8′-Hydroxyzearalanone and 2′-Hydroxyzearalanol: Resorcyclic Acid Lactone Derivatives from the Marine-Derived Fungus *Penicillium* sp.

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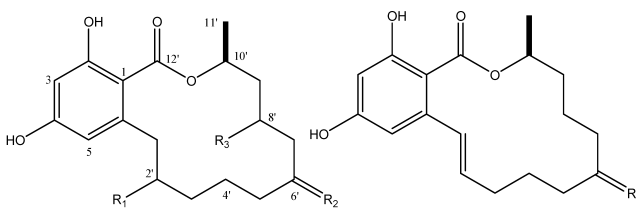
Two new 14-membered resorcyclic acid lactone derivatives, 8′-hydroxyzearalanone (1) and 2′-hydroxyzearalanol (2), and four known zearalanone derivatives (3—6) were isolated from the marine-derived fungus *Penicillium* sp. The structures of compounds 1—6 were elucidated by spectroscopic methods.

Key words marine-derived fungus; *Penicillium* sp.; 8′-hydroxyzearalanone; 2′-hydroxyzearalanol; zearalanone; zearalenone

There has recently been increased interest in the chemistry of marine fungi.[1)] Fungi are interesting organisms from an ecological prospective because they are important pathogens in the marine environment.[2)] Furthermore, since many can be cultured, they represent an important biomedical resource.[1,3—5)] In our studies on the chemistry and biology of fungi,[6)] we have investigated a marine strain of the fungus *Penicillium* sp., isolated from a drifting cotton clothing. Here, we report on the isolation and structural determination of two new and four known zearalanone analogues, 8′-hydroxyzearalanone (**1**), 2′-hydroxyzearalanol (**2**), zearalanone (**3**),[7,8)] *β*-zearalanol (**4**),[7,8)] zearalenone (**5**),[8,9)] and *β*-zearalenol (**6**),[8,9)] from a marine isolate of the fungus *Penicillium* sp.

8′-Hydroxyzearalanone (**1**), $[\alpha]_D$ −6.0 (c=0.8, acetone), was obtained as a colorless solid. Using high resolution (HR)-EI-MS and ^{13}C-NMR methods, its molecular formula was determined to be $C_{18}H_{24}O_6$. The IR spectrum of **1** exhibited bands characteristics of hydroxyl (3410 cm^{-1}), carbonyl (1700 cm^{-1}), and aryl (1644 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-NMR data for compound **1**, including correlated spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) experiments, revealed signals of an α,β-disubstituted resorcinol moiety, and also of a 14-member macrocyclic lactone ring, which includes hydroxyl, ketone, and methyl groups (Table 1, Fig. 1). The connections and positions of the functional groups in **1** were determined on the basis of COSY and HMBC correlations. Key COSY and HMBC correlations showed C1–C12′ and C6–C1′ bonds, as well as the positions of the 6′-keto, 8′-hydroxyl, and 10′-methyl groups (Fig. 1). The presence of the C1–C12′ bond was further supported by UV spectral data [272 nm (log ε 3.97), 302 (3.87)]. These spectroscopic features revealed that compound **1** had the same general structural features as compound **3**.[7,8)] NMR data for both **1** and **3** showed similar patterns, except for the presence of a hydroxyl group at C-8′ in **1**; a methylene group was observed in **3**. Thus, compound **1** was characterized as an 8′-hydroxyl derivative of **3**. Direct comparison of NMR data for **1** with those for **3**[7,8)] supported the gross structure shown for **1**.

The relative configuration between chiral centers at C-8′



8'–hydroxyzearalanone (**1**): R_1 = H, R_2 = O, R_3 = OH
2'–hydroxyzearalanol (**2**): R_1 = OH, R_2 = H, OH, R_3 = H
zearalanone (**3**): R_1, R_3 = H, R_2 = O
β–zearalanol (**4**): R_1, R_3 = H, R_2 = H, β–OH
zearalenone (**5**): R = O
β–zearalenol (**6**): R = H, β–OH

Fig. 1. Structure of Compounds **1**—**6**

Table 1. NMR Spectroscopic Data (400 MHz, DMSO-d_6) for 8′-Hydroxyzearalanone (**1**) and 2′-Hydroxyzearalanol (**2**)

Position	8′-Hydroxyzearalanone (**1**)		2′-Hydroxyzearalanol (**2**)	
	δ_C, mult.	δ_H, (J in Hz)	δ_C, mult.	δ_H, (J in Hz)
1	104.0, qC		100.0, qC	
2	164.1, qC		164.8, qC	
3	100.8, CH	6.15, d (2.1)	100.8, CH	6.16, d (2.1)
4	162.2, qC		163.4, qC	
5	110.7, CH	6.18, d (2.1)	106.9, CH	6.22, d (2.1)
6	147.1, qC		142.2, qC	
1′	36.5, CH_2	2.28, dt (4.0, 12.0)	32.2, CH_2	2.80, dd (16.5, 11.0)
		2.89, dt (4.0, 12.0)		2.90, dd (16.5, 3.0)
2′	30.8, CH_2	1.11,[a)] 1.58, m	78.8, CH	4.52, m
3′	26.7, CH_2	1.10,[a)] 1.40, m	34.2, CH_2	1.67, m
4′	21.9, CH_2	1.26,[b)] 1.88, m	21.7, CH_2	1.31, m[c)]
5′	37.5, CH_2	2.19, dt (18.0, 4.0)	37.4, CH_2	1.33, m[c)]
		2.77, dt (17.0, 4.0)		
6′	209.2, qC		69.5, CH	3.37, m[d)]
7′	54.2, CH_2	2.12, dd (11.0, 10.0)	36.8, CH_2	1.38, m
		2.84, dd (11.0, 4.0)		
8′	63.2, CH	4.00, m	20.7, CH_2	1.31, 1.58, m
9′	42.5, CH_2	1.51, 1.53, m	40.4, CH_2	1.24, 2.54, m
10′	68.8, CH	5.37, tq (4.3, 5.9)	65.8, CH	3.54, m
11′	20.6, CH_3	1.27,[b)] d (5.9)	23.6, CH_3	1.02, d (6.5)
12′	170.7, qC		169.5, qC	
2-OH		11.71, br s		11.11, br s
4-OH		10.26, br s		—[f)]
2′-OH				3.37, br s[d),e)]
6′-OH				4.29, br s[e)]
8′-OH		5.07, br s		

a—*d*) Signal partially overlapped. *e*) Interchangeable assignments. *f*) Signal unobserved.

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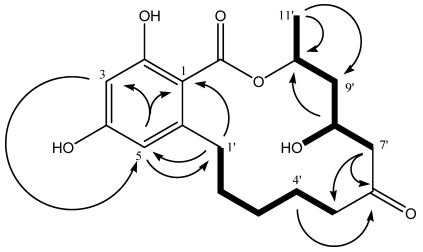

Fig. 2. Structure of **1** Elucidated by ^{1}H–^{1}H COSY (—) and HMBC (→) Correlations

and C-10′ was investigated by 1D nuclear Overhauser enhanced and exchange spectroscopy (NOESY) NMR experiments. Selective refocusing of H-8′ gave an enhancement to H-10′, while selective refocusing of H-10′ gave a weak enhancements to H-8′. Due to discrepancies between the NOESY correlations above and 3D model study of relative configuration at C-8′ and C-10′ as well as multiplicity of signal of H-8′ (δ_H 4.00, m), we have not assigned relative stereochemistry at C-8′. Because the carbon backbones of **1** and **3** are likely to be formed *via* the same biosynthetic pathway, the orientation of the 10′-methyl group of **1** is assumed to be the same as in **3**. Hence, an absolute configuration of 10′*S* is proposed for **1**.

A molecular formula of $C_{18}H_{26}O_6$ was determined for **2** by HR-EI-MS and NMR spectroscopy. ^{1}H- and ^{13}C-NMR analyses revealed that **2** lacks the ketone moiety at C-6′ that is present in **1**, and also indicated two aliphatic hydroxyl groups. The COSY, HMQC, and HMBC correlations of **2** are consistent with a structure containing 2′,6′-dihydroxyl and 8′-methylene groups. The formation of the carbon scaffold and 10′-methyl group of **2** are likely to be catalyzed by the same biosynthetic pathway as that for **3**; therefore, the configuration of C-10′ of **2** is assumed to be the same as that of **3**. NOESY data were recorded in different solvents and with different delay values to obtain additional stereochemically relevant information, but further useful cross-peaks were not observed. Because of the severe overlapping and crowding in the ^{1}H-NMR data, as well as the multiplicities of the signals, the stereochemistry of the asymmetric carbon centers, C-2′ and C-6′, remain to be assigned.

A number of 14-membered resorcyclic acid lactone analogues have been isolated from the several fungal strains, *Hypomyces subiculosus*, *H. trichothecoides*, *Coriolus versicolor*, *Aigialus parvus*[10] *Chaetomium chiversii*, *Paraphaeosphaeria quadriseptata*,[11] and *Fusarium graminarium*.[8,9] These compounds show a wide range of biological activities, such as antimalarial and antifungal activities, cytotoxicity against various murine and human cell lines,[10] anticancer activities,[11] and human estrogenic activities.[7,8]

Compounds **1**—**6** were tested for their radical scavenging activities, antibacterial activities against methycillin-resistant *Staphylococcus aureus* and multi-drug-resistant *S. aureus*, their ability to protect against ultraviolet A, and their ability to inhibit the enzyme tyrosinase. None of the compounds showed significant activity in any of these assays.

Experimental

General Optical rotation was determined on a Perkin Elmer model 341 polarimeter. 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 [DMSO-d_6: ^{1}H (δ 2.50) and ^{13}C (δ 39.5)] as reference standard. MS spectra were obtained on a JEOL JMS-700 spectrometer. HPLC was performed on a JASCO LC-2000 Plus system using a reversed-phase analytical column (Gemini C18, 4.6×250 mm, 5 μm) with UV detection.

Fungal Isolation and Culture The fungal strain, *Penicillium* sp., was isolated from the surface of the drifting cotton clothing collected in Namhae Island, Gyeongnam, Korea and identified based on the morphological evaluation and fatty acid methyl ester analysis (Korean Culture Center of Microorganism, Seoul, Korea, a similarity index of 0.919). A voucher specimen was deposited at Pukyong National University with the code MFB382. The isolate was cultured for three weeks (static) at 29 °C in SWS medium[12] consisted of soytone (0.1%), soluble starch (1.0%), and seawater (100%) (20×1 l).

Extraction and Isolation The mycelium and broth were separated by filtration through cheesecloth, and the whole broth was extracted with EtOAc (20 l) to afford crude extract (1.5 g). A portion of this extract (1.2 g) was subjected to Si gel flash chromatography. Elution was performed with *n*-hexane–EtOAc (stepwise, 0—100% EtOAc) to yield twenty collections (50 ml each). These collections were pooled on the basis of their TLC profiles to give five combined fractions. Fractions 2—5 on medium pressure liquid chromatography (MPLC) (ODS) by elution with H_2O–MeOH (gradient) afforded crude compounds **1**, **2** and **3**, **4** and **5**, and **6**, 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** (9.1 mg), **2** (31.5 mg), **3** (6.7 mg), **4** (8.4 mg), **5** (3.8 mg), and **6** (3.9 mg), respectively.

8′-Hydroxyzearalanone (**1**): A colorless solid; $[\alpha]_D$ −6.0 (c=0.8, acetone); UV λ_{max} (MeOH) nm (log ε): 272 (3.97), 302 (3.87). IR (KBr) cm^{-1}: 3410, 2945, 1700, 1644, 1262. LR-EI-MS *m*/*z*: 336 $[M]^+$ (15), 318 (5), 300 (2), 273 (4), 232 (23), 222 (14), 204 (28), 176 (32), 163 (58), 150 (53), 124 (100). HR-EI-MS *m*/*z*: 336.1583 (Calcd for $C_{18}H_{24}O_6$: 336.1573). See Table 1 for NMR spectral data.

2′-Hydroxyzearalanol (**2**): A colorless solid; $[\alpha]_D$ −9.0 (c=0.8, acetone); UV λ_{max} (MeOH) nm (log ε): 215 (7.37), 268 (7.16), 301 (6.84). IR (KBr) cm^{-1}: 3401, 2929, 1656, 1625, 1252. LR-EI-MS *m*/*z*: 338 $[M]^+$ (2), 320 (5), 302 (8), 284 (7), 251 (67), 233 (22), 222 (35), 204 (41), 179 (100), 168 (41), 150 (66), 135 (26), 112 (40), 99 (24), 81 (59), 69 (62), 55 (71). HR-EI-MS *m*/*z*: 338.1728 (Calcd for $C_{18}H_{26}O_6$: 338.1729). See Table 1 for NMR spectral data.

Zearalanone (**3**),[7,8] β-zearalanol (**4**),[7,8] zearalenone (**5**),[8,9] β-zearalenonol (**6**)[8,9] were obtained as a colorless solid. Compounds **3**—**6** showed spectral data virtually identical to those reported in the literature.[7—9]

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