[Chem. Pharm. Bull. 15(11)1776~1784(1967)]

UDC 547.466.2.02.07 : 541.653

228. Norio Takamura, Shiro Terashima, Kazuo Achiwa, and Shun-ichi Yamada*1 : Studies on Optically Active Amino Acids. XV.*2 Studies on α-Alkyl-α-amino Acids. VIII.*3 Absolute Configuration of Optically Active α-Methylserine and 2-Amino-2-methyl-3-butenoic Acid.*4

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[1])

The absolute configuration of (+)-α-methylserine ((+)-IX) obtained from natural source, has been elucidated to be S-configuration by the chemical correlation with (+)-isovaline ((+)-VII), at the same time the absolute configuration of (+)-2-amino-2-methyl-3-butenoic acid ((+)-XI) has also been proved to be S-series. Chemical schemes employed were shown in Charts 1 and 2. Optically active hydantoins (V and VIII) used in the correlation were prepared by the direct resolution of racemic hydantoins with brucine. Preliminary examinations using racemic compounds were also reported.

(Received March 9, 1967)

In preceding papers the absolute configuration of isovaline,[1] α-methylaspartic acid,[2] α-methyl-3,4-dihydroxyphenylalanine,[2a,3] α-methylphenylalanine hydrochloride[4] and α-methylphenylglycine[5] were reported by our laboratory.

In connection with the establishment of absolute configurations of these amino acids, the purpose of this paper is to present the absolute configuration of α-methylserine and 2-amino-2-methyl-3-butenoic acid.

α-Methylserine is an optically active α-methyl-α-amino acid[6a] first obtained from natural source. This amino acid occurs as a component of an antitubercular antibiotic, amicetin,[6] produced by *Streptomyces vinaceusdrappus* and by *Streptomyces fasciculatis*. α-Methylserine isolated from amicetin showed $[\alpha]_D^{22.5}$ +6.3° (C=1.0 in water). Greenstein and his collaborators proposed the absolute configuration of (+)-α-methylserine with properties identical to those of the above mentioned bacterial product, as D-isomer[7,8] since N-chloroacetyl derivative of (+)-α-methylserine resisted to hydrolyze its chloroacetyl group by L-directed acylase I enzyme,[7b] and the absolute configuration of D-isomer proposed by them was written as I in the conventional Fischer diagram[7a] without convincing evidence.

---

*1 Hongo, Bunkyo-ku, Tokyo (高村則夫, 寺島孜郎, 阿知波一雄, 山田俊一).

*2 Part XIV. This Bulletin, **15**, 1749 (1967).

*3 Part VII. *Ibid.*, **15**, 1749 (1967).

*4 This work was presented at the 86th Annual Meeting of Pharmaceutical Society of Japan, October, 1966, Sendai.

1) *a*) S. Yamada, K. Achiwa : This Bullentin, **12**, 1525 (1964). *b*) K. Achiwa, S. Yamada : *Ibid.*, **14**, 537 (1966).

2) *a*) S. Yamada, S. Terashima, K. Achiwa : *Ibid*, **13**, 227 (1965). *b*) S. Terashima, K. Achiwa, S. Yamada: *Ibid.*, **14**, 572 (1966).

3) S. Terashima, K. Achiwa, S. Yamada : *Ibid.*, **14**, 579 (1966).

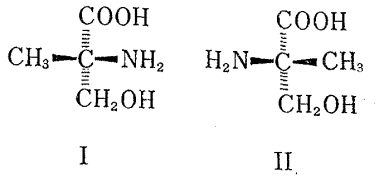
4) *Idem* : *Ibid.*, **14**, 1138 (1966).

5) H. Mizuno, S. Terashima, K. Achiwa, S. Yamada : *Ibid.*, **15**, 1749 (1967).

6) *a*) E.H. Flynn, J.W. Hinman, E.L. Caron, D.O. Woolf, Jr. : J. Am. Chem. Soc., **75**, 5867 (1953). *b*) S. Hanessian, T.H. Haskell : Tetrahedron Letters, **36**, 2451 (1964).

7) *a*) J.P. Greenstein, M. Winitz : "Chemistry of the Amino Acids" Vol. 1, 743 (1961). John Wiley and Sons, New York. *b*) *Ibid.*, Vol. 3, 2572 (1961).

8) J.P. Greenstein, M.L. Anson, K. Bailey, J.T. Edsall : Advances in Protein Chemistry, Vol. 9, 185 (1954) Academic Press, Inc., New York.



I II

Fig. 1.

On the other hand, Wilson and Snell[9] contradicted Greenstein's conclusion, and based on their findings that (+)-α-methylserine was synthesized from D-alanine by enzyme, α-methylserine-hydroxymethyltransferase, they suggested the absolute configuration of (+)-α-methylserine as II on the assumption that stereochemical inversion did not occur during the synthesis of (+)-α-methylserine from D-alanine. They gave the further support on their conclusion from the optical properties of α-methyl-α-amino acids.

These two studies for the determination of absolute configuration of α-methylserine from biochemical point of view gave different conclusions. It is impossible to assign the absolute configuration of α-alkyl-α-amino acids by biochemical methods, different from the case of naturally occuring α-amino acids having α-hydrogen atom, since α-alkyl-α-amino acids have two main skeletons, and they may be considered to belong either to the D or L-family of amino acids.[1b]

We undertook the chemical correlation between optically active α-methylserine and isovaline whose absolute configuration was clearly established,[1] aiming at the establishment of absolute configuration of α-methylserine. Correlation scheme is outlined in

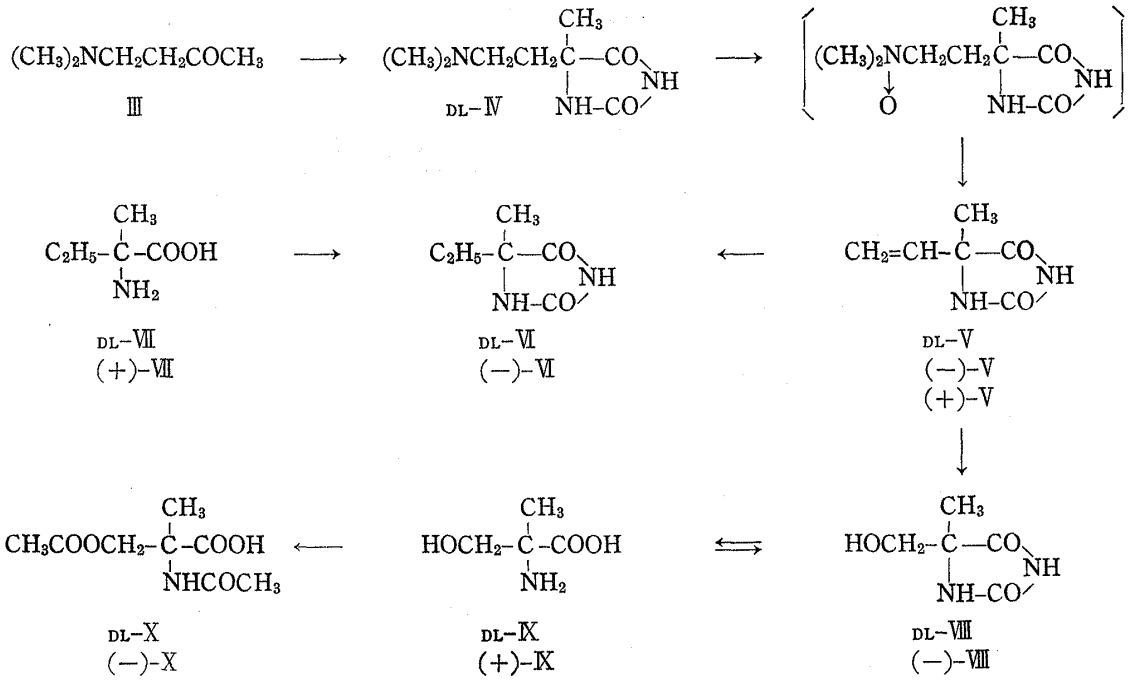


Chart 1.

Chart 1 involving the resolution of hydantoin derivatives. The reaction of 1-dimethylamino-3-butanone (III) obtained by Mannich reaction,[10] with potassium cyanide and ammonium carbonate according to Bucherer's reaction[11] gave DL-5-(2-dimethylaminoethyl)-5-methylhydantoin (DL-IV) in 59% yields. Oxidation of DL-IV with 30% hydrogen peroxide in methanol yielded N-oxide, thermal decomposition of which in N_2 atmosphere under reduced pressure yielded crude DL-5-methyl-5-vinylhydantoin (DL-V) which was purified by chromatogram over silicic acid to give pure DL-V in 70% yields. Independent

9) E.M. Wilson, E.E. Snell: J. Biol. Chem., **237**, 3180 (1962).
10) E.C. Spaeth, T.A. Geissman, T.L. Jacobs: J. Org. Chem., **11**, 399 (1946).
11) H. T. Bucherer, V.A. Lieb: J. prakt. Chem., **141**, 5 (1934).

synthesiis of DL-V from the quaternary ammonium salt of DL-Ⅳ using Hofmann degradation was unsuccessful. In the course of this reaction sequence, the resolution of racemic mixture was required. We succeeded in resolving racemic hydantoin derivative, 5-methyl-5-vinylhydantoin (DL-V), into its optically active components by means of brucine according to somewhat modified reported method.[12] Brucine forms a less soluble salt with the (+)-V than with (−)-isomer in alcohol. Several recrystallizations of a less soluble brucine salt from alcohol were unsuccessful to purify the salt. Partially resolved (+)-V, $[\alpha]_D^{23}$+4.1°(C_2H_5OH) was obtained by releasing hydantoin from a less soluble salt with hydrochloric acid. Purification of this partially resolved (+)-V by recrystallizations with ethyl acetate gave pure (+)-V, m.p. 165~166°, $[\alpha]_D^{16}$+37.8° (C_2H_5OH). Similarly, pure antipodal hydantoin (−)-V, m.p. 165~166°, $[\alpha]_D^{16}$−38.1° (C_2H_5OH) was prepared from mother liquor giving a first less soluble salt, with the same procedure as above.

Another attempt to prepare optically active V from DL-V was carried out according to the 1-menthyl ester method[13] reported from our laboratory. The reaction scheme employd is shown in Chart 2.

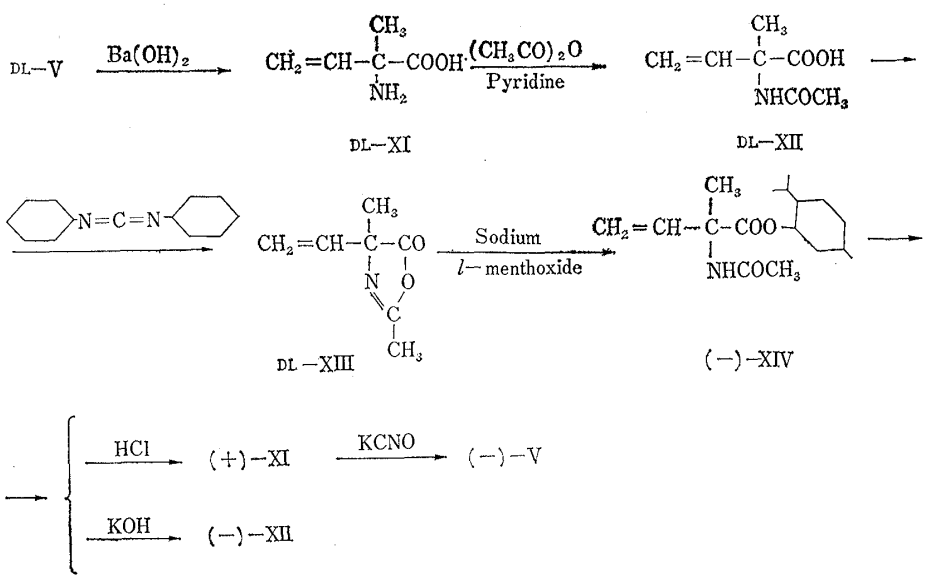


Chart 2.

DL-2-Amino-2-methyl-3-butenoic acid (DL-Ⅺ) prepared from the hydrolysis of DL-V with barium hydroxide was treated with acetic anhydride and pyridine as usual to give DL-2-acetamido-2-methyl-3-butenoic acid (DL-Ⅻ) which was cyclized to yield DL-2,4-dimethyl-4-vinyl-2-oxazolin-5-one (DL-XIII) in a moderate yield by the reflux with dicyclohexyl carbodiimide in acetonitrile. Reaction of DL-XIII with sodium 1-menthoxide in anhyd. benzene gave a mixture of two diastereoisomers, which was fractionally recrystallized several times from a mixture of benzene and *n*-hexane to give pure one isomer (−)-1-menthyl 2-acetamido-2-methyl-3-butenoate ((−)-XIV), m.p. 152~152.5°, $[\alpha]_D^{22}$−69.0° (C_2H_5OH). Pure (−)-XIV obtained above was hydrolyzed by the reflux with aqueous hydrochloric acid to give optically pure amino acid, (+)-Ⅺ, m.p. 264°, $[\alpha]_D^{27}$+33.0° (H_2O), which was converted to (−)-V, m.p. 165~166°, $[\alpha]_D^{27}$−37.3° (C_2H_5OH) by the treatment with potassium cyanate. On the other hand, reflux of (−)-XIV with potassium hydroxide in 50% aqueous ethanol gave (−)-Ⅻ, m.p. 149~150°, $[\alpha]_D^{31}$−25.4° (C_2H_5OH), in 75% yield.

12) H. Sobotka, M.F. Holzman, J. Kahn: J. Am. Chem. Soc., **54**, 4697 (1932).
13) S. Terashima, K. Achiwa, S. Yamada: This Bulletin, **13**, 1399 (1965).

Thus, optically active (−)-V could be prepared through the 1-menthyl ester method[13] by way of optically active (+)-XI, and the $[\alpha]_D$ value of (−)-V and (+)-XI thus obtained might be considered to be the purest one since these two compounds were prepared by the separation and purification of diastereoisomers XIV and no racemization took place in the following reaction sequence. The absolute configuration of (+)-XI has also been correlated to that of (−)-V by these reactions.

Reduction of (−)-V ($[\alpha]_D^{22}$ −10.2° (C_2H_5OH)) in hydrogen atmosphere over platinum oxide gave (−)-VI, m.p. 170~171.5°, $[\alpha]_D^{20}$ −23.2° (C_2H_5OH), which was found to be identical with the authentic sample, m.p. 172.5~173°, $[\alpha]_D^{25}$ −22° (C_2H_5OH) obtained from (+)-VII,[14] $[\alpha]_D^{27}$ +11.3° (H_2O) and potassium cyanate by mixed melting point and comparison of IR spectra. Subsequently, ozonolysis of (−)-5-methyl-5-vinylhydantoin ((−)-V) with ozone followed by reduction with sodium borohydride gave (−)-5-hydroxymethyl-5-methylhydantoin ((−)-VIII), m.p. 190~191°, $[\alpha]_D^{21}$ −2.9° (C_2H_5OH) in poor yields. Specific rotation of (−)-VIII was small, therefore optical rotatory dispersion measurement of (−)-VIII was carried out and negative plain curve was evidently observed. On the other hand, attempt to resolve DL-VIII with brucine in ethanol was also successful to give optically active VIII. Brucine salt with (+)-VIII gave a less soluble salt, recrystallization of which from ethanol was very difficult to yield optically pure salt. Accordingly, after treatment of the partially resolved salt of (+)-VIII with 10% sodium hydroxide and then ion exchange resin (Amberlite IR-120, H^+-form), partially active (+)-VIII, $[\alpha]_D^{19}$ +14.1° (C_2H_5OH) was obtained. Mother liquor from which first less soluble brucine salt was collected by filtration, was treated similarly to the case of (+)-VIII to give crude (−)-VIII, $[\alpha]_D^{21}$ −14.3° (C_2H_5OH), which was purified successively by recrystallization from ethanol, column chromatography using silica gel and finally repeated recrystallizations from ethanol-hexane to give pure (−)-VIII, m.p. 168~171°, $[\alpha]_D^{18}$ −45.9° (C_2H_5OH). Optical rotatory dispersion curve of pure (−)-VIII obtained from resolution of DL-VIII with brucine showed the same tendency as that of (−)-VIII from (−)-V.

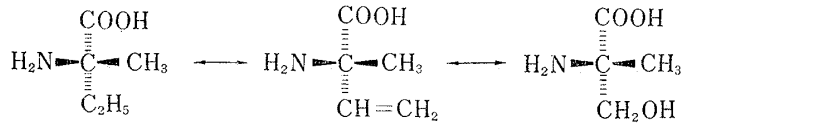
Hydrolysis of (−)-VIII in barium hydroxide solution gave (+)-α-methylserine ((+)-IX), m.p. 264°, $[\alpha]_D^{10}$ +4.7° (H_2O) after purification by chromatography over cellulose powder and recrystallization from water-ethanol-ether. This compound showed positive plain curve on optical rotatory dispersion measurement, and exhibited one single spot on paper chromatograms with two different solvent systems. The Rf values of these spots were identical with those of the authentic DL-IX[15] developed with the same solvent systems. In order to confirm this amino acid, (−)-N, O-diacetyl-α-methylserine was also prepared from (+)-IX by usual method.

Thus the absolute configuratiou of (+)-α-methylserine ((+)IX) was correlated to (+)-isovaline ((+)-VII) through (−)-5-methyl-5-vinylhydantoin ((−)-V) and (+)-α-methylserine has proved to belong to S-series as shown in Chart 3. This result is consistent with the Wilson and Snell proposal. Moreover, judging from the enzymatic finding by Wilson and Snell, we could suggest the absolute configuration of (−)-α-ethylserine as S-series with considerable certainty.

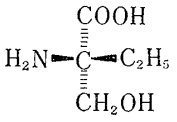
On the course of the establishment of absolute configuration of α-methylserine, the absolute configuration of (+)-2-amino-2-methyl-3-butenoic acid ((+)-XI) has also been proved to be S-series (Chart 3).

Prior to experiments with optically active compounds, racemic modifications were used to find out the favorable reaction conditions and to identify the optical active compounds by comparison with racemates. The experimental data on racemic compounds were described in detail in Experimental Part.

14) S. Akabori, T. Ikenaka, K. Matsuki: Nippon Kagaku Zasshi, **73**, 112 (1952).
15) T.T. Otani, M. Winitz: Arch. Biochem. Biophys., **90**, 254 (1960).



S(+)−Isovaline S(+)−2−Amino−2−methyl−3−butenoic acid S(+)−α−Methylserine



S(−)−α−Ethylserine

Chart 3.

Experimental*5

1-Dimethylamino-3-butanone (III)——A mixture of dimethylamine hydrochloride (245 g., 3 moles), water (480 ml.), conc. hydrochloric acid (12 ml.), acetone (1110 ml., 15 moles) and 37% formaldehyde solution (228 ml., 3 moles) was refluxed for 15 hr. The clear solution obtained was concentrated *in vacuo* to give a pale brown syrup, to which was added NaOH (150 g., 3.75 moles) dissolved in water (240 ml.). The alkaline solution was extracted with ether (150 ml. × 4). The combined ether extracts were washed with satd. NaCl and then dried over Na_2SO_4. Filtration and evaporation gave an oil, which was submitted to distillation to give colorless liquid (157 g., 45%), b.p.$_{40}$ 69~71° (lit.[10] b.p.$_{40}$ 70°). Picrate, m.p. 104.5~105° (lit.[16] m.p. 107°). Semicarbazone HCl, m.p. 154° (decomp.). *Anal.* Calcd. for $C_7H_{17}ON_4Cl$: C, 40.28; H, 8.21; N, 26.85. Found: C, 40.11; H, 8.17; N, 26.60. $IR\nu_{max}^{KBr}$ cm^{-1}: 3470,3170, 2550, 2465,1695, 1592, 1483, 1121, 964, 764.

DL-5-(2-Dimethylaminoethyl)-5-methylhydantoin (DL-IV)——A mixture of III (115 g., 1 mole), 95% potassium cyanide (82 g., 1.2 moles) and ammonium carbonate (317 g., 3.3 moles) in water (750 ml.) and EtOH (750 ml.) was stirred at 55° for 12 hr., and then warmed up to 75° to remove excess ammonium carbonate from the reaction mixture. The bright yellow solution was concentrated *in vacuo* to remove EtOH, seeded and kept standing in a refrigerator overnight to crystallize out DL-IV (110 g., 59%), m.p. 180~181°. Several recrystllizations from ethanol afforded colorless prisms, m.p. 181°. *Anal.* Calcd. for $C_8H_{15}O_2N_3$: C, 51.87; H, 8.16; N, 22.69. Found: C, 52.03; H, 8.10; N, 22.41. $IR\nu_{max}^{KBr}$ cm^{-1}: 3030, 2810, 2790, 2740, 1766, 1712.

DL-5-Methyl-5-vinylhydantoin (DL-V)——To a cooled suspension of DL-IV (74.1 g., 0.4 mole) in MeOH (150 ml.) was added slowly (five minutes) 30% hydrogen peroxide (123 ml., 1.2 moles) under stirring and ice cooling. The mixture was stirred for 11 hr. at room temperature and then kept standing overnight. The excess hydrogen peroxide was decomposed by the addition of a small amount of platinum black and stirring for 1.5 hr. under cooling in an ice bath. The platinum black was filtered off and the filtrate was evaporated to dryness *in vacuo* to afford N-oxide as a white solid, which was dried over P_2O_5 overnight. This N-oxide was submitted to thermal decomposition. A round flask containing N-oxide obtained above was placed in an oil bath preheated to 180° under a nitrogen atmosphere at 7 mm. Hg. The temperature of an oil bath was gradually raised to 200° (15 min.), and being kept between 200° and 210° for 40 min. until evolution of N,N-dimethylhydroxylamine ceased. The reddish brown residue cooled was dissolved in acetone (20 ml.)-ethyl acetate (60 ml.), and purified on column chromatography using silica gel (500 g., solvent: ethyl acetate). The fractions required were combined and evaporated to dryness *in vacuo* to give crude DL-V as pale yellow needles (39 g., 70%), m.p. 122~124°. Three recrystallizations from ethyl acetate afforded colorless needles, m.p. 129°. *Anal.* Calcd. for $C_6H_8O_2N_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.33; H, 5.64; N, 20.08. $IR\nu_{max}^{KBr}$ cm^{-1}: 3220, 1773, 1740, 1717, 1638, 995, 927.

Resolution of DL-5-Methyl-5-vinylhydantoin (DL-V) into Its Optically Active Components by Brucine——A mixture of DL-V (14.0 g., 0.1 mole) and brucine (35.5 g., 0.09 mole) in EtOH (250 ml.) was warm-

*5 All melting points are uncorrected. IR spectra measurements were performed with a Spectrometer, Model DS-402. Japan Spectroscopic Co., Ltd. Optical activities were measured with a Yanagimoto Photo Direct Reading Polarimeter, Model OR-20. Optical Rotatory dispersion curve measurements were carried on with a Spectrophotometer Model ORD/UV-5, Japan Spectroscopic Co., Ltd.

16) I.N. Nazarov, S.A. Vartanyan, S.G. Matsoyan, V.N. Zhamagortsyan: Zhur. Obscheĭ Khim., **23**, 1986 (1953) (Chem. Abstr., 3002h (1955)).

ed to become homogeneous. The EtOH solution was seeded*6 and kept standing in a refrigerator to crystallize out the brucine salt of (+)-V as prisms (27.8 g.), m.p. 157~160°, $[\alpha]_D^{16}$ −65.2° (C=0.840, EtOH), which was dissolved in 10% aq. HCl (50 ml.). The acidic aqueous solution was extracted with ethyl acetate (50 ml. × 4) and the combined extracts were washed with satd. NaCl, dried over Na_2SO_4, and evaporated to dryness *in vacuo* to give (+)-V as pale yellow needles (4.7 g.), m.p. 125~133°, $[\alpha]_D^{23}$ +4.1° (C=1.650, EtOH). Crude (+)-V obtained above was recrystallized several times from ethyl acetate to give pure (+)-V as colorless needles, m.p. 165~166°, $[\alpha]_D^{16}$ +37.8° (C=0.650, EtOH). *Anal.* Calcd. for $C_6H_8O_2N_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.50; H, 5.64; N, 19.67. IRν_{max}^{KBr} cm^{-1}: 3210, 1772, 1739, 1715, 1637, 993, 926.

The mother liquor from which the brucine salt of (+)-V crystallized out was evaporated to dryness to give a yellow oil, which was dissolved in 10% aq. HCl (50 ml.). The acidic aqueous solution was treated similarly to the above to give (−)-V (5.4 g.), m.p. 125~133°, $[\alpha]_D^{18}$ −5.7°(C=1.236, EtOH). Several recrystallizations from ethyl acetate afforded pure (−)-V as colorless needles, m.p. 165~166°, $[\alpha]_D^{16}$ −38.1° (C=0.536, EtOH). *Anal.* Calcd. for $C_6H_8O_2N_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.71; H, 5.85; N, 19.77. IRν_{max}^{KBr} cm^{-1}: 3215, 1773, 1740, 1715, 1638, 995, 927. This IR spectrum was superimposable with those of the authentic DL-V and (+)-V.

DL-5-Ethyl-5-methylhydantoin (DL-VI)——a) DL-VI from DL-5-methyl-5-vinylhydantoin (DL-V): A mixture of DL-V (1.40 g., 0.01 mole) and PtO_2 (200 mg.) in EtOH (50 ml.) was hydrogenated at room temperature and atmospheric pressure. After the theoretical amount of H_2 gas was absorbed, the catalyst was filtered and washed with EtOH. The combined ethanolic filtrate and washings were evaporated to dryness *in vacuo* to give a white solid (1.39 g., 98%), m.p. 137~139°. Several recrystallizations from H_2O and then from AcOEt–hexane afforded colorless needles, m.p. 141°. *Anal.* Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.94; H, 6.94; N, 19.58. IRν_{max}^{KBr} cm^{-1}: 3210, 1770, 1737, 1716.

b) DL-VI from DL-isovaline (DL-VII): A mixture of DL-VII (2.70 g., 0.02 mole), obtained according to the method reported by Levene and Steiger,[17] and potassium cyanate (2.60 g., 0.032 mole) in H_2O (40 ml.) was refluxed for 3 hr. The reaction mixture was cooled, acidified with conc. HCl and again refluxed for one hour. The whole was poured through column (Amberlite IR-120, H^+-form, 300 ml.). The column was eluted with H_2O. The eluates showing pH value from 1 to 7 were combined and evaporated to dryness to give pale yellow solid (1.11 g., 39%), m.p. 135~137°. Several recrystallizations from H_2O and then from ethyl acetate–hexane afforded colorless needles, m.p. 141°. The mixed melting point with an authentic DL-VI obtained from DL-V (see a)) showed no depression. *Anal.* Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.69; H, 7.02; N, 19.84. IRν_{max}^{KBr} cm^{-1}: 3215, 1770, 1733, 1715. This IR spectrum was superimposable with that of the authentic DL-VI (see a)).

(−)-5-Ethyl-5-methylhydantoin ((−)-VI)——a) (−)-VI from (−)-5-methyl-5-vinylhydantoin ((−)-V): (−)-V (0.70 g., 0.005 mole, m.p. 127.5~140°, $[\alpha]_D^{22}$ −10.2° (C=1.210, EtOH)) was reduced similary to the case of DL-V to give (−)-VI as a white solid (0.68 g., 96%), m.p. 138.5~150°, $[\alpha]_D^{19}$ −9.5° (C=0.972, EtOH). Crude (−)VI was recrystallized successively from water, ethyl acetate and then ethyl acetate–hexane to afford colorless needles, m.p. 170.5~171.5°, $[\alpha]_D^{20}$ −23.2° (C=0.630, EtOH). *Anal.* Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.51; H, 7.06; N, 19.90. IRν_{max}^{KBr} cm^{-1}: 3215, 1773, 1736, 1713. This IR spectrum was superimposable with that of DL-VI.

b) (−)-VI from S(+)-isovaline ((+)-VII): (+)−VII (1.0 g., 0.0074 mole, m.p. >280°, $[\alpha]_D^{27}$ +11.3° (C=0.900, H_2O))*7 was treated similarly to the case of DL-VII and then purified on column chromatography using silica gel (40 g., solvent: ethyl acetate) to give a white solid (0.13 g., 13%), m.p. 172.5~173°, $[\alpha]_D^{31}$ −22.9° (C=1.014, EtOH). Twice recrystallizations from ethyl acetate–hexane afforded colorless needles, m.p. 172.5~173°, $[\alpha]_D^{25}$ −22.0° (C=0.436, EtOH). This (−)-VI showed no depression on the mixed melting point with the authentic (−)-VI (see a)). *Anal.* Calcd. for $C_6H_{10}O_2N_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.65; H, 7.11; N, 19.89. IRν_{max}^{KBr} cm^{-1}: 3215, 1770, 1734, 1715. This IR spectrum was identical with that of the authentic (−)-VI (see a)).

DL-5-Hydroxymethyl-5-methylhydantoin (DL-VIII)——a) DL-VIII from DL-5-methyl-5-vinylhydantoin (DL-V): Through a solution of DL-V (2.8 g., 0.02 mole) in EtOH (30 ml.) cooled in an ice–NaCl bath was passed a stream of ozone in oxygen. The reaction was followed by the thin-layer chromatography using silica gel. After DL-V was consumed completely, $NaBH_4$ (8 g., 0.2 mole) dissolved in cold 50% aq. EtOH (100 ml.) was added slowly to the stirred reaction mixture at 10°. The mixture was stirred at room temperature for 1 hr. and then warmed at 65° for 1.5 hr. The whole was acidified with conc. HCl to destroy excess $NaBH_4$, and concentrated to remove ethanol. A small amount of water was added to the residue, and the whole was poured through the column (Amberlite IR-120, H^+-form, 200 ml.). The column was eluted with H_2O. The elu-

*6 The seeds were prepared by the following procedure. Another homogeneous ethanol solution was evaporated to dryness and the viscous oil obtained was triturated in an ice bath to give white crystals, which was recrystallized several times from ethanol to give a partially resolved brucine salt of (+)-V as prisms.

*7 N-Formyl-DL-isovaline was resolved by brucine and hydrolyzed to S(+)-isovaline as colorless needles of monohydrate according to the method of Akabori, *et al.*[14]

17) P.A. Levene, R.E. Steiger: J. Biol. Chem., **76**, 299 (1928).

ates showing pH value from 1 to 7 was combined, and evaporated to dryness to give reddish brown residue. Addition of MeOH to the residue, and evaporation to dryness was repeated to remove H_3BO_3. The residue obtained was purified on column chromatography using silica gel (50 g., solvent : ethyl acetate–acetone). The fractions required were combined evaporated to dryness *in vacuo* to give a pale yellow semisolid, which was triturated with EtOH to give a colorless solid (0.32 g., 11%), m.p. 190～191°. Several recrystallizations from ethanol–ether afforded pure DL-VIII as colorless prisms, m.p. 191°. *Anal.* Calcd. for $C_5H_8O_3N_2$: C, 41.66; H, 5.59; N, 19.44. Found : C, 41.76; H, 5.64; N, 19.36. IRν_{max}^{KBr} cm^{-1} : 3290, 3195, 3060, 1767, 1730, 1060.

b) DL-VIII from DL-α-methylserine (DL-IX) : A mixture of DL-IX (53.6 g., 0.45 mole) and potassium cyanate (58.3 g., 0.72 mole) in H_2O (600 ml.) were refluxed for 2 hr. The cooled mixture was acidified with conc. HCl and refluxed again for 30 min. The whole was evaporated to dryness *in vacuo* to give sirupy residue, which was dissolved in water (200 ml.) and poured through column (Amberlite IR-120, H^+-form, 3000 ml.). The column was eluted with H_2O. The eluates showing pH vallue from 1 to 7 was combined and evaporated to dryness to give a pale yellow solid, which was recrystallized from EtOH to give DL-VIII as colorless prisms (19.6 g., 30%), m.p. 189～190°. Several recrystallizations from EtOH afforded colorless prisms, m.p. 191°. This sample showed no depression on the mixed melting point with the authentic DL-VIII (see a)). *Anal.* Calcd. for $C_5H_8O_3N_2$: C, 41.66; H, 5.59; N, 19.44. Found : C, 41.89; H, 5.74; N, 19.16. IRν_{max}^{KBr} cm^{-1} : 3295, 3200, 3060, 1769, 1735, 1062. This IR spectrum was superimposable with that of the authentic DL-VIII (see a)).

(−)-5-Hydroxymethyl-5-methylhydantoin (−)-VIII——(−)-V (2.8 g., 0.02 mole, m.p. 128～144°, $[\alpha]_D^{15}$ −10.7° (C=0.826, EtOH)) was treated as same as DL-V to give colorless solid (0.27 g., 9.4%), m.p. 180～189°, $[\alpha]_D^{14}$ −13.2° (C=1.186, EtOH). Twice recrystallizations from EtOH–ether efforded (−)-VIII as colorless prisms, m.p. 190～191°, $[\alpha]_D^{21}$ −2.9° (C=1.086, EtOH). This sample showed m.p. 190～191° on a mixed melting point with the authentic DL-VIII and m.p. 168～190° with the authentic (−)-VIII (see below, m.p. 168～171°, $[\alpha]_D^{18}$ −45.9° (C=0.606, EtOH)). *Anal.* Calcd. for $C_5H_8O_3N_2$: C, 41.66; H, 5.59; N, 19.44. Found : C, 41.76; H, 5.53; N, 19.57. IRν_{max}^{KBr} cm^{-1} : 3295, 3200, 3060, 1769, 1738, 1729, 1062. This IR spectrum was very similar but not identical with that of DL-VIII. Optical rotatory dispersion measurement : $[\alpha]^{22.5}$ (C=1.086, EtOH) (mμ) : −41.4° (270), −19.9° (300), −10.1° (350), −6.1° (400), −4.1° (450), −2.8° (500), −2.0° (589). This optical rotatory dispersion curve showed the same tendency as that of the authentic (−)-VIII (see below).

Resolution of DL-5-Hydroxymethyl-5-methylhydantoin (DL-VIII) into Its Optically Active Components by Brucine——A mixture of DL-VIII (2.9 g., 0.02 mole) and brucine (7.9 g., 0.02 mole) in EtOH (60 ml.) was warmed until solution occurred, and was kept in a refrigerator, the brucine salt of (+)-VIII (6.1 g.) crystallized out as prisms, m.p. 166.5～189°, $[\alpha]_D^{24}$ −65.9° (C=1.096, EtOH), to which was added water (30 ml.) and NaOH (1 g.) dissolved in H_2O (5 ml.). The brucine separated was filtered and washed with water. The combined filtrate and the washings, were concentrated and poured onto a column (Amberlite IR-120, H^+-form, 50 ml.). The column was eluted with H_2O (200 ml.). The eluate was evaporated to dryness to give crude (+)-VIII as a pale yellow solid (0.9 g.), m.p. 165～188°, $[\alpha]_D^{19}$ +14.1° (C=1.080, EtOH).

The mother liquor from which the first brucine salt of (+)-VIII was obtained was evaporated to dryness to give a yellow oil, which was treated as same as above to give (−)-VIII (1.41 g.), m.p. 171～190°, $[\alpha]_D^{21}$ −143° (C=0.996, EtOH). One recrystallization from EtOH afforded white crystals (0.90 g.), m.p. 191°. $[\alpha]_D^{22}$ −4.4° (C=0.856, EtOH). The mother liquor of this recrystallization from EtOH was evaporated to dryness to give a solid, which was purified on column chromatography using silica gel (50 g., solvent : acetone–ethyl acetate). The fractions containing (−)-VIII only were evaporated to dryness to give white crystals (0.20 g.), m.p. 166.5～168°. $[\alpha]_D^{16}$ −46.3° (C=0.704, EtOH). Further twice recrystallizations from EtOH–hexane afforded pure (−)-VIII, m.p. 168～171°, $[\alpha]_D^{18}$ −45.9° (C=0.606, EtOH). *Anal.* Calcd. for $C_5H_8O_3N_2$: C, 41.66; H, 5.59; N, 19.44. Found : C, 41.88; H, 5.78; N, 19.55. IRν_{max}^{KBr} cm^{-1} : 3260, 3048, 1744, 1710, 1061. This IR spectrum was not identical with that of DL-VIII. Optical rotatory dispersion measurement : $[\alpha]^{24}$ (C=0.606, EtOH) (mμ) : −1716 (250), −354 (300), −186 (350), −115 (400), −78.4 (450), −57.7 (500), −41.7 (589).

DL-α-Methylserine (DL-IX) and Its O,N-Diacetyl Derivative (DL-X)——a) from DL-alanine : According to the method reported by Otani and Winitz,[15] DL-IX was obtained from alanine and formaldehyde in 74% yield, m.p. 255° (decomp.). *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found : C, 40.29; H, 7.62; N, 11.77. IRν_{max}^{KBr} cm^{-1} : 3370, 3050, 1609, 1529, 1462, 1413, 1370, 1062. Paper chromatograms with two different solvent systems showed one spot respectively. Rf value 0.16 (solvent A),*8 0.29 (solvent B).*8

DL-IX was treated with Ac_2O–pyridine as usual to give DL-X in 91% yield. Recrystallizations from EtOH and then three times from EtOH–hexane afforded colorless pillars, m.p. 183°. *Anal.* Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45; N, 6.89. Found : C, 47.34; H, 6.46; N, 6.78. IRν_{max}^{KBr} cm^{-1} : 3355, 1745, 1715, 1614, 1551, 1262, 1052.

b) from DL-5-hydroxymethyl-5-methylhydantoin (DL-VIII) : A mixture of DL-VIII (2.88 g., 0.02 mole), $Ba(OH)_2 \cdot 8H_2O$ (31.6 g., 0.1 mole) and water (100 ml.) was refluxed for 50 hr. $(NH_4)_2CO_3$ (14.4 g., 0.15 mole) was added to the reaction mixture and the precipitated $BaCO_3$ was removed by filtration, the filtrate was concentrated and

*8 Solvent A : *n*-BuOH–EtOH–2*N*·NH_4OH (5:1:2). Solvent B : *n*-BuOH–HCOOH–H_2O (14:3:3).

poured onto a column (Amberlite IR 120, H^+-form, 200 ml.) to take up the basic product completely. The column was washed thoroughly with water and then eluted with dil. NH_4OH. Fractions being positive for the ninhydrin test were collected and evaporated to dryness *in vacuo* to give a yellowish brown solid, which was recrystallized from H_2O-EtOH to give white crystals (1.27 g., 53%), m.p. 253~254° (decomp.). Twice recrystallizations from H_2O-EtOH afforded white crystals, m.p. 255° (decomp.), *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.15; H, 7.63; N, 11.65. IRν_{max}^{KBr} cm^{-1}: 3370, 3050, 1607, 1528, 1461, 1414, 1371, 1063. This IR spectrum was superimposable with that of the authentic DL-IX (see a)). Paper chromatograms with two different solvent systems showed one spot respectively. Rf value 0.17 (solvent A),*8 0.29 (solvent B).*8

This DL-IX was treated with Ac_2O-pyridine to give DL-X as colorless pillars, m.p. 182~182.5° in 92% yields. The mixed melting point with the authentic DL-X (see a)) showed no depression. *Anal.* Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.35; H, 6.59; N, 6.64. IRν_{max}^{KBr} cm^{-1}: 3355, 1745, 1713, 1614, 1550, 1261, 1052. This IR spectrum was identical with that of the authentic DL-X (see a)).

(+)-α-Methylserine ((+)-IX) and Its O,N-Diacetyl Derivative ((−)-X)——(−)-VIII (2.20 g., 0.153 mole, m.p. 165~171°, $[\alpha]_D^{15}$ −37.5° (C=0.886, EtOH)) was treated similary to the case of DL-VIII to give a yellow solid (1.71 g., 94%), m.p. 241° (decomp.), $[\alpha]_D^{15}$ +5.5° (C=1.198, H_2O), which was purified on column chromatography using cellulose powder (80 g., solvent: *n*-BuOH 5 : EtOH 1 : 4.15*N* NH_4OH 2) to give a white solid, m.p. 242° (decomp.), $[\alpha]_D^{12}$ +3.7° (C=0.696, H_2O). A part of this solid was recrystallized twice from H_2O-EtOH-ether to give (+)-IX as small white needles, m.p. 264° (decomp.), $[\alpha]_D^{10}$ +4.7° (C=0.888, H_2O). *Anal.* Calcd. for $C_4H_9O_3N$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.50; H, 7.65; N, 11.76. IRν_{max}^{KBr}: 3395, 3110, 1647, 1614, 1572, 1537, 1410, 1066, 1058. This IR spectrum was different from that of DL-IX. Optical rotatory dispersion measurement: $[\alpha]^{11}$ (C=0.888, H_2O) (mμ): +248° (230), +121° (250), +45.1° (300), +24.8° (350), +15.8° (400), +10.7° (450), +7.9° (500), +5.1° (589), +2.8° (700). Paper chromatograms with two different solvent systems showed one spot respectively. Rf value 0.18 (solvent A),*8 0.30 (solvent B).*8 These values were identical with those of racemic compound.

Another part of (+)-IX (0.5 g., m.p. 242° (decomp.), $[\alpha]_D^{12}$ +3.7° (C=0.696, H_2O) was treated with Ac_2O-pyridine as usual to give (−)-X as white crystals (0.64 g., 75%), m.p. 166~169°. $[\alpha]_D^{13}$ −27.7° (C=0.860, EtOH). Twice recrystallizations from EtOH-hexane afforded white crystals, m.p. 169.5~171.5°, $[\alpha]_D^{10}$ −32.5° (C=0.916, EtOH). *Anal.* Calcd. for $C_8H_{13}O_5N$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.39; H, 6.47; N, 6.71. IRν_{max}^{KBr} cm^{-1}: 3315, 1725, 1626, 1535, 1265, 1218, 1056. This IR spectrum was not identical with that of DL-X.

DL-2-Amino-2-methyl-3-butenoic Acid (DL-XI) and Its Hydrochloride Salt——A mixture of DL-V (21 g., 0.15 mole), $Ba(OH)_2 \cdot 8H_2O$ (237 g., 0.75 mole) and water (600 ml.) was refluxed for 50 hr. under stirring and then treated similarly to the case of DL-VIII to give colorless prisms (12.0 g., 70%), m.p. 256° (decomp. with sublimation). Several recrystallizations from H_2O-EtOH afforded colorless prisms, m.p. 256° (decomp. with sublimation). *Anal.* Calcd. for $C_5H_9O_2N$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.86; H, 8.04; N, 12.37. IRν_{max}^{KBr} cm^{-1}: 3035, 1633, 1582, 1535, 1356, 998, 925.

DL-XI (0.40 g.) obtained above was dissolved in 10% HCl (20 ml.) and concentrated to dryness *in vacuo* to give DL-XI hydrochloride as a yellowish white solid (0.52 g., 100%). m.p. 224~224.5° (decomp.). Twice recrystallizations from EtOH-ether afforded white crystals, m.p. 226~226.5° (decomp.). *Anal.* Calcd. for $C_5H_{10}O_2NCl$: C, 39.61; H, 6.65; N, 9.24. Found: C, 39.47; H, 6.68; N, 9.38. IRν_{max}^{KBr} cm^{-1}: 3000 (broad), 1741, 991, 926.

DL-2-Acetamido-2-methyl-3-butenoic Acid (DL-XII)——DL-XI was treated with Ac_2O-pyridine to give DL-XII. Recrystallization from ethyl acetate afforded colorless crystals, m.p. 133°, yield, 86%. *Anal.* Calcd. for $C_7H_{11}O_3N$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.30; H, 7.08; N, 8.89. IRν_{max}^{KBr} cm^{-1}: 3330, 1716, 1617, 1540, 999, 930.

DL-2,4-dimethyl-4-vinyl-2-oxazolin-5-one (DL-XIII)——A mixture of DL-XII (55 g., 0.35 mole) and dicyclohexylcarbodiimide (80 g., 0.388 mole) in CH_3CN (550 ml.) was reflued for 9 hr. under stirring. The mixture was filtered and concentrated carefully at atmospheric pressure. The residual oil was submitted to fractional distillation to give colorless liquid (26.6 g., 55%), b.p_{40} 82~83°. IRν_{max}^{Cap} cm^{-1}: 1832, 1692, 1638, 1241, 1054, 990, 938, 904. No band was observed in the range of 3400~3300 cm^{-1}. This was used immediately for the following step.

(−)-1-Menthyl 2-Acetamido-2-methyl-3-butenoate ((−)-XIV)——To a suspension of Na-powder (6.6 g., 0.287 mole) in anhyd. benzene (400 ml.) was added l-menthol (35.8 g., 0.229 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 supernatant and the washings was added a solution of DL-XIII (26.6 g., 0.191 mole) in anhyd. benzene (50 ml.). The whole was stirred for 9 hr. at room temperature and then kept standing overnight to give a clear orange-yellow solution, which was washed successively with 10% AcOH solution (300 ml. × 2), H_2O (300 ml.), 2.5% Na_2CO_3 solution (300 ml.) and H_2O (300 ml.), and then dried over Na_2SO_4. An evaporation of the solvent gave a white solid, which was dissolved in benzene-hexane on warming. The solution was seeded and kept standing to crystallize out crude (−)-XIV (32.5 g.), m.p. 112~135°, $[\alpha]_D^{26}$ −55.8° (C=0.850, EtOH). Seven recrystallizations from benzene-hexane, until specific rotation remained unchanged, afforded pure (−)-XIV as colorless small needles, m.p. 152~152.5°,

$[\alpha]_D^{22}$ −69.0° (C=0.664, EtOH). *Anal.* Calcd. for $C_{17}H_{29}O_3N$: C, 69.11; H, 9.90; N, 4.74. Found: C, 69.27; H, 9.79; N, 4.85. $IR\nu_{max}^{KBr}$ cm^{-1} 3260, 1740, 1640, 1553, 1125, 987, 916.

(+)-2-Amino-2-methyl-3-butenoic Acid ((+)-XI) and Its Hydantoin Derivative (−)-V——A mixture of (−)-XIV (6.0 g., m.p. 151.5~152.5, $[\alpha]_D^{29}$ −70.0° (C=0.980 in EtOH)) and conc. HCl (45 ml.) in H_2O (45 ml.) was refluxed for 9 hr. After addition of H_2O (45 ml.), the solution was extracted with benzene (50 ml.×2) to remove l-menthol. Aqueous layer was evaporated to dryness to give a yellowish white solid. This solid was dissolved in H_2O (20 ml.) and poured through the ion exchanger column (Amberlite IR-120, H^+-form, 100 ml.). The column was washed with H_2O until eluates became neutral, and then eluted with dil. NH_4OH until ninhydrin-test became negative. The eluates were combined and evaporated to dryness to give crude (+)-XI*9 as a pale brown solid (2.0 g.), m.p. 231~232° (decomp. with sublimation). A part of this (+)-XI was recrystallized three times from H_2O-EtOH to give colorless crystals, which was dried completely at 40~50° *in vacuo* to give an analytical sample, m.p. 264° (decomp. with sublimation), $[\alpha]_D^{27}$ +33.0° (C=0.612, H_2O). This sample was hygroscopic. *Anal.* Calcd. for $C_5H_9O_2N$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.41; H, 8.02; N, 11.94. $IR\nu_{max}^{KBr}$ cm^{-1}: 3045, 1664, 1610, 1537, 1360, 998, 939. This IR spectrum was different from that of DL-XI.

A solution of another part of crude (+)-XI (1.2 g.) and potassium cyanate (4.0 g.) in H_2O (30 ml.) were refluxed for 2 hr. The cooled solution was acidified with conc. HCl and refluxed again 30 min., and then extracted with ethyl acetate (40 ml.×3). The combined extracts were washed with satd. NaCl (40 ml.) and dried over Na_2SO_4. An evaporation of the solvent gave (−)-V as a white solid (0.33 g.), m.p. 162~164°, $[\alpha]_D^{33}$ −35.2° (C=0.762, EtOH). Twice recrystallizations from ethyl acetate afforded colorless needles, m.p. 165~166°, $[\alpha]_D^{27}$ −37.3° (C=0.782, EtOH). The mixed melting point with the authentic (−)-V showed no depression. *Anal.* Calcd. for $C_6H_8O_2N_2$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.77; H, 5.78; N, 20.17. $IR\nu_{max}^{KBr}$ cm^{-1}: 3220, 1772, 1740, 1713, 1638, 1427, 994, 926. This IR spectrum was superimposable with that of the authentic (−)-V.

(−)-2-Acetamido-2-methyl-3-butenoic Acid ((−)-XII)——A mixture of (−)-XIV (1.48 g., 0.005 mole, m.p. 151.5~152.5°, $[\alpha]_D^{29}$ −70.0 (C=0.980, EtOH)) and KOH (2.8 g., 0.05 mole) in 50% aq. EtOH (40 ml.) was refluxed for 9 hr., condensed to *ca.* 20 ml., added H_2O (5 ml.) and extracted with benzene (10 ml.×2) to remove l-menthol. An aqueous layer was acidified with conc. HCl, saturated with NaCl and extracted with ethyl acetate (30 ml.×3). The combined extracts were washed with satd. NaCl (20 ml.), dried over Na_2SO_4, and evaporated to dryness to give (−)-XII as a white solid (0.59 g., 75%), m.p. 147~148°, $[\alpha]_D^{29}$ −24.7° (C=0.866, EtOH). Several recrystallizations from ethyl acetate afforded colorless plates, m.p. 149~150°, $[\alpha]_D^{31}$ −25.4° (C=0.794, EtOH). *Anal.* Calcd. for $C_7H_{11}O_3N$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.77; H, 7.15; N, 8.73. $IR\nu_{max}^{KBr}$ cm^{-1}: 3335, 1718, 1618, 1556, 989, 934. This IR spectrum was different from that of DL-XII.

The authors are grateful to the members of the Central Analysis Room of this faculty for elemental analyses and spectral data.

*9 IR spectrum of this sample showed that it contained some crystal water, so the determination of the yield and the specific rotation of this sample was difficult.