Journal of the Korean Magnetic Resonance Society **2008**, *12* 33-39

Sarcotrine G, a New Derivative Isolated from a Marine Sponge *Sarcotragus* Species

Buyng Su Hwang[1], Su Young Park[1], Myung Gil Park[2], and Jung-Rae Rho[1,*]

[1]Department of Oceanography, Kunsan National University, Kunsan 573-701, Korea
[2]Department of Oceanography, Chonnam National University, Gwangju 500-757, Korea
Received March 30, 2008

Abstract : Four sesterterpenoids were isolated from AMPK activity-guided fraction of marine sponge *Sarcotragus* species. Their planar structures were determined from combination of extensive 1D and 2D NMR experiments and MS data, and the configuration at the chiral centers were assigned by comparison with the ^{1}H and ^{13}C chemical shifts of the known compounds. Among four compounds, compound **2** was found to be a new sarcotrine derivative. Though not strong, compounds **1-4** moderately showed AMPK activation effect on L6 myoblast cell through Western Blot analysis.
Keywords : 1D and 2D NMR, Sarcotrine G, Sponge *Sarcotragus* sp., AMPK activation

INTRODUCTION

Marine sponges have been provided structurally noble and biologically active compounds for the last forty years. However, many researchers have mainly focused on biological activities including cytotoxic, antimicrobial, antifeedant, antidiabetic activity, and antiinflammatory properties.[1-4] Recently, for the treatment of metabolic syndrome like obesity and type 2 diabetes,[5, 6] we screened the activation effect on a AMP-activated protein kinase (AMPK), which is a key sensor and regulator in the cellular energy metabolic system. AMPK stimulates downstream pathways which increase energy production (glucose transport, fatty acid oxidation) and switched off pathways which consume energy (lipogenesis, gluconeogenesis).

* To whom correspondence should be addressed. E-mail : jrrho@kunsan.ac.kr

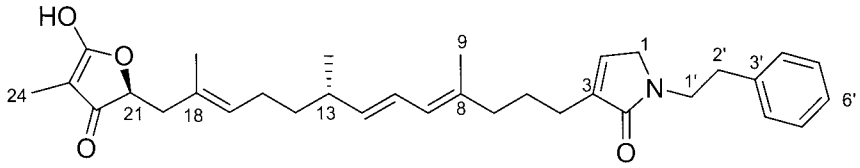
In the screening of AMP-activated protein kinase (AMPK) activator from the extracts of marine sponges with Western Blot analysis, we found moderate activity in the methanol fraction of a marine sponge *Sarcotragus* sp. collected in Jeju island. Bioassy-guided isolation afforded four sesterterpenes whose structures were elucidated by NMR spectroscopy and MS data. Based on the analysis of extensive 2D NMR data, one of these compounds were found to be a new derivative of sarcotrine, and other three compounds were confirmed by the NMR data reported in the literature.[7,8] The sesterterpenes with a tetronic acid have been frequently isolated from a marine sponge *Sarcotragus* sp.[9,10] Here, we describe isolation and structure elucidation of four sarcotrine derivation compounds **1-4**.

EXPERIMENTAL

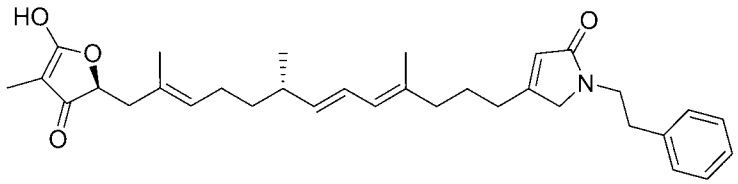
Extraction and Isolation

A marine sponge *Sarcotragus* sp. (sample no. 05J-18) was collected by hand using SCUBA at Jeju island in 2005. The freeze-dried specimen (0.5Kg) was extracted with MeOH twice at room temperature. The crude extract was first partitioned between H_2O and methylene chloride (MC), and then the aqueous layer re-partitioned between butanol and H_2O solvent. The butanolic phase was subsequently repartitioned with hexane and 15% aqueous MeOH to remove the fatty acids. The polar fraction was in turn subjected to reversed phase vacuum flash chromatography eluting with stepwise gradients of MeOH in H_2O (50%, 60%, 70%, 80%, 90%, 100%). Among them, the 100% MeOH fraction moderately showed the AMPK activation effect on L1 Myoblast cell with Western Blot analysis. For effective separation of active compounds, this fraction was partitioned into five subfractions (M1-M5) by using Sephadex LH20 open column chromatography. First, M4 fraction (20mg) was separated by reversed phase HPLC(YMC ODS-A column, 250mm × 10mm, Varian RI detector) using a solvent system (H_2O / MeOH = 15 / 85) to yield compounds **1 - 2** and **4**. Compound **1** (3.4mg), **2** (1.6mg) and **4** (8.3mg) were purified at the retention time of 37 min, 35 min, and 52 min, respectively. Second, M3 (43mg) fraction was separated in the same manner as M4 fraction to give compound **3** (2.3mg) at the retention time of 37 min.

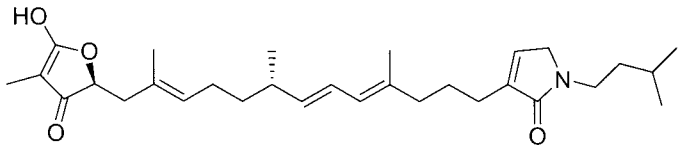
The 1D and 2D NMR spectra were obtained on a Varian NMR system working at 500MHz for proton and 125MHz for carbon. The ^{1}H and ^{13}C-NMR chemical shifts refer to CD_3OD at 3.30 and 49.0ppm, respectively. For all experiments, the temperature was stabilized at 297 K. The parameters used for 2D NMR spectra were as follows; The gradient COSY data were collected with a spectral width 2567 Hz in a 512 (t1) × 1024 (t2) matrix applying the pulse gradient of 1ms duration with a strength 10 G/m and processed with a sinebell function. The gradient HSQC spectra were measured in a 128 (t1) × 1024 (t2) matrix with J_{CH} = 140 Hz and processed in a 256(t1) × 1024(t2) matrix by a linear prediction method for a higher resolution. The gradient HMBC experiment was carried out with the optimized the long-range coupling constant of 7Hz.



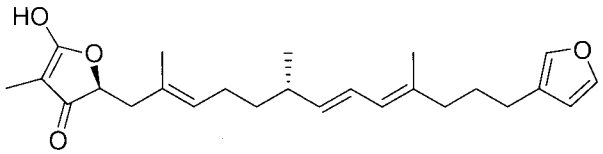
1



2



3



4

Fig. 1. Four compounds isolated from the marine sponge *Sarcotragus* sp.

RESULTS AND DISCUSSION

The 90% aqueous MeOH fraction of the extract was subjected on the LH20 open column chromatography to give five fractions (M1-M5). First, the M4 fraction was purified using ODS HPLC to afford compounds **1-3**. And the M3 fraction was separated with the same manner to give one compound **4**. Among the isolated four compounds, the compound **2** was found as a new derivative of sarcotrines.

Sarcotrine B (**1**) was isolated as a colorless oil and given as a molecular ion peak of *m/z* = 518 from the LRESIMS measurement. The ^{1}H and ^{13}C-NMR spectra measured in CD_3OD indicated four methyls (three singlets and one doublet) in the upfield region and the presence of a phenyl and olefinic groups in the downfield. Together with 1D NMR data, careful analysis of 2D NMR spectra showed that **1** contained a linear chain composed of a trisubstituted olefin and a 1, 1, 4-trisubstituted diene, and also a tetronic acid at one terminus of the chain which are characterized by typical chemical shifts (δ_H 4.68, H-20, δ_C 79.3, C-21; δ_C 179.2, C-22; δ_C 95.1, C-23; δ_H 1.62, H-24, δ_C 6.0, C-24; δ_C 180.3, C-25). Furthermore, the HMBC correlations from methylene protons at δ_H 2.19 to three carbons at δ_C 137.7, 140.2 and 173.8 revealed the linkage of α, β unsaturated–γ-lactam ring to the other end of the chain as shown by Fig. 2. Finally, based on the HMBC correlations of the methylene triplet at δ_H 3.69 to two carbons at δ_C 173.8 and 52.9, the connection of the unassigned phenyl group to the nitrogen of the lactam ring completed the planar structure of **1**. From a literature survey, this structure proved out to be known, and the ^{1}H and ^{13}C-NMR chemical shifts were very well consistent with those reported previously.[7] Also, this enabled us to deduce the configuration at C-13 and -21 to be *S* form for each center by comparison of the corresponding chemical shifts.

Sarcotrine G (**2**) was given as the same molecular weights as compound **1** and showed similar ^{1}H and ^{13}C-NMR spectra. However, one distinct difference in the ^{1}H-NMR spectra of two compounds was that the singlet proton at δ_H 6.75 in **1** shifted to the upfield of δ_H 5.78 in **2**. In the same way as **1**, the entire structure of **2** was established from a combination of 1D and 2D NMR experiments and the complete assignments was listed in Table 1. As shown in Table 1, the ^{1}H and ^{13}C-NMR chemical shifts of the linear chain and the tetronic acid moieties in **2** agreed very well with those of **1**, which also indicated the same

Table 1. NMR spectral data for compounds **1** and **2** in CD_3OD

position	δ_H (*J* in Hz, **1**)	δ_H (*J* in Hz, **2**)	δ_C, mult (**1**)	δ_C, mult (**2**)
1	3.78, brs		52.9, CH_2	163.6, qC
2	6.75, s	5.78, s	137.7, CH	121.7, CH
3			140.2, qC	136.0, qC
4		3.82, s	173.8, qC	56.6, CH_2
5	2.19, t (7.4)	2.31, t (7.3)	26.3, CH_2	29.7, CH_2
6	1.65, q (7.4)	1.65, q (7.3)	27.0, CH_2	27.0, CH_2
7	2.05, t (7.4)	2.04, t (7.3)	40.3, CH_2	40.1, CH_2
8			136.5, qC	136.0, qC
9	1.70, s	1.70, s	16.4, CH_3	16.4, CH_3
10	5.76, d (10.6)	5.75, d (10.8)	126.7, CH	126.9, CH
11	6.18, dd (15.1, 10.6)	6.18, dd (15.1, 10.4)	126.6, CH	126.5, CH
12	5.37, dd (8.2, 15.1)	5.39, dd (8.1, 15.0)	139.2, CH	139.5, CH
13	2.14, m	2.15, m	38.0, CH	38.0, CH
14	0.98, d (6.7)	0.99, d (6.6)	21.5, CH_3	21.5, CH_3
15	1.33, m	1.33, m	38.3, CH_2	38.2, CH_2
16	1.98, m	1.98, m	27.0, CH_2	27.0, CH_2
17	5.27, t (6.9)	5.29 t (6.9)	130.2, CH	130.3, CH
18			131.1, qC	130.8, qC
19	1.75, s	1.75, s	24.4, CH_3	24.4, CH_3
20	2.24, dd (8.8, 14.3)	2.26, dd (8.8, 14.3)	35.6, CH_2	35.5, CH_2
	2.60, dd (2.7, 14.3)	2.60, dd (3.1, 14.3)		
21	4.68, dd (2.7, 8.8)	4.72, dd (3.1, 8.8)	79.3, CH	79.0, CH
22			179.2, qC	178.7, qC
23			95.1, qC	95.8, qC
24	1.62, s	1.63 s	6.0, CH_3	6.0, CH_3
25			180.3, qC	178.6, qC
1′	3.69, t (7.0)	3.65, t (7.3)	45.2, CH_2	44.8, CH_2
2′	2.89, t (7.0)	2.88, t (7.3)	35.7, CH_2	35.7, CH_2
3′			140.2, qC	140.2, qC
4′, 8′	7.19, d (7.4)	7.20, d (7.4)	129.8, CH	129.8, CH
5′, 7′	7.25, dd (7.4, 7.2)	7.26, dd (7.4, 7.2)	129.6, CH	129.6, CH
6′	7.18, t (7.2)	7.18, t (7.2)	127.5, CH	127.5, CH

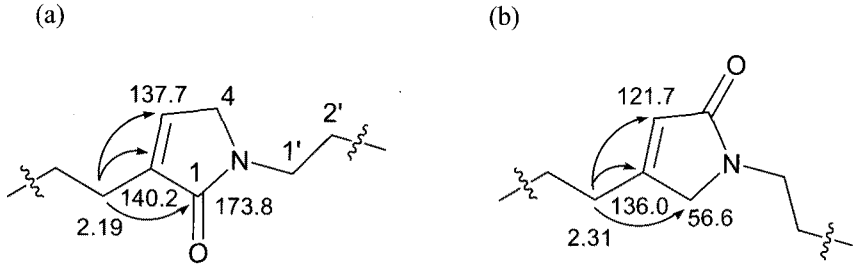


Fig. 2. Key HMBC correlations of (a) **1**, and (b) **2**

configuration at C-13 and C-21. However, the triplet protons (δ_H 2.31) at the other end of the chain correlated with two sp^2 carbons at δ_C 121.7 and 136.0, and one methylene carbon at δ_C 56.6 in the HMBC spectrum. This suggested the linkage of the chain to the position of β of the α, β-unsaturated-γ-lactam ring of **2**. Though sarcotrine G (**2**) is very similar to compound **1**, but the position of the carbonyl group in **2** was changed from C-1 to C-4. This structure was found as a new compound from a literature survey.

Sarcotrine C (**3**) was isolated from the fraction of M3 and given as a molecular ion peak *m/z* = 484 in the LRESIMS. Unlike compounds **1** and **2**, **3** showed a strong doublet methyl at δ_H 0.94, instead of the signals corresponding to phenyl group in the ^{1}H NMR spectrum. Starting from this signal, the sequential COSY correlations revealed the dimethyl propyl moiety, which is connected with the nitrogen atom in the α, β-unsaturated-γ-lactam ring on the basis of HMBC correlations between the triplet methylene and two carbons at δ_C 52.2 and 173.7. The determined planar structure of **3** was also revealed as the known compound.[7]

Sarcotin A (**4**) was isolated from the fraction of M4 with compounds **1** and **2**. The ^{1}H-NMR spectrum was characteristic of three singlet protons at δ_H 6.28, 7.24 and 7.37 in the downfield region, compared with those of compounds **1-3**. Along with this information, the corresponding ^{13}C chemical shifts at δ_C 111.9, 140.1, 143.9 and a quaternary carbon at δ_C 126.3 were reminiscent of a furan ring. The linkage of the furan ring with the main chain moiety was established by the HMBC correlation from the triplet methylene at δ_H 2.37 to three carbons at δ_C 111.0, 126.3 and 140.1. The completed structure of **4** was also reported in a literature.[8]

Unfortunately, compounds **1-4** did not exhibit a significant activation effect on AMPK, though they showed a weak effect to a concentration of 10 μM in a Western blot analysis. We are under collaboration for investigation into other biological activities.

Acknowledgement

This work was supported by the BK21 project of the Ministry of Education & Human Resources Development, Republic of Korea.

REFERENCES

1. R. G. Kerr, B. J. Baker, *Nat. Prod. Rep.*, **8**, 441-526 (1999)
2. N. Fusetani, H. Nagata, H. Hirota, T. Tsuyuki, *Tetrahedron Lett.*, **30**, 7079-7082 (1989)
3. S. Carmely, M. Loya, Y. Kashman, *J. Nat. Prod.*, **52**, 167-170 (1989)
4. J. W. Blunt, B. R. Copp, W-P. Hu, M. H. G. Munro, P. T. Northcote, M. R. Prinsep, *Nat. Prod. Rep.*, **25**, 135-194 (2008)
5. D. G. Hardie, S. A. Hawley, J. W. Scott, *J. Physio..*, **574**, 7-15 (2006
6. B. Cool, B. Zinker, W. Chiou, L. Kifle, N. Cao, M. Perham, R. Dickinson, A. Adler, G. Gagne, R. Iyengar, G. Zhao, K. Marsh, P. Kym, P. Jung, H. S. Camp, E. Frevert, *Cell Metabolism*, **3**, 403-416 (2006)
7. Y. Liu, J Hong, C-O Lee, K. S. Im, N. D. Kim, J. S. Choi, J. H. Jung, *J. Nat. Prod.*, **65**, 1307-1314 (2002)
8. Y. Liu, T. A. Mansoor, J Hong, C-O Lee, Chung Ja Sim, K. S. Im, N. D. Kim, J. H. Jung, *J. Nat. Prod.*, **66**, 1451-1456 (2003)
9. J. Shin, J-R. Rho, Y. Seo, H-S. Lee, K. W. Cho, C. J. Sim, *Tetrahedral Lett.*, **42**, 3005-3007 (2001)
10. J-R Rho, J. Shin, H-S. Lee, *J. Kor. Magn. Reson.*, **7**, 55-61 (2003)