

CHEMICAL COMPONENTS FROM MARINE SPONGE *IANTHELLA BASTA*

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Abstract

By various chromatographic separations, five known compounds, 24-oxocholesterol, indole-3-aldehyde, phenylacetic acid, thymidine, and deoxycytidine were isolated from the sponge *Ianthella basta*. Their structures were elucidated by spectroscopic methods and in comparison with the reported data. All compounds were isolated from this sponge for the first time.

Keywords: *Ianthella basta*, Ianthellidae, 24-oxocholesterol, indole-3-aldehyde, phenylacetic acid.

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. The genus *Ianthella basta* Pallas, 1766 is one of the fan-shaped sponge in the family Ianthellidae. The chemical investigations of *I. basta* led to the isolations of bastadins [1, 2] and hemibastadinols [2, 3]. They exhibited various activities such as antimicrobial [3], cytotoxic activities [2], and exhibited differential activity as SR Ca²⁺ channel agonists of the Ry1R FKBP12 complex [4]. During our search of bioactive compounds, five known compounds, 24-oxocholesterol, indole-3-aldehyde, phenylacetic acid, thymidine, and deoxycytidine were isolated from the sponge *I. basta*. Their structures were elucidated by NMR spectra.

2. MATERIAL AND METHODS

2.1. Animal materials

The specimens of *Ianthella basta* 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 (NT01) is deposited at Institute of Marine Biochemistry, VAST, Hanoi, Vietnam.

2.2. General experimental procedures

All NMR spectra were recorded on a Agilent 400-MR NMR spectrometer, 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 μ m, 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 *Ianthella basta* (1.0 kg) were well grinded and extracted with hot MeOH three times and then concentrated under reduced pressure to give MeOH extract (IB, 35 g). This extract was suspended in water and then partitioned with chloroform to obtain the CHCl₃ (IB 1, 20 g) and water (IB2, 15 g) layers after removal of the solvents *in vacuo*. The IB1 layer (20 g) was chromatographed on a silica gel column and eluting with a gradient elution of *n*-hexane-acetone (40:1 \rightarrow 0:1, v/v) to obtain four sub-fractions, IB1A (5.0 g), IB1B (4.0 g), IB1C (4.5 g), and IB1D (3.8 g). The IB1B fraction was chromatographed on a silica gel column eluting with *n*-hexane - EtOAc (6:1, v/v) to yield 1 (500 mg). The IB1C fraction was chromatographed on an YMC RP-18 column eluting with acetone - water (3:1, v/v) to yield 2 (15.0 mg)

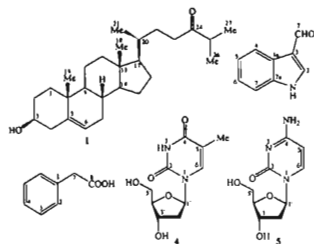


Figure 1: Chemical structure of 1-5 from *lanthella basta*

and 3 (20.0 mg). The IB2 fraction was chromatographed on a silica gel column with a gradient elution of CHCl_3 – MeOH (10:1 → 2:1, v/v) to obtain three sub-fractions, IB2A (4.8 g), IB2B (4.0 g). The IB2B fraction was chromatographed on an YMC RP-18 column eluting with MeOH – water (1:4, v/v) to yield 4 (20.0 mg) and 5 (17.0 mg).

24-Oxcholesterol (1): White amorphous powder; mp: 136 °C; $[\alpha]_D^{25} = -40$ (c 0.1, CHCl_3); $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz), see table 1.

Indole-3-aldehyde (2): Yellow amorphous powder; mp: 197 °C; $^1\text{H-NMR}$ (CD_3OD , 400 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz), see table 2.

Phenylacetic acid (3): White amorphous powder; mp: 77 °C; $^1\text{H-NMR}$ (CD_3OD , 400 MHz) and $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz), see table 2.

Thymidine (4): White amorphous powder; mp: 186 °C; $^1\text{H-NMR}$ (CD_3OD , 400 MHz) δ_{H} : 7.78 (s, H-6), 1.85 (s, H-7), 6.25 (t, $J = 6.8$ Hz, H-1'), 2.21 (m, H-2'), 4.38 (m, H-3'), 3.89 (m, H-4'), 3.70 (dd, $J = 3.2, 12.0$ Hz, H-5a'), and 3.78 (dd, $J = 3.2, 12.0$ Hz, H-5b'); $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz) δ_{C} : 152.31 (C-2), 166.35 (C-4), 111.49 (C-5), 138.13 (C-6), 12.45 (C-7), 86.17 (C-1'), 41.08 (C-2'), 72.14 (C-3'), 88.71 (C-4'), and 62.78 (C-5').

Deoxycytidine (5): White amorphous powder; mp: 210 °C; $^1\text{H-NMR}$ (CD_3OD , 400 MHz) δ_{H} : 5.67 (s, H-5), 7.96 (s, H-6), 1.85 (s, H-7), 6.24 (t, $J = 6.8$ Hz, H-1'), 2.20 (m, H-2'), 4.36 (m, H-3'), 3.90 (m, H-4'), 3.71 (dd, $J = 3.2, 12.0$ Hz, H-5a'), and 3.76 (dd, $J = 3.2, 12.0$ Hz, H-5b'); $^{13}\text{C-NMR}$ (CD_3OD , 100 MHz) δ_{C} : 152.18 (C-2), 166.22 (C-4), 102.62

(C-5), 142.50 (C-6), 12.41 (C-7), 86.57 (C-1'), 41.34 (C-2'), 72.23 (C-3'), 88.96 (C-4'), and 62.82 (C-5').

Table 1: The NMR data for 1 and reference compounds

Pos.	$\delta_{\text{C}}^{\text{a}}$	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ (J in Hz)
1	37.2	37.20	0.96 (m)/1.71 (m)
2	29.8	29.74	1.15 (m)
3	71.8	71.40	3.38 *
4	42.3	41.94	2.14 (m)
5	140.7	140.75	-
6	121.7	121.42	5.24 (br s)
7	31.6	31.20	1.71 (m)
8	31.3	31.77	1.40 (m)
9	50.1	49.99	0.82 (m)
10	36.5	36.39	-
11	21.1	20.96	1.39 (m)
12	39.7	39.65	1.05 (m)/1.87 (m)
13	42.3	42.24	-
14	56.7	56.62	0.90 (m)
15	24.2	24.16	1.46 (m)
16	28.1	28.03	1.74 (m)
17	55.9	55.76	1.00 (m)
18	11.9	11.76	0.58 (s)
19	19.4	19.28	0.90 (s)
20	35.4	35.29	1.27 (m)
21	18.3	18.18	0.81 (d, 6.0)
22	31.8	31.25	1.86 (m)
23	37.2	37.17	2.32 (m)
24	215.5	216.17	-
25	40.8	40.75	2.51 (m)
26	18.4	18.25	0.98 (d, 6.0)
27	18.5	18.39	0.98 (d, 6.0)

*Measured in CDCl_3 , ^a100 MHz, ^c400 MHz, ^d δ_{C} of 24-oxocholesterol [5].

3. RESULTS AND DISCUSSION

Compound 1 was obtained as a white amorphous powder. The $^1\text{H-NMR}$ spectrum of 1 (table 1) showed the following signals: one olefinic proton at δ_{H} 5.24 (s, 1H), one oxygenated methine protons at δ_{H} 3.38 (m, 1H), two tertiary methyl at δ_{H} 0.58 (s, 3H) and 0.90 (s, 3H), three secondary methyl groups at δ_{H} 0.81 (d, $J = 6.0$ Hz), 0.98 (d, $J = 6.0$ Hz, 6H). The $^{13}\text{C-NMR}$ and DEPT spectra showed the presence of one carbonyl (δ_{C} 216.17), two olefinic (δ_{C} 121.42 and 140.75), one oxygenated (δ_{C} 71.40), two quaternary (δ_{C} 36.39 and 42.24), six methine (δ_{C} 31.77, 35.29, 40.75, 49.99, 56.62, and 55.76), ten methylene (δ_{C} 20.96, 24.16, 28.03, 29.74, 31.20, 31.25, 37.17, 37.20, 39.65, and 41.94)

Table 2: The NMR data for 2, 3 and reference compounds

2			
Pos.	$\delta_C^{(a)}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)
2	138.5	139.68	8.04 (s)
3	118.1	120.07	-
3a	124.1	125.66	-
4	120.8	122.36	8.13 (d, 7.6)
5	122.1	123.59	7.20 (dd, 7.6, 8.0)
6	123.4	124.98	7.24 (dd, 7.6, 8.0)
7	112.4	113.11	7.45 (d, 7.6)
7a	137.1	138.89	-
8	185.0	187.38	9.84 (s)

3			
Pos.	$\delta_C^{(5)}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (J in Hz)
1	135.6	135.97	-
2	129.9	130.32	7.25*
3	129.0	129.41	7.26*
4	127.5	127.86	7.21 (t, 8.0)
5	129.0	129.41	7.26*
6	129.9	130.32	7.25*
7	41.1	41.91	3.56 (s)
8	172.8	175.61	-

*Measured in CD₃OD, ^b100 MHz, ^c400 MHz, ^a δ_C of indole-3-aldehyde [6], ^b δ_C of phenylacetic acid [7].

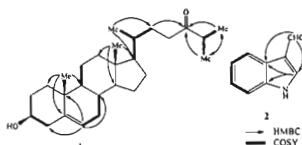


Figure 2: The important HMBC and COSY correlations of 1 and 2

and five methyl carbons (δ_C 11.76, 18.18, 18.25, 18.39, and 19.28) (table 1). Analysis of 1D- and 2D-NMR spectroscopic data indicated structure of 1 was identical to 24-oxocholesterol [5]. The HMBC correlations from methyl H-19 (δ_H 0.90) to C-1 (δ_C 37.20), C-5 (δ_C 140.75), C-9 (δ_C 49.99), and C-10 (δ_C 36.39); from H-6 (δ_H 5.24) to C-4 (δ_C 41.94), C-5 (δ_C 140.75), C-8 (δ_C 31.77), and C-10 (δ_C 36.39) confirmed the methyl group and the double bond were at C-10 and C-5/C-6, respectively. The hydroxyl group at C-3 was proved by COSY correlations of H-2 (δ_H 1.86)/H-3 (δ_H 3.38)/H-4 (δ_H 2.14). The HMBC correlations between H-26/H-27 (δ_H 0.98) and C-24 (δ_C 216.17)/C-25 (δ_C 40.75); between H-22 (δ_H 1.15)/H-23 (δ_H 2.32) and C-24 (δ_C 216.17) suggested the isopropyl and carbonyl groups at C-24. Consequently, compound 1 was determined to be 24-oxocholesterol.

Compound 2 was obtained as a white amorphous powder. The ¹H-NMR spectrum of 2 (Table 2) showed signals for one benzene ring with *ortho*-substitutes at δ_H 7.20 (1H, dd, J = 7.6, 8.0 Hz), 7.24

(1H, dd, J = 7.6, 8.0 Hz), 7.45 (1H, d, J = 7.6 Hz), and 8.13 (1H, d, J = 7.6), one singlet proton at δ_H 8.04 (1H, s), one aldehyde group at δ_H 9.84 (1H, s). The ¹³C-NMR and DEPT spectra exhibited the presence of one carbonyl (δ_C 187.38), three quaternary (δ_C 120.07, 125.66, and 138.89), five methine carbons (δ_C 113.11, 122.36, 123.59, 124.98, and 139.68). The ¹H- and ¹³C-NMR data of 2 was identical to indole-3-aldehyde, an indol alkaloid was isolated from red sea sponge *Hyrtios erectus* [6]. The HMBC correlations (Figure 2) between H-7 (δ_H 9.84) and C-2 (δ_C 139.68), C-3 (δ_C 120.07), and C-3a (δ_C 125.66); between H-2 (δ_H 8.04) and C-3 (δ_C 120.07), C-3a (δ_C 125.66), C-7 (δ_C 113.11), and C-7a (δ_C 138.89) confirmed the aldehyde group was at C-3 of indole. The *ortho*-disubstitutes of benzene ring were confirmed by COSY correlations of H-4 (δ_H 8.13)/H-5 (δ_H 7.20)/H-6 (δ_H 7.24)/H-7 (δ_H 7.45). Based on the above evidence, structure of 2 were determined to be indole-3-aldehyde.

The ¹H-NMR of 3 showed the presence of five aromatic protons for phenyl group at δ_H 7.21 (1H, s), 7.25 (2H), and 7.26 (2H) and two methylene protons at δ_H 3.56 (2H, s). The ¹³C-NMR and DEPT spectra of 3 revealed eight carbons signals including one carbonyl at δ_C 175.6, six aromatic at δ_C 127.86 (1C), 129.41 (2C), 130.32 (2C), and 135.97 (1C), and one methylene carbons at δ_C 41.91 (table 2). The ¹H- and ¹³C-NMR data of 3 were identical to those of phenylacetic acid [7]. The HMBC correlations from H-7 (δ_H 3.56) to C-1 (δ_C 135.97), C-2/C-6 (δ_C 130.32) and C-8 (δ_C 175.61) confirmed an acetic acid moiety was at C-1 of phenyl ring.

Consequently, compound 3 was determined to be phenylacetic acid.

The remaining compounds were elucidated to be thymidine (4) and deoxycytidine (5). Their structures were established based on spectral and chemical evidence, which agreed with previous studies [6]. All compounds 1-5 were isolated from *I. basta* for the first time.

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