# Bioactive compounds from the edible mushroom Cortinarius caperatus

**Note**

# Bioactive compounds from the edible mushroom *Cortinarius caperatus*

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Abstract

Six compounds, 5-(1-hydroxyethyl)dihydrofuran-2(3*H*)-one (1), 4-ethoxy-4-oxobutanoic acid (2), 4-ketononanoic acid (3), *S*-acetyl-*N*-glycyl cysteamine (4), methyl benzoate (5) and *p*-hydroxybenzoic acid (6) were isolated from the fruiting bodies of *Cortinarius caperatus*. Compounds 1, 3, 5, and 6 inhibited growth of *Flammulina velutipes* mycelia. Compounds 2, 3, 5, and 6 exhibited growth regulatory activities toward rice seedlings, while compounds 3, 5, and 6 regulated the growth of lettuce. Compound 4 was first isolated from a natural source. In addition, the activity of compound 6 against rice was compared with those of its analogs.

*Keywords*

Mycelia growth regulation; Plant growth regulation; Structural identification

Higher fungi have been recognized to produce secondary metabolites with a variety of biological activities. In recent years, our group has been searching for metabolites of mushroom-forming fungi with specialized activities, such as growth regulation toward plants and fungi. For example, erinaceolactones A to C from the culture broth of *Hericium erinaceus* and melleolide and armillarikin from *Armillaria* sp. were reported to regulate the growth of lettuce and mycelia of *Coprinopsis cinerea* and *Flammulina velutipes* (Kobori et al. 2015; Wu et al. 2015). Recently, we also reported five compounds from the edible mushroom *Russula vinosa*, and two compounds from the fruiting bodies of *Leccinum extremiorientale* that regulated the growth of lettuce, and three compounds that inhibited the root growth of bentgrass from the fairy ring-forming fungus *Lepista sordida* (Ito et al. 2017a,b; Matsuzaki et al. 2016). In our continuing attempt to search for bioactive compounds from mycelia and fruiting bodies of higher fungi, we revealed that extracts from *Cortinarius caperatus* regulated the growth of rice seedlings, lettuce, and mycelium of *F. velutipes*.

*Cortinarius caperatus* is an edible mushroom and widely grows in the temperate zone of the northern hemisphere (Falandysz 2014). This species has been reported to produce an antiviral compound, RC-183, toward herpes simplex virus (Piraino and Brandt 1999). However, further studies on secondary metabolites and the biological activities of this mushroom have not been carried out.

In this article, we describe the isolation, structural identification, and biological activities of the compounds.

***Extraction and isolation***

Fresh fruiting bodies of *C. caperatus* (38.4 kg) were collected at Narusawa village, Yamanashi Prefecture, in Japan. The crushed fruiting bodies were extracted with ethanol (EtOH) and then acetone. The solutions were combined, concentrated under reduced pressure, and partitioned between *n*-hexane and water, ethyl acetate (EtOAc) and water, and *n*-butanol and water, successively. The EtOAc soluble part (14.9 g) was subjected to silica gel column chromatography (Silica gel 60N, Kanto Chemical, Tokyo, Japan); 100% $CH_2Cl_2$ (fraction 1), 90% (fractions 2 to 3), 70% (fractions 4 to 6), 50% (fractions 8 to 11) $CH_2Cl_2$/acetone; 80% (fractions 12 to 15), 60% (fractions 16 to 18) $CH_2Cl_2$/methanol (MeOH), 100% MeOH (fraction 19) to obtain 19 fractions (fractions 1 to 19). Fraction 10 (701 mg) was fractionated by ODS gel flash chromatography (60%, 70%, 80%, 90%, 100% MeOH) to afford 12 fractions (fractions 10-1 to 10-12). HPLC separations were performed with a Jasco Gulliver system. Fraction 10-2 (48.7 mg) was separated by reverse-phase HPLC (Develosil C30-UG-5, Nomura Chemical, Seto, Japan; 40% MeOH) to obtain compounds 2 (9.9 mg) and 6 (10.7 mg), respectively. Fraction 10-2-2 (16.2 mg) was further purified by reverse-phase HPLC (Cosmosil PBr, Nacalai Tesque, Kyoto, Japan; 40% MeOH) to afford compound 1 (7.8 mg). Fraction 8 was subjected to ODS gel flash chromatography (60%, 70%, 80%, 90%, 100% MeOH) to obtain 10 fractions (fractions 8-1 to 8-10). Fraction 8-2 (44.4 mg) was further fractionated by reverse-phase HPLC (InertSustain Phenyl, GL Sciences, Tokyo, Japan; 40% MeOH) to yield compound 5 (6.2 mg). Fraction 8-3 (41.0 mg) was purified by reverse-phase HPLC (InertSustain Phenylhexyl, GL Sciences, Tokyo, Japan; 40% MeOH) to afford compound 3 (9.1 mg). Fraction 11 (620 mg) was separated by silica gel MPLC using (90%,

80%, 70%, $CHCl_3$/MeOH, 100% MeOH) to give 9 fractions (fractions 11-1 to 11-9). Fraction 11-3 (27.7 mg) was further fractionated by normal-phase HPLC (Cosmosil 5SLII, Nacalai Tesque, Kyoto, Japan; 90% $CHCl_3$/MeOH) to obtain compound 4 (1.5 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 isolated six compounds from the fruiting bodies of *C. caperatus*.

Compound 1: colorless oil; ESIMS *m/z* 153 $[M+Na]^+$; IR (neat): 3419 $cm^{-1}$; $[\alpha]\ ^{27}_D$ = +6 (*c* = 0.7, $CHCl_3$); $^1$H-NMR (in $CD_3OD$) $\delta_H$: 1.16 (d, *J* = 6.4), 2.20 (m), 2.53 (dd, *J* = 7.9, 8.8), 3.92 (m), 4.40 (m), 7.30; $^{13}$C-NMR, $\delta_C$: 18.7, 22.6, 29.4, 68.8, 85.6, 180.3.

Compound 2: pale yellow oil; ESIMS *m/z* 145 $[M-H]^-$; $^1$H-NMR (in $CD_3OD$) $\delta_H$: 1.24 (t, *J* = 8.0), 2.58 (s), 4.13 (m), 4.89 (s); $^{13}$C-NMR $\delta_C$: 14.5, 29.8, 30.1, 61.7, 174.3.

Compound 3: pale yellow oil; ESIMS *m/z* 171 $[M-H]^-$; $^1$H-NMR (in $CD_3OD$) $\delta_H$: 0.9 (t, *J* = 7.0), 1.28 (m), 1.32 (m), 1.56 (q, *J* = 7.3), 2.47 (t, *J* = 7.3), 2.51 (t, *J* = 6.7), 2.72 (t, *J* = 6.7); $^{13}$C-NMR $\delta_C$: 14.3, 23.5, 24.5, 28.8, 32.5, 38.0, 43.4, 176.6, 212.0.

Compound 4: pale yellow amorphous; ESIMS *m/z* 172 $[M+Na]^+$; IR (neat): 3584 $cm^{-1}$; $[\alpha]\ ^{22}_D$ = +4.7 (*c* = 0.22, MeOH); $^1$H-NMR (in $CD_3OD$) $\delta_H$: 1.90 (s), 2.63 (s), 2.86 (ddd, *J* = 13.4, 6.1, 5.8), 3.00 (ddd, *J* = 13.1, 6.1, 5.8), 3.51 (m), 3.55 (m); $^{13}$C-NMR $\delta_C$: 22.4, 34.6, 38.4, 54,8, 173.6.

Compound 5: white powder; ESIMS *m/z* 135 $[M-H]^-$; $^1$H-NMR (in $CD_3OD$) $\delta_H$: 4.8 (s), 6.9 (d), 7.3 (s), 7.6 (d); $^{13}$C-NMR $\delta_C$: 42.0, 127.9, 129.4, 130.3, 136.1, 175.6.

Compound 6: white amorphous; ESIMS *m/z* 137 $[M-H]^-$; $^1$H-NMR (in $CD_3OD$) $\delta_H$: 4.9 (s), 6.8 (d), 7.9 (d); $^{13}$C-NMR $\delta_C$: 116.0, 122.8, 132.9, 163.3, 170.1.

All the compounds have been previously reported and their structures were identified by interpretation of spectroscopic data (Fig. 1). Compound 1 was identified as 5-(1-hydroxyethyl)dihydrofuran-2(3*H*)-one. The stereochemistry of the compound remains undetermined. Compound 2 was identified as 4-ethoxy-4-oxobutanoic acid, which has been previously isolated from *Saccharomyces cerevisiae* (Vigentini et al. 2016). This compound also has been found as an antioxidant (Pereira et al. 2017). Compound 3, 4-ketononanoic acid, has been isolated from tobacco (Leffingwell et al. 1972). The biological activity of this compound has not been reported yet. Although compound 2 was detected by GC-MS in the fruiting bodies of *Dictyophora indusiata*, this is the first isolation of compounds 2 and 3 from mushrooms (Chao et al. 2014). Compound 4 was identified as *S*-acetyl-*N*-glycyl cysteamine. This compound has been synthesized as a radioprotectant in the treatment of tumour, however, this is the first isolation from a natural source (Maurizis et al. 1988, 1989). Compound 5 was identified as methyl benzoate and has been isolated from *Agaricus subrufucens* (Chen and Wu 1984). Methyl benzoate has been previously reported as antioxidant and insect attractant for *Euglossa cybelia* (Schiestl and Roubik 2003). Compound 6 was identified as *p*-hydroxybenzoic acid. This compound has been isolated from *Ganoderma lucidum* and exhibited antimicrobial and demelanizing activity (Heleno et al.

2013).

***Mycelial growth regulatory activity***

The mycelia of *Flammulina velutipes* were placed onto the center of a potato dextrose agar (PDA) plate. Meanwhile, each test compound solutions were poured onto autoclaved paper disks (Advantec $\phi$ 8 mm) and then air-dried. Each air-dried paper disk containing 10 μmol/paper disk of the compounds were placed directly onto the incubated plate (n = 3). Plates were further incubated at room temperature for 1 wk. After the incubation, the activity was evaluated by observation of the inhibition zones.

All the compounds except for compound 4 were evaluated for growth regulating activity against a fungus *F. velutipes* (Supplementary Fig. S1). All the tested compounds except for compound 2 inhibited growth of the mycelia at 10 μmol/paper disk. Among them, compound 1 showed the strongest activity.

***Plant growth regulatory activity***

Lettuce seeds (*Lactuca sativa* L. cv. Great Lakes 366, Takii Co., Ltd., Tokyo, Japan) were put on filter paper (Advantec No. 2, $\phi$ 55 mm; Toyo Roshi Kaisha, Ltd., Tokyo, Japan), soaked in distilled water in a Petri dish ($\phi$ 60 × 20 mm) and incubated in a growth chamber under dark at 25 ºC for 1 d. Each sample was dissolved in 1 mL of methanol (1, $10^{-1}$, $10^{-2}$ and $10^{-3}$ μmol/mL) and then poured onto filter paper ($\phi$ 55 mm) in a Petri dish ($\phi$ 60 × 20 mm). After the solvent was air-dried, 1 mL of distilled water was poured on the sample-loaded paper or intact filter paper (control). The pre-incubated lettuces (n = 9 in each petri dish) were transferred onto the filter paper and incubated in a growth chamber under dark at 25 ºC for 3 d. The elongations were measured using a ruler.

Rice seeds (*Oryza sativa* L. cv. Nipponbare) were sterilized in ethanol for 5 minutes and then 1% sodium hypochlorite for 30 min on a plastic container (18.5 × 14.5 × 4.5 cm). The seeds were washed completely in sterile water and germinated for 2 d at 30 ºC with intensive light. The seedlings (n = 5) were planted onto glass jars ($\phi$ 5.5 × 10 cm) containing 500 μL test samples (10, 100, 1000 μM) and 5 mL distilled water. For the comparison of *p*-hydroxybenzoic acid and its analogues, different concentrations (0.01, 0.1, 0.5, 1, and 5 mM) were applied (n = 15). Afterward incubated for 5 d at 30 ºC with intensive light. The elongations were measured using a ruler and digimatic caliper (Mitutoyo, Kawasaki, Japan).

We examined the plant regulatory activity using lettuce, in the assay, 2,4-dichlorophenoxyacetic acid was also used as a positive control, which inhibited lettuce growth dose-dependently (Supplemetary Fig. S2). Compounds 5 and 6 inhibited the growth of the root at 1 μmol/paper. As for the hypocotyl, compounds 3 and 5 showed inhibition at $10^{-3}$ and 1 μmol/paper, respectively. While compounds 5 and 6 promoted the plant at $10^{-2}$ and $10^{-1}$ μmol/paper, respectively. Compound 4 was not tested due to the insufficient amount.

Compounds 1–6 were examined for growth regulatory activity toward rice (Supplementary Fig. S3). The positive control, 2,4-dichlorophenoxyacetic acid, inhibited the plant significantly. Compounds 2 and 6 promoted the growth of the root at 1mM. Compound

3 promoted the root growth at 100 μM, however, inhibited the root and shoot growth at 1 mM. As for the shoot, compound 5 inhibited the growth of the shoot at 1 mM. Compound 6 has been previously reported as a wheat allelochemical (Cheng and Cheng 2015).

Since compound 6, *p*-hydroxybenzoic acid, showed the strongest promotion activity against rice among the compounds tested, we compared the activity with those of its commercially available analogues, *m*-hydroxybenzoic acid (7), *o*-hydroxybenzoic acid (8), benzoic acid (9), methyl *p*-hydroxybenzoate (10), methyl *m*-hydroxybenzoate (11), methyl *o*-hydroxybenzoate (12), and methyl benzoate (5). As shown in Fig. 2, compounds 6 and 7 promoted the root growth at 1 mM and 5 mM. Meanwhile, compounds 8 and 12 showed promotion activity at 0.1 mM. In addition, compounds 10 and 11 have the optimum concentration for promotion of the growth at 0.5 mM. Compounds 9 and 5 showed no significant effect on the growth. As for the shoot, compound 6 showed promotion activity at 0.1, 0.5, and 1 mM. In this assay, it is very difficult to discuss the structure-activity relationship. However, it is allowed us to conclude that at least *p*-hydroxybenzoic acid (6) isolated from the mushroom in this study was the strongest growth promoter and hydroxy group attached to the benzene ring tends to increase the promotive activity. The previous study with the similar bioassay toward dwarf rice (Tan-ginbozu rice) revealed that compounds with no hydroxy substitution had no effect or are slightly inhibitory for the root growth. *Ortho*-substituted compounds were highly inhibitory, *meta*-substituted compounds were less inhibitory, and *para*-substituted compounds stimulated the root growth. In addition, the presence of methoxy group exhibited root growth inhibitory activity (Ueda 1989).

Plant and mycelial growth regulatory activities of the obtained compounds have not been reported previously. Due to structures of compounds and variety of activities, it might be difficult to explain the structure-activity relationships or mechanisms among them in the plant and mycelial growth regulatory activity. However, our results will provide advantageous information for the further study on fungal bioactive compounds specifically for agricultural usage.

**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 1–6.

Fig. 2– Effect of *p*-hydroxybenzoic acid and its analogues on the growth of rice seedlings. Results are the mean ± standard deviation (n = 15). [ *p < 0.05, **p < 0.01 (growth inhibition); $^{+}p < 0.05$, $^{++}p < 0.01$ (growth promotion)].

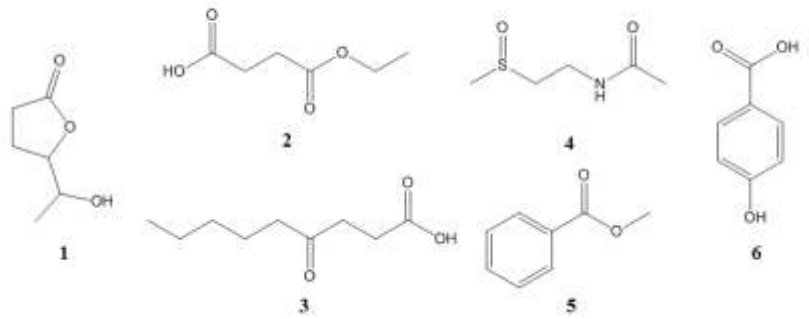


**Figure 1.** Ridwan *et al.*

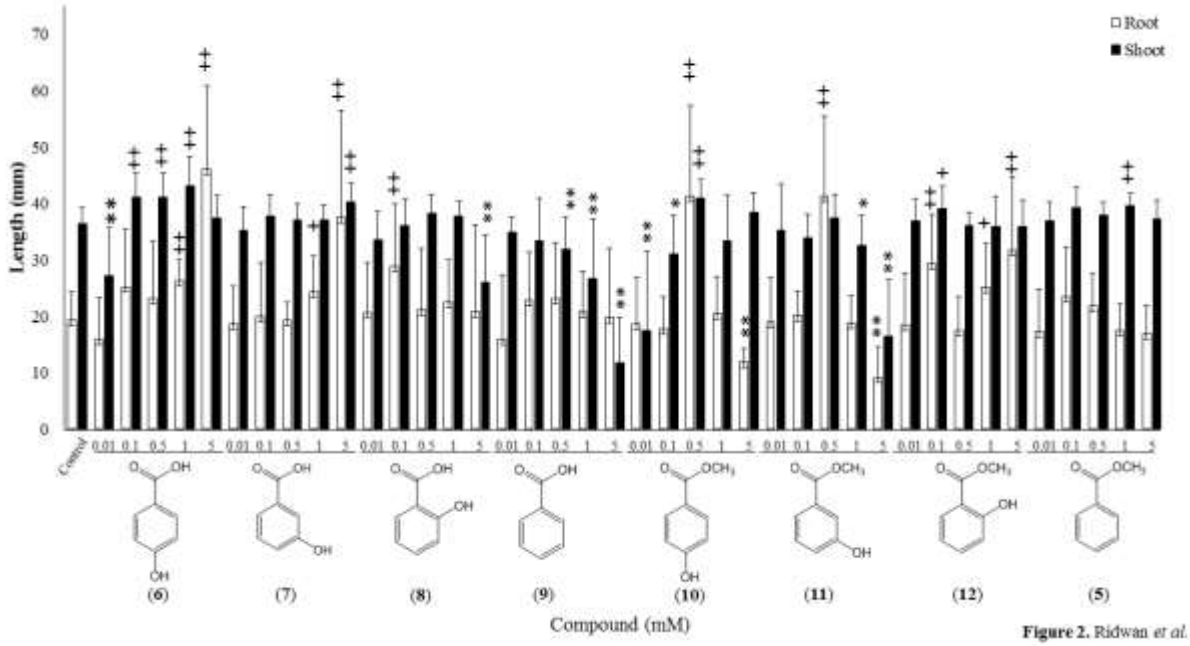


**Figure 2.** Ridwan *et al.*