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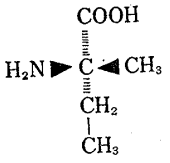
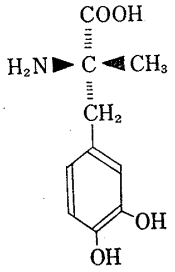
Absolute Configuration of Biochemically Active (−)-α-Methyl-3,4-dihydroxyphenylalanine

α-Methyl-3,4-dihydroxyphenylalanine (α-methyl-DOPA) is well recognized as a hypotensive agent,[1] and the isomer with negative rotation in hydrochloric acid, $[\alpha]_D$ −4° (C=2, 0.1*N* HCl), is biochemically active,[2,3] whereas the antipode is completely inactive.



(+)-I



(−)-II

In a previous communication,[4] we reported the chemical determination of absolute configuration of (+)-isovaline ((+)-I), which is most popular and biochemically very interesting in this field of α-methyl-α-amino acid series. This success prompted us to correlate the absolute configuration of (+)-I to biochemically active (−)-α-methyl-DOPA. After a completion of our work, Tristram, *et al.*[5] have suggested that the (−)-α-methyl-DOPA ((−)-II) is assigned to either the L- or *S*-configuration by its biochemical activity and the rotatory characteristics.

However, their conclusion was based on the assumption that the substitution of methyl group for the α-hydrogen of a naturally occuring L-α-amino acid which is optically active does not change the rotatory characteristics greatly. This communication presents an unequivocal correlation on the absolute configuration between (−)-α-methyl-DOPA and (+)-I.

The chemical scheme which we employed is shown in Chart. 1. (−)-Benzylether ((−)-III), obtained by the resolution of DL-III[6] using menthyl ester method,[7] was reduced over 30% Pd-C in alcohol, followed by refluxing with acetic anhydride-acetic acid to yield the (+)-lactone ((+)-IV) in 55% yield, prisms, m.p. 117~118°, $[\alpha]_D^{25}$ +18.6° (c=1.28, CH_3OH), (*Anal.* Calcd. for $C_7H_{11}O_3N$: C, 53.49; H, 7.05; N, 8.91. Found: C, 53.60; H, 6.85; N, 8.79). Refluxing (+)-IV with sodium thiophenolate in dimethylformamide for 4 hours afforded the thioether ((−)-V), in quantitative yield, white needles, m.p. 198.5~200°, $[\alpha]_D^{20}$ −19.1°, (c=1.40, CH_3OH), (*Anal.* Calcd. for $C_{13}H_{17}O_3NS$: C, 58.52; H, 6.38; N, 5.24. Found: C, 58.48; H, 6.31; N, 5.18.). Desulfurization of (−)-V with Raney nickel in alcohol yielded (−)-N-acetylisovaline ((−)-VI) in 48% yield, m.p. 193~193.5°, $[\alpha]_D^{18}$ −1.3° (c=1.32, CH_3OH), (*Anal.* Calcd. for $C_7H_{13}O_3N$: C, 52.81; H, 8.23; N, 8.80. Found: C, 53.03; H, 8.42; N, 8.66). The infrared spectrum of (−)-VI was identical with that of the authentic sample obtained by acetylation of *S*-(+)-I, whose absolute configuration was definitely established in a previous communication[4] and a mixed melting point with the authentic sample showed no depression. Refluxing (−)-V with 12% hydrochloric acid afforded (+)-2-amino-2-methyl-4-phenylthio butyric acid hydrochloride ((+)-

1) I. A. Oates, L. Gillespie, S. Udenfriend, A. Sjoerdsma: Science, **131**, 1890 (1960).
2) A. Sjoerdsma, S. Udenfriend: Biochem. Pharmacol., **8**, 164 (1961).
3) Merck & Co., Inc., South African Patent, 61/950 (1962).
4) S. Yamada, K. Achiwa: This Bulletin, **12**, 1525 (1964).
5) E. W. Tristram, T. ten Broeke, D. F. Reinhold, M. Sletzinger, D. E. Williams: J. Org. Chem., **29**, 2053 (1964).
6) *a*) J. Murata, H. Arai, M. Tanaka: Kogyo Kagaku Zasshi, **60**, 1206 (1957); *b*) J. Murata, H. Arai: *Ibid.*, **56**, 628 (1953); *c*) U. Shimodoi, M. Sashio, J. Murata: *Ibid.*, **63**, 2140 (1960); *d*) N-acetylation was accomplished by the method reported by A. Neuberger, (Biochem. J., **32**, 1452 (1938)).
7) Presented at the 84th Annual Meeting of Pharmaceutical Society of Japan, April, 1964, Tokyo.

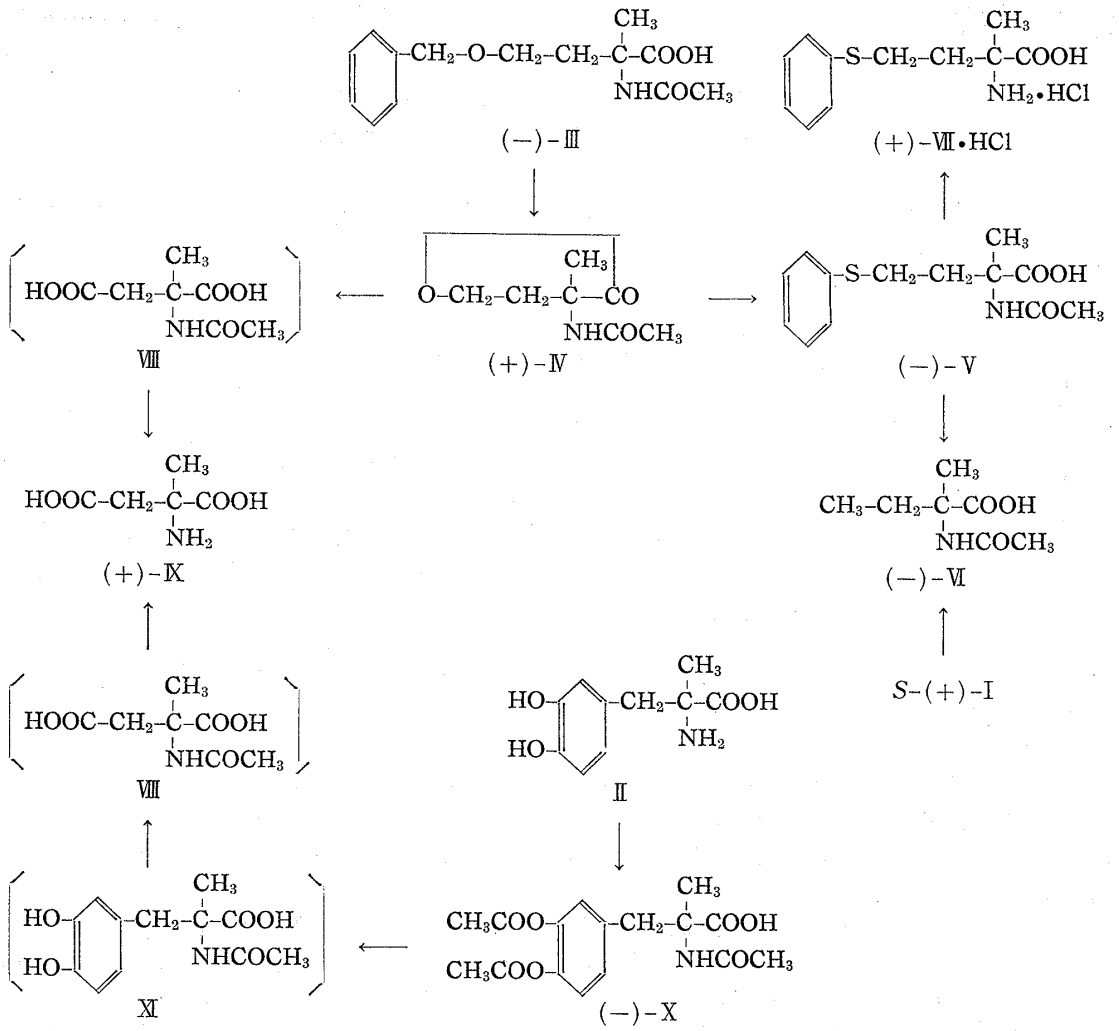


Chart 1.

Ⅶ-HCl) in 92% yield, white crystals, m.p. 251~253° (decomp.), $[\alpha]_D^{20}$ +21.2° (c=0.774, CH_3OH), (*Anal.* Calcd. for $C_{11}H_{16}O_2SNCl$: C, 50.46; H, 6.16; N, 5.35. Found: C, 50.61; H, 6.15; N, 5.18).

The oxidation of (+)-Ⅳ with potassium permanganate in alkaline solution afforded N-acetyl-α-methylaspartic acid (Ⅷ), which was hydrolyzed without separation to give (+)-α-methylaspartic acid ((+)-Ⅸ) in 30% yield from (+)-Ⅳ, white needles, m.p. 256~257° (decomp.), $[\alpha]_D^{18}$ +49.0° (c=0.518, H_2O),[8] (*Anal.* Calcd. for $C_5H_9O_4N$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.98; H, 6.23; N, 9.54.). This (+)-Ⅸ and the authentic DL-Ⅸ[9,10] showed the same Rf values developed by three different solvent systems on paper chromatography.

These results show that the absolute configuration of α-carbon in (+)-Ⅸ is exactly as same as the one in *S*-(+)-I.

The biochemically active (−)-α-methyl-DOPA was treated with acetic anhydride in pyridine to give the (−)-triacetate ((−)-X) in 82% yield, m.p. 180~181°, $[\alpha]_D^{22}$ −94.2° (c=0.716, CH_3OH), (*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.). The hydrolysis of (−)-X in water solution containing 3 moles

8) This $[\alpha]_D$ value is quite different from the value reported, $[\alpha]_D^{23}$ +3.46° (c=19.37, H_2O) by P. Pfeiffer, *et al.* (J. prakt. Chem., **146**, 105 (1936)).
9) H. T. Bucherer, V. A. Lieb: J. prakt. Chem., **141**, 5 (1934).
10) P. Pfeiffer, E. Heinrich: *Ibid.*, **146**, 105 (1936).

of potassium hydroxide gave N-acetyl derivative (Ⅺ), which was later oxidized with potassium permanganate and then hydrolyzed to afford (+)-α-methylaspartic acid ((+)-Ⅸ) in 9.6% yield from (−)-X, white needles, m.p. 258° (decomp.), $[\alpha]_D^{13}$ +50.8° (c= 0.508, H_2O),[8] (*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.). Two optically active α-methylaspartic acids obtained from (+)-Ⅳ and (−)-α-methyl-DOPA showed the identical infrared spectrum, and same Rf values developed by three different solvent systems on paper chromatography.

Accordingly, it was unequivocally proved that the absolute configuration of biochemically active (−)-α-methyl-DOPA was shown to be (−)-Ⅱ, and that both (+)-isovaline and (−)-α-methyl-DOPA belonged to such a *S*-series as the α-hydrogen of L-butyrine and L-DOPA was respectively replaced by methyl group.

Table I. Effect of Acid on the Shift in the Molar Rotation of Optically Active α-Methyl-α-amino Acids

α-Methyl-α-amino acids[a]	$[M]_D$ (*N* HCl)[b] (°C)	$[M]_D$ (H_2O)[b] (°C)	$[M]_D$ (*N* HCl)−$[M]_D$ (H_2O) (°C)
S-Ⅱ	− 3.2	−35.9	+32.7
S-Ⅰ	+ 5.7	+13.8	− 8.1
S-2-Amino-2-methyl-4-benzyloxybutyric acid	+ 5.4	−38.6	+44.0
S-Ⅶ	+67.5	0.0	+67.5
S-Ⅸ	+80.4	+80.4	0.0
S-α-Methylhomoserine[c]	− 7.7	−20.7	+13.0
R-α-Methyl-β-(3,4-dimethoxyphenyl)alanine[d]	− 6.0	+27.8	−33.8

a) Absolute configuration of all compounds have correlated chemically, *S*-series corresponds to L-series of protein-derived L-amino acids in replacing α-methyl group by hydrogen.
b) c=0.25~0.5, temperature at the measurement 15~23°.
c) This amino acid was correlated to (+)-Ⅳ (unpublished).
d) This amino acid was correlated to (−)-Ⅱ (unpublished).

In an attempt to ascertain whether Clough-Lutz-Jirgensons rule would be appliable to these α-methyl-α-amino acids whose absolute configurations were well established by the authors, the changes in the direction of rotation from water to *N* hydrochloric acid were indicated in Table I. An examination of this table disclosed that all the *S*-series amino acids tested revealed a positive value $[M]_D$ (*N* HCl)−$[M]_D$ (H_2O) with an exception of *S*-isovaline and *S*-α-methylaspartic acid. This rule may be applicable to α-methyl-α-amino acids in some cases, when the α-hydrogen of naturally occuring L-amino acids is considered to be replaced by methyl group. However, a general application of this rule to α-methyl-α-amino acid seems to be more limited than the cases of natural L-amino acids, even though in the case reported by Tristram, *et al.*,[5] their assumption has been proved to be correct in this report.

Moreover, the results of rotatory dispersion curve measurement on these *S*-α-methyl-α-amino acids showed that [α] (*N* HCl)−[α] (H_2O) values are continuously positive during the longer wave lengh, from 300 mμ to 700 mμ for *S*-Ⅱ, *S*-Ⅶ, and *S*-2-amino-4-benzyloxy-2-methylbutyric acid, successively negative for *S*-Ⅰ, and negative during the shorter wave length and nearly zero during 450~700 mμ for *S*-α-methylaspartic acid.

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