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Two New Scalaranes from a Korean Marine Sponge *Spongia* sp.

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Abstract – Intensive chemical investigation of Korean marine sponge *Spongia* sp. has led to the isolation of two new scalaranes. The planar structures of the new compounds **1** and **2** were determined through 1D and 2D NMR spectral data analysis, while the relative stereochemistry of the compounds was determined based on the analysis of ^{1}H-^{1}H coupling constants and NOESY spectroscopic data. Compounds **1** and **2** did not display any significant biological activities on farnesoid X-activated receptor (FXR) in co-transfection assay.
Keywords – Scalarane, Sesterpenoid, *Spongia* sp., Korean sponge, Marine natural product

Introduction

Marine sponge is well known as a rich source of natural products with diverse chemical skeletons.[1] Sesterterpene is a well-known chemical skeleton with the five carbon isoprene building units and has been isolated from diverse organisms including higher plants and fungus.[2] Scalarane, belonging to the sesterterpene class of natural products, is one of the marine exclusive chemical classes mainly isolated from sponges and nudibranches.[3] These scalaranes have not been isolated from terrestrial organisms. After the first report on this class of compound in 1972,[4] more than 200 derivatives were reported until 2010[3] and novel derivatives of scalaranes are still being reported.[5] Early research revealed that these natural products possessed antifeedant and antifouling activities,[6,7] as well as other variety of bioactivities had been reported such as cytotoxicity,[8] anticancer,[9] antibacterial,[10] and anti-inflammatory.[11] Biological activities of scalaranes were broadened to include the inhibition of nuclear factor,[12] protein tyrosine phosphatase,[13] and farnesoid X-activated receptor.[14] Recent efforts to discover novel marine natural products from Korean sponges resulted in the isolation of new analogs of scalarane compounds,[15] also our research group has reported several scalaranes with FXR and cytotoxic activities from two Korean sponge samples, *Spongia* and *Psammocinia* sp. repectively.[16] We further investigated chemical components to discover new natural products from *Spongia* sp., and have isolated two new scalaranes. Herein, we describe the isolation and the structure elucidation of two new scalarenes **1** and **2** from *Spongia* sp.

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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 and 500 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 – The genus *Spongia* sp. sponge was collected by SCUBA at the Geoje Island, South Sea of Korea. The frozen animal (3.4 kg, wet wt.) was lyophilized and the dried specimen (600 g) was extracted with 50% MeOH in CH_2Cl_2. The extract was partitioned between CH_2Cl_2 and water layers. The CH_2Cl_2-soluble layer was evaporated *in vacuo* and then partitioned between *n*-hexane and 90% aqueous MeOH. The 90% aqueous MeOH layer was subsequently separated into 24 fractions with Sephadex LH-20 column eluting with 50% MeOH in CH_2Cl_2. The fraction 20 containing the mixture of **1** and **2** was further

separated by reversed-phase HPLC (Phenomenex Luna C-18(2), 250 × 100 mm, 2.5 mL/min, 5 μm, 100 Å, UV = 205 nm) eluting with 70% CH_3CN in H_2O.

Compound 1: colorless oil; $[\alpha]_D^{21}$ −1° (0.002, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.98) nm; IR (film) ν_{max} 3393, 1761, 1682, 1236 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; LRFABMS *m/z* 401 $[M+H]^+$; HRFABMS *m/z* 401.3052 $[M+H]^+$ (calcd for $C_{26}H_{41}O_3$, 401.3056).

Compound 2: colorless oils; $[\alpha]_D^{21}$ +8° (0.002, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 210 (3.96) nm; IR (film) ν_{max} 3392, 1760, 1683, 1238 cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 1; LRFABMS *m/z* 417 $[M+H]^+$; HRFABMS *m/z* 417.3017 $[M+H]^+$ (calcd for $C_{26}H_{41}O_4$, 417.3005).

Results and Discussion

The molecular formula of compound **1** was established as $C_{26}H_{40}O_3$ based on the analysis of HRFABMS data (a

Table 1. ^1H and ^{13}C NMR data of **1** and **2** in $CDCl_3$.

	1[a]				**2**[b]	
No.	δ_C, m[c]	δ_H, m, *J* (Hz)	COSY	HMBC	δ_C, m[c]	δ_H, m, *J* (Hz)
1	40.1, CH_2	0.79, m	2	3, 23	40.4, CH_2	0.76, m
		1.67, m				1.67, m
2	18.8, CH_2	1.51, m	1, 3		18.7, CH_2	1.38, m
		1.55, m				1.48, m
3	42.2, CH_2	1.13, dt (9.4, 3.7)	2	5	42.2, CH_2	1.15, m
		1.39, m				1.38, m
4	33.5, C				33.4, C	
5	56.6, CH	0.78, m			56.5, CH	0.76, m
6	18.2, CH_2	1.40, m			18.2, CH_2	1.33, m
		1.51, m				1.48, m
7	41.8, CH_2	0.96, dt (13.2, 3.5)		5, 9, 24	41.8, CH_2	0.89, m
		1.69, d (10.2)				1.69, m
8	37.8, C				37.3, C	
9	61.4, CH	0.85, m			58.5, CH	0.83, m
10	37.8, C				37.3, C	
11	17.3, CH_2	1.42, m		8	26.9, CH_2	1.43, m
		1.55, m				1.69, m
12	40.7, CH_2	1.40, m		9, 18, 25	76.2, CH	3.56, dd (11.8, 4.2)
		1.78, d (9.8)				
13	37.8, C				44.0, C	
14	54.7, CH	1.30, m	15	24, 25	47.7, CH	1.33, m
15	24.2, CH_2	2.07, m	14, 16	16, 17	25.9, CH_2	2.21, d (11.4)
		2.31, dd (20.4, 4.0)				2.36, dt (20.2, 5.2)
16	136.6, CH	6.84, d (3.3)	15, 18	18, 20	143.1, CH	7.28, m
17	127.0, C				125.8, C	
18	57.9, CH	2.47, m	19		57.8, CH	3.58, s
19	105.6, CH	5.20, d (5.8)	18	19-OMe	204.0, CH	9.86, d (3.6)
20	171.0, C				167.0, C	
21	21.5, CH_3	0.78, s		22	21.4, CH_3	0.77, s
22	33.5, CH_3	0.84, s		21	33.4, CH_3	0.84, s
23	16.5, CH_3	0.84, s			17.0, CH_3	0.88, s
24	16.6, CH_3	0.91, s			16.8, CH_3	0.89, s
25	15.5, CH_3	0.76, s			16.0, CH_3	0.90, s
19-OMe	57.9, CH_3	3.56, s				
20-OMe					52.1, CH_3	3.70, s

[a]600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR. [b]500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR [c]Multiplicity was determined by the analysis of 2D NMR spectroscopic data.

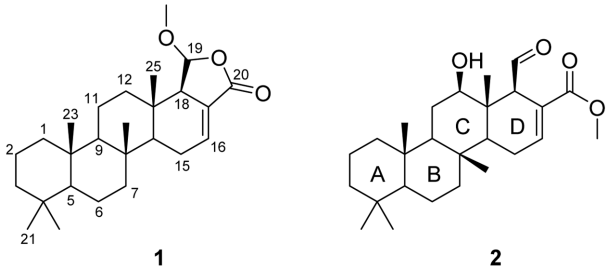

Fig. 1. Chemical structures of **1** and **2**.

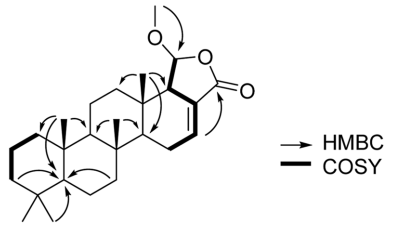

Fig. 2. Key COSY and HMBC correlations of **1**.

pseudomolecular ion peak at *m/z* 401.3052 $[M + H]^+$) and on the interpretation of ^{13}C NMR data. This molecular formula indicated that **1** had seven degrees of unsaturation. The ^1H NMR spectrum of **1** revealed the presence of two downfield methine protons H-16 (δ 6.84, d, $J = 3.3$ Hz), H-19 (δ 5.20, d, $J = 5.8$ Hz), one methoxy group at 19-OMe (δ 3.56), and five methyl groups with all singlets [H-21 (δ 0.78), H-22 (δ 0.84), H-23 (δ 0.84), H-24 (δ 0.91), H-25 (δ 0.76)]. The ^1H and ^{13}C NMR spectroscopic data indicated the presence of carboxylic carbon [C-20 (δ 171.0)] and a tri-substituted double bond system [H-16 (δ 6.84), C-17 (δ 127.0) and C-16 (136.6)]. The α,β-unsaturated-γ-lactone ring was constructed by the analysis of long-range HMBC correlations. HMBC correlations from H-16 (δ 6.84) to carbons C-18 (δ 57.9), and C-20 (δ 171.0), and from H-19 (δ 5.20) to a carbon C-20 (δ 171.0) allowed the construction of the α,β-unsaturated-γ-lactone ring moiety. Further interpretation of 2D NMR spectroscopic data revealed that **1** was based on a scalarane skeleton, having a methoxy group at C-19 (Fig. 1). In particular, HMBC correlations from five methyl singlets to carbons (H-22/C-5, H-23/C-1, C-5, C-9; H-24/C-9, C-14; H-25/C-12, C-14, C-18) were helpful to assign the tetracyclic ring system of the scalarane skeleton (Fig. 2). The HMBC correlations to C-5 (from H-3, H-7, H-21, and H-23) and C-9 (from H-7, H-23, and H-24) in combination with the interpretation of COSY cross peaks of H-1 and H-3 constructed A and B ring of scalarane. The presence of C and D rings was supported by HMBC correlations from the methyl singlet H-24 to carbons C-9, and C-14) and from the methyl singlet H-25 to carbons C-12 and C-14, and by the COSY correlations between H-15 and H-16, and between H-18 and H-19, respectively. Lastly, tetrahydrofuran ring was established from HMBC correlations from H-25 to C-18, from H-16 to C-20. The interpretation of ^1H-^1H coupling constants and NOESY correlations was deployed to determine the relative stereochemistry of compound **1**. NOESY cross-peaks between H-22 and H-23, H-23 and H-24 implied the axial orientations of C-22, C-23, and C-24. Finally, the α-configuration of the methoxy group at C-19 was determined by correlations between H-19 and H-25 in NOESY spectra.

The molecular formula of compound **2** was established as $C_{26}H_{40}O_4$ based on the analysis of HRFABMS data (a pseudomolecular ion peak at *m/z* 417.3017 $[M + H]^+$) and on the interpretation of ^{13}C NMR data. The ^1H NMR spectra of the compound **2** were similar to those of compound **1** except for the presence of aldehyde [H-19 (δ 9.86)] and oxygenated methine protons [H-12 (δ 3.56)]. The aldehyde group was positioned at C-18 based on HMBC correlations from H-19 (δ_H 9.86) to carbons C-13 (δ_C 44.0), C-17 (δ_C 125.8), and C-18 (δ_C 57.8). The position of a methyl ester at C-15 was also determined by HMBC correlations of H-16 (δ_H 7.28) and H-20-OMe (δ_H 3.70) to C-20 (δ_C 167.0). Analysis of 2D NMR spectroscopic data allowed the planar structure of **2** to be established as shown in Fig. 1. The coupling constant of H-12 ($J = 11.8$ Hz) indicated the axial oriented to the plane, suggesting the β-orientation of the hydroxy group at C-12.

The FXR antagonistic effect and cytotoxicity of compounds **1** and **2** were tested with published method,[16a] but no significant antagonistic or cytotoxicity activities were observed. Previously reported scalaranes with the FXR antagonistic activity have hydroxyl/methoxy functional group at C-12 and possess the substituted furan moiety in the molecules.[16a,b] The presence of hydroxyl group at C-12 and the furan ring in scalaranes could be crucial for FXR inhibitory activity. The common functional group with cytotoxic scalaranes is the oxygen atom at C-23[16c], however compound **1** and **2** possess a carbon atom at that position. These structural differences may explain the no bioactivities of the isolated compounds.

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