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SHORT REPORTS

A CHLOROHYDRIN AMINO ACID FROM *AMANITA ABRUPTA*

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Key Word Index—*Amanita abrupta*; Basidiomycetes; toxic mushroom; unusual amino acids; (2*S*,4*Z*)-2-amino-5-chloro-6-hydroxy-4-hexenoic acid.

Abstract—A new amino acid, (2*S*,4*Z*)-2-amino-5-chloro-6-hydroxy-4-hexenoic acid, has been isolated from *Amanita abrupta*. Three other unusual amino acids were also isolated from the same fungus.

INTRODUCTION

Although the name and the toxicity are not commonly known, the white mushroom *Amanita abrupta*, which shows fatal toxicity, can be found occasionally in Japanese forests in autumn. Quite recently Yamaura *et al.* [1] reported some toxic effects of the aqueous extract of this mushroom. They also identified 2-amino-4,5-hexadienoic acid and allylglycine from the fungus and this compound exhibited similar biochemical effects to those associated with extracts. The stereochemical nature of the amino acids was not reported. Our independent investigation of physiologically active components of this mushroom led to the isolation of a new amino acid along with three unusual amino acids, (*S*)-2-amino-4,5-hexadienoic acid [2], L-allylglycine [3] and L-propargylglycine [4].

RESULTS AND DISCUSSION

TLC indicated that the amino acid fraction from the fruit-bodies of *A. abrupta* contained several unsaturated amino acids giving yellowish ninhydrin reactions. The major one (0.26%) was identified as (*S*)-2-amino-4,5-hexadienoic acid [2] which is known to be toxic to guinea-pigs [5]. The new amino acid (5.9×10^{-3}%) was isolated from the allene amino acid fraction by HPLC and cellulose chromatography. The observed highest peaks at *m/z* 180 and *m/z* 182 in the FD mass spectrum of the amino acid and those at *m/z* 396 and *m/z* 398 in the CI mass spectrum of the trimethylsilyl derivatives implied the existence of a chlorine atom. This was confirmed by the high resolution mass spectrum of the trifluoroacetyl derivative of the amino acid methyl ester (**2**) which showed the molecular formula $C_{11}H_{10}NO_5ClF_6$. The ^{1}H NMR spectrum indicated the sequence of α-methine [δ3.80 (1H, *t*, *J* = 6.0 Hz)], methylene [δ2.77 (2H, *m*)], trisubstituted olefin [δ5.86 (1H, *t*, *J* = 7.2 Hz)], and an isolated carbinol [δ4.18 (2H, *s*)].

The above partial structure and the physicochemical properties led to structural formula **1** for the new amino acid. Treatment with L-amino acid oxidase from Habu snake venom revealed the (*S*)-configuration of the amino acid (**1**). The (*Z*)-configuration of the double bond was determined from the observed NOE between the 4- and 6-hydrogen atoms in ^{1}H NMR spectrum of the diacetate **3**. Thus, it is concluded that the new amino acid is represented as (2*S*, 4*Z*)-2-amino-5-chloro-6-hydroxy-4-hexenoic acid (**1**).

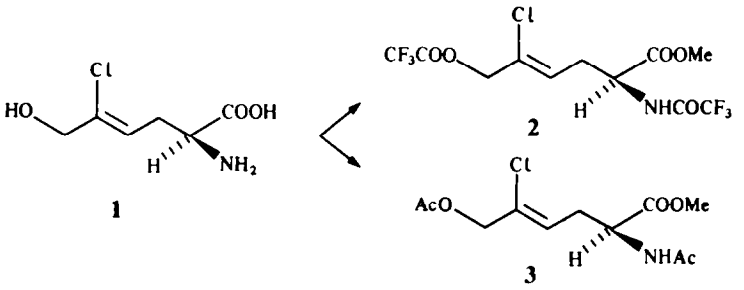
Two other unusual amino acids, L-allylglycine [3] (2.8×10^{-3}%) and L-propargylglycine [4] (4.1×10^{-3}%) were also isolated. Although the biological role of these unsaturated amino acids is not clear, the new amino acid **1** may be formed from (*S*)-2-amino-4,5-hexadienoic acid by biological chlorohydrin formation.

EXPERIMENTAL

General. TLC plates used were 'Avicel' (Funakoshi Pharmaceutical Co.) and chromatographic solvents were (1) *t*-BuOH–MeCOEt–HCO_2H–H_2O (10:10:1:2); (2) *n*-BuOH–HOAc–H_2O (63:10:27). Cellulose powder (100–200 mesh) was purchased from Toyo Roshi Co. Amino acid analysis (AA) was performed using Hitachi Gel No. 2618 (500 × 2.6 mm). Amino acids were detected using a refractive index monitor. Elution buffers were 0.2 M ammonium formate, pH 2.9 (buffer A), pH 3.02 (buffer B), pH 3.54 (buffer C) and pH 3.59 (buffer D). Flow rate was 0.5 ml/min and the pressure was 135–140 kg/cm^2. Prep. HPLC was performed under the same conditions. NMR signals recorded in D_2O were referred to TMS as an ext. std.

Fungus. Fruit-bodies of *A. abrupta* Peck were collected in Sendai city and identified by Prof. K. Yokoyama, Shiga University. Voucher specimens are preserved in the Pharmaceutical Institute, Tohoku University, Sendai, Japan.

Isolation. A MeOH extract of fruit-bodies of *A. abrupta* (320 g), soaked immediately after collection, was partitioned



between EtOAc and H_2O. The H_2O fraction was passed through a column of Amberlite IR-120B (H^+ form, 900 ml). Neutral and acidic amino acids adsorbed were eluted with 1 M pyridine (800 ml). The mixture of amino acids (14 g) was put on Dowex 50 W × 4 columns [200–400 mesh, (800 × 15 mm) × 4] which were buffered with 0.2 M ammonium formate, pH 3.02. Amino acids were eluted with the same buffer and eluates (25 ml fractions) were monitored by HPLC. Fractions 26–28 which gave a single peak on AA, were combined and lyophilized to give (*S*)-2-amino-4,5-hexadienoic acid. Recrystallization from MeOH–H_2O gave 830 mg of colourless needles, mp 200–205° (decomp). IR ν_{max}^{KBr} cm^{-1}: 3350, 1950, 1570, 1450, 830. ^1H NMR (100 MHz, D_2O): δ2.60 (2H, *m*, H-3), 3.84 (1H, *dd*, J = 5, 6.5 Hz, H-2), 4.8–5.4 (3H, *m*, H-4, H-6). ^{13}C NMR (25 MHz, D_2O): δ30.1 (*t*, C-3), 54.4 (*d*, C-2), 76.3 (*t*, C-6), 84.0 (*d*, C-4), 181.6 (*s*, C-1), 209.6 (*s*, C-5). The compound was completely oxidized with L-amino acid oxidase using procedure A. Fractions 7–11 were combined and subjected to prep. HPLC (buffer A) giving L-propargylglycine (24 mg) which was lyophilized and crystallized from MeOH–H_2O, mp 230° (decomp, sublimed > 190°). CD (H_2O): $[\theta]_{208}$ + 1250. ^1H NMR (100 MHz, D_2O): δ2.58 (1H, *m*, H-3), 2.90 (2H, *m*, H-5), 3.96 (1H, *t*, J = 5.4 Hz, H-2). Fractions 18–25 contained 2-amino-4,5-hexadenoic acid, allylglycine and a new amino acid (**1**) using AA. The latter two amino acids were sepd from allene amino acid by prep. HPLC (buffer C). The lyophilized mixture was applied to cellulose columns [(1100 × 8 mm) × 2] and fractionated with the solvent 1 to yield allylglycine (9 mg) and the amino acid (**1**) (19 mg). Both amino acids were lyophilized and crystallized from MeOH–H_2O. L-*Allylglycine*. Mp 232° (decomp, sublimed > 170°). FDMS *m/z* (rel. int.): 116 $[M+H]^+$ (100), 74 $[NH_2CHCOOH]^+$ (17). ^1H NMR (100 MHz, D_2O): 2.77 (2H, *m*, H-3), 3.94 (1H, *dd*, J = 5.5, 6.5 Hz, H-2), 5.2–5.7 (2H, *m*, H-5), 5.6–6.2 (1H, *m*, H-4). The compound was completely oxidized with L-amino acid oxidase by procedure B. (2*S*, 4*Z*)-2-*Amino*-5-*chloro*-6-*hydroxy*-4-*hexenoic acid* (**1**). Mp 200–212° (decomp). $[\alpha]_D^{20}$ − 17.9° (H_2O, *c* 0.19). FDMS *m/z* (rel. int.): 182 $[M+H]^+$ (17), 180 $[M+H]^+$ (100), 74 $[NH_2CHCOOH]^+$ (35). IR ν_{max}^{KBr} cm^{-1}: 3050, 2600, 1615, 1405, 855, 795, 701. ^1H NMR (100 MHz, D_2O): δ2.77 (2H, *m*, H-3), 3.80 (1H, *t*, J = 6.0 Hz, H-2), 4.18 (2H, *s*, H-6), 5.86 (1H, *t*, J = 7.2 Hz, H-4). The compound was completely oxidized with L-amino acid oxidase by procedure A.

Methyl 5-chloro-2-trifluoroacetamido-6-trifluoroacetoxy-4-hexenoate (**2**). Amino acid **1** (2 mg) in MeOH (1 ml) which contained *ca* 10% HCl gas was left for 1 day at 40°. The MeOH was evapd and the residue dried by addition of CH_2Cl_2 (3 ml) and evapn. TFAA (0.5 ml) and CH_2Cl_2 (0.5 ml) were added to the residue and the mixture was left for 1 day at room temp. After evapn of solvents, the residue was passed through silica gel (1 g) eluting with CH_2Cl_2 to give the trifluoroacetate (3 mg). EI-high resolution-MS (probe) 70 eV, *m/z*: 385 $[M^+]$ (found: 385.0166, $C_{11}H_{10}NO_5{}^{35}ClF_6$ requires: 385.0151). 356 $[M-OMe]^+$ (found: 355.9892, $C_{10}H_7NO_4{}^{37}ClF_6$ requires: 355.9937). 354 $[M-OMe]^+$ (found: 353.9968, $C_{10}H_7NO_4{}^{35}ClF_6$ requires: 353.9968).

Methyl 5-chloro-2-acetamido-6-acetoxy-4-hexenoate (**3**). Amino acid **1** (9.3 mg) in 10% HCl–MeOH (1 ml) and 2,2-dimethoxypropane (1.1 ml) was left for 4 hr at room temp. After the solvents were evapd the residue was dissolved in Ac_2O (1 ml) and pyridine (0.5 ml) and the mixture left overnight at room temp. The reaction mixture was quenched with ice-H_2O and extd with EtOAc. The EtOAc extract was passed through silica gel (1 g) eluting with CH_2Cl_2–EtOAc (9:1) to afford the diacetate (11.5 mg, 81%). EIMS (probe) 70 eV, *m/z* (rel. int.): 280 $[M+H]^+$ (7.1), 278 $[M+H]^+$ (21.6), 131 $[AcNHCHCOOMe]^+$ (43.1), 116 $[AcNHCH{=}C(OH)OMe{-}Me]^+$ (100). ^1H NMR (100 MHz, $CDCl_3$): δ2.03 (3H, *s*, H-Ac), 2.12 (3H, *s*, H-Ac), 2.75 (2H, *m*, H-3), 3.76 (3H, *s*, H-OMe), 4.64 (2H, *s*, H-6), 4.75 (1H, *m*, H-2), 5.80 (1H, *t*, J = 7.2 Hz, H-4), 6.16 (1H, *m*, H-NH).

Oxidation with L-amino acid oxidase. (A) Crude L-amino acid oxidase prepared from Habu snake (*Trimeresurus flavoviridis*) venom in 0.05 M NH_4OAc buffer, pH 7.2 (150 μl) was added to an amino acid soln (*ca* 100 μg in 50 μl H_2O) at 0°. Immediately after the addition, a few μl of the reaction mixture was withdrawn, spotted on TLC and heated to destroy the activity of the enzyme. The remaining mixture was incubated at 37° for 24 hr with vigorous stirring, and spotted on TLC. TLC was developed with solvent 2. (B) Procedure as given in ref. [6].

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