New Production of a Monoterpene Glycoside, 1-*O*-(α-D-Mannopyranosyl) geraniol, by the Marine-derived Fungus *Thielavia hyalocarpa*

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The application of microbial transformation to natural products has been shown to be a powerful tool for the generation of new, active, and less toxic derivatives.[1,2] We are currently exploring the microbial transformation of bioactive natural products by marine-derived microorganisms. We have identified marine-derived bacteria and fungi that regioselectively oxidize bioactive natural products to new, more bioactive compounds.[3–9] In this study, many growing cultures were initially screened for their abilities to catalyze interesting biotransformation reactions with geraniol (**1**) as substrate. A culture of the mudflat-derived *Thielavia hyalocarpa* was able to metabolize and transform compound **1** to a more polar metabolite. Therefore, this strain was selected for preparative-scale fermentation of **1**.

Examination of the biotransformation-culture broth led to the isolation of a metabolite (**2**) (Figure 1).

High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis of **2** suggested a quasi-molecular formula of $C_{16}H_{28}O_6Na$ ($[M + Na]^+ = 339.1718$, $\Delta = 0$ mDa) (Figure S4, Supporting Information), which was in accordance with the structural information provided by the ^{13}C-NMR spectrum (Figure S2, Supporting Information). The IR spectrum of **2** exhibited strong absorptions at 3372 and 1062 cm^{-1} indicative of hydroxyl and glycosidic functionalities, respectively, suggesting that **2** was a glycoside of **1**. Detailed analyses of 1-D NMR (^{1}H- and ^{13}C-NMR, DEPT) of **2** revealed the presence of two sp^2 quaternary carbons, two sp^2 methines, five sp^3 oxymethines, two sp^3 oxymethylenes, two sp^3 methylenes, and three olefinic methyls (Table 1).

COSY spectral data suggested the presence of partial structures consisting of two sequential methylene groups with 1,1,2-trisubstituted vinyl group, 1-oxy-3,3-disubstituted allylic group, and sequentially oxygenated five methines and one methylene. Connectivities of those partial structures and quaternary carbons were determined by analyses of HMBC spectral data. The HMBC correlations of the olefinic protons H-2 with C-10 and H-6 with C-8 and C-9 established the geraniol moiety, and further HMBC correlation of the oxymethylene proton H_2-1 with C-1′ aided the assignment of the glycosidic linkage. The ^{13}C-NMR spectrum showed carbon resonances at δ_c 62.8 (CH_2), 68.5 (CH), 72.2 (CH), 72.5 (CH), 74.5 (CH), and 100.2 (CH), indicative of the presence of an α-mannopyranoside.[8,10]

The α configuration of the mannose moiety was further confirmed by the coupling constant of the anomeric proton at 4.77 (d, $J_{H1'-H2'} = 1.5$ Hz), as well as the anomeric carbon signal at δ 100.2 (d, C-1′) with a $^1J_{C-H}$ value of 175 Hz,[11] deduced from DEPT nondecoupling measurements.

The absolute configuration of the mannose moiety was found to be D (*vide infra*). Acid hydrolysis of **2** with 9% aq. HCl yielded **1** and mannose (Supporting Information, p. 8). The specific rotation of mannose ($[\alpha]_D^{20} = +28.8$) indicated a D-configuration (reference: $[\alpha]_D^{20} = +29.3$).[12]

Based on these results, the structure of compound **2** is 1-*O*-(α-D-mannopyranosyl)geraniol (Figure 1).

In order to clarify the structure of **2**, we synthesized **2** from **1** and 1-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)trichloroacetimide in the same manner as described for the synthesis of 1-*O*-(α-D-mannopyranosyl)chlorogentisyl alcohol[8] (Scheme S1 and Supporting Information p. 8). Values of $[\alpha]_D$, ESI-MS, and ^{1}H-NMR for the synthetic compound were identical to those of compound **2**.

Although some examples of microbial glucosidation have been reported,[13–15] microbial mannosidation is very rare.[5,8]

Geraniol (**1**) is an important component of the essential oil from the rhizome of *Alpinia galangal*,[16] and is also a biosynthetic precursor of many types of acyclic and cyclic monoterpenoids.[17] Furthermore, geraniol (**1**) is an important flavor component of some grape juices and wines, for which flavor development is important.

The production of useful flavor compounds using a marine-derived fungus is a potentially important application of microbial technology.

Experimental

General. The instruments used to obtain the physical data were the same as those described in our previous paper.[9]

Isolation of the Marine-derived Fungus *Thielavia hyalocarpa*. The fungal strain, *T. hyalocarpa*, was isolated from the mudflat collected at Suncheon Bay, Korea, and identified based on 18S rRNA analyses (SolGent Co., Ltd., Daejeon,

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Korea), with an identity of 99%. A voucher specimen is deposited at Pukyong National University (code MSA018).

Biotransformation of 1. Organisms were cultivated in two stages in the modified SWS medium composed of soytone (0.1%), soluble starch (1.0%), mannose (0.1%), and seawater (100%). The media were sterilized in an autoclave at 121 °C for 15 min. Cultures were grown in 3 L culture flasks containing one-third of their volumes of culture medium. Incubations were conducted at 29 °C on a rotary shaker (130 rpm) for 1 week. Stage I cultures were inoculated from fresh *T. hyalocarpa* slants and were grown as described. A 10% (v/v) inoculum derived from a 24-h-old, first-stage culture was used to initiate the second-stage culture, which was incubated as described. After 24 h, compound **1** dissolved in *N,N*-dimethyl formamide (DMF) (30 mg/mL) was added to second-stage cultures to a final concentration of 0.5 mg/mL. The progress of microbial transformation reactions was monitored by thin-layer chromatography (TLC). Substrate control consisted of sterile medium and substrate incubated under the same conditions but without microorganism. Also, culture control was composed of fermentation blanks in which the microorganism was grown under identical condition but without the addition of substrate. After 2 weeks of incubation, each control was harvested and analyzed by TLC. The culture was filtered through cheesecloth, and the filtrate was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered through sintered glass, and vacuum-concentrated to yield a crude extract (78 mg).

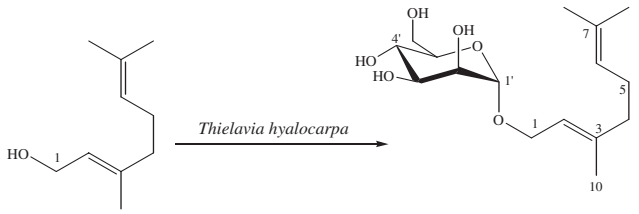

Figure 1. Microbial mannosidation of geraniol (**1**) to the metabolite 1-*O*-(α-D-mannopyranosyl)geraniol (**2**).

Isolation of the metabolite (2). The extract (78 mg) was subjected to silica gel flash column chromatography eluting with *n*-hexane-EtOAc (stepwise, 0–100% EtOAc). From the 80% EtOAc/*n*-hexane and 100% EtOAc fractions, semipure **1** and **2** were obtained by reverse phase C_{18} MPLC eluting with 50% methanol/water, respectively. Final purification by repeated C_{18} HPLC (Gemini C18, 4.6 × 250 mm, 5 μm) with a 30 min gradient program of 50–100% MeOH in H_2O provided the substrate (**1**) (5.0 mg) and the metabolite (**2**) (11.5 mg), respectively.

1-*O*-(α-D-Mannopyranosyl)geraniol (**2**): Colorless oil; $[\alpha]_D^{20} = +45.6$ (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 274 (3.4), 225 (0.8) nm; IR (neat) ν_{max} 3372, 1667, 1448, 1379, 1124, 1062, 1029, 976 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) (see Table 1); LR-ESI-MS: *m/z* 339 $[M + Na]^+$; HR-ESI-MS: *m/z* 339.1778 $[M + Na]^+$ (calcd. for $C_{16}H_{28}O_6$, 339.1778) ($\Delta = 0$ mDa).

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Table 1. NMR spectroscopic data for geraniol (**1**)[a] and 1-*O*-(α-D-mannopyranosyl)geraniol (**2**).[b]

	Geraniol (**1**)		1-*O*-(α-D-mannopyranosyl)geraniol (**2**)	
Carbon position	δ_H (mult., *J* in Hz)	δ_C (mult.)	δ_H (mult., *J* in Hz)	δ_C (mult.)
1	4.13 (d, 7.0)	59.2 (t)	4.05 (dd, 12.0, 7.5)	64.2 (t)
			4.18 (dd, 12.0, 6.5)	
2	5.39 (d, 7.0)	123.5 (d)	5.31 (dd, 7.5, 6.5)	121.3 (d)
3		139.2 (s)		142.0 (s)
4	2.02 (t, 7.0)	39.6 (t)	2.04 (t-like, 6.5)	40.7 (t)
5	2.09 (dt, 8.0, 7.0)	26.4 (t)	2.10 (dt-like, 7.0, 6.5)	27.4 (t)
6	5.09 (t, 7.0)	124.0 (d)	5.09 (t, 7.0)	125.0 (d)
7		131.6 (s)		132.5 (s)
8	1.60 (s)	17.6 (q)	1.60 (s)	17.7 (q)
9	1.67 (s)	25.6 (q)	1.67 (s)	25.9 (q)
10	1.66 (s)	16.2 (q)	1.69 (s)	16.5 (q)
1′			4.77 (d, 1.5)	100.2 (d)
2′			3.75 (dd, 3.0, 1.5)	72.2 (d)
3′			3.68 (dd, 10.0, 3.0)	72.5 (d)
4′			3.60 (dd, 10.0, 9.0)	68.5 (d)
5′			3.51 (ddd, 9.0, 5.5, 2.0)	74.5 (d)
6′			3.71 (dd, 11.8, 5.5)	62.8 (t)
			3.82 (dd, 11.8, 2.0)	

[a] Recorded in DMSO-d_6 at 400 MHz (^{1}H) and 100 MHz (^{13}C).
[b] Recorded in CD_3OD at 400 MHz (^{1}H) and 100 MHz (^{13}C).

Supporting Information. ^{1}H-NMR, ^{13}C-NMR, and LR- and HR-ESI-MS spectra of **2**, Scheme S1 for synthesis of **2** from **1**, and Experimental.

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