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# Plant growth regulators from the fruiting bodies of Tricholoma flavovirens



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1 **Plant growth regulators from *Tricholoma flavovirens***
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1 **Abstract**

2 **A novel indole derivative (1) and three known compounds (2–4) were isolated**
3 **from the fruiting bodies of *Tricholoma flavovirens*. Their structures were**
4 **determined or identified by the interpretation of spectroscopic data. Compounds 1**
5 **and 2 promoted root growth of lettuce and inhibited hypocotyl growth at 1**
6 **μmol/paper. Compound 3 inhibited hypocotyl and root growth at 100 nmol/paper.**

7

8 **Key words**: *Tricholoma flavovirens*; structural determination; plant growth regulating
9 activity; mushroom

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1 We have been continuing to search for bioactive compounds from mushrooms
2 using various bioassays. In our previous works, we have isolated several plant growth
3 regulators from several kinds of fruiting bodies or culture broth of higher fungi.[1-3)]
4 We also reported the isolation and structural determination of a novel compound
5 and a known one from the mushroom *Tricholama flavovirens*.[4)] During the further
6 search, we succeeded in isolation of plant growth regulating compounds from this
7 mushroom.
8 Here we describe the isolation and structural determination of a novel indole
9 derivative and three known compounds, and their activity.
10 Fresh fruiting bodies of *T. flavovirens* were extracted with EtOH and then with
11 acetone. After the solutions were combined and concentrated, they were partitioned
12 between *n*-hexane and $H_2O$, EtOAc and $H_2O$, and then *n*-BuOH and $H_2O$. The
13 *n*-hexane- and EtOAc-soluble parts were fractionated by repeated chromatography. As a
14 consequence, four compounds (**1** – **4**) were purified (Fig. 1A).
15 Compound **1** was isolated as a white amorphous, mp 178-180 °C (decomp.). Its
16 molecular formula was determined as $C_{12}H_{15}NO_2$ by HRESIMS at *m/z* 206.1165 $[M +$
17 $H]^+$ (calcd. for $C_{12}H_{16}NO_2$ 206.1181), indicating the presence of six degrees of
18 unsaturation in the molecule. The structure of **1** was elucidated by interpretation of
19 NMR spectra including DEPT, COSY, HMQC, and HMBC (Fig. 1B). The DEPT
20 experiment indicated the presence of four methyls, two methines and six quaternary
21 carbons. The structure of 2,4-dimethylindole skeleton was elucidated by the HMBC
22 correlations (H-2-Me/C-2, C-3; H-4-Me/C-3a, C-4, C-5, H-6/C-4, C-5, C-7, C-7a) and
23 the COSY correlations (H-2-Me/H-3). The HMBC correlations (H-5-OMe/C-5;
24 H-7-OMe/C-7) indicated the position of 5-OMe and 7-OMe on the 2, 4-dimethylindole
25 skeleton. The complete assignment of protons and carbons of NMR was accomplished
26 as shown in Table 1. As a result, the structure of **1** was determined to be
27 5,7-dimethoxy-2,4-dimethylindole.
28 Compound **2** was isolated as a white amorphous. It was identified as
29 5-methoxy-2,4-dimethylindole. This compound has been reported as a degradation
30 product when the bitter principle of *Tricholoma lascivum*, lascivol, was treated with
31 strong acid, and has been isolated from the same genus mushroom *Tricholoma*
32 *sciodes*.[5,6)]
33 Compound **3** was isolated as a white crystal. Its molecular formula was determined

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1 as $C_{10}H_9NO_3$ by HRESIMS at *m/z* 190.0510 $[M - H]^-$ (calcd. for $C_{10}H_8NO_3$ 190.0504),
2 indicating the presence of 7 degrees of unsaturation in the molecule. The structure of **3**
3 was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and
4 HMBC (Fig. 1B). The DEPT experiment indicated the presence of one methyl, four
5 methines and five quaternary carbons. The structure of phthalide skeleton was
6 elucidated by the HMBC correlations (H-3/C-1, C-3a, C-4, C-7a; H-4/C-3, C-3a, C-5,
7 C-6, C-7, C-7a; H-5/C-3a, C-4, C-6, C-7, C-7a; H-6/C-3a, C-4, C-5, C-7, C-7a) and the
8 COSY correlations (H-4/H-5, H-5/H-6). The HMBC correlations (H-1'/C-2';
9 H-3'/C-2') indicated the presence of acetamido group. The connection between
10 acetamido group and phthalide was confirmed by the HMBC correlations (H-3'/C-6,
11 C-7a). The complete assignment of protons and carbons of NMR was accomplished as
12 shown in Table 1. As a result, **3** was identified to be 7-acetamidophthalide. This
13 compound has been synthesized, but this is the first report as a natural compound.[7)]
14 Compound **4** was isolated as a white amorphous. It was identified as
15 4-methoxymethyl- 3-[(2-methyl-4-indolyl)methyl]-2-methylindole. It has also been
16 isolated from *T. sciodes* together with **2**.[6)]
17 Biological activities of compounds **2** to **4** have not been reported yet.
18 Compounds **1** to **3** were evaluated in the plant growth regulatory assay using
19 lettuce (Fig. 2). 2,4-Dichlorophenoxyacetic acid was used as positive control, which
20 inhibited the hypocotyl and root growth of lettuce dose-dependently. In order to know
21 structure-activity relationship, **3** was compared with phthalide. As a result, **1** and **2**
22 promoted the root growth and inhibited hypocotyl growth at 1 μmol/paper. **3** and
23 phthalide inhibited the root growth dose-dependently. In addition, phthalide inhibited
24 the hypocotyl growth at 1 μmol/paper, while **3** showed inhibition activity at 100
25 nmol/paper.
26
27 **Experimental**
28 *General experiments.* $^1$H-NMR spectra (one-and two-dimensional) were
29 recorded on a Jeol lambda-500 spectrometer (Jeol Ltd., Tokyo, Japan) at 500 MHz,
30 while $^{13}$C-NMR spectra were recorded by the same instrument at 125 MHz. HRESIMS
31 data were measured by a JMS-T100LC mass spectrometer (Jeol Ltd., Tokyo, Japan).
32 HPLC separation was performed with a Jasco Gulliver system (Jasco Co., Tokyo,



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1 Japan) using a reverse-phase HPLC column (Cosmosil $\pi$NAP Waters, 10×250 mm,
2 Nacalai tesque, Kyoto, Japan) and two normal phase HPLC columns (YMC-pack
3 Diol-60-NP, 20×250 mm, YMC Co., Ltd., Kyoto, Japan; Senshu Pak AQ, 20×250 mm,
4 Senshu Scientific Co., Ltd., Tokyo, Japan). Silica cartridges and C18 cartridges (Nihon
5 Waters K.K., Tokyo, Japan) were used in the pro-processing of the samples. Silica gel
6 plate (TLC Silica gel 60 $F_{254}$, Merck KGaA, Darmstadt, Germany) and silica gel 60N
7 (Kanto Chemical Co.,Inc., Tokyo, Japan) were used for analytical TLC and for flash
8 column chromatography, respectively.

9 *Fungal strain and plant materials.* Fresh fruiting bodies of *T. flavovirens* were
10 collected at Narusawa village, Yamanashi Prefecture in Japan. Lettuce seeds (*Lactuca*
11 *sativa* L. cv. Great Lakes 366; Takii Co., Ltd., Tokyo, Japan) were used in this study.

12 *Extraction and isolation*. The fresh fruiting bodies of *T. flavovirens* (20.6 kg)
13 were extracted with EtOH (42 L, 3 times) and then with acetone (20 L, 3 times). After
14 the solutions were combined and concentrated under reduced pressure, the concentrate
15 was partitioned between *n*-hexane and $H_2O$, EtOAc and $H_2O$, and then *n*-BuOH and
16 $H_2O$. The *n*-hexane-soluble part (39.8 g) was fractionated by silica gel flash column
17 chromatography ($CH_2Cl_2$; 90/10, 80/20 $CH_2Cl_2$/acetone; 90/10, 80/20 $CH_2Cl_2$/MeOH;
18 MeOH; 95/5 MeOH/$H_2O$; 2.0 L each) to obtain 20 fractions (fractions 1 to 20). Fraction
19 7 (6.78 g) was further separated by silica gel flash column chromatography ($CH_2Cl_2$;
20 95/5, 90/10, 80/20 $CH_2Cl_2$/acetone; MeOH; 2 L each) to give 14 fractions (fractions 7-1
21 to 7-14). Fraction 7-4 (23.3 mg) was further separated by normal-phase HPLC
22 (YMC-pack Diol-60-NP, UV 245 nm, 5 mL/min, 30/70 hexane/$CHCl_3$) to afford **1** (1.5
23 mg). Fraction 7-3 (28.5 mg) was separated by reverse-phase HPLC (Cosmosil $\pi$NAP
24 Waters, UV 254 nm, 2 mL/min, 80/20 MeOH/$H_2O$) to afford **2** (1.5 mg).

25 The EtOAc soluble part (16.4 g) was fractionated by silica gel flash column
26 chromatography ($CH_2Cl_2$; 95/5, 90/10, 80/20, 70/30, 50/50 $CH_2Cl_2$/EtOAc; MeOH; 2L
27 each) to obtain 17 fractions (fractions 1 to 17). Fraction 8 (426 mg) was fractionated by
28 C18 cartridges to give two fractions (fractions 8-1 and 8-2). Fraction 8-1 (214 mg) was
29 separated by silica gel flash column chromatography ($CH_2Cl_2$; 95/5, 90/10, 80/20
30 $CH_2Cl_2$/acetone; MeOH; 500 mL each) to give eight fractions (fractions 8-1-1 to 8-1-8).
31 Fraction 8-1-5 was further fractionated by preparative TLC to give ten fractions
32 (fractions 8-1-5-1 to 8-1-5-10). Fraction 8-1-5-1-5 (10.9 mg) was separated by
33 reverse-phase HPLC (Cosmosil $\pi$NAP Waters, UV 255nm, 2 mL/min, 75/25

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1 MeOH/$H_2O$) to afford **3** (7.7 mg). Fraction 10 (740 mg) was fractionated by silica gel
2 flash column chromatography ($CH_2Cl_2$; 90/10 $CH_2Cl_2$/acetone; MeOH; 500 mL each) to
3 give 11 fractions (fractions 10-1 to 10-11). Fractions 10-4 (5.5 mg), 10-5 (7.2 mg) and
4 10-6 (20.5 mg) were separated by normal-phase HPLC (Senshu Pak AQ, UV 270 nm, 5
5 mL/min, 70/30 hexane/$CHCl_3$) respectively to afford **4** (0.8 mg) in total.
6 *Bioassay.*[2,3] Lettuce seeds were put on filter paper (Advantec No. 2, $\phi$ 55 mm;
7 Toyo Roshi Kaisha, Ltd., Japan), soaked in distilled water in a Petri dish ($\phi$ 60×20 mm)
8 and incubated in a growth chamber under dark at 25˚C for 1 day. Each sample was
9 dissolved in 1 mL of methanol (1, 10, $10^2$ and $10^3$ nmol/mL) and then poured on filter
10 paper ($\phi$ 55 mm) in a Petri dish ($\phi$ 60×20 mm). After the solvent was air-dried, 1mL of
11 distilled water was poured on the sample-loaded paper or intact filter paper (control).
12 The pre-incubated lettuces (n = 9 in each petri dish) were transferred onto the filter
13 paper and incubated in a growth chamber under dark at 25˚C for 3 days. The lengths of
14 the hypocotyl and the root were measured using a ruler.
15
16 **Author contribution**
17 H. Ka. designed the experiments. W. Q., H. Ko. and J. W. performed the
18 experiments. J. C., H. H. and H. Ka. contributed to discussions. W. Q. and H. Ka. wrote
19 the manuscript.
20
21 **Disclosure statements**
22 No potential conflict of interest was reported by authors.
23
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Legend to figure

Fig. 1. Structures of compounds **1**–**4** (A) and COSY and HMBC correlations in **1** and **3** (B).

Fig. 2. Growth regulating activity against lettuce of compounds **1** to **3**.
Notes: white and black columns indicate the length of the hypocotyl and the root, respectively. 2,4-Dichlorophenoxyacetic acid (2,4-D) was used as positive control. Results are the mean ± standard deviation (n = 9). [ $^{*}p < 0.05$, $^{**}p < 0.01$ (growth inhibition); $^{+}p < 0.05$, $^{++}p < 0.01$ (growth promotion)].

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Table 1. $^{1}$H and $^{13}$C NMR Data for **1** and **3** (in $CDCl_3$)

| Position | Compound **1** | | Compound **3** | |
|---|---|---|---|---|
| | $^{1}$H | $^{13}$C | $^{1}$H | $^{13}$C |
| | (δ; multiplicity; *J* in Hz) | δ | (δ; multiplicity; *J* in Hz) | δ |
| 1 | 7.29 (br. s) | | | 172.1 |
| 2 | | 135.0 | | |
| 3 | 6.14 (br.s) | 99.4 | 5.28 (s) | 69.9 |
| 3a | | 130.6 | | 146.6 |
| 4 | | 109.2 | 7.08 (d; 7.6) | 115.9 |
| 5 | | 151.1 | 7.60 (dd; 7.6, 8.2) | 136.3 |
| 6 | 6.36 (s) | 92.3 | 8.50 (d; 8.2) | 118.3 |
| 7 | | 143.5 | | 138.8 |
| 7a | | 121.7 | | 111.4 |
| 2-Me | 2.41 (s) | 13.7 | | |
| 4-Me | 2.31 (s) | 11.4 | | |
| 5-OMe | 3.83 (s) | 58.6 | | |
| 7-OMe | 3.91 (s) | 55.5 | | |
| 1’ | | | 2.24 (s) | 24.9 |
| 2’ | | | | 169.1 |
| 3’ | | | 9.56 (br. s) | |

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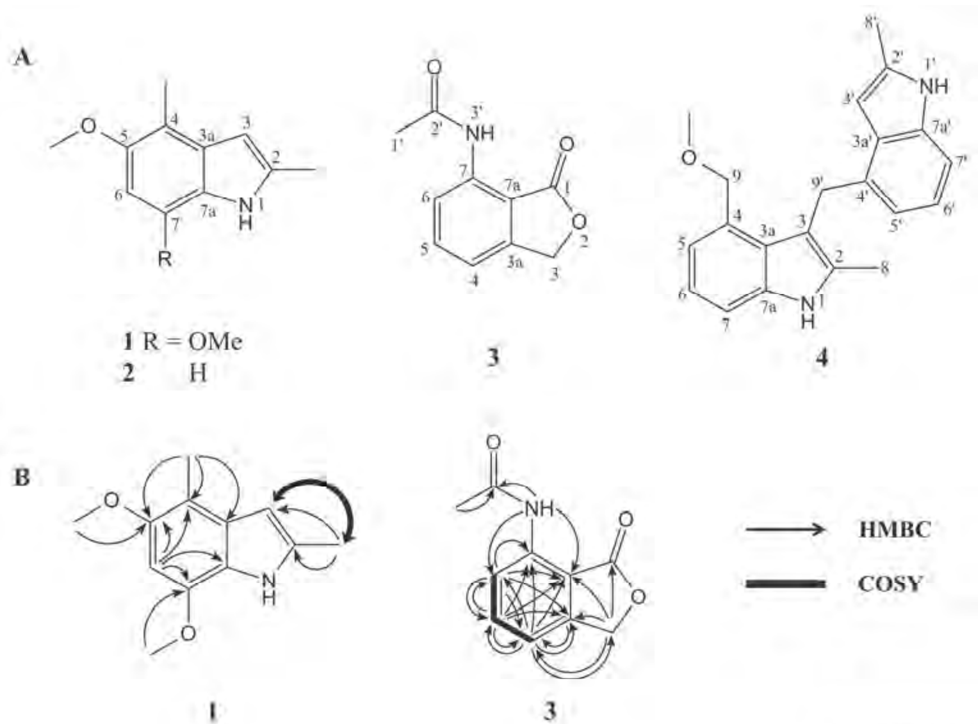


Figure 1

238x183mm (300 x 300 DPI)

view

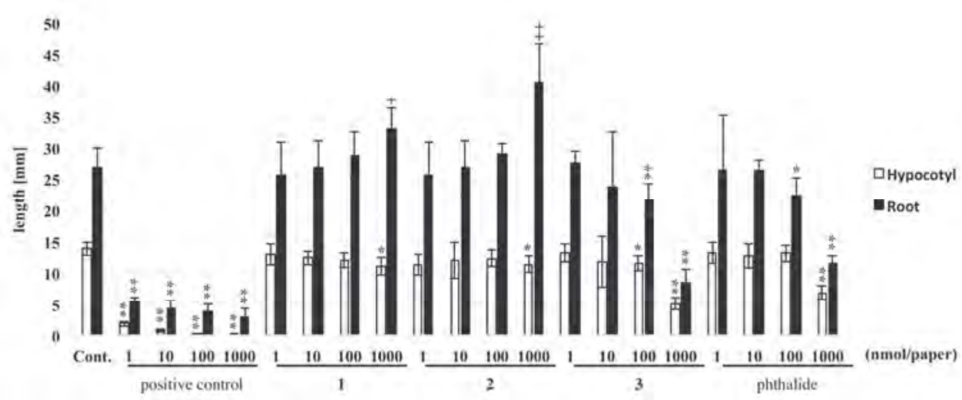


Fig. 2 Qiu et al

Figure 2

236x143mm (300 x 300 DPI)

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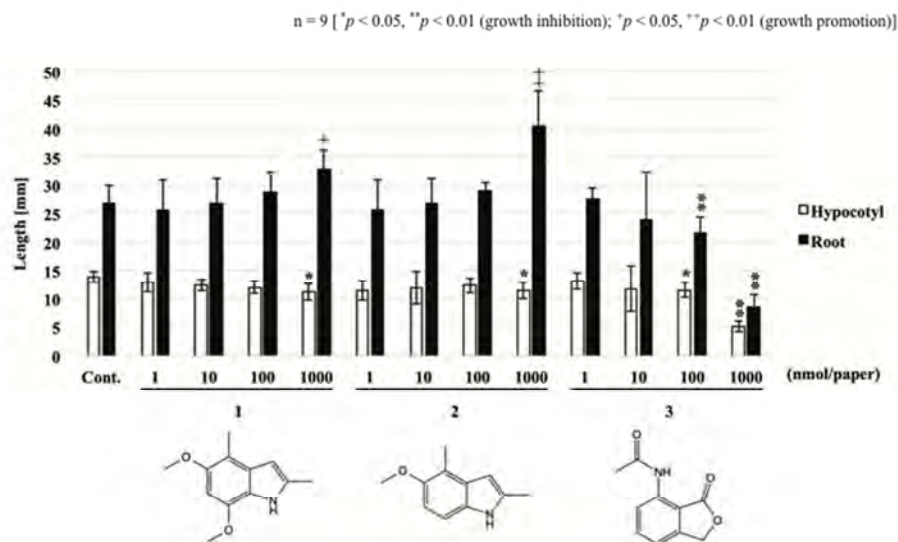


Graphic abstract

254x190mm (72 x 72 DPI)

eview

Plant growth regulators **1**, **2** and **3** were isolated from the mushroom *Tricholoma flavovirens*.

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