Steroid constituents from the soft coral *Sinularia microspiculata*

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**ABSTRACT**

A methanol extract of the soft coral *Sinularia microspiculata* revealed five sterols, including two new compounds. Using combined chromatographic and spectroscopic experiments, the new compounds were found to be 7-oxogorgosterol (1) and 16α-hydroxysarcosterol (2). Their structures were determined on the basis of spectroscopic data (\(^1\)H and \(^1\)C NMR, HSQC, HMBC, \(^1\)H-\(^1\)H COSY, NOESY, and FT-ICR-MS) and by comparing obtained results to the values indicated in previous studies. Among the isolated compounds, 3 showed weak cytotoxic effects against HL-60 (IC\(_{50}\) = 89.02 ± 9.93 μM) cell line, whereas 5 was weakly active against HL-60 (IC\(_{50}\) = 82.80 ± 13.65 μM) and SK-Mel2 (IC\(_{50}\) = 72.32 ± 1.30 μM) cell lines.

1. Introduction

*Sinularia* soft corals (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea) are a rich source of steroids and terpenoids [1–3]. Steroids are a highly diverse group of metabolically active compounds. The typical sterols have a 3β-hydroxy-∆\(^5\)-(or ∆\(^7\)-) cholestane nucleus and a C\(_8\)-C\(_{10}\) side chain. Marine organisms are of particular interest for research due to their high content of oxysterols, which are involved in a variety of biological activities [3].

In continuation of our ongoing research on the steroid constituents of Vietnamese corals [4,5], we report the isolation and structure elucidation of five sterols (Figure 1), including two new compounds, from the soft coral *Sinularia microspiculata*.

2. Results and discussion

Using combined chromatographic separations, two new and three known steroids were isolated from the methanol extract of the soft coral *S. microspiculata*. By detailed analysis of the
spectroscopic data (1D, 2D NMR, and MS) and comparison with previously reported values, the known compounds were identified as sarcophytosterol (3) [6], 3β-hydroxypregna-5, 16-dien-20-one (4) [7], and 3β,7α-dihydroxyergosta-5,24(28)-diene (5) [8]. This is the first report of compounds 3–5 from S. microspiculata.

Compound 1 was isolated as a white powder. Its molecular formula, C_{30}H_{48}O_{2}, was determined by a quasi-molecular ion peak at m/z 463.3539 [M + Na]^+ on Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). The 13C NMR spectrum showed 30 carbon signals including 7 methyls, 8 methylenes, 10 methines, and 5 quaternary carbons. The seven methyl groups were identified by signals at \( \delta_C 12.0 \) (C-18), 17.3 (C-19), 21.3 (C-21), 21.5 (C-26), 22.2 (C-27), 15.4 (C-28), and 14.3 (C-29). Moreover, one oxymethine group [\( \delta_C 70.5 \) (C-3)], a trisubstituted double bond [\( \delta_C 165.0 \) (C, C-5)/126.1 (CH, C-6)], and a ketone group [\( \delta_C 202.3 \) (C-7)] were also observed. The carbon signal from the ketone group was strongly shifted upfield, suggesting it is in a conjugated position with the double bond. In 1H NMR spectrum, the presence of four high-field protons at \( \delta_H 0.18–0.21 \) (1H, m, H-22), 0.23–0.26 (1H, m, H-24), −0.13 (1H, t, J = 5.0 Hz, H_β-30), and 0.46 (1H, dd, J = 5.0, 9.0 Hz, H_α-30) is characteristic of a gorgosterol-type side chain possessing a cyclopropane ring [9]. Three tert-methyl protons [\( \delta_H 0.65 \) (H-18), 1.20 (H-19), and 0.91 (H-29); each 3H, s], three sec-methyl protons [\( \delta_H 0.86 \) (H-26), 0.95 (H-27), and 0.93 (H-28); each 3H, d, J = 6.5 Hz], one olefinic proton [\( \delta_H 5.69 \) (1H, br s, H-6)], and one oxymethine proton [\( \delta_H 3.65–3.69 \) (1H, m, H-3)] were also identified. A sec-methyl signal appeared as a broad singlet at \( \delta_H 1.02 \), which was assigned to H-21 by HSQC, HMBC, and COSY experiments, is also typical for sterols possessing a gorgosterol-type side chain [9,10]. The 1H-1H COSY experiment revealed the proton–proton correlations of H_{-1}/H_{-2}/H_{-3}/H_{-4}, H_{-2}/H_{-11}/H_{-9}/H_{-8}/H_{-14}/H_{-2}/H_{-15}/H_{-16}/H_{-17}/H_{-20}/H_{-22}/H_{-30}, and H_{-28}/H_{-24}/H_{-25}/H_{-26}. These data, together with HMBC cross peaks of H-19 (\( \delta_H 1.20 \)) with C-1 (\( \delta_C 36.4 \)), C-5 (\( \delta_C 165.0 \)), C-9 (\( \delta_C 50.0 \)), and C-10 (\( \delta_C 38.3 \)); H-6 (\( \delta_H 5.69 \)) with C-4 (\( \delta_C 41.8 \)), C-8 (\( \delta_C 45.5 \)), and C-10 (\( \delta_C 38.3 \)); and H-8 (\( \delta_H 2.21 \)) with C-7 (\( \delta_C 202.3 \)), confirmed positions of the double
bond at C-5/C-6 and the ketone at C-7. Detailed analysis of other HMBC correlations clearly identified the planar structure of compound 1 (Figure 2).

The proton signals of H-3 at $\delta_H 3.65-3.69$ (1H, m, $J_{1/2} = 11.0$ Hz) is indicative for its $\alpha$-orientation [11] (versus t, $J = 2.5$ or 3.0 Hz for $\beta$-orientation of H-3 without a large $J$ value attributable to an axial–axial coupling [12,13]). This was further confirmed by an agreement of the $^{13}C$ NMR chemical shift for C-3 ($\delta_C 70.5$) of 1 with that of 3$\beta$-hydroxycholest-5-en-7-one at $\delta_C 70.61$ [11] which is quite different from that of aragusterol G at $\delta_C 66.4$ [14]. Moreover, the $^{13}C$-NMR data for the side chain of 1 were essentially identical to those of crassumsterol [15] and 7$\beta$-hydroxygorgosterol [16], suggesting the same configurations in the side chain of these three compounds, which was also assigned by nuclear overhauser effect spectroscopy (NOESY, see Figure 3). Thus, the structure of 1 was determined to be 7-oxogorgosterol.

The molecular formula of compound 2 was defined as C$_{29}$H$_{48}$O$_2$ by a FT-ICR-MS quasi-molecular ion peak at $m/z$ 451.3552 [M + Na]$^+$. The NMR features indicated a C$_{29}$-sterol containing two oxymethines [$\delta_C 71.7$ (C-3) and 72.3 (C-16)/$\delta_H 3.51-3.55$ (1H, m, H-3) and 4.65 (1H, d, $J = 5.0$ Hz, H-16)], two double bonds [$\delta_C 140.8$ (C, C-5), 121.6 (CH, C-6), 149.1 (C, C-17), and 133.0 (C, C-20)/$\delta_H 5.37$ (1H, t, $J = 2.5$ Hz, H-6)], three tert-methyls [$\delta_C 16.9$ (C-18), 19.3 (C-19), and 17.1 (C-21)/$\delta_H 0.88$ (H-18), 1.03 (H-19), and 1.72 (H-21), each 3H, s], and four sec-methyls [$\delta_C 19.1$ (C-26), 21.6 (C-27), 11.5 (C-28), and 13.8 (C-29)/$\delta_H 0.84$ (H-26), 0.90 (H-27), 0.78 (H-28), and 0.70 (H-29), each 3H, d, $J = 6.5$ Hz]. The $^1H$ and $^{13}C$ NMR data of 2 were similar to those of sarcosterol [17], except for the presence of an oxymethine at $\delta_C 72.3$ in 2 instead of a methylene in sarcosterol. HMBC cross-peaks of the additional oxymethine proton at $\delta_H 4.65$ with C-13 ($\delta_C 44.2$), C-14 ($\delta_C 52.9$), and C-20 ($\delta_C 133.0$) confirmed the position of this hydroxy group at C-16. Detailed analysis of other HMBC correlations clearly defined the planar structure of 2 (Figure 2).

Similar to compound 1, the signals of H-3 at $\delta_H 3.51-3.55$ (1H, m) and C-3 at $\delta_C 71.7$ confirmed a $\beta$-orientation of the hydroxy group at C-3 [17]. In addition, the coupling constant of the oxymethine proton H-16 (d, $J = 5.0$ Hz) indicated that H-16 was equatorial on the five-membered ring [18], which was further confirmed by a NOESY correlation of H-16 ($\delta_H 4.65$) with H-18 ($\delta_H 0.88$) versus that of H-16 with H-14 for (3$\beta$,16$\beta$,17Z,23R)-16-methoxy-23-methylergosta-5,17-dien-3-ol [19]. The spatial proximity of H$_b$-12 ($\delta_H 2.35$) with H-21 ($\delta_H 1.72$) and H-16 ($\delta_H 4.65$) with H$_b$-22 ($\delta_H 2.25$) confirmed a Z configuration of the double bond at C-17/C-20 (Figure 2) [19]. The relative configurations at C-23 and C-24 of

Figure 2. Key COSY and HMBC correlations of compounds 1 and 2.
2 were assumed to be identical to those of sarcosterol [17] and \((3\beta,16\beta,17Z,23R)-16\text{-methoxy-}23\text{-methylergosta-}5,17\text{-dien-}3\text{-ol}\) [19], based on the agreement of the \(^{13}\text{C}\) NMR chemical shifts from the side chain as well as the coexistence of 2 and sarcophytosterol (3) in the soft coral \(S.\ microspiculata\). Consequently, compound 2 was identified as \(16\alpha\text{-hydroxysarcosterol}\).

All isolated compounds were evaluated for their cytotoxic activity against a panel of eight human cancer cell lines including HepG2 (hepatoma cancer), HL-60 (acute leukemia), KB (epidermoid carcinoma), LNCaP (prostate cancer), LU-1 (lung cancer), MCF7 (breast cancer), SK-Mel2 (melanoma), and SW480 (colon adenocarcinoma) using the sulforhodamine B method [20]. Weak cytotoxic effects were observed for 3 against the HL-60 \((IC_{50} = 89.02 \pm 9.93 \mu M)\) cell line and 5 against the HL-60 \((IC_{50} = 82.80 \pm 13.65 \mu M)\) and SK-Mel2 \((IC_{50} = 72.32 \pm 1.30 \mu M)\) cell lines relative to that of the positive control (ellipticine: \(IC_{50} = 1.58 \pm 0.12\) and \(1.71 \pm 0.08 \mu M\) against HL-60 and SK-Mel2, respectively). The other compounds showed no cytotoxicity against any of the tested cancer cell lines \((IC_{50} > 100 \mu M)\).

### 3. Experimental

#### 3.1. General experimental procedures

Optical rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). The high resolution mass spectra were gained using a Varian 910 FT-ICR mass spectrometer (Varian, CA, USA). The \(^1\text{H}\) NMR \((500\text{ MHz})\) and \(^{13}\text{C}\) NMR \((125\text{ MHz})\) spectra were recorded on a Bruker AM500 (Billerica, MA, USA). TMS was used as an internal standard.
Medium pressure liquid chromatography (MPLC) was carried out on a Biotage Isolera One system (SE-751 03 Uppsala, Sweden). Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and YMC RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan). Thin layer chromatography (TLC) used pre-coated silica gel 60 F254 (1.05554.0001, Merck, Darmstadt, Germany) and RP-18 F254S plates (1.15685.0001, Merck, Darmstadt, Germany), and compounds were visualized by spraying with aqueous 10% H2SO4 and heating for 3–5 min.

3.2. Biological material

The sample of the soft coral *Sinularia microspiculata* Tixier-Durivault was collected at Da Den, Quangninh, Vietnam, in April 2014, and identified by Prof. Do Cong Thung. A voucher specimen (No. SM09) was deposited at the Institute of Marine Biochemistry and Institute of Marine Environment and Resources, VAST, Vietnam.

3.3. Extraction and isolation

The samples of *S. microspiculata* were washed, cut into small pieces, dried at 50 °C, and then powdered. The dried powder (2 kg) was extracted three times with methanol under ultrasonic condition. The resulting solutions were filtered, combined, and concentrated under reduced pressure to obtain the methanol residue (M, 200 g). The methanol residue was suspended in water and extracted in turn with *n*-hexane and dichloromethane resulting in extracts of *n*-hexane (H, 42 g), dichloromethane (D, 3 g), and an aqueous layer.

The H extract (42 g) was crude and separated on silica gel MPLC using the mobile phase of *n*-hexane-acetone (gradient 50:1→1:1, v/v) to obtain eight fractions, H1–H8. Fraction H3 (9 g) was further separated on YMC CC using a stepwise eluent of methanol-water (1.5:1, 3:1, 6:1, and 1:0, v/v) to give 13 subfractions, H3A–H3N. Subfraction H3F (110 mg) was purified on silica gel CC and eluted with *n*-hexane-ethyl acetate (3:1, v/v) to provide compound 4 (3 mg). Compounds 2 (4.5 mg) and 3 (2 mg) were obtained from subfraction H3L (1 g) after purification with YMC CC and elution with acetone-water (3:1, v/v). Compound 1 (3.5 mg) was obtained from subfraction H3N (1 g) after purification with silica gel CC and elution with dichloromethane-ethyl acetate (20:1, v/v), followed by silica gel CC with *n*-hexane-ethyl acetate (3:1, v/v). Fraction H4 (5 g) was further separated into 12 subfractions, H4A–H4M, by RP-18 MPLC using stepwise concentrations of methanol-water (1.5:1, 3:1, 6:1, 1:0, v/v). Subfraction H4M (1.3 g) was separated into five smaller fractions, H4M1–H4M5, by silica gel CC and elution with dichloromethane-ethyl acetate (1:1, v/v). Compound 5 (5 mg) was obtained from fraction H4M1 (50 mg) after purification with silica gel CC and elution with *n*-hexane-ethyl acetate.

3.3.1. 7-Oxogorgosterol (1)

Amorphous white powder; [α]D20 − 65 (c 0.05, CHCl3); IR (KBr) νmax: 3410, 2955, 1670, 1061, and 955 cm−1; UV λmax 236 nm; for 1H NMR (CDCl3, 500 MHz) and 13C NMR (CDCl3, 125 MHz) spectroscopic data, see Table 1; FT-ICR-MS: m/z 463.3539 [M + Na]+ (calcd for C30H48NaO2, 463.3547).
Table 1. $^1$H (500 MHz, CDCl$_3$) and $^{13}$C NMR (125 MHz, CDCl$_3$) spectroscopic data of compounds 1 and 2.

| Position | $\delta_c$ | $\delta_H$ mult. (J in Hz) | $\delta_c$ | $\delta_H$ mult. (J in Hz) |
|----------|------------|-----------------------------|------------|-----------------------------|
| 1        | 36.4       | 1.48–1.53 m/1.93–1.95 m     | 37.2       | 1.08–1.12 m/1.84–1.86 m     |
| 2        | 31.2       | 1.60–1.62 m/1.92–1.94 m     | 31.7       | 1.49–1.51 m/1.84–1.86 m     |
| 3        | 70.5       | 3.65–3.69 m                 | 71.7       | 3.51–3.55 m                 |
| 4        | 41.8       | 2.38–2.40 m/2.50 dd (2.5, 14.0) | 42.3       | 2.24–2.26 m/2.29–2.31 m     |
| 5        | 165.0      | –                           | 140.8      | –                           |
| 6        | 126.1      | 5.69 s                      | 121.6      | 5.37 t (2.5)                |
| 7        | 202.3      | –                           | 31.6       | 1.65–1.69 m/1.99–2.03 m     |
| 8        | 45.5       | 2.21 t (11.5)               | 30.6       | 1.56–1.58 m                 |
| 9        | 50.0       | 1.48–1.53 m                 | 50.1       | 1.01–1.06 m                 |
| 10       | 38.3       | –                           | 36.6       | –                           |
| 11       | 21.3       | 1.56–1.60 m                 | 21.4       | 1.54–1.56 m/1.61–1.63 m     |
| 12       | 38.8       | 1.13–1.17 m/2.05–2.07 m     | 37.8       | 1.53–1.55 m/2.34–2.36 m     |
| 13       | 43.5       | –                           | 44.2       | –                           |
| 14       | 49.8       | 1.33–1.35 m                 | 52.9       | 1.59–1.63 m                 |
| 15       | 26.5       | 1.22–1.24 m/2.40–2.42 m     | 35.0       | 1.42–1.44 m/1.54–1.56 m     |
| 16       | 28.6       | 1.32–1.34 m/2.08–2.10 m     | 72.3       | 4.65 d (5.0)                |
| 17       | 56.6       | 1.22–1.24 m                 | 149.1      | –                           |
| 18       | 12.0       | 0.65 s                      | 16.9       | 0.88 s                      |
| 19       | 12.3       | 1.20 s                      | 19.3       | 1.03 s                      |
| 20       | 35.2       | 1.01–1.03 m                 | 133.0      | –                           |
| 21       | 21.3       | 1.02 br s                   | 17.1       | 1.72 s                      |
| 22       | 32.2       | 0.18–0.21 m                 | 41.9       | 1.96–1.98 m/2.24–2.26 m     |
| 23       | 25.9       | –                           | 32.8       | 1.79–1.81 m                 |
| 24       | 50.8       | 0.23–0.26 m                 | 44.2       | 1.06–1.08 m                 |
| 25       | 32.0       | 1.56–1.58 m                 | 30.1       | 1.60–1.64 m                 |
| 26       | 21.5       | 0.86 d (6.5)                | 19.1       | 0.84 d (6.5)                |
| 27       | 22.2       | 0.95 d (6.5)                | 21.6       | 0.90 d (6.5)                |
| 28       | 15.4       | 0.93 d (6.5)                | 11.5       | 0.78 d (6.5)                |
| 29       | 14.3       | 0.91 s                      | 13.8       | 0.70 d (6.5)                |
| 30       | 21.3       | $\beta$–0.13 t (5.0)/$\alpha$ 0.46 dd (5.0, 9.0) |

Note: All assignments were done by HSQC, COSY, HMBC, and NOESY experiments.

3.3.2. 16α-Hydroxysarcosterol (2)
Amorphous white powder; [\(\alpha\)]$_D^{20} = 50 \, (c 0.05, CHCl$_3$); IR (KBr) \(\nu_{max}\): 3421, 2957, 1457, and 1371 cm$^{-1}$; for $^1$H NMR (CDCl$_3$, 500 MHz) and $^{13}$C NMR (CDCl$_3$, 125 MHz) spectroscopic data, see Table 1; FT-ICR-MS: \(m/z\) 451.3552 [M + Na]$^+$ (calcd for C$_{29}$H$_{48}$NaO$_2$, 451.3547).

3.4. Cytotoxic assays
Cytotoxic evaluations were performed by following the previously described protocols [21,22].

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Disclosure statement
No potential conflict of interest was reported by the authors.
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References

[1] V. Lakshmi and R. Kumar, *Nat. Prod. Res.* **23**, 801 (2009).
[2] W.T. Chen, Y. Li, and Y.W. Guo, *Acta Pharm. Sin. B.* **2**, 227 (2012).
[3] N.S. Sarma, M.S. Krishna, S.G. Pasha, T.S. Rao, Y. Venkateswarlu, and P.S. Parameswaran, *Chem. Rev.* **109**, 2803 (2009).
[4] N.P. Thao, B.T.T. Luyen, Y.N. Sun, S.B. Song, N.V. Thanh, N.X. Cuong, N.H. Nam, P.V. Kiem, Y.H. Kim, and C.V. Minh, *Bioorg. Med. Chem. Lett.* **24**, 2834 (2014).
[5] V.A. Tu, E.G. Lyakhova, C.N. Diep, A.I. Kalinovsky, P.S. Dmitrenok, N.X. Cuong, N.V. Thanh, E.S. Menchinskaya, E.A. Pislyagain, N.H. Nam, P.V. Kiem, V.A. Stonik, and C.V. Minh, *Steroids.* **104**, 246 (2015).
[6] Y. Lu, Y.-C. Lin, Z.-H. Wen, J.-H. Su, P.-J. Sung, C.-H. Hsu, Y.-H. Kuo, M.Y. Chiang, C.-F. Dai, and J.-H. Sheu, *Tetrahedron*. **66**, 7129 (2010).
[7] B.B. Shingate, B.G. Hazra, D.B. Salunke, V.S. Pore, F. Shirazi, and M.V. Deshpande, *Chem. Biol. Interface.* **1**, 198 (2011).
[8] F. de Riccardis, L. Minale, M. Iorizzi, C. Debitus, and C. Lévi, *J. Nat. Prod.* **56**, 282 (1993).
[9] H.T. D’Armas, B.S. Mootoo, and W.F. Reynolds, *J. Nat. Prod.* **63**, 1669 (2000).
[10] A. Rueda, E. Zubia, M.A.J. Ortega, and J. Salvá, *Steroids.* **66**, 897 (2001).
[11] G. Notaro, V. Piccialli, and D. Sica, *J. Nat. Prod.* **55**, 1588 (1992).
[12] A. Aiello, E. Fattorusso, and S. Magno, *J. Nat. Prod.* **50**, 191 (1987).
[13] X.C. Nguyen, A. Longeon, V.C. Pham, F. Urvois, C. Bressy, T.T. Trinh, H.N. Nguyen, V.K. Phan, V.M. Chau, J.F. Briand, and M.L. Bourguet-Kondracki, *J. Nat. Prod.* **76**, 1313 (2013).
[14] H. Miyaoaka, M. Shinohara, M. Shimomura, H. Mitome, A. Yano, K. Iguchi, and Y. Yamada, *Tetrahedron.* **53**, 5403 (1997).
[15] N.P. Thao, N.H. Nam, N.X. Cuong, N.X. Nhiem, P.T. Tung, T.H. Quang, N.T.T. Ngan, P.V. Kiem, C.V. Minh, and Y.H. Kim, *Bull. Korean Chem. Soc.* **34**, 249 (2013).
[16] M. Qin, X. Li, and B. Wang, *Chin. J. Chem.* **30**, 1278 (2012).
[17] M. Kobayashi, *Steroids.* **59**, 27 (1994).
[18] Z.B. Cheng, H. Xiao, C.Q. Fan, Y.N. Lu, G. Zhang, and S. Yin, *Steroids.* **78**, 1353 (2013).
[19] N.X. Zhang, X.L. Tang, L. van Ofwegen, L. Xue, W.J. Song, P.L. Li, and G.Q. Li, *Chem. Biodivers.* **12**, 273 (2015).
[20] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, and M.R. Boyd, *J. Natl. Cancer Inst.* **82**, 1107 (1990).
[21] N.H. Nam, P.V. Kiem, N.K. Ban, N.P. Thao, N.X. Nhiem, N.X. Cuong, C. Tistaert, B. Dejaeger, Y.V. Heyden, J. Quetin-Leclercq, D.T. Thao, and C.V. Minh, *Phytochem. Lett.* **4**, 348 (2011).
[22] D.T. Thao, D.T. Phuong, T.T.H. Hanh, N.P. Thao, N.X. Cuong, N.H. Nam, and C.V. Minh, *J. Asian Nat. Prod. Res.* **16**, 364 (2014).