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A new pregnane analogue from Hainan soft coral *Scleronephthya gracillimum* Kükenthal

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1. Subject and source

The soft coral *Scleronephthya gracillimum* (Kükenthal, 1906) was collected from Linchang Reef in the South China Sea in April 2006 and identified by Prof. Ren-Lin Zou, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen was deposited at the Key Laboratory of Marine Drugs, Ministry of Education, the School of Medicine and Pharmacy, Ocean University of China with the access code of HN-LCJ-20060008.

2. Previous work

To the best of our knowledge, there have been no reports on chemical constituents of *S. gracillimum*. The previous studies on the chemistry of *Scleronephthya* include the isolation of two pregnanes from *Scleronephthya pallida* (Kittakoop et al., 1999), and five other pregnanes together with two norpregnane glycosides from *Scleronephthya* sp. (Yan et al., 2004).

3. Present study

The soft coral *S. gracillimum* (540.0 g) was exhaustively extracted three times with ethanol at room temperature (2 L × 3), and the combined solution was evaporated to dryness under vacuum (15.5 g). The residue was suspended in water and partitioned with EtOAc, then followed by *n*-BuOH. Both the EtOAc and *n*-BuOH solutions were then concentrated under reduced pressure. The EtOAc extract (3.2 g) was subjected to silica gel vacuum liquid chromatography (VLC) and eluted with petroleum ether containing increasing amounts of EtOAc to yield ten fractions (Fractions 1–10). Fraction 4 was further separated on a silica gel

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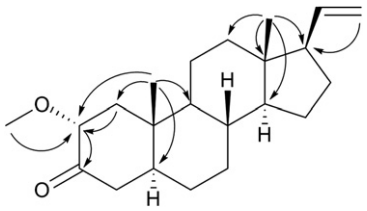

Fig. 1. Selected HMBC correlations for compound **1**.

column chromatography (CC) (petroleum ether/acetone, 20/1, v/v) and Sephadex LH-20 CC ($CHCl_3$/MeOH, 1/1, v/v) to yield compounds **1** (6.0 mg) and **5** (20.0 mg) (Yan et al., 2004). Fraction 2 was subjected to silica gel CC (petroleum ether/EtOAc, 95/5, v/v) and Sephadex LH-20 CC (petroleum ether/$CHCl_3$/MeOH, 2/1/1, v/v/v) to afford **2** (150.0 mg) (Blackman et al., 1985) and **3** (10.0 mg) (Yan et al., 2004). Fraction 9 was subjected to silica gel CC (petroleum ether/acetone, 7/3 ~ 1/1, v/v) then followed by preparative HPLC (MeOH/H_2O, 9/1, v/v) to afford **4** (6.0 mg) (Seo et al., 1995).

Compound **2** showed moderate cytotoxic activity against KB and KBv200 tumor cell lines with the IC_{50} values of 16.0 and 17.6 μg/mL, respectively. Compound **2** also exhibited evident lethality toward brine shrimp *Artemia salina* with 67% mortality at a concentration of 10 μg/mL. However, none of the compounds showed any potent antifouling activity against the larvae settlement of barnacle *Balanus amphitrite* at a concentration of 50 μg/mL.

Compound **1** was obtained as colorless crystals from $CHCl_3$. Its molecular formula was determined as $C_{22}H_{34}O_2$ by HRESIMS, possessing 6° of unsaturation. In the ^{1}H NMR spectrum, the proton signals and the coupling constants at δ_H 4.91 (1H, ddd, J = 16.9, 2.2, 0.8 Hz, H-21a), 4.89 (1H, ddd, J = 10.6, 2.2, 0.8 Hz, H-21b), and 5.69 (1H, ddd, J = 16.9, 10.6, 7.7 Hz, H-20) indicated the presence of a terminal vinyl group. In addition, two methyl group signals at δ_H 0.54 and 0.93, and one methoxyl group signal at δ_H 3.23 were also observed. The ^{13}C NMR and DEPT spectra of **1** exhibited the presence of 22 carbon signals assigned to two methyls, one methoxyl, nine methylenes, seven methines and three quaternary carbons, of which, two olefinic carbons at δ_C 139.8 and 114.5, one oxygenated carbon at δ_C 83.4, and one carbonyl at δ_C 210.4 were observed. The methylene carbon at δ_C 114.5 further confirmed the presence of a terminal vinyl group in compound **1**. Since two unsaturated degrees were accounted for, this implied the molecule should have four rings. The above NMR data as well as that reported in the literature indicated that **1** has a 3-one pregnane skeleton (Kittakoop et al., 1999), with a methoxyl group.

Comparing the ^{13}C NMR spectra of **1** in $CDCl_3$ with that of **2**, there were three additional carbon signals at δ_c 56.7, 40.6 and 83.4. The disappearance of the two double bond carbon signals at δ_c 128.0 and 158.1 in the spectrum of **1** implied the methoxyl group was attached to C-1 or C-2. HMBC correlations between H–OCH_3/C-2, H-1/C-2 and C-3, H-2/C-1 and C-3 confirmed that the methoxyl group was at C-2 (Fig. 1). The NOESY correlations between H-2/H-19, H-2/H-1β, H-1β/H-19, H-1β/H-4β, H-2/H-4β and H-4β/H-19 revealed the β-orientation of H-2 (Fig. 2). Taking all the results above into account, the structure of compound **1** was elucidated as pregna-2-α-methoxy-3-one. ^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.69 (1H, ddd, J = 16.9, 10.6, 7.7 Hz, H-20), 4.91 (1H, ddd, J = 16.9, 2.2, 0.8 Hz, H-21a), 4.89 (1H, ddd, J = 10.6, 2.2, 0.8 Hz, H-21b), 3.45 (1H, dd, J = 2.9, 2.9 Hz, H-2), 3.23 (3H, s, OCH_3), 2.59 (1H, ddd, J = 15.8, 2.9, 2.9 Hz, H-1α), 2.37 (1H, dd, J = 15.8, 2.9 Hz, H-1β), 2.15 (1H, dd, J = 15.0, 14.6 Hz, H-4β), 2.04 (1H, ddd, J = 15.0, 4.0, 2.2 Hz, H-4α), 1.98 (1H, m, H-5), 1.90 (1H, dd, J = 17.6, 8.8 Hz, H-14), 1.72 (1H, m, H-15a), 1.60 ~ 1.61 (3H, m, H-6a, 12b, 16a), 1.48 (1H, m, H-15b), 1.37 ~ 1.39 (3H, m, H-8, 9, 11a), 1.29 ~ 1.31 (3H, m, H-7, 11b), 1.11 (1H, m, H-6b), 1.03 (2H, m, H-12a, 17), 0.93 (3H, s, H-19), 0.89 (1H, m, H-16b), 0.54 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 210.4 (C-3), 139.8 (C-20), 114.5 (C-21), 83.4 (C-2), 56.7 (2-OCH_3), 55.4 (C-17), 55.3 (C-14), 46.4 (C-9), 44.7 (C-4), 43.7 (C-13), 40.6 (C-1), 40.2 (C-10), 39.6 (C-5), 37.3 (C-12), 35.5 (C-8), 31.4 (C-16), 28.6 (C-7), 27.2 (C-15), 24.8 (C-6), 20.6 (C-11), 12.9 (C-18), 12.7 (C-19). ESI-MS^+ m/z: $[M + H]^+$ 331.3, $[M + Na]^+$ 353.3, $[2M + H]^+$ 661.6, $[2M + Na]^+$ 683.6. HRESIMS m/z: $[M + H]^+$ 331.2628 (calcd 331.2637 for $C_{22}H_{35}O_2$).

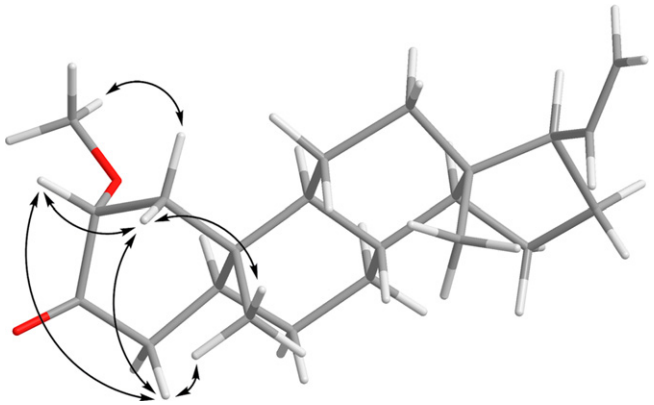

Fig. 2. Selected NOESY correlations for compound **1**.