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# 5,8-Epidioxysterols and related derivatives from a Chinese Soft Coral *Sinularia flexibilis*

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ABSTRACT

Chromatographic separation of the methanolic extract of the marine soft coral, *Sinularia flexibilis*, resulted in the isolation and characterization of four new sterols, 5α,8α-epidioxygorgosta-6-en-3β-ol (**1**), 5α,8α-epidioxygorgosta-6,9(11)-dien-3β-ol (**2**), 22α,28-epidioxycholesta-5,23(*E*)-dien-3β-ol (**3**) and its C-22 epimer (**4**), along with nine known sterols. The structures of the new compounds were determined on the basis of extensive spectroscopic data (IR, MS, $^{1}H$ and $^{13}C$ NMR, HMQC, HMBC, and NOESY) analyses.



## 1. Introduction

Soft corals (Coelenterata, Octocorallia, Alcyonaceae) are a rich source of steroids, terpenoids, and other type of secondary metabolites. The array of secondary metabolites play important roles in the complex behavioral and ecological interactions among organisms [1–5]. Soft corals of the genus *Sinularia* are prolific, with 90 known species of which about 36 have been chemically examined. The genus *Sinularia* is a rich source of a variety of polyhydroxylated steroids [6–8] and diterpenes [9,10]. The species *Sinularia flexibilis* (Alcyoniidae) occurring in different seas of South-Asia was reported to contain a range of cembranoid diterpenes, and some of the metabolites showed significant activity against human tumor cell-lines [11–13]. However, steroids frequently occurring in other *Sinularia* species has not yet been reported from *S. flexibilis*. As a part of our study on the chemical diversity from Chinese marine organisms, the soft coral *S. flexibilis* was collected off inner reef in Hainan Island of South China Sea, PR China. A chemical examination revealed that the metabolites of this species growing in South China Sea were rich in steroids whereas the cembranoid diterpenes were obtained as a minor components. This chemical variation may be due to different chemical environmental factors.

## 2. Experimental

### 2.1. General

Optional rotations were measured on a JASCO DIP-370 polarimeter. IR spectra were recorded on a Perkin-Elmer Nicol FT-50X spectrometer. $^{1}H$ and $^{13}C$ NMR as well as 2D NMR spectra were performed on a Bruker Avance DRX 500 NMR

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spectrometer using TMS as internal standard, and the probe temperature was within 298 K (25 °C). EIMS was performed with a Bruker APEII mass spectrometer while HREIMS spectra were obtained on Bruker Daltonics APEXII-FT-ICR-EIMS mass spectrometer. HRFABMS spectra were obtained on a VG Autospec spectrometer. Column chromatography was carried out on Merck Silica gel (200–400 mesh), and the HF-254 silica gel for TLC was provided by Sigma Co. Ltd. Sephadex LH-20 columns (18–110 mm) were obtained from Pharmacia. High-pressure liquid chromatography (HPLC) was performed on an Alltech-426 apparatus using Kromasil prepack column (ODS, 10 mm × 250 mm, for reverse-phase) and monitored by UV detector.

### 2.2. Collection, extraction and isolation

The soft coral *S. flexibilis* was collected by SCUBA diving off coral reef at a depth of 15–20 m at Sanya in Hainan Island, South China Sea, PR China, in June 2003. The sample was frozen immediately after collection. The species was identified by Dr. Lee P. Van Ofwengen (National Museum of National History Naturalis, The Netherlands), and a voucher specimen (HSE-11) was deposited at the same museum and also State Key Laboratory of Natural and Biomimetic Drugs of Peking University, Beijing, PR China.

The soft coral (500 g, dry weight) was homogenized and extracted with MeOH for three times under room temperature, and the MeOH extracts were combined and then concentrated under vacuo to give a dark brown residue (20 g). The crude residue was subjected to silica gel column chromatography using a gradient of petroleum ether–EtOAc (from 10:1 to EtOAc) to obtain 16 fractions (F1–F16), The sterol components mainly existed in fractions F4–F6, which were then combined. The combined residue (300 mg) was subsequently subjected on the silica gel column eluting by a gradient of petroleum ether–acetone to collect six fractions ($F_{4-6}$-1–$F_{4-6}$-6). $F_{4-6}$-6 (50 mg, 4:1) showing a major spot on TLC was purified over silica gel with petroleum ether–EtOAc (10:1) as an eluent to yield **12** (20 mg). $F_{4-6}$-1 (80 mg) was subjected to silica gel column chromatography using petroleum ether–acetone (10:1) as an eluent to obtain two parts designated part 1 (12 mg) and part 2 (65 mg), and each of them showed a main spot in TLC, but exhibited a mixture of sterols in $^1$H NMR spectrum. Part 1 was then separated through semi-preparative HPLC ($C_{18}$) using MeOH–$H_2O$ (98:2) as a mobile phase to yield **10** (4.8 mg) and **11** (2.3 mg), and part 2 was followed by the same manner as part 1 to yield **1** (15.5 mg), **2** (4.6 mg), **7** (15.0 mg), **3** (8.6 mg), **4** (10.6 mg), **5** (2.5 mg), and **6** (4.4 mg), respectively. $F_{4-6}$-2 (68 mg) showing a main spot in TLC was separated directly by semi-preparative HPLC using MeOH–$H_2O$ (97:3) as a mobile phase to obtain **8** (12.5 mg), **9** (12.2 mg), and **13** (6.9 mg). The isolation of remaining fractions $F_{4-6}$-3–5, which showed a mixture of cembranoids and lipids were excluded in this paper.

### 2.3. Spectral data of new compounds

#### 2.3.1. 5α,8α-Epidioxygorgosta-6-en-3β-ol (1)

White amorphous powder. $[\alpha]_D^{20}$ −22.7° (c 0.15, $CHCl_3$); IR (KBr) $\nu_{max}$: 3454 (OH), 3049, 2957, 2931, 1664, 1460, 1380, 1160, 1043, 958 cm$^{-1}$; EIMS *m/z* 456 $[M]^+$ (5), 438 $[M-H_2O]^+$ (9), 424 $[M-O_2]^+$ (100), 391 (13), 365 (6), 326 (5), 301 (3), 152 (11); HRFABMS *m/z* 479.3492 (calcd for $C_{30}H_{48}O_3Na$ 479.3495); $^1$H and $^{13}$C NMR data, see Table 1.

#### 2.3.2. 5α,8α-Epidioxygorgosta-6,9(11)-dien-3β-ol (2)

White amorphous powder. $[\alpha]_D^{20}$ +10.8° (c 0.54, $CHCl_3$); IR (KBr) $\nu_{max}$: 3305 (OH), 3049, 2959, 2874, 1639, 1568, 1460, 1377, 1266, 1164, 1076, 1036, 935 cm$^{-1}$; EIMS *m/z* 454 $[M]^+$ (3), 422 $[M-O_2]^+$ (25), 404 (25), 379 (7), 327 (11), 327 (8), 267 (7), 251 (59), 209 (22), 152 (30), 109 (33), 97 (62); HRFABMS *m/z* 477.3332 (calcd for $C_{30}H_{46}O_3Na$ 477.3338); $^1$H and $^{13}$C NMR data, see Table 1.

#### 2.3.3. 22α,28-Epidioxycholesta-5,23(E)-dien-3β-ol (3)

White amorphous powder. $[\alpha]_D^{20}$ −14.3° (c 0.28, $CHCl_3$); IR (KBr) $\nu_{max}$: 3461 (OH), 3040, 2962, 2931, 1671, 1461, 1379, 1262, 1101, 1056, 1025, 966 cm$^{-1}$; EIMS *m/z*: 428 $[M]^+$ (4), 410 $[M-H_2O]^+$ (25), 396 $[M-O_2]^+$ (2), 267 (6), 301 (5), 283 (15), 255 (11), 213 (10), 159 (22), 137 (100), 127 (36), 95 (40); HREIMS $[M]^+$ *m/z* 428.3277 (calcd for $C_{28}H_{44}O_3$ $[M]^+$ 428.3290); $^1$H and $^{13}$C NMR data, see Table 1.

#### 2.3.4. 22β,28-Epidioxycholesta-5,23(E)-dien-3β-ol (4)

White amorphous powder. $[\alpha]_D^{20}$ −11.4° (c 0.07, $CHCl_3$); IR (KBr) $\nu_{max}$: 3338 (OH), 3025, 2928, 2853, 1671, 1463, 1379, 1102, 1055, 966 cm$^{-1}$; EIMS *m/z*: 428 $[M]^+$ (25), 410 $[M-H_2O]^+$ (100), 396 $[M-O_2]^+$ (6), 283 (8), 255 (26), 213 (10), 159 (21), 145 (24), 137 (100), 105 (52), 95 (58); HREIMS *m/z* 428.3319 (calcd for $C_{28}H_{44}O_3$ $[M]^+$ 428.3290); $^1$H and $^{13}$C NMR data, see Table 1.

## 3. Results and discussion

A repeated chromatography of the MeOH extract of a soft coral *S. flexibilis* resulted in the isolation and characterization of four new 5α,8α-epidioxysterols (**1–4**), together with nine known sterols. The structures of sterols (**5–13**) were identical to 5,8-epidioxy-24-methylcholesta-6,24(28)-dien-3β-ol (**5**) [14], ergosterol peroxide (**6**) [15], 5,8-epidioxy-24-methylcholesta-6-en-3β-ol (**7**) [14], 5,8-epidioxy-22,23-methylene-24-methylcholesta-6-en-3β-ol (**8**) [16], 5,8-epidioxy-23,24-dimethylcholesta-6,22-dien-3β-ol (**9**) [17], 5,8-epidioxy-24-methylcholesta-6,9(11),24(28)-trien-3β-ol (**10**) [14], 5,8-epidioxy-24-methylcholesta-6,9(11),22-trien-3β-ol (**11**) [14], 24-methylcholesta-5,24(28)-dien-3β-ol (**12**) [18–19], and 24-methylcholesta-5,24(28)-diene-3,7-diol (**13**) [20], respectively (Fig. 1), by comparison of their spectroscopic data with those reported in literature. The occurrence of the $\Delta^{9(11)}$ containing 5α,7α-epidioxy-6-ene nucleus was a rare group of sterols discovered in nature, and isolated from the genus *Sinularia* for the first time.

The molecular formula of **1** was determined to be $C_{30}H_{48}O_3$ by HRFABMS and NMR data, possessing 7° of unsaturation. In $^1$H NMR spectrum, the presence of two angular methyl signals at $\delta_H$ 0.80 (3H, s, H-18) and 0.90 (3H, s, H-19), two vicinal coupled olefinic protons at $\delta_H$ 6.53 (1H, d, $J$ = 8.5 Hz, H-6) and 6.27 (1H, d, $J$ = 8.5 Hz, H-7), and a oxygenated methine at $\delta_H$ 3.99 (1H, m, H-3) were characteristic of a 3-hydroxy-6-en-5,8-epidioxysterol nucleus, which was also recognized by the $^{13}$C NMR signals at $\delta_C$ 66.5 (d, C-3), 82.2 (s, C-5), 130.8 (d, C-6), 135.4 (d, C-7), and 79.5 (s, C-8). The presence of a peroxide group