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A New Sesterterpene from the Korean *Sarcotragus* sp. Sponge

Jung-Kyun Woo[1], Ju-eun Jeon[1], Bora Kim[1], Chung J. Sim[2], Dong-Chan Oh[1], Ki-Bong Oh[3,*], and Jongheon Shin[1,*]

[1]*Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Korea*
[2]*Department of Biological Science, College of Life Science and Nano Technology, Hannam University, Daejeon 305-811, Korea*
[3]*Department of Agricultural Biotechnology, College of Agriculture and Life Science, Seoul National University, Seoul 151-921, Korea*

Abstract – Sarcotragin C (**1**), a new sesterterpene metabolite was isolated from a *Sarcotragus* sp. sponge collected from Chuja Island, Korea. On the basis of the combined spectroscopic analyses, the structure of this compound was determined to be a linear norsesterterpene containing a leucine-derived γ-lactam moiety. This compound exhibited moderate cytotoxicity against K562 and A549 cell-lines.
Keywords – Sponge, *Sarcotragus* sp., Sarcotragin C, Norsesterterpene, Cytotoxicity

Introduction

Marine sponges of the family Irciniidae (class Demospongiae, order Dictyoceratida) are widely recognized to be the prolific sources of sesterterpenes. Among these, animals of the genus *Sarcotragus* are particularly rich with linear sesterterpenoids such as variabilins and sarcotragins.[1-5] Even in early days of marine natural products chemistry, these linear sesterterpenes, varying greatly in both their carbon frameworks and functionalities, significantly contributed to the chemosystematics of sponges.[9] During the course of chemical investigation of marine sponges of Korea, we encountered the specimens of *Sarcotragus* sp. from Chuja Island. Herein, we report the structure determination of sarcotragin C (**1**), a new norsesterterpene lactam, possessing moderate cytotoxicity against K562 and A549 cancer cell-lines.

Experimental

General experimental procedures – Optical rotations were measured on a JASCO P-1020 polarimeter using a 1-cm cell. UV spectra were recorded on a Hitachi U-3010 spectrophotometer. IR spectra were recorded on a JASCO 300E FT-IR spectrometer. Proton and carbon NMRs were measured at 500 and 125 MHz, respectively, using a Bruker Avance 500 spectrometer. Mass spectrometric data were obtained at the Korea Basic Science Institute (Seoul, Korea) and were acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol (NBA) as a matrix for the FABMS. HPLC was performed on a SpectraSystem p2000 equipped with a SpectraSystem RI-150 refractive index detector. All solvents were spectroscopic grade or distilled in a glass prior to use.

Aninal material – Specimens of *Sarcotragus* sp. (Voucher number 03CH-11) were collected by hand at −25 m using SCUBA equipment off the shore of Chuja-do, Korea, 2003. The specimens were readily identified by the presence of very fine collagenous filaments with beaded ends in the mesohyl which supplemented the fiber skeleton. It also possesses pithed and concentrically laminated, primary and secondary fibers. A voucher specimen is deposited (registry No. Por. 35) at the Natural Institute of Biological Resources, Korea.

Extraction and isolation – The collected specimens were immediately frozen and kept at −25 ºC until chemically investigated. The freeze-dried sponges (869.9 g) were macerated and repeatedly extracted with MeOH (2 L × 3) and CH_2Cl_2 (2 L × 3). The combined solvents were removed *in vacuo*. The extract (158.2 g) was partitioned between water (97.9 g) and *n*-butanol (52.8 g), and then the organic layer was dried and re-partitioned with 15% aq. MeOH (20.72 g) and *n*-hexane (30.96 g). An aliquot of the latter (5.45 g) was separated by C18 reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (elution

*Author for correspondence
Jongheon Shin, Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
Tel: +82-2-880-2484; E-mail: shinj@snu.ac.kr

Ki-Bong Oh, Department of Agricultural Biotechnology, College of Agricultural and Life, Seoul National University, Seoul 151-921, Korea
Tel: +82-2-880-4646; E-mail: ohkibong@snu.ac.kr

order: 50%, 40%, 30%, 20%, 10% aq. MeOH, MeOH, and acetone). The 10% aq. MeOH (1.50 g) fraction was separated by C18 reversed-phase HPLC (YMC ODS-A column, 250 × 10 mm, 15% aq. MeOH) to yield **1** as a yellowish gum. Final purification was accomplished by normal-phase HPLC (YMC-silica column, 250 × 10 mm, 25% EtOAc in *n*-hexane) to yield 4.6 mg of **1**.

Sarcotragin C (1) – yellowish gum. $[\alpha]_D^{25}$: +8.9 (*c* 0.67, MeOH); IR ($CHCl_3$) ν_{max} cm^{-1}: 3420 (br), 2927, 1729, 1669, 1457; UV (MeOH) λ_{max} (log ε) 238 (2.21) nm; ^1H, ^{13}C NMR, see Table 1; HRFABMS *m/z* 482.3249 $[M+Na]^+$ (calcd for $C_{28}H_{45}NO_4Na$, 482.3246).

Cytotoxicity assay – The cytotoxicity assays were performed in accordance with literature protocols.[10]

Result and Discussion

The structure of sarcotragin C (**1**) was determined by extensive spectroscopic analyses and literature study. The molecular formula of **1**, a yellowish gum, was deduced as $C_{28}H_{45}NO_4$ by HRFABMS analysis. The ^{13}C NMR spectra of this compound showed signals of twenty-eight carbons; 5 × C, 8 × CH, 9 × CH_2, and 6 × CH_3. The carbonyl signals at δ_c 175.0 and 172.4, aided by the mass data and IR absorption bands at 1729 and 1669 cm^{-1}, were interpreted as an ester and a lactam functionality, respectively (or *vice versa*). In addition, the presence of four double bonds were deduced from the signals of eight olefinic carbons at δ_c 139.2-125.3 (3 × C and 5 × CH). A methyl carbon at δ_c 69.8 was indicative of a methoxy group. The presence of five additional methyl signals in upfield regions in both ^{13}C and ^1H NMR data revealed the terpene nature of this compound.

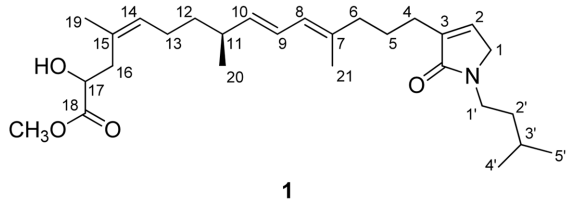
Given this information, the structure of compound **1** was determined by a combination of 2-D NMR experiments such as COSY, HMQC and HMBC. First, all of the protons and their attached carbons were precisely matched by HMQC data (Table 1). Subsequently five proton-proton spin systems were defined by COSY data. Of these, a two-proton spin system was found between an olefinic (δ_H 6.84, H-2) and a methylene proton (δ_H 3.91, H-1). The long-range HMBC correlations of these protons with neighboring carbons including a carbonyl carbon at δ_c 172.4 (C-22) indicated the presence of a five-membered ring system accommodating these functionalities as an α, β-unsaturated carbonyl group (Fig. 1). This interpretation of a cyclopentenone type moiety was supported by the small vicinal proton-proton coupling ($J_{1,2}$ vs) between the ring protons. Although the characteristic chemical shift of the C-1 methylene carbon at δ_c 50.9 suggested the attachment of a nitrogen inherent in the mass data, thus a lactam for this moiety, it was not conclusive at this stage and needed crucial evidence.

In the meanwhile, the combined 2-D NMR analyses readily defined an isopentyl group consisted of five upfield carbons (δ_c 40.5, 37.3, 25.8, 21.6 (x 2), C-1'-C-5')

Table 1. ^1H and ^{13}C NMR data of **1** in CD_3OD (δ in ppm, 500 MHz for ^1H and 125 MHz for ^{13}C)

Position	1	
	δ_H	δ_C
1	3.91 br s	50.9
2	6.84 br s	136.3
3		139.2
4	2.22 m	25.2
5	1.69 m	25.8
6	2.09 t (7.4)	39.2
7		135.3
8	5.79 d (10.7)	125.5
9	6.20 dd (15.1, 10.7)	125.3
10	5.40 dd (15.1, 8.2)	137.9
11	2.16 m	36.8
12	1.32 dt (8.0, 7.3)	37.2
13	1.98 dt (7.3, 7.1)	25.7
14	5.26 t (7.1)	128.5
15		130.5
16	2.45 dd (13.6, 5.6)	36.8
	2.38 dd (13.6, 8.1)	
17	4.24 dd (8.1, 5.6)	69.8
18		175.0
19	1.74 d (0.9)	22.8
20	1.00 d (6.7)	20.2
21	1.71 s	15.2
22		172.4
1'	3.49 t (7.4)	40.5
2'	1.48 dt (7.4, 6.2)	37.3
3'	1.56 m	25.8
4'	0.95 d (6.6)	21.6
5'	0.95 d (6.6)	21.6
OMe	3.70 s	51.1

[a] *J* values are in parentheses and reported in Hz; the assignments were based on ^1H-^1H COSY, HMQC, and HMBC experiments.



1

Fig. 1. The structure of compound **1**.

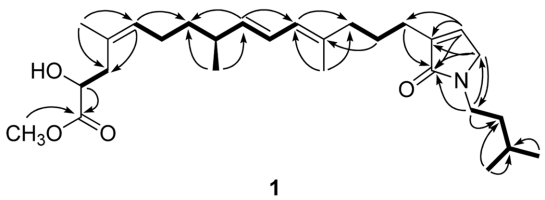


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Fig. 2. Key HMBC (H → C) and COSY (▬) correlations of **1**.

and their attached protons. The HMBC correlations between this alkyl group and the pre-described 5-membered ring at H-1/C-1', H-1'/C-1, and H-1'/C-22 secured not only the linkage between these but also the presence of a lactam moiety. Thus, a *N*-isopentyl-α, β-unsaturated-γ-lactam moiety was constructed.

The remaining part of **1** was also determined by similar combinations of 2-D NMR data including the key HMBC correlations between the upfield methyl protons at H-19, H-20, and H-21 with the neighboring carbons assigned by COSY and HMQC data (Fig. 2). In this way, a linear triprenyl moiety (C-4~C-16) was adequately deduced. The direct linkage between this and γ-lactam moiety was also established by the HMBC correlations at H-2/C-4 and H-4/C-22. Based upon the COSY data, the C-16 terminus of prenyl chain was directly connected to the C-17 oxymethine group that was further extended to a carbomethoxy group by the HMBC correlations at H-17/C-18 and OCH_3/C-18. Thus, the planar structure of **1** was defined to be a *N*-isopentyl-norsesterterpene-γ-lactam. Literature study revealed that **1** was structurally related to norsesterterpenes sarcotragins A and B from the Korean *Sarcotragus* sp. sponge.[3] Similar compounds were also reported from *Ircinia* spp. and *Sarcotragus* spp.[4-8] These compounds had structural similarity by their mixed biogenesis between a norsesterterpene and amino acid-derived moiety. However, the isopentyl amide moiety of **1**, possibly derived from a leucine, is unprecedented among these compounds.

Sarcotragin C (**1**) possessed three asymmetric double bonds and two asymmetric carbon centers. The geometries of three double bonds were determined as 7*E*, 9*E*, and 14*Z* on the basis of the proton-proton coupling constant ($J_{9,10}$ = 15.1 Hz) and carbon chemical shifts (C-19, δ_c 22.8; C-21, δ_c 15.2). The absolute configuration at C-11 was assigned to be *S* by the comparison of specific rotation (+8.9) with those of analogs (*e. g.* +16.0 and +17.5 for sarcotragins A and B, respectively) in literature.[3] Due to the highly unstable nature of compound **1** under given conditions, however, the absolute configuration at the C-17 oxymethine center was unassigned by applications of Mosher method and/or PGME method.[3]

In our measurement of bioactivities, compound **1** exhibited moderate cytotoxicity against the K562 and A549 cancer cell-lines with IC_{50} values at 18.8 and 13.1 μM, respectively, (doxorubicin as a positive control, IC_{50} 1.6 and 0.7 μM, respectively).[10]

Acknowledgments

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Author Contributions

J.-K. Woo and J.-e. Jeon contributed equally to this work.

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