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Running title: A New Compound from *Tricholoma flavovirens*

# A New Compound from the Mushroom *Tricholoma flavovirens*

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A novel compound (**1**) and a known one (**2**) were isolated from the fruiting bodies of *Tricholoma flavovirens*. Their structures were determined by the interpretation of spectroscopic data. Both compounds showed inhibition effects on the growth of hypocotyl of lettuce with significant differences. In addition, compound **1** showed a promotion effect on the growth of root with significant differences and **2** had the similar tendency to promote the growth.

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

A well known axiom is that "plants act as producers, animals act as consumers, and fungi act as restorers and decomposers". Fungi, including mushroom, play an important role in ecological balance as it can restore the nutrients used by plants and animals back to the land. We are interested in biological activity of components from mushroom towards plants and have reported isolation of some compounds that regulate lettuce growth.[1-3] Using the assay evaluating growth-regulating activity toward lettuce, we screened extracts of various mushrooms and found relatively strong inhibitory activity in hexane soluble part of the extracts of the mushroom *Tricholama flavovirens*.

Since ancient times *T. flavovirens* (English name, yellow knight; Japanese name, kishimeji) belonging to the family Tricholomataceae is known as an eatable mushroom throughout the world. Till now, few chemical studies were, there, so with the purpose to find novel constituents with activity from *T. flavovirens*, we started this study.

Here we describe the isolation, structural determination of a novel compound and a known one from the fruiting bodies of the fungus along with the biological activity of the compounds.

Fresh fruiting bodies of *T. flavovirens* were extracted with EtOH and then with acetone. After the solutions were combined and concentrated, they were partitioned between hexane and $H_2O$, EtOAc and $H_2O$, and then *n*-BuOH and $H_2O$. The

hexane-soluble part was fractionated by repeated chromatography. As a consequence, two compounds (**1** and **2**) were purified.

Compound **1** isolated as yellow oil with a molecular formula determined as $C_{12}H_{15}NO$ by HRESIMS at *m/z* 188.1053 $[M - H]^-$ (calcd. for $C_{12}H_{14}NO$ 188.1075), indicating presence of six degrees of unsaturation in the molecule. The structure of **1** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC (Fig. 1) with the complete assignment of protons and carbons of NMR was accomplished as shown in Table 1. The DEPT experiment indicated the presence of two methyls, two methylenes, four methines and four quaternary carbons. The structure of 2-methylindole was elucidated by the HMBC correlations (H-1/C-2, C-3, C-3a, C-7a; H-3/C-2, C-3a, C-7a; H-5/C-3a, C-7; H-6/C-4, C-7a; H-7/C-3a, C-5; H-8/C-2, C-3) and the COSY correlations (H-5/H-6, H-6/H-7). The HMBC correlations (H-1'/C-2'; H-2'/ C-1', C-3'; H-3'/ C-2') and the COSY correlations (H-1'/H-2') indicated the presence of the ethoxymethyl group. The connection between the 2-methylindole and ethoxymethyl moiety was confirmed by the HMBC correlations (H-3'/ C-3a, C-4, C-5). As a result, the structure **1** was determined as shown.

Fig. 1

Table 1

Compound **2** was identified as 4-methoxymethyl-2-methylindole and has been isolated from the fruiting bodies of *Tricholoma sciodes* and *Tricholoma virgatum*.[4)] However, no known biological activity of the compound has yet been reported.

Both compounds **1** and **2** showed inhibition effects on the growth of hypocotyl of lettuce at 1 μmol/paper with significant differences. In addition, compound **1** showed a promotion effect on the growth of root at $10^{-1}$ μmol/paper with significant differences and **2** had the similar tendency to promote the growth (Fig. 2). The result indicated that compounds **1** and **2** possessed similar growth regulation activity against lettuce.

Fig. 2

*Experimental*

*General experiments*. $^{1}$H-NMR spectra (one-and two-dimensional) were recorded on a Jeol lambda-500 spectrometer at 500 MHz, while $^{13}$C-NMR spectra were recorded by the same instrument at 125 MHz. A JASCO grating infrared spectrophotometer was used to record the IR spectra. The HRESIMS data were measured by a JMS-T100LC mass spectrometer. HPLC separation was performed with a Jasco Gulliver system using a reverse-phase HPLC column (Cosmosil πNAP Waters, Nacalai tesque, Japan). Silica gel plate (Merck $F_{254}$), silica gel 60N (Merck 100-200 mesh), and $C_{18}$-OPN (Cosmosil 140 μm) were used for analytical TLC and for flash column chromatography, respectively.

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

*Extraction and isolation*. The fresh fruiting bodies of *T. flavovirens* (20.6 kg) were extracted with EtOH (42 L, 3 times) and then with acetone (20 L, 3 times). After the solutions were combined and concentrated under reduced pressure, the concentrate was partitioned between hexane and $H_2O$, EtOAc and $H_2O$, and then *n*-BuOH and $H_2O$. The hexane-soluble part (39.8 g) was fractionated by silica gel flash column chromatography ($CH_2Cl_2$; 90%, 80%, 20% $CH_2Cl_2$/acetone; 90%, 80% $CH_2Cl_2$/MeOH; MeOH; 95% MeOH/$H_2O$, 2.0 L each) to obtain twenty fractions (fractions 1 to 20). Fraction 8 (11.8 g) was further separated by silica gel flash column chromatography ($CH_2Cl_2$; 95%, 90%, 80%, 50% $CH_2Cl_2$/acetone; 95% $CH_2Cl_2$/MeOH and MeOH, 2 L each) to give twenty fractions (fractions 8-1 to 8-20). Fraction 8-6 (2.49 g) was further separated by ODS flash chromatography (90% MeOH/$H_2O$ and $H_2O$, 2L each) and eight fractions (fractions 8-6-1 to 8-6-8) were obtained. Fraction 8-6-2 (90.3 mg) was separated by reverse-phase HPLC (Cosmosil *π*NAP Waters, 80% MeOH) to afford **1** (12.6 mg) and **2** (12.3 mg).

Compound **1**. Yellow oil; IR (neat) 3400, 2974, 1553, 1400, 1347, 1089 cm$^{-1}$; $^{1}$H and

$^{13}C$ NMR, see Table 1; ESIMS *m/z* 188 $[M-H]^-$; HRESIMS *m/z* 188.1053 $[M-H]^-$ (calcd. for $C_{12}H_{14}NO$ 188.1075).

*Bioassay: growth regulating activity against lettuce*.[1-3] Lettuce seeds were put on filter paper (Advantec No. 2, φ 55 mm; Toyo Roshi Kaisha, Ltd., Japan), soaked in distilled water in a Petri dish (φ 60×20 mm) and incubated in a growth chamber under dark at 25°C for 1 day. Each sample was dissolved in 1 mL of methanol (1, $10^{-1}$, $10^{-2}$ and $10^{-3}$ μmol/mL ) and then poured on filter paper (φ 55 mm) in a petri dish (φ 60× 20 mm). After the solvent was air-dried, 1mL of distilled water was poured on the sample-loaded paper or intact filter paper (control). The pre-incubated lettuces (n = 7 in each petri dish) were transferred onto the filter paper and incubated in a growth chamber under dark at 25°C for 3 days. The lengths of the hypocotyl and the root were measured using a ruler.

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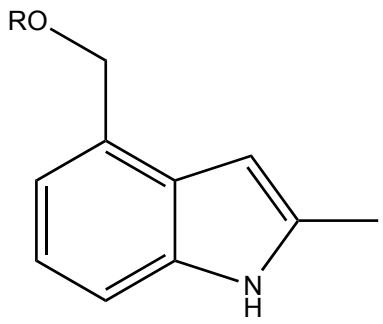
Legend to figure

**Fig. 1.** COSY and HMBC Correlations in **1**.

**Fig. 2.** Growth Regulating Activity against Lettuce.

Black and white columns indicate the elongation of the root and the hypocotyl, respectively. Each value is presented as the mean ± SD of the relative elongation compared with the control group (n=7). *$p < 0.01$ (growth inhibition); +$p < 0.01$ (growth promotion).




1 R = ethyl
2 methyl

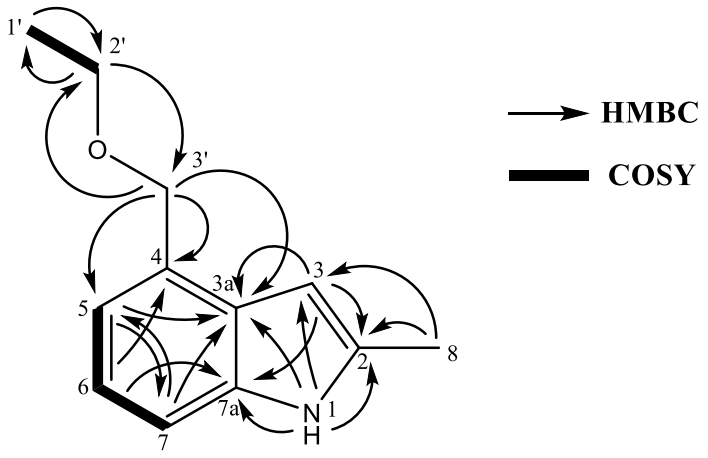



Fig. 1 Qiu et al.

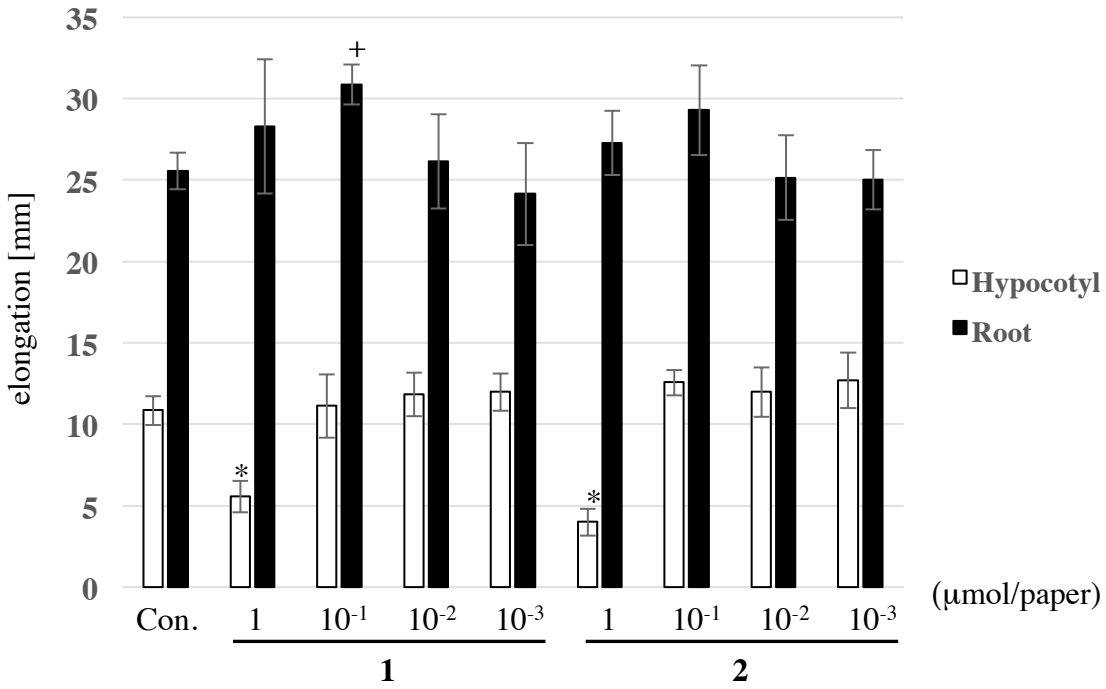



Fig. 2 Qiu et al.

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

| Position | $^{1}H$ | $^{13}C$ |
|---|---|---|
| | (δ; multiplicity; *J* in Hz) | δ |
| 1 | 7.88 (br. s) | |
| 2 | | 135.2 |
| 3 | 6.33 (s) | 98.0 |
| 3a | | 127.1 |
| 4 | | 127.9 |
| 5 | 7.02 (d, 7.3) | 118.6 |
| 6 | 7.06 (dd, 7.3, 7.6) | 120.1 |
| 7 | 7.21 (d, 7.6) | 110.0 |
| 7a | | 135.9 |
| 2-Me | 2.44 (s) | 13.0 |
| 4-$CH_2$- | 4.75 (s) | 71.0 |
| $OCH_2\underline{CH_3}$ | 1.23 (t, 7.0) | 14.9 |
| $O\underline{CH_2}CH_3$ | 3.55 (q, 7.0) | 65.2 |