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Graphical Abstract

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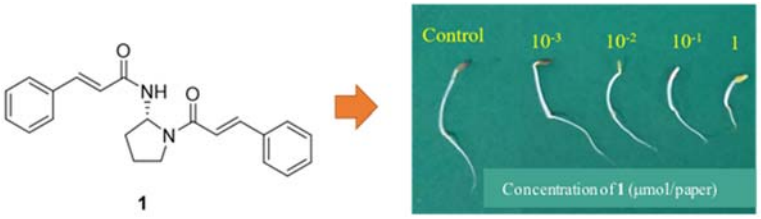

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ABSTRACT

Nine compounds including a new cinnamamide (**1**), *N*-(1-cinnamoylpyrrolidin-2-yl)cinnamamide, and two compounds (**8** and **9**) first isolated from natural sources, were obtained from the edible mushroom *Pholiota lubrica*. Their structures were determined by the interpretation of spectroscopic data. Compounds **1**, **3** and **9** exhibited the inhibitory activity against lettuce, while compounds **2** and **7** promoted the growth of lettuce.

Introduction

Mushrooms have provided a great source of bioactive secondary metabolites. Some of the metabolites have been known as plant growth regulators. In our previous studies, we have isolated plant growth regulators from some kinds of culture broth and fruiting bodies of higher fungi. [1-9] For example, we have reported the plant growth regulators, 2-azahypoxanthine and imidazole-4-carboxamide, produced by a fairy-ring forming fungus *Lepista sordida*.[4-5] These compounds increased grain yields of rice and wheat in greenhouse or field experiments.[4-8] The fruiting bodies of the edible mushroom *Leccinum extremiorientale* also produced plant growth regulatory compounds against lettuce.[9]

In our continuing screening for plant growth regulators using lettuce, we found strong activity in the extracts of the fruiting bodies of *Pholiota lubrica.* This mushroom belongs to genus *Pholiota* of family Strophariaceae and has a widespread distribution, especially in temperate regions and frequently grows on wood or at the bases of trees. It has exhibited an allelopathic activity against lettuce in the previous report.[10]

The fresh fruiting bodies of *Pholiota lubrica* were extracted with EtOH and acetone, successively. After the solution was combined and evaporated under reduced pressure, the concentrate was divided into an *n*-hexane soluble part, a $CHCl_3$ soluble part, and a water soluble part. A bioassay-guided fractionation resulted in the isolation of a new cinnamamide, *N*-(1-cinnamoylpyrrolidin-2-yl)cinnamamide (**1**), along with eight known compounds (**2**–**9**) (Fig. 1). Herein, we describe the structure determination of **1** and plant growth regulatory activities of the isolated compounds.

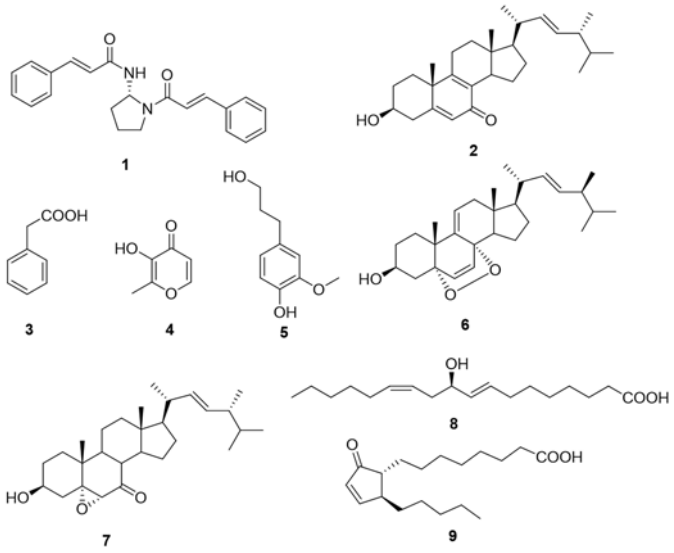

Figure 1. Structures of **1**–**9**.

Results and discussion

Compound **1** was isolated as a white amorphous material.[11] The molecular formula was determined as $C_{22}H_{22}N_2O_2$ by HRESIMS (*m/z* 369.1592 $[M+Na]^+$; calcd for $C_{22}H_{22}N_2O_2Na$, 369.1579), indicating 13 degrees of unsaturation. The structure of **1** was elucidated by interpretation of NMR spectra including DEPT, COSY, HMQC, and HMBC. The ^{13}C NMR, DEPT and HMQC data established the presence of three methylenes, fifteen methines, and four quaternary carbons (Table 1).

Table 1 ^{1}H and ^{13}C NMR data for **1** (in $CDCl_3$:CD_3OD=1:1)

Position	δ_C	δ_H (*J* in Hz)
1	166.6	-
2	118.1	6.95 (d, 15.6)
3	143.3	7.60 (d, 15.6)
4	134.9	-
5, 9	128.3	7.48 (m)
6, 8	128.9	7.27 (m)
7	130.04	7.29 (m)
2’	63.3	6.15 (d, 6.1)
3’	34.3	1.98 (m), 2.19 (m)
4’	21.6	1.93 (m), 2.00 (m)
5’	46.2	3.46 (m), 3.65 (m)
1”	165.7	-
2”	120.2	6.45 (d, 15.9)
3”	142.0	7.63 (d, 15.9)
4”	134.8	-
5”, 9”	127.9	7.43 (m)
6”, 8”	128.9	7.27 (m)
7”	129.96	7.29 (m)

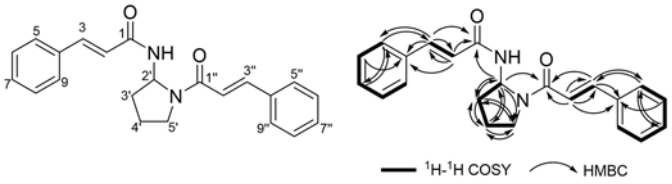

Figure 2. The COSY and HMBC correlations of **1.**

In the ^{1}H and ^{13}C NMR spectra of **1**, the cinnamoyl moieties were identified by the assignment of aromatic signals at δ_H 7.27-7.48 (10H, m); δ_C 130.04 (C-7), 129.96 (C-7”), 128.3 (C-5, C-9), 127.9 (C-5”, C-9”), 128.9 (C-6, C-8), 128.9 (C-6”, C-8”), 134.9 (C-4), 134.8 (C-4”) and the characteristic chemical shifts and coupling constants at δ_C 166.6 (C-1), 118.1 (C-2), 143.3 (C-3), 165.7 (C-1”), 120.2 (C-2”), 142.0 (C-3”); δ_H 6.95 (d, J = 15.6 Hz, H-2), 7.59 (d, J = 15.6 Hz, H-3), δ_H 6.45 (d, J = 15.9 Hz, H-2”), 7.63 (d, J = 15.9 Hz, H-3”). Moreover, the HMBC correlations (H-2/C-1, C-3, C-4; H-3/C-1, C-2, C-4, C-5, C-9; H-2”/C-1”, C-3”, C-4”; H-3”/C-1”, C-2”, C-4”, C-5”, C-9”) and the COSY correlations (H-2/H-3; H-5/H-6; H-6/H-7; H-7/H-8; H-8/H-9; H-2”/H-3”; H-5”/H-6”; H-6”/H-7”; H-7”/H-8”; H-8”/H-9”) also confirmed the presence of two cinnamoyl moieties (Fig. 2). Furthermore, pyrrolidin-2-amine was elucidated by the HMBC correlations (H-2’/C-3’, C-4’, C-5’; H-3’/C-2’, C-4’, C-5’; H-4’/C-2’, C-3’, C-5’; H-5’/C-3’, C-4’), the COSY correlations (H-2’/H-3’; H-3’/H-4’; H-4’/H-5’) and observed by the characteristic chemical shifts of a pyrrolidine ring at position 2’ (δ_H 6.15, δ_C 63.3). The connections between the two cinnamoyl groups and the pyrrolidin-2-amine moiety were determined by the HMBC correlations (H-2’/C-1, C-1”). As a result, the plane structure of **1** was determined as shown. In order to determine the absolute configuration of the compound, its specific rotation ($[\alpha]_D^{28}$ + 18, c = 0.40, $CHCl_3$), $[\alpha]_D^{28}$ + 5.9, c = 0.40, EtOH) was compared with those of a known aminopyrrolidine derivative possessing a cinnamoyl and a tiglic amide moieties, dehydroodorine ($[\alpha]_D^{18}$ + 42.5, c = 0.01, $CHCl_3$)[12] whose absolute configuration has been determined by X-ray crystallography, and piriferine containing a cinnamoyl and a methylpropanoic acid moieties ($[\alpha]_D^{28}$ + 30, c = 0.01, EtOH)[13]. All the data allowed us to conclude that **1** was *N*-((*R*)-1-cinnamoylpyrrolidin-2-yl)cinnamamide.

Compounds (**2**–**9**) were respectively identified as 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**2**)[14], benzeneacetic acid (**3**)[15], 2-methyl-3-hydroxypyran-4-one (**4**)[16], dihydroconiferyl alcohol (**5**)[17], (22*E*,24*S*)-5α,8α-epidioxy-24-methyl-cholesta-6,9,(11)22-trien-3β-ol (**6**)[18], 5α,6α-epoxy-3β-hydroxyergost-22-ene-7-one (**7**)[19], (8*E*,10*R*,12*Z*)-10-hydroxy-8,12-octadecadienoic acid (**8**)[20], and 8-((1*R*,5*S*)-2-oxo-5-pentylcyclopent-3-en-1-yl)octanoic acid (**9**)[21] by the comparison of their spectroscopic data with those of reported data. To the best our knowledge, compound **5** was the first isolation from mushroom-forming fungi. This compound has been reported as a lettuce cotyledon factor which induced lettuce elongation.[18] Compound **8** was produced from linoleic acid or α-linolenic acid as substrates by whole recombinant cells expressing PpoC, which code for fatty acid oxygenases with homology to fungal linoleate 7,8-diol synthases (7,8-LDS) and cyclooxygenases.[22] Thus, this is the first report of the isolation of **8** from a natural source. Compound **9** has been found as the oxidative metabolite of linoleic and linolenic acids in

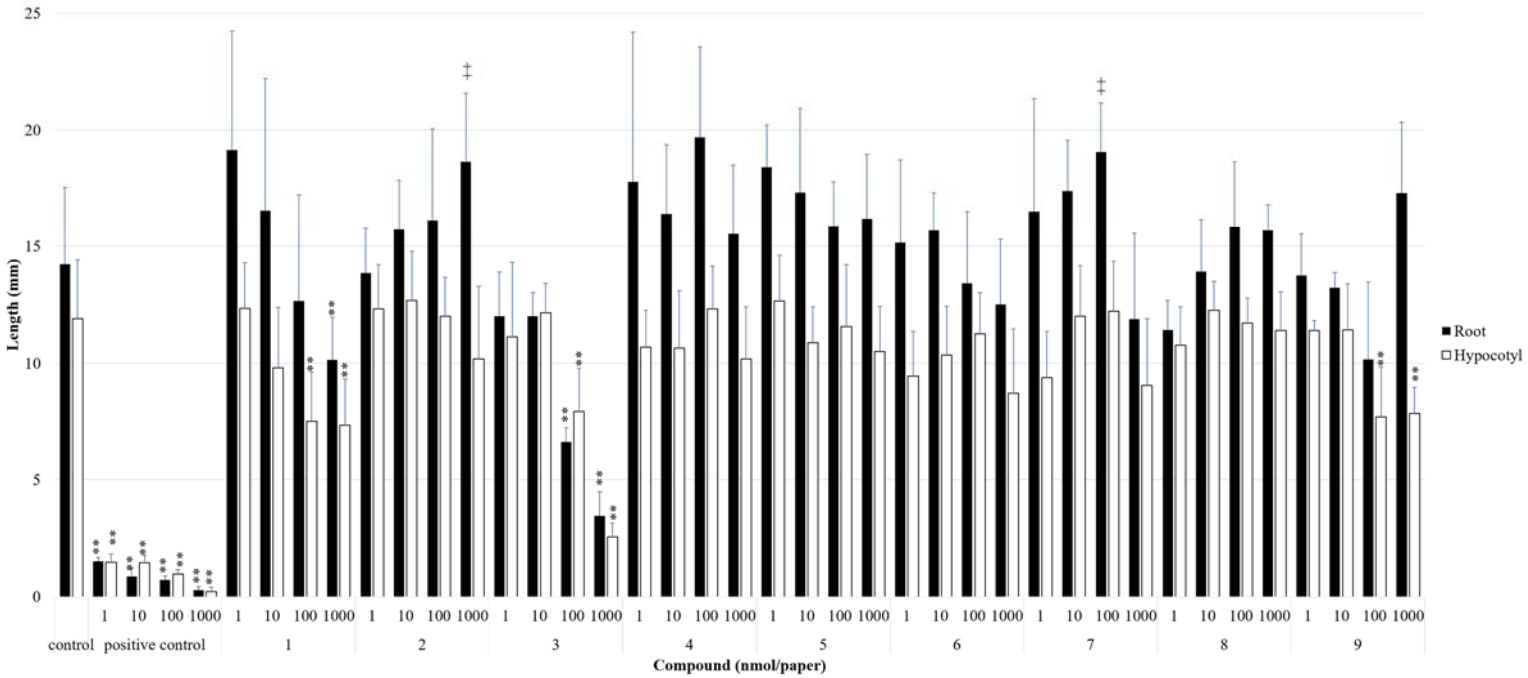

Figure 3. Growth regulatory of compounds **1** to **9** against lettuce. Results are the mean ± standard deviation (n = 9). **p < 0.01 (inhibition), ^{++}p < 0.01 (promotion).

preparations of stolons of potato. This compound was also isolated from nature for the first time.[21]

All the compounds were subjected to the plant regulatory assay against the lettuce. In this assay, the positive control, 2,4-dichlorophenoxyacetic acid significantly inhibited the growth of lettuce at all concentrations. Toward the hypocotyl, compounds **1**, **3**, and **9** inhibited the growth at 0.1 and 1 μmol/paper with a significant difference, whereas the other compounds had no activity. As for the root, we observed growth inhibitory activity of **1** at 1 μmol/paper and **3** at 0.1 and 1 μmol/paper. However, **2** and **7** exhibited promotion activity at 1 and 0.1 μmol/paper, respectively (Fig. 3). Among **2, 6** and **7**, only compounds **2** and **7** showed promotion activity toward root of lettuce. This finding indicates that the carbonyl group at C-7 of compounds **2** and **7** play an important role in the plant growth promotion activity. Cinnamamide and its derivative, betaine cinnamamide, were reported to promote and stimulate the lengths of root and shoot of wheat.[23] In order to investigate the structure activity relationship of compound **1**, commercially available compounds, cinnamamide and cinnamic acid were used for comparison of the lettuce growth regulatory assay. The results showed that compounds **1** and cinnamic acid inhibited the growth of hypocotyl at 0.01, 0.1, and 1 μmol/paper. Meanwhile, cinnamamide inhibited the growth at all concentrations. As for the root, compound **1** inhibited the growth at 1 μmol/paper, while cinnamamide and cinnamic acid showed inhibition activity at 0.1 and 1 μmol/paper (Fig. S10). These results suggested that cinnamoyl moiety in the tested compounds were essential for the plant growth inhibition activity.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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11. Compound **1**: white amorphous; IR (neat): 1650, 3260 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS (*m/z* 369.1592 $[M + Na]^{+}$; calcd for $C_{22}H_{22}N_2O_2Na$, 369.1579).
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