Polyhydroxylated Sterols from the South China Sea Gorgonian *Verrucella umbraculum*

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Two new polyhydroxylated sterols, named verumbsteroids A and B (**1** and **2**, resp.), along with seven known analog, **3**–**9**, were isolated from the gorgonian *Verrucella umbraculum* collected from the South China Sea. The structures and relative configurations of the new compounds were elucidated by comprehensive spectroscopic investigations. The absolute configuration of **1** was determined by using modified *Mosher* method with the acetonide derivative of **1**. Compounds **1** and **3**–**5** were found to show cytotoxicities against five human tumor cell lines (HL-60, K562, HeLa, A-549, and HCT-116) with the IC_{50} values ranging from 2.76 to 9.62 μM.

Introduction. – Among the various classes of secondary metabolites produced by the gorgonian corals, a large number of polyhydroxylated sterols have been described [1]. Many of them showed an array of biological features including cytotoxic, X-activated receptor inhibitory, and anti-inflammatory activities [2–6]. The study of structure–activity relationships (SARs) indicated that 3,5,6,7-tetrahydroxy steroids possess significant cytotoxicities [7]. Recently, in the course of our investigation on new bioactive substances from corals collected from the South China Sea [8–13], the gorgonian *Verrucella umbraculum* attracted our attention, because the crude AcOEt extract showed lethal activity toward the brine shrimp *Artemia salina*. Chemical investigation on the active extract led to the isolation of two new polyhydroxylated sterols, named verumbsteroids A and B (**1** and **2**, resp.), together with seven known analogs cholestane-3*β*,5*α*,6*β*,7*β*-tetrol (**3**) [4], ergost-24(28)-ene-3*β*,5*α*,6*β*,7*β*-tetrol (**4**) [4], (24*S*)-ergostane-3*β*,5*α*,6*β*,7*β*-tetrol (**5**) [4], 5*β*,6*β*-epoxycholestane-3*β*,7*β*-diol (**6**) [4], 5*β*,6*β*-epoxyergost-24(28)-ene-3*β*,7*β*-diol (**7**) [4], globosterol (**8**) [5], and 27-methyl-24-methylidenecholest-5-ene-3*β*,7*β*-diol (**9**) [6] (*Fig. 1*). Their structures were elucidated by NMR spectroscopic methods and comparison with those previously reported in the literature. Herein, we describe the isolation, structural elucidation, and bioactivity of these metabolites.

Results and Discussion. – Verumbsteroid A (**1**) was obtained as white, amorphous powder. The molecular formula was determined as $C_{27}H_{46}O_4$ on the basis of positive-ion-mode HR-ESI-MS (*m*/*z* 417.3356 ($[M+H-H_2O]^+$)), indicating five degrees of unsaturation. The IR spectrum of **1** showed strong absorptions ($\tilde{\nu}_{max}$) at 3420 (OH) and 1647 cm^{-1} (C=C). In the ^{1}H-NMR spectrum (*Table 1*), three Me *doublet*s (δ(H) 1.02 (*d*, *J* = 6.0, Me(21)), 0.88 (*d*, *J* = 6.5, Me(26)), and 0.88 (*d*, *J* = 6.5, Me(27))) and two Me

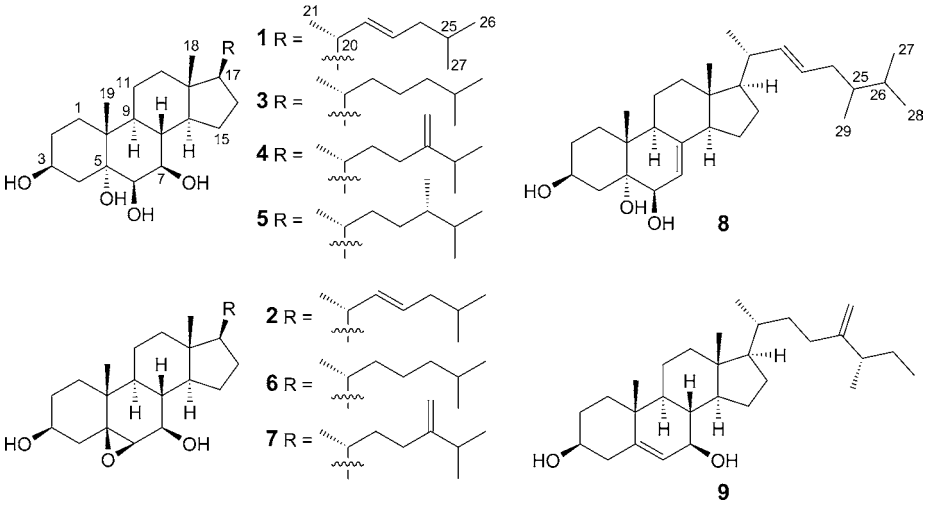


Fig. 1. *Structures of compounds* **1**–**9** *from* Verrucella umbraculum

*singlet*s (δ(H) 1.14 (*s*, Me(19)) and 0.73 (*s*, Me(18))) were attributed to the five Me groups of a steroid. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** (*Table 1*) with those of cholestane-3β,5α,6β,7β-tetrol (**3**) [4] revealed that **1** shares the same nucleus structure as **3**. The main difference was the presence of the typical signals for a C=C bond (δ(H) 5.22 (*dd*, J = 15.0, 8.0, H–C(22)), δ(C) 139.6 (C(22)) and δ(H) 5.30 (*dd*, J = 15.0, 8.0, H–C(23)), δ(C) 127.4 (C(23))) in the side chain of **1**, suggesting that the CH_2(22)–CH_2(23) moiety in **3** was replaced by a disubstituted C(22)=(23) bond in **1**. Furthermore, the position of the C=C bond was confirmed by the HMBC spectrum of **1**, which showed correlations from Me(21) to C(22), and from H–C(23) to C(24) and C(25). The coupling constant between H–C(22) and H–C(23) (J = 15.0) indicated (*E*) configuration for the C=C bond. A combination of HMQC, ^{1}H,^{1}H-COSY, and HMBC (*Fig. 2*) experiments enabled us to delineate the structure of **1**. Therefore, **1** was identified as the 22,23-didehydro derivative of **3**.

The relative configuration of **1** was determined by analysis of its NOE spectrum (*Fig. 2*). In selective 1D-NOE experiments, irradiation of Me(18) resulted in enhancements of H–C(8), Me(19), and H–C(20) signals, and irradiation of Me(19) resulted in enhancement of the H_β–C(1) signal, suggesting that these H-atoms all were β-oriented. The irradiation of H–C(3) caused the enhancement of H_α–C(1) signal and the irradiation of H–C(6) also caused the enhancement of H_α–C(1) signal. Furthermore, the irradiation of H–C(7) produced the enhancements of H–C(6), H–C(9), and H–C(14) signals. These observations suggested that H_α–C(1), H–C(6), H–C(7), H–C(9), and H–C(14) all had the same α-orientation.

The absolute configuration of **1** was determined using a modified *Mosher* method. Protection of 6,7-diol of **1** by using 2,2-dimethoxypropane and TsOH · H_2O gave the corresponding acetonide derivative **10** of **1** (*Scheme 1*). Treatment of **10** with (+)-(*S*)-α- and (−)-(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) gave the (*R*)- and (*S*)-MTPA esters, **10r** and **10s**, respectively. The ^{1}H-NMR signals of the

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.34–1.38 (*m*), 1.61–1.63 (*m*)	31.8	1.17–1.23 (*m*), 1.98–2.05 (*m*)	36.9
2	1.67–1.69 (*m*), 1.83–1.87 (*m*)	33.6	1.39–1.43 (*m*), 1.81–1.83 (*m*)	30.9
3	3.97–4.03 (*m*)	68.1	3.69–3.73 (*m*)	69.1
4	1.72–1.74 (*m*), 2.09–2.11 (*m*)	43.2	1.41–1.45 (*m*), 2.02–2.05 (*m*)	41.7
5	–	77.7	–	67.2
6	3.38 (*d*, J = 4.0)	79.4	3.13 (*s*)	67.5
7	3.69 (*dd*, J = 10.0, 4.0)	73.8	3.50 (*d*, J = 6.0)	74.8
8	1.63–1.67 (*m*)	41.5	1.38–1.44 (*m*)	38.3
9	1.57–1.61 (*m*)	45.5	0.72–0.74 (*m*)	49.7
10	–	38.9	–	34.2
11	1.09–1.15 (*m*), 1.80–1.82 (*m*)	22.5	0.88–0.92 (*m*), 1.41–1.43 (*m*)	21.9
12	1.15–1.19 (*m*), 1.73–1.75 (*m*)	41.5	1.12–1.17 (*m*), 1.96–2.00 (*m*)	39.5
13	–	44.7	–	42.9
14	1.20–1.24 (*m*)	56.8	1.03–1.08 (*m*)	55.1
15	1.34–1.40 (*m*), 1.64–1.66 (*m*)	28.2	1.35–1.39 (*m*), 1.92–1.94 (*m*)	27.3
16	1.61–1.64 (*m*), 2.00–2.02 (*m*)	30.2	1.26–1.30 (*m*), 1.85–1.87 (*m*)	28.9
17	1.15–1.17 (*m*)	57.2	1.04–1.07 (*m*)	55.5
18	0.73 (*s*)	13.1	0.66 (*s*)	12.0
19	1.14 (*s*)	17.7	1.01 (*s*)	16.8
20	1.42–1.46 (*m*)	39.6	1.96–2.02 (*m*)	39.9
21	1.02 (*d*, J = 6.0)	21.6	0.99 (*d*, J = 6.5)	21.0
22	5.22 (*dd*, J = 15.0, 8.0)	139.6	5.20 (*dd*, J = 15.5, 8.5)	137.9
23	5.30 (*dd*, J = 15.0, 8.0)	127.4	5.27 (*dd*, J = 15.5, 8.5)	126.4
24	1.92–1.97 (*m*), 2.04–2.08 (*m*)	41.8	1.84–1.91 (*m*), 1.91–1.93 (*m*)	41.9
25	1.53–1.55 (*m*)	29.8	1.45–1.49 (*m*)	28.5
26	0.88 (*d*, J = 6.5)	22.7	0.85 (*d*, J = 6.5)	22.3
27	0.88 (*d*, J = 6.5)	22.8	0.85 (*d*, J = 6.5)	22.3

[a]) Recorded in CD_3OD. [b]) Recorded in $CDCl_3$.

two MTPA esters were assigned on the basis of their ^{1}H,^{1}H-COSY spectra. The $\Delta\delta_H(S-R)$ values were then calculated. Following the MTPA rules [14], the absolute configuration at C(3) of **1** was assigned as (*S*). Therefore, the absolute configuration of **1** was determined as (3*S*,5*R*,6*R*,7*R*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*R*).

Verumbsteroid B (**2**) was also isolated as white, amorphous powder with a molecular formula $C_{27}H_{44}O_3$ as deduced from the HR-ESI-MS (*m*/*z* 439.3195 ($[M+Na]^+$)). In the ^{13}C-NMR (*Table 1*), four signals appeared at δ(C) 74.8, 69.1, 67.5, and 67.2, indicating the presence of four O-bearing C-atoms. Since the molecular formula accounted for only three O-atoms, it was deduced that two of the C-atoms mentioned must be attached to a common O-atom forming a cyclic ether ring. The oxirane structure of **2** was supported by the upfield shifts of C(5) (δ(C) 67.2 in **2** *vs.* δ(C) 77.7 in **1**) and C(6) (δ(C) 67.5 in **2** *vs.* δ(C) 79.4, in **1**) due to the shielding effect of the cyclic ether ring. Comparison of the NMR data ascertained **2** to be closely similar to 5β,6β-epoxycholestane-3β,7β-diol (**6**) [4]. The main significant difference was that two upfield C-atoms of **6** were replaced by a C(22)=C(23) in **2**. A combination of HMQC,

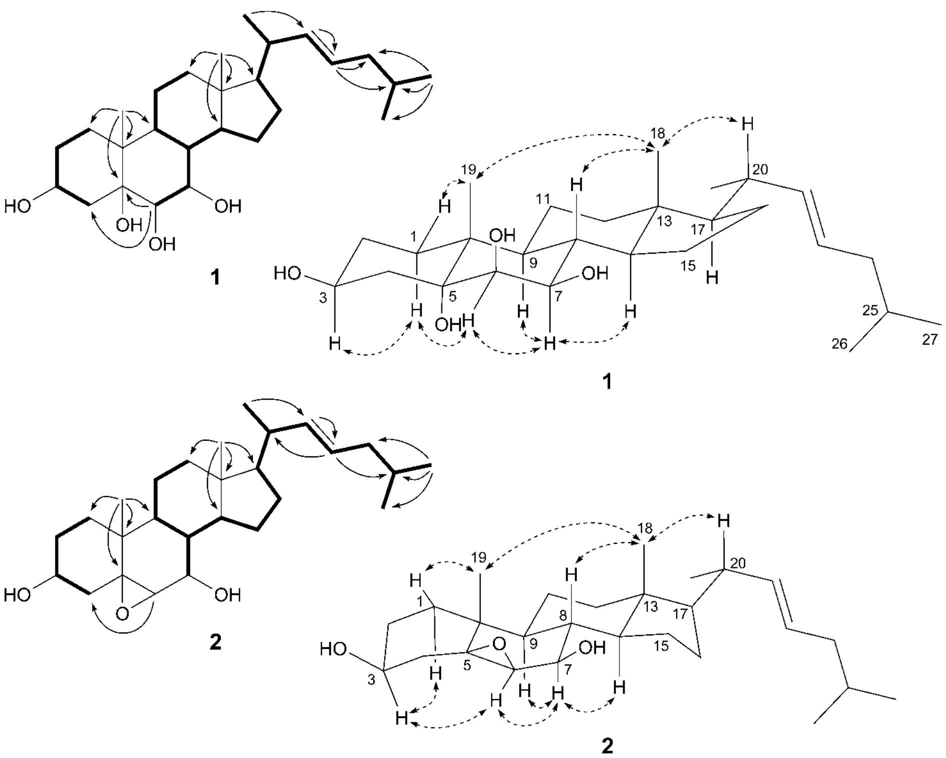


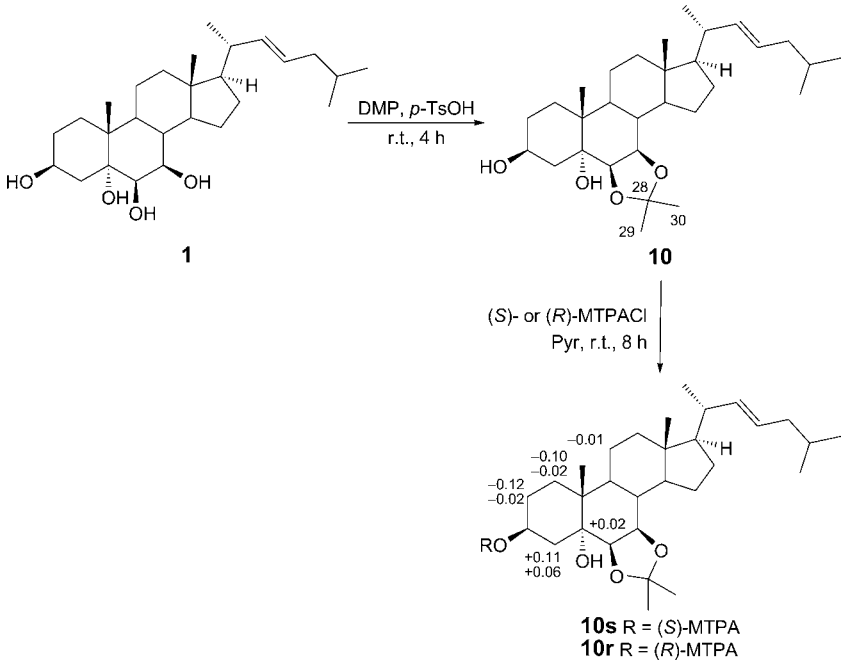
Fig. 2. *^{1}H,^{1}H-COSY* (—), *key HMB* (→), *and NOE* (⤢) *correlations of* **1** *and* **2**

^{1}H,^{1}H-COSY and HMBC (*Fig. 2*) experiments confirmed the proposed structure and allowed us to assign all ^{13}C and ^{1}H resonances. The J(22,23) value (15.5) was indicative of the (22*E*)-geometry for the C=C bond in **2**. Therefore, the structure of **2** was identified as a 22,23-didehydro analog of **6**.

The relative configuration of **2** was determined by analysis of its 1D-NOE spectrum (*Fig. 2*). In the selective NOE experiments, the irradiation of H–C(3) resulted in the enhancements of H_α–C(1) and H–C(6) signals, and the irradiation of H–C(7) resulted in the enhancements of H–C(6), H–C(9), and H–C(14) signals, indicating that these H-atoms should be on the same face of the molecule. Irradiation of Me(18) caused enhancements of H–C(8), Me(19) and H–C(20) signals, and irradiation of Me(19) cause the enhancement of H_β–C(1) signal, implying that H_β–C(1), Me(18), Me(19), and H–C(20) should be placed on the other face of the molecule.

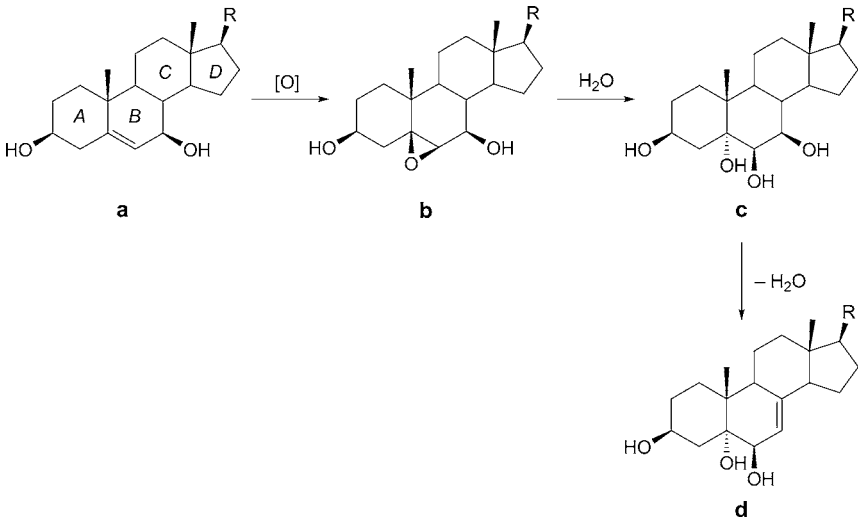
The absolute configuration of **2** was assigned on the basis of a common biogenesis with the co-isolated **1**. There were four types of nucleus structures in the isolated compounds with moieties of 5-ene-3β,7β-diol (**a** as **9**), 5β,6β-epoxy-3β,7β-diol (**b** as **2**, **6** and **7**), 3β,5α,6β,7β-tetrol (**c** as **1** and **3**–**5**), and 7-ene-3β,5α,6β-triol (**d** as **8**). An oxidation route of the *B*-ring of four nucleus structures of **1**–**9** is postulated in *Scheme 2*. The 5-ene-3β,7β-diol moiety, **a**, is oxidized at C(5) and C(6) to form the corresponding epoxidation product **b**. Then, **b** is hydrated to form tetrol sterol **c**.

Scheme 1. *Preparation of Acetonide Derivative* **10** *of* **1** *and Its MTPA Derivatives*



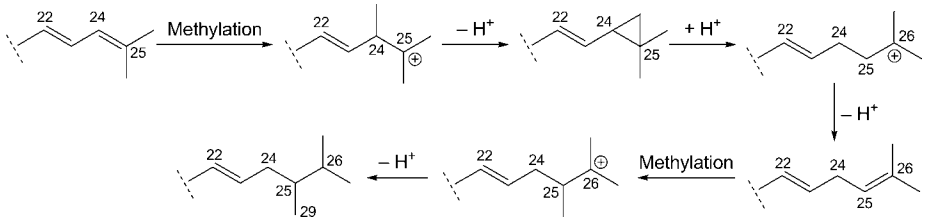
Finally, **c** is dehydrated to give the 7-ene-3β,5α,6β-triol moiety **d**. Since the relative configuration of **2** has been already established, the absolute configuration of **2** is proposed as (3*S*,5*S*,6*R*,7*R*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*,20*R*).

Scheme 2. *Oxidation Route of the* B-*Ring of the Four Nucleus Structures of* **1**–**9**



Seven known analogs, **3**–**9**, were also isolated and identified. It should be noted that globosterol (**8**) represents a rare naturally occurring sterol with an unprecedented 22,23-dihydro-25-methyl C_{10} side chain. Up to date, only two polyhydroxysterols possessing this type of side chain were isolated from natural resources [5][15]. A plausible pathway that leads to this unusual side chain is proposed in *Scheme 3*. This side chain is probably derived from the 'normal' cholesterol side chain by two biological *C*-methylation steps [5]. The introduction of Me group into sterol side chain can occur by transfer of a Me group from (*S*)-adenosylmethionine, catalyzed by a sterol methyltransferase [16].

Scheme 3. *Plausible Biosynthetic Pathway for the Unusual Terminal Side Chain of* **8**



The cytotoxic activities of compounds **1**–**9** were evaluated against a panel of human tumor cell lines (HL-60, K562, HeLa, A-549, and HCT-116). Compounds **1** and **3**–**5** showed cytotoxicities against five tumor cell lines with the IC_{50} values ranging from 2.76 to 9.62 μM (*Table 2*). Among the active compounds, **1** showed the highest activity against HL-60, K562, HeLa, A-549, and HCT-116 with the IC_{50} values of 5.57, 4.04, 3.65, 6.87, and 2.76 μM, respectively. Interestingly, **2** and **6**–**9** exhibited no cytotoxicity to any of the above cell lines. These results suggest that the cytotoxicity may be due to the presence of a 3*β*,5*α*,6*β*,7*β*-tetrol moiety in the molecules of **1** and **3**–**5**.

Table 2. *Cytotoxicities of* **1** *and* **3**–**5** *against Five Human Tumor Cell Lines*

Compounds	Cell lines IC_{50} [μM]				
	HL-60	K562	HeLa	A-549	HCT-116
1	5.57	4.04	3.65	6.87	2.76
3	6.05	4.66	9.27	8.24	3.14
4	6.22	6.71	9.62	7.24	4.88
5	8.91	6.57	9.1	8.49	5.21
Aclarubicin	0.02	0.25	0.6	0.16	0.21

All isolated compounds, **1**–**9**, were also evaluated for their antibacterial activities against a panel of pathogenic bacteria, including *Bacillus cereus*, *Tetragenococcus halophilus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas putida*, *Nocardia brasiliensis*, and *Vibrio parahaemolyticus*. None of the tested compounds displayed any activity.

Conclusions. – In this study, nine polyhydroxylated sterols, **1**–**9**, were isolated from the gorgonian *V. umbraculum*, of which **1** and **2** were new compounds. The absolute configuration of **1** was determined by application of the modified *Mosher* method. All the isolated compounds were evaluated for their cytotoxicities and antibacterial activities. Compounds **1** and **3**–**5** showed cytotoxicities against five human tumor cell lines, indicating that the 3β,5α,6β,7β-tetrol moiety in the molecules is important for cytotoxicity.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemical Group*) and *Sephadex LH-20* (*Amersham*). TLC: Precoated silica gel GF_{254} plates (*Yantai Zifu Chemical Group*). Prep. HPLC: *Hitachi L-2130* system coupled with *Hitachi L-2455* photodiode array detector. UV Spectra: *Milton-Roy* spectrophotometer. Optical rotations: *JASCO digital* polarimeter. IR Spectra: *Nicolet-Nexus-470* spectrophotometer. NMR Spectra: *AVANCE* NMR (500 (^{1}H) and 125 MHz (^{13}C)) spectrometer. ESI- and HR-ESI-MS: *Q-TOF Ultima Global GAA076 LC* mass spectrometer.

Animal Materials. The gorgonian *Verrucella umbraculum* was collected off the coral reef of Weizhou Island in the South China Sea, P. R. China, in April 2010, and was identified by Prof. *Hui Huang*, South China Sea Institute of Oceanology, Chinese Academy of Sciences, P. R. China. A voucher specimen (GXWZ-02) was deposited with the Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao, P. R. China.

Extraction and Isolation. The gorgonian *V. umbraculum* (1.1 kg, wet weight) was cut into small pieces and exhaustively extracted with EtOH (6 × 3000 ml) at r.t. by ultrasonic extraction method. The EtOH extract was evaporated to give a residue (26.0 g), which was partitioned between AcOEt and H_2O. The AcOEt soln. was concentrated under reduced pressure to give a residue (15.0 g), which was subjected to CC (SiO_2; petroleum ether (PE)/AcOEt mixtures of increasing polarity) to offer six fractions, *Frs. 1–6*. *Fr. 3* was purified by CC (SiO_2; PE/AcOEt 5:1) to yield **9** (4.0 mg). *Fr. 4* was submitted to CC (SiO_2; $CHCl_3$/MeOH 10:1), and further purified by semi-prep. HPLC (MeOH/H_2O 78:12) to give **2** (5.0 mg), **6** (4.0 mg), **7** (18.0 mg), and **8** (3.0 mg). *Fr. 5* was separated by CC (SiO_2; $CHCl_3$/MeOH 7:1; followed by *Sephadex LH-20*; $CHCl_3$/MeOH 1:1), and further purified by semi-prep. HPLC (MeOH/H_2O 85:15) to afford **1** (5.0 mg), **3** (18.0 mg), **4** (4.0 mg), and **5** (3.0 mg).

Verumbsteroid A (=*(3β,5α,6β,7β,22*E*)-Cholest-22-ene-3,5,6,7-tetrol*; **1**). White amorphous powder. $[\alpha]_D^{25} = +54.4$ ($c = 0.05$, MeOH). IR: 3420, 2853, 1647, 1384, 1075. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 457.3 ($[M + Na]^+$), 417.3 ($[M + H - H_2O]^+$), 399.3 ($[M + H - 2\,H_2O]^+$), 381.3 ($[M + H - 3\,H_2O]^+$). HR-ESI-MS: 417.3356 ($[M + H - H_2O]^+$, $C_{27}H_{45}O_3^+$; calc. 417.3363).

Verumbsteroid B (=*(3β,5β,6β,7β,22*E*)-5,6-Epoxycholest-22-ene-3,7-diol*; **2**). White amorphous powder. $[\alpha]_D^{25} = +87.2$ ($c = 0.10$, MeOH). IR: 3352, 2861, 1649, 1144, 1086. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 439.4 ($[M + Na]^+$), 399.5 ($[M + H - H_2O]^+$). HR-ESI-MS: 439.3195 ($[M + Na]^+$, $C_{27}H_{44}NaO_3^+$; calc. 439.3183).

*Preparation of the (*R*)- and (*S*)-MTPA Ester of* **1**. A soln. of **1** (2.5 mg) and TsOH (0.24 mg) in 2,2-dimethoxypropane (0.2 ml) was stirred at r.t. for 4 h. $NaHCO_3$ (0.1 mg) was added to the mixture, which was then extracted with $CHCl_3$ (3 × 3 ml), and the $CHCl_3$ extract was evaporated to dryness. The residue was purified by CC (SiO_2; PE/AcOEt 5:1) to afford the 6,7-acetonide derivative **10** (2.0 mg) [17]. To a stirred soln. of **10** (1.0 mg) in pyridine (500 µl) was added 4-(dimethylamino)pyridine (DMAP; 2 mg) and (+)-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*S*)-MTPA-Cl; 8 µl). The mixture

was stirred at r.t. for 8 h. The mixture was then passed through a disposable pipet packed with SiO_2 and eluted with PE/AcOEt 10:1 to give the corresponding (*R*)-MTPA ester **10r**. Treatment of **10** (1.0 mg) with (*R*)-MTPA-Cl (8 µl) as described above yielded the corresponding (*S*)-MTPA ester, **10s** [8][11].

*(3a*R*,3b*R*,5*S*,7a*R*,9a*R*,10*R*,12c*R*)-Hexadecahydro-2,2,7a,9a-tetramethyl-10-[(2*R*,3*E*)-6-methylhept-3-en-2-yl]-3b*H*-cyclopenta[1,2]phenanthro[9,10-*d*][1,3]dioxole-3b,5-diol* (**10**). ^{1}H-NMR (500 MHz, $CDCl_3$): 5.26 (*dd*, *J* = 15.5, 8.5, H–C(23)); 5.20 (*dd*, *J* = 15.5, 8.5, H–C(22)); 4.05 – 4.09 (*m*, H–C(3)); 3.90 (*dd*, *J* = 8.5, 5.0, H–C(7)); 3.73 (*d*, *J* = 5.0, H–C(6)); 1.46 (*s*, Me(29)); 1.30 (*s*, Me(30)); 1.17 (*s*, Me(19)); 1.00 (*d*, *J* = 6.5, Me(21)); 0.86 (*d*, *J* = 6.0, Me(26), Me(27)); 0.68 (*s*, Me(18)). ESI-MS: 457.4 ($[M + H - H_2O]^+$).

*(*S*)-MTPA Ester* (= *(3a*R*,3b*R*,5*S*,7a*R*,9a*R*,10*R*,12c*R*)-Hexadecahydro-3b-hydroxy-2,2,7a,9a-tetramethyl-10-[(2*R*,3*E*)-6-methylhept-3-en-2-yl]-3a*H*-cyclopenta[1,2]phenanthro[9,10-*d*][1,3]dioxol-5-yl (2*S*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate*; **10s**): ^{1}H-NMR (500 MHz, $CDCl_3$): 5.42 – 5.45 (*m*, H–C(3)); 5.26 (*dd*, *J* = 15.0, 8.0, H–C(23)); 5.20 (*dd*, *J* = 15.0, 8.0, H–C(22)); 3.91 (*dd*, *J* = 8.0, 5.0, H–C(7)); 3.74 (*d*, *J* = 5.0, H–C(6)); 3.56 (*s*, Me (MTPA)); 2.21 – 2.25 (*m*, H_a–C(4)); 1.90 – 1.94 (*m*, H_a–C(2)); 1.84 – 1.93 (*m*, H_b–C(4)); 1.66 – 1.68 (*m*, H_b–C(2)); 1.62 – 1.64 (*m*, H_a–C(1)); 1.46 (*s*, Me(29)); 1.40 – 1.42 (*m*, H_b–C(1)); 1.29 (*s*, Me(30)); 1.16 (*s*, Me(19)); 1.00 (*d*, *J* = 6.5, Me(21)); 0.85 (*d*, *J* = 6.0, Me(26), Me(27)); 0.67 (*s*, Me(18)). ESI-MS: 691.5 ($[M + H]^+$), 713.5 ($[M + Na]^+$).

*(*R*)-MTPA Ester* (= *(3a*R*,3b*R*,5*S*,7a*R*,9a*R*,10*R*,12c*R*)-Hexadecahydro-3b-hydroxy-2,2,7a,9a-tetramethyl-10-[(2*R*,3*E*)-6-methylhept-3-en-2-yl]-3a*H*-cyclopenta[1,2]phenanthro[9,10-*d*][1,3]dioxol-5-yl (2*R*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropanoate*; **10r**): ^{1}H-NMR (500 MHz, $CDCl_3$): 5.42 – 5.46 (*m*, H–C(3)); 5.27 (*dd*, *J* = 15.0, 8.5, H–C(23)); 5.21 (*dd*, *J* = 15.0, 8.5, H–C(22)); 3.91 (*dd*, *J* = 8.0, 5.0, H–C(7)); 3.72 (*d*, *J* = 5.0, H–C(6)); 3.55 (*s*, MeO (MTPA)); 2.09 – 2.15 (*m*, H_a–C(4)); 1.92 – 1.96 (*m*, H_a–C(2)); 1.81 – 1.83 (*m*, H_b–C(4)); 1.78 – 1.80 (*m*, H_b–C(2)); 1.72 – 1.75 (*m*, H_a–C(1)); 1.45 (*s*, Me(29)); 1.41 – 1.45 (*m*, H_b–C(1)); 1.28 (*s*, Me(30)); 1.17 (*s*, Me(19)); 1.00 (*d*, *J* = 6.5, Me(21)); 0.85 (*d*, *J* = 6.5, Me(26), Me(27)); 0.67 (*s*, Me(18)). ESI-MS: 691.5 ($[M + H]^+$), 713.5 ($[M + Na]^+$).

Cytotoxicity Assay. The cytotoxicities against HeLa (cervical cancer), HL-60 (human promyelocytic leukemia), A-549 (human lung epithelial carcinoma), HCT-116 (human colon carcinoma), and K562 (human erythroleukemia) cell lines were evaluated by using sulforhodamien B (SRB) [18] and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) [19] methods, resp., according to the protocols described in the literature.

Antibacterial Activity Assay. Antibacterial activities were evaluated by the conventional broth dilution assay [20]. Eight pathogenic bacterial strains, *B. cereus*, *T. halophilus*, *S. epidermidis*, *S. aureus*, *E. coli*, *P. putida*, *N. brasiliensis*, and *V. parahaemolyticus* were used, and ciprofloxacin was used as a positive control.

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