Plant growth regulators from the edible mushroom Leccinum extremiorientale

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Abstract: Two compounds were isolated from the edible mushroom Leccinum extremiorientale. In the bioassay examining plant growth regulatory activity using lettuce, compound 1 promoted the root growth as low as 100 nmol/paper dose-dependently and inhibited the hypocotyl growth at 1000 nmol/paper. Compound 2 inhibited the root and hypocotyl growth at 1000 nmol/paper.

Note

Plant growth regulators from the edible mushroom *Leccinum extremiorientale*

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ABSTRACT

Two compounds were isolated from the edible mushroom *Leccinum extremiorientale*. In the bioassay examining plant growth regulatory activity using lettuce, compound **1** promoted the root growth as low as 100 nmol/paper dose-dependently and inhibited the hypocotyl growth at 1000 nmol/paper. Compound **2** inhibited the root and hypocotyl growth at 1000 nmol/paper.

Keywords:

Structural identification

Fungi are good sources of plant growth regulators, and we have been searching for the regulators from various fungi. For example, we have reported the plant growth regulators, 2-azahypoxanthine and imidazole-4-carboxamide, produced by a fairy ring-forming fungus *Lepista sordida* (Choi et al. 2010a, b). Furthermore, these compounds increased yields of rice and wheat in field experiments (Tobina et al. 2014; Asai et al. 2015). We have also reported the isolations of agrocybynes A to E from *Agrocybe praecox*, erinaceolactones A to C from *Hericium erinaceus*, (1*R*,2*S*)-1-phenylpropane-1,2-diol, isolactarorufin, lactarorufin A, 8α,13-dihydroxy-marasm-5-oic acid γ-lactone, and 7α,8α,13-trihydroxy-marasm-5-oic acid γ-lactone from *Russula vinosa* (Fushimi et al. 2012; Wu et al. 2015; Matsuzaki et al. 2016). All these compounds showed the growth regulatory activity against lettuce. During further screening for plant growth regulators using lettuce, we found the activity in the extracts of the fruiting bodies of *Leccinum extremiorientale*.

The edible mushroom *L. extremiorientale* (Japanese name, Akayamadori) belongs to the genus *Leccinum* in the family Boletaceae, and it can be seen from summer to autumn. The mushroom has a red brown areolate cap and distributes mainly in the northern temperate zone. The exhaustive studies on chemical constituents of some species in this family Boletaceae have been carried out (Kovganko et al. 1999; Hellwig et al. 2002; Kim et al. 2006; Kukovinets et al. 2006). In our previous research on the bioactive compounds from the mushroom, two sterols showed the ability to suppress the formation of osteoclasts, and leccinine A (**2**) showed protective activity against the endoplasmic reticulum stress-dependent cell death (Choi et al. 2010c, 2011). In order to find plant growth regulatory compounds from the mushroom, we carried out a chemical investigation of the mushroom.

Here, we describe the isolation, structural identification, and growth regulatory activity against lettuce of compounds **1** and **2**.

Extraction and isolation

The fresh fruiting bodies of *L. extremiorientale* were collected at Narusawa village, Yamanashi Prefecture in Japan, in Aug 2007. The fresh fruiting bodies of *L. extremiorientale* (14.9 kg) were extracted with EtOH (20 L, three times) and then with acetone (10 L, once). After the solution was combined and evaporated under reduced pressure, the concentrate of the extracts was divided into *n*-hexane-soluble, EtOAc-soluble and water-soluble parts. The *n*-hexane-soluble part (58.0 g) and the EtOAc-soluble part (32.4 g) showed the growth regulatory activity against lettuce. In this study, the *n*-hexane soluble part was fractionated by silica gel flash column chromatography (50%, 20% *n*-hexane/CH_2Cl_2; 90%, 50% CH_2Cl_2/acetone; acetone and MeOH, 2.0 L each) to obtain 16 fractions (fractions 1 to 16). Fraction 14 (814 mg) was further separated by reverse-phase HPLC (Develosil C30-UG-5; column size, ϕ20×250 mm; UV wavelength, 210 nm; flow rate, 5 mL/min; eluent, 95% MeOH) and 21 fractions were obtained (fractions 14-1 to 14-21). Fraction 14-8 (42.8 mg; retention time, 16 min) was subjected to recrystallization in diethyl ether and *n*-hexane, and compound **1** (24.2 mg) was obtained from this fraction.

Identification of active compounds

^{1}H NMR spectra (one-and two-dimensional) were recorded on a JEOL lambda-500 spectrometer (JEOL Ltd., Tokyo, Japan) at 500 MHz, while ^{13}C NMR spectra were recorded by the same instrument at 125 MHz. HRESIMS data were measured by a JMS-T100LC mass spectrometer (JEOL Ltd., Tokyo, Japan). HPLC separation was performed with a JASCO Gulliver system (JASCO Co., Tokyo, Japan) using a reverse-phase HPLC column (Develosil C30-UG-5, ϕ20×250 mm, Nomura Chemical, Seto, Japan). IR spectrum was recorded on a

FT/IR-4100 (JASCO Co., Tokyo, Japan). The specific rotation value was measured with a JASCO DIP-1000 polarimeter (JASCO Co., Tokyo, Japan). Silica cartridges and C18 cartridges (Nihon Waters K.K., Tokyo, Japan) were used in the pro-processing of the samples. Silica gel plate (TLC Silica gel 60 F_{254}, Merck KGaA, Darmstadt, Germany) and silica gel 60N (Kanto Chemical Co., Inc., Tokyo, Japan) were used for analytical TLC and for flash column chromatography, respectively.

Compound **1** was purified as colorless crystals. We measured the NMR spectra including DEPT, COSY, HMQC and HMBC. The structure of compound **1** was identified to be (8*E*, 12*Z*)-10,11-dihydroxyoctadeca-8,12-dienoic acid by comparison of their spectroscopic data with those that were reported previously (Fig. 1). This compound has been isolated from *Aspergillus flavus*, and exhibited low inhibitory activity to acetylcholinesterase that is involved in progressing Alzheimer's disease (Lopez et al. 2002; Qiao et al. 2011). The value of specific rotation of compound **1** (+ 37.0) was similar to that reported previously (+ 28.2) and the NMR data of compound **1** were also identical to that in the previous report, suggesting that absolute configuration of compound **1** was the same as that in the report (Qiao et al. 2011). However, the absolute configurations at C-10 and -11 of compound **1** has been still unknown. There is no other report of isolation of compound **1** from natural sources.

Compound **1**: colorless crystals; ESIMS *m/z* 335 $[M+Na]^+$; $[\alpha]^{28}_D$ + 37.0 (*c* 0.10, MeOH); ^{1}H NMR (in CD_3OD) δ_H: 0.86 (t, 7.0, 3H), 1.27 (m), 1.27 (m), 1.35 (m), 1.37 (m), 1.37 (m), 1.37 (m), 1.61 (m, 2H), 2.03 (m, 2H), 2.06 (m, 2H), 2.31 (t, 7.3, 2H), 4.04 (dd, 7.3, 4.3, 1H), 4.42 (dd, 8.5, 4.3, 1H), 5.37 (dd, 11.3, 8.5, 1H), 5.46 (dd, 15.6, 7.3, 1H), 5.61 (m, 1H), 5.71 (m, 1H); ^{13}C NMR (in CD_3OD) δ_C: 14.6, 22.5, 24.5, 28.0, 28.5, 28.6, 28.7, 29.3, 31.5, 32.2, 33.9, 70.5, 75.7, 127.3, 127.7, 135.1, 135.2, 178.8.

Leccinine A (**2**) has been isolated as an endoplasmic reticulum stress-suppressive compound from *L. extremiorientale* by us, however, activity towards plant growth has not been examined (Choi et al. 2011).

Plant growth regulatory activity

Lettuce seeds (*Lactuca sativa* L. cv. Cisko; Takii Co., Ltd., Tokyo, Japan) were put on filter paper (Advantec No. 2, ϕ 55 mm; Toyo Roshi Kaisha, Japan), soaked in distilled water in a Petri dish (ϕ 60 × 20 mm), and incubated in a growth chamber in the dark at 23 ˚C for 1 d. Compounds **1**, **2** and 2,4-dichlorophenoxyacetic acid (2,4-D, positive control) were dissolved in 1 mL of dichloromethane (1, 10, 100 and 1000 nmol/mL) and then poured on filter paper (ϕ 55 mm) in a Petri dish (ϕ 60 × 20 mm). After the sample-loaded paper had been air-dried, 1 mL of distilled water was poured on the sample-loaded paper or intact filter paper (control). The preincubated lettuces (n = 23 in each Petri dish) were transferred onto the sample-loaded filter paper or control filter paper and incubated in a growth chamber in the dark at 23 ˚C for 3 d. The lengths of the hypocotyl and the root were measured using a ruler.

Effect of the compounds on the growth of lettuce was examined (Fig. 2). 2,4-D inhibited the root and hypocotyl growth of lettuce dose-dependently. Compound **1** promoted the root growth as low as 100 nmol/paper dose-dependently and inhibited the hypocotyl growth at 1000 nmol/paper. Compound **2** inhibited the root and hypocotyl growth at 1000 nmol/paper.

We isolated (8*E*, 12*Z*)-10,11-dihydroxyoctadeca-8,12-dienoic acid (**1**) from *L. extremiorientale*. Although compound **1** was a known compound, this was the first time to isolate from mushroom. In the previous reports, (12*Z*,15*Z*)-9-hydroxy-10-oxo-octadeca-12,15-dienoic acid (KODA) was isolated as a stress-mediated compound from *Lemna paucicostata*. The reaction products of KODA with norepinephrine or epinephrine showed strong flower-inducing activities toward *Lemna* (Yokoyama et al. 2000, 2009; Murata et al. 2012). As this example shows, oxidized fatty acids show various activities towards plants.

Although leccinine A (**2**) has been isolated from this mushroom, this is the first report of biological activity of the compound towards plant growth. The taxon-specific germination-inducing factor (GIF) was purified from the ectomycorrhizal fungus *Leccinum aurantiacum* (Bjurman et al. 1984). Mushrooms belonging to the genus *Leccinum* may be good sources of plant growth regulators. Our result will provide useful information for the development of plant-growth regulators.

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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Figure legends

Fig. 1– Structures of compounds **1** and **2**.

Fig. 2– Effect of compounds **1** and **2** on the growth of lettuce. Lettuce seedlings were treated with compounds **1** and **2**. Respective length of growth compared with the control ± standard deviation (**p* < 0.01 vs control, n = 21–23).

Highlights

• Two compounds were isolated from the fruiting bodies of *Leccinum extremiorientale*.

• This is the first reported isolation of compound **1** from mushroom.

• Compound **1** strongly promoted the root growth of lettuce.

• Compound **2** strongly inhibited the root and hypocotyl growth of lettuce.

• The biological activity of the compounds against plant was first found in this study.

Figure
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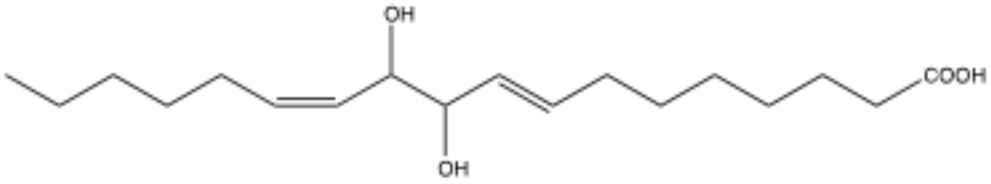

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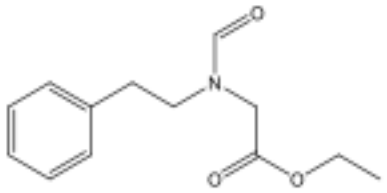

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Fig. 1 Ito *et al.*

Figure
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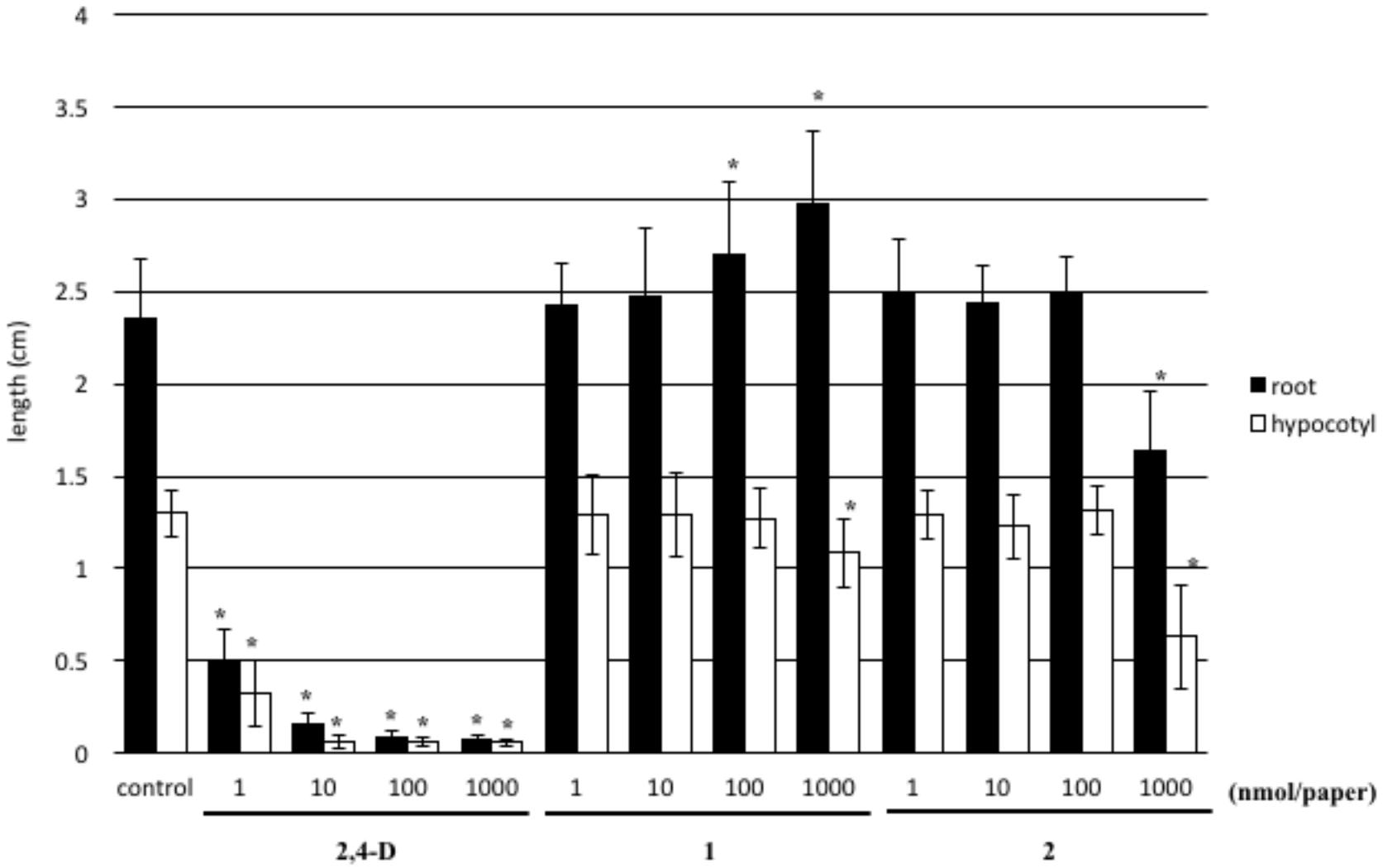

Fig. 2 Ito *et al.*