Bromomyrothenone B and Botrytinone, Cyclopentenone Derivatives from a Marine Isolate of the Fungus *Botrytis*

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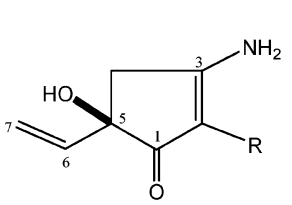
Received February 6, 2006

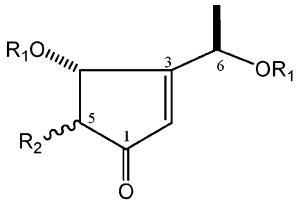
New cyclopentenones, bromomyrothenone B (**1**) and botrytinone (**2**), and the known cyclonerodiol (**3**) were isolated from the marine algicolous fungus of the genus *Botrytis*. The absolute stereostructures of compounds **1** and **2** were elucidated on the basis of chemical and physicochemical evidence including quantum chemistry calculation, X-ray analysis, and CD exciton chirality method.

Marine microorganisms such as bacteria and fungi inhabit virtually every environment in the sea, and they are rich sources of chemically and biologically diverse compounds.[2] During a search for bioactive constituents from marine microorganisms,[3] we isolated two new cyclopentenone derivatives, bromomyrothenone B (**1**) and botrytinone (**2**), and the known cyclonerodiol (**3**)[3,4] from the marine-derived fungus *Botrytis* sp. We report here on the isolation and structural elucidation of these new compounds.



bromomyrothenone B (**1**): R= Br

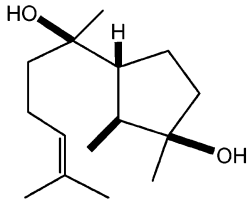
myrothenone B (**1a**): R= H



botrytinone (**2**): R_1= H, R_2= β-OH

(**2a**): R_1= *p*-BrC_6H_4CO, R_2= β-Cl

(**2b**): R_1= *p*-BrC_6H_4CO, R_2= α-Cl



cyclonerodiol (**3**)

Bromomyrothenone B (**1**) was a red oil with an isotopic cluster at *m*/*z* 217 and 219 with the ratio 1:1, suggesting the presence of a bromine atom. The molecular formula of **1** was established as $C_7H_8BrNO_2$ (4 unsaturations) by HREIMS and ^{13}C NMR methods. The IR absorption spectrum of **1** showed the bands characteristic of the hydroxyl and amino groups (3321 cm^{-1}) and enone (1630 cm^{-1}) functionalities. The general features of the UV, IR, and NMR spectra of **1** closely resembled those of myrothenone B (**1a**),[3] except for the NMR signals at C-2, which were changed from an sp^2-methine [δ 4.70 (s, H-2), 95.8 (C-2)] in myrothenone B (**1a**) to an sp^2-quaternary carbon [δ 86.1 (C-2)] in **1**. The ^1H and ^{13}C NMR spectra of **1**, including DEPT, showed a monosubstituted double bond, a tetrasubstituted double bond, a primary amine, an oxygenated quaternary carbon, a carbonyl carbon, and a diastereotopic methylene. The presence of a 1,2,3,3-tetrasubstituted enone chromophore was further supported by the UV spectral data [210 nm (log ϵ 3.7), 277 (4.2)] and by the characteristic double-bond carbon signals [δ 86.1 (C-2), 169.6 (C-3)] located considerably upfield and downfield, respectively.[5] Detailed analyses of the ^1H and ^{13}C NMR spectra of **1**, including the results from DEPT, HMQC, and HMBC experiments, suggest that metabolite **1** is a 2-bromide derivative of myrothenone B (**1a**).

The configuration illustrated for **1** is based on the optical rotation, which had established that myrothenone B (**1a**) and **1** possessed an identical *R* configuration at C-5 from the same sign of the optical rotation ($[\alpha]^{20}_D$ +61), induced mainly by the substituents at C-5, as that of myrothenone B (**1a**) ($[\alpha]^{20}_D$ +35). This conclusion was further supported by the comparison of the CD data between myrothenone B (**1a**) and **1**. The CD spectrum of **1** was similar to that of myrothenone B (**1a**) and showed a positive Cotton effect at 289 nm ($\Delta\epsilon$, +9.8) and a negative Cotton effect at 265 (−5.2), indicating that both compounds shared the same configuration. On the basis of the evidence described above, the stereostructure of bromomyrothenone B was determined to be (5*R*)-3-amino-2-bromo-5-ethenyl-5-hydroxy-2-cyclopenten-1-one (**1**).

Botrytinone (**2**) was a colorless oil isolated from the broth extract. A molecular formula of $C_7H_{10}O_4$, which gave three degrees of unsaturation, was established by HREIMS and ^{13}C NMR methods. The IR absorption spectrum of **2** showed bands that are characteristic of hydroxyl (3335 cm^{-1}) and enone (1716 cm^{-1}) functionalities.

The ^1H and ^{13}C NMR spectra, including DEPT, showed one methyl, three oxygenated methines, one trisubstituted double bond, and one carbonyl carbon. Detailed analyses of the ^1H and ^{13}C NMR spectra of **2**, including the results from COSY and TOCSY experiments, suggest the presence of 1,2-disubstituted ethylene glycol and 1,3-dialkyl-3-(1-hydroxyethyl)-2-propen-1-one. The presence of a 1,3,3-trisubstituted enone chromophore was further supported by UV data [225 nm (log ϵ 3.8)]. The connection of functional groups in **2** was achieved on the basis of HMBC. Diagnostic HMBC correlations, from H-2 to C-4 and C-5, from H-4 to C-2 and C-3, from H-5 to C-1, from H-6 to C-2, C-3, and C-4, and from H_3-7 to C-3, showed the connections of C1−C5,

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10.1021/np0600548 CCC: $37.00
Published on Web 01/18/2007

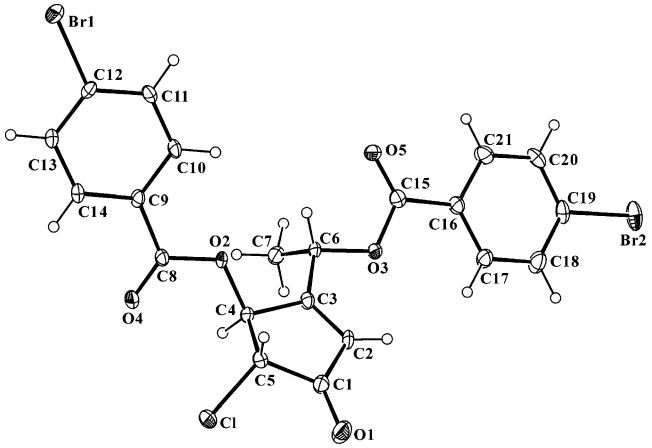

Figure 1. ORTEP III drawing of *p*-bromobenzoate derivative (**2a**). Displacement ellipsoids are drawn at the 30% probability level.

C3−C4, and C3−C6 in **2**. On the basis of all the foregoing evidence, we propose that the structure of botrytinone is 4,5-dihydroxy-3-(1-hydroxyethyl)-2-cyclopenten-1-one (**2**).

The configuration illustrated for **2** is based on NOE experiments. Key NOE correlations between H-2 and 6-OH, between H-4 and H_3-7, and between 4-OH and H-5 were critical in establishing the relative structure of compound **2**. A *trans* relationship of both hydroxyls at C-4 and C-5 was further supported by comparing the coupling constant between H-4 and H-5 in **2** ($J_{H4-H5} = 3.0$ Hz) with values reported for the *trans* stereoisomers of (+)-terrein ($J = 2.3$ Hz),[6,7] (+)-terrein diacetate ($J = 2.9$ Hz),[8] and dihydroterrein diacetate ($J = 2.9$ Hz)[8]and the *cis* stereoisomers of (+)- and (−)-isoterrein ($J = 5.7$ Hz).[6]

To confirm our assumptions for the configuration of botrytinone (**2**), a quantum chemistry calculation for **2** and X-ray diffraction analysis using its *p*-bromobenzoyl derivative (**2a**) were performed. The quantum chemistry calculation results of optimized geometry, molecular energy, and NMR spectra suggest the relative stereostructure of **2** with a great probability (see Table S1 and Figure S3 in the Supporting Information). *p*-Bromobenzoylation of **2** with *p*-bromobenzoyl chloride in pyridine yielded two *p*-bromobenzoate-5-chloride derivatives, **2a** and its 5-epimer (**2b**). We suppose that these two products were formed from the nucleophilic substitution of the 5-OH by chloride ion through an S_N1 reaction during the benzoylation of **2**. Two products (**2a** and **2b**) showed the coupling constants of $J_{H4-H5} = 3.0$ Hz in **2a** and $J_{H4-H5} = 5.9$ Hz in **2b**, indicating *trans* and *cis* conformation between H-4 and H-5, respectively.[6] The stereochemistry and reaction mechanism were further supported by CD of both products (**2a**, **2b**). The CD spectra of **2a** and **2b** revealed the same sign of Cotton effect at 243 nm ($\Delta\epsilon$, −39.9) and 245 nm ($\Delta\epsilon$, −1.07), indicating that both compounds not only shared the same configuration at C-4 but also retained this configuration during the reaction. Recrystallization of **2a** and **2b** from *n*-hexane−acetone only afforded a crystal of **2a** (see Supporting Information). The crystal structure of **2a** with relative configuration is shown in Figure 1.[9]

The absolute configuration of **2** was determined by the CD exciton chirality method.[10] The CD spectrum of **2a** showed a negative first Cotton effect at 243 nm ($\Delta\epsilon$ −39.9) attributed to the enone−benzoate chromophores. The $\pi-\pi^*$ excitation of the enone group is expected to be perturbed mainly by the two allylic 4- and 6-benzoate groups. The dihedral angles between enone and 4- and 6-benzoate are 45.3° and 0.9°, respectively. Therefore, the 4-benzoate interacts mainly with enone $\pi-\pi^*$ maxima to give the negative first Cotton effect, which gives the 4*R* and 6*R* configuration in **2a** and consequently the 4*R*, 5*S*, and 6*R* configuration in botrytinone (**2**). On the basis of these data, the absolute stereostructure of botrytinone was determined to be (4*R*,5*S*,6*R*)-4,5-dihydroxy-3-(1-hydroxyethyl)-2-cyclopenten-1-one (**2**).

Table 1. NMR Spectroscopic Data (400 MHz, DMSO-d_6) for Bromomyrothenone B (**1**) and Botrytinone (**2**)

	1		**2**	
position	δ_H (J in Hz)	δ_C, mult.	δ_H (J in Hz)	δ_C, mult.
1		193.3, qC		195.7, qC
2		86.1, qC	6.12, s	124.9, CH
3		169.6, qC		184.2, qC
3-NH_2	8.00, br, s			
	7.54, br, s			
4	2.79, d (16.9)	42.1, CH_2	4.69, dd (7.3,3.0)	77.1, CH
	2.56, d (16.9)			
4-OH			6.36, d (7.3)	
5		76.5, qC	4.48, d (3.0)	65.0, CH
6	5.79, dd (17.2, 10.6)	140.5, CH	4.61, dq (4.9, 6.7)	64.1, CH
6-OH			5.40, d (4.9)	
7	5.27, dd (17.2, 1.6)	113.3, CH_2	1.30, d (6.7)	21.2, CH_3
	5.08, dd (10.6, 1.6)			

The known compound **3** was identified by spectroscopic analysis (^{1}H and ^{13}C NMR, LREIMS, and $[\alpha]_D$) and comparison to literature data.[3,4]

Cyclopentenones have been isolated from liverwort,[11] fern,[12] fungi,[8,13−15] and the actinomycete family of bacteria.[16] These compounds show a wide range of biological activities, such as tyrosinase inhibitory activities,[3,15] antibacterial,[11] leishmanicidal,[12] plant growth inhibitory,[13] glucose-6-phosphate translocase T1 inhibitory,[14] and melanogenesis inhibitory activities.[15] However, compounds **1** and **2** were virtually inactive in radical scavenging, tyrosinase inhibitory, and antimicrobial assays (IC_{50} and MIC, over 100 μg/mL, respectively).

Experimental Section

General Experimental Procedures. Optical rotation was determined on a Perkin-Elmer model 341 polarimeter. CD spectra were taken on a JASCO J-715 spectropolarimeter. 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. ^{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 (δ 2.50 in ^{1}H and δ 39.5 in ^{13}C NMR) as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. Single-crystal X-ray measurements were performed on a Bruker SMART CCD diffractometer.

Fungal Isolation and Culture. The fungal strain was isolated from the surface of the marine green alga *Enteromorpha compressa* collected at Baegunpo, Busan, in 2002, and identified as a *Botrytis* sp. (family Sclerotiniaceae) on the basis of 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 MFA58-2. The fungus was cultured (20 L) for three weeks (static) at 29 °C in SWS medium consisting 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, 3, and 4 on medium-pressure liquid chromatography (MPLC) (ODS) by elution with H_2O−MeOH (from 1:1 to 1:5) afforded crude compounds **1**, **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 H_2O to furnish **1** (8.0 mg), **2** (5.5 mg), and **3** (20.0 mg), respectively.

Bromomyrothenone B (1): red oil; $[\alpha]^{20}_D$ +61 (*c* 0.9, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 289 (+9.8), 265 (−5.2) nm; UV (MeOH) λ_{max} (log ϵ) 210 (3.7), 277 (4.2) nm; IR (KBr) ν_{max} 3321, 3188, 1630, 1559, 1423, 1262, 1024 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 219 $[M]^+$ (45), 217 $[M]^+$ (45), 138 $[M - Br]^+$ (70), 110 $[M - Br - CO]^+$ (45), 82 (40), 68 (41), 55 (100); HREIMS *m*/*z* 218.9725 (calcd for $C_7H_8{}^{81}BrNO_2$, 218.9718), 216.9721 (calcd for $C_7H_8{}^{79}BrNO_2$, 216.9738).

Botrytinone (2): colorless oil; $[\alpha]^{20}_{D}$ −5.3 (*c* 1.3, MeOH); CD (MeOH) λ_{max} ($\Delta\epsilon$) 322 (+2.5), 228 (−12.7) nm; UV (MeOH) λ_{max} (log ϵ) 225 (3.8) nm; IR (neat) ν_{max} 3335, 1716, 1616, 1265, 1160, 1075 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 158 $[M]^+$ (3), 141 $[M - OH]^+$ (100), 123 (50), 115 (15), 107 (23), 95 (95), 79 (25), 67 (62), 55 (59); HREIMS *m*/*z* 158.0607 (calcd for $C_7H_{10}O_4$, 158.0579).

Cyclonerodiol (3): colorless oil; spectral data virtually identical to those reported in the literature.[3,4]

Quantum Chemistry Calculation of Compound 2 and X-ray Crystallographic Analysis of Compound 2a: Supporting Information.

Acknowledgment. Xifeng Li acknowledges the support of the Brain Korea 21 (F020). Circular dichroism (CD) and mass spectral data were kindly provided by the Korea Basic Science Institute. This work was supported by Grant No. R01-2004-000-10467-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

Supporting Information Available: *p*-Bromobenzoylation of botrytinone, quantum chemistry calculation result, X-ray analysis and crystal data, ^1H and ^{13}C NMR spectra of **1** and **2**, NOESY spectrum of **2**, and CD spectrum of **2a**. These materials are available free of charge via the Internet at http://pubs.ac.org.

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NP0600548