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109. Shun-ichi Yamada, Tozo Fujii, and Takayuki Shioiri: Studies on Optically Active Amino Acids. I. Preparation of 3-(3,4-Methylenedioxyphenyl)-D-, and -L-alanine.

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For many years the significance of steric factors to biological activities has been realized.[1] Consequently the studies on the absolute configuration of numerous optically active compounds such as amino acids and their related compounds,[2] alkaloids,[3] and other natural and synthetic products, have been extensively made by many researchers.

The hypothetical biogenesis of some alkaloids[4] has prompted the present authors to take an interest in synthesizing optically active alkaloids starting from optically active, biogenetically related amino acids. Thus, the preparation of 3-(3,4-methylenedioxyphenyl)-D-, and -L-alanine which could be starting materials for the syntheses of optically active 1- or 3-substituted 6,7-methylenedioxy-1,2,3,4-tetrahydroisoquinolines was attempted by the following three methods.

The first method adopted was the chemical resolution of N-acetyl-3-(3,4-methylenedioxyphenyl)-DL-alanine (DL-(IV)). 3,4-Methylenedioxybenzyl chloride (II) was allowed to react with diethyl acetamidomalonate (I) as shown in Chart 1 by the method of Snyder, *et al.*,[5] whereby diethyl acetamido(3,4-methylenedioxybenzyl)malonate (III) was obtained in a yield of ca. 60%, which was then converted into (IV) in 87% yield by aqueous sodium hydroxide solution followed by decarboxylation. The condensation of (I) and (II) was also effected with sodium hydride in benzene by the method of Shapira, *et al.*[6] The compound (IV) thus obtained was identical with the one prepared from piperonal and N-acetylglycine, via the azlactone (VI) and α-acetamido-3,4-methylenedioxycinnamic (VII), according to the method of Okuda and Fujii.[7] In order to confirm its structure, (IV) was converted by boiling it with 10% hydrochloric acid solution for 1 hour into 3-(3,4-methylenedioxyphenyl)-DL-alanine (DL-(V))[8] in a yield of 79%.

The resolution of DL-(IV) was effected in a satisfactory manner by means of cinchonine in ethanol and gave two diastereoisomeric salts, the sparingly soluble cinchonine·D-(IV) salt and the easily soluble cinchonine·L-(IV) salt. After recrystallization from ethanol, the former was treated with dil. hydrochloric acid to give D-(IV), m.p. 158~159°,

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1) A.H. Beckett: Angew. Chem., **72**, 686 (1960).
2) *a*) A. Neuberger: Advances in Protein Chem., **4**, 298 (1948). *b*) T. Kaneko: "Chemistry of Proteins," Ed. by S. Akabori, S. Mizushima (in Japanese), Vol. 1, 494 (1954), Kyoritsu Shuppan Co., Tokyo.
3) *a*) R.H.F. Manske (Ed.): "The Alkaloids, Chemistry and Physiology," Vol. 1~6 (1950~1960), Academic Press Inc., New York and London. *b*) E. Leete: Book reviews on Vol. 6 of ref. 3a, J. Am. Chem. Soc., **82**, 4754 (1960).
4) R. Robinson: "The Structural Relations of Natural Products," (1955), Oxford Univ. London and New York.
5) H.R. Snyder, J.F. Shekleton, C.D. Lewis: J. Am. Chem. Soc., **67**, 310 (1945).
6) J. Shapira, R. Shapira, K. Dittmer: *Ibid.*, **75**, 3655 (1953).
7) T. Okuda, Y. Fujii: Bull. Chem. Soc. Japan, **30**, 698 (1957).
8) *a*) C.Gränacher, M. Gerö, A. Ofner, A. Klopfenstein, E. Schlatter: Helv. Chim. Acta, **6**, 465 (1923). *b*) V. Deulofeu, J. Mendive: Z. physiol. Chem., **211**, 1 (1932). *c*) K.S. Narang, J.N. Ray, T.D. Sachdeva: J. Indian Chem. Soc., **13**, 260 (1936). *d*) H. Barry, A.M. Mattocks, W.H. Hartung: J. Am. Chem. Soc., **70**, 693 (1948). *e*) H. Behringer, K. Schmeidl: Chem. Ber., **90**, 2515 (1957). *f*) K. Mori: Nippon Kagaku Zasshi, **81**, 464 (1960).

$[\alpha]_D^{18}$ −53.4° (EtOH), in a yield of ca. 70% based on DL-(IV) used. On the other hand, the latter salt seemed difficult to purify, and so, it was liberated from cinchonine without any purification to give crude L-(IV), which was repeatedly recrystallized from water to obtain pure needles, m.p. 158~159°, $[\alpha]_D^{13}$ +53.4° (EtOH) in a yield of ca. 40% based on DL-(IV) used. The compound (D-(IV)), when refluxed with 10% HCl for 1 hour, afforded 3-(3,4-methylenedioxyhpenyl)-D-alanine (D-(V)), m.p. 235~237° (decomp.), $[\alpha]_D^{20}$ +13.8° (*N* HCl) in a fair yield. The absolute configuration of D-(IV) was proved by converting it into 3-(3,4-dihydroxyphenyl)-D-alanine, the detailed data of which will be described in the succeeding paper.[9]

As the second method, the enzymatic resolution of DL-(IV) was investigated. It is well known that the racemic N-acyl derivatives of many natural α-amino acids[10] as well as those of synthetic α-amino acids[11] smoothly undergo the asymmetric hydrolysis to give L-amino acids and N-acylated D-amino acids using acylase such as that of rat and hog kidney,[10,11d,12] soil bacteria,[11e,13] mold,[14] and commercial amidase preparations.[11j,15]

Kameda, *et al.*[11e] reported that N-benzoyl-3-(3,4-methylenedioxyphenyl)-DL-alanine was asymmetrically hydrolyzed by using a soil bacterial suspension to afford L-(V) and N-benzoylated D-(V), although their data lacked the substantial proof for the absolute configurational assignment to the products.

Among these enzymatic techniques, the usage of Takadiastase[15] which is convenient in a laboratory operation seemed preferable for the asymmetric hydrolysis of N-acylated DL-amino acids. Thus, several conditions which should be optimum to incubate DL-(IV) with Takadiastase were surveyed according to the method reported[15d] by one (S. Y.) of the present authors. The results are shown in the Experimental section. Finally, when the sodium salt of DL-(IV) was incubated at 37° and pH 6.9 (1/15*M* phosphate buffer) for 90~135 hours with its 4/100~8/100 weight of Takadiastase in the presence of Co^{2+} ion[15e,f,23] in a concentration of $10^{-4}M$, the L-amino acid (L-(V)), m.p. 235~237°

9) Part III. S. Yamada, T. Fujii, T. Shioiri: This Bulletin, **10**, 693 (1962).

10) *a*) J. P. Greenstein: Advances in Protein Chem., **9**, 174 (1954). *b*) J. P. Greenstein, S. M. Birnbaum, M. C. Otey: J. Biol. Chem., **204**, 307 (1953). *c*) S. C. J. Fu, S. M. Birnbaum: J. Am. Chem. Soc., **75**, 918 (1953).

11) *a*) J. P. Greenstein, L. Levintow: J. Am. Chem. Soc., **72**, 2812 (1950). *b*) J. P. Greenstein, L. Levintow, C. G. Baker, J. White: J. Biol. Chem., **188**, 647 (1951). *c*) C. G. Baker, A. Meister: J. Am. Chem. Soc., **73**, 1336 (1951). *d*) S. M. Birnbaum, L. Levintow, R. B. Kingsley, J. P. Greenstein: J. Biol. Chem., **194**, 455 (1952). *e*) Y. Kameda, E. Toyoura, H. Yamazoe, Y. Kimura, Y. Yasuda: Nature, **170**, 888 (1952); E. Toyoura: This Bulletin **7**, 787 (1959). *f*) S. M. Birnbaum, J. P. Greenstein: Arch. Biochem. Biophys., **39**, 108 (1952). *g*) L. Berlinguet, R. Gaudry: J. Biol. Chem., **198**, 765 (1952). *h*) S. M. Birnbaum, S.-C. J. Fu, J. P. Greenstein: *Ibid.*, **203**, 333 (1953). *i*) L. Benoiton, M. Winitz, S. M. Birnbaum, J. P. Greenstein: J. Am. Chem. Soc., **79**, 6192 (1957); *Ibid.*, **78**, 2423 (1956). *j*) Y. Murase, K. Okawa, S. Akabori: Bull. Chem. Soc. Japan, **33**, 123 (1960). *k*) M. Jaeger, S. Iskrić, M. Wickerhauser: Croat. Chem. Acta, **28**, 5 (1956). (C. A., **51**, 1840 (1957)).

12) P. J. Fodor, V. E. Price, J. P. Greenstein: J. Biol. Chem., **178**, 503 (1949); S. Utzino, T. Yoneya: Chem. Ber., **85**, 860 (1952).

13) *a*) Y. Kameda, E. Toyoura, Y. Kimura, *et al.*: Yakugaku Zasshi, **78**, 748, 754, 759, 763, 765, 767, 769 (1958), and the earlier works cited therein. *b*) *Idem*: Nature, **182**, 453 (1958). *c*) *Idem*: This Bulletin **7**, 702 (1959). *d*) E. Toyoura: *Ibid.*, **7**, 785, 789 (1959).

14) *a*) K. Michi, H. Nonaka: Nippon Nôgei-Kagaku Kaishi, **28**, 346 (1954). *b*) *Idem*: Bull. Agr. Chem. Soc. Japan, **19**, 153 (1955). *c*) K. Michi, H. Tsuda: *Ibid.*, **21**, 235 (1957). *d*) *Idem*: *Ibid.*, **22**, 283 (1958). *e*) I. Chibata, T. Ishikawa, S. Yamada: *Ibid.*, **21**, 304 (1957). *f*) N. Sugimoto, H. Watanabe, A. Ide: Tetrahedron, **11**, 231 (1960).

15) *a*) C. Neuberg, K. Linhardt: Biochem. Z., **147**, 372 (1924). *b*) C. Hoppert: *Ibid.*, **149**, 510 (1924). *c*) C. Neuberg, I. Mandl: Enzymologia, **14**, 128 (1950). (C. A., **45**, 7163 (1951). *d*) S. Yamada, I. Chibata, S. Yamada: Yakugaku Zasshi, **75**, 113 (1955). *e*) I. Chibata, A. Watanabe, S. Yamada: Bull. Agr. Chem. Soc. Japan, **21**, 291 (1957). *f*) *Idem*: *Ibid.*, **21**, 296 (1957). *g*) S. Utzino, T. Yoneya, T. Murachi, S. Yoshimoto: Ann., **607**, 190 (1957).

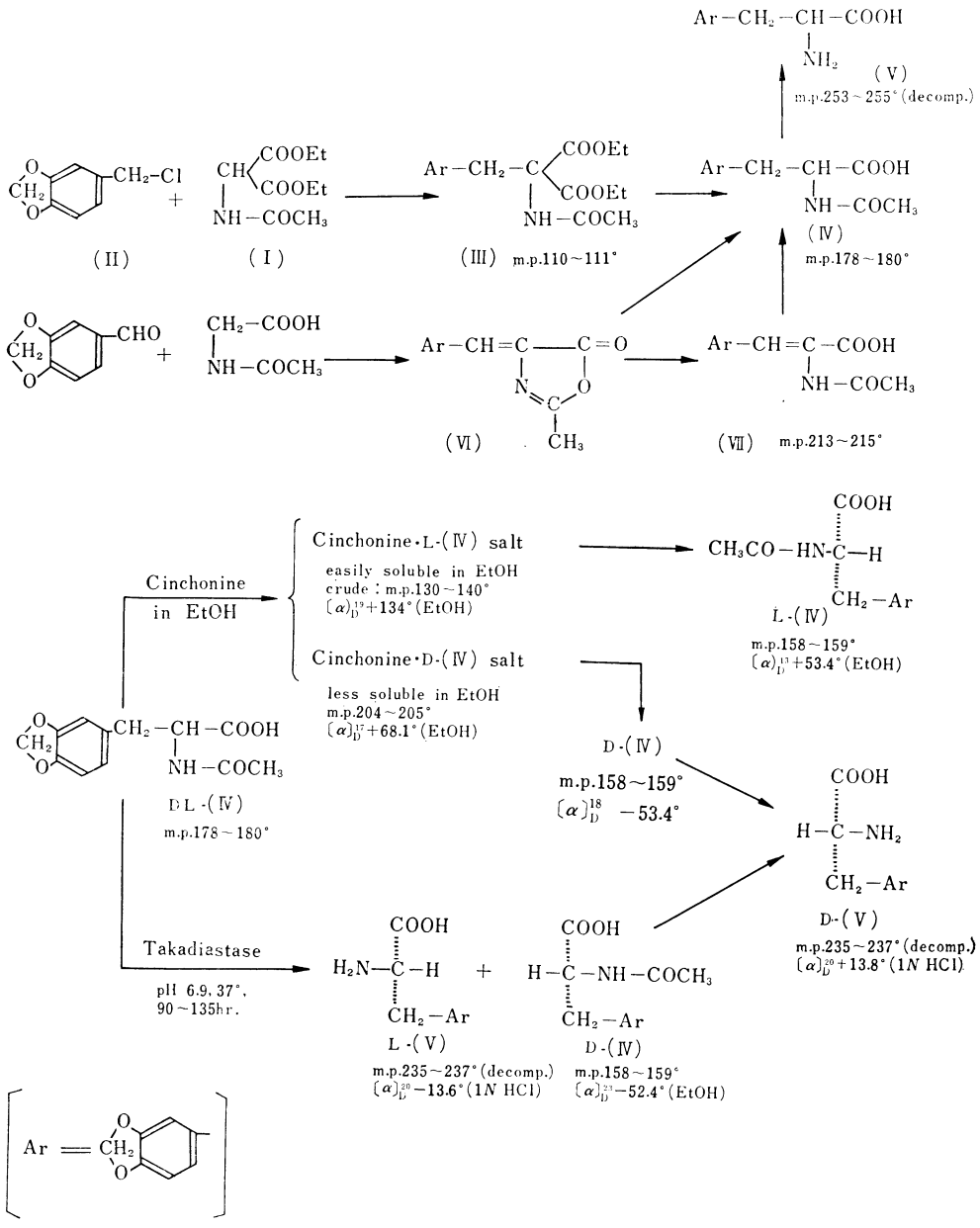


Chart 1.*2

(decomp.), $[\alpha]_D^{20}$ −13.6° (*N* HCl), and the N-acetyl-D-amino-acid (D-(IV)), m.p. 158~159°, $[\alpha]_D^{23}$ −52.4° (EtOH), were obtained in respective yields of 45~47% and 70~74% after one recrystallization. The infrared spectrum of the L-amino acid (L-(V)) thus obtained was superimposable with that of the D-isomer derived from the chemical resolution. The compound (D-(IV)) was also shown to be identical by mixed melting point determination and the infrared spectra comparison with a sample obtained by the chemical

*2 The projection formulas in this Chart conform to the Fischer convention and represent absolute configuration.

resolution, and was convertible into the D-amino acid (D-(V)), m.p. 235~237° (decomp.), $[\alpha]_D^{12}$ +13.8° (*N* HCl), in a fair yield, which was identical with the authentic sample mentioned above. Judging from their specific optical rotation values, the samples of L-, D-(V) and D-(IV), thus obtained, seem to be practically pure, although a biological method of evaluation [10b,16] has not been applied to them.

The absolute configuration of L-(V) and D-(V) obtained by the enzymatic resolution was proved by converting them into L-Dopa and D-Dopa, respectively, the detailed data of which will be reported in the succeeding paper.[9] The above results serve as an additional example to that in which Greenstein, *et al.*[11d] pointed out that the use of the acylases in the resolution of the synthetic amino acids led directly to an identification of the D- and L-enantiomorphs.

As the third method, asymmetric reduction of the *l*-menthyl ester of (VII) is also a method of choice for preparation of the optically active amino acid (V). The result of this scheme will be reported in the following paper.[17]

The preparation of 3-(3,4-methylenedioxyphenyl)-D- and -L-alanine (D- and L-(V)) has now become possible and the investigation of their biological activity and the synthetic scheme starting from them are now under progress.

Experimental*3

3,4-Methylenedioxybenzyl Chloride (II)——This compound (II) was prepared according to Akaboshi and Achiwa's data.[18] To powdered 3,4-methylenedioxybenzyl alcohol[19] (11.2 g.) was added conc. HCl (18 cc.) and the whole was vigorously shaken in a separatory funnel. The crystals of the alcohol went into solution within 3~5 min. After shaking for 20 min., a nearly colorless oil was separated from the upper aqueous layer, and the aqueous layer was extracted twice with benzene. The benzene extracts were combined with the oil and washed with H_2O, satd. $NaHCO_3$ solution, and then satd. NaCl solution, dried over anhyd. Na_2SO_4. The nearly colorless benzene solution thus obtained was evaporated *in vacuo* to leave a slightly yellowish oil (II) which thoroughly solidified to needles on being kept standing at 0° to 3° for 2 days and melted again at room temperature (ca. 20°). Yield, 12.0 g. (95%). The IR spectrum of (II) thus obtained showed no band between 3050~4000 cm^{-1}. The oil (II) was directly used in the next step.

Diethyl Acetamido(3,4-methylenedioxybenzyl)malonate (III)——To a solution of metallic Na (11.4 g.) in anhyd. EtOH (750 cc.) was added diethyl acetamidomalonate*4 (I : 107.4 g.) with stirring to give a slightly yellowish clear solution. When the chloride (II : 84.3 g.) was added dropwise to the above solution, an exothermic reaction occurred and the solution became turbid. After stirring for 2 hr., the whole was heated under reflux and stirring for 12 hr. in an oil bath kept at 100~105°. The reaction mixture was filtered and the precipitate (NaCl) was washed with EtOH. The combined filtrates were evaporated *in vacuo*. The pale yellowish oily residue which solidified on being kept standing was dissolved in benzene (ca. 1 L.), washed with 10% Na_2CO_3 solution, H_2O and dried. On evaporating the benzene solution *in vacuo*, there remained a yellowish oil (153 g.), which solidified on being kept standing in a refrigerator and formed colorless plates (III), m.p. 109~111° after one recrystallization from EtOH (ca. 60 cc.) followed by washing with a small amount of Et_2O. Yield, 111 g. (64%). Repeated recrystallization from EtOH gave (III) as colorless plates, m.p. 110~111°. *Anal.* Calcd. for $C_{17}H_{21}O_7N$: C, 58.11; H, 6.02; N, 3.99. Found : C, 58.00; H, 5.62; N, 4.08. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε) : 236 (3.63), 287 (3.60). UV $\lambda_{min}^{95\%\ EtOH}$ mμ (log ε) : 223 (3.52), 256 (2.65). IR ν_{max}^{Nujol} cm^{-1} : 3315 (NH), 1750, 1726 (COOEt), 1640 (CONH), 1032 (-O-CH_2-O-).

The above condensation was also effected with sodium hydride in benzene.

*3 All m.p.s are uncorrected. For the measurement of optical rotation a "Zeiss Kreispolarimeter" was used. For the paper chromatography the samples were applied on Toyo Roshi No. 50 filter paper and run ascendingly for 17 hr. with a solvent system of BuOH-AcOH-H_2O (4:1:1). Spots were detected as usual by spraying with 5% solution of ninhydrin in Me_2CO. Rf values in this report indicate the values under this condition, unless otherwise stated.

*4 The authors are grateful to Mr. M. Nagata for a donation of this compound.

16) A. Meister, L. Levintow, R. B. Kingsley, J. P. Greenstein : J. Biol. Chem., **192**, 535 (1951).
17) Part II. S. Yamada, T. Shioiri, T. Fujii : This Bulletin, **10**, 688 (1962).
18) S. Akaboshi, K. Achiwa : Unpublished work.
19) D. Davidson, M. T. Bogert : J. Am. Chem. Soc., **57**, 905 (1935).

α-Acetamido-3,4-methylenedioxycinnamic Acid (VII)——A mixture of the crude azlactone[7] (VI: 14.4 g.), Me_2CO (420 cc.) and H_2O (140 cc.) was heated under reflux for 6 hr., and then evaporated to remove the Me_2CO. The yellowish brown residue was extracted four times with boiling water, and the combined extracts (ca. 1.8 L.) were boiled with charcoai, and filtered. After standing in a refrigerator overnight the colorless silky needles that separated were collected and dried. The yield was 9.5 g. (61.3%) of the needles (VII), m.p. 213~215° (reported m.p. 220~221°,[20] 119~120°,[21] 210°[22]), which were found to be identical by admixture and IR comparison with a sample prepared by the method of Heard.[21] The m.p. did not rise after recrystallization from H_2O. *Anal.* Calcd. for $C_{12}H_{11}O_5N$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.61; H, 4.54; N, 5.59. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 230 (shoulder) (4.08), 292 (4.06), 321.5 (4.17). UV $\lambda_{min}^{95\%\ EtOH}$ mμ (log ε): 260 (3.62), 301 (4.02). IR ν_{max}^{Nujol} cm^{-1}: 3290 (NH), 1695 (COOH), 1657 (CONH), 1038, 932 (-O-CH_2-O-).

N-Acetyl-3-(3,4-methylenedioxyphenyl)-DL-alanine (DL-(IV))——i) Reduction of (VII): The unsatd. acid (VII; 14.0 g.) was hydrogenated catalytically over 10% Pd-C (2.0 g.) in 0.5*N* NaOH solution (114 cc.) at room temperature (23°) under atmospheric pressure, 1430 cc. of H_2 being smoothly absorbed in 2 hr. (Required, 1370 cc.) After removing the catalyst, the pale yellowish filtrate was acidified to pH 2 with conc. HCl and kept standing in a refrigerator overnight. The colorless crystals that separated were collected on a filter, washed with H_2O, then recrystallized from H_2O (charcoal), giving DL-(IV) as colorless needles, m.p. 178~180° (reported[7] m.p. 164~166°). Yield, 12.5 g. (88%). Repeated recrystallization did not raise their m.p. *Anal.* Calcd. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22; N, 5.58. Found: C, 57.22; H, 5.42; N, 5.43. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 236 (3.63), 287 (3.59). UV $\lambda_{min}^{95\%\ EtOH}$ mμ (log ε): 223 (3.49), 256 (2.56). IR ν_{max}^{Nujol} cm^{-1}: 3355 (NH), 1705 (COOH), 1630 (CONH). IR $\nu_{max}^{Dioxane}$ cm^{-1}: 3380 (NH), 1745 (COOH), 1684 (CONH).

Recrystallization of DL-(IV) from H_2O sometimes gave colorless plates of m.p. 176~178°. *Anal.* Calcd. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22; N, 5.58. Found: C, 57.30; H, 5.55; N, 5.30. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 236 (3.63), 287 (3.59). UV $\lambda_{min}^{95\%\ EtOH}$ mμ (log ε): 223 (3.49), 256 (2.58). IR ν_{max}^{Nujol} cm^{-1}: 3426, 3349 (shoulder), 3217 (NH), 1728 (shoulder), 1710 (shoulder), 1696 (COOH), 1640, 1620 (shoulder) (CONH). IR $\nu_{max}^{Dioxane}$ cm^{-1}: 3380 (NH), 1745 (COOH), 1684 (CONH).

The IR spectrum of the plates, m.p. 176~178°, in dioxane was superimposable with that of the needles, m.p. 178~180°, although not superimposable in Nujol phase. The UV absorption spectra of them were also superimposable. Furthermore, both the crystals were interconvertible to each other by seeding alternative one to a hot aqueous solution of another one. Therefore, the samples of m.p. 178~180° and of m.p. 176~178° seemed probably to be dimorphic. This dimorphism was also observed when they were prepared by direct reduction of the azlactone (VI) according to Okuda and Fujii.[7]

The hydrogenation of (VII) with Raney Ni (W-2) also proceeded smoothly in an equimolar amount of 5% NaOH solution at room temperature under 50~100 atm. of H_2 to afford DL-(IV) in a yield of 81% after one recrystallization from H_2O.

Methyl ester of DL-(IV): The acid (DL-(IV); 3.00 g.) was heated under reflux with 10% methanolic HCl (45 cc.) for 2.5 hr. and treated as usual to give the crude ester (2.36 g.). Recrystallization from benzene-hexane (2:3) or dil. MeOH gave N-acetyl-3-(3,4-methylenedioxyphenyl)-DL-alanine methyl ester as colorless minute needles, m.p. 113~114° (reported[22] m.p. 107~108°). *Anal.* Calcd. for $C_{13}H_{15}O_5N$: C, 58.86; H, 5.70; N, 5.28. Found: C, 59.07; H, 6.07; N, 5.31. UV $\lambda_{max}^{95\%\ EtOH}$ mμ (log ε): 236 (3.63), 287 (3.59). UV $\lambda_{min}^{95\%\ EtOH}$ mμ (log ε): 223 (3.49), 256 (2.58). IR ν_{max}^{Nujol} cm^{-1}: 3346 (NH), 1731 (COOMe), 1635 (CONH).

ii) Hydrolysis and decarboxylation of (III): The diester (III: 8.1 g.) was heated with a solution of NaOH (4.3 g.) in H_2O (39 cc.) under reflux for 4 hr. to give a yellowish clear solution. The cooled solution was heated with 10% HCl (39 cc.) under reflux for 1 hr. The evolution of CO_2 subsided within 1 hr. On being kept standing in a refrigerator, the colorless pillars that separated were collected, washed with H_2O and dried, giving 5.0 g. (87%) of DL-(IV), m.p. 170~176°. On recrystallization, it formed colorless needles of m.p. 178~180°, which were found to be identical by admixture and IR comparison with a sample obtained by the method (i).

N-Acetyl-3-(3,4-methylenedioxyphenyl)-D-alanine Cinchonine Salt (Cinchonine·D-(IV) Salt)——N-Acetyl-3-(3,4-methylenedioxyphenyl)-DL-alanine (DL-(IV): 18.8 g.) and cinchonine (22.0 g.) were dissolved together in dehyd. EtOH (160 cc.) under warming and the solution was kept standing in a refrigerator for 3 days. The colorless prisms that separated were filtered off, washed with EtOH (ca. 20 cc.) and dried, giving 19.1 g. (93.6%) of the salt, m.p. 200~203°. The salt was recrystallized from EtOH (ca. 270 cc.) to form 15.2 g. (74.5%) of colorless prisms, m.p. 204~205°, $[\alpha]_D^{11}$ +68.7 (c=1.452,

20) H. D. Dakin: J. Biol. Chem., **82**, 439 (1929).
21) R. D. H. Heard: Biochem. J., **27**, 54 (1933). The melting point of this acid seems to have been misprinted in the reference cited here.
22) M. Ohara: Yakugaku Zasshi, **72**, 146 (1952).

EtOH, $l=1$). After three recrystallizations from EtOH, m.p. and $[\alpha]_D$ of them became constant: m.p. 204~205°, $[\alpha]_D^{17}$ +68.1° (c=1.145, EtOH, $l=1$). Yield, 9.7 g. (47.6%). *Anal.* Calcd. for $C_{31}H_{35}O_6N_3$: C, 68.24; H, 6.47; N, 7.70. Found: C, 68.10; H, 6.71; N, 7.61.

The resolution was also effected by using MeOH as a solvent.

N-Acetyl-3-(3,4-methylenedioxyphenyl)-L-alanine Cinchonine Salt (Cinchonine·L-(IV) Salt)——The alcoholic mother liquor after removal of the crude insoluble cinchonine·D-(IV) salt was treated with charcoal and then concentrated under diminished pressure to a syrup. This was dissolved in Me_2CO (100 cc.) and the solution was kept standing at room temperature overnight. The colorless crystals, the salt of D-(IV), that separated was filtered, and the filtrate was evaporated *in vacuo* to give 18.5 g. (90%) of a slightly yellowish powder which sintered at ca. 120° and melted at 130~140°, $[\alpha]_D^{19}$ +134° (c=1.972, EtOH, $l=1$). The purification of this salt seemed so difficult that it was used directly in the next step.

N-Acetyl-3-(3,4-methylenedioxyphenyl)-D-, and -L-alanine (D- and L-(IV)) from the Corresponding Cinchonine Salts——D-(IV): To a suspension of 6.9 g. of the powdered cinchonine·D-(IV) salt (m.p. 204~205°, $[\alpha]_D^{17}$ +68.1°) in H_2O (5 cc.) was added dropwise *N* HCl (28 cc.) under cooling and shaking, resulting in a pale yellow, clear solution which soon separated out colorless crystals. After standing in a refrigerator overnight they were filtered off, washed with H_2O (20 cc.), and dried, affording 3.0 g. (93.8%) of D-)IV), m.p. 158~159°, $[\alpha]_D^{19}$ −53.3° (c=1.491, EtOH, $l=1$). Recrystallization from H_2O gave pure D-(IV) as colorless needles, m.p. 158~159°, $[\alpha]_D^{18}$ −53.4° (c=1.841, EtOH, $l=1$). *Anal.* Calcd. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22; N, 5.58. Found: C, 57.31; H, 5.18; N, 5.48. IR ν_{max}^{Nujol} cm^{-1}: 3316 (NH), 1710 (COOH), 1610 (CONH). From the filtrate and washings of the acid (D-(IV)), cinchonine was nearly quantitatively recovered as colorless minute crystals, m.p. 260~262°, $[\alpha]_D^{11}$ +231° (c=0.614, EtOH, $l=1$).

L-(IV): When the crude cinchonine·L-(IV) salt (18.4 g.) was treated with *N* HCl (75 cc.) as in the case of the D-isomer, the crude L-acid was obtained as colorless crystals (7.5 g., 84.7%), m.p. 155~157°, $[\alpha]_D^{19}$ +47.1° (c=2.640, EtOH, $l=1$), which were recrystallized twice from H_2O to give L-(IV) as colorless needles, m.p. 158~159°, $[\alpha]_D^{13}$ +53.4° (c=2.262, EtOH, $l=1$). Yield, 3.9 g. *Anal.* Calcd. for $C_{12}H_{13}O_5N$: N, 5.58. Found: N, 5.39. The IR spectrum of this sample was superimposable with that of the D-acid. Mixed m.p. test with a sample of the D-isomer showed m.p. 178~180° sintering from 155°.

Cinchonine was also recovered in good yield.

3-(3,4-Methylenedioxyphenyl)-DL-, -D-, and -L-alanine (DL-, D- and L-(V))——DL-(V): The N-acetylated DL-amino acid (DL-(IV): 2.00 g.) was heated with 10% HCl (5 cc.) under reflux for 1 hr. The crystals dissolved within 50 min. After evaporation under diminished pressure, the colorless crystalline residue was dissolved in H_2O (ca. 30 cc.), treated with charcoal, and then adjusted to pH 5.6~5.8 with dil. NH_4OH, whereupon colorless crystals were separated. After standing in a refrigerator overnight the crystals were filtered, washed with H_2O, and dried, giving 950 mg. (57.2%) of DL-(V), m.p. 248~252° (decomp.) with sintering from 210°. The combined solution of the filtrate and the washings was evaporated *in vacuo* and the residue was recrystallized from H_2O giving an additional amount (360 mg., of 21.7%) of DL-(V). Recrystallization from H_2O gave DL-(V) as colorless minute leaflets of m.p. 253~255° (decomp.). (Reported m.p. 249~250°,[8f] 250~255°,[8a] 262~264°[8b]). *Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.14; H, 5.23; N, 6.57. Rf 0.45.*3 UV $\lambda_{max}^{0.01N\ HCl}$ mμ (log ε): 236 (3.55). 286 (3.55). UV $\lambda_{min}^{0.01N\ HCl}$ mμ (log ε): 220 (3.24), 256 (2.62). IR ν_{max}^{KBr} cm^{-1}: 3040, 2580 (NH_3^+), 1590 (strong, broad) (NH_3^+, COO^-), 1038 (-O-CH_2-O-).

The ninhydrin test gave a purplish blue coloration.

Hydrochloride: A mixture of the N-acetylated derivative (DL-(IV); 10.05 g.) and 10% HCl (30 cc.) was heated under reflux for 1 hr., and the resulting colorless clear solution was kept standing in a refrigerator overnight. The colorless plates that separated out were filtered, washed with EtOH (10 cc.), then with Et_2O to give 7.78 g. of the hydrochloride, m.p. 230~235° (decomp.). Recrystallization from dil. EtOH (EtOH:H_2O=3:2) gave DL-(V)·½HCl salt as colorless minute leaflets, m.p. 245~246° (decomp.). *Anal.* Calcd. for $C_{10}H_{11}O_4N\cdot\frac{1}{2}HCl$: C, 52.81; H, 5.10; N, 6.16; Cl, 7.80. Found: C, 52.88; H, 5.12; N, 5.98; Cl, 7.53. IR ν_{max}^{KBr} cm^{-1} 3160~2900 (medium, broad) (NH_3^+, COOH), 1730 (medium, broad) (COOH), 1600 (mdeium,broad) (NH_3^+, COO^- ?). When this sample was dissolved in 10% methanolic HCl, and then mixed with anhyd. Et_2O, DL-(V)·HCl salt was obtained as colorless minute crystals after recrystallization from EtOH-Et_2O. It melted at 246~247° with decomposition (reported m.p. 278~280°,[8c] 284°,[8d] 235°[8e]). *Anal.* Calcd. for $C_{10}H_{11}O_4N\cdot HCl$: C, 48.89 H, 4.92, N, 5.70; Cl, 14.43. Found: C, 49.13; H, 4.84; N, 5.98; Cl, 14.37. IR ν_{max}^{KBr} cm^{-1}: 3100~2860 (strong, broad) (NH_3^+, COOH), 1990 (weak) (NH_3^+) 1740, 1193 (strong) (COOH).

D-(V): When the N-acetyl-D-amino acid (D-(IV): 1.20 g.), m.p. 158~159°, $[\alpha]_D^{18}$ −53.4°, obtained by the above-mentioned chemical resolution, was heated with 10% HCl (4 cc.) and worked up as in the case of the racemate, 730 mg. (73%) of colorless crystals (D-(V)), m.p. 227~230° (decomp.) with sintering from 210°, were obtained. Repeated recrystallization from H_2O or dil. EtOH gave D-(V) as colorless

fluffy needles, m.p. 235~237° (decomp.), $[\alpha]_D^{20}$ +13.8° (c=1.486, *N* HCl, *l*=2), *Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30; N, 6.70. Found: C, 57.74; H, 5.22; N, 6.67. Rf 0.45.*3 IR ν_{max}^{KBr} cm^{-1}: 3080~3020, 2560, 2480, 2040 (NH_3^+), 1600 (strong, broad) (NH_3^+, COO^-), 1040 (-O-CH_2-O-). The IR spectrum of the D-isomer in KBr disc was not superimposable with that of the racemate (DL-(V)).

On similar treatment with 10% HCl (6 cc.), the N-acetylated derivative (D-(IV); 2.20 g.), obtained by the enzymatic hydrolysis procedure which will be described later, gave D-(V) (1.46 g., 79%) as colorless fluffy needles, m.p. 232° (decomp.). Recrystallization from H_2O furnished colorless needles, m.p. 235~237° (decomp.), $[\alpha]_D^{12}$ +13.8° (c=1.523, *N* HCl, *l*=1). *Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30; N,

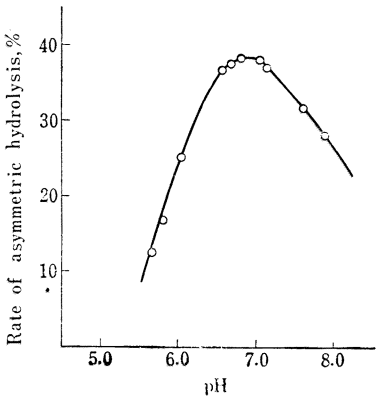


Fig. 1. Relation between Enzyme Activity and pH of Medium

A mixture of the substrate (Na-salt of DL-(IV)) (0.001 mole), 1/15*M* phosphate buffer (pH 4.6~8.3) (5 cc.), Takadiastase (0.02 g.) and 0.1% formalin (1 cc.) was diluted with H_2O to 10 cc., and incubated at 37° for 21 hr. In such a concentration of phosphate buffer as above, pH of the reaction mixture did not change from a starting value at the end of incubation. It seems that optimum pH is 6.8~6.9.

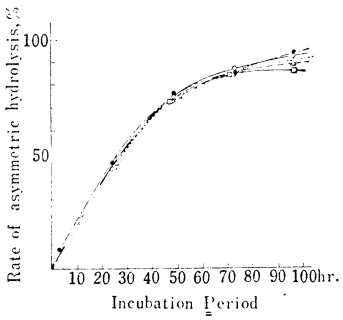


Fig. 2. Effect of Buffer Concentration on Rate of Reaction

A mixture of the substrate (Na-salt of DL-(IV)) (0.001 mole), 1/15*M* phosphate buffer (pH 6.81) (5 cc., 2 cc., 1 cc., 0.5 cc., and 0 cc.), Takadiastase (0.02 g.) and 0.1% formalin (1 cc.) was diluted with H_2O to 10 cc., and incubated at 37°.

Usage of 1 cc. of the buffer seems preferable in this case.

Volume of the buffer

-○—○-: 5 cc. --×---×--: 2 cc. -·•-·-·•-·-: 1 cc.
-△--△-: 0.5 cc. -□—□-: 0 cc.

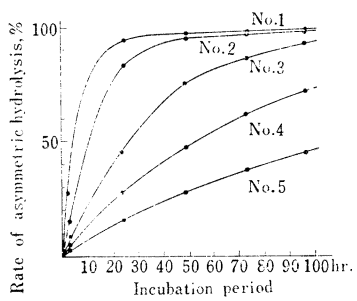


Fig. 3. Effect of Enzyme Concentration on Rate of Reaction

A mixture of the substrate (Na-salt of DL-(IV)) (0.001 mole), 1/15*M* phosphate buffer (pH 6.81) (1 cc.), Takadiastase (0.1 g. (No. 1), 0.05 g. (No. 2), 0.02 g. (No. 3), 0.01 g. (No. 4), and 0.005 g. (No. 5)) and 0.1% formalin (1 cc.) was diluted with H_2O to 10 cc., and incubated at 37°.

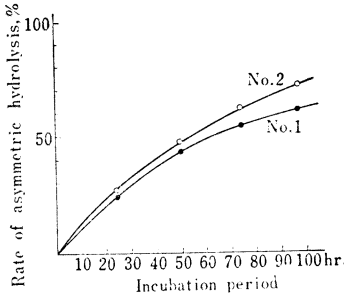
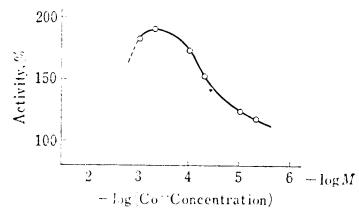


Fig. 4. Effect of Substrate Concentration on Rate of Reaction

A mixture of the substrate (Na-salt of DL-(IV)) (0.001 mole), 1/15*M* phosphate buffer (pH 6.81) (1 cc.), Takadiastase (0.01 g.) and 0.1% formalin (1 cc.) was diluted with H_2O to 5 cc. (No. 1) and to 10 cc. (No. 2), and incubated at 37°. It seems preferable to adopt the condition such as that of No. 2.



A mixture of the substrate (Na-salt of DL-(IV)) (0.001 mole), $^1/_{15}M$ phosphate buffer (pH 6.81) (1 cc.), Takadiastase (0.01 g.), 0.1% formalin (1 cc.) and $10^{-2}\sim10^{-4}M$ $CoCl_2$ solution (required volume) was diluted with H_2O to 10 cc., and incubated at 37° for 20 hr. Activity was calculated by comparing the rate of hydrolysis with that of a standard which was carried out without $CoCl_2$ in the same condition. It seems very advantageous to use $CoCl_2$ for this hydrolysis in a concentration of $10^{-4}\sim10^{-3}M$.

Fig. 5. Effect of Co^{++} Concentration on the Enzymatic Hydrolysis

6.70. Found: C, 57.16; H, 5.05; N, 6.84. Rf 0.45.*3 The IR spectrum of this sample was superimposable with that of the one derived from the chemical resolution.

L-(V): The data will be shown in the procedure of the asymmetric hydrolysis of DL-(IV).

Asymmetric Hydrolysis of DL-(IV) by Takadiastase——As preliminary experiments, the effects of pH, buffer, enzyme, substrate concentrations and concentration of activating agents,[15e,f,23] such as Co^{++}, Mg^{++}, were investigated by tracing the hydrolyzed amino acid with the photometric ninhydrin method reported by Troll and Cannan.[24] The results are shown in Figs. 1~5.*5

$MgCl_2$ slightly inhibited the hydrolysis in a concentration of $10^{-3}M$. Among these conditions tested, it seemed preferabale to use the following combination for the preparative purpose: a mixture of the substrate (Na salt) (2 milimole), 1/15M phoshate buffer (pH 6.81) (2 cc.), 0.1% formalin (2 cc.), Takadiastase (0.02~0.04 g.), and $10^{-3}M$ $CoCl_2$ solution (2 cc.), was diluted with H_2O to 20 cc., and incubated at 37° and pH 6.8~6.9. This combination of conditions was tested, with the results as shown in Fig. 6.

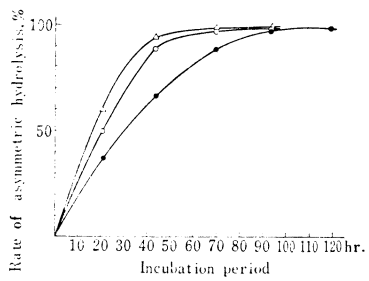


Fig. 6. Hydrolysis Curve under the Condition for Preparation

A mixture of the substrate (Na-salt of DL-(IV)) (0.002 mole), 1/15M phosphate buffer (pH 6.81) (2 cc.), Takadiastase (0.02 g. (No. 1), 0.03 g. (No. 2), 0.04 g. (No. 3)), 0.1% formalin (2 cc.) and $10^{-3}M$ $CoCl_2$ solution (2 cc.), was diluted with H_2O to 20 cc., and the resulting solution (pH 6.9) was incubated at 37°.

-•—•-: No. 1
-○—○-: No. 2
-△—△-: No. 3

Since the conditions, No. 1, No. 2 and No. 3 in Fig. 6 were found to be preferable, the preparation of L-(V) and D-(IV) was carried out as follows.

A mixture of the N-acetyl-DL-amino acid (DL-(IV): 5.02 g. (0.02 mole)), N NaOH solution (20 cc.), 1/15M phosphate buffer (pH 6.81, 20 cc.), $10^{-3}M$ $CoCl_2$ solution (20 cc.), 0.1% formalin (20 cc.) and Takadiastase (0.30 g.) was diluted with H_2O to 200 cc. to give a slightly yellowish clear solution (pH 6.9). After being kept standing at 37° for 110 hr. the yellowish clear solution (pH 6.9) was adjusted to pH 5.0 with AcOH, and heated at 90~95° for 5 min. with charcoal (0.5 g.), then filtered. The resulting colorless clear solution was evaporated at 50° *in vacuo* to dryness, leaving a nearly colorless crystalline solid. The solid was heated with abs. EtOH (100 cc.) in a water bath under stirring for some time, and then kept standing at room temperature overnight. The insoluble crystals were filtered off, washed with abs. EtOH (20 cc.), then with H_2O (25 cc.) to remove the Na salt of D-(IV), and dried, furnishing 1.56 g. (74.6%) of L-(V), m.p. 232~236° (decomp. with sintering at 210°.). The crystals thus obtained were dissolved in boiling H_2O (ca. 40 cc.) and the solution was boiled with a small amount of charcoal for 10 min., filtered, and kept standing in a refrigerator overnight to give L-(V) as colorless fluffy needles, m.p. 235~237° (decomp.), $[\alpha]_D^{17}$ −13.6° (c=1.547, NHCl, l=2), $[\alpha]_D^{28}$ +5.4° (c=2.075, NNaOH, l=1). Yield, 940 mg. (45%), *Anal.* Calcd. for $C_{10}H_{11}O_4N$: C, 57.41; H, 5.30; N, 6.70.

*5 The authors are grateful to Miss S. Fujii for her technical assistance.

23) *a*) K. R. Rao, S. M. Birnbaum, R. B. Kingsley, J. P. Greenstein: J. Biol. Chem., **198**, 507 (1952). *b*) R. Marshal, S. M. Birnbaum, J. P. Greenstein: J. Am. Chem. Soc., **78**, 4636 (1956). *c*) K. Michi, H. Tsuda: Bull. Agr. Chem. Soc. Japan, **21**, 18 (1957). *d*) *Idem*: J. Biochem. (Tokyo), **45**, 745 (1958). *e*) I. Chibata, M. Kisumi, S. Yamada: Bull. Agr. Chem. Soc. Japan, **22**, 24 (1958).
24) W. Troll, R. K. Cannan: J. Biol., Chem, **200**, 803 (1953).

Found : C, 57.39; H, 4.92; N, 6.34. Rf 0.45.*3 The IR spectrum of this sample was superimposable with that of D-(V) derived from the chemical resolution of DL-(IV).

The ethanolic mother liquor after removal of the insoluble L-(V) was evaporated *in vacuo* to dryness. The residue was dissolved in H_2O (20 cc.) and combined with the aqueous washings obtained in the isolation of L-(V). This aqueous solution was treated with 10% HCl to bring its pH to 1~2. After standing in a refrigerator overnight, the crystatls that separated was filtered, washed with H_2O, and dried, giving 2.32 g. (94%) of D-(IV), m.p. 155~158°. It was recrystallized from H_2O (ca. 50 cc.) (charcoal) to give D-(IV) as colorless needles, m.p. 158~159°, $[\alpha]_D^{23}$ −52.4° (c=3.584, EtOH, l=1). Yield. 1.74 g. (69.3%). *Anal.* Calcd. for $C_{12}H_{13}O_5N$: C, 57.37; H, 5.22°; N, 5.58. Found : C, 57.10; H, 4.84; N, 5.64. This sample was found to be identical by admixture and IR comparison with D-(IV) obtained by the chemical resolution of DL-(IV).

The absolute configurations of these sampes, L-(V) and D-(IV), were confirmed by converting them into 3-(3,4-dihydroxyphenyl)-L-, and -D-alanine. The data will be described in the following paper.[9]

When the above asymmetric hydrolysis was carried out with 0.4 g. or 0.2 g. of Takadiastase during an incubation period 90 hr. or 135 hr. instead of 0.3 g. of the former and 110 hr. of the latter, the result was as good as when with 0.3 g. of Takadiastase during an incubation period of 110 hr.

The authors are grateful to Dr. S. Tsurufuji of this Faculty for advices on the photometric ninydrin method. Thanks are also due to the members of the Central Analysis Room of this Faculty for elemental analyses and spectral data.

Summary

The preparation of 3-(3,4-methylenedioxyphenyl)-D-, and -L-alanine (D- and L-(V)) was carried out by the chemical or biological resolution of N-acetyl-3-(3,4-methylenedioxyphenyl)-DL-alanine (DL-(IV)). The N-acetyl-DL-amino acid prepared from 3,4-methylenedioxybenzyl chloride (II) and diethyl acetamidomalonate (I), via the diester (III), was resolved into two forms, D- and L-(IV), by means of fractional recrystallization of their cinchonine salts from ethanol, followed by the liberation of cinchonine base. Asymmetric hydrolysis of DL-(IV) was also smoothly effected by Takadiastase to give the L-amino acid (L-(V)) and N-acetyl-D-amino acid (D-(IV)). The latter as well as the one derived from the chemical resolution was converted by boiling it with 10% hydrochloric acid into the D-amino acid (D-(V)) in good yield.

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110. Shun-ichi Yamada, Takayuki Shioiri, and Tozo Fujii : Studies on Optically Active Amino Acids. II.*1 Partially Asymmetric Synthesis of 3-(3,4-Methylenedioxyphenyl)alanine.

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In the preceding paper*1 the authors described the preparation of optically active 3-(3,4-methylenedioxyphenyl) alanine (V) by two resolution methods; chemical resolution and enzymatic asymmetric hydrolysis. Now we wish to report that the optically active amino acids (V) can also be prepared conveniently by the partially asymmetric synthesis, which seems to be one of the most important problems in amino acids syntheses.

*1 Part I : This Bulletin, **10**, 680 (1962).

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