Cytotoxic 19-oxygenated steroids from the South China Sea gorgonian, *Pacifigorgia senta*

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**ABSTRACT**

Bioassay guided chemical investigation of the gorgonian *Pacifigorgia senta* led to the discovery of a new 19-oxygenated steroid, cholesta-5,24-diene-3β,7β,19-triol (1), as well as three known steroids (2–4). The structure of 1 was determined by extensive spectroscopic analysis, including NMR and MS spectra. All of the compounds exhibited cytotoxicities against HepG2, Hep3B, MCF-7/ADR, PC-3 and HCT-116 cell lines, with the IC_{50} values ranging from 7.0 to 29.7 μM. It is the first report on the chemical constituents of the coral species *P. senta*.

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**GRAPHICAL ABSTRACT**

*Pacifigorgia senta*

Cytotoxicity:
against HepG2, Hep3B, MCF-7/ADR, PC-3, and HCT-116
IC_{50} values: 7.0–29.7 μM

**1. Introduction**

Gorgonians, a diverse group of Cnidaria, are emerging as important sources for bioactive natural products. *Pacifigorgia senta*, a marine octocoral often occurring along the coast of tropical eastern Pacific and western Atlantic, has not been reported on its chemical constituents so far. Only one research group has conducted a chemical investigation of genus *Pacifigorgia* (*Pacifigorgia adamsii, Pacifigorgia pulchra exilis* and *Pacifigorgia media* Verrill) which led to the discovery of an ichthyotoxic sesquiterpene (Izac, Poet et al. 1982) and four germacrene-type sesquiterpenes (Izac, Bandurraga et al. 1982). As part of our ongoing...
research for biologically active secondary metabolites from marine organisms (Li et al. 2011; Cao et al. 2014; Chen et al. 2014; Cao et al. 2015; Sun et al. 2015), the gorgonian \( P. \) \( s \)enta was taken for chemical investigation because of the evident cytotoxicity against a series of tumor cell lines of its EtOAc extract. Bioassay guided fractionation led to the isolation of four 19-oxygenated steroids (1–4) (Figure 1). We describe herein the isolation, structure elucidation and cytotoxicity of these compounds.

2. Results and discussion

Compound 1 was obtained as colourless crystals. Its molecular formula was proposed as \( \text{C}_{27}\text{H}_{44}\text{O}_{3} \) (6° of unsaturation) on the basis of the sodium adduct [M + Na]+ at \( m/z \) 439.3185 (calcd. for \( \text{C}_{27}\text{H}_{44}\text{O}_{3} \text{Na} \), 439.3188) in HRESI-MS. In 1D NMR spectra, the presence of two olefinic proton signals at \( \delta_{H} \) 5.61 (brs) and 5.09 (brt, \( J = 7.2 \text{ Hz} \)), and four olefinic carbon signals at \( \delta_{C} \) 138.3 (C), 131.1 (C), 130.6 (CH) and 125.2 (CH) suggested two trisubstituted double bonds were presented in 1, which accounted for 2° of unsaturation. Consequently, the molecule should have four rings. The rest of the signals from \( ^{13}\text{C} \) NMR and DEPT spectra revealed the presence of two oxymethines, one oxymethylene, nine methylenes, five methines, two quaternary carbons and four methyls. All of the NMR data suggested a typical steroid framework for 1. A careful comparison of its \( ^{1}\text{H} \) NMR spectral data with that of the known compound 2 previously identified from a formosan soft coral \( \text{Nephthea erecta} \) (Cheng et al. 2007) indicated a very close structural relationship between them. In \( ^{1}\text{H} \) NMR spectra, the significant downfield chemical shifts of Me-26 (\( \delta_{H} \) 1.68 (s) in 1 vs 0.88 (d, \( J = 6.6 \text{ Hz} \)) in 2) and Me-27 (\( \delta_{H} \) 1.60 (s) in 1 vs 0.88 (d, \( J = 6.6 \text{ Hz} \)) in 2) revealed a 24-trisubstituted double bond appeared in 1. HMBC correlations from Me-26 to C-24, C-25 and C-27; and from Me-27 to C-24, C-25 and C-26 further confirmed the presence of 24-trisubstituted double bond for 1 (Supplementary Figure S1). A detailed analysis of 1D and 2D NMR spectra led to the establishment of the full planar structure of 1.

The relative configuration of 1 was determined based on coupling constants and biogenetic considerations. Firstly, the relative configurations of C-3 and C-7 were determined by an analysis of the coupling constants. The axial proton at C-4 (\( \delta_{\text{Hax-4}} \) 2.21 (t, \( J = 11.4 \text{ Hz} \))) showed a large \( \text{trans} \)-diaxial coupling (11.4 Hz) with H-3, indicating that H-3 must be axially oriented, thereby placing the 3–OH group at an equatorial position. The olefinic proton signal H-6 (\( \delta_{H} \) 5.61 (brs)) showed a very small coupling, while the oxymethine signal H-7 (\( \delta_{H} \) 3.78 (d, \( J = 7.8 \text{ Hz} \))) showed a large coupling to H-8 (axial), demonstrating that H-7 occupied an axial position; consequently, 7–OH adopted an equatorial orientation. Similar 19-oxygenated steroids containing 3\( \beta \),7\( \beta \),19-triol or 3\( \beta \),7\( \alpha \),19-triol were reported in the literature (Cheng et al. 2007). Furthermore, the full relative configurations of 1 were proposed to be consistent with the configurations of the co-isolated

![Figure 1. Structures of compounds 1–4.](image-url)
known analogues 2–4 on the basis of biogenetic considerations. Compound 1 was determined as cholest-5,24-diene-3β,7β,19-triol.

The known secondary metabolites cholest-5-ene-3β,7β,19-triol (2), cholest-5,22-diene-3β,7β,19-triol (3) and ergosta-5,24(28)-diene-3β,7β,19-triol (4) were identified under the direction of comprehensive spectroscopic methods, including 1H NMR, 13C NMR and MS, and also by a careful comparison of their spectroscopic data with those reported in literature (Aiello et al. 1992; Cheng et al. 2007). Steroids are very abundant components in corals, but 19-oxygenated steroids have been relatively rarely reported. To the best of our knowledge, totally less than forty steroids with a hydroxyl or an acyl moiety at C-19 have been found from corals. In the present study, four 19-oxygenated steroids were obtained from the gorgonian P. senta, which is the first report on the chemical constituents of the coral species P. senta.

The cytotoxicities of 1–4 against human hepatoma HepG2 and Hep3B, human breast cancer MCF-7/ADR, human prostatic cancer PC-3 and human colon carcinoma HCT-116 cell lines were evaluated by the MTT method (Scudiero et al. 1988) with epirubicin as a positive control. All of the compounds revealed cytotoxic activities against all of the tested cell lines, of which 2 with a saturated side chain is the most active compound (Table 1). Moreover, compounds 1–4 showed stronger cytotoxic activities against MCF-7/ADR, PC-3, and HCT-116 cell lines with the IC50 values ranging from 7.0 to 14.7 μM than towards HepG2 and Hep3B.

3. Experimental

3.1. General experimental procedures
Optical rotations were measured on a JASCO P-1020 digital polarimeter. IR spectrum was recorded on a Nicolet-Nexus-470 spectrometer (Nicolet Corp., Madison, WI, USA) using KBr pellets. NMR spectra were acquired using a JEOL JEM-ECP NMR spectrometer (JEOL Ltd., Tokyo, Japan) (600 MHz for 1H and 150 MHz for 13C), using TMS as internal standard. ESI-MS spectra were obtained from a Micromass Q-TOF spectrometer (Waters Corp., Milford, MA, USA). HPLC was performed on a Waters 1525 system coupled with a Waters 2996 photodiode array detector (Waters Corp., Milford, MA, USA), using a C18 column (Kromasil, 5 μm, 250 × 10 mm) for semi-preparation HPLC. Silica gel (Qing Dao Hai Yang Chemical Group Co., Qingdao, China) (200–300 mesh) and Sephadex LH-20 (Amersham Biosciences Inc. Piscataway, NJ, USA) were used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., Yantai, China) (G60, F-254) were used for thin layer chromatography.

3.2. Gorgonian material
A sample of gorgonian coral P. senta was collected from Xisha islands coral reef in the South China Sea in April 2009. The coral was identified by Prof. Hui Huang, South China Sea Institute.

Table 1. IC50 value (μM) of compounds 1–4 against tumor cell lines in vitro.

| Cell line | HepG2 | Hep3B | MCF-7/ADR | PC-3 | HCT-116 |
|-----------|-------|-------|-----------|------|---------|
| 1         | 27.1  | 27.9  | 12.9      | 12.7 | 10.5    |
| 2         | 13.2  | 11.9  | 8.2       | 7.1  | 7.0     |
| 3         | 25.4  | 29.7  | 7.1       | 8.7  | 10.9    |
| 4         | 23.6  | 19.2  | 10.0      | 14.7 | 12.1    |
| Epirubicin | 1.66  | 0.97  | 1.66      | 0.47 | 0.83    |

Epirubicin is the positive control.
of Oceanology, Chinese Academy of Sciences. A voucher specimen (HN-XS-20090003) was deposited at the Key Laboratory of Marine Drugs, Ministry of Education, the School of Medicine and Pharmacy, Ocean University of China.

3.3. Extraction and isolation
The gorgonian P. senta (1.5 kg, wet weight) was repeatedly extracted with ethanol (98%, 10 L) at room temperature. The combined extracts were evaporated to dryness under vacuum. The resulting brown residue was partitioned between H₂O (1 L × 3) and EtOAc (1 L × 3). The EtOAc layer exhibited cytotoxicity against a series of tumour cell lines. The EtOAc extract (14.2 g) was subjected to silica gel vacuum liquid chromatography eluted with petroleum ether containing increasing amounts of EtOAc, to yield five fractions (Fractions 1–5). Fr.3 and Fr.4 showed evident cytotoxicities. Then, Fr.3 was chromatographed on silica gel (petroleum ether/acetone, 70/30–30/70, v/v), then Sephadex LH-20 (CHCl₃/MeOH, 1:1, v/v), and finally semi-preparative HPLC (MeOH/H₂O, 9/1, v/v) to obtain 1 (4.2 mg) and 2 (15.6 mg). Fr.4 was subjected to silica gel column chromatography (petroleum ether/acetone, 60/40–25/75, v/v) and then semi-preparative HPLC (MeOH/H₂O, 9/1, v/v), to yield 3 (50.0 mg) and 4 (8.8 mg).

3.3.1. Cholest-5,24-diene-3β,7β,19-triol (1)
Colourless crystals. [α]D²⁵ +19 (c = 0.6, CHCl₃). IR (KBr) νmax: 3405, 2940, 1726, 1670, 1465, 1365, 1245, 1056, 757 cm⁻¹. ¹H NMR (CDCl₃, 600 MHz, δ, ppm): 5.61 (1H, brs, H-6), 5.09 (1H, brt, J = 7.2 Hz, H-24), 3.83 (1H, d, J = 11.4 Hz, Ha-19), 3.78 (1H, d, J = 7.8 Hz, H-7), 3.59 (1H, d, J = 11.4 Hz, Hb-19), 3.56 (1H, m, H-3), 2.41 (1H, brd, J = 11.4 Hz, Hα-4), 2.21 (1H, t, J = 11.4 Hz, Hβ-4), 2.02 (1H, m, Ha-23), 1.99 (1H, m, Ha-12), 1.90 (1H, m, Ha-1), 1.88 (1H, m, Hb-23), 1.85 (1H, m, Ha-15), 1.83 (1H, m, Hb-2), 1.80 (1H, m, H-8), 1.68 (3H, s, H-26), 1.62 (1H, m, Ha-11), 1.60 (3H, s, H-27), 1.53 (1H, m, Hb-11), 1.49 (1H, m, Ha-16), 1.41 (1H, m, Ha-2), 1.37 (1H, m, H-20), 1.33 (1H, m, Hb-16), 1.28 (1H, m, Hb-15), 1.12 (1H, m, H-17), 1.10 (2H, m, H-22), 1.08 (1H, m, Hb-12), 1.06 (1H, m, H-14), 1.04 (1H, m, Hb-1), 0.98 (1H, m, H-9), 0.94 (3H, d, J = 6.6 Hz, H-21), 0.73 (3H, s, H-18). ¹³C NMR (CDCl₃, 150 MHz, δ, ppm): 138.3 (C, C-5), 131.1 (C, C-25), 130.6 (CH, C-6), 125.2 (CH, C-24), 72.5 (CH, C-7), 71.1 (CH, C-3), 62.8 (CH₂, C-19), 57.0 (CH, C-14), 55.5 (CH, C-17), 48.7 (CH, C-9), 43.3 (C, C-13), 42.2 (CH, C-8), 41.8 (CH₂, C-4), 41.4 (C, C-10), 40.0 (CH₂, C-12), 36.2 (CH₂, C-22), 35.6 (CH, C-20), 33.4 (CH₂, C-1), 31.9 (CH₂, C-2), 28.6 (CH₂, C-16), 26.2 (CH₂, C-15), 25.8 (CH₂, C-27), 24.8 (CH₂, C-23), 21.8 (CH₃, C-11), 18.8 (CH₂, C-21), 17.7 (CH₂, C-26), 12.3 (CH₂, C-18). HRESI-MS: 439.3185 [M + Na]^+ (calcd. for C₂₇H₄₄O₃Na, 439.3188).

3.4. Cytotoxicity assay
Cytotoxic activity was evaluated by the MTT method as described previously (Scudiero et al. 1988). Five tumor cell lines, including HCT-116, MCF-7/ADR, PC-3, HepG2, and Hep3B were used. Epirubicin was used as a positive control.

4. Conclusions
In summary, one new 19-oxygenated steroid, cholesta-5,24-diene-3β,7β,19-triol (1), together with three known steroids (2–4), was isolated from the South China Sea gorgonian P. senta. Their structures were elucidated using comprehensive spectroscopic methods. All of the compounds exhibited moderate to strong cytotoxicities against HepG2, Hep3B, MCF-7/ADR, PC-3 and HCT-116 cell lines.
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Supplementary material
The selected 1H,1H-COSY and HMBC correlations of 1, the 1H NMR, 13C NMR, 1H-1H COSY, HMQC, HMBC and HRESI-MS spectra of 1 are available.

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