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.

Keywords: *Petrosia* sp.; sterol; cytotoxicity

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], polycyprostolic 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), 1H-1H 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 H2O, 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).

1 R₁,R₄=H; R₂,R₃=OH; R₅=Me
2 R₁,R₃=R₅=H; R₂=Me
3 R₁,R₃=R₅=H; R₂=OMe
4 R₁,R₂=OMe; R₃,R₅=H; R₆=Me
5 R₁,R₂=OMe; R₃,R₅=H; R₆=CH₂OH
6 R₁,R₂=OMe; R₃,R₅=H; R₆=Me
7 R₁,R₂=OMe; R₃,R₅=H; R₆=Me
8 R₁,R₂=OMe; R₃,R₅=H; R₆=Me
9 R₁,R₂=OMe; R₃=R₅=H
10 R₁,R₂=OMe; R₃=R₅=H
11 R₁,R₂=OMe; R₃=R₅=H
12 R₁,R₂=OMe; R₃=R₅=H
13 R₁,R₂=OMe; R₃,R₅=H
14 R₁,R₂=OMe; R₃,R₅=H
15 R₁,R₂=OMe; R₃,R₅=H
16 R₁,R₂=OMe; R₃,R₅=H

Figure 1. Chemical structures of compounds 1–16.

Compound 1 was obtained as a white amorphous powder, and the molecular formula was established as C₂₉H₅₀O₄ by the atmospheric pressure chemical ionization-time of flight-mass spectrometer (APCI-TOF MS) at m/z 497.3414 [M + Cl]⁻ (calcd. for C₂₉H₅₀ClO₄ 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 ¹H NMR spectrum of 1 (Table 1, Figure S1) showed the upfield resonances of a cyclopropene ring at δ 0.20 (2H, m, H-25 and Hb-26), 0.10 (1H, m, Hb-26), and 0.54 (1H, m, H-27), three oxymethines at δ 4.27 (bxs, H-3), 4.13 (bxs, H-7), 3.76 (dd, J = 10.8, 2.6 Hz, H-12), three singlet methyls at δ 0.91 (CH₃-19), 1.23 (CH₃-18), and 1.35 (CH₃-21), and two doublet methyls at δ 1.02 (J = 6.6 Hz, CH₃-28) and 1.06 (J = 5.7 Hz, CH₃-29). The ¹³C NMR (Table 2, Figure S2), DEPT, and HSQC spectra revealed the presence of twenty-nine carbons, comprising five methyls, eleven methines, and three quaternary carbons. These data established the compound 1 as a C₂₉+ 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 (δH 4.13, s, H-3) 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 ¹H and ¹³C NMR signals of 1 (Tables 1 and 2, Figures S1 and S2) were established by the HSQC, HMBC, ¹H-¹H COSY, and NOESY spectral analysis. The orientations of three hydroxy groups at C-3, C-7, and C-12 were established by the ¹H-¹H 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 ($\delta$ 65.8) with those reported for aragusterol I ($\delta$ 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. $^1$H NMR (600 MHz) data of compounds 1–4 from marine sponge Petrosia sp.

| Position | 1$^a$ | 2$^b$ | 3$^a$ | 4$^b$ |
|----------|-------|-------|-------|-------|
| 1        | 1.50, m | 1.39, m | 1.06, m | 1.23, ddd (13.6, 13.6, 3.6) |
|          | 1.53, m | 1.80, m |       | 1.75, m |
| 2        | 1.28, m | 1.29, m | 1.81, m | 1.46, m |
|          | 1.64, m | 1.90, 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, m | 2.63, t (12.1) | 1.37, m |
|          | 1.50, m |       | 2.69, dd (13.0, 4.4) | 1.69, m |
| 5        | 1.92, m | 1.56, m |       | 2.30, t (13.3) |
| 6        | 1.65, m | 1.40, m | 5.91, d (4.9) | 1.52, m |
|          | 1.64, m |       |       | 1.62, m |
| 7        | 4.13, s | 1.26, m | 4.15, s | 4.06, s |
|          | 1.56, m |       |       |       |
| 8        | 1.59, m | 1.79, m | 1.61, m | 1.51, m |
| 9        | 2.92, m | 1.40, m | 1.85, m | 1.75, m |
| 10       | 1.63, m | 1.27, m | 1.80, m | 1.57, m |
|          | 2.10, m | 1.77, m | 2.07, m | 2.00, m |
| 11       | 3.76, ddd (10.8, 2.6) | 3.97, ddd (11.0, 4.9) | 3.84, ddd (10.9, 4.1) | 3.72, ddd (10.9, 4.1) |
| 12       | 1.93, m | 2.05, d, m | 1.85, m |       |
| 13       | 1.43, m | 1.47, m | 1.48, m | 1.40, m |
|          | 2.12, m | 1.62, m | 2.23, m | 2.10, m |
| 14       | 1.67, m | 1.53, m | 1.70, m | 1.63, m |
|          | 1.78, m | 1.82, m | 1.82, m | 1.76, m |
| 15       | 1.99, m | 2.62, t (9.9) | 2.05, d, m | 1.95, m |
| 16       | 1.23, s | 0.92, s | 1.22, s | 1.17, s |
| 17       | 0.91, s | 0.82, s | 1.07, s | 0.83, s |
| 18       | 1.35, s | 1.37, s | 1.32, s |       |
| 19       | 1.78, m | 1.60, m | 1.80, m | 1.52, m |
|          | 2.12, m | 1.81, m | 2.15, t (12.9) | 2.11, m |
| 20       | 1.78, m | 1.29, m | 1.49, m | 1.48, m |
|          | 1.97, m | 1.54, m | 2.00, m | 1.97, m |
| 21       | 0.70, m | 0.65, m | 0.72, m | 0.69, m |
| 22       | 0.20, m | 0.16, d, m | 0.21, m | 0.20, d, m |
|          | 0.10, m | 0.16, d, m | 0.11, m | 0.10, m |
| 23       | 0.54, m | 0.49, m | 0.54, m | 0.53, m |
|          | 1.02, d (6.6) | 0.92, d (6.0) | 1.03, d (6.2) | 1.01, d (6.7) |
| 24       | 1.06, d (5.7) | 1.03, d (6.0) |       | 1.06, d (6.0) |
| 25       | 3-OMe |       | 3.07, s |       |
| 26       | 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 | $\delta_C$ | 2a | 2b | 3a | 4b | 5a | 11b | 12a | 13b |
|----------|------------|----|----|----|----|----|-----|-----|-----|
| 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 | 28.3c |
| 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 | 28.4c |
| 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 | 36.1c | 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 | 36.2c | 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 | 22.5c | 12.5 |
| 27       | 13.2       | 12.8 | 13.2 | 13.2 | 12.7 | 12.7 | 22.8c | 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 | 47.38d |

3-OMe | 47.4 | 47.51 | 47.5 | 47.43d |

* 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$_{20}$H$_{30}$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 $\delta$H 4.13/$\delta_C$ 67.0 in 1 to $\delta$H 1.56 and 1.26 (m, each)/$\delta_C$ 26.5 in 2 and resonances at C-14 position from $\delta$H 1.93/$\delta_C$ 50.0 in 1 to $\delta_C$ 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 ($\delta$ 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 ΔδC 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-hydroxyaragusteral I).

Compound 3 was isolated as a white amorphous powder. The 1H and 13C 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 (δC 145.1 (C-5) and δH 5.91/125.5 (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 CH3-19 to C-5 (δ 145.1), C-10 (δ 137.9), 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, C29H46O4, 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 δC 65.7 in 1 to 71.1 [22] in 3 and the multiplicity of H-3 at δH 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-dimethylcholestan-5-ene-3β,7α,12β,20β-tetraol.

Compound 4 was obtained as an amorphous powder, and its molecular formula of C31H54O5 was established by the ESI-TOF MS at m/z 529.3858 [M + Na]+ (calcld for C31H54NaO5, 529.3864). After comparing the 1H and 13C 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 1H signals of two singlets at δ 3.16 and 3.07 and the 13C 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-triolen (or 7α-hydroxyaragusteralketal I).

The molecular formula C31H52O4 of compound 5 was deduced from APCI-TOF MS. The 1H and 13C 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 (δH 0.81 and 1.65) and a hydroxymethyl (δH 3.65 and 4.02/12C 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-triolen.

The molecular formula of compound 11 was determined as C31H52O4 by APCI-TOF MS. The 1H and 13C 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 (δH 4.33/12C 74.2) and the hydroxy-substituted quaternary carbon at C-20 (δC 74.4) in 4 was replaced by the methine (δH 1.85/12C 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 1H-1H COSY together with the correlation between H-16 and CH3-18 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 | 5 a | 11 b | 12 a | 13 b |
|----------|-----|------|------|------|
| 1        | 1.11, ddd (13.6, 13.6, 3.7) | 1.30, m | 1.30, ddd (13.4, 13.4, 4.8) | 1.05, ddd (13.6, 13.6, 3.7) |
|          | 1.62, m | 1.55, m | 1.88, m | 1.60, m |
| 2        | 1.48, m | 1.40, m | 2.28, m | 1.43, ddd (14.0, 14.0, 4.0) |
|          | 1.89, ddd (12.9, 12.9, 3.8) | 1.89, dq (14.1, 3.2) | 2.36, m | 1.88, br dd (2.9, 14.0) |
| 4        | 1.30, m | 1.12, m | 2.12, m | 1.28, m |
|          | 1.30, 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 | 1.56, ddd (13.7, 13.7, 2.0) | 1.25, m |
|          | 1.28, m | 1.65, m |
| 7        | 0.81, m | 0.88, m | 4.07, brs | 0.88, m |
|          | 1.65, m | 1.64, 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 | 1.25, m | 1.65, m | 1.26, m |
|          | 1.93, ddd (13.6, 4.0, 4.0) | 1.67, m | 2.04, 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 | 1.42, m | 1.40, m | 1.50, m |
|          | 1.66, m | 1.67, m | 2.10, m | 1.75, m |
| 16       | 1.75, m | 4.33, t (7.4) | 1.76, m | 1.20, m |
|          | 1.75, m | 1.40, m | 2.03, m | 0.96, m |
| 18       | 3.65, d (12.0) | 0.71, s | 1.07, s | 0.80, s |
|          | 4.02, d (12.0) |      |      |      |
| 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 | 0.88, m | 1.26, m | 6.00, d (15.5) |
|          | 1.90, m | 1.26, m | 6.00, d (15.5) | 3.82, d (10.6) |
| 23       | 1.28, m | 1.44, m | 1.99, m | 1.35, m |
|          | 1.47, m | 1.66, m | 2.03, m | 1.67, m |
|          | 5.94, ddd (15.0, 7.5, 7.5) |      |      |      |
| 24       | 0.70, m | 0.65, m | 1.99, m | 0.93, m |
| 25       | 0.19, m | 0.16 c, m | 1.66, m | 0.25, m |
| 26       | 0.11, m | 0.13 c, m | 0.92, d (6.6) | 0.16, m |
|          | 0.18, m | 0.13 c, 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 of xestokerol 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-22E-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 aragustereketal (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 araguestereketals 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 cholangiocarcinoma), 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.
1.2 L/min; evaporation temperature, 90 °C with PL-ELS 2100 evaporating light-scattering detector from Polymer Laboratories (settings: gas flow, was performed using a Thermoseparation products with spectra SYSTEM P4000 pump and coupled

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 | AS49 | HsCCA-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) * | 44.61 ± 9.76 | 9 (54.35) * | 47.48 ± 6.96 | ND | ND | 24.70 (54.35) * |
| 4         | 0 (12.35) * | 20.32 (24.70) * | 2 (12.35) * | 0 (12.35) * | ND | ND | 5.45 (24.70) * |
| 5         | 20.07 ± 0.52 | 54.89 ± 2.04 | 34.73 ± 17.71 | 37.06 ± 0.36 | ND | ND | 63.36 ± 8.30 |
| 6         | 16.33 ± 18.02 | 24.66 ± 15.32 | 23.58 ± 2.00 | 25.07 ± 2.27 | 11.23 ± 0.05 | 27.03 ± 7.79 | ND |
| 7         | 43 (108.70) * | 18.23 ± 18.6 | 32.62 ± 3.64 | 103.35 ± 2.29 | ND | ND | 90.11 ± 6.88 |
| 8         | 2 (29.34) * | 49.75 ± 3.96 | 52.15 ± 3.35 | 44 (58.69) * | ND | ND | 44.77 ± 1.36 |
| 9         | 36.57 ± 11.1 | 56.50 ± 2.15 | 54.26 ± 3.84 | 66.11 ± 0.90 | ND | ND | 76.94 ± 10.65 |
| 10        | 14.90 ± 0.25 | 12.53 ± 0.84 | 17.91 ± 2.26 | 20.79 ± 2.32 | ND | ND | 37.68 ± 10.65 |
| 11        | 24.45 ± 3.11 | 41.41 ± 3.24 | 23.76 ± 1.87 | 34.41 ± 1.83 | ND | ND | 65.90 ± 4.54 |
| 12        | 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 |
| 13        | 14.58 ± 0.36 | 11.31 ± 9.29 | 29.11 ± 8.25 | 34.60 ± 3.85 | ND | ND | 49.60 ± 6.67 |
| 14        | 0.55 ± 0.12 | 0.92 ± 0.06 | 2.24 ± 0.15 | 0.17 ± 0.10 | 1.78 ± 0.54 | 24.85 (50.00) * |
| 15        | 0.07 ± 0.005 | 31.50 ± 15.56 | ND | ND | ND | ND | ND |

* % 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 (ATR) attachment on a Perkin–Elmer Spectrum One spectrometer (PerkinElmer, Waltham, MA, USA). 1H- and 13C- and 2D-NMR spectra were obtained at 600 and 150 MHz for 1H and 13C, respectively, on a Bruker AVANCE 600 spectrometer (Bruker Corporation, Billerica, MA, USA) with tetramethylsilane (TMS) (for CDCl3) and residual solvent peaks (for pyridine-d5) 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 (Darmstadt, Germany) silica gel 60 (70–230 mesh ASTM) and PF254, 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 F254 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$_2$Cl$_2$, 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$_2$O (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$_2$Cl$_2$ (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$_2$O (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$_3$CN/H$_2$O (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$_2$Cl$_2$ (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$_2$Cl$_2$/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 subjected to preparative RP18-HPLC with CH$_3$CN/H$_2$O (60:40, flow rate 12 mL/min) as eluent, to give compound 12 (18 mg). Fraction F19 (10.4 mg) was chromatographed on preparative RP18-HPLC (eluent: MeOH/H$_2$O (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$_2$O (85:15, flow rate 12 mL/min) as mobile phase to provide twelve fractions (F1–F22). Fraction F11 was subjected to preparative RP18-HPLC with MeOH/H$_2$O (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$_2$O (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; [α]$_D^{25}$ = −15.30 (c 0.63, MeOH); IR (ATR) $\nu_{\text{max}}$: 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; [α]$_D^{29}$ = −6.87 (c 0.53, CHCl$_3$); IR (ATR) $\nu_{\text{max}}$: 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; [α]$_D^{29}$ = −70.06 (c 0.53, MeOH); IR (ATR) $\nu_{\text{max}}$: 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; [α]$_D^{29}$ = −18.28 (c 0.90, CHCl$_3$); IR (ATR) $\nu_{\text{max}}$: 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$_{49}$Na$_2$O$_5$, 529.3864).

**Compound 5.** White amorphous powder; [α]$_D^{29}$ = −11.97 (c 0.30, CH$_2$Cl$_2$); $^1$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) $\nu_{\text{max}}$: 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) $\nu_{\text{max}}$: 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 $\mu$M against HepG-2 and HeLa cell lines, respectively.

Supplementary Materials: The following are available online at www.mdpi.com/1660-3397/15/3/54/s1: Figure S1: $^1$H NMR spectrum of 1 in pyridine-$d_5$, Figure S2: $^{13}$C NMR spectrum of 1 in pyridine-$d_5$, Figure S3: $^1$H–$^1$H COSY spectrum of 1 in pyridine-$d_5$, Figure S4: HMBC spectrum of 1 in pyridine-$d_5$, Figure S5: NOESY spectrum of 1 in pyridine-$d_5$, Figure S6: HRMS spectrum of 1, Figure S7: $^1$H NMR spectrum of 2 in CDCl$_3$, Figure S8: $^{13}$C NMR spectrum of 2 in CDCl$_3$, Figure S9: $^1$H NMR spectrum of 3 in pyridine-$d_5$, Figure S10: $^{13}$C NMR spectrum of 3 in pyridine-$d_5$, Figure S11: $^1$H NMR spectrum of 4 in CDCl$_3$, Figure S12: $^{13}$C NMR spectrum of 4 in CDCl$_3$, Figure S13: $^1$H NMR spectrum of 5 in pyridine-$d_5$, Figure S14: $^{13}$C NMR spectrum of 5 in pyridine-$d_5$, Figure S15: $^1$H NMR spectrum of 11 in CDCl$_3$, Figure S16: $^{13}$C NMR spectrum of 11 in CDCl$_3$, Figure S17: $^1$H NMR spectrum of 12 in pyridine-$d_5$, Figure S18: $^{13}$C NMR spectrum of 12 in pyridine-$d_5$, Figure S19: $^1$H NMR spectrum of 13 in CDCl$_3$, Figure S20: $^{13}$C NMR spectrum of 13 in CDCl$_3$.

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