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Pregnane steroids from a gorgonian coral *Subergorgia suberosa* with anti-flu virus effects

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ABSTRACT

Five new pregnane-type steroids namely subergorgols T–X (**1**–**5**) and three known analogues (**6**–**8**) were isolated from a gorgonian coral *Subergorgia suberosa*. The structures of new compounds were determined on the basis of extensive spectroscopic (IR, MS, 1D and 2D NMR) data analyses, in association with photochemical transformation and ECD methods for the configurational assignment. Compounds **1**–**8** were evaluated for the inhibitory effects against H1N1 virus infected in MDCK cells, while subergorgols T–U and 1,2-dehydroprogesterone exerted potent inhibition against A/WSN/33 virus.

1. Introduction

Marine invertebrates are recognized to be the rich source of steroids with structural diversity [1–3]. Apart from steroid glycosides and sulfated polyoxide steroids obtained from starfishes [4–7], the steroids with unique scaffolds such as dimeric steroid (crellastatin A) [8], hemiketal steroid (cladiellin A) [9], 24-*N*-imidazolyl steroidal alkaloid (amaranzole A) [10], and steroid glycosides with an isopropyl side chain (sokodosides) [11] were generated by marine sponges and soft corals. In addition, gorgonian corals appear to be the prolific sources to produce steroids. For instance, the gorgonian coral *Subergorgia suberosa* has been investigated extensively, while a profile of sterol derivatives including 9,11-secosteroids [12–15], pregnane steroids [16,17], and polyhydroxy steroids were isolated [18,19]. It was noted that the structural variety of *S. suberosa* was related to their ecological locations. Among the abundance of marine sterols, pregnane steroids are a rare group in the marine environment. Although the ecological role of pregnane sterols derived from marine organisms is unclear, some analogues exhibiting cytotoxicity against tumor cell lines and antibacterial activity [20] conducted to be the potential leads for pharmaceutical usage. In the course of our discovery for bioactive metabolites derived from marine benthic organisms, a gorgonian coral *S. suberosa* was collected from the coral reef near Yongxin island of South China Sea. Analyses of the HPLC chromatographic spectrum [18,19] of the EtOAc extract in association with the NMR and MS features revealed that the EtOAc extract contained a number of components with unreported structures. In addition, the EtOAc extract exhibited the inhibitory effect against A/WSN/33 virus. Thus, a separation protocol was designed for the isolation and purification of the bioactive compounds. Chromatographic separation of the EtOAc extract resulted in the isolation of eight pregnane sterols including five new compounds (Fig. 1). This paper reports the structural elucidation of the new compounds and their antiviral effects.

2. Experimental

2.1. General methods

Optical rotations were measured on a Rudolph IV Autopol automatic polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer. ^{1}H and ^{13}C NMR, and 2D NMR spectra were recorded on Bruker Advance 400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively). Chemical

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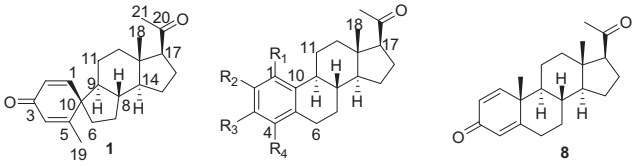

Fig. 1. Structures of compounds **1–8**.

shifts are expressed in δ (ppm) referenced to the solvent peaks at δ_H 7.28 and δ_C 77.0 for $CDCl_3$, and coupling constants are in Hz. HREIMS spectra were obtained from a Autospee Ultima-TFO spectrometer. Silica gels (160–200 and 200–300 mesh, Qingdao Marine Chemistry Co. Ltd.) and ODS (50 μm, YMC) were used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis. Semi-preparative HPLC chromatography was performed on an Alltech instrument (426-HPLC pump) equipped with an Alltech uvis-200 detector at 210 nm, and semi-preparative reversed-phase columns (YMC-packed C_{18}, 5 μm, 250 mm × 10 mm) were purchased from YMC Co.

2.2. Animal material

The gorgonian coral *S. suberosa* was collected from the coral reef at a depth of around 8 m near Yongxin island of South China Sea, in May 2013. Samples were frozen immediately after collection. The specimen was identified by Leen van Ofwegen (National Museum of National History Naturalis). The voucher specimens (YXQ-07 (9)) are deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University, China.

2.3. Extraction and isolation

Gorgonian coral *S. suberosa* (2.3 kg) was homogenized and extracted with 95% EtOH (7 L ×3). The concentrated extract (39.6 g) was desalted by dissolving in MeOH to obtain a residue, which was further partitioned between H_2O and EtOAc to obtain an EtOAc extract (10.0 g). The EtOAc extract (2.0 g) was subjected to column chromatography (2.5 × 20 cm) using silica gel (160–200 mesh, 50 g) with a gradient of petroleum ether PE/acetone (gradient from 20:1 to 1:1) to obtain seven fractions (FA–FG). FC (50 mg) was further separated on ODS column eluting with MeOH/H_2O (7:3) as a mobile to yield compounds **3** (10.0 mg), **4** (2.0 mg), and **5** (2.0 mg). FD (100 mg) was chromatographed by semipreparetive HPLC (C_{18}) using MeOH/H_2O (82:18) as a mobile phase to yield compounds **2** (5.4 mg), **6** (24.60 mg), and **7** (3.5 mg). FE (619 mg) was purified on an ODS column eluting with MeOH/H_2O (68:32) as a mobile phase to yield compounds **1** (27 mg) and **8** (582 mg).

2.3.1. Subergorgol T (**1**)

White amorphous powder; $[\alpha]_D^{24}$ +84.0 (*c* 0.10, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 283 (2.85) nm; IR (KBr) ν_{max} 3428, 1700, 1659 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 312.2083 $[M]^+$ (calcd for $C_{21}H_{28}O_2$, 312.2089).

2.3.2. Subergorgol U (**2**)

White amorphous powder; $[\alpha]_D^{24}$ +37.6 (*c* 0.54, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 283 (3.47) nm; IR (KBr) ν_{max} 3428, 1697, 1611 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 312.2087 $[M]^+$ (calcd for $C_{21}H_{28}O_2$, 312.2089).

2.3.3. Subergorgol V (**3**)

White amorphous powder; $[\alpha]_D^{24}$ + 51.9 (*c* 0.32, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 283 (3.19) nm; IR (KBr) ν_{max} 3444, 1683 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 312.2088 $[M]^+$ (calcd for $C_{21}H_{28}O_2$, 312.2089).

2.3.4. Subergorgol W (**4**)

White amorphous powder; $[\alpha]_D^{24}$ + 93.0 (c 0.20, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 283 (3.53) nm; IR (KBr) ν_{max} 3444, 1683 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HREIMS *m/z* 312.2075 $[M]^+$ (calcd for $C_{21}H_{28}O_2$, 312.2089);

2.3.5. Subergorgol X (**5**)

White amorphous powder; $[\alpha]_D^{24}$ +60.0 (*c* 0.20, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 283 (3.38) nm; IR (KBr) ν_{max} 3399, 1690,

Table 1
^1H NMR data of compounds **1–5** ($CDCl_3$, 400 MHz).[a]

Position	**1**		**2**		**3**		**4**		**5**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	154.7	6.88 (d, 10.0)	109.8	6.71 (d, 1.5)	118.4	6.76, s	154.7		122.6	
2	126.6	6.23 (d, 10.0)	153.3		136.2		114.2	6.36, s	152.4	
3	186.6		114.5	6.56 (d, 1.5)	113.1	6.51, s	136.4		112.5	6.61,d (8.2)
4	128.6	6.20 (s)	137.9		153.4		122.7	6.54, s	127.4	6.85,d (8.2)
5	163.3		127.2		119.7		140.1		130.6	
6	34.8	2.01 (m), 1.80 (m)	26.5	2.72 (m), 2.57 (m)	22.8	2.82 (m), 2.59 (m)	31.4	2. 84 (m), 2.72 (m)	31.7	2.84 (m), 2.69 (m)
7	30.4	2.01 (m), 1.39 (m)	27.9	1.99 (m), 1.36 (m)	27.2	2.01 (m), 1.38 (m)	26.4	1.78 (m), 1.26 (m)	26.1	1.75 (m), 1.23 (m)
8	42.3	1.82 (m)	37.9	1.40, m	38.0	1.41, m	40.8	1.48, m	41.5	1.51, m
9	56.2	1.59 (ddd, 4.5, 12.0, 12.0)	44.4	2.28, m	44.2	2.28, m	44.3	2.44, m	47.1	2.49, m
10	52.5		141.6		141.6		124.1		139.9	
11	21.9	1.27 (m), 1.19 (m)	26.8	2.32 (m), 1.55 (m)	26.6	2.38 (m), 1.56 (m)	26.4	3.10 (m), 1.35 (m)	28.4	2.42 (m), 1.35 (m)
12	38.4	1.98 (m), 1.33 (m)	39.1	2.19 (m), 1.65 (m)	39.1	2.18 (m), 1.66 (m)	39.8	2.09 (m), 1.72 (m)	39.8	2.09 (m), 1.77 (m)
13	45.1		44.2		44.3		44.1		45.2	
14	56.6	1.33 (m)	55.9	1.38, m	56.0	1.40, m	55.9	1.52, m	56.2	1.59, m
15	24.8	1.72 (m), 1.36 (m)	24.1	1.82 (m), 1.36 (m)	24.2	1.83 (m), 1.38 (m)	24.2	1.78 (m), 1.36 (m)	24.0	1.78 (m), 1.38 (m)
16	23.3	2.22 (m), 1.75 (m)	23.0	2.24 (m), 1.75 (m)	22.9	2.25 (m), 1.74 (m)	22.7	2.24 (m), 1.72 (m)	22.9	2.26 (m), 1.73 (m)
17	62.8	2.54 (t, 9.6)	63.9	2.63, t, (9.6)	64.0	2.64, t, (9.6)	64.1	2.65, t, (9.6)	63.9	2.67, t, (9.6)
18	13.6	0.62 (s)	13.4	0.67, s	13.4	0.68, s	13.9	0.70, s	14.3	0.72, s
19	19.5	1.99 (s)	19.8	2.19, s	21.2	2.29, s	20.6	2.23, s	14.7	2.24, s
20	209.3		209.6		210.1		209.5		209.5	
21	31.4	2.12 (s)	31.5	2.18, s	31.5	2.18, s	31.4	2.16, s	31.4	2.17, s

[a] δ(ppm), *J* (Hz).