Parasitenone, a New Epoxycyclohexenone Related to Gabosine from the Marine-Derived Fungus *Aspergillus parasiticus*

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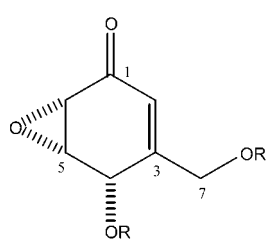
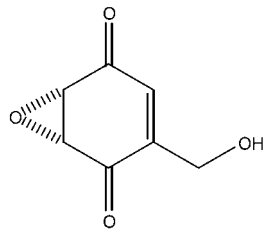
Bioassay-guided fractionation of an organic extract of the broth from the marine-derived fungus culture of *Aspergillus parasiticus* led to the isolation and subsequent structural elucidation of a new gabosine derivative, parasitenone (**1**), and two known benzyl alcohols, 3-chloro-4,5-dihydroxybenzyl alcohol (**2**) and gentisyl alcohol (**3**). The benzyl alcohols (**2**, **3**) were identified as the principal free radical scavenging components. Parasitenone (**1**) also showed moderate activity in the free radical scavenging assay.

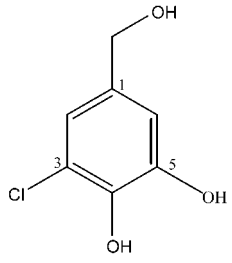
As part of an effort to discover biologically active natural products from marine organisms,[1] we investigated bioactive constituents of the marine algicolous fungus *Aspergillus parasiticus* and isolated a fairly unstable epoxycyclohexenone, parasitenone (**1**), and two known benzyl alcohols, 3-chloro-4,5-dihydroxybenzyl alcohol (**2**)[2] and gentisyl alcohol (**3**).[3]



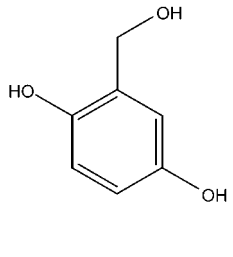
1: R=H
1a: R=$COCH_3$



1b (-)-phyllostine



2



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Parasitenone (**1**) was isolated as a very unstable colorless oil, which was deduced to have the molecular formula $C_7H_8O_4$ from the HREIMS of its diacetate (**1a**) and ^{13}C NMR data of **1** and **1a**. The IR spectrum of **1** suggested the presence of hydroxyl (3356 cm^{-1}), enone (1680, 1027 cm^{-1}), and epoxy (1236, 903, 867 cm^{-1}) groups. The UV spectrum of **1** showed the presence of a $\beta\beta$-disubstituted enone chromophore [203 nm (ϵ 5300), 237 (4800)]. Since **1a** showed six degrees of unsaturation from HREIMS analysis, it was implied that **1a** contains two rings. In the ^1H NMR spectrum, two proton signals [δ 5.79 (1H, d, J = 6.2 Hz, 4-OH), 5.01 (1H, t, J = 5.5 Hz, 7-OH)] were exchanged by D_2O, suggesting that **1** had two hydroxyl groups. The ^1H and ^{13}C NMR spectra of **1** show signals attributable to one ketone (δ 193.9, C-1), one trisubstituted double bond [(δ 6.39, H-2), (δ 141.4, C-2), (δ 133.8, C-3)], one oxygenated methylene [(δ 4.07, 3.96, H_2-7), (δ 57.3, C-7)], and three oxygenated methines [(δ 4.70, H-4), (δ 63.7, C-4), (δ 3.40, H-5), (δ 52.9, C-5), (δ 3.76, H-6), (δ 54.0, C-6)] bearing oxygen. The upfield oxygenated ^{13}C NMR signals (δ 52.9, C-5; δ 54.0, C-6) were assigned to an epoxy group.

Interpretation of 2D NMR (COSY, HMQC, HMBC) confirmed the presence of functional groups noted above and led to structure **1**.

The physicochemical data of **1** were not consistent with the data for the 4-epimer of **1**, which was previously reported as a synthetic intermediate in the synthesis of (−)-phyllostine (**1b**).[4] So, we elucidated the stereostructure of **1**.

The relative stereochemistry of **1** was determined by coupling constants ($J_{4,5}$ = 0, $J_{5,6}$ = 4.2 Hz, $J_{4,6}$ = 2.8 Hz) and NOESY data. Key NOE correlations from H-4 to H-6 and from H-5 to H-6 indicated that H-4, H-5, and H-6 were all of the *cis* configuration. The three stereocenters in parasitenone (**1**) were assigned *S* configurations by interpretation of its CD data. Parasitenone (**1**) showed a positive first Cotton effect at 338 nm ($\Delta\epsilon$ + 0.95), which from the octant rule indicated 4*S*, 5*S*, and 6*S* configurations for **1**.[5] The absolute configuration of **1** was further supported by chemospecific oxidation of the secondary allylic hydroxyl of **1** with pyridinium dichromate (PDC) in DMF to **1b**, which was identified by direct comparison of its spectral data with those of natural (−)-phyllostine (**1b**).[4,6] On the basis of the above evidence, the structure of parasitenone was determined to be (4*S*,5*S*,6*S*)-5,6-epoxy-4-hydroxy-3-hydroxymethylcyclohex-2-en-1-one (**1**).

The polyoxygenated cyclohexenone derivatives known as gabosines have been previously reported from natural sources.[7,8] Since they are biologically interesting for their phytotoxic, antibiotic, antitumor, and antigermination activities, they have been used as targets or intermediates for the synthesis of biologically active compounds.[8,9]

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There is considerable recent evidence that free radicals induce oxidative damage to biomolecules. This oxidative damage is considered to play a causative role in aging and several degenerative diseases such as Alzheimer's disease, rheumatoid arthritis, cancer, and atherosclerosis.[10] So far, a number of free radical scavengers have been found by using various screening systems.[10−12]

Parasitenone (**1**) and benzyl alcohols (**2**, **3**) showed scavenging activities toward free radicals, 1,1-diphenyl-2-picrylhydrazyl (DPPH), peroxy nitrite ($ONOO^-$), superoxide radical ($^{\bullet}O_2^-$), and nitric oxide radical ($^{\bullet}NO$). Parasitenone (**1**) exhibited mild activity against DPPH and $ONOO^-$, with IC_{50} values of 57.0 and 52.6 μM, respectively. In contrast, **2** and **3** showed strong activity, and the corresponding IC_{50} values (μM) for each radical are as follow: **2**, DPPH (0.6), $ONOO^-$ (3.1), $^{\bullet}O_2^-$ (11.0), $^{\bullet}NO$ (0.5); **3**, DPPH (1.4), $ONOO^-$ (2.2), $^{\bullet}O_2^-$ (50≪, inactive), $^{\bullet}NO$ (0.4). Among the assay results obtained, gentisyl alcohol (**3**) exhibited more potent activity (300-fold) than the positive control (carboxy-PTIO), with an IC_{50} value of 137.7 μM against nitric oxide radical ($^{\bullet}NO$). Details of the radical scavenging activity of compounds **1**−**3** and its mode of action are currently under investigation and will be reported in due course.

Experimental Section

General Experimental Procedures. Melting points were determined on a Electrothermal model IA 9100 micro-melting point apparatus and are uncorrected. Optical rotation was determined on a Perkin-Elmer model 341 polarimeter. The IR spectrum was recorded on a Bruker FT-IR model IFS-88 spectrometer. ^{1}H (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 spectra were obtained on a JEOL JMS-700 spectrometer. UV/visible spectra were measured on a Hitachi U-2001 UV/vis spectrometer. CD spectra were taken on a JASCO J-715 spectropolarimeter.

Fungal Isolation and Culture. The fungal strain (culture # MFA 153) was isolated from the surface of the marine red alga *Carpopeltis cornea* collected in the Golmae village, Ulsan City, in 1999 and identified as a *Aspergillus parasiticus* on the basis of fatty acid methyl ester analysis (Korean Culture Center of Microorganisms, Seoul, Korea), similarity index 0.858. 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 Parasitenone (1) and Benzyl Alcohols (2, 3). The resulting broth and mycelium were separately extracted with EtOAc and CH_2Cl_2−MeOH (1:1) to afford crude extracts of 0.6 and 2.2 g, respectively. The broth extract showed strong radical scavenging activity against DPPH, and the active components were purified by assay-guided isolation using repeated silica gel (*n*-hexane−EtOAc) and HPLC (10:1 EtOAc−MeOH) methods to yield a new gabosine derivative, parasitenone (**1**, 8 mg), as well as the known benzyl alcohols, 3-chloro-4,5-dihydroxybenzyl alcohol (**2**, 22 mg) and gentisyl alcohol (**3**, 12 mg).

Parasitenone (1): unstable colorless oil; $[\alpha]_D$ + 71.6° (*c* 0.3, MeOH); IR (neat) ν_{max} 3356, 1680, 1400, 1236, 1027, 903, 867 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 203 (3.72), 237 (3.68) nm; CD (MeOH) ($\Delta\epsilon$) 338 (+0.95), 245 (−1.76) nm; CIMS *m*/*z* 156 $[M]^+$ (100), 138 $[M - H_2O]^+$ (7), 122 $[M - H_2O - O]^+$ (2), 110(3), 86(3); ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.39 (1H, dddd, *J* = 2.7, 2.6, 2.2, 2.1 Hz, H-2), 4.70 (1H, ddddd, *J* = 6.2, 2.8, 2.8, 2.6, 2.6 Hz, H-4), 5.79 (1H, d, *J* = 6.2 Hz, 4-OH), 3.40 (1H, d, *J* = 4.2 Hz, H-5), 3.76 (1H, ddd, *J* = 4.2, 2.8, 2.7 Hz, H-6), 3.96 (1H, dddd, *J* = 15.2, 5.5, 2.8, 2.2 Hz, H-7), 4.07 (1H, dddd, *J* = 15.2, 5.5, 2.6, 2.1 Hz, H-7), 5.01 (1H, t, *J* = 5.5 Hz, 7-OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 193.9 (s, C-1), 141.4 (d, C-2), 133.8 (s, C-3), 63.7 (d, C-4), 52.9 (d, C-5), 54.0 (d, C-6). 57.3 (t, C-7).

Acetylation of Parasitenone (1). Acetylation of **1** (10 mg) in the usual manner (acetic anhydride−pyridine) furnished the diacetate (**1a**) (6 mg).

1a: colorless oil; IR (neat) ν_{max} 1737, 1367, 1208, 1023 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.49 (1H, dddd, *J* = 2.5, 2.2, 1.5, 1.5 Hz, H-2), 5.90 (1H, dddd, *J* = 2.5, 2.2, 1.5, 1.5 Hz, H-4), 3.58 (1H, d, *J* = 4.2 Hz, H-5), 3.93 (1H, ddd, *J* = 4.2, 2.5, 2.5 Hz, H-6), 4.65, 4.59 (each 1H, ddd, *J* = 13.5, 1.5, 1.5 Hz, H_2-7), 2.15, 2.03 (each 3H, s, 4- and 7-OAc); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.1 (s, C-1), 139.6 (d, C-2), 130.9 (s, C-3), 66.5 (d, C-4), 51.6 (d, C-5), 54.9 (d, C-6), 59.9 (t, C-7), 169.9, 169.8 (each s, 4- and 7-OAc), 20.8, 20.7 (each q, 4- and 7-OAc); EIMS *m*/*z* 240 $[M]^+$ (5), 180 $[M - AcOH]^+$ (15), 138 $[M - AcOH - CH_2{=}C{=}O]^+$ (100); HREIMS *m*/*z* 240.0641 (calcd for $C_{11}H_{12}O_6$, 240.0634).

Oxidation of Parasitenone (1). Pyridinium dichromate (67 mg, 0.17 mmol) was added to a solution of **1** (13 mg, 0.08 mmol) in DMF (1 mL) at 0 °C, and the mixture was stirred for 3 h at the same temperature, then at room temperature for 3 h. The reaction mixture was filtered through a Celite pad. The filtrate was reduced under vacuum, and the residue was chromatographed on a Si gel column eluted with *n*-hexanes−EtOAc (1:1) to afford (−)-phyllostine (**1b**) (2.7 mg).

(−)-Phyllostine (1b): reddish solid. The compound exhibits spectral data (mp, $[\alpha]_D$, IR, ^{1}H NMR) comparable to published values.[4,6]

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Supporting Information Available: The physicochemical data of 3-chloro-4,5-dihydroxybenzyl alcohol (**2**) and methods of the radical scavenging assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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