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Microbial Transformation of a Monoterpene, Geraniol, by the Marine-derived Fungus *Hypocrea* sp.

Leutou, Alain S.[1], Guohua Yang[1], Viviane N. Nenkep[1], Xavier N. Siwe[1], Zhile Feng[1], Thang T. Khong[1], Hong Dae Choi[2], Jung Sook Kang[3], and Byeng Wha Son[1]*

[1]*Department of Chemistry, Pukyong National University, Busan 608-737, Korea*
[2]*Department of Chemistry, Dongeui University, Busan 614-714, Korea*
[3]*College of Dentistry, Pusan National University, Yangsan, Gyeongnam 626-770, Korea*

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Geraniol (1) is the biogenetic precursor of a number of monoterpenes. We tested various marine-derived microorganisms to determine their ability to biotransform 1. Only *Hypocrea* sp. was capable of transforming 1 into its oxidized derivative, 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene (2). The structure of the metabolite obtained was assigned on the basis of detailed spectroscopic data analyses.

Keywords: Biotransformation, geraniol, monoterpene, 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene

Selectivity is an essential requirement in synthetic organic chemistry. The regioselectivity of enzymes is a fundamental strength of biocatalysis, and enzymes can modify complex or symmetric molecules without any need for protecting groups. Biocatalysis is a powerful tool for regioselective and enantioselective synthesis of bioactive compounds. It can also be applied to generate new, active, and less toxic derivatives of bioactive natural products, because it produces significant quantities of metabolites that would be difficult to obtain from either biological systems or chemical synthesis [2, 7, 8]. The aim of our program is to explore the biological transformation of bioactive metabolites produced by microorganisms isolated from marine habitats. As part of this program, we identified two species of marine-derived *Streptomyces* (MFAac18 and 67) and one *Penicillium* species (MFAac49) that regioselectively biotransformed terreusinone into the unsymmetrical alcohol derivative terreusinol [5]; 6-*n*-pentyl-α-pyrone into two oxidized metabolites, 6-*n*-(4-oxopentyl)-α-pyrone and 6-*n*-[(*S*)-1-hydroxypentyl]-α-pyrone [3]; and cyclonerodiol into three metabolites, 10(*Z*)-, 10(*E*)-cyclonerotriol, and cyclonerodiol mannopyranoside [4]. In our continuing studies on applications of biological transformation [4], we screened 50 cultures for their ability to biotransform geraniol (**1**). The marine-derived ascomycete *Hypocrea* sp. was selected as the biotransforming target strain.

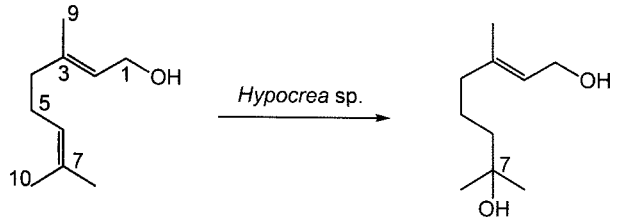
*Corresponding author
Phone: +82-51-629-5592; Fax: +82-51-629-5583;
E-mail: sonbw@pknu.ac.kr

Isolation of the Fungus *Hypocrea* sp.

The *Hypocrea* sp. isolated from the surface of the edible marine brown alga *Undaria pinnatifida* (Korean name: Miyeog) had light gray, downy, and soft white vegetative mycelia, and was identified as *Hypocrea* sp. based on 18S rRNA analyses (SolGent Co., Ltd., Daejeon, Korea), identity of 99%. The culture, designated as MFAac46-2, is deposited at Pukyong National University, South Korea.

Biotransformatin of 1, and Isolation of the Metabolite, 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene (2)

A two-stage fermentation protocol [10] was used to obtain metabolites of **1** on a preparative scale. The SWS medium contained soytone (0.1%), soluble starch (1.0%), and seawater (100%), and was autoclaved at 121°C for 15 min. The preparative culture (stage 1) was incubated in 1 l of sterile medium in a 3-l culture flask on a rotary shaker (130 rpm) at 29°C for 1 week. A 10% inoculum derived from the 1-week-old preparative culture was used to initiate the stage II culture, which was incubated for a further 24 h under the same conditions before addition of 20 mg of **1** in 0.75 ml of *N,N*-dimethyl formamide (DMF). Incubation was continued for 72 h in the same manner as described above. The substrate control consisted of sterile medium and substrate incubated under the same conditions, but without microorganisms. The culture control consisted of the microorganism grown under the same condition, but without substrate. After 72 h, each control was harvested and analyzed by TLC. The



geraniol (**1**) 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene (**2**)

Fig. 1. Biotransformation of geraniol (**1**) by the marine-derived fungus *Hypocrea* sp.

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 (75 mg) that was subjected to Si gel flash column chromatography using *n*-hexane-EtOAc (1:2) as eluent to give substrate **1** (5.5 mg) and metabolite **2** (9.0 mg).

1,7-Dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene (**2**): colorless oil; IR (Neat) ν_{max} 3,346, 2,939, 1,667, 1,377, 1,204, 1,151, 1,003 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.11 (2H, d, *J*= 7.0 Hz, H_2-1), 5.38 (1H, t, *J*=7.0 Hz, H-2), 1.46 (2H, m, H_2-4), 1.41 (2H, m, H_2-5), 2.00 (2H, t, *J*=6.5 Hz, H_2-6), 1.19 (6H, s, H_3-8, 10), 1.65 (3H, s, H_3-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 58.9 (CH_2, C-1), 123.8 (CH, C-2), 138.8 (qC, C-3), 39.8 (CH_2, C-4), 22.3 (CH_2, C-5), 43.2 (CH_2, C-6), 70.8 (qC, C-7), 29.1 (CH_3, C-8, 10), 16.1 (CH_3, C-9); LR-EI-MS *m/z* 154 $[M-H_2O]^+$ (0.8), 136 $[M-2H_2O]^+$ (27), 121 (32), 109 (6), 93 (32), 83 (58), 69 (97), 59 (100); LR-ESI-MS *m/z* 195 $[M+Na]^+$, 171 $[M-H]^+$).

Structural Determination

Biotransformation of geraniol (**1**) was carried out with *Hypocrea* sp. using a two-stage fermentation protocol [10]. After fermentations of *Hypocrea* sp. with **1** (20 mg) for 72 h, the culture broth was harvested and filtered, and extracted with EtOAc to yield crude extracts. The extract was purified by repeated Si gel flash chromatography (*n*-hexane in ethyl acetate) to yield the oxidized metabolite, 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene (**2**). The ESI mass spectrum of **2** showed two prominent fragment ions corresponding to the quasimolecular ions (*m/z* 195 $[M+Na]^+$ and 171 $[M-H]^+$), and EI mass spectrum of **2** showed the parent molecule with loss of two H_2O (*m/z* 136 $[M-2H_2O]^+$) and the base fragment, 2-hydroxypropyl group (*m/z* 59). The IR spectrum of **2** suggested the presence of a hydroxyl group (3,346 cm^{-1}). The ^1H and ^{13}C NMR data of **2** closely resembled those of **1** except for the appearance of an additional sp^3 methylene [δ 2.00 (t, *J*=6.5 Hz, H_2-6), 43.2 (t, C-6)], an sp^3 quaternary carbon [δ 70.8 (s, C-7)] with an oxygen function rather than a loss of an olefinic proton [δ 5.09 (d, *J*=7.0 Hz, H-6)], an sp^2 methine [δ 124.0 (d, C-6)], and sp^2 quaternary [δ 131.6 (s, C-7)] carbons. Thus, compound **2** was characterized as a 7-hydroxylated derivative of **1**. The hydration at the C6-C7 double bond of **1** is supported by the fragment ions, *m/z* 136 $[M-2H_2O]^+$ and *m/z* 59 $[CH_3C(OH)CH_3]^+$ (base fragment), in the mass spectrum. Direct comparison of ^1H and ^{13}C NMR data for **2** with those for **1** supported the gross structure shown for **2**. Based on these data, the structure of **2** was determined to be 1,7-dihydroxy-3,7-dimethyl-(*E*)-oct-2-ene. To our knowledge, compound **2** is the first example of a compound derived from biological transformation of **1**. Geraniol (**1**) is an important component of essential oil from the rhizome of *Alpinia galangal* [12], and is also a biosynthetic precursor of many types of acyclic and cyclic monoterpenoids [1]. Furthermore, geraniol (**1**) is an important flavor component of some grape juices and wines. In wines and other beverages, flavor development is important to the consumer. Compound **2** is a product of both acidic hydrolysis of **1** [9] and enzymatic hydrolysis of glycoside [6], and is a synthesized product [11]. The production of useful flavor compounds using marine-derived fungi is a potentially important application of microbial technology.

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