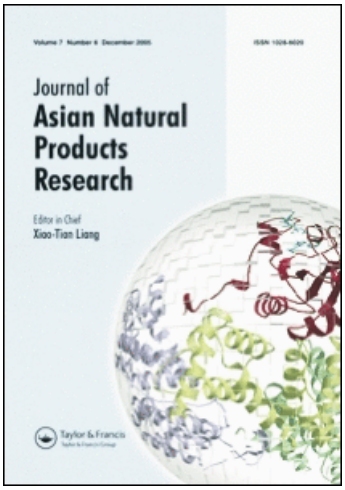

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6-Hydroxy-4-en-3-one sterols from the marine sponge *Iotrochoto birotulata*

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Five sterols with a nucleus skeleton of 6-hydroxy-4-en-3-one, namely cholesta-6β-hydroxy-4-en-3-one (**1**), ergosta-6β-hydroxy-4,24(28)-dien-3-one (**2**), ergosta-6α-hydroxy-4,24(28)-dien-3-one (**3**), ergosta-6α-hydroxy-4,22-*E*-dien-3-one (**4**), and ergosta-28-methyl-6β-hydroxy-4,24(28)-dien-3-one (**5**) have been isolated from the marine sponge *Iotrochoto birotulata*, collected from the southern China sea. Sterols **2–4** are new compounds, and **1** has been isolated from marine organisms for the first time. The structures have been elucidated on the basis of extensive spectroscopic and chemical properties.

Keywords: Marine sponge; *Iotrochoto birotulata*; Ergosta-6β-hydroxy-4,24(28)-dien-3-one; Ergosta-6α-hydroxy-4,24(28)-dien-3-one; Ergosta-6α-hydroxy-4,22-*E*-dien-3-one

1. Introduction

The marine sponge *Iotrochoto birotulata* (Demospongiae) is widely distributed in tropical and subtropical oceans. Previous chemical investigations on this species have revealed several halogenated derivatives [1–5], ecdysteroids [6], and an alkaloid purpurone [7–9]. In our ongoing program to investigate bioactive secondary metabolites from Chinese marine organisms, the sponge *Iotrochoto birotulata* was collected from the tropical area in Hainan Island of the southern China sea. Extensive chromatography on the EtOH extract of this sponge has yielded five sterols, each with a 6-hydroxy-4-en-3-one skeleton (**1**–**5**).

2. Results and discussion

Repeated column chromatography and semi-preparative HPLC separation of EtOH the extract from the sponge *I. birotulata* afforded five 6-hydroxy-4-en-3-one type sterols (**1**–**5**). **1** was identified as cholesta-6β-hydroxy-4-en-3-one, a chemical conversion product that

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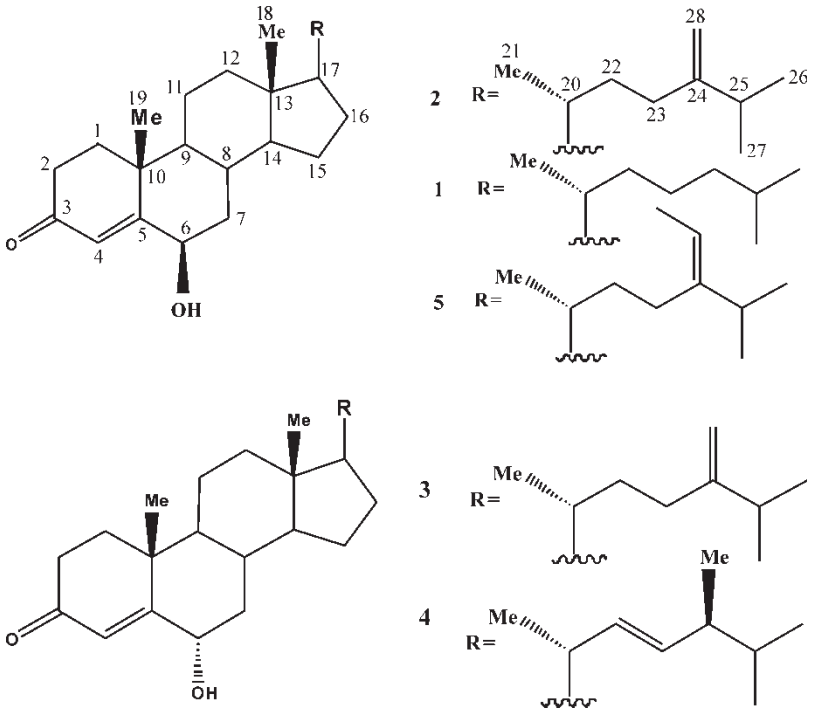
originated from cholesterol [10], but this is the first time it has been obtained from a marine organism. **5** is a known sterol originally isolated from the brown alga *Turbinaria conoides* [11], and reported to possess significant activity against the growth of P-388, A-549 and HT-29 cancer cells and moderate cytotoxicity toward KB cells [11].



Compound **1** was isolated as a white powder. Its molecular formula $C_{27}H_{44}O_2$ was established by HR-EIMS, with six degrees of unsaturation. The IR spectrum showed absorptions at 3422 and 1689 cm^{-1}, suggesting hydroxyl and conjugated carbonyl groups. The ^1H and ^{13}C (table 1) NMR data of **1** agree with those of sterols possessing a 6β-hydroxy-4-en-3-one skeleton [12]. In the ^1H NMR spectrum, a broad singlet at δ 4.15 (br) is attributed to H-6, a singlet at δ 5.66 (s) is due to the olefinic proton H-4, and two sharp singlets at δ 0.70 (s, 3H) and 1.29 (s, 3H) are attributed to methyl groups at C-18 and C-19. The broad carbinyl proton signal for H-6 suggests a β-axial hydroxyl group at C-6 according to a Dreding structure model, and this is supported by the H-19 (δ 1.29, s) of **1** which is shifted 0.20 ppm downfield that of stigmasta-4-en-3-one [12], owing to a 1,3 diaxial interaction with the hydroxyl group [13]. The ^1H NMR spectrum of the side chains has a doublet at δ 0.93 (d, $J = 6.5$ Hz, 3H) for methyl group Me-21, and two doublets at δ 0.85 (d, $J = 6.5$ Hz, 3H), 0.86 (d, $J = 6.5$ Hz, 3H) for methyl groups Me-26 and Me-27 respectively. The DEPT spectrum indicates three methyls [δ 19.40 (q, C-21), 23.54 (q, C-26), 23.27 (q, C-27)], three methylenes [δ 36.48 (t, C-22), 24.00 (t, C-23), 28.26 (t, C-24)], and two methines [δ 36.03 (d, C-20), 39.64 (d, C-25)] in the side chain. HMBC and HMQC analysis revealed that side chain has a 1,5-dimethylhexyl moiety. Thus, the structure of **1** was identified as cholesta-6β-hydroxy-4-en-3-one, a chemical conversion product that originates from cholesterol [10] but isolated from a natural resource for the first time; its NMR data have been fully assigned by extensive use of 2D NMR spectra.

Table 1. ^{13}C NMR data of compounds **1**–**4**.

C	*2*	*1*	*3*	*4*	*5*
1	37.51,t	37.46,t	36.38,t	36.68,t	37.47,t
2	34.67,t	34.74,t	34.56,t	34.56,t	34.73,t
3	200.78,s	200.07,s	199.79,s	199.83,s	200.09,s
4	126.76,d	125.95,d	120.07,d	120.07,d	125.96,d
5	168.78,s	170.00,s	171.77,s	171.77,s	170.01,s
6	73.70,d	71.93,d	69.09,d	69.11,d	71.95,d
7	38.95,t	39.64,t	41.91,t	41.89,t	39.20,t
8	30.14,d	30.22,d	34.20,d	34.22,d	30.46,d
9	54.02,d	53.96,d	54.15,d	54.17,d	53.96,d
10	38.39,s	38.41,s	39.44,s	39.73,s	39.00,s
11	21.38,t	21.40,t	21.44,t	21.34,t	21.41,t
12	40.01,t	40.00,t	39.85,t	40.65,t	40.23,t
13	42.96,s	42.90,s	42.90,s	42.74,s	42.91,s
14	56.29,d	56.18,d	55.96,d	56.07,d	56.17,d
15	28.55,t	28.63,t	28.50,t	29.13,t	28.64,t
16	24.54,t	24.68,t	24.55,t	24.13,t	24.05,t
17	56.41,d	56.47,d	56.31,d	56.23,d	56.70,d
18	12.42,q	12.64,q	12.34,q	12.53,q	12.65,q
19	19.92,q	19.77,q	18.69,q	18.69,q	19.78,q
20	36.13,d	36.03,d	36.08,d	39.74,d	36.50,d
21	19.07,q	19.40,q	19.02,q	21.42,q	18.81,q
22	35.05,t	36.48,t	34.98,t	132.57,d	35.31,t
23	31.38,t	24.00,t	31.34,t	136.16,d	25.90,t
24	157.20,s	28.26,t	157.15,s	43.48,d	147.00,s
25	34.21,d	39.64,d	34.21,d	33.62,d	34.82,d
26	22.39,q	23.54,q	22.40,q	20.04,q	21.89,q
27	22.27,q	23.27,q	22.26,q	20.58,q	21.90,q
28	106.40,t		106.43,t	18.47,q	116.25,d
29					13.35,q

Compound **2** was isolated as needles, and the molecular formula $C_{28}H_{44}O_2$ was established on the basis of HR-EIMS. The IR spectrum shows absorptions for hydroxyl group (3500 cm^{-1}) and a conjugated carbonyl group (1692 cm^{-1}). The ^{1}H NMR spectrum displays an olefinic proton at δ 5.84 (s, H-4), an oxygenated proton at δ 4.38 (br, H-6), five methyl groups at δ 0.77 (s, Me-18), 0.98 (d, $J = 6.5$ Hz, Me-21), 1.04 (d, $J = 6.5$ Hz, Me-26), 1.06 (d, $J = 6.5$ Hz, Me-27) and 1.40 (s, Me-19), as well as an olefinic methylene at δ 4.68 (br, H-28a) and 4.75 (br, H-28b). The ^{13}C NMR spectrum of **2** revealed twenty-eight carbons in the molecule. Comparing the ^{1}H and ^{13}C (table 1) NMR data of **2** with those of **1** and a known sterol stigmast-4-en-6β-ol-3-one [12,14], the partial structure of **2** was proved to be identical to 6β-hydroxy-4-en-3-one skeleton. The DEPT spectrum implies that side chain is composed of nine carbons, namely three methyls [δ19.07 (q, C-21), 22.39 (q, C-26) and 22.27 (q, C-27)], three methylenes [δ 35.05 (t, C-22), 31.38 (t, C-23) and 106.40 (t, C-28)], two methines [36.13 (d, C-20) and 34.21 (d, C-25)] and a quaternary carbon (δ 157.20, s, C-24). The HMQC spectrum assigned the protons and their associated carbons. In the HMBC spectrum, two methyl protons at δ1.04 (d, $J = 6.5$ Hz, Me-26) and 1.06 (d, $J = 6.5$ Hz, Me-27) correlate with C-24 and C-25, an olefinic methylene at δ4.68 (br, H-28a) and 4.75 (br, H-28b) correlates with C-24, C-25 and C-23, and a methyl proton at δ0.98 (d, $J = 6.5$ Hz, Me-21) correlates with C-20, C-21 and C-17 (δ 56.41, d), and a methylene at δ 2.10 (m, H-23) correlates with C-24, C-28, C-25, C-22 and C-20, which all lead to confirmation of a 20,25-dimethyl-24 (28)-en-hexyl moiety [15] that is considered to annex to C-17 with C-20 due to a long-range correlation between H-21 and C-17 in the HMBC

spectrum. Accordingly, the structure of **2** was determined as ergosta-6β-hydroxy-4,24(28)-dien-3-one.

The ^{1}H and ^{13}C (table 1) NMR data of **3** as well as its IR absorptions closely resemble those of **2**, and are characteristic of a 6-hydroxyl-4-en-3-one steroid. The side chain of **3** agrees with that of **2** due to the close identity of the spectral data of both compounds. Compound **3** differs from **2** in the ^{1}H NMR spectrum where the H-4 of **3** is shifted downfield to δ 6.19 (s) due to the spatial proximity of the 6-OH group, and H-6 appears as a dd coupling at δ 4.36 (dd, $J = 5.5$, 11.0 Hz) due to its β-axial orientation [12]. The stereochemistry of **3** at C-6 was further supported by a NOESY spectrum, in which H-6 shows NOE correlation with Me-19 (δ 1.20, s, 3H), indicating spatial proximity between H-6 and H-19. Therefore, the structure of **3** was identified as a C-6 epimer of 2, namely ergosta-6α-hydroxy-4,24(28)-dien-3-one.

The ^{1}H and ^{13}C (table 1) NMR spectra of compound **4** closely resemble those of **3** in the nucleus skeleton, but differ in the side chain. The relative configuration of the hydroxyl group at C-6 of **4** was identified as the same as that of **3** according to the NOE correlation between H-6 (δ 4.36, dd, $J = 4.5$, 13.0 Hz) and H-19 (δ 1.21, s, 3H) in the NOESY spectrum. The ^{1}H NMR spectrum of **4** presented two additional olefinic protons at δ 5.21 (dd, $J = 9.5$, 15.0 Hz) and 5.17 (dd, $J = 7.5$, 15.0 Hz), attributed to H-22 and H-23, while the methylene group at C-24 of **3** is replaced by a methyl group (δ 1.03, d, $J = 6.5$ Hz, H-28). The position of double band in the side chain was confirmed by the HMBC correlations between H-21 (δ 0.94, d, $J = 7.0$ Hz, 3H) and C-22 (δ 132.57, d) and between H-28 and C-23 (δ 136.16, d), and the geometry of the double bond between C-22 and C-23 is *E*-configuration due to the coupling constant $J_{H22/H23} = 15$ Hz. The full NMR data of **4** have been assigned on the basis of HMQC, HMBC and DQFCOSY correlations. The structure of **4** was thus determined as ergosta-6α-hydroxy-4,22-*E*-dien-3-one.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a WZZ-15 digital polarimeter. Melting points were determined using a XT_4A melting point apparatus and are uncorrected. UV spectra were recorded on a UV spectrophotometer, and IR spectra were measured on a NEXUS FT-470IR spectrophotometer. NMR spectra were recorded on an AVENCE-500 FT NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$ and DMSO using TMS as internal standard. ESI-MS were obtained on a MDS-SCIEX QSTAR mass spectrometer. LKB-2150 HPLC Pump and LKB-2151 Variable Wavelength Monitor were employed for sample purification. Silica gel (200–300 mesh) was used for column chromatography and HF254 silica gel for TLC, and the silica gels used in the experiments were purchased from Qingdao Marine Chemical Co.

3.2 Sponge material

The specimen of *Iotrochoto birotulata* was collected by scuba diving in the southern China sea, close to Xidao Island, Sanya, Hainan Island in June 1999. The species was identified by Dr R. van Soest at the Institute of Systematic Population Biology, Amsterdam University,

The Netherlands. A voucher specimen (HS-20) has been deposited at the State Key Laboratory of Natural and Biomimetic Drugs, Peking University.

3.3 Extraction and isolation

The sponge *Iotrochoto birotulata* (243 g, dry wt) was kept frozen before being soaked in 95% EtOH; the EtOH extract was then evaporated under reduced pressure, and the sponge residue extracted with MeOH to obtain a MeOH extract after evaporation *in vacuo*. The combined MeOH and EtOH extracts were partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (3.5 g) was subjected to silica-gel column chromatography eluting with a gradient of acetone-light petroleum (bp 60–90°C). A fraction (acetone–light petroleum = 1:4) was collected and subjected to a silica-gel column with EtOAc–light petroleum (EtOAc–light petroleum = 1:4) as eluting solvent to afford needle crystals (20 mg) which showed two main peaks in analytical HPLC. The mixture crystals were separated by semi-preparative HPLC on an ODS column with 90% MeOH as mobile phase to yield compounds **2** (5 mg) and **1** (3 mg). The remaining fractions (60 mg) (1:4) were combined and separated on a silica-gel column repeatedly, using $CHCl_3$–acetone (15:1) as eluent. The collected fractions which showed one TLC spot were collected and analyzed by HPLC (ODS) and found to display three main peaks which were further separated by semi-preparative HPLC (ODS), eluting with MeOH–H_2O (9:1), to yield compounds **3** (4 mg), **4** (2 mg) and **5** (1.5 mg).

Cholesta-6β-hydroxy-4-en-3-one (**1**): a colorless solid, mp 180–182°C. $[\alpha]_{20}^{D}$ +50.0 (*c* 0.51, MeOH). UV λ_{max} 245 nm (MeOH). IR (KBr) ν_{max} (cm^{-1}): 3442, 2953, 2868, 1688, 1468, 1383, 1267, 1244, 1038, 1017, 878. ^{1}H NMR ($CDCl_3$) δ_H (ppm): 2.07 (m, H-1a), 1.76 (m, H-1b), 1.20 (m, H-2a), 2.51 (m, H-2b), 5.66 (s, H-4), 4.15 (br, H-6), 1.30 (m, H-7a), 2.00 (m, H-7b), 2.05 (m, H-8), 0.96 (m, H-9), 1.50 (m, H-11), 1.98 (m, H-12a), 1.26 (m, H-12b), 1.10 (m,H-14), 1.77 (m, H-15), 1.05 (m, H-16), 1.14 (m, H-17), 0.70 (s, H-18), 1.29 (s, H-19), 1.45 (m, H-20), 0.93 (d, J = 6.5 Hz, H-21), 2.10, 1.85 (m, H-22), 1.54 (m, H-23), 1.87 (m, H-24), 1.77 (m, H-25), 0.85 (d, J = 6.5 Hz, H-26), 0.86 (d, J = 6.5 Hz, H-27). HR-EIMS *m*/*z*: 400.3355 $[M]^+$ (calcd for $C_{27}H_{44}O_2$, 400.3341).

Ergosta-6β-hydroxy-4,24(28)-dien-3-one (**2**): needle crystals, mp 193–195°C. $[\alpha]_{20}^{D}$ +56.0 (*c* 0.32, MeOH). UV λ_{max} 245 nm (MeOH). IR (KBr) ν_{max} (cm^{-1}): 3500, 2957, 2868, 1691, 1624, 1615, 1467, 1377, 1265, 1248, 1192, 1038, 1017, 886. ^{1}H NMR ($CDCl_3$) δ_H (ppm): 2.08 (m, H-1a), 1.75 (m, H-1b), 1.18 (m, H-2a), 2.55 (m, H-2b), 5.84 (s, H-4), 4.38 (br, H-6), 1.28 (m, H-7a), 2.02 (m, H-7b), 2.00 (m, H-8), 0.95 (m, H-9), 1.56 (m, H-11), 2.10 (m, H-12a), 1.22 (m, H-12b), 1.04 (m,H-14), 1.75 (m, H-15), 1.07 (m, H-16), 1.18 (m, H-17), 0.77 (s, H-18), 1.40 (s, H-19), 1.47 (m, H-20), 0.98 (d, J = 6.5 Hz, H-21), 2.40, 1.59 (m, H-22), 2.10 (m, H-23), 2.06 (m, H-25), 1.04 (d, J = 6.5 Hz, H-26), 1.06 (d, J = 6.5 Hz, H-27), 4.68 (br, H-28a), 4.75 (br, H-28b). ESI-MS *m*/*z*: 413 $[M + H]^+$, 279. HR-EIMS *m*/*z*: 412.3318 $[M]^+$ (calcd for $C_{28}H_{44}O_2$, 412.3341).

Ergosta-6α-hydroxy-4,24(28)-dien-3-one (**3**): needle crystals, mp 152-155°C. $[\alpha]_{20}^{D}$ +74.0 (*c* 0.62, MeOH). UV λ_{max} 245 nm (MeOH). IR (KBr) ν_{max} (cm^{-1}): 3508, 2960, 2856, 1653, 1608, 1444, 1377, 1274, 1220, 1077, 887. ^{1}H NMR ($CDCl_3$) δ_H (ppm): 98 (m, H-1a), 1.67 (m, H-1b), 1.20 (m, H-2a), 2.48 (m, H-2b), 6.19 (s, H-4), 4.36 (dd, J = 5.5, 11.0 Hz, H-6), 1.32 (m, H-7a), 2.00 (m, H-7b), 1.97 (m, H-8), 0.96 (m, H-9), 1.71 (m, H-11), 1.99 (m, H-12a), 1.26 (m, H-12b), 1.01 (m,H-14), 1.80 (m, H-15), 1.05 (m, H-16), 1.20 (m, H-17), 0.74 (s, H-18), 1.20 (s, H-19), 1.50 (m, H-20), 0.96 (d, J = 6.5 Hz, H-21), 2.38, 1.62

(m, H-22), 2.11 (m, H-23), 2.08 (m, H-25), 1.06 (d, $J = 6.5$ Hz, H-26), 1.05 (d, $J = 6.5$ Hz, H-27), 4.74 (br, H-28a), 4.68 (br, H-28b). HR-EIMS *m/z*: 412.3359 $[M]^+$ (calcd for $C_{28}H_{44}O_2$, 412.3341).

Ergosta-6α-hydroxy-4,22-*E*-dien-3-one (**4**): needle crystals, mp 129–132°C. $[\alpha]_{20}^{D}$ +156.0 (*c* 0.43, MeOH). UV λ_{max} 245 nm (MeOH). IR (KBr) ν_{max} (cm^{-1}): 3422, 2930, 1666. ^1H NMR ($CDCl_3$) δ_H (ppm): 1.95 (m, H-1a), 1.70 (m, H-1b), 1.26 (m, H-2a), 2.41 (m, H-2b), 6.19 (s, H-4), 4.36 (dd, $J = 4.5$, 13.0 Hz, H-6), 1.35 (m, H-7a), 2.02 (m, H-7b), 1.95 (m, H-8), 0.95 (m, H-9), 1.74 (m, H-11), 2.01 (m, H-12a), 1.28 (m, H-12b), 1.03 (m, H-14), 1.75 (m, H-15), 1.04 (m, H-16), 1.24 (m, H-17), 0.75 (s, H-18), 1.21 (s, H-19), 2.04 (m, H-20), 0.94 (d, $J = 7.0$ Hz, H-21), 5.21 (dd, $J = 9.5$, 15.0 Hz, H-22), 5.17 (dd, $J = 7.5$, 15.0 Hz, H-23), 2.39 (m, H-24), 2.04 (m, H-25), 0.87 (d, $J = 6.5$ Hz, H-26), 0.85 (d, $J = 6.5$ Hz, H-27), 1.03 (d, $J = 6.5$ Hz, H-28). HR-EIMS *m/z*: 412.3355 $[M]^+$ (calcd for $C_{28}H_{44}O_2$, 412.3341).

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