

TWO BISCEMBRANIDS, LOBOPHYTONES K AND L FROM THE SPONGE *PETROSIA NIGRICANS*

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Abstract

By various chromatographic separations, two biscebranoids were isolated from the sponge *Petrosia nigricans*. Their structures were elucidated as lobophytone K (1) and lobophytone L (2) by spectroscopic methods and in comparison with the reported data. Both biscebranoids were the first time isolated from this sponge.

Keywords: *Petrosia nigricans*.

1. INTRODUCTION

Sponges 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* sp. are known to be a rich source of biologically polyacetylenes [1], steroid [2], and quinone [3]. Cembranoids were mainly found in the soft corals [4] and few cases were found in the sponge [5]. Chemical investigations of *Petrosia nigricans* Lindgren, 1897 led to isolate purine analogues [6], batilol, 5,8-epidioxycholest-6-en-3-ol, and cholesterol [7]. During our search of bioactive compounds, two biscebranoids were isolated from the sponge *P. nigricans*. Their structures were elucidated by 1D-, 2D-NMR, and mass spectra.

2. MATERIAL AND METHODS

2.1. Animal materials

The specimens of *Petrosia nigricans* Lindgren, 1897 were collected in Langco, Da Nang, Vietnam during April, 2012 and deep frozen until used. The scientific name was identified by Dr.

Do Cong Thung, Institute of Marine Resources and Environment, Vietnam Academy of Science and Technology. A voucher specimen (LANGCO 08) is deposited at Institute of Marine Biochemistry and Institute of Marine Resources and Environment, VAST, Hanoi, Vietnam.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ¹H- and 125 MHz for ¹³C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. 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 60 F₂₅₄ (Merck 1.05715) or RP₁₈ F_{254s} (Merck) plates. Compounds were visualized by spraying with aqueous 10% H₂SO₄ and heating for 5 minutes.

2.3. Extraction and isolation

Fresh frozen samples of the sponge *P. nigricans* (2 kg) were well grinded and extracted with hot MeOH three times and then concentrated 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 and eluting with a gradient elution of *n*-hexane – acetone (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 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 fraction PN1B2 was chromatographed on a YMC RP-18 column eluting with acetone – water (3:1, v/v) to yield **1** (15.0 mg) and **2** (20.0 mg).

Lobophytone K (1): White amorphous powder

$[\alpha]_D^{25} = +50.2$ (c 0.5, MeOH); IR (KBr) ν_{max} 3450, 1705, 1680, 1208, 1064 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) see Table 1; HR-ESI-MS m/z 723.4440 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{41}\text{H}_{64}\text{O}_9\text{Na}$: 723.4442).

Lobophytone L (2): White amorphous powder

$[\alpha]_D^{25} = +65.2$ (c 0.5, MeOH); IR (KBr) ν_{max} 3451, 1706, 1679, 1208, 1063 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) see table 1; HR-ESI-MS m/z 723.4445 $[\text{M}+\text{Na}]^+$ (Calcd for $\text{C}_{41}\text{H}_{64}\text{O}_9\text{Na}$: 723.4442).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $\text{C}_{41}\text{H}_{64}\text{O}_9$, by HR-ESI-MS at m/z 723.4440 (Calcd for $\text{C}_{41}\text{H}_{64}\text{O}_9\text{Na}$: 723.4442). The

$^1\text{H-NMR}$ spectrum of **1** (table 1) showed the following signals: one olefinic proton at δ_{H} 4.98, two oxygenated methine protons at δ_{H} 3.27 and 5.05, one methoxy at δ_{H} 3.50, four tertiary methyl at δ_{H} 1.17, 1.19, 1.60, and 1.74, four secondary methyl groups at δ_{H} 0.67, 0.84, 0.98, and 1.12 (each 3H, d, $J = 7.0$ Hz). The $^{13}\text{C-NMR}$ and DEPT spectra exhibited the presence of four carbonyl, one quaternary, four olefinic, five oxygenated, six methine, twelve methylene, and nine methyl carbons. Analysis of 1D- and 2D- NMR spectroscopic data indicated structure of **1** was identical to lobophytone K [8], a biscebranoids isolated from the soft coral *Lobophytum pauciflorum*. The HMBC correlations from methyl H-16 (δ_{H} 0.98) and methyl H-17 (δ_{H} 0.67) to C-12 (δ_{C} 51.79) and C-15 (δ_{C} 28.95); from H-18 (δ_{H} 1.12) to C-8 (δ_{C} 33.95), C-9 (δ_{C} 47.66), and C-10 (δ_{C} 213.93); from H-19 (δ_{H} 0.84) to C-4 (δ_{C}

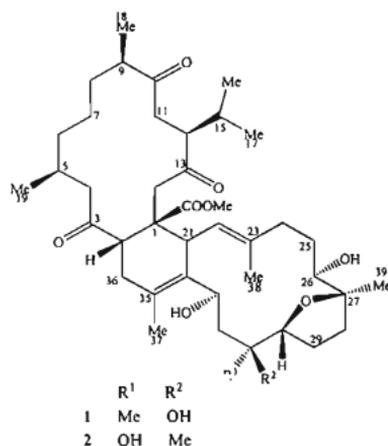


Figure 1: Structures of compounds **1** and **2**

54.01), C-5 (δ_{C} 27.52), and C-6 (δ_{C} 37.41) suggested that two methyl and one isopropyl groups were at C-5, C-9, and C-12, respectively (see figure 2). The carboxylate methyl group at C-1 was confirmed by HMBC correlations from H-2 (δ_{H} 3.47), H-14 (δ_{H} 2.76 and 3.02), H-21 (δ_{H} 3.68), and methoxy protons (δ_{H} 3.50) to C-20 (δ_{C} 174.98). In addition, the HMBC correlations from H-38 (δ_{H} 1.74) to C-22 (δ_{C} 127.85), C-23 (δ_{C} 136.92), and C-24 (δ_{C} 36.77); from H-37 (δ_{H} 1.60) to C-34 (δ_{C} 131.95), C-35 (δ_{C} 125.36), and C-36 (δ_{C} 32.88) confirmed that two double bonds were at C-22/C-23 and C-34/C-35 and two methyl groups were at C-23 and C-35. Moreover, the HMBC correlations between H-39 (δ_{H} 1.17) and C-26 (δ_{C} 73.97), C-27 (δ_{C} 85.85), and C-28 (δ_{C} 35.96); between methyl H-40 (δ_{H} 1.19) and C-30 (δ_{C} 88.28), C-31 (δ_{C} 76.10), and C-32 (δ_{C} 39.33), suggested two methyl to be at C-27 and C-30. The epoxy ring was confirmed by high chemical shifts of C-27 (δ_{C} 85.85) and C-30 (δ_{C} 88.28) and HMBC correlation between H-30 (δ_{H} 3.96) and C-27 (δ_{C} 85.85). The NOESY correlations between H-2 (δ_{H} 3.47) and H-22 (δ_{H} 4.98); between H-14 (δ_{H} 2.76) and H-21 (δ_{H} 3.68) and H-38 (δ_{H} 1.74); between H-22 (δ_{H} 4.98) and methoxy (δ_{H} 3.50) and H-2 (δ_{H} 3.47) confirmed the double bond at C-22/C-23 to be *E*; the carboxylate methyl, H-2, and C-22 at the same side of cyclohexene ring. The configuration of proton at C-33 was suggested to be β by no NOESY correlation between H-33 (δ_{H} 5.05) and H-21 (δ_{H} 3.68). For detail, the important NOESY correlations were shown in figure 2. Consequently, compound was determined to be lobophytone K.

Table 1: NMR data of compounds 1 and 2 and reference compounds

C	$^a\delta_c$	1		$^e\delta_c$	2	
		$\delta_c^{a,b}$	$\delta_H^{a,c}$ mult. ($J = \text{Hz}$)		$\delta_c^{a,b}$	$\delta_H^{a,c}$ mult. ($J = \text{Hz}$)
1	49.3	49.70	-	49.3	49.99	-
2	43.3	43.48	3.47 (dd, 7.5, 8.5)	43.6	44.22	3.62 (dd, 7.5, 8.5)
3	213.6	213.51	-	213.6	213.38	-
4	53.9	54.01	3.01 (d, 19.0) 2.38 (d, 10.0)	53.8	53.80	3.01 (d, 19.0) 2.38 (d, 10.0)
5	27.3	27.52	1.80 (m)	27.3	27.51	1.77 (m)
6	37.7	37.41	1.05 (m) 1.12 (m)	37.7	37.53	1.05 (m) 1.12 (m)
7	25.8	25.52	1.10 (m) 1.26 (m)	25.8	25.63	1.10 (m) 1.25 (m)
8	33.8	33.95	1.50 (m)	33.9	34.19	1.50 (m)
9	47.1	47.66	2.52 (m)	47.2	48.16	2.49 (m)
10	213.2	213.93	-	213.3	213.92	-
11	31.2	31.16	2.95 (dd, 10.0, 17.5)	31.1	31.38	2.95 (dd, 10.0, 17.5)
12	52.1	51.79	3.07 (dd, 2.0, 10.5)	52.0	51.26	3.05 (dd, 2.0, 10.5)
13	209.5	209.03	-	209.7	209.55	-
14	45.3	45.33	2.76 (d, 19.0) 3.02 (d, 19.0)	45.5	46.41	2.76 (d, 19.0) 3.02 (d, 19.0)
15	28.8	28.95	2.34 (m)	28.6	29.02	2.23 (m)
16	21.5	21.36	0.98 (d, 7.0)	21.2	21.32	0.97 d (6.5)
17	17.9	17.41	0.67 (d, 7.0)	17.6	17.51	0.70 d (6.5)
18	17.6	17.48	1.12 (d, 7.0)	17.6	17.65	1.12 d (7.0)
19	22.7	22.29	0.84 (d, 7.0)	22.8	21.76	0.85 d (7.0)
20	174.7	174.98	-	174.9	174.76	-
21	43.4	43.09	3.68 (d, 10.0)	43.6	43.69	3.42 d (10.0)
22	126.9	127.85	4.98 (d, 10.0)	127.2	129.05	4.92 (10.0)
23	137.4	136.92	-	136.6	138.10	-
24	37.1	36.77	2.13 (m)	36.6	36.77	2.13 (m)
25	28.7	26.83	1.30 (m) 1.84 (m)	28.0	29.70	1.30 (m) 1.84 (m)
26	72.6	73.97	3.27 (d, 10.0)	71.8	71.92	3.21 (d, 11.0)
27	85.8	85.85	-	85.9	84.78	-
28	35.5	35.96	1.68 (m); 2.38 (m)	35.5	36.17	1.60 (m); 2.35 (m)
29	27.7	26.83	1.57 (m); 1.87 (m)	26.3	27.33	1.55 (m); 1.80 (m)
30	87.9	88.28	3.96 (dd, 6.5, 10.5)	89.2	88.58	3.96 (dd, 5.0, 11.0)
31	74.7	76.10	-	72.0	74.42	-
32	39.1	39.33	2.20 (dd, 10.5, 14.0)	42.0	40.00	1.34 (dd, 6.5, 15.0) 2.10 (dd, 8.5, 15.0)
33	66.8	67.33	5.05 (d, 10.5)	64.7	65.58	5.12 (d, 5.5)
34	133.4	131.95	-	135.6	133.55	-
35	123.9	125.36	-	122.2	127.57	-
36	32.8	32.88	1.95 (m) 2.53 (m)	32.7	32.67	1.95 (m) 2.29 (m)
37	17.6	17.88	1.60 (s)	18.1	18.33	1.75 (s)
38	16.6	16.27	1.74 (s)	16.7	20.68	1.76 (s)
39	21.4	20.42	1.17 (s)	21.5	19.34	1.14 (s)
40	23.6	21.24	1.19 (s)	28.4	21.76	1.16 (s)
41	51.1	50.98	3.50 (s)	51.0	51.26	3.53 (s)

^aMeasured in CDCl₃, ^b125 MHz, ^c500 MHz, ^e δ_c of lobophytone K [8], ^f δ_c of lobophytone L [8] in DMSO-d₆.

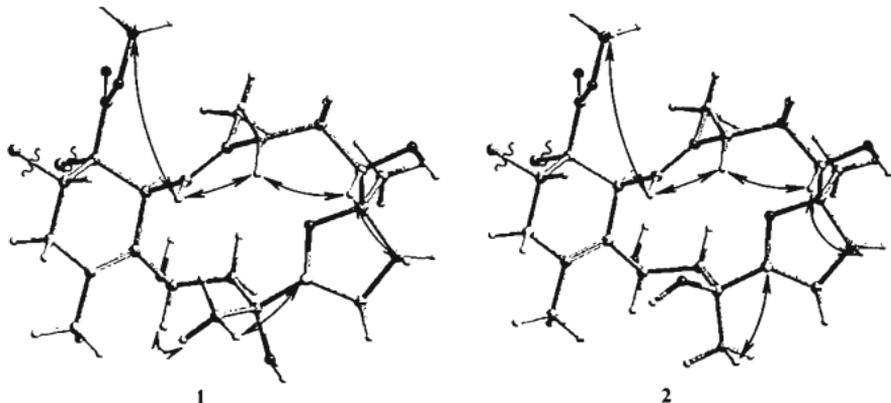


Figure 2: Important NOESY correlations of compounds 1 and 2

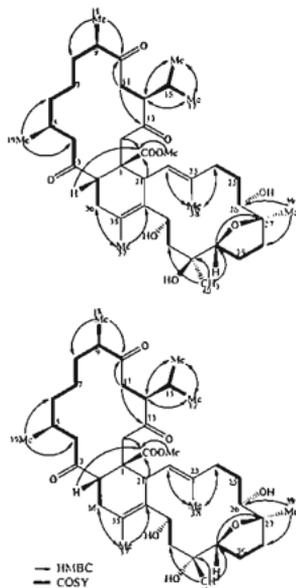


Figure 3: Important HMBC and COSY correlations of compounds 1 and 2

Compound 2 was also obtained as a white amorphous powder and its molecular has the same these of 1 as determined by HR-ESI-MS and NMR data. The ^1H - and ^{13}C -NMR data of 2 (table 1) were identical of 1, suggested that 2 could be the stereoisomer of 1. The different observation was

found to be chemical shifts of C-31 (δ_{C} 74.42) and C-33 (δ_{C} 65.58) of 2 moved to strong field in comparison to those of compound 1 [C-31 (δ_{C} 76.10) and C-33 (δ_{C} 67.33)]. This was due to hydrogen bond appeared when two hydroxyl group at C-31 and C-33 at the same side. These finding implied that 2 was an epimer of 1 at C-31 (see Figure 1). In addition, the NOESY correlation between H-33 (δ_{H} 5.12) and H-40 (δ_{H} 1.16), but no NOESY correlation between H-1 (δ_{H} 3.42) and H-33 (δ_{H} 5.12) were observed (see figure 2), confirming both methyl at C-31 and proton at C-33 at the same side. Consequently, compound 2 was determined to be lobophytone L, previously reported from *Lobophytum pauciflorum* [8]. To our best knowledge, compounds 1 and 2 were the first time isolated from *P. nigricans*.

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REFERENCES

1. Y. Hitora, K. Takada, S. Okada, S. Matsunaga, Miyakosynes A-F, cytotoxic methyl branched acetylenes from a marine sponge *Petrosia* sp. *Tetrahedron*, 67, 4530-4534 (2011).
2. H. H. Sun, S. S. Gross, M. Gunasekera, F. E. Koehn. *Weinbersterol disulfates A and B, antiviral steroid sulfates from the sponge Petrosia weinbergi*. *Tetrahedron*, 47, 1185-1190 (1991).

3. P. Ramesh, N. S. Reddy, Y. Venkateswarlu. *A new 1,2-dihydroisoquinoline from the sponge Petrosia similis*, *J. Nat. Prod.*, **62**, 780-781 (1999).
4. T. H. Quang, T. T. Ha, C. V. Minh, P. V. Kiem, H. T. Huong, N. T. T. Ngan, N. X. Nhiem, N. H. Tung, B. H. Tai, D. T. T. Thuy, S. B. Song, H. -K. Kang, Y. H. Kim. *Cytotoxic and anti-inflammatory cebranoids from the Vietnamese soft coral Lobophytum laevigatum*, *Bioorg. Med. Chem.*, **19**, 2625-2632 (2011).
5. A. Fontana, M. L. Ciavatta, P. Amodeo, G. Cimino. *Single solution phase conformation of new antiproliferative cebranones*, *Tetrahedron*, **55**, 1143-1152 (1999).
6. M. Ashour, R. Edrada-Ebel, R. Ebel, V. Wray, S. R. W. M. van, P. Proksch. *New purine derivatives from the marine sponge Petrosia nigricans*, *Natural Products Communication*, **3**, 1889-1894 (2008)
7. T. T. Huong, T. A. Nguyen, T. T. Quang, C. N. Huy, T. T. Minh, C. V. Minh, P. V. Kiem. *Study on chemistry of the sponge Petrosia nigricans living in Vietnamese sea*, *Vietnam Journal of Chemistry*, **45**, 141-144 (2007).
8. P. Yan, Z. Deng, L. van Ofwegen, P. Proksch, W. Lin. *Lobophytone H-N biscebranoids from the Chinese soft coral Lobophytum pauciflorum*, *Chem. Pharm. Bull.*, **58**, 1591-1595 (2010).

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