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α-Amino acids from a mushroom, *Amanita castanopsidis Hongo*, with growth-inhibiting activity

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Abstract

Two α-amino acids were isolated from a mushroom, *Amanita castanopsidis Hongo*. Spectroscopic analyses revealed that one was 2-amino-3-cyclopropyl-butanoic acid and the other the known 2-amino-5-chloro-4-pentenoic acid. Both inhibited root elongation in lettuce seedlings.

Keywords: *Amanita castanopsidis Hongo*; α-Amino acid; 2-Amino-3-cyclopropyl-butanoic acid; 2-Amino-5-chloro-4-pentenoic acid; Root growth; Lettuce; *Lactuca sativa*

1. Introduction

The α-amino acids, (S)-*cis*-amino-5-chloro-4-pentenoic acid and (2S)-2-amino-3-cyclopropylpropionic acid have been isolated from a mushroom, *Amanita vergineoides* (Ohta, Matsuda, Takahashi, Nakajima & Nozoe, 1995; Ohta et al., 1986). During our search for novel plant growth regulators in *Amanita castanopsidis Hongo* (Koshiroonitake in Japanese) using a lettuce seedling assay, two α-amino acids were isolated with both having biological activity like indole-3-acetic acid (Okamoto, Isogai & Koizumi, 1967). In this report, we describe the isolation, structure elucidation and biological activity of these α-amino acids.

2. Results and discussion

Fresh mushrooms, *A. castanopsidis Hongo*, were extracted twice with MeOH. The MeOH extract was partitioned with *n*-BuOH. The *n*-BuOH layer was purified with a preparative HPLC equipped with an ODS column.

Substances crystallized from the *n*-BuOH layer were identified by spectroscopic methods as 2-amino-3-cyclopropyl-butanoic acid (**1**) and 2-amino-5-chloro-4-pentenoic acid (**2**), respectively.

Crude **1** was dissolved in water–MeOH (1:1) and allowed to stand for 1–2 days at room temperature to yield colorless plates (317 mg of **1**), mp 267–272° (decomp.), $[\alpha]_D^{25}$ −51° (H_2O; *c* 0.5). **1** gave an ion peak at *m/z* 144.1036 (mmu + 1.1) $M+H^+$ (HR-FABMS) and its molecular formula was determined as $C_7H_{14}O_2N$. The ^1H and ^{13}C NMR spectral data of **1** and **2** are shown in Table 1 and scheme 1. **1** had a cyclopropane ring [δ_H 0.47 (m), 0.21 (m) and 0.10 (m); δ_C 3.38 and 2.45]. Furthermore, **1** possessed an amino group [δ_H 3.64 (*br d, J* = 4.5 Hz); δ_C 15.92]. The absolute configuration of the α-carbon of **1** was deduced to be in the (S)-configuration by comparison of the positive Cotton effect ($[\theta]_{203}$ + 1388) of the CD spectrum of **1** with those of some (S)-α-amino acids reported by Craig and Roy (1965). However, the absolute configuration of β-carbon of **1** has not yet been established, so that further efforts are required to

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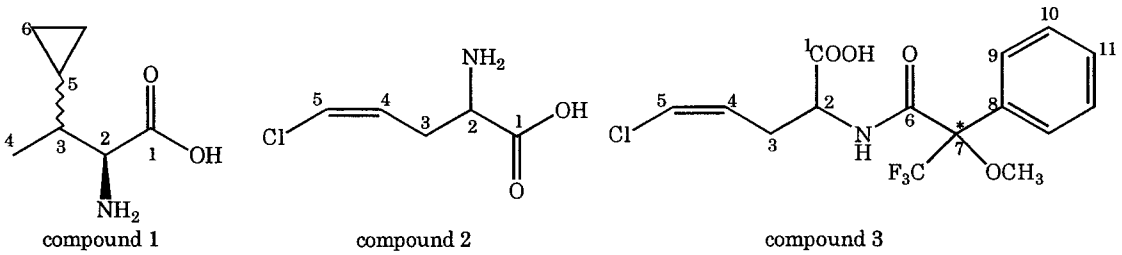
Table 1
NMR data of **1**, **2** and **3**

	Compound **1**		Compound **2**		Compound **3**	
No.	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift	^{13}C shift	^{1}H shift
1	174.1		174.3		173.6	
2	60.0	3.64 (*br, d, J* = 4.5 Hz)	54.6	3.81 (*dd, J* = 7.0, 5.5 Hz)	55.5	
					55.8	
3	39.4	1.32 (*m*)	29.1	2.79 (*m*)	30.0	2.66 (*m*), 2.77 (*m*)
					30.1	2.88 (*m*)
4	15.9	1.07 (*d, J* = 7.2 Hz)	125.6	5.83 (*q, J* = 7.0 Hz)	126.7	5.67 (*q*)
					126.8	5.82 (*q*)
5	12.7	0.66 (*m*)	123.5	6.34 (*dt, J* = 7.0, 1.5 Hz)	123.8	6.16 (*dt, J* = 7.1, 1.5 Hz)
					123.9	6.26 (*dt, J* = 7.1, 1.5 Hz)
6	3.4	0.47 (*m*), 0.21 (*m*)			168.6	
7	2.5	0.47 (*m*), 0.10 (*m*)			134.5	
8					133.9	
9					130.6	7.41
10					129.4	7.56
11					129.3	7.56
CF_3					128.7	
OCH_3					52.8	



Scheme 1. Structure of compound 1, 2 and 3.

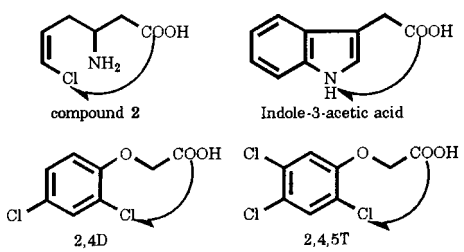
obtain crystalline derivatives of **1** adequate to X-ray analysis.

Compound **2** was identified as 2-amino-5-chloro-4-pentenoic acid based on the NMR spectrum. (S)-*cis*-2-amino-5-chloro-4-pentenoic acid was reported by Ohta et al. (1995). But our mp and IR data of **2**, mp 272° (decomp.) and $[\alpha]_D^{25} \pm 0°$ (H_2O; *c* 0.52), were very different from those of (S)-*cis*-2-amino-5-chloro-4-pentenoic acid, mp 186° (decomp.) and $[\alpha]_D^{25}$ −79.0° (H_2O; *c* 0.1). Also, the (S)-α-methoxy-α-trifluoromethylprophenylacetic acid derivative of **2** (**3**) shows coupling of vinyl proton peaks (δ_H 5.67 and 5.82, 6.16 and 6.26), whose intensities are almost same, as indicated in Table 1. Therefore, **2** is considered to exist as a racemic compound in this mushroom, because the conditions in the extraction process were relatively mild and the presence of racemic compounds has been observed sometimes in naturally occurring products (Kozlov et al., 1996).

Growth experiments were carried out according to the method of Kamisaka (1973). **1** and **2** did not affect germination in Grand Rapids lettuce seeds (data not shown). On the other hand, **1** and **2** at 10^{-4} M substantially inhibited root elongation (Fig. 1), while they had no effect on hypocotyl elongation (data not shown).

The action mechanism of these α-amino acids has not yet been elucidated.

The analysis of the relationship between structure and activity in native and synthetic auxins such as indole-3-acetic acid, 2,4-dichlorophenoxyacetic acid (2,4D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5T) suggests that the relative spatial configurations between NH or Cl and COOH groups govern their auxin-like activities (Thimann, 1963): compound **2** exhibited configurational similarity to auxins (Scheme



Scheme 2. Configurational similarity of compound 2 to auxins.

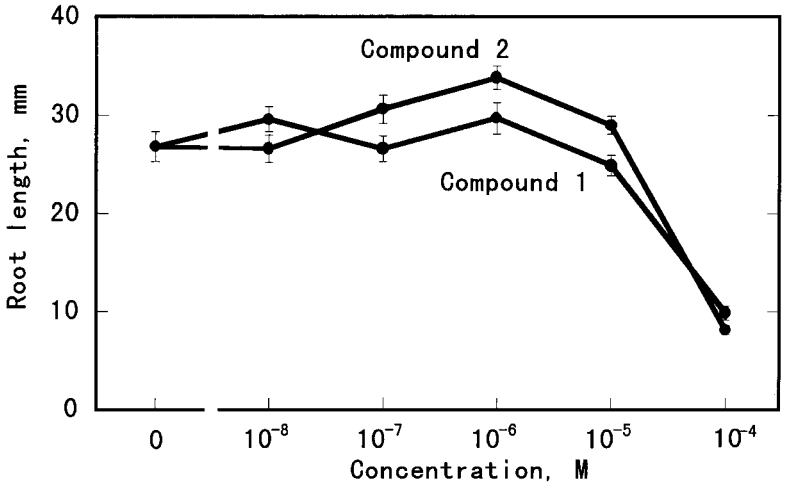


Fig. 1. Effect of compounds **1** and **2** on lettuce root elongation. One-day-old seedlings were grown for 3 days with or without varying concentrations of compounds **1** and **2**. Values are means with standard error ($n = 30$).

2). The fact that exogenous auxin strongly inhibits root growth in lettuce seedlings may support our idea. (2S)-2-amino-3-cyclopropylpropionic acid, which has a very similar structure to **1**, is known to be active against rice blast disease (Ohta et al., 1986).

3. Experimental

General. ^{1}H and ^{13}C NMR spectra were measured at 400 and 100 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent peak: δ_H 3.30 and δ_C 49.0 for MeOH. FAB–MS were taken at ionization voltage of 5 eV.

Plant material. Mushrooms, *A. castanopsidis Hongo*, were collected from a forest near Matsuo Temple, Izumi City, Osaka, Japan, in July 1996. A voucher specimen is deposited in Department of Chemistry, Faculty of Science, Osaka City University.

Extraction and isolation. Fresh whole mushrooms (810 g) were extracted twice with MeOH (2 liters) and filtered. The filtrate was concentrated in vacuo to give an aqueous solution, which was partitioned with CH_2Cl_2 and *n*-BuOH, respectively. The *n*-BuOH layer was concentrated in vacuo to yield an oily material, which was dissolved in a small volume of water–MeOH (1:1) solution and purified using a preparative HPLC equipped with an ODS column (Inertsil ODS-3 column, 5 μm, 250 × 20.0 mm column). Fractions containing **1** and **2** were collected and concentrated in vacuo.

Crude **1** was dissolved in water–MeOH (1:1) and allowed to stand for 1–2 days at room temperature to yield colorless plates of **1** (317 mg). Crude **2** was dissolved in water and allowed to stand for 1–2 days at room temperature to yield colorless needles of **2** (73 mg).

Preparation of (S)-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) ester of **2**. To a solution of **2** (5.2 mg) in pyridine (0.3 ml) was added a large excess of (S)-MTPA-Cl (100 mg) and allowed to stand for 12 h at room temperature under Ar. Ice was added to the solution and a quenched mixture was extracted with diethyl ether. The ethereal solution, after washing successively with dilute hydrochloric acid, was dried ($MgSO_4$). The solvent was evaporated, and purification by HPLC (MeOH–H_2O, 3:1) gave pure (S)-MTPA ester of **2**.

Plant growth experiment. Seeds of lettuce (*Lactuca sativa* L., cv. Grand Rapids) were germinated on 2 layers of a 7 cm filter paper, moistened with distilled H_2O, and kept for 2 days under continuous fluorescent light (3000 lux at plant level) at $25.0 \pm 0.5^{\circ}$. After 1 day, 10 seedlings selected for uniformity were placed on 2 layers of a 7 cm filter paper in a 9 cm Petri dish containing 2.7 ml of test solution. Seedlings were allowed to grow under the same light and temperature conditions used for germination. After 3 days of cultivation the lengths of the lettuce hypocotyl and their roots were measured and an average was taken for 30 seedlings from three Petri dishes.

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