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A Radical Scavenging Farnesylhydroquinone from a Marine-Derived Fungus *Penicillium* sp.

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Farnesylhydroquinone (**1**) has been isolated from the mycelium of a marine-derived fungus of the genus *Penicillium*. The structure of the compound (**1**) has been elucidated by spectral method. The compound **1** exhibits potent radical scavenging activity (IC_{50} 12.5 μM) against the DPPH.

Key words: Marine fungus, *Penicillium* sp., Sesquiterpene quinone, Farnesylhydroquinone, Radical scavenging, DPPH

INTRODUCTION

Marine microorganisms, such as bacteria and fungi, inhabit virtually every environment in the sea, and are rich sources of chemically and biologically diverse compounds (Faulkner, 2001; Pietra, 1997).

As part of our search for new bioactive marine natural products from the marine organisms (Son *et al.*, 2001), we have investigated radical scavenging constituents from the marine isolate of the fungus of the genus *Penicillium*. In this paper, we report the isolation and structural elucidation of farnesylhydroquinone (**1**), a radical scavenging hydroquinone, and the sesquiterpene quinone (**2**) (Bohlmann *et al.*, 1975).

MATERIALS AND METHODS

General experimental

Melting point was determined on a Electrothermal model IA 9100 micro-melting point apparatus and is uncorrected. Optical rotation was determined on a Perkin Elmer model 341 polarimeter. IR spectrum was recorded on a Bruker FT-IR model IFS-88 spectrometer. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks as reference standard. MS spectrum was obtained on a JEOL JMS-700 spectrometer. UV/visible spectrum was measured on a Hitachi U-2001 UV/Vis spectrometer.

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Fungal isolation and culture

The fungal strain (culture # MFA 577) was isolated from the surface of the polymeric cord collected in Bijin Island, Gyeongnam Prefecture in 2000 and identified as a *Penicillium* sp. based on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea), similarity index 0.862. The fungus was cultured (20 L) for 30 days (static) at 29°C in SWS medium : soytone (0.1%), soluble starch (1.0%), agar (1.5%), and seawater (100%).

Isolation of farnesylhydroquinone (1) and sesquiterpene quinone (2)

The mycelium and broth were separated by filtration. The mycelial mat was freeze-dried and extracted twice with CH_2Cl_2 - MeOH (1:1). The combined extract (1.1 g) was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A), eluting with H_2O/MeOH (100→0%), to obtain 5 fractions. These fractions were evaluated for radical scavenging activity, and fraction 3 was found active. Further purification of fraction 3 (110 mg) over silica gel column chromatography using *n*-hexane/EtOAc (20:1→15:1), followed by HPLC (YMC ODS-A, 10 × 250 mm) (MeOH) yielded a farnesylhydroquinone (**1**, 21 mg)

and sesquiterpene quinone (**2**, 4.0 mg).

1: Colorless solid; IR (KBr): 3468, 3238, 1656, 1634, 1429, 1187 cm^{-1}; UV (MeOH): 206 (ε 12000), 294 (ε 2000) nm; HREIMS *m/z* 328.2407 (calcd for $C_{22}H_{32}O_2$, 328.2402); LREIMS *m/z* 328[M]$^+$, 259[M-69]$^+$, 191[M-137]$^+$, 137[3,7-dimethyl-2,6-octadienyl]$^+$, 69[2-methyl-3-pentenyl]$^+$, 55[C_4H_7]$^+$. See Table 1 for NMR spectral data.

2: Isolated as a yellowish oil which showed spectral data virtually identical to those reported in the literature (Bohlmann *et al.*, 1975).

Radical (DPPH) scavenging assay

Samples to be tested were dissolved in MeOH and the solution (160 μL) was dispensed into wells of a 96-well microtiter tray. 40 μL of the DPPH solution in MeOH (1.5×10^{-4} M) was added to each well. The mixture was shaken and left to stand for 30 min, and the absorbance of the resulting solution was measured at 520 nm with microplate reader (Packard Co., Spectra CountTM). The scavenging activity on DPPH radical was expressed as IC_{50}, which is the concentration of the tested compound required to give a 50% decrease of the absorbance from that of the blank solution [consisting of MeOH (160 μL) and DPPH solution (40 μL)].

Table I. ^1H (δ, mult, *J*) and ^{13}C (δ, mult) NMR data of farnesylhydroquinone (1)[a]

Carbon No.	δ_H	δc	HMBC (H to C)
1		147.4 (s)	
OH-1	4.43 (br. s)		
2		122.5 (s)	
3	6.59 (s)	118.1 (d)	1, 4, 5, 7
4		147.8 (s)	
OH-4	4.74 (br. s)		3
5		125.3 (s)	
6	6.54 (s)	116.2 (d)	1, 2, 4, 5, 1'
7	2.17 (s)	15.4 (q)	1-3
1'	3.27 (d, 7.0)	29.3 (t)	4-6, 2' 3'
2'	5.29 (dd, 7.0, 6.2)	121.6 (d)	1', 4', 15'
3'		138.3 (s)	
4'	2.08 (m)[b]	39.7 (t)	
5'	2.08 (m)[b]	26.4 (t)	
6'	5.09 (dd, 7.0, 6.5)	123.7 (d)	4', 5', 8', 12'-14'
7'		135.5 (s)	
8'	1.97 (dd-like, ca7.0, 7.0)	39.7 (t)	6', 7', 9', 14'
9'	2.08 (m)[b]	26.7 (t)	
10'	5.09 (dd, 7.0, 6.5)	124.3 (d)	4', 5', 8', 12'-14'
11'		131.3 (s)	
12'	1.67 (s)	25.7 (q)	10', 11', 13'
13'	1.59 (s)	17.7 (q)	
14'	1.59 (s)	16.0 (q)	
15'	1.75 (s)	16.2 (q)	2'-4'

[a]Recorded in $CDCl_3$ at 400 MHz (^1H) and 100 MHz (^{13}C).
[b]Overlapped.

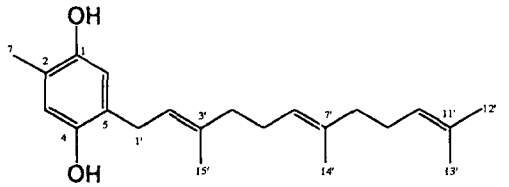
RESULTS AND DISCUSSION

Farnesylhydroquinone (**1**) was isolated as a colorless solid and was found to have an elemental composition $C_{22}H_{32}O_2$ on the basis of HREI-MS and ^{13}C NMR methods. The IR spectrum of **1** revealed absorption bands for hydroxyl (3468 cm^{-1}) and double bonds (1656, 1634 cm^{-1}). The ^1H and ^{13}C NMR data for **1**, including results from DEPT, COSY, HMQC, and HMBC experiments, showed 2,5-disubstituted hydroquinone, three trisubstituted double bonds, one aromatic methyl, and four olefinic methyls (Table I). The farnesyl unit was deduced from a detailed comparison of the ^{13}C NMR data for **1** with those of sesquiterpene quinone (**2**) (Bohlmann *et al.*, 1975), grifolin (Zechlin *et al.*, 1981), and euplexides C, F and G (Shin *et al.*, 1999; Seo *et al.*, 2001), and from the fragment ions, *m/z* 69[2-methyl-3-pentenyl]$^+$, 137[3,7-dimethyl-2,6-octadienyl]$^+$, 191[M-137]$^+$, and 259[M-69]$^+$.

These data suggested that **1** was a triprenylated hydroquinone analogue to sesquiterpene quinone (**2**) (Bohlmann *et al.*, 1975). This conclusion was further supported by HMBC correlations between H_3-7 and C-1, C-2 and C-3, OH-4 and C-3, H-6 and C-1', and H_2-1' and C-4, C-5 and C-6.

Based on all of the foregoing evidence, the structure of farnesylhydroquinone was determined to be 2-methyl-5-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-1,4-hydroquinone (**1**) (Fig. 1).

To rule out the possibility of **1** and **2** being artifacts formed as a result of the extraction and purification process, a careful TLC analysis of the extract and purified fractions



1

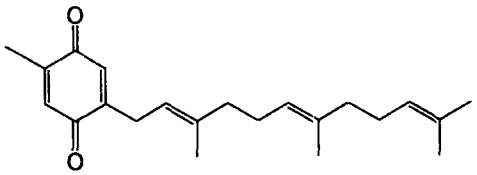


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Fig. 1. Structures of farnesylhydroquinone (**1**) and sesquiterpene quinone (**2**).

was carried out. Both farnesylhydroquinone (**1**) and its benzoquinone (**2**) were detected in the fresh original organic crude extract and the fractions obtained after chromatography. Also, the farnesylhydroquinone (**1**) and its benzoquinone (**2**) were dissolved separately in MeOH and both solutions were exposed to air for 3 days. The farnesylhydroquinone (**1**) was partly oxidized to benzoquinone (**2**), but the TLC of benzoquinone (**2**) did not reveal the presence of the reduced product, the farnesylhydroquinone (**1**). These experiments provided enough evidence to establish the natural origin of the farnesylhydroquinone (**1**).

Various prenylated quinones, including ubiquinone that is in charge of an important role in photosynthetic pathways of plants, have been isolated as bioactive constituents of the terrestrial plants, the marine algi and invertebrates, and microorganisms (Mothana *et al.*, 2000; Seo *et al.*, 2001; Shin *et al.*, 1999; Thomson *et al.*, 1987). Although farnesylhydroquinone (**1**) has been reported as the reduction-product of sesquiterpene quinone (**2**) (Bohlmann *et al.*, 1975), compound **1** is the first example, to the best of our knowledge, from a natural source. Farnesylhydroquinone (**1**) exhibits potent radical scavenging activity (IC_{50} 12.5 μM) against the DPPH radical, which was more potent than vitamin C (IC_{50} 22.7 μM).

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