

## BISCSEMBRANOIDS FROM VIETNAMESE MARINE SPONGE *Petrosia nigricans*

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### Abstract

Three biscembranoids, lobophytone H (**1**), lobophytone U (**2**), and methyl tortuoate B (**3**), were isolated from the methanol extract of Vietnamese marine sponge *Petrosia nigricans*. Their structures were determined on the basis of 1D and 2D-NMR, HR-ESI-MS and in comparison with the reported data.

**Keywords.** *Petrosia nigricans*, biscembranoids.

### 1. INTRODUCTION

The sea has played an important basis for the development of natural bioactive compounds [1]. Sponge constitutes the phylum Porifera and have been defined as sessile metazoans that have water intake and outlet openings connected by chambers lined choanocyte cells with whip-like flagella. Genus *Petrosia* is known to be a biological source of polyacetylenes [2], alkaloids [3], and quinones [4].

Cembranoids have been mainly found in soft corals [5] and rarely found in the sponge [6]. During our study on chemical constituents from the sponge *Petrosia nigricans*, three biscembranoids were isolated and elucidated.

### 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.

#### 2.2. General experimental procedure

All NMR spectra were recorded on a Bruker

Advance 500 FT-NMR spectrometer (500 MHz for <sup>1</sup>H, and 125 MHz for <sup>13</sup>C-NMR), and chemical shifts ( $\delta$ ) 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  $\mu$ m, Fujisilisa Chemical Ltd.). Thin layer chromatography was performed on DC-Alufolien 60F<sub>254</sub> (Merck 1.05715) or RP<sub>18</sub> F<sub>254</sub>, (Merck) plates. Compounds were appeared by spraying with aqueous 10 % H<sub>2</sub>SO<sub>4</sub> and heating for 5 minutes.

#### 2.3. Extraction and isolation

The fresh sample of the sponge *Petrosia nigricans* (2.0 kg) was homogeneous grinded and extracted with hot MeOH three times 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<sub>3</sub> (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 *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 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 PN1B4 fraction was chromatographed on a RP-18 column, eluting with acetone–water (2:1, v/v) to yield compounds **1** (42.0 mg) and **2** (50.0 mg). The PN1B3 fraction was chromatographed on a RP-18 column, eluting with acetone–water (2:1, v/v) to yield compound **3** (5.0 mg).

**Lobophytone H (1):** white amorphous powder;  $[\alpha]_D^{25} +70.5$  ( $c = 0.5$ , MeOH);  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ), see table 1; HR-ESI-MS found  $m/z$

705.43422  $[\text{M}+\text{Na}]^+$  (Calcd. for  $\text{C}_{41}\text{H}_{62}\text{O}_8\text{Na}^+$ , 705.43369).

**Lobophytone U (2):** white amorphous powder;  $[\alpha]_D^{25} +50.4$  ( $c = 0.5$ , MeOH);  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR ( $\text{CD}_3\text{OD}$ ), see table 1; HR-ESI-MS found  $m/z$  723.44482  $[\text{M}+\text{Na}]^+$  (Calcd. for  $\text{C}_{41}\text{H}_{64}\text{O}_9\text{Na}^+$ , 723.44426).

**Methyl turtuote B (3):** white amorphous powder;  $[\alpha]_D^{25} +84.5$  ( $c = 0.5$ , MeOH);  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ ), see table 1; HR-ESI-MS found  $m/z$  683.45226  $[\text{M}+\text{H}]^+$  (Calcd. for  $\text{C}_{41}\text{H}_{63}\text{O}_8^+$ , 683.45175).

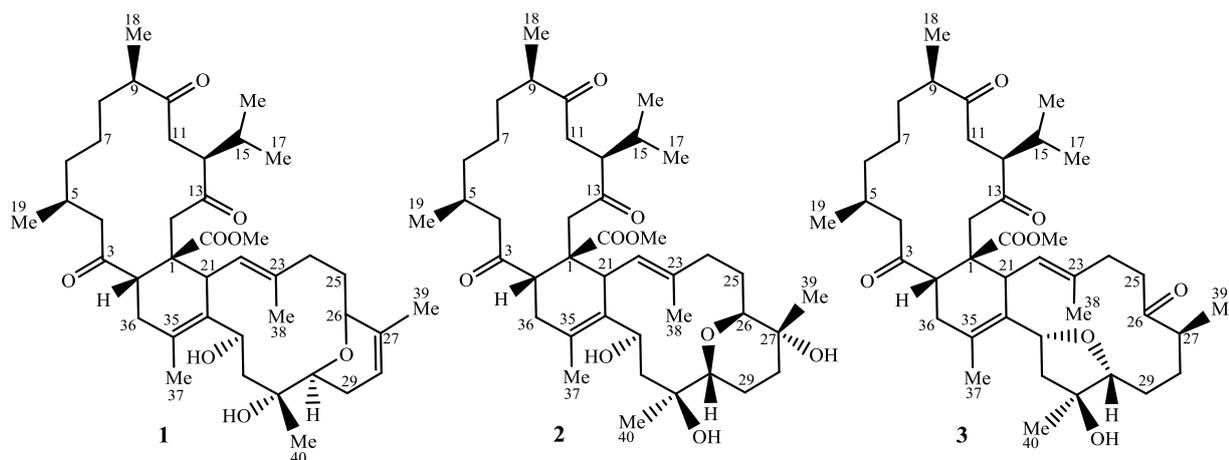


Figure 1: Structures of **1-3** from *Petrosia nigricans*

### 3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be  $\text{C}_{41}\text{H}_{62}\text{O}_8$  by HR-ESI-MS at  $m/z$  705.43422  $[\text{M}+\text{Na}]^+$  (Calcd. for  $\text{C}_{41}\text{H}_{62}\text{O}_8\text{Na}^+$ : 705.43369). The  $^1\text{H}$ -NMR spectrum of compound **1** (table 1) showed the signals: two olefinic protons at  $\delta_{\text{H}}$  5.12 (d,  $J = 11.0$  Hz), 5.56 (d,  $J = 3.5$  Hz); one methoxy at  $\delta_{\text{H}}$  3.56 (s); four secondary methyl groups at  $\delta_{\text{H}}$  0.75 (d,  $J = 7.0$  Hz), 0.84 (d,  $J = 7.0$  Hz), 0.96 (d,  $J = 7.0$  Hz), 1.13 (d,  $J = 7.0$  Hz); four methyl tertiary groups at  $\delta_{\text{H}}$  1.09, 1.64, 1.86, 1.91 (each, 3H, s); three oxygenated methine protons at  $\delta_{\text{H}}$  4.02 (br d,  $J = 7.0$  Hz), 3.68 (dd,  $J = 4.0, 10.0$  Hz), and 4.81 (br d,  $J = 10.5$  Hz). The  $^{13}\text{C}$ -NMR and DEPT spectra of compound **1** exhibited present the signals of 41 carbons, including four carbonyl, six quaternary, eleven methine, eleven methylene, and nine methyl carbons. Analysis of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data indicated that structure of compound **1** (figure 1) was identical to lobophytone H [7]. The HMBC correlations between H-16 ( $\delta_{\text{H}}$  0.96)/H-17 ( $\delta_{\text{H}}$  0.75) and C-12 ( $\delta_{\text{C}}$  51.44)/C-15 ( $\delta_{\text{C}}$  29.10); between H-18 ( $\delta_{\text{H}}$  1.13) and C-8 ( $\delta_{\text{C}}$  33.00)/C-9 ( $\delta_{\text{C}}$  47.37)/C-10 ( $\delta_{\text{C}}$

213.67); between H-19 ( $\delta_{\text{H}}$  0.84) and C-4 ( $\delta_{\text{C}}$  50.38)/C-5 ( $\delta_{\text{C}}$  26.48)/C-6 (36.37) were observed suggesting one isopropyl and two methyl groups at C-12, C-5, and C-9, respectively (figure 2). The position of carboxylate methyl group at C-1 was confirmed by HMBC correlations between H-2 ( $\delta_{\text{H}}$  3.82)/H-14 ( $\delta_{\text{H}}$  2.66 and 3.07)/H-21 ( $\delta_{\text{H}}$  3.62), methoxyl proton ( $\delta_{\text{H}}$  3.56) and C-20 ( $\delta_{\text{C}}$  174.86). In addition, the HMBC correlations between H-38 ( $\delta_{\text{H}}$  1.86) and C-22 ( $\delta_{\text{C}}$  122.92)/C-23 ( $\delta_{\text{C}}$  141.12)/C-24 ( $\delta_{\text{C}}$  38.34); H-37 ( $\delta_{\text{H}}$  1.91) and C-34 ( $\delta_{\text{C}}$  132.86)/C-35 ( $\delta_{\text{C}}$  129.14)/C-36 ( $\delta_{\text{C}}$  34.68); H-39 ( $\delta_{\text{H}}$  1.64) and C-26 ( $\delta_{\text{C}}$  79.71)/C-27 ( $\delta_{\text{C}}$  134.58)/C-28 ( $\delta_{\text{C}}$  120.22), suggested the positions of three double bonds at C-22/C-23, C-27/C-28 and C-34/C-35 and three methyl groups at C-23, C-27 and C-35. Moreover, the HMBC correlations between H-40 ( $\delta_{\text{H}}$  1.09) and C-30 ( $\delta_{\text{C}}$  68.34)/C-31 ( $\delta_{\text{C}}$  74.19)/C-32 ( $\delta_{\text{C}}$  42.19) proved the methyl and hydroxyl groups at C-31. The C-26/C-30 epoxy ring was confirmed by HMBC correlation between H-30 ( $\delta_{\text{H}}$  3.68) and C-26 ( $\delta_{\text{C}}$  79.71). Consequently, the structure of compound **1** was elucidated to lobophytone H, a biscembranoid from the soft coral *Lobophytum pauciflorum* [7].

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

C	<b>1</b>			<b>2</b>			<b>3</b>		
	<sup>*</sup> δ <sub>C</sub>	δ <sub>C</sub> <sup>a,c</sup>	δ <sub>H</sub> <sup>a,d</sup> (mult., J = Hz)	#δ <sub>C</sub>	δ <sub>C</sub> <sup>b,c</sup>	δ <sub>H</sub> <sup>b,d</sup> (mult., J = Hz)	<sup>&amp;</sup> δ <sub>C</sub>	δ <sub>C</sub> <sup>a,c</sup>	δ <sub>H</sub> <sup>a,d</sup> (mult., J = Hz)
1	49.2	49.21	-	49.2	49.62	-	49.5	49.10	-
2	47.6	47.48	3.82 (dd, 7.5, 8.5)	45.2	45.79	3.71 (dd, 7.5, 8.5)	47.4	47.67	3.82 (dd, 7.5, 8.5)
3	212.0	212.07	-	212.0	212.18	-	212.0	213.53	-
4	50.4	50.38	2.31 (d, 18.5)/ 3.18 (d, 10.0)	53.4	50.88	2.28 (d, 18.5) 2.91 (d, 10.0)	50.3	50.61	2.31 (d, 18.5) 3.19 (d, 10.5)
5	26.4	26.48	2.02 (m)	27.2	28.31	1.79 (m)	26.4	26.54	2.01 (m)
6	36.4	36.37	0.99 (m), 1.12 (m)	37.8	37.02	1.05 (m), 1.13 (m)	36.2	36.38	0.99 (m), 1.12 (m)
7	25.4	24.72	1.11 (m), 1.25 (m)	25.4	25.02	1.11 (m), 1.24 (m)	25.5	24.72	1.11 (m), 1.25 (m)
8	32.9	33.00	1.62 (m)	33.9	33.88	1.53 (m)	33.8	33.19	1.51 (m)
9	47.3	47.37	2.53 (m)	47.6	47.47	2.50 (m)	47.5	47.77	2.52 (m)
10	213.6	213.67	-	213.4	216.67	-	213.8	213.71	-
11	32.6	32.52	2.98 (dd, 10.0, 17.0)	32.9	32.68	2.98 (dd, 10.0, 17.0)	32.4	32.61	2.98 (dd, 10.0, 16.5)
12	50.9	51.44	3.03 (dd, 2.0, 10.5)	51.4	51.74	3.02 (dd, 2.0, 10.5)	51.6	51.72	3.03 (dd, 1.5, 10.5)
13	212.6	212.73	-	210.6	215.54	-	231.5	208.71	-
14	46.6	46.73	2.66 (d, 19.0) 3.07 (d,19.0)	43.2	43.62	2.59 (d, 19.0) 3.07 (d, 19.0)	45.8	45.90	2.58 (d, 19.0) 3.02 (d, 19.0)
15	29.1	29.10	2.34 (m)	28.9	28.31	2.33 (m)	29.1	29.04	2.34 (m)
16	21.3	21.87	0.96 (d, 7.0)	21.4	21.49	0.98 (d, 7.0)	21.2	21.29	0.99 (d, 7.0)
17	18.1	18.56	0.75 (d, 7.0)	17.8	18.16	0.76 (d, 7.0)	17.6	18.71	0.75 (d, 7.0)
18	17.0	17.54	1.13 (d, 7.0)	17.5	17.63	1.14 (d, 7.0)	17.4	17.53	1.13 (d, 7.0)
19	21.7	21.72	0.84 (d, 7.0)	22.8	22.46	0.85 (d, 7.0)	21.2	21.38	0.84 (d, 7.0)
20	174.8	174.86	-	174.7	176.63	-	174.4	174.47	-
21	42.1	42.08	3.62 (d, 10.0)	42.3	42.61	3.53 (d, 6.0)	42.7	42.24	3.56 (d, 10.0)
22	122.9	122.92	5.12 (d, 11.0)	129.2	129.33	5.13 (d, 11.0)	127.4	127.47	4.88 (d, 5.5)
23	141.0	141.12	-	138.7	138.50	-	136.5	136.58	-
24	38.3	38.34	1.85 (m), 2.56 (m)	38.8	38.39	1.81 (m), 2.51 (m)	30.3	30.41	1.30 (m), 2.30 (m)
25	26.7	26.83	1.32 (m), 1.61 (m)	25.7	26.59	1.31 (m), 1.60 (m)	37.7	37.35	2.28 (m), 2.77 (m)
26	79.7	79.71	4.01 (br d, 7.0)	79.5	79.65	3.35 (br d, 7.0)	215.9	215.83	-
27	134.4	134.58	-	71.0	71.35	-	45.0	45.66	2.36 (d, 1.5)
28	120.2	120.22	5.56 (d, 3.5)	34.9	34.64	1.54 (m), 2.25 (m)	31.5	31.56	1.36 (m), 1.87 (m)
29	24.7	24.05	1.98 (m)	20.1	20.67	1.80 (m)	25.4	25.46	1.96 (m)
30	68.3	68.34	3.68 (dd, 4.0, 10.0)	74.9	75.06	3.19 (dd, 6.5, 10.5)	80.2	80.34	3.47 (dd, 6.0, 10.5)
31	74.2	74.19	-	74.0	74.06	-	87.0	87.11	-
32	42.2	42.19	1.62 (dd, 4.0, 7.5)	41.0	41.15	1.43 (dd, 4.0, 10.5)	31.5	31.59	1.73 (dd, 4.0, 7.5)
33	70.1	70.19	4.81 (br d, 10.5)	65.4	68.00	4.99 (dd, 3.0, 9.5)	75.3	75.34	5.04 (dd, 7.0, 10.0)
34	132.8	132.86	-	138.5	133.04	-	135.2	135.33	-
35	129.1	129.14	-	126.2	129.33	-	126.1	126.20	-
36	34.6	34.68	2.02 (m), 2.58 (m)	33.0	34.30	2.01 (m), 2.48 (m)	33.1	33.96	2.01 (m), 2.56 (m)
37	19.9	19.88	1.91 (s)	19.1	19.19	1.82 (s)	19.0	19.11	1.84 (s)
38	19.8	19.78	1.86 (s)	18.1	18.16	1.74 (s)	19.2	19.10	1.65 (s)
39	20.3	20.29	1.64 (s)	27.0	26.25	1.10 (s)	17.4	17.53	1.08 (d, 7.0)
40	23.1	23.10	1.09 (s)	25.1	24.40	1.14 (s)	23.5	23.64	1.28 (s)
COOMe	51.4	51.00	3.56 (s)	51.2	51.74	3.55 (s)	51.1	51.18	3.52 (s)

<sup>a</sup>Measured in CDCl<sub>3</sub>, <sup>b</sup>measured in CD<sub>3</sub>OD, <sup>c</sup>125 MHz, <sup>d</sup>500 MHz, <sup>\*</sup>δ<sub>C</sub> of lobophytone H [7], #δ<sub>C</sub> of lobophytone U [8] &δ<sub>C</sub> of methyl tortuoate B [9].

Compound **2** was also obtained as a white amorphous powder and its molecular formula was determined to be C<sub>41</sub>H<sub>64</sub>O<sub>9</sub> by HR-ESI-MS *m/z* 723.44482 [M+Na]<sup>+</sup> (Calcd. for C<sub>41</sub>H<sub>64</sub>O<sub>9</sub>Na<sup>+</sup>,

723.44426). The <sup>1</sup>H-NMR spectrum of compound **2** showed the following signals: one olefinic proton at δ<sub>H</sub> 5.13 (d, *J* = 11.0 Hz); four secondary methyl groups at δ<sub>H</sub> 0.76 (d, *J* = 7.0 Hz), 0.85 (d, *J* = 7.0 Hz),

0.98 (d,  $J = 7.0$  Hz), and 1.14 (d,  $J = 7.0$  Hz); four tertiary methyl groups at  $\delta_{\text{H}}$  1.10, 1.14, 1.74, 1.82 (each, 3H, s); one methoxy group at  $\delta_{\text{H}}$  3.55 (s). The  $^{13}\text{C}$ -NMR and DEPT spectra of compound **2** exhibited the presence of 41 carbons: four carbonyl, six quaternary, ten methine, twelve methylene, nine methyl carbons. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data of compound **2** suggested the structure of compound **2** to be lobophytone U [8]. The HMBC correlations from H-39 ( $\delta_{\text{H}}$  1.10) to C-26 ( $\delta_{\text{C}}$  79.65)/C-27 ( $\delta_{\text{C}}$  71.35)/C-28 ( $\delta_{\text{C}}$  34.64); from H-40 ( $\delta_{\text{H}}$  1.14) to C-30 ( $\delta_{\text{C}}$  75.06)/C-31 ( $\delta_{\text{C}}$  74.06)/C-32 ( $\delta_{\text{C}}$  41.15), suggested both methyl and hydroxy groups at C-27 and C-31. The epoxy ring at C-26 ( $\delta_{\text{C}}$  79.65) and C-30 ( $\delta_{\text{C}}$  75.06) were proved by the HMBC correlations between H-26 ( $\delta_{\text{H}}$  3.33) and C-30 ( $\delta_{\text{C}}$  75.06) as well as between H-30 ( $\delta_{\text{H}}$  3.19) and C-26 ( $\delta_{\text{C}}$  79.65). Thus, the structure of compound **2** was defined as lobophytone U, a biscembranoid from the soft coral *Lobophytum pauciflorum* [8].

The molecular formula of compound **3** was determined to be  $\text{C}_{41}\text{H}_{62}\text{O}_8$  by HR-ESI-MS  $m/z$

683.45226  $[\text{M}+\text{H}]^+$  (Calcd. for  $\text{C}_{41}\text{H}_{63}\text{O}_8^+$ , 683.45175). The  $^1\text{H}$ -NMR spectrum of compound **3** (table 1) showed the signals of one olefinic proton  $\delta_{\text{H}}$  4.88 (d,  $J = 5.5$  Hz); one methoxy group at  $\delta_{\text{H}}$  3.52 (s); five secondary methyl groups at  $\delta_{\text{H}}$  0.75 (d,  $J = 7.0$  Hz), 0.99 (d,  $J = 6.5$  Hz), 1.13 (d,  $J = 7.0$  Hz), 0.84 (d,  $J = 7.0$  Hz), and 1.08 (d,  $J = 7.0$  Hz); three tertiary methyl groups at  $\delta_{\text{H}}$  1.84, 1.65, and 1.28 (each, 3H, s); two signals of oxygenated methine protons at  $\delta_{\text{H}}$  3.47 (dd,  $J = 6.0, 10.5$  Hz) and 5.04 (dd,  $J = 7.0, 10.0$  Hz). The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of compound **3** indicated that structure of **3** was identical to those of methyl tortuoate B [9]. The HMBC correlations of H-40 ( $\delta_{\text{H}}$  1.28) and C-30 ( $\delta_{\text{C}}$  80.34)/C-31 ( $\delta_{\text{C}}$  87.11)/C-32 ( $\delta_{\text{C}}$  31.59) as well as H-30 ( $\delta_{\text{H}}$  3.47) and C-33 ( $\delta_{\text{C}}$  75.34) suggested the both methyl and hydroxyl groups at C-31 and epoxy group at C-30/C-33. So, the structure of compound **3** was determined to be methyl tortuoate B [9], a biscembranoid from the soft coral *Sarcophyton tortuosum*.

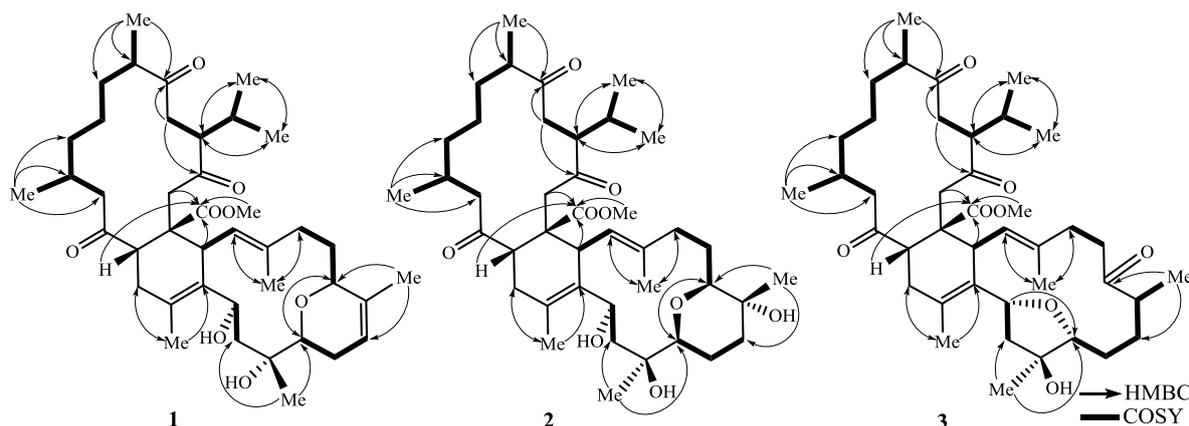


Figure 2: The important HMBC and COSY correlations of **1-3**

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