Plant growth regulatory compounds from the mushroom Russula vinosa

doi: 10.1016/j.myc.2016.07.001

Note

Plant growth regulatory compounds from the mushroom *Russula vinosa*

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Text: 7 pages; figures: 2

Abstract

Five compounds, (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 were isolated from the fruiting bodies of *Russula vinosa*. In the bioassay examining plant-growth regulatory activity using lettuce, all the compounds regulated the growth.

Keywords

Plant growth regulator; Structural identification

We have reported the isolation of plant growth regulators, 2-azahypoxanthine and imidazole-4-carboxamide, from the fungus *Lepista sordida* that form "fairy rings" (Choi et al. 2010a, b). These compounds increased yields of rice and wheat in field experiments (Tobina et al. 2014; Asai et al. 2015). Based on these studies, we are screening for plant growth regulators from fungi that interact with plants. During the screening using lettuce, we found strong activity in the extracts of the fruiting bodies of *Russula vinosa*.

Russula vinosa is an edible wild mushroom with high medicinal value (Chen et al. 2007). The extracts of *R. vinosa* have an inhibition effect on bacteria, yeasts and molds (Li et al. 1998).

Here, we describe the isolation and identification of plant growth regulators from the fruiting bodies of this species.

Extraction and isolation

Fruiting bodies of *R. vinosa* were collected in Wuyi Mountains of Fujian Province in China in Aug 2010. There were *Cyclobalanopsis chungii* nearby the fruiting bodies. The collected fruiting bodies were air-dried soon after collection. The dried fruiting bodies were extracted and fractionated twice. In the first time, 1.5 kg of the fruiting bodies was crushed in a blender and the crushed fruiting bodies were extracted with hexane and then EtOAc. The EtOAc-soluble part (25.1 g) was fractionated by silica gel flash column chromatography (Silica gel 60 N, Kanto Chemical, Tokyo, Japan; CH_2Cl_2 (fraction 1); 90% (factions 2 to 3), 80% (fractions 4 to 5), 70% (fractions 6 to 7), 60% (fraction 8), 40% (fractions 9 to 10), 30% (fraction 11), 10% (fractions 12 to 13) CH_2Cl_2/EtOAc; 90% (fraction 14), 70% (fraction 15), 50% (fractions 16 to 17), 40% (fractions 18 to 20), 30% (fraction 21), 20% (fractions 22 to 23), 10% (fraction 24) EtOAc/MeOH; MeOH; 80% (fraction 25), 70% (fraction 26) MeOH/acetic acid) to obtain 26 fractions (fractions 1 to 26). HPLC separations were performed with a Jasco Gulliver system. Fraction 14 (301 mg) was separated by reverse-phase HPLC (Develosil C30-UG-5, Nomura Chemical, Seto, Japan; 40% MeOH) to afford 1 (2.0 mg). Fraction 15 (173 mg) was fractionated by ODS gel flash column chromatography (Cosmosil 140 C18-OPN, Nacalai Tesque, Kyoto, Japan; 90%, 98%, 99% MeOH, MeOH) to obtain 10 fractions (fractions 15-1 to 15-10). Fraction 15-3 (59.0 mg) was further separated by reverse-phase HPLC (Develosil C30-UG-5, 55% MeOH) to obtain 9 fractions (fractions 15-3-1 to 15-3-9). Fraction 15-3-4 (5.9 mg) was fractionated by reverse-phase HPLC (Cosmosil πNAP, Nacalai Tesque, Kyoto, Japan; 50% MeOH) to afford 2 (2.7 mg). In the second time, 5.0 kg of the fruiting bodies was crushed in a blender and the crushed fruiting bodies were extracted with hexane and then EtOAc. The EtOAc-soluble part (41.5 g) was fractionated by silica gel flash column chromatography (Silica gel 60 N, Kanto Chemical, Tokyo, Japan; CH_2Cl_2 (fraction 1'); 90% (fraction 2'), 80% (fraction 3'), 60% (fraction 4'), 40% (fraction 5'), 30% (fraction 6'), 10% (fraction 7') CH_2Cl_2/EtOAc; 90% (fraction 8'), 70% (fraction 9'), 60% (fraction 10'), 50% (fraction 11'), 40% (fraction 12'), 30% (fraction 13'), 20% (fraction 14') EtOAc/MeOH; MeOH (fractions 15' to 16'); 70% (fraction 17') MeOH /acetic acid) to obtain 17 fractions (fractions 1'-17'), respectively. Fraction 8' (333 mg) and fraction 9' (1.19 g) were separated by ODS gel flash column chromatography (70%, 80%, 90% MeOH, MeOH) to give 13 and 19 fractions (fractions 8'-1 to 8'-13 and fractions

9'-1 to 9'-19), respectively. Fraction 8'-2 (16.7 mg) was separated by reverse-phase HPLC (Develosil C30-UG-5, 55% MeOH) to afford 4 (1.8 mg) and 5 (1.4 mg). Fraction 9'-2 (40.9 mg) was separated by reverse-phase HPLC (Develosil C30-UG-5, 55% MeOH) to obtain 23 fractions (fractions 9'-2-1 to 9'-2-23) and fraction 9'-2-13 (5.1 mg) was further separated by reverse-phase HPLC (Cosmosil πNAP, 50% MeOH), affording 3 (1.1 mg).

^{1}H NMR spectra (one- and two-dimensional) were recorded on a Jeol lambda-500 spectrometer at 500 MHz, and ^{13}C NMR spectra were recorded on the same instrument at 125 MHz (Jeol, Tokyo, Japan). ESIMS spectra were measured on a JMS-T100LC mass spectrometer (Jeol, Tokyo, Japan). IR spectra were recorded on a FT/IR-4100 (Jasco, Tokyo, Japan).

We obtained five compounds from the fruiting bodies of *R. vinosa*.

Compound 1: white crystals; ESIMS *m/z* 175 $[M+Na]^+$; IR (neat): 3351 cm^{-1}; ^{1}H-NMR (in CD_3OD) δ_H: 1.10 (d, J = 6.4), 2.03, 4.00 (dq, J = 4.0, 6.4), 2.64, 4.48 (d, J = 5.2), 7.30 (m, J = 7.6); ^{13}C-NMR, δ_C: 18.1, 72.4, 79.1, 128.0, 128.3, 129.0, 143.4.

Compound 2: white crystals; ESIMS *m/z* 289 $[M+Na]^+$; IR (neat): 3392 cm^{-1}; ^{1}H-NMR (in CD_3OD) δ_H: 0.95 (s), 1.0 (d), 1.08 (s), 1.18 (dd), 1.45 (ddd), 1.53 (s), 1.65 (dd), 1.70 (d), 1.81 (ddd), 1.93 (m), 2.12 (m), 4.16 (d), 4.25 (d), 4.28 (d); ^{13}C-NMR δ_C: 18.6, 24.4, 27.3, 29.8, 34.6, 39.1, 39.4, 41.3, 44.3, 47.7, 48.8, 72.1, 73.4, 75.6, 178.1.

Compound 3: white amorphous; ESIMS *m/z* 289 $[M+Na]^+$;IR (neat): 3221 cm^{-1}; ^{1}H-NMR (in CD_3OD) δ_H: 1.03 (s), 1.09 (s), 1.21 (s), 1.37 (d), 1.40 (m), 1.60 (d), 1.70 (m), 2.46 (d), 2.60 (m), 2.62 (dd), 2.69 (d), 4.32 (d), 4.74 (d), 4.88 (d); ^{13}C-NMR δ_C: 28.3, 29.3, 30.4, 36.7, 37.4, 45.0, 46.3, 47.1, 52.2, 69.1, 72.6, 74.0, 124.1, 164.3, 177.4.

Compound 4: white amorphous; ESIMS *m/z* 273 $[M+Na]^+$;IR (neat): 3392 cm^{-1}; ^{1}H-NMR (in CD_3OD) δ_H: 1.04 (s), 1.06 (d), 1.13 (s), 1.24 (d), 1.25 (s), 1.50 (m), 1.54 (m), 1.62, (d), 1.65 (m), 1.85 (m), 2.41 (d), 2.64 (m), 3.23 (t), 4.17 (m), 4.69 (m); ^{13}C-NMR δ_C: 17.8, 30.2, 30.9, 32.5, 32.7, 36.7, 37.8, 42.7, 44.7, 45.6, 46.2, 46.7, 73.2, 73.8, 180.6.

Compound 5: white amorphous; ESIMS *m/z* 289 $[M+Na]^+$;IR (neat): 3335 cm^{-1}; ^{1}H-NMR (in CD_3OD) δ_H: 0.74 (d), 0.93 (s), 1.03 (s), 1.41 (s), 1.47 (q), 1.60(d), 1.80 (q), 1.82 (d) 2.17 (d), 2.25 (d), 2.76 (m), 3.65 (d), 4.12(d), 4.51 (d); ^{13}C-NMR δ_C: 17.8, 27.4, 28.9, 29.6, 30.0, 33.6, 38.4, 45.0, 45.2, 46.9, 50.6, 74.5, 76.3, 79.6, 180.5.

Their structures were identified by interpretation of their spectroscopic data and all the compounds have been reported previously (Fig. 1). Compound 1, (1*R*,2*S*)-1-phenylpropane-1,2-diol, has been isolated from *Trametes* sp. (Brambilla et al. 1995). Compound 2 was identified as isolactarorufin and has been isolated from *Lactarius piperatus* (Wang et al. 2003). Compound 3 was lactarorufin A, which has been isolated from *Lactarius vellereus* (Kamo et al. 2006), *Strobilurus stephanocystis* (Hiramatsu et al. 2008) and *Russula emetica* (Kobata et al. 1995). Lactarorufin A showed selective cytotoxicity against the A549, and HCT-15 cell lines (Kim et al. 2010) and anti-feedant activity of insects *Sitophilus granarius* (Daniewski et al. 1993). Compound 4, 8α,13-dihydroxy-marasm-5-oic acid γ-lactone, has been isolated from *L. vellereu*s (Daniewski et al. 2003) and *Russula foetens* (Wang et al. 2006). Compound 5, 7α,8α,13-trihydroxy-marasm-5-oic acid γ-lactone, was isolated from *R. foetens* (Wang et al. 2006) and *L. piperatus* (Wang et al. 2003).

Plant growth regulatory activity

Lettuce seeds (*Lactuca sativa* L. cv. Cisko; Takii Co., Ltd., Tokyo, Japan) were put on filter paper (Advantec No. 2, ϕ55 mm; Toyo Roshi Kaisha, Japan), soaked in distilled water in a Petri dish (ϕ60 × 20 mm), and incubated in a growth chamber in the dark at 25 ˚C for 1 d. Each sample was dissolved in 1 mL of MeOH (1, 10^{-1}, 10^{-2} and 10^{-3} μmol/mL) and then poured on filter paper (ϕ55 mm) in a Petri dish (ϕ60 × 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 = 9 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 25 ˚C for 3 d. The lengths of the hypocotyl and the root were measured using a ruler.

Effects of all the compounds on the growth of lettuce were examined (Fig. 2). Compound 4 weakly inhibited the root and hypocotyl growth of lettuce at 1 μmol/paper, while compound 3 showed an inhibition as low as 100 nmol/paper. As for the root growth of lettuce, compound 2 showed promotion at 10 and 100 nmol/paper, but showed inhibition at 1 nmol/paper and 1 μmol/paper. As for the hypocotyl growth of lettuce, compound 1 showed inhibition at 10 and 100 nmol/paper and promotion at 1 nmol/paper, compound 2 showed a promotion at 100 nmol/paper, compound 5 showed promotion at lower doses (1 and 10 nmol/paper).

We isolated five compounds from the mushroom *R. vinosa*. The structures were identified by spectroscopic analyses. Although these compounds have been already known as mentioned above, their biological activity against plant was first found in this study. In the previous report (Kamo et al. 2006), compound 3 at 3.6×10^2 μM showed no growth activity against lettuce, while this compound at 100 nmol/paper inhibited lettuce growth in this study. The reason of the difference between the two results might be due to the difference of the procedures of the bioassay. However, our results 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.

Acknowledgments

We thank Dr. V. K. Deo (Shizuoka University) for valuable discussion, and Mr. H. Oka for collecting the mushroom.

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Figure legends

Fig. 1– Structures of compounds 1–5.

Fig. 2– Effect of 1 to 5 on the growth of lettuce. Lettuce seedlings were treated with 1 to 5. Respective length of growth compared with the control ± standard deviation (*$p < 0.05$, **$p < 0.01$ vs control, n = 7)

Highlights

- Five compounds were isolated from the fruiting bodies of *Russula vinosa*.
- All the compounds regulated lettuce growth.
- The biological activity of the compounds against plant was first found in this study.

Figure
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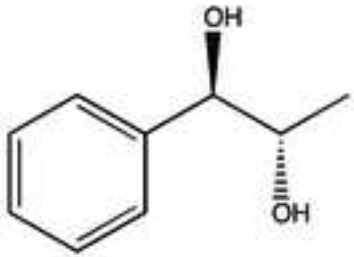

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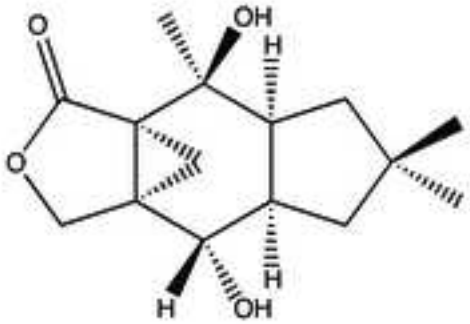

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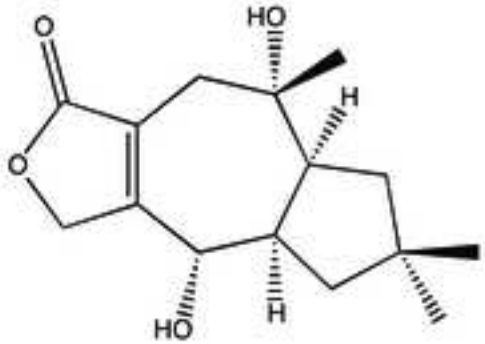

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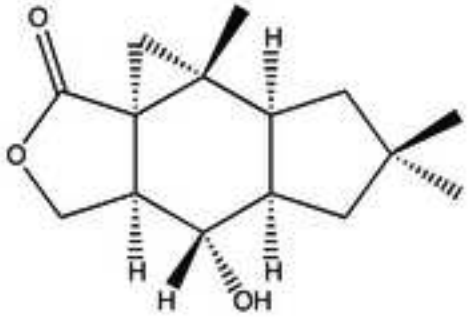

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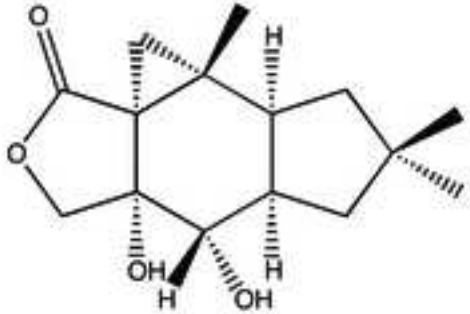

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Figure 1. Matsuzaki *et al.*

Figure
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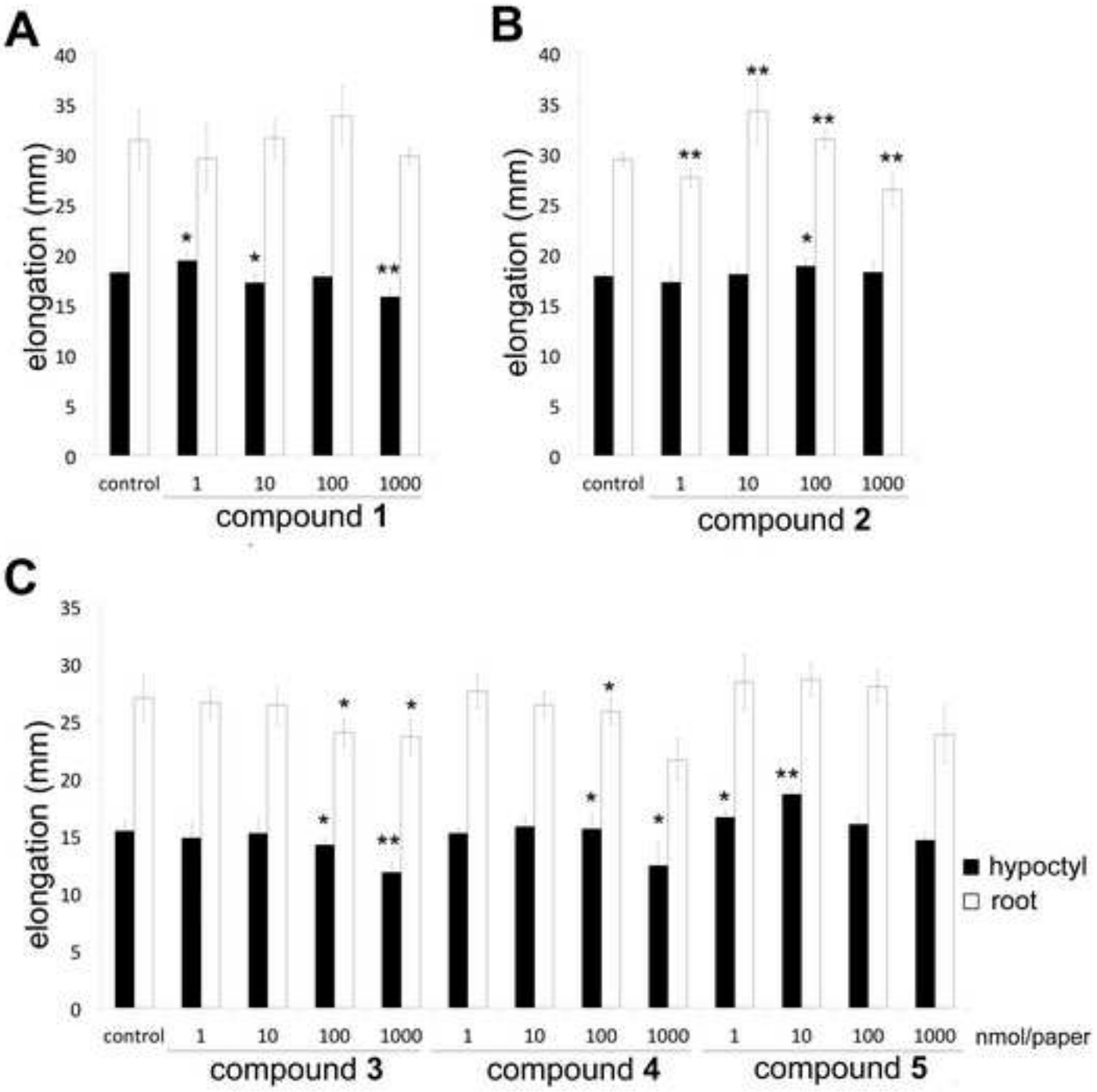

Figure 2 Matsuzaki *et al.*