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Sterols from Thai Marine Sponge
Petrosia (*Strongylophora*) sp. and Their Cytotoxicity

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Abstract: Eight new sterols (**1–5** and **11–13**), together with eight known compounds (**6–10** and **14–16**) were isolated from marine sponge *Petrosia* sp. The structures of these compounds were elucidated on the basis of extensive spectroscopic analysis. The cytotoxicity of some compounds against a panel of human cancer cell lines is also reported.

1. Introduction

As part of our on-going search for biologically active metabolites from marine organisms [1–3], we have investigated a sponge species belonging to the genus *Petrosia* (*Strongylophora*) collected from the Similan Island, Thailand. Previously, various studies on the chemical constituents of *Petrosia* sp. led to the isolation of cyclosterols [4–6], polyacetylenic alcohols [7–12], meroditerpenes [13–15], 1,2-dihydroisoquinolines [16], halenaquinones [17], and pyridoacridine alkaloids [18]. Among them, some exhibited significant biological effects such as cytotoxicity [9,10,12,19–21], neurotrophic [11], antifouling [22] and antimicrobial activities [21], inhibitions against proteasome [15], protein Tyrosine phosphate 1B [13], cholinesterase [18], as well as inhibitory effects of the receptor activator of nuclear factor κB ligand (RANKL) induced osteoclastogenesis [17]. In this paper, we report the isolation and structure determination of eight new (**1–5** and **11–13**) and eight known (**6–10** and **14–16**) steroids from marine sponge *Petrosia* sp. and several of them were evaluated for their cytotoxicity against a panel of human cancer cell lines. The structures of eight new sterols have been established by extensive spectroscopic analysis, including 1D and 2D nuclear magnetic resonance (NMR) (distortionless enhancement by polarization transfer (DEPT), [1]H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear overhauser effect spectroscopy (NOESY)) spectroscopy.

2. Results

Petrosia sp. (6.4 kg wet wt.) was collected by hand via scuba diving from the Similan Island in Thailand. The MeOH extract of the frozen sponge was partitioned between EtOAc and H_2O, the EtOAc-soluble portion of the MeOH extract of *Petrosia* sp. exhibited significant cytotoxicity against various cancer cell lines (>67% inhibition of cell proliferation) at a concentration of 30 µg/mL.

The EtOAc-soluble portion was then separated by sequential chromatographic techniques to afford eight new (**1**–**5** and **11**–**13**) and eight known (**6**–**10** and **14**–**16**) steroids (Figure 1).
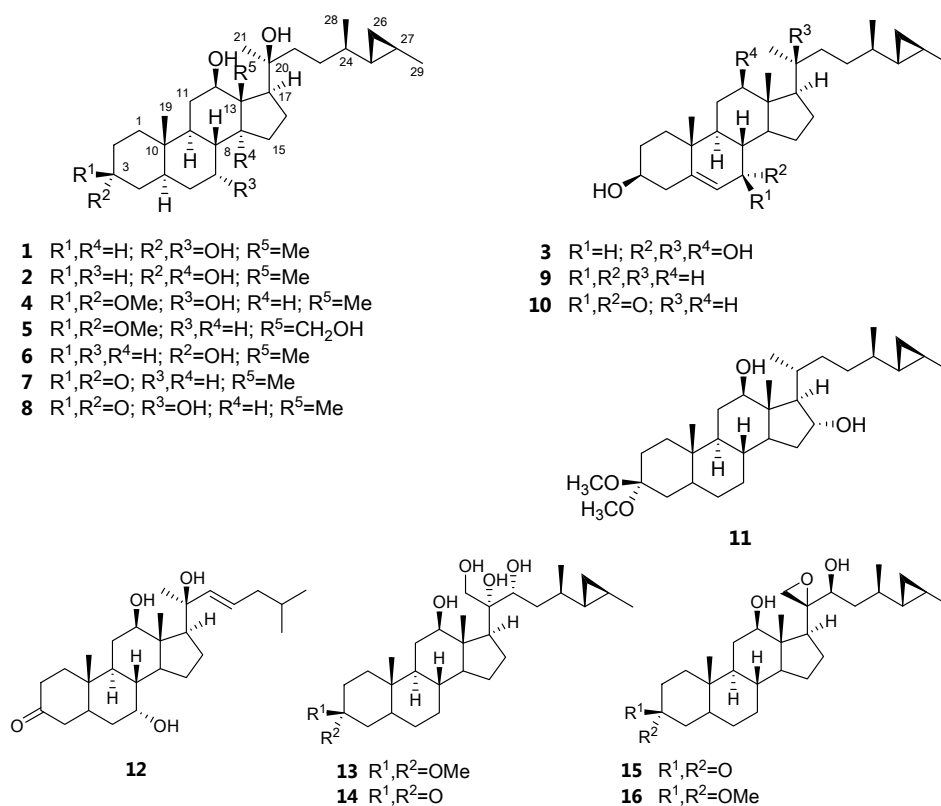

Figure 1. Chemical structures of compounds **1**–**16**.

Compound **1** was obtained as a white amorphous powder, and the molecular formula was established as $C_{29}H_{50}O_4$ by the atmospheric pressure chemical ionization-time of flight-mass spectrometer (APCI-TOF MS) at m/z 497.3414 $[M + Cl]^-$ (calcd. for $C_{29}H_{50}ClO_4$, 497.3403, see Figure S6). The infrared spectrophotometer (IR) spectrum exhibited a broad absorption band at 3280 cm^{-1}, suggesting the presence of hydroxyl groups. The ^{1}H NMR spectrum of **1** (Table 1, Figure S1) showed the upfield resonances of a cyclopropane ring at δ 0.20 (2H, m, H-25 and Hb-26), 0.10 (1H, m, Ha-26), and 0.54 (1H, m, H-27), three oxymethines at δ 4.27 (brs, H-3), 4.13 (brs, H-7), 3.76 (dd, *J* = 10.8, 2.6 Hz, H-12), three singlet methyls at δ 0.91 (CH$_3$-19), 1.23 (CH$_3$-18), and 1.35 (CH$_3$-21), and two doublet methyls at δ 1.02 (*J* = 6.6 Hz, CH$_3$-28) and 1.06 (*J* = 5.7 Hz, CH$_3$-29). The ^{13}C NMR (Table 2, Figure S2), DEPT, and HSQC spectra revealed the presence of twenty-nine carbons, comprising five methyls, ten methylenes, eleven methines, and three quaternary carbons. These data established the compound **1** as a C$_{29}$-steroidal structure with a cyclopropane ring at C-25–C-27, and its NMR spectra revealed close similarity to those of aragusterol I (**6**), which was also isolated in this study and reported previously from the marine sponge *Xestospongia testudinaria* [22]. The only difference was the presence of signals for oxymethine (δ$_H$ 4.13, s/δ$_C$ 67.0) at C-7 in **1** instead of the signal for methylene in aragusterol I (**6**), suggesting that the C-7 position of **1** was substituted with the hydroxyl group. This assignment was supported by HMBC correlations (Figure 2 and Figure S4) from H-7 to C-5 (δ 45.6) and C-9 (δ 32.1) and H-6 to C-7 (δ 67.0). All of the ^{1}H and ^{13}C NMR signals of **1** (Tables 1 and 2, Figures S1 and S2) were established by the HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY spectral analysis. The orientations of three hydroxyl groups at C-3, C-7, and C-12 were established by the ^{1}H-^{1}H coupling constants of H-3, H-7, and H-12, respectively. Two broad singlets of H-3 and H-7 were deduced as 3α-OH and 7α-OH configurations [21,22] while the dd (*J* = 10.8 and 2.6 Hz) of H-12 was assigned as 12β-OH-configuration [21]. In addition, α-orientation of hydroxyl group at C-3 was confirmed

based on the similarity of ^{13}C chemical shift of **1** (δ 65.8) with those reported for aragusterol I (δ 66.5, 3α-OH) [22]. The NOESY correlations (Figure 3 and Figure S5) among H-12 and H-17 and CH_3-21 were assigned as β-orientations for both side chain at C-17 and hydroxyl group at C-20. The cyclopropane ring was assigned as possessing an *E*-geometry due to its NOESY correlation between H-25 and CH_3-29. Moreover, the proton and carbon resonances of C-20–C-29 side chain of **1** were similar to those of aragusterols B (**7**) [23], I (**6**) [22], and xestokerol B (**8**) [21], indicating their identical relative configuration. In addition, the relative stereochemistry of the rings A–D (from C-1 to C-19) of **1** was deduced by the NOESY experiments as shown in Figure 3 and Figure S5. Therefore, compound **1** was identified as 26,27-cyclo-24,27-dimethylcholestan-3α,7α,12β,20β-tetraol (or 7α-hydroxyaragusterol I).

**Table 1.** ^1H NMR (600 MHz) data of compounds **1**–**4** from marine sponge *Petrosia* sp.

Position	δ$_H$, mult. (*J* in Hz)			
	1 [a]	**2** [b]	**3** [a]	**4** [b]
1	1.50, m	1.39, m 1.53, m	1.06, m 1.80, m	1.23, ddd (13.6, 13.6, 3.6) 1.75, m
2	1.28, m 1.82, m	1.29, m 1.64, m	1.81 [c], m 1.90, m	1.46, m 1.89, m
3	4.27, s	4.05, s	3.76, dddd (10.6, 10.6, 5.6, 5.6)	
4	1.61, m	1.40 [c], m 1.50, m	2.63, t (12.1) 2.69, dd (13.0, 4.4)	1.37, m 1.69, m
5	1.92, m	1.56, m		2.30, t (13.3)
6	1.65, m	1.40 [c], m 1.64, m	5.91, d (4.9)	1.52 [c], m 1.62, m
7	4.13, s	1.26, m 1.56, m	4.15, s	4.06, s
8	1.59, m	1.79, m	1.61, m	1.51 [c], m
9	2.92, m	1.40 [c], m	1.85, m	1.75, m
11	1.63, m 2.10, m	1.27, m 1.77, m	1.80 [c], m 2.07, m	1.57, m 2.00, m
12	3.76, dd (10.8, 2.6)	3.97, dd (11.0, 4.9)	3.84, dd (10.9, 4.1)	3.72, dd (10.9, 4.1)
14	1.93, m		2.05 [d], m	1.85, m
15	1.43, m 2.12, m	1.47, m 1.62, m	1.48, m 2.25, m	1.40, m 2.10, m
16	1.67, m 1.78, m	1.53, m 1.82, m	1.70, m 1.82 [c], m	1.63, m 1.76, m
17	1.99, m	2.62, t (9.9)	2.05 [d], m	1.95, m
18	1.23, s	0.92, s	1.22, s	1.17, s
19	0.91, s	0.82, s	1.07, s	0.83, s
20				
21	1.35, s		1.37, s	1.32, s
22	1.78, m 2.12, m	1.60, m 1.81, m	1.80 [c], m 2.15, t (12.9)	1.52, m 2.11, m
23	1.78, m 1.97, m	1.29, m 1.54, m	1.49, m 2.00, m	1.48, m 1.97, m
24	0.70, m	0.65, m	0.72, m	0.69, m
25	0.20 [c], m	0.16 [d], m	0.21 [e], m	0.20 [d], m
26	0.10, m 0.20 [c], m	0.10, m 0.16 [d], m	0.11, m 0.21 [e], m	0.10, m 0.20 [d], m
27	0.54, m	0.49, m	0.54, m	0.53, m
28	1.02, d (6.6)	0.92, d (6.0)	1.03, d (6.2)	1.01, d (6.7)
29	1.06, d (5.7)	1.03, d (6.0)		1.06, d (6.0)
3-OMe				3.07, s
3-OMe				3.16, s

[a] Measured in pyridine-d_5; [b] Measured in $CDCl_3$; [c–e] overlapped with other signals.

Table 2. ^{13}C NMR (150 MHz) data of compounds **1–5** and **11–13** from marine sponge *Petrosia* sp.

Position	δ_C							
	1 [a]	2 [b]	3 [a]	4 [b]	5 [a]	11 [b]	12 [a]	13 [b]
1	32.9	32.3	37.6	35.4	34.9	35.4	38.7	35.0
2	29.9	29.0	32.4	28.9	28.25	28.3	38.4	28.3 [c]
3	65.8	66.4	71.1	100.7	100.3	100.3	210.3	100.3
4	36.75	35.8	43.3	35.8	35.3	34.9	44.66	35.4
5	45.6	38.9	145.1	35.23	42.3	42.4	39.7	42.4
6	38.0	28.33	125.5	37.6	28.34	28.4	38.0	28.4 [c]
7	67.0	26.5	64.6	66.7	31.6	31.5	66.3	31.4
8	39.1	36.8	37.1	39.0	34.6	33.7	38.8	33.9
9	32.1	46.4	42.6	45.2	53.1	52.8	44.71	52.7
10	36.79	36.3	37.9	36.3	35.7	35.7	36.1	35.7
11	29.7	28.27	30.0	30.0	31.2	31.1	30.3	29.1
12	78.4	71.8	78.1	78.2	80.6	79.8	78.0	78.0
13	49.1	52.7	48.8	49.0	50.5	49.3	49.1	49.3
14	50.0	87.2	49.1	49.8	54.9	51.3	49.5	54.5
15	23.5	33.1	24.0	23.5	23.4	36.1 [c]	23.6	22.8
16	25.6	24.0	25.8	25.6	23.9	74.2	25.1	23.5
17	66.0	57.6	65.8	65.9	61.2	67.9	64.1	54.2
18	10.2	12.4	10.2	10.2	60.8	9.1	10.0	9.7
19	10.6	10.9	18.5	10.9	11.58	11.49	10.4	11.5
20	74.4	75.5	74.5	74.4	74.6	32.3	73.9	76.3
21	28.6	28.9	28.6	28.6	29.0	22.5	31.6	66.4
22	35.2	34.4	35.2	35.15	37.0	32.7	137.6	75.1
23	32.5	31.9	32.5	32.5	32.0	36.2 [c]	125.8	37.7
24	39.5	39.0	39.5	39.5	38.8	38.7	42.4	34.7
25	27.7	27.1	27.7	27.7	27.1	27.3	28.9	27.9
26	11.9	11.5	11.9	11.9	11.64	11.53	22.5 [c]	12.5
27	13.2	12.8	13.2	13.2	12.7	12.7	22.8 [c]	12.3
28	20.6	20.1	20.6	20.7	19.8	19.9		18.7
29	19.3	19.1		19.3	19.1	19.1		19.1
3-OMe				47.3	47.45	47.4		47.38 [d]
3-OMe				47.4	47.51	47.5		47.43 [d]

[a] Measured in pyridine-d_5; [b] Measured in CDCl$_3$; [c,d] interchangeable.
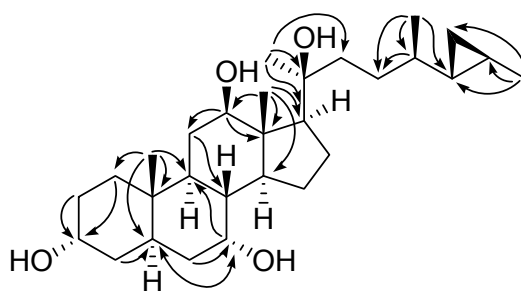
Figure 2. HMBC correlations of compound **1**.

Compound **2** possessed the same molecular formula of C$_{29}$H$_{50}$O$_4$ as that of **1**. Analysis of the ^{1}H and ^{13}C NMR spectral data of **1** and **2** (Tables 1 and 2, Figures S1, S2, S7 and S8) revealed that a hydroxyl group signal present at C-7 in **1** was now present at C-14 in **2**, as the resonances at C-7 position were shifted from δ_H 4.13/δ_C 67.0 in **1** to δ_H 1.56 and 1.26 (m, each)/δ_C 26.5 in **2** and resonances at C-14 position from δ_H 1.93/δ_C 50.0 in **1** to δ_C 87.2 in **2**. The location of the hydroxyl group at C-14 in **2** was assigned based on the HMBC correlations from H-15 and CH$_3$-18 to C-14 (δ 87.2). Concerning the configuration of 14-OH in **2**, the ^{13}C chemical shifts of both C-9 and C-12 were

significantly shifted upfield by $\Delta\delta C$ by approximately 6 ppm when compared with those reported for aragusterols I (**6**) [22] and B (**7**) [23] due to the γ-gauche effect [24]. These results established the hydroxyl group at C-14 as having an α-orientation. Consequently, compound **2** was identified as 26,27-cyclo-24,27-dimethylcholestan-3α,12β,14,20β-tetraol (or 14-hydroxyaragusterol I).
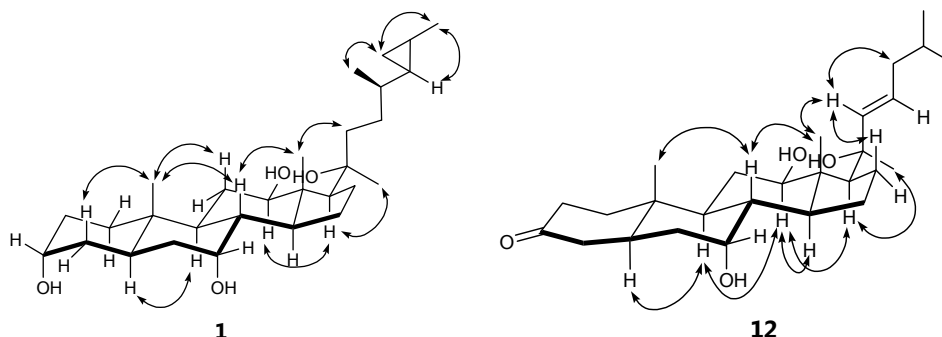
Figure 3. NOESY correlations of compounds **1** and **12**.

Compound **3** was isolated as a white amorphous powder. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 1 and 2, Figures S9 and S10) were closely similar to those of **1**, except for the orientation of the 3-OH group and the presence of signals for an additional double bond (δ_C 145.1 (C-5) and δ_H 5.91/δ_C 125.5 (C-6)) at C-5/C-6 in **3**. The HMBC correlations from H-4 and H-7 to C-5 (δ 145.1) and C-6 (δ 125.5) and CH$_3$-19 to C-5 (δ 145.1), C-10 (δ 37.9), and C-9 (δ 42.6) indicated that the methylene group at C-6 in **1** was replaced by the double bond at C-5/C-6. The molecular formula of **3**, C$_{29}$H$_{48}$O$_4$, had two mass units less than that of **1**, as determined from APCI-TOF MS at *m/z* 495.3252; [M + Cl]$^-$ also supported this result. The orientation of 3-OH in **3** was determined by the carbon chemical shifts at C-3 and coupling constants of H-3. The downfield chemical shift of C-3 from δ_C 65.7 in **1** to 71.1 [22] in **3** and the multiplicity of H-3 at δ_H 3.77 as dddd (*J* = 10.6, 10.6, 5.6, 5.6 Hz) were assigned as 3β-OH orientation [4]. Thus, the new compound **3** was identified as 26,27-cyclo-24,27-dimethylcholest-5-ene-3β,7α,12β,20β-tetraol.

Compound **4** was obtained as an amorphous powder, and its molecular formula of C$_{31}$H$_{54}$O$_5$ was established by the ESI-TOF MS at *m/z* 529.3858 [M + Na]$^+$ (calcd for C$_{31}$H$_{54}$NaO$_5$, 529.3864). After comparing the ^{1}H and ^{13}C NMR spectral data with that of compound **1** (Tables 1 and 2), the proton and hydroxyl groups at C-3 in **1** were replaced by two methoxy groups in **4**, based on the ^{1}H signals of two singlets at δ 3.16 and 3.07 and the ^{13}C signal of ketal at C-3 (δ 100.7). Therefore, compound **4** was established as 26,27-cyclo-24,27-dimethylcholestan-3,3-dimethoxy-7α,12β,20β-triol (or 7α-hydroxyaragusterolketal I).

The molecular formula C$_{31}$H$_{54}$O$_5$ of compound **5** was deduced from APCI-TOF MS. The ^{1}H and ^{13}C NMR spectral data closely resembled those of **4** except that the secondary hydroxyl group at C-7 and methyl group at C-13 in **4** were replaced by a methylene (δ_H 0.81 and 1.65) and a hydroxymethyl (δ_H 3.65 and 4.02/δ_C 60.8) group, respectively in **5**. The HMBC correlations from H-18 to C-12 (δ 80.6) and C-17 (δ 61.2) supported the position of the hydroxymethyl at C-13 of the sterol. Thus, compound **5** was elucidated as the new steroid 26,27-cyclo-24,27-dimethylcholestan-3,3-dimethoxy-12β,18,20β-triol.

The molecular formula of compound **11** was determined as C$_{31}$H$_{54}$O$_4$ by APCI-TOF MS. The ^{1}H and ^{13}C NMR spectra of **11** (Tables 2 and 3, Figures S15 and S16) were similar to those of compound **4**, suggesting a 12β-hydroxy group and dimethyl ketal at C-3. The main differences were that the position of the β-hydroxy group at C-7 in **4** was now at C-16 (δ_H 4.33/δ_C 74.2) and the hydroxy-substituted quaternary carbon at C-20 (δ_C 74.4) in **4** was replaced by the methine (δ_H 1.85/δ_C 32.3) group. The 16α-OH group in **11** was assigned on the basis of its correlations of H-16 with C-20 (δ 32.3) and C-14 (δ 51.3) in the HMBC correlations and the cross peak of H-17 and H-16 in the ^{1}H-^{1}H COSY together with the correlation between H-16 and CH$_3$-18 in the NOESY

spectrum. In addition, the relative stereochemistry of C-20–C-29 side chain at C-17 of compound **11** was confirmed by the similarity of the ^{1}H and ^{13}C chemical shifts to those of known petrosterol (**19**) [25] and 7-oxopetrosterol (**10**) [4]. The NOESY correlation between H-17 and H-12 further established the orientation of C-17 side chain as β-orientation. Thus, the structure of **11** was elucidated as 26,27-cyclo-24,27-dimethylcholestan-3,3-dimethoxy-12β,16α-diol.

Table 3. ^{1}H NMR (600 MHz) data of compounds **5** and **11**–**13** from marine sponge *Petrosia* sp.

Position	δ_H, mult. (*J* in Hz)			
	5 [a]	**11** [b]	**12** [a]	**13** [b]
1	1.11, ddd (13.6, 13.6, 3.7) 1.62, m	1.30, m 1.55, m	1.30, ddd (13.4, 13.4, 4.8) 1.88, m	1.05, ddd (13.6, 13.6, 3.7) 1.60, m
2	1.48, m 1.89, ddd (12.9, 12.9, 3.8)	1.40, m 1.89, dq (14.1, 3.2)	2.28, m 2.36, m	1.43, ddd (14.0, 14.0, 4.0) 1.88, br dd (2.9, 14.0)
4	1.30, m	1.12, m	2.12, m 2.32, m	1.28, m
5	1.31, m	1.30, m	2.43, m	1.29, m
6	1.28, m	1.20, m 1.28, m	1.56, ddd (13.7, 13.7, 2.0) 1.65, m	1.25, m
7	0.81, m 1.65, m	0.88, m 1.64, m	4.07, brs	0.88, m 1.67, m
8	1.32, m	1.31, m	1.51, ddd (11.2, 11.2, 2.2)	1.30, m
9	0.85, m	0.88, m	1.76, m	0.84, m
11	1.57, m 1.93, ddd (13.6, 4.0, 4.0)	1.25, m 1.67, m	1.65, m 2.04, m	1.26, m 1.73, m
12	3.50, dd (11.0, 4.0)	3.53, dd (10.9, 4.5)	3.75, dd (10.9, 4.3)	3.38, dd (11.0, 4.4)
14	1.02, m	1.30, m	1.87, m	1.76, m
15	1.01, m 1.66, m	1.42, m 1.67, m	1.40, m 2.10, m	1.50, m 1.75, m
16	1.75, m	4.33, t (7.4)	1.76, m	1.20, m 1.69, m
17	1.75, m	1.40, m	2.03, m	0.96, m
18	3.65, d (12.0) 4.02, d (12.0)	0.71, s	1.07, s	0.80, s
19	0.84, s	0.79, s	1.00, s	0.80, s
20		1.85, m		
21	1.44, s	1.09, d (6.9)	1.45, s	3.83, d (11.5) 3.98, d (11.5)
22	1.61, m 1.90, m	0.88, m 1.26, m	6.00, d (15.5)	3.82, d (10.6)
23	1.28, m 1.47, m	1.44, m 1.66, m	5.94, ddd (15.0, 7.5, 7.5)	1.35, m 1.67, m
24	0.70, m	0.65, m	1.99, m 2.03, m	0.93, m
25	0.19, m	0.16 [c], m	1.66, m	0.25, m
26	0.11, m 0.18, m	0.13 [c], m	0.92, d (6.6)	0.16, m 0.25, m
27	0.51, m	0.46, m	0.91, d (6.6)	0.53, m
28	0.93, d (6.7)	0.91, d (6.7)		0.95, d (6.0)
29	1.02, d (6.0)	1.00, d (6.0)		1.03, d (6.0)
3-OMe	3.14, s	3.14, s		3.14, s
3-OMe	3.19, s	3.19, s		3.19, s

[a] Measured in pyridine-d_5; [b] Measured in CDCl$_3$; [c] overlapped with other signals.

Compound **12** was isolated as a white amorphous powder, and the molecular formula was established as $C_{27}H_{44}O_4$ by APCI-TOF MS at *m/z* 467.2941 [M + Cl]$^-$ (calcd for $C_{27}H_{44}ClO_4$, 467.2934). The IR spectrum showed characteristic absorption bands of a hydroxyl group at 3518 cm^{-1} and a carbonyl group at 1713 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of the rings A–D of **12** (Tables 2 and 3,

Figures S17 and S18) were similar to those ofxestokerol B (**8**) [22], suggesting the presence of the carbonyl group at C-3 (δ 210.3), α-OH at C-7 (δ 66.3), and β-OH at C-12 (δ 78.0). The main difference was the signals due to the side chain (C-20–C-27) at C-17, which was assigned by the analysis of ^{1}H-^{1}H COSY and HMBC correlations. The ^{1}H-^{1}H COSY spectrum showed the cross peak between the olefinic H-23 (δ 5.94) and methylene H-24 (δ 1.99 and 2.03), which in turn coupled to a methine H-25 (δ 1.66) of the isopropyl group and showed the HMBC correlations from CH$_3$-21 to C-17 (δ 64.1); 20-OH to C-20 (δ 73.9), C-21 (δ 31.6), and C-17 (δ 64.1); and both H-22 and H-23 to C-20 (δ 73.9). All of these suggested that the side chain of **12** was 1-hydroxy-1,5-dimethyl-2-hexenyl unit. The *trans* geometry of the double bond at C-22 and C-23 was established from the large coupling constants of 15.5 Hz. In addition, the orientation of the hydroxy group at C-20 could be established as β-OH from the cross peak between H-22 and CH$_3$-18, H-17 and CH$_3$-21 in NOESY analysis (Figure 3). Thus, compound **12** was identified as 7α,12β,20β-trihydroxycholesta-22*E*-en-3-one.

Compound **13** had the molecular formula C$_{31}$H$_{54}$O$_6$ as determined by APCI-TOF MS. The NMR spectra of **13** (Tables 2 and 3, Figures S19 and S20) were similar to those of a known xestokerol A (**14**), except for the replacement of the ketone at C-3 in **14** with the dimethyl ketal (δ_C 100.3). The HMBC correlations from 3-OMe, H-1, H-2, H-4, and H-5 to C-3 supported the location of the dimethyl ketal. Thus, compound **13** was identified as 26,27-cyclo-24,27-dimethylcholestan-3,3-dimethoxy-12β,21,20α,22α-tetraol (or 3-dimethyl ketal analogue of xestokerol A (**14**)) [21].

Besides these eight new compounds, the eight known structures, aragusterol I (**6**) [22], aragusterol B (**7**) [23], xestokerol B (**8**) [21], petrosterol (**9**) [25], 7-oxopetrosterol (**10**) [4], xestokerol A (**14**) [21], aragusterol A (**15**) [20], and aragusterolketal (**16**) [19] were isolated and identified by NMR techniques and comparison of their spectral data (^{1}H and ^{13}C NMR and [α]$_D$) with literature values.

Although the 3-dimethyl ketal functionality of compounds **4** and **5** were assumed to have been artificially formed during the isolation and purification procedures, there have been some examples that the aragusteroketals A (**16**) [19] and B [24] possessing the 3-dimethyl ketal functionality have also been isolated from a marine sponge of *Xestospongia* sp. As an additional proof, the xestokerol B (**8**) and the aragusterol A (**15**) with carbonyl group at C-3 were subjected to conditions similar to those during the process of the isolation and purification for one month. No change in the thin layer chromatography (TLC) analyses was observed, suggesting that all isolated dimethyl ketal derivatives are naturally occurring.

In a previous study, sterols with a cyclopropane ring were reported to possess cytotoxicity toward various cancer cell lines [19,20,23]. In our study, several compounds (**1–4**, **6–8**, **10**, and **12–16**) were evaluated for their cytotoxicity using a panel of human cancer cell lines, including MOLT-3 (acute lymphoblastic leukemia), HepG2 (hepatocarcinoma), A549 (human lung cancer), HuCCA-1 (human chlolangiocarcinoma), HeLa (cervical carcinoma), and MDA-MB-231 (hormone-independent breast cancer) as well as a normal cell line, MRC-5 (normal human embryonic lung fibroblasts). As shown in Table 4, all of the tested compounds, except for sterol **15**, exhibited weak to moderate cytotoxicity, with the IC$_{50}$ values in the range of 11.23–103.5 μM. The most potent, compound **15,** was cytotoxic, with the IC$_{50}$ values of 7.10 and 6.11 μM against HepG-2 and HeLa cell lines, respectively, while exhibiting moderate cytotoxicity with the IC$_{50}$ values of 12.84, 37.93, 37.58, and 18.01 μM against the other four cancer cell lines, MOLT-3, A549, HuCCA-1, and MDA-MB-231, respectively. It was noted that all of the tested compounds exhibited weaker cytotoxic activity than the positive control (etoposide or doxorubicin) and were noncytotoxic towards a normal cell line (MRC-5), with IC$_{50}$ values greater than 37.68 μM.

Table 4. Cytotoxicity data of pure compounds from the marine sponge *Petrosia* sp.

Compounds	Cell Lines (IC$_{50}$, μM); Values Are Expressed as Mean ± S.D. (*n* = 3).						
	MOLT-3	HepG-2	A549	HuCCA-1	HeLa	MDA-MB-231	MRC-5
1	17.86 ± 0.26	12.71 ± 1.67	20.04 ± 1.52	21.32 ± 1.84	ND	ND	40.13 ± 1.23
2	32.36 ± 1.08	44.78 ± 3.79	38.59 ± 0.24	37.92 ± 3.01	ND	ND	60.61 ± 12.75
3	8 (27.17) [a]	44.61 ± 9.76	9 (54.35) [a]	47.48 ± 6.96	ND	ND	24.70 (54.35) [a]
4	0 (12.35) [a]	20.32 (24.70) [a]	2 (12.35) [a]	0 (12.35) [a]	ND	ND	5.45 (24.70) [a]
6	20.07 ± 0.52	54.89 ± 2.04	34.73 ± 17.71	37.06 ± 0.36	ND	ND	63.36 ± 8.30
7	16.33 ± 18.02	26.46 ± 5.32	23.58 ± 2.00	25.07 ± 2.27	11.23 ± 0.05	27.03 ± 7.79	ND
8	43 (108.70) [a]	18.23 ± 1.86	32.62 ± 3.64	103.35 ± 2.29	ND	ND	90.11 ± 6.88
10	2 (29.34) [a]	49.75 ± 3.96	52.15 ± 3.35	44 (58.69) [a]	ND	ND	44.77 ± 1.36
12	36.57 ± 1.11	56.50 ± 2.15	54.26 ± 3.84	66.11 ± 0.90	ND	ND	76.94 ± 10.65
13	14.90 ± 0.25	12.53 ± 0.84	17.91 ± 2.26	20.79 ± 2.32	ND	ND	37.68 ± 10.65
14	24.45 ± 3.11	41.41 ± 3.24	23.76 ± 1.87	34.41 ± 1.83	ND	ND	65.90 ± 4.54
15	12.84 ± 0.98	7.10 ± 4.76	37.93 ± 0.07	37.58 ± 1.40	6.11 ± 0.02	18.01 ± 5.74	ND
16	14.58 ± 0.36	11.31 ± 9.29	29.11 ± 8.25	34.60 ± 3.85	ND	ND	49.60 ± 6.67
Doxorubicin [b]	ND	0.55 ± 0.12	0.92 ± 0.06	2.24 ± 0.15	0.17 ± 0.10	1.78 ± 0.54	24.85 (50.00) [a]
Etoposide [b]	0.07 ± 0.005	31.50 ± 15.56	ND	ND	ND	ND	ND

[a] % inhibition (at concentration, μM); [b] positive control; ND: not determined.

3. Materials and Methods

3.1. General Experimental Procedures

UV–Vis spectra were obtained on a Shimadzu UV-1700 PharmaSpec Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Optical rotations were measured at the sodium D line (590 nm) on a JASCO 1020 digital polarimeter (Japan Spectroscopic Corporation, Tokyo, Japan). Fourier Transform infrared (FTIR) spectra were recorded with a universal attenuated total reflectance (UATR) attachment on a Perkin–Elmer Spectrum One spectrometer (PerkinElmer, Waltham, MA, USA). ^{1}H-, and ^{13}C- and 2D-NMR spectra were obtained at 600 and 150 MHz for ^{1}H and ^{13}C, respectively, on a Bruker AVANCE 600 spectrometer (Bruker Corporation, Billerica, MA, USA) with tetramethylsilane (TMS) (for CDCl$_3$) and residual solvent peaks (for pyridine-d_5) as internal standards. APCI-TOF MS were determined using a Bruker MicroTOF$_{LC}$ spectrometer (Bruker Corporation, Billerica, MA, USA). Column chromatography and preparative TLC were performed on normal-phase with Merck (Merck, Darmstadt, Germany) silica gel 60 (70–230 mesh ASTM) and PF$_{254}$, respectively, and RP-18 reverse-phase silica gel (40–63 μM). Sephadex LH-20 (GE Healthcare, Uppsala, Sweden) was also used for column chromatography. TLC was carried out on silica gel 60 F$_{254}$ plates (Merck, 0.2 mm). Medium pressure liquid chromatography (MPLC) was performed using a Büchi Pump Module C-605 and Büchi UV Monitor C-630 (Büchi, Flawil, Switzerland). High performance liquid chromatography (HPLC) was performed using a Thermoseparation products with spectra SYSTEM P4000 pump and coupled with PL-ELS 2100 evaporating light-scattering detector from Polymer Laboratories (settings: gas flow, 1.2 L/min; evaporation temperature, 90 °C; nebulizer temperature, 50 °C). For columns, Hichrom C18, 5 μm (21.2 × 250 mm) (Hichrom, Berkshire, UK), and Waters Symmetry C-18 prep (19 × 300 mm) stainless steel columns (Waters Corporation, Milford, MA, USA)were used.

3.2. Animal Material

The sponge, *Petrosia* sp., was collected from the Similan Islands in the Andaman Sea, Phang Nga, Thailand in February 2011, by hand via scuba diving. It was identified by Dr. Sumaitt Putchakarn, Institute of Marine Science, Burapha University, Bangsaen, Chonburi, Thailand. A voucher specimen (No. CRI 589) was deposited at the Laboratory of Natural Products, Chulabhorn Research Institute.

3.3. Extraction and Isolation

The sponge (wet weight ca. 6.4 kg) was extracted with MeOH and concentrated under reduced pressure. The MeOH extract was partitioned between EtOAc and water. The EtOAc-soluble portion

(10 g) was chromatographed on silica gel column chromatography (CC), eluting with a gradient of increasing polarity (hexane, CH_2Cl_2, and MeOH) to afford thirteen fractions (A1–A13). Compound **9** (900 mg) was obtained from fraction A6. Fraction A8 (3.2 g) was further subjected to silica gel column eluted with hexane and acetone in a polarity gradient to provide twelve fractions (B1–B12). The combined fractions B4 (132 mg) and B5 (20 mg) were purified by preparative RP18-HPLC with $MeOH/H_2O$ (94:6, flow rate 12 mL/min) as eluent to give compound **10** (17 mg). Fraction B6 (1.0 g) was further chromatographed on Sephadex LH-20 CC (3 × 120 cm) by eluting with $MeOH/CH_2Cl_2$ (1:1) to yield five fractions (C1–C5). Fraction C3 (439.0 mg) was chromatographed on preparative RP-18 MPLC with solvents of $MeOH/H_2O$ (60% to 100% MeOH) over 240 min to give compounds **7** (74 mg) and **15** (34 mg). Compounds **11** (2.9 mg), **16** (15 mg), and **5** (2.9 mg) were obtained after purification of fraction B7 by preparative RP18-HPLC (eluent: CH_3CN/H_2O (90:10), flow rate 8.5 mL/min). Fraction A9 (2.5 g) was purified by gel permeation over a Sephadex LH-20 CC (3 × 120 cm), eluting with $MeOH/CH_2Cl_2$ (1:1) as mobile phase, to provide three fractions (D1–D3). A mixture of sterols (823 mg) was obtained from fraction D2 (2.2 g) after crystallization with CH_2Cl_2/MeOH. The mother liquor solution from fraction D2 was further purified by gel permeation over a Sephadex LH-20 CC (3 × 120 cm) with MeOH as eluent to yield six fractions (E1–E6). Fraction E4 (1.16 g) was chromatographed on preparative RP-18 MPLC with solvents of $MeOH/H_2O$ (70% to 100% MeOH) over 240 min to give twenty-two fractions (F1–F22). Fraction F11 was subjected to preparative RP18-HPLC with CH_3CN/H_2O (60:40, flow rate 12 mL/min) as eluent, to give compound **12** (18 mg). Fraction F19 (104.2 mg) was chromatographed on preparative RP18-HPLC (eluent: $MeOH/H_2O$ (85:15)) to provide compounds **8** (5 mg), **13** (10 mg), and **14** (6 mg). Compound **6** (17 mg) was obtained from fraction F22. Fraction A10 (716.3 g) was chromatographed on gel permeation over a Sephadex LH-20 CC (3 × 120 cm), eluting with MeOH as mobile phase, and then purified by chromatography on a silica gel column, eluting with a gradient of hexane and EtOAc (70%–100%) to provide five fractions (G1–G5). Fraction G4 (46.8 mg) was purified by preparative RP18-HPLC eluting with $MeOH/H_2O$ (85:15, flow rate 12 mL/min) as mobile phase to provide compound **2** (5 mg). Fraction G5 (139.3 mg) was chromatographed on preparative RP-18 MPLC with solvents of $MeOH/H_2O$ (65% to 100% MeOH) over 360 min to give twelve fractions (H1–H12). Compounds **3** (11 mg) and **1** (8 mg) were obtained after purification of fraction H6 and H10, respectively, by preparative RP-18 HPLC with $MeOH/H_2O$ (80:20 for compound **3** and 85:15 for compound **4**, flow rate 12 mL/min for both **3** and **4**) as eluent.

Compound **1**. White amorphous powder; $[\alpha]_D^{25}$ −15.30 (*c* 0.63, MeOH); IR (ATR) ν_{max}: 3280, 2925, 2856, 2310, 1951, 1722, 1666, 1452, 1372, 1145, 1005 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); APCI-TOF MS *m/z*: 497.3414 [M + Cl]$^-$ (calcd. for $C_{29}H_{50}ClO_4$, 497.3403).

Compound **2**. White amorphous powder; $[\alpha]_D^{29}$ −6.87 (*c* 0.53, CHCl$_3$); IR (ATR) ν_{max}: 3304, 2927, 2926, 2857, 1449, 1372, 1004 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); APCI-TOF MS *m/z*: 497.3384 [M + Cl]$^-$ (calcd. for $C_{29}H_{50}ClO_4$, 497.3403).

Compound **3**. White amorphous powder; $[\alpha]_D^{29}$ −70.06 (*c* 0.53, MeOH); IR (ATR) ν_{max}: 3239, 2926, 2855, 1734, 1455, 1374, 1056, 1015 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); APCI-TOF MS *m/z*: 495.3252 [M + Cl]$^-$ (calcd. for $C_{29}H_{48}ClO_4$, 495.3247).

Compound **4**. White amorphous powder; $[\alpha]_D^{28}$ −18.28 (*c* 0.90, CHCl$_3$); IR (ATR) ν_{max}: 3230, 2951, 2918, 2870, 1720, 1452, 1373, 1362, 1243, 1175, 1141, 1116, 1044, 1018, 921, 750 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); ESI-TOF MS *m/z*: 529.3858 [M + Na]$^+$ (calcd. for $C_{31}H_{54}NaO_5$, 529.3864).

Compound **5**. White amorphous powder; $[\alpha]_D^{26}$ −11.97 (*c* 0.30, CH_2Cl_2); ^{1}H and ^{13}C NMR data (see Tables 2 and 3); APCI-TOF MS *m/z*: 507.4044 [M + H]$^+$ (calcd. for $C_{31}H_{55}O_5$, 507.4036).

Compound **11**. White amorphous powder; ^{1}H and ^{13}C NMR data (see Tables 2 and 3); APCI-TOF MS *m/z*: 525.3708 [M + Cl]$^-$ (calcd. for $C_{31}H_{54}ClO_4$, 525.3716).

Compound **12**. White amorphous powder; $[\alpha]_D^{26}$ +33.70 (*c* 1.82, MeOH); IR (ATR) ν_{max}: 3518, 3248, 2955, 2871, 1713, 1450, 1384, 1226, 1156, 1063, 1041, 1019, 974, 748 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 2 and 3); APCI-TOF MS *m/z*: 467.2941 [M + Cl]$^-$ (calcd. for $C_{27}H_{44}ClO_4$, 467.2934).

Compound **13**. White amorphous powder; $[\alpha]_D^{26}$ +20.43 (*c* 0.80, CHCl$_3$); IR (ATR) ν_{max}: 3274, 2946, 2871, 1716, 1456, 1360, 1178, 1055, 970 cm^{-1}; ^{1}H and ^{13}C NMR data (see Tables 2 and 3); APCI-TOF MS *m/z*: 557.3624 [M + Cl]$^-$ (calcd. for $C_{31}H_{54}ClO_6$, 557.3614).

3.4. Cytotoxicity Assays

The cytotoxic activity toward a panel of mammalian cancer cell lines (HepG2, A549, HuCCA-1, HeLa, MDA-MB-231) were tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [26], while the activity toward MOLT-3 cancer cell line was performed by the 2,3-bis-(2-methoxy-4-nitro-5-sulphenyl)-(2H)-tetrazolium-5-carboxanilide (XTT) assay [27]. Etoposide and doxorubicin were used as positive controls (Table 4).

4. Conclusions

The chemical investigation of Thai marine sponge *Petrosia* sp. led to the isolation of eight new (**1–5** and **11–13**) and eight known (**6–10** and **14–16**) sterols. Their structures were established by the basis of spectroscopic method. Some compounds (**1–4**, **6–8**, **10**, and **12–16**) were evaluated for their cytotoxicity using a panel of human cancer cell lines. The most potent, compound **15**, was cytotoxic, with the IC$_{50}$ values of 7.10 and 6.11 μM against HepG-2 and HeLa cell lines, respectively.

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