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SHORT REPORTS

E-2(*S*)-AMINO-3-METHYL-3-PENTENOIC ACID FROM *CONIOGRAMME INTERMEDIA*

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Key Word Index—*Coniogramme intermedia*; Pteridaceae; *E*-2(*S*)-amino-3-methyl-3-pentenoic acid; structural determination.

Abstract—A new amino acid, *E*-2(*S*)-amino-3-methyl-3-pentenoic acid was isolated from *Coniogramme intermedia*. The structure was elucidated by elementary analysis, optical rotation, catalytic hydrogenation, ^1H and ^{13}C NMR spectra.

INTRODUCTION

Among many nonprotein amino acids known to be distributed in plants, several uncommon monoaminodicarboxylic acids have been reported, mainly by Virtanen and his co-workers. Thus, α-aminopimelic acid [1,2] and its γ-OH compound [3] were identified from *Asplenium septentrionale*, γ-methylglutamic acid from *Phyllitis scolopendrium* [4,5], γ-methyl-γ-hydroxyglutamic acid from *Adiantum pedatum* [6,7] and also from *Asplenium septentrionale* [5, 8].

We now report a novel neutral amino acid, *E*-2(*S*)-amino-3-methyl-3-pentenoic acid, from the fern *Coniogramme intermedia* which is one of the unsaturated forms of L-isoleucine.

RESULTS AND DISCUSSION

The amino acid gave a yellow spot on PC and isolation was carried out by repeated CC with anion exchange resin and cellulose powder. Elementary analysis of the purified crystals was in good agreement with the formula $C_6H_{11}NO_2$. Its extremely high specific rotation in water +251°, together with the ninhydrin coloration, suggested the structure of a β-unsaturated amino acid [9]. In acid solution, the $[\alpha]_D$ value showed a definite though small shift to a more positive value, suggesting that it belongs to the L-series. This assignment was confirmed by oxidative deamination with L-amino acid oxidase prepared from Habu snake venom [10]. On catalytic hydrogenation, the amino acid completely disappeared and was replaced by a mixture of isoleucine and alloisoleucine (7:5).

From the above results the structure of the new amino acid should be 2(*S*)-3-methylenenorvaline or 2(*S*)-amino-3-methyl-3-pentenoic acid. The former is known to be a constituent of fruit bodies of *Lactarius helvus* (Basidiomycetes) [9]. Since this amino acid isolated from the above fungus in our laboratory did not correspond to our amino acid, the only possible structure is 2(*S*)-amino-3-methyl-3-pentenoic acid. ^1H NMR supported the structure, showing signals for one olefin, one methyne and two Me protons. Furthermore, for the structure, 2(*S*)-amino-3-methyl-3-pentenoic acid, there are two geometrical isomers, *E*(*cis*)- and *Z*(*trans*)-forms. We prepared the *Z*-form by Strecker synthesis and it migrated slightly faster on cellulose TLC than the natural amino acid in solvents A and B.

As the final step for the elucidation of the structure, ^{13}C NMR spectra of the natural amino acid and synthetic *Z*-2-amino-3-methyl-3-pentenoic acid (racemate) were determined. The C-2 signal of the natural amino acid was higher by 8.6 ppm, while the C-6 signal was considerably lower, than those of the corresponding carbons of the synthetic amino acid. Since this high-field shift for C-2 of the former is clearly due to the well-documented steric compression effect [11], it can be concluded that C-2 is *cis* and C-6 is *trans* to C-5 with respect to the double bond of the natural amino acid.

EXPERIMENTAL

General. Evaporation of solvent was carried out in a rotary evaporator below 40°. Cellulose powder and TLC plates used were, if not otherwise stated. 'Avicel' of Funakoshi Pharmaceutical Co. and chromatographic solvents were *n*-BuOH–HOAc–H_2O (63:10:27) (A), PhOH–H_2O (25:8) (B), MeOH–pyrdine–H_2O (5:5:1) (C) and *t*-AmOH satd with H_2O (D).

Plant. Fronds of *C. intermedia* Hieron (8.5 kg) were collected in Sept. 1979 in Hanno City, Saitama Prefecture, and the vouchers have been deposited in the Department of Biology, College of General Education, The University of Tokyo.

Isolation. The fronds were kept at 4° for 18 hr after collection, cut into small pieces and immediately soaked in 80% EtOH. After 2 days they were homogenized in a mixer, filtered and the residue soaked again in aq. EtOH for 3 days.

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The combined filtrate (130 l.) was passed through a column of Daiaion SK 1B (Mitsubishi Chemical Industries Ltd). The resin was then washed throughly with 80% EtOH and H_2O, successively, and the amino acids adsorbed were eluted with 2 M NH_4OH (20 l.). The syrup obtained on conc of the above eluate was applied to a cellulose column (112 × 6 cm) and developed with solvent (A). The relevant fractions were combined and fractionated further on a Dowex 1 × 4 column (200–400 mesh, OAc^- form, 95 × 2.5 cm) using 0.2 M HOAc, yielding a crystalline mass which contained an equal amount of valine. Repeated fractionation on a cellulose column (184 × 3.4 cm) and solvent (C) gave pure crystals of the new amino acid (300 mg). They were recrystallized ×3 from H_2O–Me_2CO, mp 182° (decomp). $[\alpha]_D^{23}$ +251° (H_2O; *c* 0.68), +257° (3 M HCl; *c* 0.34). Found: C, 55.65; H, 8.97; N, 10.86. $C_6H_{11}NO_2$ requires: C, 55.80; H, 8.58; N, 10.84%. ^1H NMR (100 MHz, D_2O): δ1.6–1.85 (6H, *m*, H-5, H-6), 4.76 (1H, *s*, H-2), 5.64–6.0 (1H, *m*, H-4). ^{13}C NMR (22.5 MHz, D_2O): δ13.7 (*q*, C-5), 18.3 (*q*, C-6), 54.4 (*d*, C-2), 128.1 (*s*, C-3), 130.4 (*d*, C-4), 174.0 (*s*, C-1).

Oxidative deamination. Crude L-amino acid oxidase prepared from Habu snake (*Trimeresurus flavoviridis*) venom [10] in 0.05 M $MeCO_2NH_4$ buffer, pH 7.2 (30 μl) was added to the amino acid soln (25 μg in 10 μl H_2O). After the mixture was incubated at 37° for 30 hr, the reaction was stopped by heating at 100° for 1 min, 3 μl of the supernatant were applied to cellulose-TLC (DC-Alufolien, Merck) and developed with solvent (B) in the presence of NH_3 vapour.

Hydrogenation. A small amount of the pure sample was hydrogenated over Pt catalyst. The products were analysed on DC-Alufolien Cellulose (Merck) using two developments (20 cm) in solvent (D).

Synthesis. *Z*-2-amino-3-methyl-3-pentenoic acid (racemate) was synthesized using the Strecker method from tiglic aldehyde (Fluka AG, Chemische Fabrik), NH_4Cl and NaCN. ^{13}C NMR (22.50 MHz, D_2O): δ 11.8 (*q*, C-6), 13.8 (*q*, C-5), 63.0 (*d*, C-2), 128.9 (*s*, C-3), 130.9 (*d*, C-4), 174.3 (*s*, C-1).

Chromatographic data. R_{Nle} values of the new amino acid in solvents (A), (B), and (C) were 0.69, 0.98 and 1.03 and R_{Val} values were 1.01, 1.07 and 1.23, respectively.

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