyl amino acids (−)-IIIa, m.p. 152.5~153.5°, $[\alpha]_D^{25}$ −7.3° (CH_3OH) and (+)-IIIa, m.p. 153.5~154.5°, $[\alpha]_D^{26}$ +8.0° (CH_3OH) in a quantitative yield, respectively. Both of these N-acetyl amino acids, (−)-IIIa and (+)-IIIa, are enantiomorphic, *i.e.* they showed the same magnitude of optical rotation with the opposite sign, and identical melting points, infrared spectra in the solid state were quite superimposable. The resolution of DL-isovaline (IIb) and DL-α-methyl-β-(3,4-dimethoxyphenyl)alanine (IIc) was achieved, following to the same procedure, to afford the corresponding optically active N-acyl-α-methyl amino acids. The results

15) A Part of this work concerning the absolute configurations of isovaline, α-methyl-DOPA and some other amino acids of this series was preliminarily communicated as follows. (S. Yamada, K. Achiwa: This Bulletin, **12**, 1525 (1964)[a]; S. Yamada, S. Terashima, K. Achiwa : *Ibid.*, **13**, 227 (1965).[b]



16) S. Yamada, T. Shioiri, T. Fujii : This Bulletin, **8**, 688 (1962).
17) J. Murata, H. Arai, M. Tanaka : Kôgyô Kagaku Zasshi, **60**, 1206 (1957).
18) J. Murata, H. Arai : *Ibid.*, **56**, 628 (1953).
19) U. Shimodoi, M. Sashio, J. Murata : *Ibid.*, **63**, 2140 (1960).
20) U. Shimodoi, K. Masuda, J. Murata : *Ibid.*, **65**, 1664 (1962).

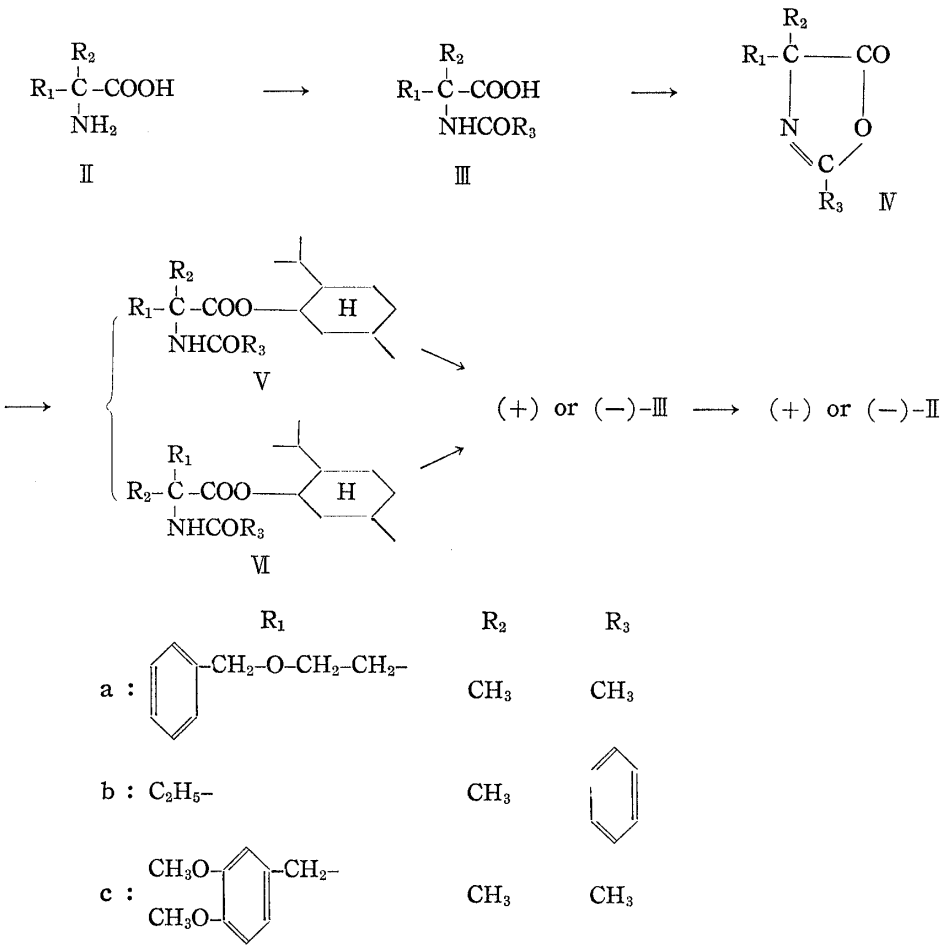


Chart 1.

TABLE I.

Compds.		m.p. or b.p.* (mm. Hg) (°C)	Yield (%)	$[\alpha]_D$ (CH_3OH)
DL-III	a	136～137	96	
	b	195～196	44	
	c	212～213	97	
DL-IV	a	170～171 (8)*	92	
	b	46～47, 118 (9)*	82	
	c	176 (6)*	85	
V	a	103～104	52	$[\alpha]_D^{30}$ −51.6°
	b	72.5～75	15	$[\alpha]_D^{12}$ −41.9°
	c	139～140	20	$[\alpha]_D^{31}$ −107°
VI	a	72.5～73.5	12	$[\alpha]_D^{28}$ −29.2°
	b	110～111.5	38	$[\alpha]_D^{18}$ −54.7°
	c	154.5～155.5	47	$[\alpha]_D^{33}$ +36.8°
(−)-IIIa	from V	152.5～153.5	quantitative	$[\alpha]_D^{25}$ −7.3°
(+)-IIIb	from V	176～178	79	$[\alpha]_D^{15}$ +10.3°
(−)-IIIc	from V	189～190	90	$[\alpha]_D^{21}$ −58.2°
(+)-IIIa	from VI	153.5～154.5	quantitative	$[\alpha]_D^{26}$ +8.0°
(−)-IIIb	from VI	176～178	90	$[\alpha]_D^{16}$ −10.4°
(+)-IIIc	from VI	189～190	90	$[\alpha]_D^{18}$ +60.6°

are summarized in Table I. In case of IIb, N-benzoyl derivative was chosen in order to make easier separation of diastereomeric *l*-menthyl esters.

The optically active N-acyl-α-methyl-α-amino acids, (+) or (−)-IIIa, b, c, thus obtained by the procedure described above, were converted into optically active α-methyl-α-amino acid respectively by the reactions illustrated in Chart 2. Alkaline hydrolysis

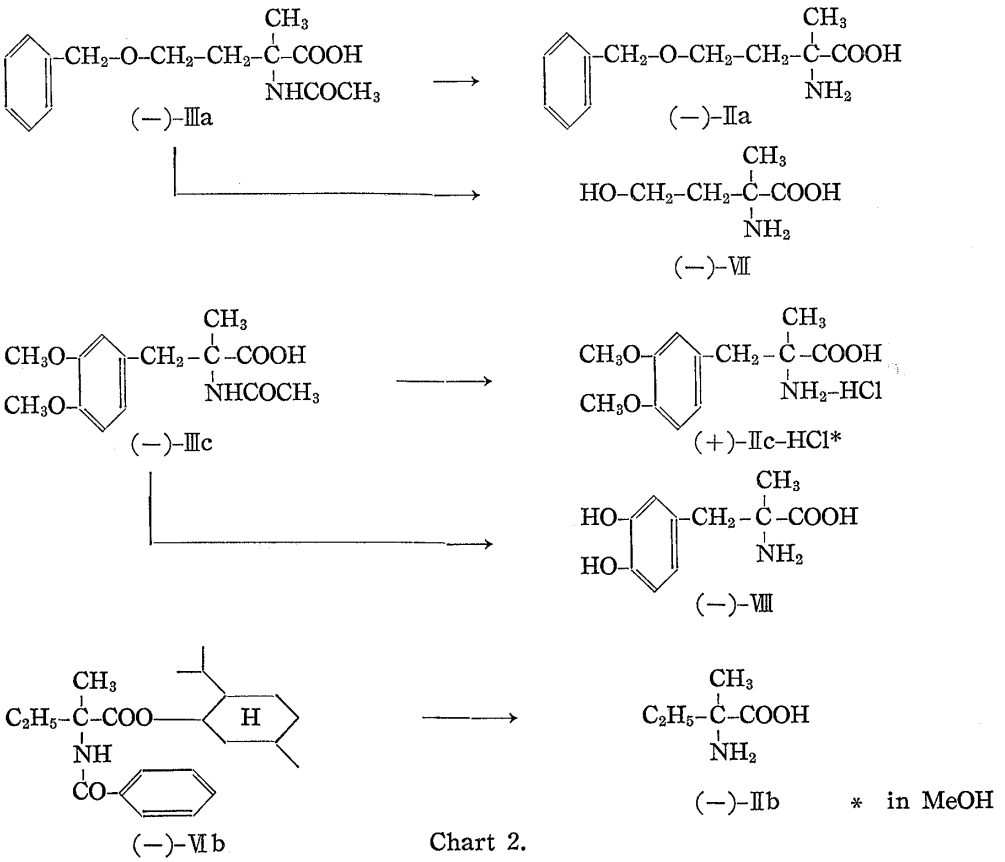


Chart 2.

of (−)-IIIa under a reflux in 20% sodium hydroxide solution for 40 hours underwent the deacetylation of (−)-IIIa to afford (−)-IIa.*[5] The free amino acid, (−)-IIa, thus obtained in 26% yield afforded small needles, m.p. 254°(decomp.). $[\alpha]_D^{25}$ −16.9°(H_2O). Because of the easy debenzylation in acidic condition, it was difficult to accomplish only deacetylation without any attack at benzyl group. Accordingly, when submitted to acidic hydrolysis with 48% hydrobromic acid under a reflux for 9 hours, (−)-IIIa was converted to (−)-α-methylhomoserine ((−)-VII*[5]) which gave colorless needles m.p. 213.5~216°. $[\alpha]_D^{27}$ −15.3°(H_2O).

Hydrolysis of (−)-IIIc with 12% hydrochloric acid by 3 hours reflux underwent only deacetylation of (−)-IIIc, and (+)-IIc hydrochloride*[5,21] was obtained in 79% yield as a monohydrate of colorless needles m.p. 169~171°(decomp.), $[\alpha]_D^{14}$ +8.8°(MeOH). However, when (−)-IIIc was submitted to hydrolysis of refluxing with 48% hydrobromic

*[5] All of the optically active α-methyl-α-amino acids in this report are indicated by the sign of either (+) or (−). The absolute configuration of all amino acids described here has already been established in our laboratory (ref. 15). Details concerning the absolute configuration besides ref. 15 will be published in near future.

21) H.L. Slate, D. Taub, C.H. Kuo, N.L. Wendler : J. Org. Chem., **29**, 1424 (1964). In this report, melting point of L-(−)-α-methyl-β-(3,4-dimethoxyphenyl)alanine hydrochloride was described to be 235~238°, data of IR spectrum published is relatively concomitant with our data, even though $[\alpha]_D$ value was not reported.

acid for 8 hours, hydrolysis and simultaneous demethylation occurred and thus (+)-α-methyl-β-(3,4-dihydroxyphenyl)alanine ((−)-Ⅷ)*6~*8 was obtained in 59% yield as a sesquihydrate of colorless powdery crystals, m.p. >250°. $[\alpha]_D^{24}$ −1.5° (*N* HCl). (+)-Ⅲc, the antipode of (−)-Ⅲc, was treated with hydrochloric acid under the similar condition to the case of (−)-Ⅲc and (−)-Ⅱc hydrochloride[21] was obtained in 82% yield as a monohydrate m.p. 169~171° (decomp.), $[\alpha]_D^{17}$ −8.8° (CH_3OH), which was converted to free amino acid (−)-Ⅱc, a sesquihydrate, m.p. 256~258° (decomp.), $[\alpha]_D^{25}$ −2.6° (*N* HCl) by passing through ion exchanger column (Amberlite IR-120, H^+-form).

Instead of stepwise hydrolysis from Ⅴ to Ⅱ *via* Ⅲ, Ⅱ could be obtained directly from Ⅴ by allowing simultaneous ester hydrolysis and deacylation with acids. The reaction of N-benzoyl isovaline *l*-menthyl ester ((−)-Ⅵb) with 48% hydrobromic acid under a reflux for 6 hours provided (−)-isovaline ((−)-Ⅱb)[15] in 61% yield, m.p. >250°, $[\alpha]_D^{21}$ −11.9° (H_2O).

All amino acids thus obtained showed only a single spot on paper chromatography developed by two different solvent systems.

Alkaline hydrolysis of menthyl ester of amino acids containing a hydrogen substituent at a α-carbon atom was accompanied by the considerable racemization[16] and the acid hydrolysis was taken place in poor yield or otherwise producing an unknown reddish resin.[16] However, in the case reported here, no racemization was occurred even by alkaline hydrolysis, since α-methyl-α-amino acids do not have a hydrogen at the α-carbon atom which induces racemization. Moreover, a mixture of two diastereoisomers of menthyl esters (Ⅴ and Ⅵ) is separated into two crystalline forms by a relatively simple procedure described in experimental part, and pure Ⅴ and Ⅵ can be obtained in moderatedly good yield. Therefore optical purity of amino acids obtained by hydrolysis of pure Ⅴ or Ⅵ is essentially optical pure, the purest $[\alpha]_D$ value of amino acids in this series can be ascertain by this procedure. The reaction seems to be general and has the potentiality to be utilized to resolve various kinds of amino acids of this series.

Experimental*9

DL-2-Amino-2-methyl-4-benzyloxybutyric acid (DL-IIa) and its N-acetyl derivative (DL-IIIa)——According to the report of Murata, *et al*[17~19]., DL-Ⅱa was obtained from acetone by way of 4-hydroxy-2-butanone $b.p_{10\sim13}$ 69~76°; yield 26% (lit.,[17] $b.p_{15}$ 76~78°, yield 73%), methyl vinyl ketone, b.p. 79~83°, yield 56% (lit.,[17] b.p. 81.4°, yield 63~70%), 4-benzyloxy-2-butanone, $b.p_7$ 118~125°, yield 68% (lit.,[18] $b.p_5$ 116~126°, yield 89%) and 5-(2-benzyloxyethyl)-5-methylhydantoin, m.p. 147~148.5°, yield 86% (lit.,[19] m.p. 146~147°, yield 68%). DL-Ⅱa, m.p. 238~238.5° (decomp.), yield 74% (lit.,[19] m.p. 244° (decomp.), yield 98%). IR ν_{max}^{KBr} cm^{-1} : 1660, 1620, 1599, 1545, 735, 699.

DL-Ⅱa thus obtained was treated with Ac_2O-pyridine to give DL-Ⅲa in 96% yield. Recrystallization from aq. EtOH afforded white needles, m.p. 136~137° (lit.,[20], m.p. 133.5~135°). IR ν_{max}^{KBr} cm^{-1} : 3435, 1737, 1614, 1531, 740, 698.

DL-Isovaline (DL-IIb) and its N-Benzoyl Derivative (DL-IIIb)——DL-5-Ethyl-5-methylhydantoin, m.p. 143~144.5° obtained in 95% yield according to the report of Bucherer, *et al*.[22] (lit.,[22] m.p. 146°, yield 90%)

*6 Ref. 6 reported $[\alpha]_D$ value of L-α-methyl-β-(3,4-dihydroxyphenyl) alanine as $[\alpha]_D$ −4±2° (c=1, *N* HCl), even though melting point was not mentioned.

*7 Ref. 7 mentioned $[\alpha]_D^{25}$ −4° (c=1, *N* HCl) for L-α-methyl-β-(3,4-dihydroxyphenyl)alanine.

*8 In ref. 5, $[\alpha]_D$ values of L-α-methyl-β-(3,4-dihydroxyphenyl)alanine in various conditions were reported. For example, $[\alpha]_D^{25}$ −4° (c=2, 0.1*N* HCl), $[\alpha]_D^{25}$ −13.5° (c=2, phosphate buffer, pH 6.5), etc.

*9 All melting points are uncorrected. IR spectra measurements were carried on with a Spectrophotometer, Model DS-301. Japan Spectroscopic Co., Ltd. Optical activities were measured with a Yanagimoto Photo Magnetic Direct Reading Polarimeter, Model OR-20.

22) H. T. Bucherer, L. A. Lieb : J. prak. Chem., **141**, 5 (1934).

was hydrolyzed under reflux in aqueous solution containing 2.5 molar equivalents of $Ba(OH)_2$[23,24] to give DL-IIb. Recrystallization from aq. EtOH afford colorless needles, m.p. >250°(lit.,[25a] m.p. 317~318° (decomp.), lit.,[25b] m.p. 307.5°(in sealed tube)) yield 69%. IR ν_{max}^{KBr} cm^{-1} : 3510, 3230, 3070, 1641, 1613, 1587, 1559. DL-IIb was submitted to the Schotten-Baumann reaction in 2*N* NaOH with benzoyl chloride at 30° to yield DL-IIIb. Recrystallization from 50% MeOH afforded colorless needles, m.p. 195~196°, yield 44% (lit.,[25b] m.p. 198~199°, yield 20~30% lit.,[23] m.p. 204~205°, yield 54%, lit[26]., m.p. 196°, yield 76%). IR ν_{max}^{KBr} cm^{-1} : 3390, 1718, 1625, 1541, 762, 715.

DL-α-Methyl-β-(3,4-dimethoxyphenyl)alanine (DL-IIc) and its N-Acetyl Derivative (DL-IIIc)——DL-IIc, white powder, sesquihydrate, m.p. 254~255°(decomp.) (lit.,[27] m.p. 282~283.5°(decomp.)) was supplied to us through the kindness of Taisho Pharmaceutical Co., Ltd. IR ν_{max}^{KBr} cm^{-1} : 3520~3470, 1593, 857, 817, 765.

DL-IIc was treated with Ac_2O-pyridine to give DL-IIIc. Recrystallization from aq. EtOH afforded colorless crystals m.p. 212~213°, yield 97% (lit.,[5] m.p. 213~215°). IR ν_{max}^{KBr} cm^{-1} : 3305, 1712, 1641, 1552, 1521, 861, 819, 797, 766.

DL-4-(2-Benzyloxyethyl)-2,4-dimethyl-2-oxazolin-5-one (DL-IVa)——A mixture of DL-IIIa (70.0 g., 0.264 mole) and Ac_2O (420 ml.) was refluxed for 6 hr. After cooling, volatile material was evaporated under the reduced pressure to give reddish yellow residue (75.0 g.), which was submitted to fractional distillation *in vacuo* to afford DL-IVa as colorless oil (59.4 g., 92%), b.p_8 170~171°. IR ν_{max}^{cap} cm^{-1} : 1825, 1685, 1599, 737, 702. No band was observed in the region of 3400~3300 cm^{-1}. The hydrolysis of IVa was taken place even in the atmospheric moisture to give the starting material,[20] then IVa was used immediately for the following step.

DL-2-Phenyl-4-ethyl-4-methyl-2-oxazolin-5-one(DL-IVb)——DL-IIIb(8.7 g., 0.039 mole) was treated with Ac_2O as same conditions as described above to give DL-IVb as a colorless oil (6.54 g., 82%) b.p_9 118° which was solidified on standing at room temperature, m.p. 46~47°(lit.,[26] m.p. 48° colorless rhombic plates). IR ν_{max}^{cap} cm^{-1} : 1821, 1657, 1603, 740, 695. No band was shown in the range 3400~3300 cm^{-1}. DL-IVb was immediately employed for the following step because of the easy hydrolysis.

DL-4-(3,4-Dimethoxybenzyl)-2,4-dimethyl-2-oxazolin-5-one (DL-IV)——DL-IIIc (50.0 g., 0.178 mole) was treated with Ac_2O as same procedure as described above to yield DL-IVc as colorless oil (40.7 g., 85%), b.p_6 178°. IR ν_{max}^{cap} cm^{-1} : 1805, 1699, 1609, 1594, 810, 761. No band was observed in the range of 3400~3300 cm^{-1}. This was used immediately for the following step.

Preparation and Separation of Diastereoisomeric *l*-Menthyl Esters——1) *l*-Menthyl 2-acetamido-2-methyl-4-benzyloxybutyrate (Va and VIa) : To a suspension of Na-powder (8.3 g., 0.36 atom) in anhyd. benzene (400 ml.) was added *l*-menthol*10 (48.7 g., 0.312 mole), the reaction mixture was kept standing overnight avoiding moisture and then refluxed for 2 hr. Unreacted Na-powder was decanted off, and washed with benzene (50 ml.). To the combined solution of the supernatant and the washings was added a solution of DL-IVa (59.8 g., 0.242 mole) in anhyd. benzene (50 ml.). It was stirred for 5 hr. at room temperature and then kept standing overnight to give a clear orange-yellow solution, which was washed successively with 10% AcOH solution (400 ml.×2), H_2O (400 ml.) 2.5% Na_2CO_3 solution (400 ml.) and H_2O (400 ml.), and then dried over Na_2SO_4. An evaporation of the solvent gave yellow oil which was dissolved in hexane (500 ml.) on warming. Hexane solution was seeded and kept standing in a refrigerator to crystallize out crude Va (66.5 g.), which was washed twice with hexane, m.p. 91~97°, $[\alpha]_D^{24}$ −45.8° (c=2.56, MeOH). Three recrystallizations from hexane afforded colorless small needles (25.4 g., 52%) m.p. 103~104°, $[\alpha]_D^{25}$ −51.7° (c=2.38, MeOH). Pure Va was obtained by the further recrystallization from hexane, m.p. 103~104°, $[\alpha]_D^{30}$ −51.6° (c=2.02, MeOH). *Anal.* Calcd. for $C_{24}H_{37}O_4N$: C, 71.43; H, 9.24; N, 3.47. Found : C, 71.36; H, 9.38; N, 3.33. IR ν_{max}^{KBr} cm^{-1} : 3370, 1721, 1681, 1530, 727, 693.

The combined filtrate and washings from which crude Va was obtained, was evaporated to dryness on a water bath to give a yellow oil (70.2 g.). The oil was distilled fractionally under high reduced pressure to afford an oil (47.6 g.) b.$p_{0.75}$ 202~210° which was dissolved in iso-Pr_2O (40 ml.). The solution was seeded and kept in a refrigerator for 3 days. Crude VIa (16.3 g.) was crystallized out, m.p. 68~72°, $[\alpha]_D^{28}$ −33.4°(c=4.40, MeOH), which were recrystallized from hexane (30 ml). The crystals initially appeared were removed by decantation, the supernatant was seeded and kept in a refrigerator to crystallize out VIa as colorless needles (5.2 g., 12%), m.p. 69~71°, $[\alpha]_D^{27}$ −30.5°(c=2.66, MeOH). Recrystallization from hexane afforded needles, m.p. 72.5~73.5°, $[\alpha]_D^{28}$ −29.2° (c=2.02, MeOH). *Anal.* Calcd. for $C_{24}H_{37}O_4N$: C, 71.43; H, 9.24; N, 3.47. Found : C, 71.79; H, 9.55; N, 3.47. IR ν_{max}^{KBr} cm^{-1} : 3340, 1744, 1641, 1540, 753, 704. IR spectra of Va and VIa showed a remarkable difference in a solid state, whereas in $CHCl_3$

*10 *l*-Menthol used was a sample, $[\alpha]_D^{12}$ −51.3°(c=3.06, EtOH).

23) S. D. Uphan, O. C. Dermer : J. Org. Chem., **22**, 799 (1957).
24) K. T. Potts : J. Chem. Soc., **1955**, 1632.
25) *a*) W. Cocker, A. Lapworth : *Ibid.*, **1931**, 1391; *b*) M. D. Slimmer : Ber., **35**, 400 (1902).
26) A. Kjaer : Acta Chem. Scand., **7**, 889 (1953).
27) G. A. Stein, H. A. Bronner, K. Pfister, III : J. Am. Chem. Soc., **77**, 700 (1955).

and benzene solution, both were found to be quite similar but not identical.

2) *l*-Menthyl 2-benzamido-2-methylbutyrate (Vb and VIb): To a solution prepared from Na-powder (1.01 g., 0.044 atom) and *l*-menthol*[10] (6.86 g., 0.044 mole) in anhyd. benzene (200 ml.) followed by previous manner, DL-IVb (6.54 g., 0.032 mole) was added and treated as above to give a diastereoisomeric mixture of Vb and VIb as a brownish yellow oil. The oil was submitted to a high vacuum distillation to afford a fraction (9.16 g.) b.$p_{0.06}$ 171~175°. Repeated recrystallization from hexane yielded VIb (1.9 g., 38%), m.p. 110~111.5°, $[\alpha]_D^{18}$ −54.7°(c=1.28, MeOH) as colorless pillars. *Anal.* Calcd. for $C_{22}H_{33}O_3N$: C, 73.50; H, 9.25; N, 3.90. Found: C, 72.95; H, 9.02; N, 4.06. IR ν_{max}^{KBr} cm^{-1}: 3320, 1722, 1658, 1536, 722, 691.

Mother liquor of the initial recrystallization from hexane mentioned above was kept standing for 2 days at room temperature, crystals separated were filtered off and the filtrate was kept standing at room temperature to yield crystals. Recrystallization from hexane and 80% aq. EtOH successively afforded Vb (0.75 g., 15%) m.p. 72.5~75°, $[\alpha]_D^{12}$ −41.9°(c=1.48, MeOH). *Anal.* Calcd. for $C_{22}H_{33}O_3N$: C, 73.50; H, 9.25; N, 3.90. Found: C, 73.55; H, 9.08; N, 4.19. IR ν_{max}^{KBr} cm^{-1}: 3405, 1709, 1658, 1524, 710, 692. IR spectra of Vb and VIb in the solid state were remarkably different and quite alike but not identical in $CHCl_3$ solution.

3) N-Acetyl-α-methyl-β-(3,4-dimethoxyphenyl) alanine *l*-menthyl ester (Vc and VIc): A diastereomeric mixture of Vc and VIc was prepared from Na-powder (4.2 g., 0.180 atom), *l*-menthol*[10] (26.6 g., 0.170 mole) and DL-IVc (40.7 g., 0.154 mole) in benzene solution as described above. The bright yellow oil obtained was dissolved in benzene (50 ml.) on warming, by adding hexane (450 ml.) and seeding, then white crystals (51.5 g.) were separated out, m.p. 123~127°, $[\alpha]_D^{30}$ −31.3°(c=4.26, MeOH). Three recrystallizations from benzene-hexane afforded VIc (15.2 g., 47%), m.p. 154~154.5°, $[\alpha]_D^{31}$ +36.1°(c=4.24, MeOH). Further recrystallization from a mixture of benzene and hexane yielded colorless prisms, m.p. 154.5~155°, $[\alpha]_D^{33}$ +36.8°(c=2.56, MeOH). *Anal.* Calcd. for $C_{24}H_{37}O_5N$: C, 68.70; H, 8.89; N, 3.34. Found: C, 69.01; H, 9.16; N, 3.31. IR ν_{max}^{KBr} cm^{-1}: 3380, 1745, 1677, 1536, 1523.

Mother liquor of the first recrystallization of crude solid (51.5 g.) gave crystals after kept standing, which were recrystallized from benzene and hexane to afford needles (7.0 g.), m.p. 130~136°. On the other hand, the mother liquor from which crystals were afforded after kept standing as cited above was evaporated to dryness to give a yellowish white substance, which was recrystallized from iso-Pr_2O-hexane to give yellowish white needles (3.9 g.), m.p. 136.5~138°. Two lots of needles were combined and recrystallized twice from benzene-hexane. Vc was obtained as needles (6.5 g., 20%), m.p. 136~137.5°, $[\alpha]_D^{30}$ −104°(c=2.02, MeOH). Twice recrystallizations from a mixture of benzene and hexane afforded needles, m.p. 139~140°, $[\alpha]_D^{31}$ −107°(c=2.22, MeOH). *Anal.* Calcd. for $C_{24}H_{37}O_5N$: C, 68.70; H, 8.89; N, 3.34. Found: C, 68.94; H, 8.90; N, 3.37. IR ν_{max}^{KBr} cm^{-1}: 3315, 1740, 1641, 1553, 1519. IR spectra of Vc and VIc in the solid state were observed a remarkable difference and they were, in $CHCl_3$ solution, quite alike but not identical.

(−)-2-Acetamido-2-methyl-4-benzyloxybutyric Acid ((−)-IIIa)——A mixture of Va (24.0 g., 0.059 mole) and KOH (30.5 g., 0.544 mole) in 50% aq. EtOH (400 ml.) was refluxed for 5 hr., condensed to ca. 150 ml. and extracted with benzene (50 ml.×4) to remove *l*-menthol. An aqueous layer was acidified with conc. HCl to separate crude product out, (−)-IIIa (15.8 g., quantitative), m.p. 150~152°, $[\alpha]_D^{23}$ −6.2° (c=2.04, MeOH). Repeated recrystallization from aq. EtOH afforded colorless needles, m.p. 152~153.5°, $[\alpha]_D^{25}$ −7.3°(c=1.98, MeOH). *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.40; H, 7.39; N, 5.16. IR ν_{max}^{KBr} cm^{-1}: 3400, 1715, 1619, 1516, 734, 693.

(+)-2-Acetamido-2-methyl-4-benzyloxybutyric Acid ((+)-IIIa)——VIa was treated as same as Va to give (+)-IIIa as colorless needles, m.p. 153~154.5°, $[\alpha]_D^{26}$ +8.0°(c=1.88, MeOH). Quantitative yield. *Anal.* Calcd. for $C_{14}H_{19}O_4N$: C, 63.38, H, 7.22, N, 5.28. Found: C, 62.78, H, 7.00, N, 5.03. IR ν_{max}^{KBr} cm^{-1}: 3415, 1716, 1620, 1518, 738, 697. The IR spectra was identical with that of (−)-IIIa.

(−)-N-Benzoylisovaline ((−)-IIIb)——A mixture of VIb (0.50 g., 0.0014 mole) and KOH (0.78 g., 0.014 mole) in 50% aq. EtOH (7 ml.) was refluxed for 6 hr., and the solvent was evaporated and then H_2O (10 ml.) was added. It was extracted with benzene to remove *l*-menthol. Aq. layer was acidified with conc. HCl to separate out crystals (0.28 g., 90%), m.p. 170~172°. Recrystallization from dil. EtOH afforded (−)-IIIb as plates, m.p. 176~178°, $[\alpha]_D^{16}$ −10.4°(c=1.82, MeOH). *Anal.* Calcd. for $C_{12}H_{15}O_3N$: C, 65.14; H, 6.83. Found: C, 65.31; H, 6.56. IR ν_{max}^{KBr} cm^{-1}: 3395, 1731, 1628, 1543.

(+)-N-Benzoylisovaline ((+)-IIIb)——A mixture of Vb (0.30 g., 0.00084 mole), KOH (0.47 g., 0.0084 mole) in 50% aq. EtOH (6 ml.) was refluxed for 6 hr. and treated similarly to the above, to give (+)-IIIb (0.15 g., 79%) as plates, m.p. 176~178°, $[\alpha]_D^{15}$ +10.3°(c=1.44, MeOH). *Anal.* Calcd. for $C_{12}H_{15}O_3N$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.43; H, 6.79; N, 6.72. IR ν_{max}^{KBr} cm^{-1}: 3395, 1727, 1626, 1540. The IR spectra of both IIIa and IIIb were essentially superimposable.

(+)-N-Acetyl-α-methyl-β-(3,4-dimethoxyphenyl)alanine ((+)-IIIc)——A mixture of VIc (2.0 g., 0.0048 mole) and KOH (2.7 g., 0.048 mole) in 50% aq. EtOH (30 ml.) was refluxed for 5 hr. The reaction mixture was evaporated to one-half of its volume and extracted with benzene (20 ml.×4) to remove *l*-menthol. Aqueous layer was acidified with conc. HCl to separate crystals (+)-IIIc (1.2 g., 90%), m.p. 188~189°, $[\alpha]_D^{28}$ +46.2°(c=1.06, MeOH). Repeated recrystallization from 20% aq. EtOH gave colorless needles m.p. 189~190°, $[\alpha]_D^{18}$ +60.6°(c=1.024, MeOH). *Anal.* Calcd. for $C_{14}H_{19}O_5N$: C, 59.77; H, 6.81; N, 4.98.

Found : C, 59.74; H, 6.84; N, 4.98. IR ν_{max}^{KBr} cm^{-1} : 3390, 1721, 1630, 1566, 1519.

(−)-N-Acetyl-α-methyl-β-(3,4-dimethoxyphenyl)alanine ((−)-IIIc)——Vc was treated as same as Ⅵc to give (−)-Ⅲc, yield 90%, m.p. 188～189°, $[\alpha]_D^{27}$ −50.5°(c=1.10, MeOH). Repeated recrystallization from 20% aq. EtOH afforded colorless crystals, m.p. 189～190°, $[\alpha]_D^{21}$ −58.2°(c=1.110, MeOH).*11 *Anal.* Calcd. for $C_{14}H_{19}O_5N$: C, 59.77; H, 6.81; N, 4.98. Found : C, 59.84; H, 6.71; N, 5.16. IR ν_{max}^{KBr} cm^{-1}; 3390, 1725, 1631, 1565, 1517. The IR spectrum was completely superimposable with (+)-Ⅲc.

(−)-2-Amino-2-methyl-4-benzyloxybutyric Acid ((−)-IIa)——A solution of (−)-Ⅲa (m.p. 151～153°, $[\alpha]_D$ −6.7°(c=2.46, MeOH)) in 20 w/w% NaOH (25 g.) was refluxed for 40 hr., and then neutralized with 10% HCl. An insoluble substance was filtered off and the filtrate was concentrated to ca. 10 ml. *in vacuo*, to which MeOH (50 ml.) was added, and crystals separated were filtered off. The filtrate was diluted with the same volume of H_2O, and the solution was poured through the column (Amberlite IR-120, H^+-form 66 ml.). The column was washed with H_2O until eluates became neutral and eluted with ca. 10% NH_4OH until ninhydrin test became negative. The eluates were combined and evaporated to dryness to give a white solid (0.56 g.) which was submitted to a cellulose powder column chromatography (20 g.) employing EtOH–conc. NH_3–H_2O (4:1:1) as eluting solvent system, 25 ml. each of the eluted fraction was collected and the fourth fraction was evaporated to dryness to give a solid (0.41 g.). Recrystallization from MeOH–acetone afforded (−)-Ⅱa as colorless crystals (0.22 g., 26%), m.p. 244°(decomp.) $[\alpha]_D^{25}$ −15.9° (c=1.02, H_2O). Further recrystallization from H_2O gave small needles m.p. 254°(decomp.), $[\alpha]_D^{25}$ −16.9° (c=0.886, H_2O). *Anal.* Calcd. for $C_{12}H_{17}O_3N$: C, 64.55; H, 7.68; N, 6.27. Found : C, 64.66; H, 7.76; N, 6.49. IR ν_{max}^{KBr} cm^{-1} : 3040～2920, 1642, 1611, 1570, 1531, 738, 695. The IR spectrum was different from that of DL-Ⅱa. Paper chromatograms by two different solvent systems showed one spot respectively. Rf value*12 0.81 (solvent A), 0.75 (solvent B). These values were identical with those of racemic compound.

(−)-α-Methylhomoserine ((−)-VII)——A mixture of (−)-Ⅲa (m.p. 148.5～150.5°, $[\alpha]_D^{22}$ −7.6° (c=1.60, MeOH)) (0.60 g., 0.0023 mole) and 48% HBr (20 ml.) was refluxed for 9 hr. and diluted with the same volume of H_2O and extracted with benzene (20 ml.) to remove benzyl alcohol. Aqueous layer was evaporated to dryness to give reddish brown oil which was dissolved in H_2O (20 ml.). The solution was poured through the column of ion exchanger (Amberlite IR-120, H^+-form, 26 ml.). The column was washed with H_2O until eluates became neutral, and then eluted with ca. 10% NH_4OH until ninhydrin test became negative. The eluates were combined and evaporated to dryness to give a white residue (0.35 g.). Two recrystallizations from H_2O–EtOH–ether afforded (−)-Ⅶ (0.17 g.) as colorless needles, m.p. 213.5～216°, $[\alpha]_D^{27}$ −15.3° (c=0.90, H_2O). *Anal.* Calcd. for $C_5H_{11}O_3N$-1/2H_2O: C, 42.24; H, 8.51; N, 9.84. Found : C, 42.50; H, 8.52; N, 10.21. IR ν_{max}^{KBr} cm^{-1} : 3460, 3240, 1656, 1640, 1617, 1589, 1052. Its IR spectrum was different from that of DL-α-methylhomoserine.*13 Paper chromatograms with two different solvent systems showed one spot respectively. Rf value*12 0.30 (solvent A), 0.37 (solvent B). These values were identical with those of racemic compound.*13

(−)-α-Methyl-β-(3,4-dihydroxyphenyl)alanine ((−)-VIII)——A mixture of (−)-Ⅲc (m.p. 188～189°, $[\alpha]_D^{27}$ −50.5°(c=1.10, MeOH)) (1.2 g., 0.0043 mole) in 48% HBr (24 ml.) was refluxed for 8 hr. in N_2 atmosphere and evaporated to dryness *in vacuo* under N_2 stream to give a reddish brown oil. On adding H_2O (10 ml.), H_2O was repeatedly evaporated *in vacuo* to give an amber residue which was dissolved in H_2O (12 ml.) and adjusted the pH around 6.4 with aq. NH_4OH. SO_2 was passed into the solution to decolorize, it was evaporated *in vacuo* to ca. 10 ml. in N_2 atmosphere and again adjusted to pH 6.4 with aq. NH_4OH to separate a crude (−)-Ⅷ (0.60 g. 59%), m.p. >250°. The crude product was suspended in H_2O and dissolved by passing SO_2 into the solution. After filtrating the insoluble material, the filtrate was evaporated *in vacuo* in N_2 atmosphere to ca. 3 ml. and kept standing overnight to yield white powder (0.40 g.) m.p. >250°, $[\alpha]_D^{24}$ −1.5°(c=0.904, *N* HCl), $[\alpha]_D^{13}$ −16.5°(c=0.379, H_2O),*14,*15 (lit.,[7] m.p. 299.5～300°(decomp.), $[\alpha]_D^{25}$ −4°(c=1, *N* HCl), lit.,[5] m.p. 306～308° (decomp.) $[\alpha]_D^{25}$ −4°(c=2, 0.1 *N* HCl), $[\alpha]_D^{25}$ −0.1° (c=2, *N* HCl), $[\alpha]_D^{25}$ −14° (c=2, pH=6.5 phosphate)). *Anal.* Calcd. for $C_{10}H_{13}O_4N \cdot 1\frac{1}{2}H_2O$: C, 50.41; H, 6.77; N, 5.89. Found : C, 50.66; H, 6.88; N, 5.81. IR ν_{max}^{KBr} cm^{-1}: 3540, 3250, 1661, 1635, 1604, 1566. Its IR spectrum was very similar to that of DL-α-methyl-3,4-dihydroxyphenylalanine,*16 but not superimpo-

*11 *a*) In ref. 7, $[\alpha]_D$ value of this compound was reported to be $[\alpha]_D$ −56°(c=1, MeOH), m.p. being not described. *b*) In ref. 5, this compound was reported having m.p. 192～194° and $[\alpha]_D^{25}$ −55°(c=1, MeOH).

*12 solvent A, BuOH–AcOH–H_2O(4:1:2), solvent B, BuOH–Pyridine–H_2O(1:1:1).

*13 Racemic compound m.p. 228°(decomp.), was prepared according to the method of H. Brockmann, *et al.* (Ber., 87, 856 (1954). lit., m.p. 228°(decomp.)).

*14 Absolute configuration of this compound was proposed as *L*-series from optical properties. cf. ref. 5. On the other hand, absolute configuration was established chemically as *S*- or *L*-series in our laboratory. cf. ref. 15.

*15 This optical activity was calculated from the optical rotatory dispersion chart measured with a Spectrophotometer model ORD/UV-5. Japan Spectroscopic Co., Ltd.

sable. Paper chromatograms with different two solvent systems showed one spot in each case Rf value*12 0.37 (solvent A), 0.58 (solvent B).

(+)-**α-Methyl-β-(3,4-dimethoxyphenyl)alanine Hydrochloride** ((+)-**IIc-HCl**)——A mixture of (−)-IIIc (m.p. 187~188°, $[\alpha]_D^{12}$ −52.0°(c=1.25, MeOH)) (10.0 g., 0.036 mole) and ca. 12% HCl (150 ml.) was refluxed for 3 hr., cooled and kept in an ice bath for 2 hr. to separate (+)-IIc-HCl (8.2 g. 79%) as colorless needles, m.p. 169~172°(decomp.), $[\alpha]_D^{13}$ +8.3°(c=0.840, MeOH). Repeated recrystallization from dil. HCl afforded colorless needles m.p. 169~171°(decomp.)[21] $[\alpha]_D^{14}$ +8.8°(c=1.026, MeOH).*17 *Anal.* Calcd. for $C_{12}H_{18}O_4NCl \cdot H_2O$: C, 49.06; H, 6.86; N, 4.77. Found : C, 49.25; H, 6.86; N, 4.74. IR ν_{max}^{KBr} cm^{-1} : 3470, 3380, 3120, 1753, 1591, 1521.

(−)-**α-Methyl-β-(3,4-dimethoxyphenyl)alanine Hydrochloride** ((−)-**IIc-HCl**) **and** (−)-**α-Methyl-β-(3,4-dimethoxyphenyl)alanine** ((−)-**IIc**)——A mixture of (+)-IIIc (m.p. 188~189°, $[\alpha]_D^{32}$ +50.3° (c=1.18, MeOH)) (0.85 g., 0.0030 mole) and 12% HCl (15 ml.) was refluxed for 3 hr., and treated as above to give (−)-IIc-HCl as colorless needles (0.73 g., 82%), m.p. 167~169°(decomp.), $[\alpha]_D^{20}$ −9.0°(c=1.04, MeOH). Recrystallization from dil. HCl gave colorless needles, m.p. 169~171°(decomp.),[21] $[\alpha]_D^{17}$ −8.8°(c=0.844, MeOH). *Anal.* Calcd. for $C_{12}H_{18}O_4NCl \cdot H_2O$: C, 49.06; H, 6.86; N, 4.77. Found : C, 48.79; H, 6.67; N, 4.74. IR ν_{max}^{KBr} cm^{-1} : 3550, 3440, 3130, 1756, 1611, 1591, 1518.

(+)-IIc-HCl thus obtained, was dissolved in H_2O and aq. solution was passed through the ion exchanger column (Amberlite IR 120 H^+-form) which was washed with H_2O until eluate became neutral. The column was eluted with ca. 10% NH_4OH. Eluates were combined and evaporated to dryness to give crude (−)-IIc. Repeated recrystallizations from H_2O yielded white powder m.p. 256~258°(decomp.), $[\alpha]_D^{25}$ −2.6°(c=1.01, *N* HCl).*18 *Anal.* Calcd. for $C_{12}H_{17}O_4N \cdot 1\frac{1}{2}H_2O$: C, 54.08; H, 7.51; N, 5.25. Found : C, 54.72, H, 7.56, N, 5.43. IR ν_{max}^{KBr} cm^{-1} : 3500, 3240, 1647, 1624, 1600, 1583, 1514. Its IR spectrum was almost superimposable with that of DL-IIc.

Paper chromatograms with two different solvent systems*12 showed a single spot in each case. Rf value of (−)-IIc-HCl, 0.59 (solvent A), 0.61 (solvent B), and of (−)-IIc, 0.60 (solvent A), 0.62 (solvent B).

(−)-**Isovaline** ((−)-**IIb**)——A mixture of VIb (m.p. 110~111.5°, $[\alpha]_D^{20}$ −54.3°(c=0.652, MeOH)) (0.96 g., 0.0030 mole) and 48% HBr (12 ml.) was refluxed for 6 hr., after adding H_2O (24 ml.) the solution was extracted with ether (20 ml. × 3) to remove *l*-menthol and benzoic acid. Aqueous layer was evaporated to dryness to give a yellowish white solid (1.0 g.). The residue was dissolved in H_2O (20 ml.) and poured through the ion exchanger column (Amberlite IR-120 H^+-form 71 ml.), which was washed with H_2O until eluates became neutral. Amino acids was eluted from a column with ca. 7% NH_4OH, until no ninhydrin-test was identified in the eluates. The eluates were combined and evaporated to dryness to give a white solid. Recrystallization from H_2O-EtOH afforded (−)-IIb as colorless needles of monohydrate (0.22 g., 61%), m.p.>250°, $[\alpha]_D^{21}$ −11.9°*19 (c=0.786, H_2O) (lit.,[1] $[\alpha]_D^{19}$ +11.0° (c=8.9, H_2O) as *d*-isovaline, lit.,[8] $[\alpha]_D^{25}$ −11.28° (c=5, H_2O) as D-isovaline). IR ν_{max}^{KBr} cm^{-1} : 3520, 3230, 3070, 1642, 1619, 1589, 1557. Its IR spectrum was quite superimposable in that of DL-IIb. Rf value*12 0.46 (solvent A), 0.45 (solvent B).

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Summary

New resolution method of α-methyl-α-amino acid has been accomplished by way of *l*-menthyl ester *i.e.* (−)-IIa, (−)-IIb, (+)and (−)-IIc-HCl, (−)-VII and (−)-VIII were obtained in pure state from DL-IIa, IIb, and IIc by way of the scheme shown in Chart 1 and 2.

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*16 This compound was prepared from hydrolysis of DL-IIIc. cf. ref. 27.

*17 Absolute configuration of (−)-VIII has been chemically established as *S*-series (ref. 15), therefore the absolute configuration of (+)-IIc-hydrochloride in methanol proved to be manifested by way of (−)-IIIc as *S*-series.

*18 Absolute configuration of (−)-IIc in *N* HCl proved to be manifested as *R*-series on the relationship between (+)-IIc-HCl as *S*-series and (−)-IIc-HCl as *R*-series.

*19 Absolute configuration of this compound was established as *R*-series, cf. ref 15.