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Furanosesterterpenes from the marine sponge *Ircinia echinata* (Keller, 1889)

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Abstract

Four furanosesterterpene, (7*E*,12*E*,20*Z*,18*β*)-variabilin (**1**), (12*E*,20*Z*,18*β*)-8-hydroxyvariabilin (**2**), (7*E*,11*E*,3*β*)-3,7,11-trimethyl-14-(furan-3-yl)tetradec-7,11-dienoic acid (**3**), and furoscalarol (**4**), and one sterol, 3*β*-hydroxycholest-5-en-7-one (**5**) were isolated from the methanol extract of the sponge *Ircinia echinata* (Keller, 1889). Their structures were elucidated by 1D and 2D-NMR spectra and in comparison with those reported in the literature.

Keywords. Sponge, *Ircinia echinata*, furanosesterterpene, sterol.

1. INTRODUCTION

Ircinia is a genus of marine demosponge in the family Irciniidae providing a number of furanosesterterpenoids and steroids. These compounds exhibited a wide spectrum of bioactivities such as anticancer [1-4], antimicrobial [5], and antiviral activities [6]. However, chemical and biological studies of *Ircinia echinata* have not been studied yet. In this paper, we report the isolation and structural determination of four furanosesterterpenes and one sterol from the Vietnamese sponge, *I. echinata* (figure 1).

2. MATERIAL AND METHODS

2.1. Animal material

The sponge *Ircinia echinata* was collected at Coto Island, Quangninh, Vietnam in April, 2014. Its scientific name was identified by one of the authors, Prof. Do Cong Thung. A voucher specimen (HM04) was deposited at the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

Optical rotations were measured on a Jasco DIP-370 automatic polarimeter. The 1D- and 2D-NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Column chromatography was performed using either silica-gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Whitehouse Station, NJ) or reverse phase (RP-18 resins, 150 μm, YMC Co. Ltd.). Thin layer chromatography (TLC) was carried out using pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck). Spots were detected under UV radiation (254 and 365 nm), sprayed with 10% H_2SO_4 solution followed by heating with heat gun.

2.3. Extraction and isolation

Frozen dried sample of *I. echinata* (10 kg) was ground and ultrasonically extracted with methanol at 40 °C (10 L × three times, 5h each) to give MeOH extract (230.0 g) after removal the solvent *in vacuo*. Then, MeOH extract was suspended in water and partitioned with dichloromethane to give dichloromethane extract (IED, 90.0 g) and water layer (IEW, 140.0 g). The IED was roughly separated on a silica gel column chromatography, eluting with gradient solvent system of *n*-hexane/acetone (100/1 to 0/1, v/v) to give six fractions, IED1-IED6. IED2 (19.3 g) was repeatedly subjected to a silica gel column and eluted with *n*-hexane/acetone (6/1, v/v) to furnish five fractions, IED2A-IED2E. Compound **4** (12.0 mg) was obtained from IED2A by a silica gel column, eluting with *n*-hexane/ethyl acetate (5/1, v/v). IED2C (1.3 g) was purified on a RP-18 column, eluting with methanol/water (6/1, v/v) to yield compound **3** (8.0

mg). IED2D was chromatographed on a silica gel column, eluting with *n*-hexane/acetone (2.5/1, v/v) to yield compound **2** (11.0 mg). IED3 (12.6 g) was chromatographed on a silica gel column, eluting with *n*-hexane/acetone (6/1, v/v) to give four fractions, IED3A-IED3D. Compound **1** (30.0 mg) was obtained from IED3A (1.7 g) by a RP-18 column, eluting with methanol/water (5/1, v/v). Compound **5** (20.0 mg) was isolated from IED5A fraction on a RP-18 column, eluting with acetone/water (1/1, v/v).

(7*E*,12*E*,20*Z*,18*β*)-Variabilin (1): colorless oil,$[\alpha]_D^{25}$: -25.9 (*c* = 0.1, MeOH), molecular formula $C_{25}H_{34}O_4$, ^{1}H- and ^{13}C-NMR data, see table 1.

(12*E*,20*Z*,18*β*)-8-Hydroxyvariabilin (2): colorless oil, $[\alpha]_D^{25}$: -19.6 (*c* = 0.1, MeOH), molecular formula $C_{25}H_{36}O_5$, ^{1}H- and ^{13}C-NMR data, see table 1.

(7*E*,11*E*,3*β*)-3,7,11-Trimethyl-14-(furan-3-yl)tetradec-7,11-dienoic acid (3): colorless oil, optical rotation $[\alpha]_D^{25}$: -21.5 (*c* = 0.1, MeOH), molecular formula $C_{21}H_{32}O_3$, ^{1}H- and ^{13}C-NMR data, see table 2.

Furoscalarol (4): colorless oil, $[\alpha]_D^{25}$: +58.2 (*c* = 0.1, MeOH), molecular formula $C_{27}H_{40}O_4$, ^{1}H- and ^{13}C-NMR data, see table 2.

3β-Hydroxycholest-5-en-7-one (5): colorless oil, $[\alpha]_D^{25}$: +45.1 (*c* = 0.1, MeOH), molecular formula $C_{27}H_{44}O_2$, ^{1}H- and ^{13}C-NMR data, see table 2.

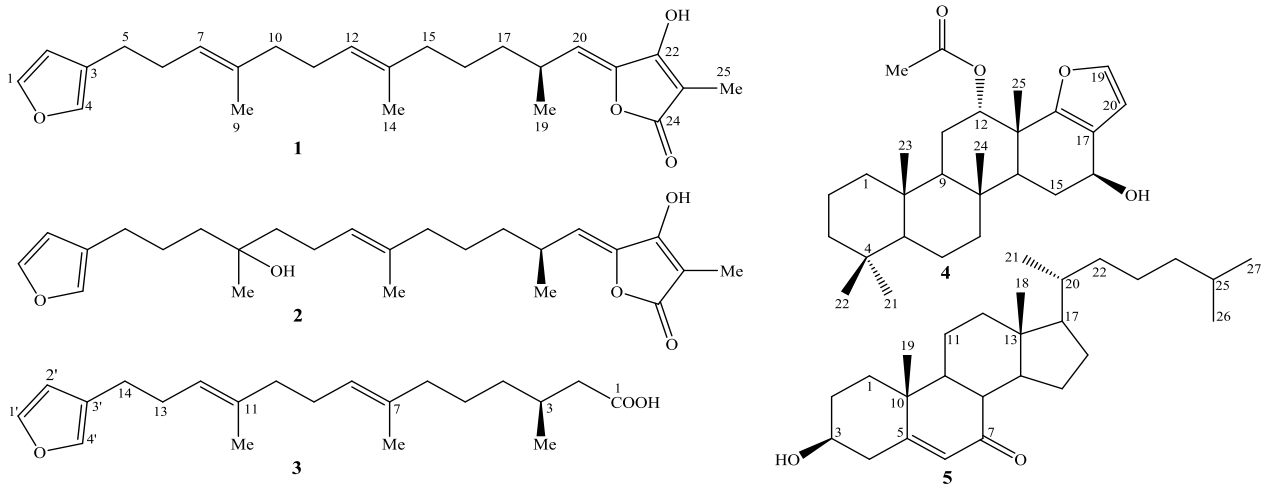

Figure 1: Chemical structures of **1-5**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a colorless oil. The ^{1}H-NMR spectrum of **1** (in CD_3OD) showed six olefinic protons at δ_H 5.11 (t, *J* = 6.5 Hz), 5.17 (t, *J* = 7.5 Hz), 5.30 (d, *J* = 10.0 Hz), 6.31 (br s), 7.25 (br s), and 7.38 (br s), four methyl groups at δ_H 1.08 (d, *J* = 7.0 Hz), 1.58 (s), 1.59 (s), and 1.76 (s). The ^{13}C-NMR and DEPT spectra of **1** exhibited the presence of 25 carbons, including seven non-protonated carbons (δ_C 99.01, 126.20, 135.81, 136.52, 144.94, 164.52, and 173.46), seven methines (δ_C 31.87, 143.73, 112.01, 140.06, 125.21, 125.60, and 115.84), seven methylenes (δ_C 25.98, 26.81, 27.43, 29.59, 37.62, 40.71, and 40.42), and four methyl groups (δ_C 6.09, 15.90, 16.11, and 21.06). Analytical ^{1}H- and ^{13}C-NMR data of **1** indicated its NMR data were very similar to those of variabilin [7]. The HMBC correlations from H-5 (δ_H 2.45) to C-2 (δ_C 112.01)/C-3 (δ_C 126.20)/C-4 (δ_C 140.06)/C-6 (δ_C 29.59)/C-7 (δ_C 125.21) indicated the furan ring with C-3-substituted. Moreover, the HMBC correlations between H-9 (δ_H 1.59) and C-7 (δ_C 125.21)/C-8 (δ_C 136.52)/C-10 (δ_C 40.71); H-14 (δ_H 1.58) and C-12 (δ_C 125.60)/C-13 (δ_C 135.81)/C-15 (δ_C 40.42); H-19 (δ_H 1.08) and C-17 (δ_C 37.62)/C-18 (δ_C 31.87)/C-20 (δ_C 115.84); H-25 (δ_H 1.76) C-22 (δ_C 164.52)/C-23 (δ_C 99.01)/C-24 (δ_C 173.46); and between H-20 (δ_H 5.30) and C-21 (δ_C 144.94)/C-22 (δ_C 164.52) suggested the positions of double bonds at C-7/C-8, C-12/C-13, C-20/C-21, and C-22/C-23. In addition, comparison of ^{13}C-NMR data of **1** to those of variabilin [7] showed the similarity (recorded in the same solvent $CDCl_3$) [8]. Thus, the structure of **1** was determined to be (7*E*,12*E*,20*Z*,8*β*)-variabilin, a compound was already reported from the sponge *Iricinia variabilis* [9].

Table 1: ^{1}H- and ^{13}C-NMR data of compounds **1** and **2**

	1				**2**			
C	δ_C [#]	δ_C [a]	δ_C [b]	δ_H [b] (mult., *J*, Hz)	δ_C [$]	δ_C [a]	δ_C [b]	δ_H [b] (mult., *J*, Hz)
1	142.4	142.52	143.73	7.38 (br s)	142.7	142.76	143.90	7.39 (br s)
2	111.0	111.13	112.01	6.31 (br s)	110.9	110.91	111.89	6.31 (br s)
3	124.9	125.03	126.20	-	124.8	124.87	126.37	-
4	138.7	138.83	140.06	7.25 (br s)	138.8	138.87	140.08	7.28 (br s)
5	25.0	25.05	25.98	2.45 (t, 7.5)	25.1	25.18	26.19	2.43 (t, 7.5)
6	28.4	28.45	29.59	2.25 (q, 7.5)	24.4	24.42	25.60	1.63 (m)
7	123.7	123.72	125.21	5.17 (t, 7.5)	41.2	41.32	42.17	1.49 (m)
8	135.7	135.79	136.52	-	73.9	73.93	73.23	-
9	16.0	16.06	16.11	1.59 (s)	26.5	26.61	26.87	1.15 (s)
10	39.5	39.55	40.71	2.01 (m)	41.2	41.20	42.62	1.43 (m)
11	26.5	26.57	27.43	2.10 (q, 6.5)	22.5	22.58	23.51	2.00 (m)
12	124.4	124.35	125.60	5.11 (t, 6.5)	124.3	124.35	126.02	5.17 (t, 7.0)
13	134.7	134.88	135.81	-	135.6	135.71	135.74	-
14	15.8	15.83	15.90	1.58 (s)	15.9	16.01	15.81	1.60 (s)
15	39.6	39.69	40.42	2.01 (m)	39.3	39.24	40.41	2.00 (m)
16	25.6	25.72	26.81	1.40 (m)	25.5	25.57	26.78	1.40 (m)
17	36.5	36.66	37.62	1.38 (m)	36.5	36.52	37.62	1.40 (m)
18	30.9	30.87	31.87	2.77 (m)	30.7	30.73	31.79	2.76 (m)
19	20.6	20.66	21.06	1.08 (d, 7.0)	20.5	20.62	21.06	1.08 (d, 6.5)
20	117.1	115.62	115.84	5.30 (d, 10.0)	115.7	115.59	115.61	5.29 (d, 10.0)
21	142.9	142.77	144.94	-	142.0	143.10	145.20	-
22	162.4	n.d.	164.52	-	162.3	n.d.	165.00	-
23	99.0	99.49	99.01	-	99.2	99.31	98.50	-
24	172.5	n.d.	173.46	-	172.1	n.d.	173.60	-
25	6.0	6.17	6.09	1.76 (s)	6.2	6.21	6.09	1.76 (s)

[a)]recorded in $CDCl_3$, [b)]recorded in CD_3OD, [#]δ_C of (7E,12E,20Z,18β)-variabilin [8],
[$]$\delta_C$ of (12E,20Z,18β) 8-hydroxyvariabilin [8], n.d., not determined.

Compound **2** was also obtained as a colorless oil. The ^{1}H-NMR spectrum of **2** showed the signals of five olefinic protons at δ_H 5.17 (t, *J* = 7.0 Hz), 5.29 (d, *J* = 10.0 Hz), 6.31 (br s), 7.28 (br s), and 7.39 (br s); four methyl groups at δ_H 1.08 (d, *J* = 6.5 Hz), 1.15 (s), 1.60 (s), and 1.76 (s). The ^{13}C-NMR and DEPT spectra of **2** exhibited the signals of 25 carbons, including seven non-protonated carbons (δ_C 73.23, 98.50, 126.37, 135.74, 145.20, 165.00, and 173.60), six methines (δ_C 31.79, 111.89, 115.61, 126.02, 140.08, and 143.90), eight methylenes (δ_C 23.51, 25.60, 26.19, 26.78, 37.62, 40.41, 42.17, and 42.62), and four methyl groups (δ_C 6.09, 15.81, 21.06, and 26.87). Analytical ^{1}H- and ^{13}C-NMR data of **2** indicated that its structure was similar to those of 8-hydroxyvariabilin [8]. The HMBC correlations from H-5 (δ_H 2.43) to C-2 (δ_C 111.89)/C-3 (δ_C 126.37)/C-4 (δ_C 140.08)/C-6 (δ_C 25.60)/C-7 (δ_C 42.17); from H-9 (δ_H 1.15) to C-7 (δ_C 42.17)/C-8 (δ_C 73.23)/C-10 (δ_C 42.62) confirmed the position of 3-substituted furan ring at C-5 and hydroxyl group at C-8. The HMBC correlations between H-14 (δ_H 1.60) and C-12 (δ_C 126.02)/C-13 (δ_C 135.74)/C-15 (δ_C 40.41); H-19 (δ_H 1.08) and C-17 (δ_C 37.62)/C-18 (δ_C 31.79)/C-20 (δ_C 115.61); H-20 (δ_H 5.29) and C-21 (δ_C 145.20)/C-22 (δ_C 165.00); and between H-25 (δ_H 1.76) and C-22 (δ_C 165.00)/C-23 (δ_C 98.50)/C-24 (δ_C 173.60) confirmed the positions of three double bonds at C-12/C-13, C-20/C-21, and C-23/C-24. Thus, the structure of **2** was defined as (12*E*,20*Z*,18*β*)-8-hydroxyvariabilin, a furanosesterterpene from the sponge *Sarcotragus* sp. [8].

The ^{1}H-NMR spectrum of **3** showed the signals: five olefinic protons at δ_H 5.11 (1H, t, *J* = 7.5 Hz), 5.19 (1H, t, *J* = 7.0 Hz), 6.31 (1H, br s), 7.26 (1H, br s), and 7.38 (br s); three methyl groups at δ_H 0.95 (3H, d, *J* = 6.5 Hz), 1.60 (6H, s)]. The ^{13}C-NMR and DEPT spectra of **3** exhibited the signals of 21 carbons: four non-protonated carbons (δ_C 126.20, 136.12, 136.57, and 180.03), six methines (δ_C 31.95, 112.01, 125.18, 125.29, 143.75, and 140.06), eight methylenes (δ_C 25.98, 26.54, 27.49, 29.59, 37.79, 40.79, 40.94, and 45.46), and three methyl groups (δ_C 15.96, 16.11, and 20.32). The ^{1}H- and ^{13}C-NMR data of **3** were similar to those of 7*E*,11*E*,3*β*)-3,7,11-

Table 2: ^{1}H- and ^{13}C-NMR data of compounds **3-5**

3				**4**				**5**		
C	δ_C [#]	δ_C [b]	δ_H [b] (*J*, Hz)	C	δ_C [$]	δ_C [a]	δ_H [a] (*J*, Hz)	δ_C [¥]	δ_C [a]	δ_H [a] (*J*, Hz)
1	186.7	180.03	-	1	39.6	39.69	0.62 (m)/ 1.58 (m)	36.4	36.37	1.21 (m)/ 1.95 (m)
2	41.2	45.46	2.00 (m)/ 2.23 (m)	2	18.1	18.13	1.41 (m) 1.60 (m)	31.2	31.22	1.61 (m) 1.94 (m)
3	30.1	31.95	1.94 (m)	3	41.3	41.35	1.08 (m)/ 1.88 (m)	70.6	70.54	3.67 (m)
4	36.2	37.79	1.18 (m)/ 1.31 (m)	4	33.2	33.31	-	41.9	41.83	2.40 (m)/2.50 (m)
5	25.2	26.54	1.43 (m)	5	56.5	56.69	0.83 (m)	165.2	165.04	-
6	39.7	40.94	2.00 (m)	6	18.5	18.50	1.42 (m)/ 1.60 (m)	126.2	126.14	5.68 (s)
7	134.9	136.12	-	7	41.9	42.00	1.15 (m)/ 1.40 (m)	202.4	202.27	-
8	124.3	125.29	5.19 (t, 7.0)	8	36.9	37.00	-	45.5	45.43	2.23 (m)
9	26.6	27.49	2.10 (m)	9	53.0	53.12	1.29 (m)	50.0	49.99	1.35 (m)
10	39.7	40.79	2.00 (m)	10	37.1	37.20	-	38.3	38.29	-
11	135.8	136.57	-	11	21.7	21.73	1.76 (m)/ 1.85 (m)	21.3	21.24	1.59 (m)
12	123.8	125.18	5.11 (t, 7.5)	12	73.5	73.48	5.41 (br s)	38.8	38.73	1.12 (m)/ 2.03 (m)
13	28.5	29.59	2.26 (m)	13	40.7	40.78	-	41.9	43.12	-
14	25.1	25.98	2.45 (t, 7.5)	14	49.8	49.91	1.75 (m)	50.0	49.99	1.50 (m)
3-Me	19.7	20.32	0.95 (d, 6.5)	15	29.3	29.60	1.45 (m)/2.21(m)	26.4	26.33	1.25 (m)/ 2.40 (m)
7-Me	15.9	15.96	1.60 (s)	16	66.5	66.99	4.68 (m)	28.6	28.55	1.29 (m)/ 1.90 (m)
11-Me	16.1	16.11	1.60 (s)	17	120.0	119.96	-	54.9	54.83	1.11 (m)
1′	142.5	143.75	7.38 (br s)	18	156.6	157.08	-	12.0	11.98	0.68 (s)
2′	111.1	112.01	6.31 (br s)	19	140.9	141.33	7.19 (d, 2.0)	17.4	17.33	1.20 (s)
3′	125.0	126.20	-	20	108.1	108.06	6.33 (d, 2.0)	35.8	35.72	1.38 (m)
4′	138.8	140.06	7.26 (br s)	21	21.3	21.31	0.82 (s)	18.9	18.88	0.92 (d, 6.0)
				22	33.2	33.26	0.86 (s)	36.2	36.20	1.21 (m)/ 1.96 (m)
				23	15.9	15.93	0.83 (s)	23.9	23.84	1.16 (m)/ 1.33 (m)
				24	17.2	17.27	0.94 (s)	39.5	39.49	1.12 (m)
				25	22.1	22.17	1.29 (s)	28.0	28.00	1.52 (m)
				26				22.6	22.80	0.87 (d, 6.5)
				27				22.8	22.56	0.87 (d, 6.5)
				1′	170.0	170.21	-			
				2′	21.0	21.14	1.89 (s)			

[a)]recorded in $CDCl_3$, [b)]recorded in CD_3OD, [#]δ_C of (7E,11E,3β)-3,7,11-trimethyl-14-(furan-3-yl)tetradec-7,11-dienoic acid [8], [$]$\delta_C$ of furoscalarol [10], [¥]δ_C of 3β-hydroxycholest-5-en-7-one [11].

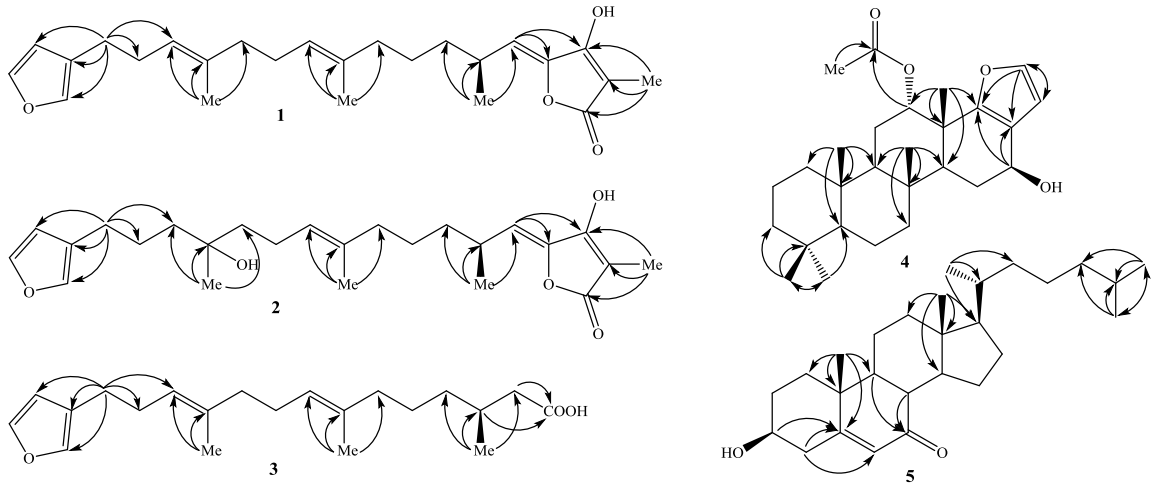

Figure 2: The key HMBC correlations of **1-5**

trimethyl-14-(furan-3-yl)tetradec-7,11-dienoic acid [8]. The positions of functional groups were determined based on analysis HSQC and HMBC spectra, as well as in comparison of similar compound in the literature. Thus, compound **3** was identified as (7*E*,11*E*,3*β*)-3,7,11-trimethyl-14-(furan-3-yl)tetradec-7,11-dienoic acid. This compound was already reported from the marine sponge *Sarcotragus* sp. [8].

The ^{1}H-NMR spectrum of compound **4** showed the signals of two olefinic protons at δ_H 6.33 (d, J = 2.0 Hz) and 7.19 (d, J = 2.0 Hz); two oxymethine protons at δ_H 5.41 (br s), and 4.68 (m), six methyl groups at δ_H 0.82 (s), 0.83 (s), 0.86 (s), 0.94 (s), 1.29 (s), and 1.89 (s).

The ^{13}C-NMR and DEPT spectra of **4** exhibited the signals of 27 carbons, including seven non-protonated carbons, seven methines, seven methylenes, and six methyl groups, indicated the presence of furano-tetracyclic sesterterpene. Moreover, the signal of carbonyl (δ_C 170.21) and methyl [δ_C 21.14 and δ_H 1.89 (s)] indicated the presence of acetyl group. The signals of four olefins at δ_C 108.06 (CH)/ δ_H 6.33 (d, J = 2.0 Hz), 119.96 (C), δ_C 141.33 (CH)/δ_H 7.19 (d, J = 2.0 Hz), and δ_C 157.08 (C)] featured for 2,3-disubstituted furan. ^{1}H- and ^{13}C-NMR data of **4** were identical to those of furoscalrol [10]. The HMBC correlations from H-21 (δ_H 0.82) to C-3 (δ_C 41.35)/C-4 (δ_C 33.31)/C-5 (δ_C 56.69)/C-22 (δ_C 33.26); from H-22 (δ_H 0.86) to C-3 (δ_C 41.35)/C-4 (δ_C 33.31)/C-5 (δ_C 56.69)/C-21 (δ_C 21.31); from H-24 (δ_H 0.94) to C-8 (δ_C 37.00)/C-9 (δ_C 53.12)/C-11 (δ_C 21.73)/C-14 (δ_C 49.91); from H-23 (δ_H 0.83) to C-1 (δ_C 39.69)/C-5 (δ_C 56.69)/C-9 (δ_C 53.12)/C-10 (δ_C 37.20); from H-25 (δ_H 1.29) to C-12 (δ_C 73.48)/C-13 (δ_C 40.78)/C-14 (δ_C 49.91)/C-18 (δ_C 157.08), confirmed the position of two methyl groups at C-4 and the remaining methyl groups at C-8, C-10, and C-13. The position of acetoxy group at C-12 was confirmed by HMBC correlation from H-12 (δ_H 5.41)/H-2′ (δ_H 1.89) to C-1′ (δ_C 170.21). Moreover, the HMBC correlations between H-16 (δ_H 4.68)/H-19 (δ_H 7.19) and C-17 (δ_C 119.96)/C-18 (δ_C 157.08) suggested the position of hydroxyl group at C-16 and furan ring at C-17/C-18. Thus, compound **4** was determined to be furoscalrol [10].

The ^{1}H-NMR of **5** exhibited the presence of five methyl groups at δ_H 0.68 (3H, s), 0.87 (6H, d, J = 6.5 Hz), 0.92 (3H, d, J = 6.0 Hz), and 1.20 (3H, s), one oxymethine proton at δ_H 3.67 (1H, m), and one olefinic proton at δ_H 5.69 (1H, s). The ^{13}C-NMR and DEPT spectra of **5** showed the signals of 27 carbons, including 1 carbonyl, 3 non-protonated carbons, 8 methines, 10 methylenes, and 5 methyl groups. Analysis of ^{1}H- and ^{13}C-NMR data indicated the strucutre of **5** to be a steroid, a class commonly found in the sponge [11]. The HMBC correlations from H-6 (δ_H 5.68)/H-8 (δ_H 2.23)/H-9 (δ_H 1.35)/to C-7 (δ_C 202.27) confirmed the carbonyl group at C-7. In addition, the ^{1}H- and ^{13}C-NMR data of **5** were similar to those of 3*β*-hydroxycholest-5-en-7-one [11].

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