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Scalaran-type sesterterpenes from a Marine Sponge *Smenospongia* species showing the AMPK activation

Buyng Su Hwang and Jung-Rae Rho*

Department of Oceanography, Kunsan National University, Kunsan 573-701, Korea
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Abstract : A new scalaran class sesterterpenoid with five known ones was isolated from a marine sponge *Smenospongia* species collected from the Gageo island, Korea. Chemical structure of all of compounds was determined on the basis of a combination of extensive 1D and 2D NMR experiments and MS data. The new compound exhibited a new functional group on a common scalaran sesterterpene skeleton, identified as 12-deacetoxy-23-deacetoxyscalarin. The compound **1** moderately showed the effect of the activation of AMP-activated protein kinase (AMPK) in L6 myoblast cell.

Keywords: 1D and 2D NMR, 12-deacetoxy-23-deacetoxyscalarin, sponge *Smenospongia* sp., AMP-activated protein kinase (AMPK)

INTRODUCTION

Scalaran sesterterpenes are one of the most frequently encountered metabolites in sponges of the order Dictyoceratides. Moreover, these compounds showed a wide range of biological activities including cytotoxic, antimicrobial, antifeedant, platelet-aggregation inhibitory, and anti-inflammatory properties.[1-6] Recently, In an effort of searching for candidates treating the metabolic syndrome, we tested the effect of the activation of AMP-activated protein kinase (AMPK) in L6 myoblast cell on

Department of Oceanography, Kunsan National University, Miryong-dong, Gusan, Jeonbuk 573-701, Korea
Tel: + 82.63.469.4606 Fax: +82.63.469.4990 email: jrrho@kunsan.ac.kr
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the extracts from Korean marine sponges. AMPK is a key sensor and regulator in the cellular energy metabolic system. The activated AMPK stimulates downstream pathways which increase energy production (glucose transport, fatty acid oxidation) and switched off pathways which consume energy (lipogenesis, gluconeogenesis). Accordingly, AMPK has been an interesting molecular target to develop a drug for curing metabolic diseases.[7, 8] We found that acetoxyscalarin compounds isolated from the sponge *Smenospongia* sp. activated AMPK enzyme in L6 myoblast cell.

The sponge was extracted twice with methanol at room temperature. Bioactivity-screened fractionation yielded six scalaran type sesterterpenes including a new derivative (**1**). Their chemical structures were completely determined by a combination of extensive NMR techniques and mass spectrometry. In this paper, we will present the structure determination of **1** and the effect of AMPK activation on isolated compounds using Western Blot analysis.

EXPERIMENTAL

Extraction and Isolation

The marine sponge *Smenospongia* sp. (00G-19) was collected from Gageo Island, South Korea and extracted twice with MeOH at room temperature. The methanolic extract was partitioned between CH_2Cl_2 and H_2O solvents and then the organic layer repartitioned between *n*-hexane and

15% aqueous MeOH for defatting. The MeOH fraction was performed on the vacuum column chromatography eluting with seven stepped solvent mixtures of MeOH and water. Among them, the fraction of 100% MeOH and 10% aqueous MeOH solvent showed moderate AMPK activation effect. The active 10% aqueous MeOH fraction (250 mg) was separated by reversed phase HPLC (YMC ODS-A column, 250 mm × 10 mm, Varian RI detector) using a solvent system (H_2O / MeOH = 17 / 83) to yield compound **1** and **2**. Similarly, another fraction (700 mg) afforded compound **3**, **4**, **5**, and **6** under the solvent condition (H_2O / MeOH = 12 / 88).

NMR experiment

The 1D and 2D NMR spectra were obtained on a Varian NMR system working at 500MHz for proton and 125 MHz for carbon. The ^{1}H and ^{13}C NMR chemical shifts refer to CD_3OD at 3.30 and 49.0 ppm, respectively. For all experiments, the temperature was stabilized at 297 K. The parameters used for 2D NMR spectra were as follows; a gradient COSY spectrum was collected with a spectral width 2800 Hz in a 512 (t1) × 1024 (t2) matrix applying the pulse gradient of 1 ms duration with a strength 10 G/m and processed with a sinebell function. The gradient HSQC and HMBC spectra were measured with J_{CH} = 140 Hz and $^{n}J_{CH}$ = 7 Hz, respectively, and processed in a 256 (t1) × 1024 (t2) matrix by a linear prediction method for a higher resolution.

Assay of AMPK activation

Sample preparation : For AMPK phosphorylation assay, L6 myoblast cells were plated 24 h in advance in 6-well plates at 3×10^5 cells/well in 2 ml of DMEM culture medium.

Western blot analysis: L6 cells were harvested, pelleted, washed twice with 2 ml of PBS, and then lysed by adding SDS sample buffer (62.5 mM Tris-HCl[pH 6.8], 6% [wt/vol] SDS, 30% glycerol, 125 mM dithiothreitol [DTT], 0.03% [wt/vol] bromophenol blue). Total cell lysates were denatured by boiling for 5 min, and then electrophoresed in SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were blocked in TBS containing 5% (wt/vol) skim milk and 0.1% Tween 20 for 1 h at room temperature. Phosphorylation of AMPKα was determined with anti-phospho-AMPKα(Thr172) antibody (1:1000, cell signaling). Anti-AMPKα antibody was used as a loading control.

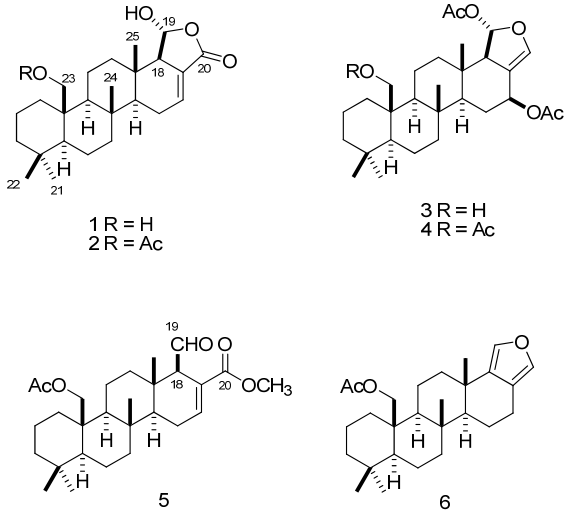


Figure 1. Six compounds isolated from the marine sponge *Smenospongia* sp.

RESULTS AND DISCUSSION

The 10% aqueous MeOH and 100% MeOH fractions of the extract were separated by reversed phase HPLC to yield six scalaran-type sesterterpenes including a new derivative. Combined spectroscopic analysis identified the structures of five known compounds **2**-**6**: 12-deacetoxy-23-acetoxyscalarin (**2**)[9], 12-deacetoxy-23-hydroxyheteronemin (**3**)[9,10], 12-deacetoxy-23-acetoxyheteronemin (**4**)[10], 12-deacetoxy-23-acetoxy-20-methoxyscaladial (**5**)[10], and 12-deacetoxy-23-acetoxyscalarafuran (**6**)[10]. The spectroscopic data were in good agreement with those reported previously.

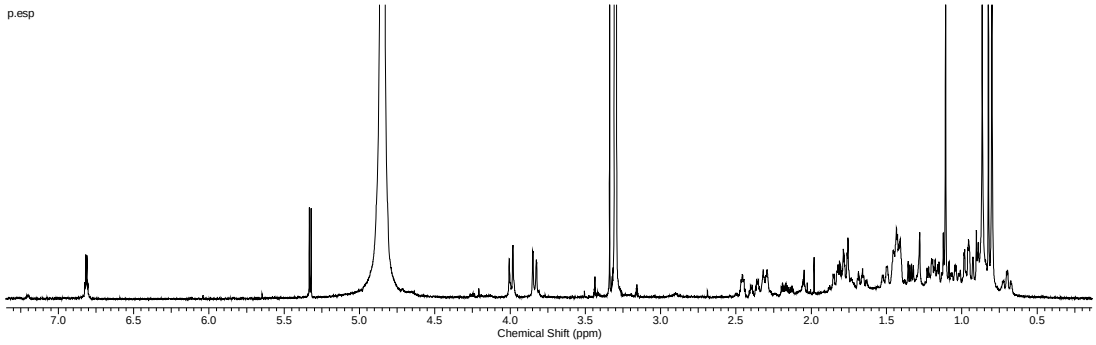
Compound **1** was isolated as an amorphous white solid and deduced to be $C_{25}H_{38}O_4$ on the basis of the ^{13}C NMR data and HRFABMS with the ion peak of $[M + H]^+$ at *m*/*z* 403.2854 (Δ +0.6 mmu), consistent with seven degrees of unsaturation. The ^1H NMR spectrum showed signals for one oxymethylene unit at δ_H 3.89 and 3.99 (C-23), one olefinic proton at δ_H 6.81 (C-16), and four methyl singlets at δ_H 0.86 (C-21), 0.80 (C-22), 1.11 (C-24), and 0.82 (C-25) [Fig. 2 (a)]. The ^{13}C and HSQC-DEPT NMR spectra indicated that **1** was composed of nine methylenes, six methines, and six quaternary carbons including one carbonyl (δ_C 169.8) along with four methyls [Fig. 2(b)]. The carbonyl and two olefinic carbons (δ_C 128.7 and 138.1) formed an α, β-unsaturated moiety, which was supported by a characteristic UV absorption band at 210 nm (log ε 3.7) and the absorption band

at 1739 cm^{-1} in the IR spectrum. This information induced that **1** possessed five rings from the remaining five degrees of unsaturation.

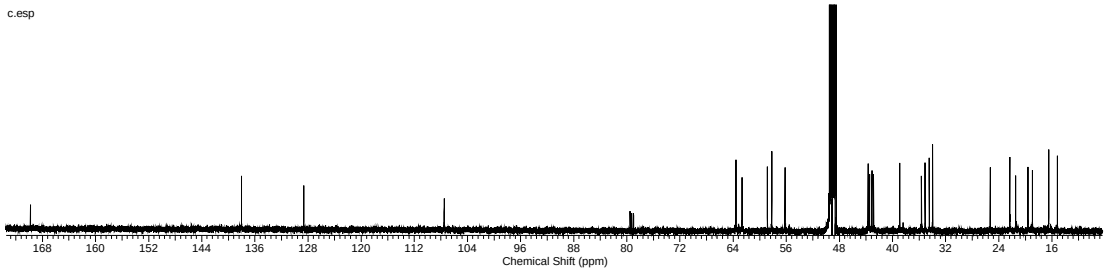
Detailed interpretation of HSQC, COSY, TOCSY and HMBC spectra led to a scalaran-type skeleton consisting of four rings A-D with an aid of the intensive HMBC correlations of four methyl singlet protons with their neighboring carbons (Table 1). An acetal group was defined by characteristic chemical shifts of the proton and carbon (δ_H 5.33, δ_C 107.5). The doublet acetal proton was coupled to the methine proton at δ_H 5.33 (H-18) in the COSY spectrum, and also showed the long range correlations with the carbonyl carbon at δ_C 169.8 and the quaternary carbon at δ_C 35.1(C-13) in the HMBC spectrum. This implied that γ-hydroxy five-membered lactone was fused to ring D at C-17 and 18. The remaining oxymethylene group was located at the C-23 of the ring A/B juncture, which was apparent from the long range correlations with carbon resonances at δ 35.7 (C-1), 63.6 (C-9) and 43.7 (C-10) (Fig. 3). Furthermore, the upfield shifts of the H-23 oxymethylene protons were assessed from the hydroxy group at the C-23 position, by comparison with the acetyl group, indicating that **1** was 23-hydroxy-scalarin. Thus, the gross structure of compound **1** could be determined. The structure of **1** was similar to that of 12-deacetoxy-23-acetoxyscalarin (**2**), except for the absence of an acetyl group in **1**, implying that **1** was the deacetyl derivative of **2**.

The relative stereochemistry at the asymmetric carbon centers of **1** was determined by the ROESY experiment. The junctions of rings A-D were determined as *trans* orientation on the basis of a series of ROESY cross-peaks: H-22/H-23, H-23/H-24, H-24/H-25, and H-25/H-19 (Fig. 3). H-18

was assigned as α orientation, an opposite direction to H-19, because the NOE cross peak was observed between H-18 and H-14. Therefore, the structure of compound **1** was established to be 12-deacetoxy-23-deacetoxyscalarin.



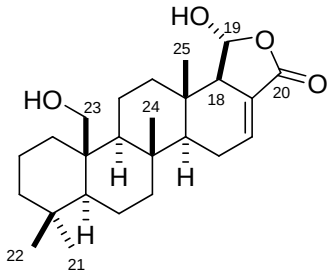
(a)



(b)

Figure 2. (a) Proton and (b) carbon NMR spectra of **1**.

Table 1. NMR spectral data for compound **1** in CD_3OD (500 MHz for ^1H)



No	d_c, mult	d_H (*J* in Hz)	HMBC
1	35.7, CH_2	2.31, br d (13.2)	C-2
		0.70, ddd (13.2, 13.2, 2.7)	
2	19.6, CH_2	1.66, m	
		1.45, m	
3	43.1, CH_2	1.42, m	C-1, 4
		1.21, dd (13.7, 4.9)	
4	34.0, C		
5	58.2, CH	0.96, dd (12.5, 2.2)	C-7, 9
6	19.0, CH_2	1.51, br d (12.5)	
		1.42, m	
7	43.5, CH_2	1.80, m	C-8, 14
		1.04, ddd (12.5, 12.5, 4.4)	
8	38.9, C		
9	63.6, CH	0.93 br d (12.2)	C-8
10	43.7, C		
11	21.5, CH_2	1.82, m	
		1.76, m	
12	42.9, CH_2	1.77, br d (12.2)	C-11
		1.17, dd (12.2, 3.7)	
13	35.1, C		
14	56.2, CH	1.34, dd (11.3, 5.6)	C-8,15,16,18,24,25
15	25.3, CH_2	2.35, dddd (20.5, 5.6, 3.8, 3.8)	
		2.15, dddd (20.5, 11.3, 3.8, 3.8)	
16	138.1, CH	6.81, ddd (7.1, 3.8, 3.8)	
17	128.7, C		
18	58.8, CH	2.45, m	
19	107.5, CH	5.33, d (5.9)	C-13, 18, 20
20	169.8, C		
21	34.5, CH_3	0.86, s	C-3, 4, 5, 22
22	22.4, CH_3	0.80, s	C-3, 4, 5, 21
23	62.7, CH_2	3.99, d (11.9), 3.84, d (11.9)	C-1, 9,10
24	16.5, CH_3	1.11, s	C-7, 8, 9, 14
25	15.2, CH_3	0.82, s	C-12,13,14,18

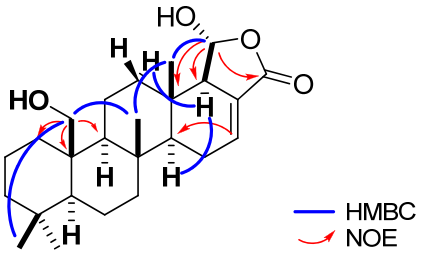


Figure 3. Key HMBC and NOE correlations of **1**.

We screened the activation effect on six compounds by monitoring the phosphorylated AMPK of antibody directly, Compound **5** showed dark band, which indicates the creation of phosphorylated AMPK compared with DMSO (control) at a concentration of 10mM as shown in Fig. 4.

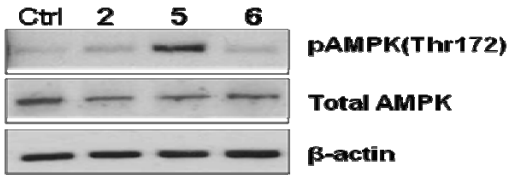


Figure 4. Western Blot analysis of total AMPK and the phosphate AMPK for compound **2**, **5**,and **6**.

Acknowledgement

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REFERENCES

1. R.A. Keyzers, M.T. Davies-Coleman, *Chem. Soc. Rev*. **34**, 355-365 (2005).

2. G.R. Pettit, R. Tan, Z.A Cichaz, *J. Nat. Prod*. **67**, 1767-1770 (2004).
3. G. Ryu, S. Matsunaga, N. Fuseyani, *J. Nat. Prod*. **59**, 515-517 (1998).
4. L.C. Chang, S. Otero-Quintero, G.M. Nicholas, C.A. Breley, *Tetrahedron* **57**, 5731-5738 (2001).
5. H. Kikuchi, Y. Tsukitani, I. Schimizu, M. Kobayashi, I. Kitagawa, *Chem. Pharm. Bull*, **31**, 552-556 (1983).
6. P. Crews, P. Bescansa, *J. Nat. Prod*. **49**, 1041-1052 (1986).
7. D.G. Hardie, S.A. Hawley, J.W. Scott, *The Journal of Physiology* **574**, 7-15 (2006).
8. C. Barbara, et al, *Cell Metabolism* **3**, 403-416 (2006).
9. J.-R. Rho, H.-S. Lee, H. J. Shin, J.-W. Ahn, J.-Y. Kim, C.-J. Sim, J. Shin, *J. Nat. Prod*. **67**, 1748-1751 (2004).
10. J. Song, W. Jeong, N. Wang, H-S. Lee, C-J. Sim, K-B. Oh, J. Shin, *J. Nat. Prod*. **71**, 1866-1871 (2008).