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New polyoxygenated steroids from the South China Sea gorgonian *Echinogorgia aurantiaca*

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Three new polyoxygenated steroids, named 3β,7α,9α-trihydroxy-cholestan-6-one (**1**), 3β,5α,6β-trihydroxycholestan-1-one (**2**) and cholestane-3β,5α,6β,11β–tetrol (**3**), along with four known steroids (**4**–**7**) were isolated from the South China Sea gorgonian *Echinogorgia aurantiaca*. The structures of **1**–**3** were established by extensive spectroscopic analysis, including 1D and 2D NMR data.

1. Introduction

Marine organisms, especially gorgonians, have been well-recognized as a natural source of polyhydroxy sterols. Previous chemical investigations on the gorgonian have led to the isolation and identification of many polyhydroxy steroids (Tanaka et al. 2002; Zhang et al. 2004; Sheu et al. 2000; Fusetani et al. 1987; Garrido et al. 2000; Shen et al. 2001; González et al. 2001). Some of them exhibited cytotoxic activity against various cancer cell lines (Tanaka et al. 2002; Fusetani et al. 1987; Garrido et al. 2000; González et al. 2001). During the course of further searching for novel active compounds from gorgonians (Qi et al. 2004, 2005), we undertook the investigation of the South China Sea gorgonian coral *Echinogorgia aurantiaca*, and three new oxygenated steroids, named 3β,7α,9α-trihydroxy-cholestan-6-one (**1**), 3β,5α,6β-trihydroxycholestan-1-one (**2**) and cholestane-3β,5α,6β,11β-tetrol (**3**), along with four known steroids (**4**–**7**) chelestane-3β,5α,6β-triol (Yamada et al. 1980) (**4**), chelestane-1β,3β,5α,6β-tetrol (Kobayashi et al. 1983) (**5**), ergosta-7,22-diene-3,5,6-triol (Iorizzi et al. 1988) (**6**) and cheolest-5-ene-3,7,19-triol (Aiello et al. 1992) (**7**) were obtained. This paper deals with the isolation and structure elucidation of these new compounds.

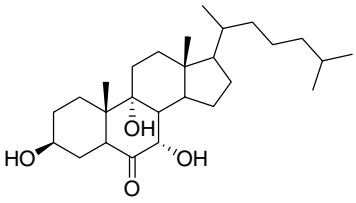
2. Investigations, results and discussion

Compound **1** exhibited a molecular ion peak at m/z 434 $[M]^+$ in its EIMS. Together with ^1H and ^{13}C NMR spectral data (Tables 1 and 2), a molecular formula of $C_{27}H_{46}O_4$ was established and confirmed by HREIMS. Its ^1H NMR spectrum exhibited signals for two tertiary methyls at δ_H 1.17, 1.58 (each 3 H, s), three secondary methyls at δ_H 0.95 (3 H, d, J = 6.3 Hz), 0.89 (6 H, d, J = 6.6 Hz), and two oxymethine protons at δ_H 4.72 (1 H, m), 4.63 (1 H, brs). The ^{13}C (DEPT) NMR spectra showed 27 carbon signals, including 5 methyls, 10 methylenes, 8 methines (two of which were oxygenated), 3 quaternary carbons (δ_C 80.7, 49.6, 42.9) and a ketone group (δ_C 213.5). These data suggested that **1** was a polyoxygenated cholest-type steroid (Yamada et al. 1980; Kobayashi et al. 1983).

In the HMBC spectrum (Fig.) of **1**, HMBC correlations of δ_H 4.72 (1 H, m) with δ_C 42.5 (C-1), 37.4 (C-4), 47.5 (C-5), δ_H 2.58 (1 H, m, H-5) with δ_C 16.8 (C-19), 42.9 (C-10), 66.8 (C-3), 213.5 (s), 68.4 (d), and δ_H 4.63 (1 H, brs) with δ_C 213.5, 47.5 (C-5), 33.6 (C-8), 58.2 (C-14), indicated the assignments of C-3 (δ_C 66.8, d), C-6 (δ_C 213.5, s), C-7 (δ_C 68.4, d), with corresponding protons of δ_H 4.72 (1 H, m, H-3), 4.63 (1 H, brs, H-7). HMBC correlations of H-19 (δ_H 1.58, 3 H, s), H-8 (δ_H 2.47, 1 H, m) and H-14 (δ_H 1.35, 1 H, m) with δ_C 80.7 (s) suggested the oxygenation of C-9 (δ_C 80.7). In the NOESY spectrum of **1**, NOE interactions of H-3 with H-4a (δ_H 2.65, m), and H-19 with both H-7 and H-4b (δ_H 2.54, m), indicated the α-orientation of H-3 and β-orientation of H-7. Thus, the structure of **1** was established as 3β,7α,9α-trihydroxycholestan-6-one.

Compound **2** also had the molecular formula of $C_{27}H_{46}O_4$ as deduced from NMR spectra and HREIMS spectrum. The ^{13}C and ^1H NMR spectral data of **2** (Tables 1 and 2) were very similar to those of **4** (Yamada et al. 1980), with the only difference of the presence of a ketone group (δ_C 212.1) instead of a methylene (δ_C 33.3). The assignment of C-1 (δ_C 212.1) was supported by the HMBC spectrum showing correlations of H-3 (δ_H 4.33, 1 H, m), H-19 (δ_H 1.44, 3 H, s) with C-1. In the NOESY spectrum of **2**, NOE correlations of H-19 with H-4b (δ_H 1.80, 1 H, m), and H-4a (δ_H 2.52, 1 H, m) with H-3/H-6 (δ_H 3.67, 1 H, brs), suggested the α-orientation of H-3 and H-6. Thus, the structure of **2** was determined as 3β,5α,6β-trihydroxycholestan-1-one.

Compound **3** was assigned the molecular formula of $C_{27}H_{48}O_4$ on the basis of its EIMS and ^{13}C (DEPT) NMR spectra. Both the ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) were closely similar to those of compound **4** (Yamada et al. 1980). The only difference between them was the oxygenation of C-11 (δ_C 69.5, d), which was supported by the HMBC spectrum showing correlations of H-11 [δ_H 4.14, 1 H, (brd, J = 2.85 Hz)] with C-9 (δ_C 49.6,



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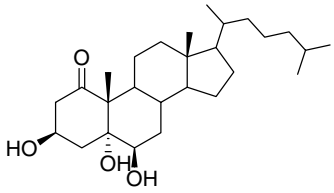

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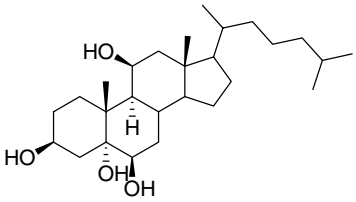

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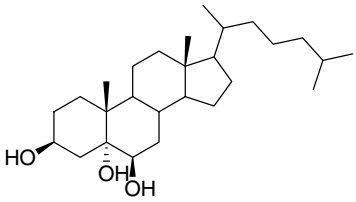

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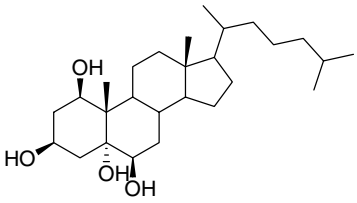

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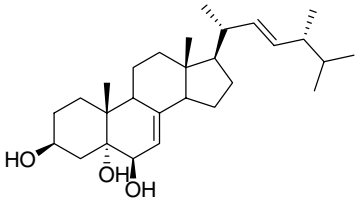

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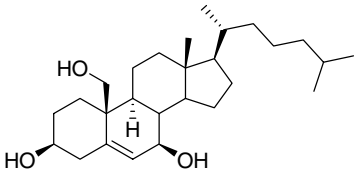

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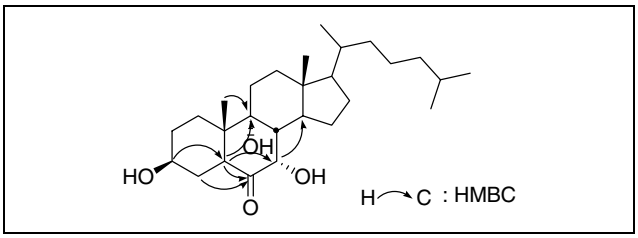

Fig.: Key HMBC Correlations for Compound **1**

d), C-10 (δ_C 40.0, s), C-8 (δ_C 28.4, d) and C-13 (δ_C 43.1, s). In the NOESY spectrum of **3**, correlations of H-19 (δ_H 1.40, 3 H, s) with H-4b (δ_H 2.17, 1 H, m), H-4a (δ_H 1.53, 1 H, m) with H-3 (δ_H 4.02, 1 H, m)/H-6 (δ_H 3.45, 1 H, brs), and H-11 with H-9 (δ_H 1.52, 1 H, m) suggested the α-orientation of H-3, H-6 and H-11. Based on the above data, compound **3** was established as cholestane-3β,5α,6β,11β-tetrol.

3. Experimental

3.1. General procedures

Optical rotations were measured with a Polartronic HNQW5 high resolution polarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as internal standard. Si gel (200–300 mesh) for column chromatography was obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China. Precoated Si gel plates were used for analytical TLC.

3.2. Animal material

The South China Sea gorgonian coral *E. aurantiaca* was collected in Sanya, Hainan province, China in October 2003 and identified by Prof. Zou R. L., the South China Sea Institute of Oceanology, *Academia Sinica*. A voucher specimen (No. 0320) was deposited in the South China Sea Institute of Oceanology, *Academia Sinica*, Guangzhou, China.

3.3. Extraction and isolation

The fresh gorgonian was exhaustively extracted with EtOH-CH_2Cl_2 at room temperature, and the solvent was evaporated *in vacuo*. The residue was partitioned in H_2O and extracted with chloroform, *n*-butanol, respectively, to provide the chloroform fraction (20 g) and the *n*-butanol fraction (7.7 g). The chloroform fraction was chromatographed on Si gel using chloroform-acetone (stepwise, 0–50% acetone), followed by chloroform-MeOH (stepwise, 60–100% MeOH), to yield 12 fractions. Fraction 7 eluted with 20% acetone was further chromatographed on Si gel to yield **1** (5 mg). Fraction 9 eluted with 30% acetone afforded **2** (6 mg), **4** and **6**. Fraction 10 eluted with 40% acetone yielded **3** (10 mg). Fraction 11 eluted with 50% acetone yielded **5** and **7**.

3.4. 3β,7α,9α-Trihydroxycholestan-6-one (**1**)

White powder; $[\alpha]_{589}^{20}$ −0.8° (c = 1.3 × 10^{-3}, Me_2CO); UV (Me_2CO) λ_{max} 197 nm; IR (KBr) ν_{max} 3510, 2900, 1715, 1465, 1190 cm^{-1}; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; EIMS(+) m/z 434 $[M]^+$; HREIMS m/z 434.3389 $[M]^+$ (calcd for $C_{27}H_{46}O_4$, 434.3396).

3.5. 3β,5α,6β-Trihydroxycholestan-1-one (**2**)

Colorless needle crystal; $[\alpha]_{589}^{20}$ 34.3° (c = 2.1 × 10^{-3}, Me_2CO); UV (Me_2CO) λ_{max} 197 nm; IR (KBr) ν_{max} 3438, 2880, 1720, 1460, 1185 cm^{-1}; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; HREIMS m/z 434.3396 $[M]^+$ (calcd for $C_{27}H_{46}O_4$, 434.3396).

3.6. Cholestane-3β,5α,6β,11β-tetrol (**3**)

Colorless needle crystal; $[\alpha]_{589}^{20}$ 12.4° (c = 4.6 × 10^{-3}, MeOH); UV (MeOH) λ_{max} 196 nm; IR (KBr) ν_{max} 3480, 2900, 1460, 1375, 1200 cm^{-1}, ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 2; HREIMS m/z 436.3549 $[M]^+$ (calcd for $C_{27}H_{48}O_4$, 436.3553).

Table 1: ^{1}H NMR Spectral data of compounds 1–3$^{\alpha}$

H	1	2	3
1	3.13 (t, 13.55, 3.85), 2.48 (m)	–	1.6 ~ 1.7 (m)
2	2.18, 2.33 (each m)	2.83 (m), 2.58 (m)	1.79, 1.57 (each m)
3	4.72 (m)	4.33 (m)	4.02 (m)
4	2.65, 2.54 (each m)	2.52, 1.80 (each m)	2.17, 1.53 (each m)
5	2.58 (m)	–	–
6	–	3.67 (brs)	3.45 (brs)
7	4.63 (brs)	1.55 ~ 1.75 (m)	1.71 (m)
8	2.47 (m)	1.73 (m)	2.08 (m)
9	–	1.74 (m)	1.52 (m)
11	1.30 ~ 1.40 (m)	1.86, 1.27 (each m)	4.14 (brd, 2.85)
12	2.44, 1.47 (each m)	1.99, 1.31 (each m)	2.23, 1.36 (each m)
14	1.35 (m)	1.15 (m)	1.07 (m)
15	1.30 (m)	1.80, 1.31 (each m)	1.38, 1.18 (each m)
16	1.53, 1.16 (each m)	1.53 ~ 1.72 (m)	1.30 (m)
17	1.12 (m)	1.20 m	1.09 (m)
18	1.17 (s)	0.69 s	0.94 (s)
19	1.58 (s)	1.44 s	1.40 (s)
20	1.40 (m)	1.39 (m)	1.40 (m)
21	0.95 (d, 6.3)	0.91 (d, 6.2)	0.98 (d, 6.45)
22	1.38 (m)	1.33 ~ 1.48 (m)	1.02 (m)
23	1.27 (m)	1.37, 1.16 (each m)	1.65 (m)
24	1.16 (m)	1.15 (m)	1.16 (m)
25	1.53 (m)	1.55 (m)	0.90 (m)
26	0.89 (d, 6.55)	0.86 (brd, 6.3)	0.90 (d, 6.6)
27	0.89 (d, 6.55)	0.86 (brd, 6.3)	0.90 (d, 6.6)

$^{\alpha}$ All compounds were determined at 500 MHz; **1** was determined in pyridine-d_5, and **2** was determined in $CDCl_3$, while **3** was measured in MeOD; chemical shifts values δ are in ppm, and coupling constant values J in Hz

Table 2: ^{13}C NMR Spectral data of compounds 1–4$^{\alpha}$

carbon	1	2	3	4
1	42.5 (t)	212.1 (s)	33.2 (t)	33.3 (t)
2	30.1 (t)	47.2 (t)	31.6 (t)	32.5 (t)
3	66.8 (d)	66.5 (d)	68.3 (d)	67.3 (d)
4	37.4 (t)	39.7 (t)	41.1 (t)	42.9 (t)
5	47.5 (d)	76.2 (s)	77.5 (s)	75.8 (s)
6	213.5 (s)	75.2 (d)	76.5 (d)	76.3 (d)
7	68.4 (d)	33.2 (t)	36.4 (t)	35.7 (t)
8	33.6 (d)	29.7 (d)	28.4 (d)	31.2 (d)
9	80.7 (s)	40.9 (d)	49.6 (d)	45.9 (d)
10	42.9 (s)	53.4 (s)	40.0 (s)	39.1 (s)
11	24.1 (t)	28.2 (t)	69.5 (d)	21.7 (t)
12	49.5 (t)	40.1 (t)	50.6 (t)	40.6 (t)
13	49.6 (s)	42.9 (s)	43.1 (s)	43.0 (s)
14	58.2 (d)	56.3 (d)	59.3 (d)	56.6 (d)
15	29.9 (t)	22.7 (t)	25.0 (t)	24.6 (d)
16	24.2 (t)	24.2 (t)	29.1 (t)	21.7 (t)
17	56.9 (d)	55.9 (d)	58.3 (d)	56.6 (d)
18	14.8 (q)	12.4 (q)	14.9 (q)	12.3 (q)
19	16.8 (q)	17.2 (q)	20.3 (q)	17.2 (q)
20	36.0 (d)	35.9 (d)	37.2 (d)	36.1 (d)
21	18.8 (q)	18.7 (q)	19.3 (q)	18.9 (q)
22	36.4 (t)	36.2 (t)	37.4 (t)	36.4 (t)
23	28.1 (t)	24.0 (t)	25.3 (t)	24.1 (t)
24	39.7 (t)	39.6 (t)	40.8 (t)	39.7 (t)
25	28.2 (d)	28.1 (d)	29.2 (d)	28.2 (d)
26	22.6 (q)	22.9 (q)	23.0 (q)	22.6 (q)
27	22.9 (q)	22.6 (q)	23.2 (q)	22.9 (q)

$^{\alpha}$ All compounds were determined at 125 MHz with TMS as internal standard; **1** and **4** were determined in pyridine-d_5, and **2** was determined in $CDCl_3$ with one drop of MeOD, whereas **3** was measured in MeOD; chemical shifts are in ppm

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