5,6:8,9-Diepoxy and Other Cytotoxic Sterols from the Marine Sponge *Homaxinella* sp.

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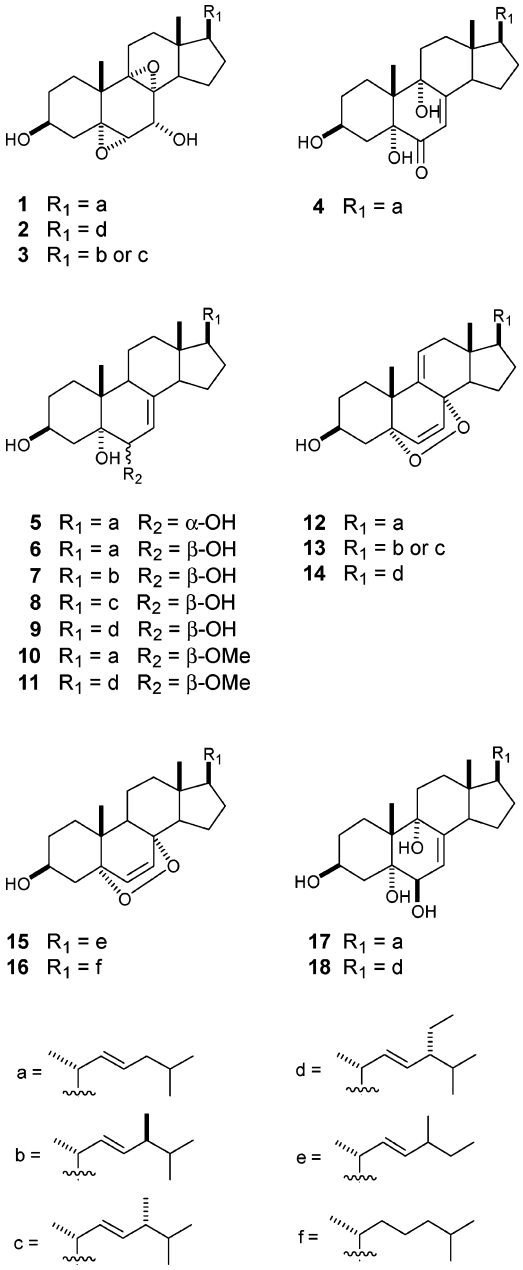
Four new (**1**, **2**, **4**, and **5**) and 14 known (**3** and **6**−**18**) polyoxygenated sterols have been isolated from the MeOH extract of the marine sponge *Homaxinella* sp. by bioactivity-guided fractionation. The planar structures of the sterols were established by 1D and 2D NMR and MS spectroscopic analysis. 5,6:8,9-Diepoxy sterols (**1**−**3**) were isolated from a marine organism for the first time. The isolated sterols were tested against a panel of five human solid tumor cell lines and exhibited varying degrees of cytotoxicity.

In a continuation of our search for bioactive metabolites from the marine sponge *Homaxinella* sp. (family Axinellidae, order Halichondrida),[1−3] we isolated additional polyoxygenated sterols by bioactivity-guided fractionation from its MeOH extract. Although many polyoxygenated sterols isolated from *Homaxinella* sp. have been chemically defined,[4−15] their biological activities have not been fully studied. Herein we describe the isolation, structure elucidation, and cytotoxicity evaluation of these new and known sterols. The brine shrimp-active MeOH extract (LD_{50} 57 μg/mL) of the sponge was partitioned between CH_2Cl_2 and H_2O, and the CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH layer (LD_{50} 170 μg/mL) was chromatographed on a reversed-phase flash column, followed by RP-HPLC of some of the subfractions, to yield four new (**1**, **2**, **4**, and **5**) and 14 known (**3** and **6**−**18**) sterols.

Homaxisterol B_1 (**1**) was isolated as an amorphous powder. Its molecular formula was established as $C_{27}H_{42}O_4$ on the basis of the HRFABMS and ^{13}C NMR spectroscopic data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 453.2966) matched well with the expected molecular formula of $C_{27}H_{42}O_4Na$ (Δ −1.5 mmu). The ^1H NMR spectrum contained two tertiary methyl signals at δ 0.72 (H_3-18) and 1.34 (H_3-19) and three secondary methyl signals at δ 0.99 (H_3-21), 0.87 (H_3-26), and 0.87 (H_3-27), suggesting the chemical nature of **1** as a sterol. In addition, the ^1H NMR spectrum featured an epoxymethine proton at δ 3.04 (H-6, d, J = 2.5 Hz) and two oxymethine protons at δ 3.78 (H-3, m) and 4.11 (H-7, d, J = 2.5 Hz). Two disubstituted olefinic protons at δ 5.19 (dd, J = 15.0, 6.0 Hz, H-22) and 5.30 (dt, J = 15.0, 7.0 Hz, H-23) were also observed. The COSY spectrum showed a correlation between H-6 and H-7. In the HMBC spectrum, H-6 showed correlations to C-4 and C-8, supporting the connectivities between the epoxymethine proton and its neighboring carbons. Other key correlations from H_3-19 to C-5 and -9, from H-14 to C-7, -8, and -9, and from H-4 to C-5 were observed (Figure 1). The ^1H NMR signals for H-6 at δ 3.04, H-7 at δ 4.11, and H_3-19 at δ 1.34 aided in the assignment of an α-configuration for the hydroxyl group at C-7. In the case of its 7β-epimer, these signals were reported to appear at δ 2.94 (H-6), 4.52 (H-7), and 1.43 (H_3-19), respectively.[16] The α-orientation of the C-7 hydroxyl group was further corroborated by the cross-peak between H_3-19 and H-7 in the NOESY spectrum (Figure 2). This correlation also implied that the B-ring of **1** adopts a boat-type conformation as a result of incorporation of the 5α,6α-epoxide moiety.[16] In addition, the NOESY spectrum showed correlations between H_3-18 and H_3-19 and between H_3-18 and H-20, showing that these are all oriented on the same side of the molecule (Figure 2). The chemical shift of H_3-21 (δ 0.99) also supported the 20*R* configuration.[17,18] The downfield shifted broad methine multiplet



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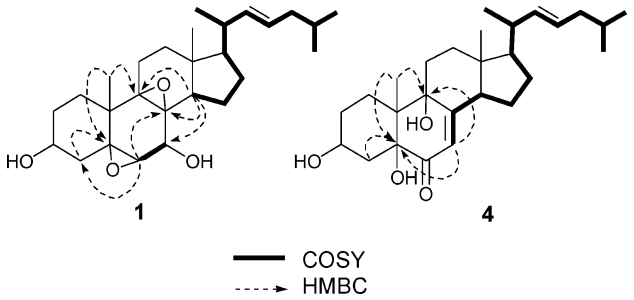

Figure 1. Key COSY and HMBC correlations of compounds **1** and **4**.

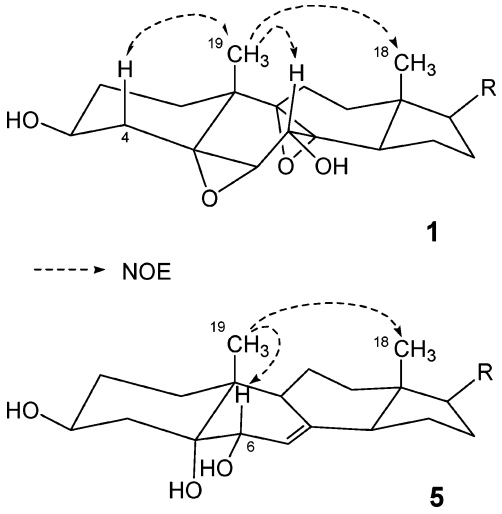

Figure 2. Key NOESY correlations of **1** and **5**.

of H-3 at δ 3.78 ($W_{1/2}$ = 14.0 Hz) supported the fact that **1** is a 3β,5α-oxygenated A/B *trans* sterol.[19,20] The geometry of the double bond of the side chain was assigned as *E* on the basis of a characteristic coupling constant (J = 15.0 Hz) of olefinic protons H-22 and -23. On the basis of these data, the structure of **1** was defined as (*E*)-5α,6α:8α,9α-diepoxycholest-22-ene-3β,7α-diol.

Homaxisterol B_2 (**2**) was isolated as an amorphous powder. Its molecular formula was established as $C_{29}H_{46}O_4$ on the basis of the HRFABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 481.3310) matched well with the expected molecular formula of $C_{29}H_{46}O_4Na$ (Δ +1.6 mmu). The ^{1}H and ^{13}C NMR data of the nucleus of **2** were almost the same as those of **1**, with the only differences being in the side chain. A triplet at δ 0.83 (J = 7.5 Hz) in the ^{1}H NMR spectrum indicated that it has an ethyl group attached at C-24, which was confirmed by HMBC correlations of H_2-24[1] (δ 1.43 and 1.20) to C-22, C-23, and C-24. The *R* configuration at C-24 was assigned tentatively on the basis of the identical chemical shift differences between H_3-26/27 and H_3-24[2] ($\Delta\delta$ 0.03 and 0.03) compared to the distinct chemical shift differences of the *S* isomer ($\Delta\delta$ 0.04 and 0.01).[21] Thus, the structure of **2** was defined as (*E*)-(24*R**)-5α,6α:8α,9α-diepoxy-24-ethylcholest-22-ene-3β,7α-diol.

Compound **3** was isolated as an amorphous powder. The molecular formula was established as $C_{28}H_{44}O_4$ on the basis of its HRFABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 467.3139) matched well with the expected molecular formula of $C_{28}H_{44}O_4Na$ (Δ +0.2 mmu). Compound **3** is a known compound, previously isolated from several mushrooms.[16] The ^{1}H and ^{13}C NMR data for the sterol nucleus of **3** were almost the same as those of **1** and **2**, with the only differences occurring in the side chain. A doublet at δ 0.92 (J = 6.5 Hz) in the ^{1}H NMR spectrum indicated that it has a methyl group attached at C-24. The stereochemistry at C-24 could not be assigned by ^{1}H NMR spectroscopy because only one isomer was available (isolated). On the basis of these data, the structure of **3** was established as (*E*)-5α,6α:8α,9α-diepoxy-24ϵ-methylcholest-22-ene-3β,7α-diol.

Homaxisterol C_1 (**4**) was isolated as an amorphous powder. Its molecular formula was established as $C_{27}H_{42}O_4Na$ on the basis of the HRFABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 453.2952) matched well with the expected molecular formula of $C_{27}H_{42}O_4Na$ (Δ −2.9 mmu). The ^{13}C NMR spectrum showed the presence of 27 carbons, including a ketone carbonyl carbon at δ 200.1 (C-6), four olefinic carbons at δ 120.9 (C-7), 165.0 (C-8), 128.1 (C-22), and 138.8 (C-23), and three oxygenated carbon signals at δ 67.8 (C-3), 80.2 (C-5), and 76.2 (C-9). The COSY spectrum showed a long-range correlation between H-7 and H-14. The 3β,5α-configurations of the hydroxyl groups were determined on the basis of the presence of a downfield shifted broad oxymethine multiplet (H-3) at δ 3.92 ($W_{1/2}$ = 16.0 Hz).[19,20] In the HMBC spectrum, key correlations were observed from H-7 to C-5 and C-9, from H-14 and H-15$_{a,b}$ to C-8, and from H-4$_{a,b}$ and H-11 to C-5 and C-9, respectively (Figure 1). The chemical shift of H_3-21 (δ 1.04) supported the 20*R* configuration.[17,18] All natural sterols have a *trans* B/C ring fusion,[22] so the configuration at C-9 was presumed to be α.[23] On the basis of these data, the structure of **4** was established as (*E*)-3β,5α,9α-trihydroxycholesta-7,22-dien-6-one. The new structural feature of this sterol is the difference of side chain, while the same sterol nucleus has been reported from five mushrooms.[19] It is speculated that the ketone group (C-6) in **4** might be incorporated by microbial dehydrogenation of similarly OH-6 substituted sterols (**17** and **18**).[24]

Homaxisterol D_1 (**5**) was also isolated as an amorphous powder. Its molecular formula was established on the basis of NMR and MS data. The FABMS of **5** showed the $[M + Na]^+$ ion at *m*/*z* 439. The ^{1}H NMR spectrum of **5** featured five methyl, two hydroxyl, and two olefinic proton signals characterizing its nature as a polyhydroxy sterol. The comparison of its ^{1}H NMR data for H_3-18 (δ 0.61), H_3-19 (δ 1.02), H-3 (δ 3.89), H-6 (δ 3.87), and H-7 (δ 5.04) with literature suggested the 6α-orientation of the hydroxyl group.[6,8,25] In the case of the 6β epimer, [5−8,25] H_3-18, H_3-19, H-3, H-6, and H-7 signals were reported to appear at δ 0.64, 1.05, 3.96, 3.54, and 5.26, respectively. Assignment of the stereochemistry at C-6 was further corroborated by the optical rotation data. For similarly substituted OH-6α sterol isomers, positive optical rotations were observed, while their 6β-epimers showed negative optical rotations.[6,8,25] Compound **5** showed a positive optical rotation ($[\alpha]^{23}_D$ +12). Its 6β-epimer (**6**), previously isolated from the same sponge[2] and other marine organisms such as a bryozoan[5] and a scallop,[8] showed a negative optical rotation ($[\alpha]^{23}_D$ −8). We have reported in our previous paper that the optical rotation values for 3β,5α,6β-oxygenated sterols were not diagnostic in terms of determining isomerism at C-24, as both epimers showed the same sign of optical rotation.[2] This finding is consistent with the other reported 3β,5α,6β-oxygenated sterols, as they all showed negative optical rotations,[5−8,25] and can be helpful in defining the stereochemistry of similarly substituted sterols. In addition, the NOESY correlation between H-6 and H_3-19 also supported the 6α-orientation of the hydroxyl group (Figure 2). On the basis of these data, the structure of **5** was established as (*E*)-cholesta-7,22-diene-3β,5α,6α-triol. Not only in this present study but also in previous literature, the isolation of both stereoisomers (6α- and 6β-OH) from the same organism is evident.[25] This implies that compounds **5** and **6** might be the artifacts of nonenzymatic hydrolysis of the epoxide precursor.

This is the first report on the isolation of 5α,6α:8α,9α-diepoxy sterols (**1**−**3**) from a marine source. Their counterparts have been previously reported from several mushrooms,[15,26] and it suggests possible involvement of symbiotic microorganisms in the biogenesis of the sterols **1**−**3**.

Thirteen known polyoxygenated sterols **6**−**18**[4−15] were also isolated from the same MeOH extract. The structures of these compounds were defined by comparison of their MS and NMR

Table 1. ^{1}H NMR Data of Compounds **1**, **2**, **4**, and **5** (CD_3OD, 500 MHz)[a]

position	1	2	4	5
1	1.72 (m)	1.74 (m)	1.78 (m)	1.51 (m)
	1.79 (m)	1.79 (m)	1.95 (m)	
2	1.62 (m)	1.62 (m)	1.44 (m)	1.42 (m)
	1.94 (m)	1.99 (m)	1.86 (m)	1.84 (m)
3	3.78 (m)	3.78 (m)	3.92 (m)	3.89 (m)
4	1.14 (m)	1.14 (m)	1.62 (m)	1.81 (m)
	2.18 (dd, 13.0, 11.5)	2.18 (dd, 13.0, 11.5)	2.10 (m)	2.14 (m)
5				
6	3.04 (d, 2.5)	3.04 (d, 2.5)		3.87 (m)
7	4.11 (d, 2.5)	4.11 (d, 2.5)	5.58 (d, 1.5)	5.04 (m)
8				
9				2.06 (m)
10				
11	1.79 (m)	1.79 (m)	1.52 (m)	1.55 (m)
	2.02 (m)	2.02 (m)	1.60 (m)	
12	1.05 (m)	1.05 (m)	1.70 (m)	1.32 (m)
	1.75 (m)	1.75 (m)	1.88 (m)	1.89 (m)
13				
14	1.54 (m)	1.54 (m)	2.76 (m)	1.95 (m)
15	1.43 (m)	1.43 (m)	1.50 (m)	1.50 (m)
	2.12 (m)	2.12 (m)	2.29 (m)	
16	1.32 (m)	1.32 (m)	1.39 (m)	1.28 (m)
	1.72 (m)	1.72 (m)	1.72 (m)	
17	1.19 (m)	1.05 (m)	1.45 (m)	1.32 (m)
18	0.72 (s)	0.73 (s)	0.66 (s)	0.61 (s)
19	1.34 (s)	1.34 (s)	1.00 (s)	1.02 (s)
20	2.00 (m)	2.00 (m)	2.04 (m)	2.00 (m)
21	0.99 (d, 7.0)	1.02 (d, 7.0)	1.04 (d, 7.0)	1.02 (d, 6.5)
22	5.19 (dd, 15.0, 6.0)	5.16 (dd, 15.0, 7.0)	5.24 (dd, 15.0, 9.0)	5.21 (dd, 15.0, 6.5)
23	5.30 (dt, 15.0, 7.0)	5.05 (dt, 15.0, 6.5)	5.34 (dt, 15.0, 8.0)	5.31 (dt, 15.0, 7.0)
24	1.82 (m)	1.55 (m)	1.84 (m)	1.89 (m)
25	1.55 (m)	1.60 (m)	1.59 (m)	1.58 (m)
26	0.87 (d, 6.5)	0.80 (d, 7.0)	0.88 (d, 6.5)	0.88 (d, 6.5)
27	0.87 (d, 6.5)	0.86 (d, 7.0)	0.88 (d, 6.5)	0.87 (d, 6.5)
24^1		1.43 (m)		
		1.20 (m)		
24^2		0.83 (t, 7.5)		

[a] Multiplicities and coupling constants are in parentheses.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, **4**, and **5**[a]

position	1[b]	2[c]	4[b]	5[d]
1	28.9	28.9	29.2	33.0
2	31.1	31.1	29.8	30.8
3	68.8	68.9	67.8	67.2
4	41.4	41.4	37.2	43.1
5	67.5	67.5	80.2	75.3
6	62.7	62.7	200.1	70.8
7	66.8	66.7	120.9	121.4
8	67.1	67.1	165.0	140.0
9	70.4	70.5	76.2	43.0
10	36.8	36.9	42.8	41.8
11	23.2	23.2	23.4	24.1
12	33.8	33.9	36.2	36.0
13	41.7	43.2	46.2	42.8
14	54.3	54.4	52.9	51.1
15	24.2	24.3	26.6	24.2
16	29.2	29.5	29.4	22.0
17	54.1	54.1	57.4	56.1
18	12.8	12.8	12.5	13.1
19	21.6	21.9	20.6	21.1
20	41.8	41.9	41.6	43.0
21	21.8	21.2	21.5	21.5
22	127.9	139.1	128.1	138.9
23	139.0	131.2	138.8	127.1
24	43.1	52.7	45.8	44.3
25	29.8	33.1	29.8	34.4
26	22.7	19.9	22.7	22.7
27	22.7	21.3	22.7	22.7
24^1		26.5		
24^2		15.0		

[a] Measured in CD_3OD. [b]Spectrum was measured at 50 MHz. [c]Spectrum was measured at 75 MHz. [d]Assignments were made on the basis of HMBC and HSQC data (500 MHz).

Table 3. Cytotoxicity Data of Compounds **1**−**5**, **8**, **10**, and **11**[a,b]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	3.9	3.5	3.1	3.4	3.2
2	1.4	1.3	1.1	1.0	1.1
3	7.1	6.3	2.7	3.4	4.8
4	5.0	7.0	3.9	3.7	3.7
8	3.9	6.0	2.8	3.3	3.0
10	4.8	7.1	3.4	4.0	4.2
11	4.4	7.0	5.2	3.8	4.9
doxorubicin	0.01	0.03	0.01	0.01	0.05
5	3.5	3.9	3.1	3.1	3.1
doxorubicin	0.04	0.13	0.04	0.06	0.06

[a] Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer. [b]Compounds **6**, **7**, **9**, **17**, and **18** were inactive ($ED_{50} > 5$ μg/mL) for all cell lines in the panel.

data with those reported. The stereochemistry at chiral carbons of these sterols was defined by comparison of NMR data with literature values.

The isolated sterols were evaluated for cytotoxicity against a panel of five human solid tumor cell lines (Table 3). Most of the compounds showed cytotoxicity to human lung cancer cell lines (A549), human skin cancer cell lines (XF498), and human colon cancer cell lines (HCT15). Compounds **1**−**5**, **8**, **10**, and **11** showed cytotoxic profiles to all tumor cell lines tested, while compound **2** was the most broadly cytotoxic test compound. It is interesting to note here that the previously isolated highly degraded sterol, demethylincisterol A_4,[2] having the same side chain as that of **2**, was the most potent among a group of other demethylincisterols.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Varian Unity Plus 300, and Varian INOVA 500 spectrometers. Chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A. HRFABMS data were obtained on JEOL JMS SX-101A. HPLC was performed with an YMC packed ODS column (250 × 10 mm, 5 μm, 120 Å) and a C_{18}-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) using a Gilson 133-RI detector.

Animal Material. The sponge was collected in August 1998 at a depth of 20 m off Jeju Island, Korea. The specimen was identified as *Homaxinella* sp. by Prof. Chung Ja Sim, Hannam University. A voucher specimen (J98J-1) of this sponge (registry No. Spo. 39) was deposited in the Natural History Museum, Hannam University, Daejon, Korea, and has been described elsewhere.[1]

Extraction and Isolation. The frozen sponge (7 kg) was extracted with MeOH at room temperature. The MeOH extract showed toxicity against brine shrimp larvae (LD_{50} 57 μg/mL). The MeOH extract was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH fraction was subjected to stepped gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 400/500 mesh), with a solvent system of 60 to 100% MeOH, to afford 22 fractions. Fraction 11 (506.8 mg), one of the bioactive fractions (LD_{50} 10 μg/mL), was again subjected to reversed-phase flash column chromatography (YMC ODS-A, 120 Å, 30/50 μm), eluting with a stepped gradient solvent system of 60−100% MeOH, to afford 10 fractions. Subfraction 6 from fraction 11 was subjected to reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 90% MeOH, followed by another reversed-phase HPLC separation (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 81% MeOH, to afford compounds **1** (1.3 mg), **3** (1.3 mg), and **17** (1.0 mg). Subfraction 7 from fraction 11 was subjected to reversed-phase HPLC (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 90% MeOH,

followed by another reversed-phase HPLC separation (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 81% MeOH, to afford compounds **4** (0.7 mg), **6** (4.5 mg), and **18** (1.0 mg). Subfraction 8 from fraction 11 was subjected to reversed-phase HPLC separation (C_{18}-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å), eluting with 90% MeOH, followed by another reversed-phase HPLC separation (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 81% MeOH, to afford compounds **7** (0.9 mg) and **8** (2.1 mg). Fraction 12 (1.2 g), another bioactive fraction (LD_{50} 27 μg/mL), was subjected to further reversed-phase flash column chromatography (YMC ODS-A, 120 Å, 30/50 μm), eluting with a stepped gradient solvent system of 65 to 100% MeOH/H_2O, to afford 10 fractions. Compounds **2** (1.8 mg), **5** (1.0 mg), and **9** (0.8 mg) were obtained by separation of subfraction 4 (52.2 mg) using a reversed-phase HPLC system (C_{18}-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å), eluting with 84% MeOH. Subfraction 5 from fraction 12 was subjected to reversed-phase HPLC (C_{18}-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å), eluting with 90% MeOH, followed by further reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 81% MeOH, to afford compound **10** (3.7 mg). Fraction 13 was subjected to reversed-phase HPLC (C_{18}-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å), eluting with 97% MeOH, followed by further reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 91% MeOH, to afford pure compound **11** (1.8 mg). Fraction 14 was separated by a successive reversed-phase HPLC process (C_{18}-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å), eluting with 97% MeOH, followed by another reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å), eluting with 91% MeOH, to afford pure compounds **12** (1.7 mg), **13** (2.0 mg), **14** (1.5 mg), **15** (0.7 mg), and **16** (1.0 mg).

Homaxisterol B_1 (1): white amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m*/*z* 453 $[M + Na]^+$; HRFABMS *m*/*z* 453.2966 (calcd for $C_{27}H_{42}O_4Na$, 453.2981).

Homaxisterol B_2 (2): white amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m*/*z* 481 $[M + Na]^+$; HRFABMS *m*/*z* 481.3310 (calcd for $C_{29}H_{46}O_4Na$, 481.3294).

Compound 3: white amorphous solid; ^{1}H NMR data (CD_3OD, 500 MHz) δ 5.20 (m, H-23), 5.18 (m, H-22), 4.11 (d, J = 2.5 Hz, H-7), 3.77 (m, H-3), 3.04 (d, J = 2.5 Hz, H-6), 1.34 (s, H_3-19), 0.99 (d, J = 7.0 Hz, H_3-21), 0.92 (d, J = 6.5 Hz, H_3-24[1]), 0.86 (d, J = 7.0 Hz, H_3-27), 0.83 (d, J = 7.0 Hz, H_3-26), 0.72 (s, H_3-18); FABMS *m*/*z* 467 $[M + Na]^+$; HRFABMS *m*/*z* 467.3139 (calcd for $C_{28}H_{44}O_4Na$, 467.3137).

Homaxisterol C_1 (4): white amorphous solid; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m*/*z* 431 $[M + H]^+$, 453 $[M + Na]^+$; HRFABMS *m*/*z* 453.2952 (calcd for $C_{27}H_{42}O_4Na$, 453.2981).

Homaxisterol D_1 (5): white amorphous solid; $[\alpha]^{23}_D$ +12 (*c* 0.1, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m*/*z* 439 $[M + Na]^+$.

Compound 6: white amorphous solid; $[\alpha]^{23}_D$ −8 (*c* 0.33, MeOH); ^{1}H NMR data (CD_3OD, 500 MHz) δ 5.31 (dd, J = 15.0, 7.0 Hz, H-23), 5.26 (m, H-7), 5.22 (dd, J = 15.0, 6.5 Hz, H-22), 3.96 (m, H-3), 3.54 (m, H-6), 1.05 (s, H_3-19), 1.03 (d, J = 6.5 Hz, H_3-21), 0.88 (d, J = 6.5 Hz, H_3-26), 0.87 (d, J = 6.5 Hz, H_3-27), 0.63 (s, H_3-18).

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