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BRIEF COMMUNICATION

# Mannonerolidol, a new nerolidol mannoside from culture broth of *Schizophyllum commune*

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## Abstract

To discover antimicrobial agents from higher fungi, mannonerolidol (**3**), a new nerolidol mannoside, together with known schizostatin (**1**) and nerolidol (**2**) were isolated from an antimicrobial fraction of the culture broth of *Schizophyllum commune*. Structures of these compounds were determined through spectroscopic methods. Compounds **1** and **3** exhibited antimicrobial activities against plant pathogenic fungi *Rhizoctonia solani*, *Diaporthe* sp., *Botrytis cinerea*, and *Alternaria solani* and bacteria *Bacillus subtilis* and *Staphylococcus aureus*.

The higher fungi produce various antibiotics to protect themselves from external environment and pathogens. Many antifungal and antibacterial compounds such as strobilurins, ganodermin, hypsin, and xylarinic acids have been isolated from mushrooms [1–4]. In our ongoing effort to search for antimicrobial compounds from fungal metabolites, we found that the culture broth of a higher fungus *Schizophyllum commune* exhibited significant antimicrobial activity. *S. commune* belonging to family Schizophyllacea is known to produce β-glucans such as schizophyllan, an immunostimulatory anticancer agent, schizines A and B that can inhibit cancer cell growth, schizostatin known to be a squalene synthase inhibitor, and schizocommunin that exhibits cytotoxicity [5–8]. In this study, we isolated a new nerolidol mannoside, designated as mannonerolidol (**3**), together with known schizostatin (**1**) and nerolidol (**2**) from an antimicrobial fraction of the culture broth of *S. commune* (Fig. 1).

**Supplementary material** The online version of this article (https://doi.org/10.1038/s41429-018-0130-3) contains supplementary material, which is available to authorized users.

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Fungal strain *S. commune* NAAS 04688 was obtained from Rural Development Administration, Korea. To determine the phylogenetic position of NAAS 04688 strain (GenBank accession number: MH279792), ribosomal DNA internal transcribed spacer (ITS) region sequences containing the 5.8s rDNA gene previously reported in Genbank were aligned with NAAS 04688 strain using MEGA 7.0 software. Phylogenetic analysis showed that NAAS 04688 strain was closely related to *S. commune* strains, sharing sequence similarities of ≥99.3% with *S. commune* strains (see Supporting Information).

We cultured a fungal strain of *S. commune* NAAS 04688 in ten 4 l flasks (each containing 2 l of potato dextrose broth) at 27 °C in a stationary culture incubator for two weeks. The culture broth (20 l) was extracted with acetone at room temperature. After removing acetone under reduced pressure, the resulting extract was partitioned between chloroform and water. The chloroform-soluble layer showed significant antibiotic activity. The chloroform-soluble layer was concentrated and subjected to silica gel column chromatography eluted with $CHCl_3$:MeOH (25:1, 5:1, MeOH only, v/v, stepwise). $CHCl_3$:MeOH (5:1) fraction and MeOH fraction were combined, concentrated under reduced pressure, and subjected to reversed-phase medium pressure liquid chromatography eluted with 50% aq. MeOH to afford compound **1** (50 mg) and an active fraction. The active fraction was subjected to a reversed-phase Sep-pak cartridge eluted with 50-100% aq. MeOH followed by silica gel column chromatography eluted with $CHCl_3$:MeOH (10:1, 5:1, v/v) to give two fractions. Each fraction was further separated by Sephadex LH-20 column

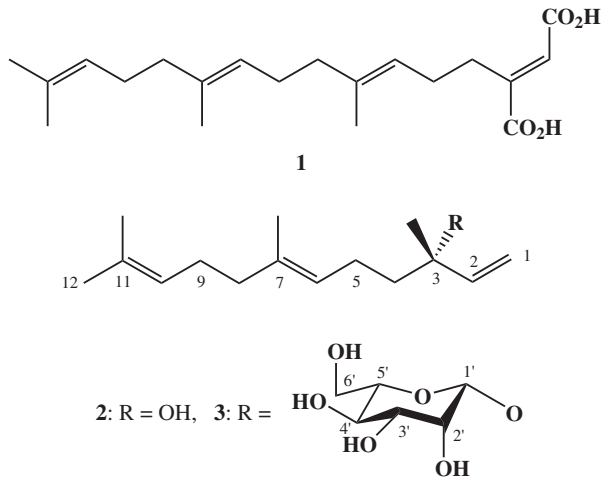


**Fig. 1** Structures of compounds **1–3**

chromatography eluted with MeOH to afford compounds **2** (8.3 mg) and **3** (4.6 mg) (see Supporting Information).

Compounds **1** and **2** were identified as schizostatin and (+)-*trans*-nerolidol, respectively, by comparing spectroscopic data with previously published data [9–12].

Compound **3** was obtained as a brown oil with specific rotation value of -83.0° ($[\alpha]_D$; 25 °C, $c = 0.1$, MeOH). It exhibited UV maxima (log ε) at 203 (3.9) nm. Its molecular formula was determined to be $C_{21}H_{36}O_6$ through a high-resolution ESI-mass measurement (*m/z* 407.2407 $[M + Na]^+$, Δ −0.3 mmu). The $^1$H NMR spectrum of compound **3** in $CD_3OD$ showed signals due to three olefinic methines at *δ* 6.03, 5.11, and 5.09, one terminal methylene at *δ* 5.23/5.16, five oxygenated methines at *δ* 4.59, 3.72, 3.57, 3.43, and 3.11, one oxygenated methylene at *δ* 3.80/3.69, four methylenes at *δ* 2.07, 2.04, 1.97, and 1.64, and four methyls at *δ* 1.67, 1.60, 1.60, and 1.33. Its $^{13}$C NMR spectrum showed 21 carbons, including two $sp^2$ quaternary carbons at *δ* 136.1 and 132.2, three $sp^2$ methine carbons at *δ* 144.4, 125.9, and 125.6, one $sp^2$ terminal methylene carbon at *δ* 115.1, one anomeric methine carbon at *δ* 96.6, four $sp^3$ oxygenated methine carbons at *δ* 78.0, 75.7, 74.2, and 68.5, one $sp^3$ oxygenated quaternary carbon at *δ* 81.3, one oxygenated methylene carbon at *δ* 63.0, four $sp^3$ methylene carbons at *δ* 41.4, 40.9, 27.9, and 23.7, and four methyl carbons at *δ* 26.0, 23.9, 17.9, and 16.3 (Table 1). All proton-bearing carbons were assigned by HMQC spectrum. Interpretation of $^1$H-$^1$H COSY spectrum established three partial structures and a mannose moiety as shown in Fig. 2. The anomeric configuration of the mannose was considered as β because of the heteronuclear coupling constant ($^1J_{CH}$) of 154.0 Hz between the anomeric carbon and the respective proton [13]. Three partial structures were connected by the HMBC spectrum which showed long-range correlations from

**Table 1** $^1$H and $^{13}$C NMR spectral data of compound **3** in $CD_3OD$[a]

| No. | $\delta_C$ | $\delta_H$ (*J* in Hz) |
|---|---|---|
| 1 | 115.1 | 5.16 (dd, *J* = 11.0, 1.3)[b] |
| | | 5.23 (dd, *J* = 17.3, 1.3) |
| 2 | 144.4 | 6.03 (dd, *J* = 17.3, 11.0) |
| 3 | 81.3 | |
| 3-$CH_3$ | 23.9 | 1.33 (s) |
| 4 | 41.4 | 1.64 (t, *J* = 8.5) |
| 5 | 27.9 | 2.07 (m) |
| 6 | 125.9 | 5.11 (m) |
| 7 | 136.1 | |
| 7-$CH_3$ | 16.3 | 1.60 (s) |
| 8 | 40.9 | 1.97 (t, *J* = 7.5) |
| 9 | 23.7 | 2.04 (m) |
| 10 | 125.6 | 5.09 (m) |
| 11 | 132.2 | |
| 11-$CH_3$ | 17.9 | 1.60 (s) |
| 12 | 26.0 | 1.67 (s) |
| 1′ | 96.6 | 4.59 (d, *J* = 1.0) |
| 2′ | 74.2 | 3.72 (dd, *J* = 3.3, 1.0) |
| 3′ | 75.7 | 3.43 (dd, *J* = 9.3, 3.3) |
| 4′ | 68.5 | 3.57 (dd, *J* = 9.3, 9.3) |
| 5′ | 78.0 | 3.11 (m) |
| 6′ | 63.0 | 3.80 (dd, *J* = 11.7, 2.5) |
| | | 3.69 (dd, *J* = 11.7, 5.5) |

[a]NMR spectra were recorded at 600 MHz for $^1$H and 150 MHz for $^{13}$C

[b]Proton multiplicity and coupling constants in parenthesis

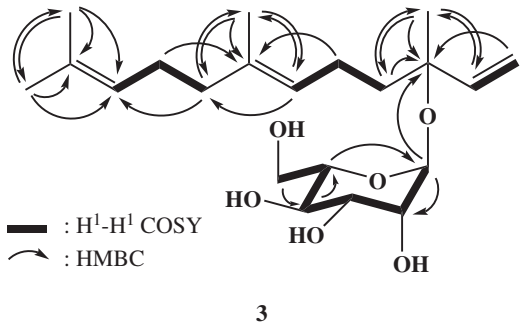


**Fig. 2** Two-dimensional NMR correlations of compound **3**

the methyl protons at *δ* 1.33 (3-$CH_3$) to C-2, C-3, and C-4, from the methyl protons at *δ* 1.60 (7-$CH_3$) to C-6, C-7, and C-8, and from two methyl protons at *δ* 1.60 (11-$CH_3$) and *δ* 1.67 (C-12) to C-10 and C-11. These HMBC correlations established a sesquiterpene nerolidol moiety. In addition to HMBC correlations, chemical shift values of $^1$H and $^{13}$C

NMR peaks were consistent with those of *trans*-nerolidol [10–12]. Finally, the chemical structure of compound **3** was confirmed by its HMBC spectrum which showed long-range correlations from anomeric proton of mannose at $\delta$ 4.59 (H-1‘) to a quaternary carbon at $\delta$ 81.3 (C-3) (Fig. 2). Therefore, compound **3** was determined to be nerolidol mannoside. The stereochemistry of C-6 was determined as *S* by specific rotation value (+36.1° ($[\alpha]_D$; 23°C, $c = 0.05$, MeOH) of nerolidol isolated from acid hydrolysate of **3** [11, 12].

Antimicrobial activities of compounds **1**–**3** were determined with conventional paper disk (6 mm in diameter) diffusion method. Test microorganisms used in this study included four fungi (*Rhizoctonia solani*, *Diaporthe* sp., *Botrytis cinerea*, and *Alternaria solani*) and two bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). These fungi were incubated at 27°C for 4 days while these bacteria were incubated at 37°C for 30 h. Compound **1** at 50 μg $disk^{-1}$ showed significant antifungal activities against *R. solani*, *Diaporthe* sp., *B. cinerea*, and *A. solani*, showing clear zone diameters of 15.6, 19.5, 12.7, and 20.2 mm, respectively. Compound **3** displayed moderate antifungal activities against *R. solani* and *Diaporthe* sp. with clear zone diameters of 11.4 and 10.9 mm, respectively. It has been reported that schizostatin possesses no antibacterial activity [7]. In this study, schizostatin at 50 μg $disk^{-1}$ displayed antibacterial activities against *B. subtilis* and *S. aureus*, showing clear zone diameters of 11.2 and 21.2 mm, respectively. Nerolidol exhibited no antimicrobial activity.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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