[Chem. Pharm. Bull. 14(6) 579~582 (1966)] UDC 547.466.02.07 : 541.653

81. Shiro Terashima, Kazuo Achiwa, and Shun-ichi Yamada : Studies on Optically Active Amino Acids. X.*1 Studies on α-Alkyl-α-amino Acids. V.*2 Absolute Configuration of Biochemically Active (−)-α-Methyl-3,4-dihydroxyphenylalanine.*3

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α-Methyl-3,4-dihydroxyphenylalanine (α-methyl-DOPA) (I) is well known as a hypotensive agent, and the isomer[1,5] with negative rotation in hydrochloric acid, $[\alpha]_D$ −4° (0.1 *N* HCl), is pharmacologically active, whereas the antipode is completely inactive. The former also inhibits the decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) by mammalian decarboxylase, while the latter is inactive.[2,5]

In our previous paper,[3] the absolute configuration of α-methylaspartic acid (V) has been established by the chemical correlation with isovaline, α-methylbutyrine, whose absolute configuration had been chemically correlated to D-(−)-quinic acid[4] in our laboratory.

An attempt has been made to correlate the absolute configuration of α-methyl-DOPA chemically with α-methylaspartic acid. Tristram, *et al.*[5] have suggested that (−)-α-methyl-DOPA ((−)-I) is to be assigned to either the L- or *S*-configuration by its biochemical activities and optical rotatory characteristics. The assignment of the L-configuration for (−)-I from the biological point of view is meaningless from the chemical point of view, since this type of amino acids is written as both an L- and a D-series by Fischer convention.[4] The assignment of (−)-I as L- or *S*-configuration from the optical rotatory point of view was under the assumption that the substitution of methyl group for the α-hydrogen of a naturally occurring L-amino acid does not greatly change the rotatory characteristics, although it was obscure at that time whether or not the Clough-Lutz-Jirgensons rule was applicable to (+)-isovaline, so called "L-isovaline".[3,4,6]

This paper presents an unequivocal correlation of the absolute configuration of (−)-I with (+)-α-methylaspartic acid ((+)-V) aiming at the determination of the absolute configuration of (−)-I. The chemical scheme employed is shown in Chart 1.

In order to find out the optimum reaction conditions each reaction was performed with racemic compounds before using optically active compounds. DL-α-Methyl-3,4-

*1 Part IX : This Bulletin, **14**, 572 (1966).
*2 Part IV : *Ibid.*, **14**, 572 (1966).
*3 A brief report of this work was published as a part of communication to the Editor in This Bulletin : **13**, 227 (1965).
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1) S. Sjoerdsma, S. Udenfriend : Biochem. Pharmacol., **8**, 164 (1961).
2) S. M. Hess, R. H. Connamacher, M. Ozaki, S. Udenfriend : J. Pharmacol. Exptl. Therap., **134**, 129 (1961); C. C. Porter, J. A. Totaro, C. M. Leiby : *Ibid.*, **134**, 139 (1961).
3) S. Yamada, S. Terashima, K. Achiwa : This Bulletin, **13**, 227 (1965); S. Terashima, K. Achiwa, S. Yamada : *Ibid.*, **14**, 572 (1966).
4) S. Yamada, K. Achiwa : *Ibid.*, **12**, 1525 (1964); K. Achiwa, S. Yamada : *Ibid.*, **14**, 537 (1966).
5) E. W. Tristram, J. ten Broeke, E. F. Reinhold, M. Sletzinger, D. E. Williams : J. Org. Chem., **29**, 2053 (1964).
6) J. P. Greenstein, M. Winitz : "Chemistry of the Amino Acids," John Wiley & Sons, Inc., New York. London, 1961, vol. 1, p. 85.

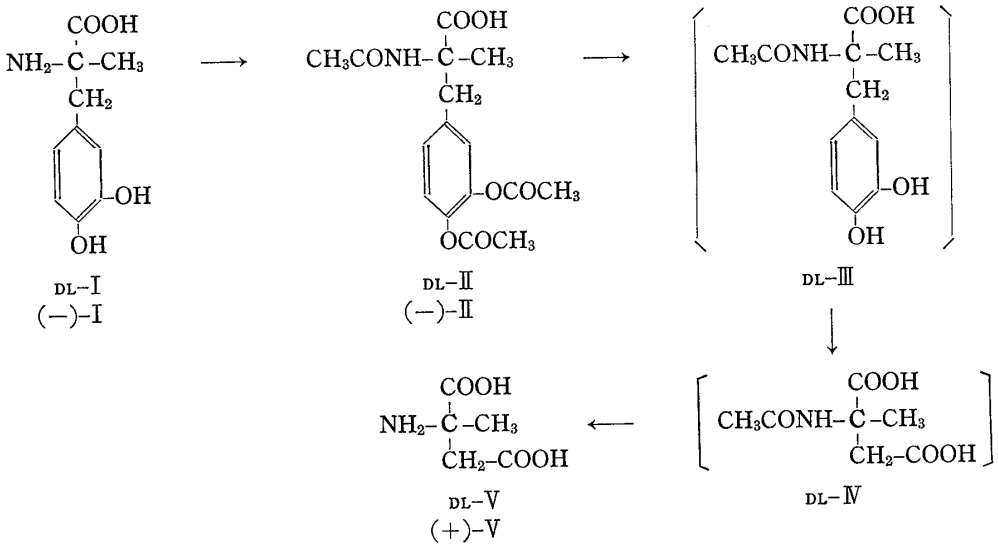

Chart 1.

dihydroxyphenylalanine (DL-I) was treated with acetic anhydride and pyridine to give N-acetyl-DL-α-methyl-3,4-diacetoxyphenylalanine (DL-II) m.p. 187.5~188.5° in a 90% yield. Selective hydrolysis of triacetate DL-II was carried out in dissolving it to an aqueous solution containing three molar equiv. of potassium hydroxide to afford a N-acetyl derivative DL-III which was oxidized with about eight molar equiv. of potassium permanganate without separation. DL-N-Acetyl-α-methylaspartic acid (DL-IV) obtained was hydrolyzed without purification with ca. 12% hydrochloric acid being followed by the purification through a cellulose powder column to afford DL-α-methylaspartic acid hydrochloride (DL-V·HCl). Free DL-V, m.p. 230~232° (decomp.), was obtained as a monohydrate in a 8.6% yield based on DL-II by dissolving the hydrochloride in ethanol and neutralizing with pyridine. The elemental analysis and IR spectrum of DL-V thus obtained agreed with those of an authentic sample[3] and moreover, the Rf values with development of three different solvent systems are consistent with those of an authentic sample.[3]

Following to the racemic compound, the same procedures were performed with optically active compounds. (−)-II, m.p. 180~181°, $[\alpha]_D^{12}$ −94.2° (methanol) was obtained from (−)-I which shows a negative rotation in *N* hydrochloric acid in a 82% yield. (−)-II was led under the same reaction conditions as those for the racemic compounds to (+)-V, m.p. 258° (decomp.), $[\alpha]_D^{13}$ +50.8° (water) in a 9.6% yield based on (−)-II. In this case, the crystals obtained did not contain water of crystallization different from the racemic V, IR spectrum of (+)-V was completely superimposed with that of (+)-V independently prepared by the procedure described in our former paper,[3] and Rf values of (+)-V were in good agreement of those of DL-V developed by three different solvent systems.

Consequently, the absolute configuration of α-asymmetric center of (−)-I was demonstrated to be the same as that of (+)-V, whose absolute configuration had been elucidated by correlating with *S* (+)-isovaline[4] in our laboratory.[3] The relationships of the absolute configuration and optical rotations among (−)-I, (+)-V and (+)-isovaline are shown in Chart 2.

Even though the $[\alpha]_D$ value of (−)-I is extremely small, it was found that it clearly shows a negative rotation in water and also negative in *N*-hydrochloric acid. The assumption proposed by Tristram, *et al.*[5] that Clough-Lutz-Jirgensons rule is applicable to (−)-α-methyl-DOPA, was manifested by the present work although in the case of (+)-isovaline this rule is not applicable.

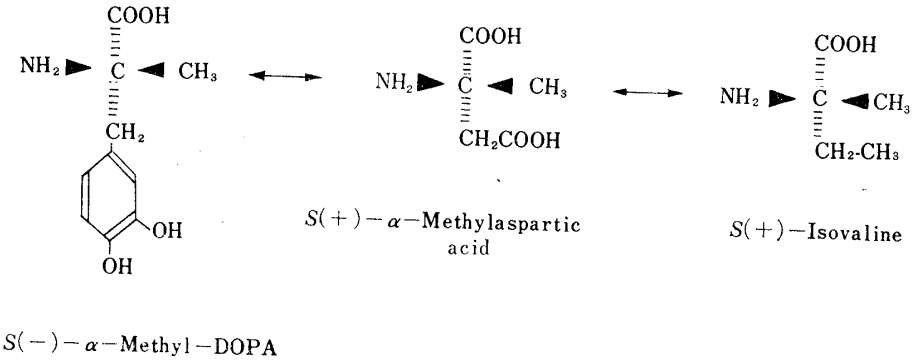

Chart 2.

Experimental[7]

N-Acetyl-DL-α-methyl-3,4-diacetoxyphenylalanine (DL-II)——A suspension of DL-α-methyl-3,4-dihydroxyphenylalanine[8,9] (DL-I) (10.0 g., 0.0474 mole) in Ac_2O (60 ml.) and pyridine (60 ml.) was refluxed for 2 hr. in an oil bath, and evaporated to dryness *in vacuo* after being kept standing overnight. H_2O (50 ml.) was added to the residue and evaporated to dryness *in vacuo*. The pale yellow oil obtained was dissolved in 20% aq. EtOH (45 ml.) on warming. The EtOH solution was acidified with 10% HCl and stimulated. After being kept standing in an ice bath for 5 hr., the filtration and drying gave white crystals (13.7 g., 90%), m.p. 185.5~187.5°. Several recrystallizations from aq. EtOH afforded pure DL-II, m.p. 187.5~188.5° (lit.,[5] m.p. 197~199°). *Anal.* Calcd. for $C_{16}H_{19}O_7N$: C, 56.97; H, 5.68; N, 4.15. Found: C, 56.75; H, 5.55; N, 4.24. IR ν_{max}^{KBr} cm^{-1}: 3290, 1782, 1716, 1640, 1555, 1222.[10] $FeCl_3$ test showed this sample contained no phenolic OH groups.

(−)-N-Acetyl-α-methyl-3,4-diacetoxyphenylalanine ((−)-II)——(−)-α-Methyl-3,4-dihydroxyphenylalanine[3,11] ((−)-I) ($[\alpha]_D^{28}$ −1.3°(c=0.922, *N* HCl)) was treated as described above to afford white powder in a 82% yield, m.p. 173~175°, $[\alpha]_D^{17}$ −93.9°(c=1.37, MeOH). Pure sample was obtained as white powder after several recrystallizations from aq. EtOH. m.p. 180~181°, $[\alpha]_D^{22}$ −94.2°(c=0.716, MeOH) (lit.,[12] m.p. 178~180°, $[\alpha]_D$ −45.6°(acetone); lit.,[13] m.p. not described, $[\alpha]_D^{23}$ −71.7°; lit.,[5] m.p. 181~183°, $[\alpha]_D^{25}$ −74.5° (c=1, 96% EtOH)). *Anal.* Calcd. for $C_{16}H_{19}O_7N$: C, 56.97; H, 5.68; N, 4.15. Found: C, 56.75; H, 5.87; N, 4.36. IR ν_{max}^{KBr} cm^{-1}: 3360, 1767, 1711, 1625, 1550, 1230.[14] This IR spectrum in a solid state was different from that of DL-II.

DL-α-Methylaspartic Acid (DL-V)——To a suspension of DL-II (m.p. 185.5~187.5°) (5.0 g., 0.0148 mole) in H_2O (40 ml.) was added KOH (2.5 g., 0.045 mole) under stirring which was continued for 15 min. at 30~40°, and then $KMnO_4$ (19.0 g., 0.121 mole) was added portionwise to the stirred alkaline solution over a 1.25 hr. The temperature was kept at 40~50°. After the addition of $KMnO_4$, the mixture was stirred for 3 hr. on a water bath, and then kept standing at room temperature overnight after a little amount of $KMnO_4$ (0.5 g.) was added, and then further $KMnO_4$ (0.5 g.) was added to the mixture, which was stirred at ca. 60° for 2 hr. An excess of $KMnO_4$ was converted to MnO_2 using EtOH. MnO_2 was filtered and washed with warm H_2O (ca. 60°). The combined filtrate and the washings were acidified with conc. HCl and evaporated to dryness *in vacuo* to give a mixture of white crystals and yellow oil. This mixture was extracted three times with EtOH (20 ml.) containing conc. HCl (2 ml.). The combined EtOH extract was evaporated to dryness *in vacuo* to give a brown oil (2.6 g.), which was again dissolved in ca. 12% HCl (20 ml.) and the whole was refluxed for 1 hr. After an evaporation to dryness *in vacuo*, the reddish brown oil obtained was dissolved in EtOH (40 ml.) on heating. The EtOH solution was treated twice with charcoal and evaporated to dryness *in vacuo* to afford reddish brown caramel (1.8 g.), which showed two spots (Rf value: 0.05, 0.14)

7) All melting points are uncorrected. IR spectra measurements were performed 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.
8) G. A. Stein, H. A. Bronner, K. Pfister, 3rd.: J. Am. Chem. Soc., **77**, 700 (1955).
9) This sample was supplied us as sesquihydrate from Taisho Pharmaceutical Co., Ltd.
10) Similar but not identical IR spectrum was reported in ref. 5.
11) A sample containing $1\frac{1}{2}H_2O$ was used directly.
12) H. L. Slate, D. Taub, C. H. Kuo, N. L. Wendler: J. Org. Chem., **29**, 1424 (1964).
13) Merck & Co., Inc., South African Patent 61/950 (1962).
14) This IR spectrum was similar but not identical with the one described in ref. 12.

on paper chromatography.[15] A substance of showing Rf value 0.05 was thought to be as DL-V by the comparison with the result of the paper chromatography using authentic DL-V.[3,16] Then, the reddish brown caramel was dissolved in EtOH (ca. 15 ml.), which was submitted to a cellulose powder column chromatography (100 g.) employing *n*-BuOH-EtOH-2*N* NH_4OH (5:1:2) as an eluting solvent. Fractions containing only DL-V were found out using ninhydrin spot test and paper chromatography.[16] All these fractions were combined and evaporated to dryness *in vacuo* to give a reddish brown oil (0.41 g.). A solution of NaOH (0.5 g.) in H_2O (30 ml.) was added to this oil and the resulting alkaline solution was concentrated to one third of the initial volume. After the addition of conc. HCl to make strong acidic pH <1 and the solution was evaporated to dryness, the residue was extracted with EtOH (10 ml. $\times$ 3). The combined EtOH solution was evaporated to dryness *in vacuo* to give a reddish brown oil (0.57 g.), which was dissolved in a mixture of EtOH (14 ml.) and H_2O (1 ml.) and treated with charcoal. Addition of pyridine (20 drops) to the EtOH solution afforded white powder, crude DL-V (0.21 g., 8.6%), m.p. 226~227°(decomp.). Several recrystallizations from H_2O-acetone gave pure DL-V as white small needles, m.p. 230~232° (decomp.) (lit.,[3] m.p. 234~235°(decomp.)). *Anal.* Calcd. for $C_5H_9O_4N \cdot H_2O$: C, 36.36; H, 6.71; N, 8.48. Found : C, 36.25; H, 6.70; N, 8.44. IR ν_{max}^{KBr} cm^{-1} : 3580, 3270, 1697, 1630 (sh), 1611, 1596, 1566 (sh). This IR spectrum was superimposable with that of the authentic DL-V.[3] Paper chromatograms developed by three different solvent systems[17] showed one spot respectively. Rf value : 0.27 (solvent A), 0.24 (solvent B), 0.05 (solvent C). These Rf values were identical with those of the authentic sample[3] developed on the same conditions.

(+)-α-Methylaspartic Acid ((+)-V)——(−)-N-Acetyl-α-methyl-3,4-diacetoxyphenylalanine ((−)-II) (m.p. 173~175°, $[\alpha]_D^{17}$ −93.9°(c=1.37, MeOH)) (5.0 g., 0.0148 mole) was treated as described on DL-II to give crude (+)-V (0.21 g., 9.6%), m.p. 240~241°(decomp.), $[\alpha]_D^{20}$ +45.0°(c=0.484, H_2O). Several recrystallizations from H_2O-acetone gave pure (+)-V as white small needles, m.p. 258°(decomp.), $[\alpha]_D^{13}$ +50.8°(c=0.508, H_2O) (lit.,[3] m.p. 256~257°(decomp.), $[\alpha]_D^{18}$ +49.0°(c=0.518, H_2O)). *Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found : C, 41.04; H, 6.45; N, 9.76. IR ν_{max}^{KBr} cm^{-1} : 3200, 1734, 1641 (sh), 1620, 1605 (sh), 1570. This IR spectrum was completely superimposable with that of the authentic (+)-V reported in the preceeding paper,[3] but different from that of DL-V. Paper chromatograms by three different solvent systems[17] showed single spot respectively. Rf value : 0.29 (solvent A), 0.23 (solvent B), 0.04 (solvent C). These Rf values were identical with those of DL-V developed on the same conditions.

The authors are indebted to the members of the Central Analysis Room of this Faculty for elemental analysis and spectral data, and to Taisho Pharmaceutical Co., Ltd. for the supply of DL-α-methyl-3,4-dihydroxyphenylalanine. A part of this work was supported by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, to which our grateful acknowledgment is made.

Summary

The absolute configuration of biologically active (−)-α-methyl-3,4-dihydroxyphenylalanine has been elucidated to be L- or *S*-configuration by the chemical correlation with (+)-α-methylaspartic acid, whose absolute configuration was confirmed in the previous report. Preriminary examinations using racemic compounds were also described.

(Received September 14, 1965)

15) Solvent *n*-BuOH-EtOH-2*N* NH_4OH (5:1:2).
16) A substance showing Rf value 0.14 was not studied at all.
17) Solvent A : *n*-BuOH-AcOH-H_2O (4:1:2). Solvent B : *n*-BuOH-Pyridine-H_2O (1:1:1). Solvent C : *n*-BuOH-EtOH-2*N* NH_4OH (5:1:2).