VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2) 256-260 APRIL 2015
DOI: 10.15625/0866-7144.2015-00126

A NEW ISOMALABARICANE FROM VIETNAMESE MARINE SPONGE *PETROSIA NIGRICANS*

Ngo Van Quang[2], Nguyen Xuan Nhiem[1], Dan Thi Thuy Hang[1], Hoang Le Tuan Anh[1]
Pham Hai Yen[1], Duong Thi Dung[1], Nguyen Thi Cuc[1], Chau Van Minh[1]
Nguyen Thi Mai Phuong[3], Phan Van Kiem[1*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)*

[2]*Institute of Chemistry, VAST*

[3]*Institute of Biotechnology, VAST*

Received 13 March 2015; Accepted for Publication 20 April 2015

Abstract

A new isomalabaricane triterpene, nigricanic acid A (**1**), and two known compounds (24*S*)-ergostane 3β,5α,6β,25-tetraol-25-monoacetate (**2**) and (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol-25-monoacetate (**3**), was isolated from Vietnamese marine sponge *Petrosia nigricans*. Their structures were determined on the basis of 1D and 2D-NMR spectra and HR-ESI-MS, and in comparison with the reported data. The cytotoxicity of all compounds were evaluated by MTT assay on four human cancer cell lines, including Hep-G2, KB, LU-1, and MCF-7. As the results, compound **1** exhibited moderate cytotoxic activity on the four human cancer cell lines with IC_{50} values ranging of 15.47÷19.34 μg/mL.

Keywords. *Isomalabaricane, Petrosia nigricans.*

1. INTRODUCTION

Of the different natural sources, the sea has become an important basis for the collection of natural bioactivity compounds [1]. Sponge constitute the phylum Porifera, and have been defined as sessile metazoans that have water intake and outlet openings connected by chambers lined with choanocyte, cells with whip-like flagella. Marine sponges of the genus *Petrosia* are known to be a rich source of biological constituents as polyacetylenes [2], steroid [3], and quinine [4]. Chemical investigations of *Petrosia nigricans* Lindgren, 1897 led to isolate purine analogues [5], batilol, and cholesterol [6]. Isomalabaricanes, tricyclic terpenoids isolated from many genera of marine sponges, have been found their inhibitory activities towards the cyclin-dependent kinases and controlling tumor cell cycle proliferation, implicating the potential application of marine compounds for chemotherapy [7].

During our study on chemical constituents from marine sponge *Petrosia nigricans*, one new isomalabaricane tritertepoids, and two known compounds were isolated. Their structures were elucidated by 1D, 2D NMR spectra and HR-ESI-MS.

2. MATERIAL AND METHODS

2.1. Animal materials

The sponge *Petrosia nigricans* was collected in Danang, Vietnam, in April 2012 and was kept in freezer until use. The scientific name was identified by Prof. Do Cong Thung (Institute of Marine Resources and Environment, VAST). A voucher specimen (LANGCO 08) was deposited at the Institute of Marine Biochemistry, VAST, Hanoi, Vietnam.

2.2. Gerenal experimental procedure

All NMR spectra were recorded on a Bruker Advance 500 FT-NMR spectrometer (500 MHz for ^{1}H, and 125 MHz for ^{13}C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. HR-ESI-MS spectra were recorded on Varian 910 FT-ICR-MS 7 tesla. Optical rotations were determined on a Jasco DIP-370 automatic

polarimeter (Jasco, Tokyo, Japan). Column chromatography (CC) was performed on silica gel 230-400 mesh (0.040-0.063 mm, Merck) or YMC RP-18 resins (30-50 µm, Fujisilisa Chemical Ltd.). Thin layer chromatography was performed on DC-Alufolien $60F_{254}$ (Merck 1.05715) or RP_{18} F_{254}, (Merck) plates. Compounds were appeared by spraying with aqueous 10 % H_2SO_4 and heating for 5 minutes.

Cytotoxic assays: Effects of compounds **1**–**3** on the growth of human cancer cells were determined by measuring the cytotoxic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [8]. Four human cancer cell lines, including HepG2 (human liver hepatocellular), KB (human KB cancer), LU-1 (human lung cancer) and MCF-7 (human breast cancer), were obtained from the Milan University, Italy and Hawaii University, USA and were grown in Dulbecco's modified eagle medium (DMEM) (2 mM L-glutamine, 10mM Hepes, and 1.0 mM sodium pyruvate) supplemented with 10 % fetal bovine serum at 37 °C in a humidified 5 % CO_2 atmosphere. The exponentially growing cells were used throughout the experiments. The MTT assays were performed as follows: human cancer cells (2.0×10^5 cells/ml) were treated for 3 days with 0.08, 0.4, 2.0, 10.0, and 100 µg/ml of compounds. Ellipticine was used as a positive control. After incubation, 180 µl DMEM was added to each well and then the cells were incubated at 37 °C for three days. The plates were centrifuged at 1000 rpm for 5 min at room temperature and the media was then carefully aspirated. Dimethylsulfoxide (150 µl) was added to each well to dissolve the formazan crystals. The plates were read immediately at 515 nm on a microplate reader (Amersham Pharmacia Biotech., USA). All the experiments were performed three times and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of crude extract or solvent fractions compared to the untreated controls. A dose-response curve was generated and the inhibitory concentration of 50 % cell growth (IC_{50}) was determined for each compound as well as each cell line.

2.3. Extraction and isolation

Fresh sample of the sponge *Petroisia nigricans* (2.0 kg) was homogeneous grinded and extracted three times repeat with hot MeOH and then evaporated under reduced pressure to give MeOH extract (PN, 120 g). This extract was suspended in water and then partitioned with chloroform to obtain the $CHCl_3$ (PN1, 45 g) and water (PN2, 75 g) layers after removal of the solvents *in vacuo*. The PN1 layer (45 g) was chromatographed on a silica-gel column eluting with *n*-hexane – acetone gradient (40 :1 → 0:1, v/v) to obtain four sub-fractions PN1A (10.0 g), PN1B (8.0 g), PN1C (5.0 g), and PN1D (13.0 g). The PN1D fraction was chromatographed on a RP-18 column eluting with methanol – water (1:1, v/v) to give two smaller fractions PN1D1 (1.8 g) and PN1D2 (2.4 g). The PN1D2 fraction was chromatographed on a silicagel column eluting with dichlomethane – methanol (2.5:1, v/v) to yield **1** (7.5 mg). The PN1B fraction was chromatographed on a silica-gel column eluting with *n*-hexane – EtOAc (6:1, v/v) to give four smaller fractions PN1B1 (1.0 g), PN1B2 (2.0 g), PN1B3 (1.8 g) and PN1B4 (0.8 g). The PN1B1 fraction was chromatographed on a RP-18 column eluting with acetone – water (2.5:1, v/v) to yield **2** (10.0 mg) and **3** (20.0 mg).

Nigricanic acid A (1): white amorphous powder, $[\alpha]_D^{25}$ + 30.5 (*c* = 1.0, CH_3OH); ^{1}H-NMR (CD_3OD, 500 MHz), ^{13}C-NMR (CD_3OD, 125 MHz); see table 1; HR-ESI-MS found *m/z* 355.18851 $[M+Na]^+$ (Calcd. for $C_{20}H_{28}O_4Na^+$, 355.18798).

(24*S*)-Ergostane 3β,5α,6β,25tetraol-25-mono acetate (2): white amorphous powder, $[\alpha]_D^{25}$ – 16.9 (*c* = 1.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 500 MHz), ^{13}C-NMR ($CDCl_3$, 125 MHz); see Table 1; HR-ESI-MS found *m/z* 493.38934 $[M+H]^+$ (Calcd. for $C_{30}H_{53}O_5$, 493.38875).

(24*S*)-Ergostane-1β,3β,5α,6β,25-pentaol-25-monoacetate (3): white amorphous powder, $[\alpha]_D^{25}$ – 3.9 (*c* = 0.65, CH_3OH); ^{1}H-NMR (CD_3OD, 500 MHz), ^{13}C-NMR (CD_3OD, 125 MHz); see Table 1); HR-ESI-MS *m/z* 509.38425 $[M+H]^+$ (Calcd. for $C_{30}H_{53}O_6$, 509.38367).

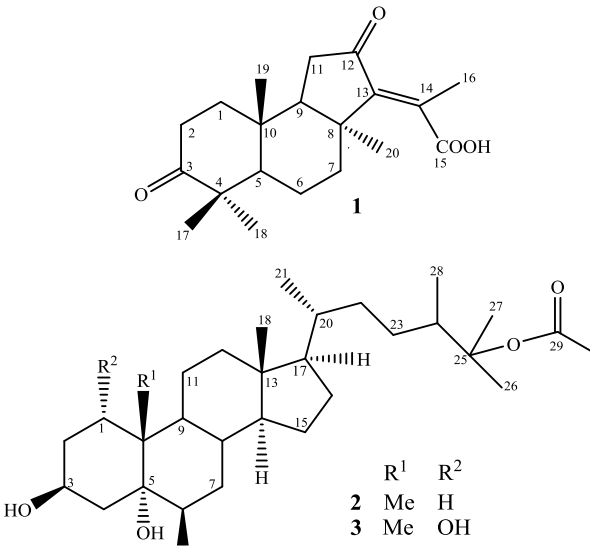

Figure 1: Structures of compound **1–3**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{20}H_{28}O_4$ by HR-ESI-MS at *m/z* 355.18851 $[M+Na]^+$ (Calcd. for $C_{20}H_{28}O_4Na$: 355.18798).

The ^{1}H-NMR spectrum of **1** showed the following signals: five methyl tertiary groups at δ_H 0.87, 1.06, 1.14, 1.43, 2.03 (each, 3H, s); five methylene groups at δ_H 1.52 (m)/2.29 (m), δ_H 2.31 (m)/2.89 (m), δ_H 1.61 (m)/1.67 (m), δ_H 2.08 (m)/2.55 (m), and at δ_H 2.18 (m); two methine groups at δ_H 2.52 (dd, *J* = 2.5 Hz; *J* = 13.0 Hz), δ_H 1.95 (d, *J* = 6.5 Hz).

The ^{13}C-NMR and DEPT spectra exhibited the present of twenty carbons, including three carbonyl groups at δ_C 222.46, 205.79, 180.00; four quaternary carbons at δ_C 35.95, 44.00, 48.10, 140.97; two methine groups at δ_C 46.47 and 49.78; five methylene groups at δ_C 20.20, 32.36, 34.43, 36.04, 37.04; five methyl groups at δ_C 18.08, 19.81, 23.83, 24.91, and 29.41. Analysis of ^{1}H and ^{13}C-NMR spectroscopic data of **1** (table 1) resulted that these data were similar to the corresponding data of (13Z)-globostelletin B [9], a isomalabaricane triterpene fom the marine sponge *Rhabdastrella globostellata*. The proton signals are assigned to the corresponding carbon signals directly on the basis of analyzing the interactions get on HSQC spectrum (see table 1). The HMBC correlations between methyl protons H-17 (δ_H 1.15)/H-18 (δ_H 1.06) and C-3 (δ_C 222.46)/C-4 (δ_C 48.10)/C-5 (δ_C 46.47); between H-19 (δ_H 0.87) and C-1 (δ_C 32.36)/C-10 (δ_C 35.95)/C-9 (δ_C 49.78)/C-5 (δ_C 46.47); between H-20 (δ_H 1.43) and C-7 (δ_C 37.04)/C-8 (δ_C 44.0)/C-9 (δ_C 49.78)/C-13 (δ_C 146.0); between H-16 (δ_H 2.03) and C-13 (δ_C 146.0)/C-14 (δ_C 140.97)/C-15 (δ_C 180.0) suggested the methyl groups were at C-4, C-8, C-10, and C-14 (see figure 1). Interpretation of HMBC spectra revealed chemical shifts at C-8 (δ_C 44.0), C-14 (δ_C 140.97), C-15 (δ_C 180.0) of **1** were moved about 4 ppm to low field toward in comparison with the corresponding data of (13*Z*)-globostelletin B. These evident suggested that the configuration at C-13 of **1** should be (13*E*). In addition, to confirm the configuration, the NOESY spectrum was measured. The NOESY corrrelations between H-19 (δ_H 0.87) and H-17 (δ_H 1.15)/H-1 (δ_H 1.52)/H-9 (δ_H 1.97); between H-18 (δ_H 1.06)/H-20 (δ_H 1.43)/H-5 (δ_H 2.55), but no NOESY correlation between H-20 and H-16 were observed (see figure 2) confirming that both protons H-19 and H-17 were at the same side, and protons H-20 and H-16 were at the different side. From the above evidence and comparative data reported in literature, structure of **1** was determined as a new compound (Fig. 1) and named nigricanic acid.

Table 1: NMR data of compounds **1-3** and references compounds

C	**1**			**2**			**3**		
	$^*\delta_C$	$\delta_C{}^{a,c}$	$\delta_H{}^{a,d}$ (mult., *J* = Hz)	$^\#\delta_C$	$\delta_C{}^{b,c}$	$\delta_H{}^{b,d}$ (mult., *J* = Hz)	$^\&\delta_C$	$\delta_C{}^{a,c}$	$\delta_H{}^{a,d}$ (mult., *J* = Hz)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	31.2	32.36	1.52 (m), 2.29 (m)	32.6	32.39	1.42 (m), 1.56 (m)	73.7	74.32	4.09 (m)
2	33.3	34.43	2.31 (m), 2.89 (m)	33.9	30.87	1.88	44.0	42.48	1.57 (m), 1.84 (m)
3	219.1	222.46	-	67.5	67.62	4.09 m	65.4	65.98	3.95 (m)
4	46.8	48.10	-	41.8	40.77	1.18 (m), 2.01 (m)	43.1	41.97	2.12 (d, 3.5)
5	45.3	46.47	2.55 (dd, 2.5, 13.0)	75.9	76.09	-	77.0	77.51	-
6	19.1	20.20	1.61 (m), 1.67 (m)	76.6	76.07	3.53 (m)	76.9	77.09	4.06 (m)
7	35.3	37.04	2.08 (m), 2.25 (m)	34.9	34.56	1.64 (m), 0.92 (m)	35.8	35.21	1.76 (m)
8	40.1	**44.00**	-	31.3	30.24	1.72 (m)	32.0	32.12	1.99 (m)
9	48.2	49.78	1.97 (dd, 6.5, 15.0)	46.8	45.87	1.28 (m)	47.1	47.38	1.65 (m)
10	34.8	35.95	-	39.8	38.32	-	44.9	44.89	-
11	34.8	36.04	2.14 (m)	20.9	21.19	1.29 (m), 1.45 (m)	47.1	47.38	1.29 (m), 1.45 (m)

12	204.3	205.79	-	41.6	39.95	1.63 (m), 2.10 (m)	24.0	25.02	1.23 (m), 1.95 (m)
13	144.9	146.00	-	43.7	42.76	-	42.8	44.89	-
14	136.5	**140.97**	-	56.4	55.94	1.12 (m)	56.7	57.54	1.05 (m)
15	174.4	**180.00**	-	24.6	24.14	1.20 (m), 1.60 (m)	25.1	25.55	1.20 (m), 1.31 (m)
16	18.9	18.08	2.03 (s)	29.4	28.12	1.84 (m)	28.5	29.05	1.33 (m), 1.81 (m)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
17	29.2	29.41	1.15 (s)	56.4	55.98	1.12 (m)	57.4	56.80	1.13 (m)
18	19.3	19.81	1.06 (s)	12.4	12.17	0.67 (s)	12.6	12.61	0.73 (s)
19	23.4	23.83	0.87 (s)	17.8	16.86	1.17 (s)	10.8	10.17	1.14 (s)
20	24.6	24.91	1.43 (s)	36.1	36.22	1.36 (m)	36.7	37.53	1.39 (m)
21				19.4	18.94	0.91 (d, 6.5)	19.2	19.49	0.96 (d, 6.5)
22				35.6	34.68	1.64 (m), 0.92 (m)	35.2	36.01	0.85 (m), 1.17 (m)
23				27.9	27.78	0.80 (m), 1.58 (m)	28.1	28.77	1.22 (m), 1.47 (m)
24				42.4	41.98	1.90 (m)	42.4	43.34	1.58 (m)
25				85.5	85.95	-	85.6	87.42	-
26				23.1	23.34	1.38 (s)	23.6	23.82	1.42 (s)
27				23.5	22.53	1.38 (s)	23.2	23.19	1.40 (s)
28				14.9	14.49	0.85 (d, 7.0)	14.8	14.88	0.92 (d, 7.0)
29				170.0	170.46	-	170.0	172.45	-
30				22.3	22.92	1.96 (s)	22.3	22.43	1.97 (s)

[a]Measured in CD_3OD, [b]measured in $CDCl_3$, [c]125 MHz, [d]500 MHz, [*]δ_C of (13Z)-globostelletin B [9], [#]δ_C of (24*S*)-ergostane 3β,5α,6β,25tetraol-25-monoacetate [10], [&]δ_C of (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol-25-monoacetate [11].

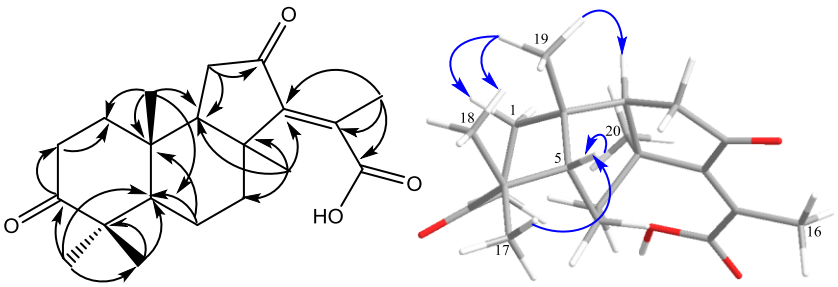

Figure 2: Important HMBC and NOESY correlations of compound **1**

Table 2: Cytotoxicity of compounds **1**–**3**

Compounds	IC_{50} (μg/mL)			
	Hep-G2	KB	LU-1	MCF-7
1	19.34	18.56	15.47	17.37
2	>100	>100	>100	>100
3	>100	>100	>100	>100
Pos.[(*)]	1.1	1.0	0.9	1.5

[(*)]Ellipticine was used as a positive control.

The known compounds were characterized as (24*S*)-ergostane 3β,5α,6β,25tetraol-25-monoacetate (**2**) [10], and (24*S*)-ergostane-1β,3β,5α,6β,25-pentaol-25-monoacetate (**3**) [11] (see figure 1), by comparing of their NMR spectroscopic data with the literature values.

Cytotoxic activities of all isolated compounds were evaluated by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay on four human cancer cell lines, including HepG2, KB, LU-1, and MCF-7 [12]. Ellipticine was used as a positive control. Ellipticine exhibited cytotoxicity on four human cancer cell lines, HepG2, KB, LU-1, and MCF-7 with IC_{50} values of 1.1, 1.0, 0.9, and 1.5 μg/mL, respectively. As the results, compound **1** exhibited moderate cytotoxic activities on all human

cancer cell lines with IC_{50} values ranging from 15.47÷19.34 μg/mL. The remaining compounds exhibited inactivity (IC50 > 100 μg/mL).

Acknowledgement. *This research was supported by Vietnam Academy of Science and Technology under grant number VAST.TĐ.ĐAB.01/13-15.*

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Corresponding author: **Phan Van Kiem**
Institute of Marine Biochemistry
Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: phankiem@yahoo.com.