

A New α -Pyrone Derivative, 6-[(*E*)-Hept-1-enyl]- α -pyrone, with Tyrosinase Inhibitory Activity from a Marine Isolate of the Fungus *Botrytis*

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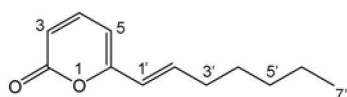
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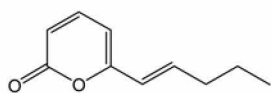
Microorganisms such as bacteria, fungi, and blue-green algae have proven to be a rich source of new biologically active secondary metabolites.¹ Marine microorganisms, particularly marine fungi, have recently drawn much attention as an important source of biologically active secondary metabolites.² Among marine fungi, those living in association with marine algae are promising sources of novel natural products due to their unique ecological niche. The association between algae and fungi appears to be highly developed since nearly one-third of all higher marine fungi described are namely algicolous or algae-associated organisms.³ Recently, marine-derived fungi have yielded unique biologically active metabolites, such as myrothenones,⁴ gliotoxin derivatives,⁵ and asperflavin ribofuranoside,⁵ suggesting that these organisms would be valuable producers of potential therapeutic agents.

As part of our search for bioactive substances from marine microorganism,⁵ the fungus was studied because the broth extract showed potent tyrosinase inhibitory activity.⁷ In order to identify the active compounds, the broth was further separated into single ones. Careful bioassay-guided fractionations resulted in isolation of a new α -pyrone derivative, 6-[(*E*)-hept-1-enyl]- α -pyrone (**1**), and two known compounds, 6-[(*E*)-pent-1-enyl]- α -pyrone (**2**)⁸ and 4-hydroxyphenethyl alcohol (**3**)⁹ from the marine isolate of fungus *Botrytis* sp. We herein report the isolation and structural elucidation of compounds **1-3**.

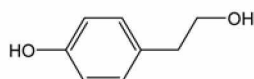
6-[(*E*)-Hept-1-enyl]- α -pyrone (**1**) was a colorless oil isolated from the broth extract. A molecular formula of C₁₂H₁₆O₂, which gave five degrees of unsaturation, was



6-[(*E*)-hept-1-enyl]- α -pyrone (**1**)



6-[(*E*)-pent-1-enyl]- α -pyrone (**2**)



4-hydroxyphenethyl alcohol (**3**)

established by HREIMS and ¹³C NMR methods. The ¹H and ¹³C NMR spectra, including DEPT, showed one methyl, four sp³ methylenes, five sp² methines, and two sp² oxygenated quaternary carbons. Detailed analyses of the ¹H and ¹³C NMR spectra of **1**, including the results from COSY and TOCSY experiments, suggest the presence of (*E*)-hept-1-enyl group and 6-substituted α -pyrone, which were further supported by the fragment ion, *m/z* 95 [M-C₇H₁₃]⁺, in the mass spectrum and IR data (1735, 1648, 1541 cm⁻¹), respectively. The connection of functional groups in **1** was achieved on the basis of HMBC. Diagnostic HMBC correlations, from H-5 to C-1', from H-1' to C-5, C-6 and C-3', and from H-2' to C-6, C-3' and C-4', showed the connection of C6-C1' in **1**. The coupling constant between H-1' and H-2' (*J*_{H1'-H2'} = 15.5 Hz) was critical in establishing the (*E*) geometry of double bond of compound **1**. On the basis of all the foregoing evidence, we propose that the structure of compound **1** is 6-[(*E*)-hept-1-enyl]- α -pyrone.

The known compounds **2** and **3** were identified by spectroscopic analysis (¹H and ¹³C NMR, and LREIMS) and comparison to literature data.^{8,9}

Compound **1** exhibited a tyrosinase inhibitory activity with IC₅₀ value of 4.5 μ M, which is more active than kojic acid (IC₅₀, 15.5 μ M) currently being used as a functional personal-care compound. But compound **2** showed a weak tyrosinase inhibitory activity with IC₅₀ value of 155.5 μ M.

Experimental Section

General. UV/visible spectra were measured on a Hitachi U-2001 UV/Vis spectrometer. IR spectra were recorded on a Bruker FT-IR model IFS-88 spectrometer. ¹H (400 MHz) and ¹³C NMR (100 MHz) spectra were obtained on a JEOL JNM-ECP 400 NMR spectrometer, using TMS or solvent peaks (δ 2.50 in ¹H and δ 39.5 in ¹³C NMR) as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer.

Fungal Isolation and Culture. The fungal strain was isolated from the surface of the marine red alga *Hyalosiphonia caespitosa* collected at Dadaepo, Busan in 2004, and identified as a *Botrytis* sp. (Family: Sclerotiniaceae) based

Table 1. NMR Spectroscopic Data (400 MHz, DMSO-*d*₆) for 6-[(*E*)-hept-1-enyl]- α -pyrone (**1**)

6-[(<i>E</i>)-hept-1-enyl]- α -pyrone (1)		
position	δ_c , mult	δ_H , (<i>J</i> in Hz)
2	160.8, qC	
3	113.2, CH	6.18, d (9.0)
4	145.0, CH	7.52, dd (9.0, 6.7)
5	103.7, CH	6.29, d (6.7)
6	158.8, qC	
1'	122.0, CH	6.20, d (15.5)
2'	138.1, CH	6.50, dt (15.5, 7.0)
3'	31.9, CH ₂	2.19, dt (7.0, 7.2)
4'	27.7, CH ₂	1.42, tt (7.2, 7.2)
5'	30.7, CH ₂	1.28, m
6'	21.8, CH ₂	1.28, m
7'	13.8, CH ₃	0.86, t (7.0)

on fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea, similarity index of 0.62). A voucher specimen is deposited at Pukyong National University with the code MFB604. The fungus was cultured (20 L) for three weeks (static) at 29 °C in SWS medium consisted of soytone (0.1%), soluble starch (1.0%), and seawater (100%).

Extraction and Isolation. The mycelium and broth were separated by filtration. The filtered broth was extracted with EtOAc to afford broth extract (0.9 g), which was subjected to Si gel flash chromatography. Elution was performed with *n*-hexane-EtOAc (stepwise, 0-100% EtOAc) to yield four fractions. Fractions 2 and 3 on medium pressure liquid chromatography (MPLC) (ODS) by elution with H₂O-MeOH (from 1:1 to 1:5) afforded crude compounds **1** and **2**, and **3**, respectively, which were further purified by HPLC (YMC, ODS-A) utilizing a 30 min gradient program of 50% to 100% MeOH in H₂O to furnish **1** (10.2 mg), **2** (1.5 mg),

and **3** (3.5 mg), respectively.

6-[(*E*)-Hept-1-enyl]- α -pyrone (**1**) was obtained as a colorless oil: UV (MeOH) λ_{max} (log ϵ) 336 (6.1) nm; IR (neat) ν_{max} 2932, 2928, 2857, 1735, 1648, 1541, 1459, 1223, 1097, 793 cm⁻¹; ¹H and ¹³C NMR, see Table 1; EIMS *m/z* 192 [M]⁺ (45), 123 (100), 110 (39), 107 (37), 95 [M-C₇H₁₃]⁺ (47), 79 (38); HREIMS *m/z* 192.1149 (calcd for C₁₂H₁₆O₂, 192.1150).

6-[(*E*)-Pent-1-enyl]- α -pyrone (**2**) and 4-hydroxyphenethyl alcohol (**3**) were obtained as a colorless oil, and showed spectral data virtually identical to those reported in the literature.^{8,9}

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