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Merosesquiterpenes from marine sponge *Smenospongia cerebriformis*

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Abstract

Using various chromatography methods, three merosesquiterpenes belonging to sesquiterpene quinone type, neodactyloquinone (**1**), dactyloquinone D (**2**), and dactyloquinone C (**3**) together with two indole derivatives indole-3-aldehyde (**4**) and indole-3-cacboxylic methyl ester (**5**) were isolated from the methanol extract of the Vietnamese marine sponge *Smenospongia cerebriformis*. Their structures were determined by 1D-, 2D-NMR spectra, HR-ESI-MS and in comparison with those reported in the literature.

Keywords. *Smenospongia cerebriformis*, merosesquiterpene, sesquiterpene quinone, indole derivative.

1. INTRODUCTION

Marine sponges are regarded as a rich source of secondary metabolites with chemically diverse structures and potential biological benefits. Merosesquiterpenes and indole alkaloid derivatives were found to be the main components of sponges, particularly, the genus *Smenospongia.* A huge variety of compounds belonging to these two chemical structure classes have been reported from sponges and possessed a broad range of interest bioactivities, such as antimalarial [1, 2], antimicrobial [1-3], anticancer [3, 4] antidepressant [5], as well as inhibition of the neuronal isozyme of nitric oxide synthase (nNOS) [6]. Herein, we report the isolation and structure determination of three merosesquiterpenes and two indole alkaloid derivatives from sponge *Smenospongia cerebriformis*.

2. MATERIALS AND METHODS

2.1. Sponge materials

The sponge *Smenospongia cerebriformis* (Duchassaing & Michelotti, 1864) was collected in Vinhmoc, Quangtri in August 2015 and identified by Prof. Do Cong Thung, Institute of Marine Environment and Resources, VAST. A voucher specimen (HM08.2015-2) was deposited at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

2.2. General experimental procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography was performed using a silica gel (Kieselgel 60, 70–230 mesh and 230-400 mesh, Merck, Whitehouse Station, NJ) or RP-18 resins (30-50 µm, Fuji Silysia Chemical Ltd.), and thin layer chromatography (TLC) using pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

Fresh frozen dried samples of sponge *Smenospongia cerebriformis* (15.0 kg) were well grinded and sonicated with hot MeOH three times and then concentrated under reduced pressure to give MeOH extract (SP, 360 g). This extract was suspended in water and then partitioned with CH_2Cl_2 to give the CH_2Cl_2 (SPD, 102 g) and water (SPW, 250 g) extracts after removal of the solvents *in vacuo*. Fraction SPD (100 g) was subjected to silica gel column chromatography and eluted with an *n*-hexane - acetone stepwise gradient to give five fractions SPD1 (39.0 g), SPD2 (5.8 g), SPD3 (12.9 g), SPD4 (20.0 g), and SPD5 (2.8 g). SPD2 was chromatographed on a RP-18 column eluting with acetone - water (1.5:1, v/v) to give four smaller fractions SPD2A-D. Fraction SPD2B was subjected

to silica gel column chromatography and eluted with a *n*-hexane-ethyl acetate (1.5:1, v/v) to give compound **4** (ASP2, 11.0 mg). Fraction SPD3 was chromatographed on a silica gel column eluting with *n*-hexane-ethyl acetate (3:1, v/v) to give five smaller fractions, SPD3A-E. Fraction SPD3D (2.2 g) was applied to a silica gel column eluting with *n*-hexane - ethyl acetate (2:1, v/v) to give compounds **1** (ASP16A, 10.0 mg) and **2** (ASP15A, 12.0 mg). Fraction SPD3E (1.8 g) was chromatographed on a RP-18 column eluting with acetone - water (1:1, v/v) to yield compound **5** (16 mg). Fraction SPD5 was subjected to a silica gel column using dichloromethane-ethyl acetate (10:1, v/v) as eluent to give five smaller fractions, SPD5A-D. Furthermore, fraction SPD5B (0.4 g) was firstly chromatographed on a RP-18 column eluting with acetone-water (2:1, v/v) and then further purified on a silica gel column eluting with dichloromethane - acetone (12:1, v/v) to yield compound **3** (ASP27, 11.0 mg).

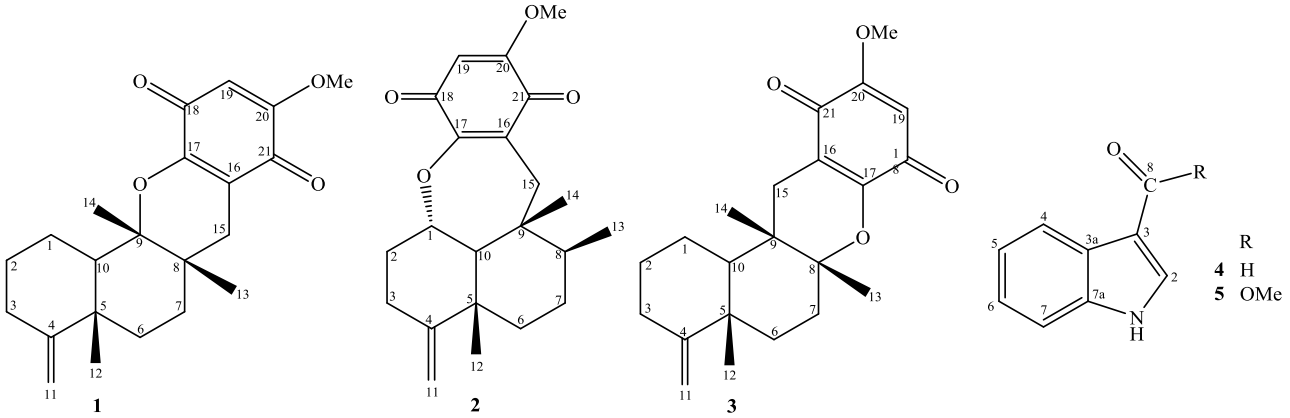

Figure 1: Chemical structures of compounds **1-5** from *S. cerebriformis*

Neodactyloquinone (**1**): White amorphous powder; α_D^{25}: +25.4 (*c* = 0.1, in $CDCl_3$); ^{1}H- and ^{13}C-NMR ($CDCl_3$), see table 1.

Dactyloquinone C (**2**): White amorphous powder; α_D^{25}: +30.2 (*c* = 0.1, in $CDCl_3$); ^{1}H- and ^{13}C-NMR ($CDCl_3$), see table 2.

Dactyloquinone D (**3**): White amorphous powder; α_D^{25}: +21.6 (*c* = 0.1, in $CDCl_3$); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H: 5.76 (s, H-19), 4.52 and 4.49 (each 1H, br s, H-11), 3.80 (3H, s, 20-OMe), 1.24 (3H, s, H-13), 1.11 (3H, s, H-12), 1.07 (3H, s, H-14); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C: 22.0 (C-1), 28.3 (C-2), 32.6 (C-3), 158.6 (C-4), 39.4 (C-5), 31.2 (C-6), 30.4 (C-7), 84.8 (C-8), 37.1 (C-9), 44.9 (C-10), 103.5 (C-11), 20.9 (C-12), 23.1 (C-13), 21.1 (C-14), 27.8 (C-15), 113.9 (C-16), 152.6 (C-17), 181.7 (C-18), 104.9 (C-19), 159.4 (C-20), 181.0 (C-21), and 56.4 (20-OMe).

Indole-3-aldehyde (**4**): White amorphous powder; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 8.04 (s, H-2), 8.13 (d, *J* = 7.5 Hz, H-4), 7.20 (dd, *J* = 7.5, 8.0 Hz, H-5), 7.24 (dd, *J* = 7.5, 8.0 Hz, H-6), 7.45 (d, *J* = 7.5 Hz, H-7), 9.84 (s, H-8); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 139.7 (C-2), 120.1 (C-3), 125.7 (C-3a), 122.4 (C-4), 123.6 (C-5), 125.0 (C-6), 113.1 (C-7), 138.9 (C-7a), 187.4 (C-8).

Indole-3-carboxylic methyl ester (**5**): White amorphous powder, ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 7.93 (s, H-2), 8.20 (dd, *J* = 3.0, 9.0 Hz, H-4), 7.28 (overlapped signals, H-5 and H-6), 7.42 (dd, *J* = 3.0, 9.0 Hz, H-7), 3.93 (s, 8-OMe); ^{13}C-NMR (CD_3OD, 125 Hz) δ_C: 133.1 (C-2), 131.0 (C-3), 136.1 (C-3a), 121.6 (C-4), 122.1 (C-5), 123.2 (C-6), 111.5 (C-7), 125.8 (C-7a), 165.6 (C-8), 51.1 (8-OMe).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. It had a molecular formula $C_{22}H_{28}O_4$ which was derived from a pseudo-molecular $[M+H]^+$ ion peak at *m/z* 357.2039 (calcd. for $C_{22}H_{29}O_4$, 357.1988) in the HR-ESI-MS and in conjunction with ^{13}C NMR data. ^{1}H NMR and HSQC spectroscopic analysis of **1** showed the presence of three tertiary methyl groups at δ_H 0.93, 1.08 and 1.41 (each 3H, s), exocyclic methylene signal at δ_H 4.54 (2H, br s), methoxy group at δ_H 3.80 (3H, s) and an olefinic proton at δ_H 5.74 (1H, s). The ^{13}C NMR of **1** revealed signals of 22 carbons which were classified by DEPT as nine non-protonated carbons, two methines, seven methylenes, and four methyl carbons. ^{1}H NMR and ^{13}C NMR disclosed the presence of a dialkoxy-1,4-benzoquinone moiety

Table 1: NMR spectral data for **1-2** and reference compounds

C	δ_C [#,a]	**1**		δ_C [$,a]	**2**	
		δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)		δ_C [a,b]	δ_H [a,c] (mult., *J* in Hz)
1	21.2	21.2	1.56 (m) 1.77 (m)	78.5	78.5	4.15 (ddd, 5.5, 10.5, 10.5)
2	27.7	27.7	1.27 (m) 1.83 (m)	35.5	35.5	1.80 (m) 2.52 (m)
3	32.7	32.7	2.10 (br d, 14.0) 2.23 (ddd, 5.5, 14.0, 14.0)	30.6	30.7	2.26 (m) 2.40 (ddd, 5.0, 14.0, 14.0)
4	158.3	158.3	-	157.2	157.2	-
5	41.3	41.4	-	40.2	40.3	-
6	30.8	30.9	1.41 (m) 2.00 (ddd, 3.5, 3.5, 14.0)	37.4	37.4	1.64 (m)
7	32.3	32.4	1.58 (m) 1.78 (br d, 14.0)	27.8	27.8	1.53 (m) 1.60 (m)
8	34.6	34.6	-	41.8	41.8	1.39 (m)
9	86.4	86.4	-	37.4	37.4	-
10	47.8	47.8	1.44 (dd, 2.5, 12.0)	60.2	60.2	1.50 (d, 10.5)
11	103.7	103.7	4.54 (br s)	104.1	104.1	4.62 (br s)
12	20.8	20.9	1.08 (s)	21.7	21.8	0.96 (s)
13	24.3	24.3	0.93 (s)	16.4	16.4	1.03 (d, 6.5)
14	19.1	19.1	1.41 (s)	15.0	15.0	0.73 (s)
15	26.8	26.8	2.04 (d, 16.5) 2.72 (d, 16.5)	34.8	34.8	1.99 (d, 14.0) 3.13 (d, 14.0)
16	114.6	114.5	-	130.2	130.3	-
17	151.2	151.3	-	156.5	156.3	-
18	181.5	181.5	-	182.6	183.0	-
19	104.7	104.7	5.74 (s)	105.2	105.2	5.80 (s)
20	159.5	159.5	-	159.0	159.0	-
21	181.5	181.5	-	182.6	182.6	-
20-OMe	56.3	56.4	3.80 (s)	56.4	56.4	3.81 (s)

Measured in [a)]$CDCl_3$, [b)]125 MHz, [c)]500 MHz. [#)]δ_C of neodactyloquinone [8], [$)]$\delta_C$ of dactyloquinone C [9].

(δ_H: 3.80, 5.74; δ_C: 56.4, 104.7, 114.5, 151.3, 159.5, 181.5, 181.5) [7]. The HMBC correlations between H-11 (δ_H 4.54) and C-3 (δ_C 32.7)/C-4 (δ_C 158.3)/C-5 (δ_C 41.4) suggested an exocyclic olefinic methylene forming at C-11/C-4. Methyl protons H-12 (δ_H 1.08) have HMBC correlations with C-4/C-5/C-6 (δ_C 30.9)/C-10 (δ_C 47.8), indicating the location of a methyl group at C-5. The HMBC correlations between H-14 (δ_H 1.41) and C-8 (δ_C 34.6)/C-9 (δ_C 86.4)/C-10 indicated a methyl group at C-9. The last tertiary methyl group located at C-8 which was indicated by HMBC correlations between proton H-13 (δ_H 0.93) and carbons C-7 (δ_C 32.4)/C-8/C-9/C-15 (δ_C 26.8). The 1,4-benzoquinone moiety linked to sesquiterpene skeleton at C-15 confirmed by HBMC correlations between methylene protons H-15 (δ_H 2.04, 2.72) and carbons C-7/C-8/C-9/C-13 (δ_C 24.3)/C-16 (δ_C 114.5)/C-17 (δ_C 151.3)/C-21 (δ_C 181.5). The HMBC correlations from protons H-19 (δ_H 5.74) and methoxy (δ_H 3.80) to C-20 (δ_C 159.5) demonstrated for a methoxy group at C-20. Furthermore, carbon chemical shifts of C-9 (δ_C 86.4) and C-17 (δ_C 151.3) suggested an ether bridge between C-9 and C-17 which was agreed with molecular formula of **1** $C_{22}H_{28}O_4$. Consequently, structure of **1** was established to be neodactyloquinone, a sesquiterpene quinone previously isolated from the sponge *Dactylospongia elegans* [8]. Its ^1H and ^{13}C NMR data were identical with those reported in the literature (table 1) and found to match well [8].

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was established as $C_{22}H_{28}O_4$ on the basis of HR-ESI-MS (*m/z*: 357.2074, $[M+H]^+$; calcd. for $C_{22}H_{29}O_4$, 357.1988) and ^{13}C-NMR analysis. ^1H NMR and HSQC spectroscopic analysis of **2** showed the presence of two tertiary methyl groups at δ_H 0.73, 0.96 (each 3H,

s) and a secondary methyl group at δ_H 1.03 (3H, d, *J* = 6.5 Hz), exocyclic methylene signal at δ_H 4.62 (2H, br s), oxygenated methine signal at δ_H 4.15 (1H, ddd, *J* = 5.5, 10.5, 10.5 Hz), methoxy group at δ_H 3.81 (3H, s) and an olefinic proton at δ_H 5.80 (1H, s). The ^{13}C NMR of **2** revealed signals of 22 carbons which were classified by DEPT as eight non-protonated carbons, three methines, seven methylenes, and four methyl carbons. ^{1}H NMR and ^{13}C NMR disclosed the presence of a dialkoxy-1,4-benzoquinone moiety [δ_H: 3.81, 5.80; δ_C: 56.4, 105.2, 130.3, 156.3, 159.0, 182.6, 183.0] [9]. The HMBC correlations between protons H-11 (δ_H 4.62) and C-3 (δ_C 30.7)/C-4 (δ_C 157.2)/C-5 (δ_C 40.3) suggested an exocyclic olefinic methylene forming at C-11/C-4. Methyl protons H-12 (δ_H 0.96) have HMBC correlations with C-4/C-5/C-6 (δ_C 37.4)/C-10 (δ_C 60.2), indicating the location of a methyl group at C-5. The HMBC correlations between proton H-14 (δ_H 0.73) and C-8 (δ_C 34.6)/C-9 (δ_C 37.4)/C-10/ C-15 (δ_C 34.8) confirmed the methyl group at C-9. The last secondary methyl group located at C-8 which was indicated by HMBC correlations between H-13 (δ_H 1.03) and C-7 (δ_C 27.8)/C-8/C-9. The 1,4-benzoquinone moiety linked to sesquiterpene skeleton at C-15 confirmed by HBMC correlations between methylene protons H-15 (δ_H 1.99, 3.13) and C-9/C-10/C-14 (δ_C 15.0)/C-16 (δ_C 130.3)/C-17 (δ_C 156.3)/C-21 (δ_C 182.6). The HMBC correlations from protons H-19 (δ_H 5.80) and methoxy (δ_H 3.81) to C-20 (δ_C 159.0) demonstrated for a methoxy group at C-20. Carbon chemical shifts of C-1 (δ_C 78.5) and C-17 (δ_C 156.3) suggested an ether bridge between C-1 and C-17 which was agreed with molecular formula of **2** $C_{22}H_{28}O_4$. In addition, the ^{1}H- and ^{13}C-NMR data of **2** were identical with those of dactyloquinone C, a compound also isolated from sponge *Dactylospongia elegans* [9] (table 1) and found to match. Consequently, the structure of **2** was established.

Compound **3** was isolated as a white amorphous powder. The molecular formula $C_{22}H_{28}O_4$ was deduced on the basis of HR-ESI-MS (*m/z*: 357.2039, $[M+H]^+$; calcd. for $C_{22}H_{29}O_4$, 357.1988) and ^{13}C-NMR analysis. ^{1}H NMR data of **3** also showed the presence of three tertiary methyl groups at δ_H 1.07, 1.11 and 1.23 (each 3H, s), exocyclic methylene signal at δ_H 4.49 and 4.52 (each 1H, br s), methoxy group at δ_H 3.80 (3H, s), and an aromatic proton at δ_H 5.76 (1H, s). The ^{13}C NMR and DEPT spectra of **3** revealed signals of 22 carbons including nine non-protonated carbons, two methines, seven methylenes, and four methyl carbons. ^{1}H NMR and ^{13}C NMR indicated the presence of a dialkoxy-1,4-benzoquinone moiety [δ_H: 3.80, 5.76; δ_C: 56.4, 103.9, 114.5, 152.6, 159.4, 181.0, 181.7]. In comparison with **2**, the 1D-NMR spectra of **3** showed the presence signals of an oxygenated tertiary carbon (84.8), a saturated methylene group, and a tertiary methyl group instead of an oxygenated secondary carbon (δ_C 78.5), a saturated methine group, and a secondary methyl group in the 1D-NMR of **2**, suggesting for the re-arrangement of ether bridge from C-1/C-17 in **2** to C-8/C-17 in **3**. Thus, compound **3** was determined to be dactyloquinone D, a known compound isolated from the sponge *Dactylospongia elegans* [9].

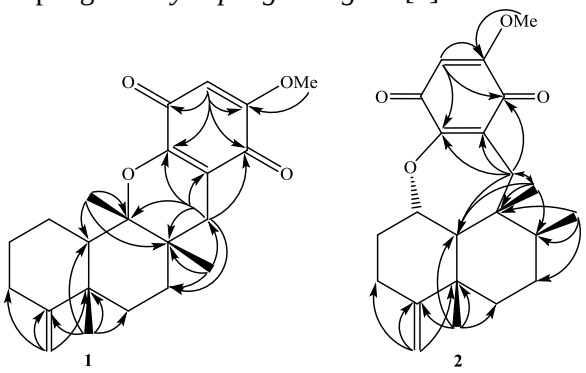

Figure 2: The key HMBC correlations of **1** and **2**

The remaining compounds were elucidated to be indole-3-aldehyde (**4**) and indole-3-cacboxylic methyl ester (**5**). Their structures were established based on spectral and chemical evidence, which agreed with previous studies [10, 11].

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