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An Antibacterial 9,11-Secosterol from a Marine Sponge *Ircinia* sp.

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Marine sponges are a rich source of structurally diverse natural products and about 35% of marine natural products have been found from this chemically rich phylum of marine organisms.[1] Many of the sponge metabolites have been reported as potent cytotoxins against cancer cell lines and several of them were used as a lead of FDA approved drugs such as Cytosar-U®, and Halaven®.[2] A number of antibacterial compounds have also been reported from the marine sponge-derived metabolites including discorhabdin Z,[3] agelasine D,[4] 7,20-diisocyanoadociane,[5] motualevic acid F.[6] In this regard, we have been investigating the anti-bacterial compounds from extracts of Korean marine sponges. An extract of a marine sponge in the genus of *Ircinia* showed antibacterial activities and its bioactive constituents have been investigated.

The genus *Ircinia* has been known as a rich source of biologically active natural products including antibacterial compounds,[7,8] cytotoxin,[9] ichthyotoxin,[10] analgesic compound,[11,12] multidrug resistance modulator,[13] thrombin inhibitor,[14] angiotensin converting enzyme/aldose reductase inhibitor,[15] and inosine monophosphate dehydrogenase inhibitor.[16,17]

In particular, this genus of marine sponge has been known to be extremely rich in terpenes, mainly furanoterpenes such as ircinin-1,7 variabilin,[10] fasciculatin,[16] and strobilinin.[18] In addition, several hydroquinones,[11,19] chromans,[20,21] and sterols[22-24] have been reported from the genus *Ircinia*. However, secosterol has not been reported from the organism in this genus. Herein, we report the structure of a unprecedented secosterol with the 2-ene-1,4-dione as well as its antibacterial activity.

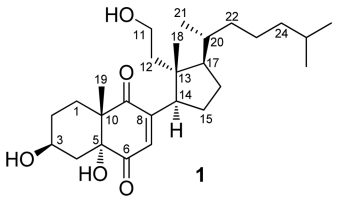

Figure 1. Chemical structure of **1**.

The molecular formula of **1** was deduced as $C_{27}H_{44}O_5$, based on the analysis of HRFABMS data (a pseudomolecular ion peak at *m/z* 449.3271 $[M+H]^+$) and on the interpretation of ^{13}C NMR data. The ^1H NMR spectrum of **1** displayed an oxygenated methine proton [δ 4.04 (m)], an olefinic proton [δ 6.49 (br s)], and one downfielded methylene protons [δ 3.84 (m), 3.70 (m)]. The ^1H NMR spectrum also showed two methyl singlets [δ 1.23, 0.70] and three methyl doublets [δ 0.97 (d, J = 6.6 Hz), 0.88 (d, J = 2.3 Hz), 0.86 (d, J = 2.3 Hz)]. The ^{13}C NMR and HSQC spectra revealed five methyl, ten methylene, six methine, and six fully-substituted carbons. The 27 carbons, five methyl protons, and an oxygenated methine proton are characteristic of a cholesterol carbon skeleton. Furthermore, ^1H NMR signals of an olefinic proton δ 6.49 (s, 1H), oxymethylene protons [δ 3.70 (m, 1H), δ 3.84 (m, 1H)], a downfield proton [δ 3.52 (dd, 1H, J = 11.0, 8.5 Hz)], and five methyls [δ 0.70 (s, 3H), δ 1.23 (s, 3H), δ 0.97 (d, 3H, J = 6.6 Hz), δ 0.88 (d, 3H, J = 6.6 Hz), δ 0.86 (d, 3H, J = 6.6 Hz)] suggested that **1** was a 9,11-secosterol.

Interpretation of 2D NMR spectroscopic data permitted the structure assignment of **1**. Analysis of COSY spectroscopic data of **1** revealed three fragments (a, b, and c) as shown in Figure 2. In addition, the carbon chemical shifts of C-6 (δ 197.5), C-7 (δ 134.4), C-8 (δ 152.1), and C-9 (δ 203.1), and the HMBC correlations from an olefinic proton H-7 to carbons C-6, C-8, and C-9 supported the construction of an ene-dione moiety.

The connectivity of A/B ring including the fragment a and the ene-dione moiety for **1** was secured from HMBC correlations. The long-range HMBC correlations from H-19 to carbons C-1, C-5, C-9, and C-10, and from H-4 to carbons

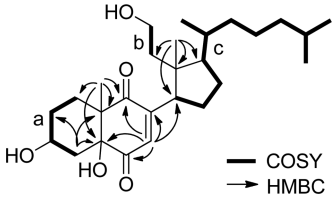

Figure 2. COSY and key HMBC correlations of **1**.

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C-2, C-5, and C-10, and from H-7 to the carbon C-5 allowed the A/B ring connectivity. The fragments b and c were also connected from the interpretation of HMBC correlations. A two-bond HMBC correlation from a methyl singlet proton H-18 to a carbon C-13, and three-bond HMBC correlations from H-18 to carbons C-12, C-17 permitted the C-12/C-13/C-17 connectivity. Lastly, the establishment of C-8/C-14 attachment based on the interpretation of three-bond HMBC correlations from the olefinic proton H-7 to a carbon C-14, and from the methyl singlet proton H-18 to a carbon C-14 allowed the completion of structure assignment of **1**.

The relative stereochemistry of the side chain and rings of **1** was identical to that of reported secosterols, which was determined by comparison with NMR data of known secosterols and by interpretation of NOESY correlations.[25,26] Briefly, NOESY correlations [H-7/H-14, H-14/H-12, H-12/H-21, H-18/H-20] were well corresponded to previously reported NOE correlations.[27] The β-configuration of 3-hydroxy group at C-3 was defined from the coupling constants of H-4α (δ 2.16, dd, J = 11.2, 3.3 Hz) and NOESY correlations [H3/H4α, H4β (δ 1.77)/H19].

Table 1. NMR spectroscopic data of **1**[a] ($CDCl_3$)

No.	δ_C, m[b]	δ_H, m, J (Hz)	COSY	HMBC (10 Hz)
1	25.9, t	1.77 m 2.21 dt (11.2, 3.7)	2	3, 5, 10, 19
2	29.8, t	1.55 m 2.00 m	1, 3	
3	66.6, d	4.04 m	2, 4	
4	35.6, t	1.77 dd (11.2, 11.1) 2.16 dd (11.2, 3.3)	3	2, 5, 10
5	80.5, s			
6	197.5, s			
7	134.4, d	6.49, s		5, 6, 8, 9, 14
8	152.1, s			
9	203.1, s			
10	52.1, s			
11	59.9, t	3.70 m 3.84 m	12	
12	41.1, t	1.11 m 1.73 m	11	
13	47.5, s			
14	43.9, d	3.52 dd (11.0, 8.5)	15	7, 8, 9, 12, 13, 15, 18
15	26.3, t	1.75 m 1.84 m	14, 16	
16	26.6, t	1.68 m 1.75 m	15, 17	
17	50.1, d	1.73 m	16, 20	
18	17.7, q	0.70 s		12, 13, 14, 17
19	20.6, q	1.23 s		1, 5, 9, 10
20	34.5, d	1.41 m	17, 21, 22	
21	18.8, q	0.97 d (6.6)	20	17, 20, 22
22	35.6, t	0.99 m 1.35 m	20, 23	24
23	24.4, t	1.15 m 1.35 m	22, 24	
24	39.4, t	1.13 m 1.15 m	23, 25	
25	27.9, d	1.51 m	24, 26, 2723	
26	22.5, q	0.86 d (6.6)	25	24, 25, 27
27	22.7, q	0.88 d (6.6)	25	24, 25, 26
5-OH		2.36 br s		

[a]600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR. [b]Multiplicity was determined by the analysis of 2D NMR spectroscopic data.

Table 2. Antibacterial activity of **1**

Strain	**1**[a] (IC_{50}, μg/mL)	Gentamicin
S. epidermidis ATCC 12228	25	0.2
Micrococcus lutes ATCC 9341	3.1	3.1
Bacillus subtilis ATCC 6633	25	0.2
Staphylococcus aureus ATCC 65381	> 200	0.2
Escherichia coli ATCC 11775	> 200	0.8
Salmonella typhimurium ATCC 14028	> 200	1.6
Klebsiella pneumonia ATCC 4352	> 200	0.8

[a]Each experiment was repeated more than three times.

Compound **1** was evaluated for antibacterial activity against seven pathogenic strains (Table 2). Compound **1** displayed the most potent activity on *Micrococcus lutes* ATCC 9341 and also showed the moderate activity against *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* ATCC 6633 with IC_{50} values of 3.1, 25 and 25 μg/mL, respectively. Meanwhile, **1** did not show any activity against gram negative strains include *Escherichia coli* ATCC 11775 *Salmonella typhimurium* ATCC 14028 and *Klebsiella pneumonia* ATCC 4352 up to 200 μg/mL. Interestingly, growth of one of the gram positive strain *Staphylococcus aureus* ATCC 65381 was not inhibited by **1** up to 200 μg/mL.

In conclusion, a new 9,11-Secosterol (**1**) with the 2-ene-1,4-dione moiety was isolated from the genus *Ircinia* and this compound displayed the most potent activity against *Micrococcus lutes* ATCC 9341 with the IC_{50} value of 3.1 μg/mL.

Experimental

General Experimental Procedures. The optical rotation was measured using a Rudolph Research Autopol III polarimeter with a 5 cm cell. The UV spectrum was recorded in a Scinco UVS-2100 with a path length of 1 cm. Infrared spectra were recorded on a Thermo Electron Corporation spectrometer. NMR spectral spectroscopic data were obtained using Bruker Avance 600 MHz spectrometer [$CDCl_3$ (δ_H 7.26; δ_C 77.0) was used as an internal standard]. HRFAB-MS data were measured on a JEOL, JMS-AX505WA mass spectrometer.

Isolation of Compound 1. The genus *Ircinia* sponge was collected by SCUBA at Yeongdeok-Gun in the East Sea. The wet animal (3 kg) was extracted three times with 50% methanol (MeOH) in dichloromethane. These extracts were concentrated and partitioned three times between hexanes and MeOH. Then the MeOH-soluble layer was partitioned three times between ethylacetate (EtOAc) and water. The

water-soluble fraction was further extracted thrice with *n*-butanol. The EtOAc-soluble layer (7.0 g) was subjected to silica flash column chromatography using step-gradient elution of EtOAc in hexanes (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%) to afford seven fractions (Fr 1-Fr 11). Fr 5 (68.3 mg), which contained the mixture of **1**, was further purified by reversed-phase HPLC (Polar-RP, 250 × 10 mm, 5 μm, 80 Å, 2.5 mL/min, UV detection = 210 nm), eluting with 70% acetonitrile in H_2O to afford compound **1** (2.7 mg), as colorless needles.

Compound 1: Colorless needles; $[\alpha]_D^{21} = -6.5^o$ (0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 274 (3.96) nm; IR (film) ν_{max} 3422, 2951, 2851, 1718, 1681, 1464, 1633 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; LRFABMS *m/z* 449 $[M+H]^+$; HRFABMS *m/z* 449.3271 $[M+H]^+$ (calcd for $C_{27}H_{45}O_5$, 449.3275).

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Supporting Information. 2D NMR spectroscopic data of **1** were available in the supporting information.

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