Cytotoxic Sterol Derivatives from a Marine Sponge *Homaxinella* sp.

Tayyab A. Mansoor,[†] Jongki Hong,[‡] Chong-O. Lee,[§] Song-Ja Bae,[⊥] Kwang Sik Im,[†] and Jee H. Jung*[,†]

College of Pharmacy, Pusan National University, Busan 609-735, Korea, Korea Basic Science Institute, Seoul, Korea, Korea Research Institute of Chemical Technology, Daejon, Korea, and Silla University, Busan, Korea

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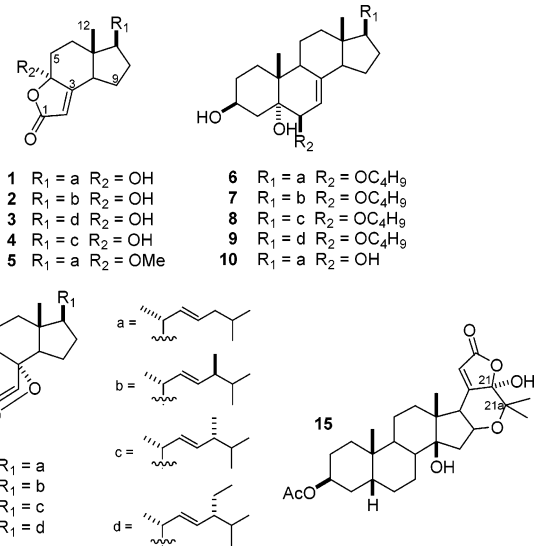
A bioactivity-guided fractionation of a marine sponge *Homaxinella* sp. has led to the isolation of three new (**1**−**3**) highly degraded sterols and four new 6-*O*-alkylated (**6**−**9**) sterols, along with known sterol derivatives. The degraded sterols (**1**−**5**) belong to the class incisterols, previously isolated from the marine sponge *Dictyonella incisa*. Mainly NMR and MS spectroscopic analyses established the gross structures of the new compounds. The relationship between the stereoisomerism of the side chain and HPLC retention time has also been discussed. The compounds were tested against a panel of five human solid tumor cell lines, and especially the degraded sterols (**1**−**4**) displayed significant cytotoxicity.

Marine sponges, including the genus *Homaxinella*,[1] are reported to contain numerous unusual sterols. These sterols have unique variations both in the side chain[2] and nucleus;[3] contain a variety of oxygenated functionalities such as polyhydroxy,[4−8] epoxide,[9] epidioxy,[10−14] and mono- or polyenone systems;[15−18] and possess modified or degraded structures with the loss of carbon atoms from the nucleus skeleton.[1,19] Some of the polyoxygenated and degraded sterols have provided very interesting results in cytotoxicity,[20] MDR (multidrug resistance) reversal,[21] and DNA polymerase-α inhibitory studies.[22]

In continuation of our search for cytotoxic metabolites from a marine sponge *Homaxinella* sp. (family Axinellidae, order Halichondrida) collected from Korean waters,[23] we have isolated three new (**1**−**3**) and two known (**4**, **5**)[19,22] highly degraded sterols, four new 6-*O*-alkylated sterols (**6**−**9**), a known 3*β*,5α,6*β*-trihydroxy sterol (**10**),[4] and four known 5α,8α-epidioxy sterols (**11**−**14**),[13] from the MeOH extract of the sponge. The degraded sterols (**1**−**5**) belong to the class incisterols, previously isolated from the marine sponge *Dictyonella incisa*.[19] Herein, we describe the structure elucidation and cytotoxicity evaluation of these sterol derivatives.



Results and Discussion

Demethylincisterol A_1 (**1**) was isolated as a colorless oil. Its molecular formula was established as $C_{20}H_{30}O_3$ on the basis of the ^{13}C NMR and HRFABMS data. The exact mass of the $[M + Na]^+$ ion (*m/z* 341.2142) matched well with the expected molecular formula $C_{20}H_{30}O_3Na$ (Δ +4.9 mmu). The upfield methyl region in the ^1H NMR spectrum indicated the presence of characteristic steroidal methyl signals, except the H_3-19 methyl singlet. However, its ^{13}C NMR data showed only 20 carbons, indicating its chemical nature as a degraded sterol. In addition, IR absorption at 1751 cm^{-1}, UV absorption at 220 nm, a ^1H NMR signal at δ 5.67 (d, J = 1.5 Hz), and ^{13}C NMR signals at δ 173.6, 172.9, 112.6, and 104.1 suggested the presence of a γ-hydroxy-α,β-unsaturated γ-lactone system in the molecule. The presence and position of the proposed lactone moiety were further corroborated by the HMBC and COSY experiments (Figure 1). In the HMBC spectrum, key correlations from H-2 to C-1, 3, and 4, from H-8 to C-3, 6, 7, and 11, and from H-5 to C-4 were observed. In the COSY spectrum, H-2 showed a long-range correlation with H-8 (Figure 1). A doublet of triplets at δ 5.36 and a doublet of doublets at δ 5.24 in the ^1H NMR spectrum were assigned to the olefinic protons H-15 and H-16 of the side chain. The stereochemistry of the double bond was defined as *E* on the basis of the coupling constant (15.0 Hz). The relative stereochemistry of the degraded sterol nucleus was deduced by NOESY experiment, which demonstrated key correlations depicted in Figure 2. A cross-peak was observed between H_3-12 and H_{ax}-5, while H-8 showed correlations with H_{ax}-6 and H-11, thus indicating a trans fusion of the cyclohexane and cyclopentane rings. A NOESY correlation between H_3-12 and H-13 and the chemical shift of H_3-14 (δ 1.05) also supported its 13*R* configuration (H_3-21 signal appears at δ 1.04 and 0.94 for 20*R* and 20*S* Δ^{22}-sterols, respectively).[24,25] The absolute configuration at C-4 was defined on the basis of CD spectral data. In the α,β-unsaturated γ-lactone, the Cotton effects associated with the $\pi-\pi^*$ transition in the region 205−235 nm were used to determine the stereochemistry of the substituent at the γ-carbon atom. The model compound 16,21a-epoxy-21-hydroxy-21-isopropyldigitoxigenin-3-acetate (**15**) showed a positive $\pi-\pi^*$ Cotton effect at 223 nm and was assigned the *R* configuration at C-21.[26] The same stereochemistry

* To whom correspondence should be addressed. Tel: 82-51-510-2803. Fax: 82-51-510-2803. E-mail: jhjung@pusan.ac.kr.
[†] Pusan National University.
[‡] Korea Basic Science Institute.
[§] Korea Research Institute of Chemical Technology.
[⊥] Silla University.

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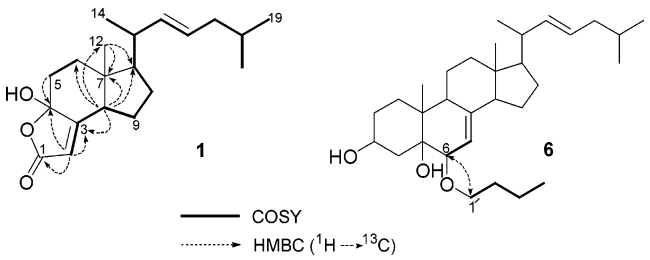

Figure 1. Key COSY and HMBC correlations of compounds **1** and **6**.

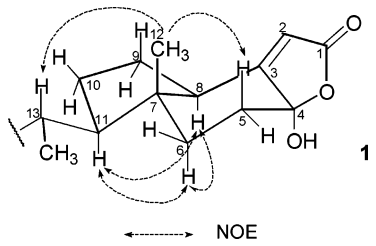

Figure 2. Key NOESY correlations of **1**.

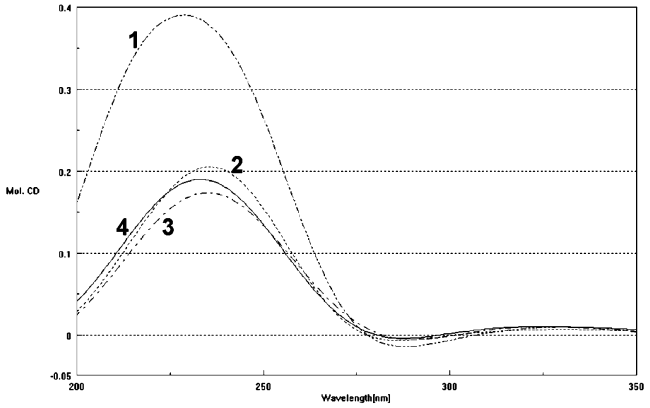

Figure 3. CD spectra of **1**−**4**.

was assigned to C-4 of **1**, as it showed a positive $\pi-\pi^*$ Cotton effect at 229 nm (Figure 3), but due to different functional priorities around the chiral carbon as compared to those of **15**, the absolute configuration was assigned as *S*.

Demethylincisterol A_2 (**2**) was also isolated as a colorless oil, and its molecular formula was established as $C_{21}H_{32}O_3$ on the basis of FABMS and ^{13}C NMR data. The FABMS spectrum of **2** showed the $[M + H]^+$ and $[M + Na]^+$ ions at *m/z* 333 and 355, respectively. The NMR data of **2** were same as those of **1** for the nucleus. The only difference was in the side chain. The ^{1}H NMR spectrum showed an additional doublet in the upfield methyl region (δ 0.94), indicating that it has an additional methyl group. The doublet at δ 0.94 showed correlation to H-17 in the COSY spectrum and long-range correlations to C-16 and C-18 in HMBC spectrum, locating the methyl group at C-17. The configuration of the C-21 methyl group was defined by comparison of the ^{1}H NMR data with that of compound **4** (vide infra). Compound **2** showed a positive $\pi-\pi^*$ Cotton effect at 235 nm and hence was assigned the same configuration at C-4 as that of **1**.

Demethylincisterol A_4 (**3**) was also isolated as a colorless oil. Its molecular formula was established as $C_{22}H_{34}O_3$ on the basis of FABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 369.2340) matched well with the expected molecular formula of $C_{22}H_{34}O_3Na$ (Δ −6.6 mmu). The ^{1}H and ^{13}C NMR data of the nucleus of **3** were almost the same as those of **1** and **2**, with the only difference in the side chain. A triplet at δ 0.84 in the ^{1}H NMR spectrum indicated that it has an ethyl group attached at C-17 that was confirmed by HMBC correlations of H-21 to C-16, C-17, and C-18. The *R* configuration at C-17 was tentatively assigned on the basis of the similar chemical shift differences between H_3-19/20 and H_3-22 ($\Delta\delta$ 0.02 and 0.03) compared to the distinct chemical shift differences of the *S* isomer ($\Delta\delta$ 0.04 and 0.01).[27] Compound **3** also showed a positive $\pi-\pi^*$ Cotton effect similar to those of **1** and **2** (235 nm) in the CD spectrum, and the same *S* configuration was assigned to C-4.

Demethylincisterol A_3 (**4**) was also isolated as a colorless oil. Its molecular formula was established as $C_{21}H_{32}O_3$ on the basis of FABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 355.2292) matched well with the expected molecular formula of $C_{21}H_{32}O_3Na$ (Δ +4.2 mmu). The ^{13}C NMR and MS data of **4** showed that it is an isomer of **2**. The comparison of the ^{1}H NMR spectra of **4** and **2** revealed that they are 17 *S*/*R* isomers. The H_3-14 methyl proton signal of **2** appeared at δ 1.04, upfield shifted compared to that of **4** (δ 1.05), suggesting that **2** and **4** are 17*S* and 17*R* isomers, respectively.[3,28] Compound **4** was previously reported as an intermediate in the synthesis of (17*R*)-17-methylincisterol.[29] Compound **4** also showed a pattern of $\pi-\pi^*$ Cotton effect in the CD spectrum (234 nm) similar to those of **1**−**3** and was assigned the same *S* configuration at C-4.

Incisterol (**5**), a colorless oil, was a known compound previously isolated from the marine sponge *Dictyonella incisa*.[19] It showed ^{1}H and ^{13}C NMR data almost identical to those of **1**, except that **1** contained a hydroxyl group at C-4, while **5** contained a methoxyl group at the same position. The 4-methoxylated incisterols were earlier described as artifacts of methanol extraction.[19,29] However, the isolation of 4-hyroxylated (**1**−**4**) and 4-methoxylated forms (**5**), employing the same isolation and purification procedures, suggests that the 4-methoxylated forms actually might be a natural product. The 4-hydroxylated compound (**4**) was kept at 50 °C in MeOH for 7 days without showing any sign of chemical change or transformation to the methoxylated form.

Homaxisterol A_1 (**6**) was isolated as a light yellow oil. Its molecular formula was established as $C_{31}H_{52}O_3$ on the basis of HRFABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 495.3800) matched well with the expected molecular formula of $C_{31}H_{52}O_3Na$ (Δ −1.4 mmu). The ^{13}C NMR data also supported the presence of 31 carbons, including four oxygenated carbons. The ^{1}H NMR, HSQC, and HMBC experiments confirmed the above functionalities. The downfield-shifted broad methine multiplet of the H-3 carbinol proton at δ 3.95 ($w_{1/2}$ = 16.0 Hz) indicated that **6** is a 3β,5α-hydroxy A/B trans sterol.[30] The pyridine-induced shift of the H-3α signal to δ 4.81 was also indicative of 1,3-diaxial correlation with the 5α-oriented hydroxyl group.[31,32] The ^{1}H NMR also showed an olefinic proton at δ 5.36 (bd, 5.0 Hz) coupled with a broad singlet at δ 3.22 (H-6) in the COSY spectrum. These data and the upfield-shifted resonance of the C-18 methyl signal (δ 0.62) suggested the presence of the 6-oxygenated-Δ^7 system.[6] The ^{1}H NMR spectrum also revealed the presence of a butoxyl group at C-6, which was confirmed by the COSY and HMBC experiments. The geminal proton signals at δ 3.61 and δ 3.39 of the butoxyl group showed long-range correlations to the C-6 signal in the HMBC spectrum (Figure 1). The configuration of the butoxyl group at C-6 was defined as β on the basis of the coupling constant between H-6 and H-7 (5.0 Hz). Its α-epimer is expected to show a near zero coupling constant.[6] In the NOESY spectrum, a

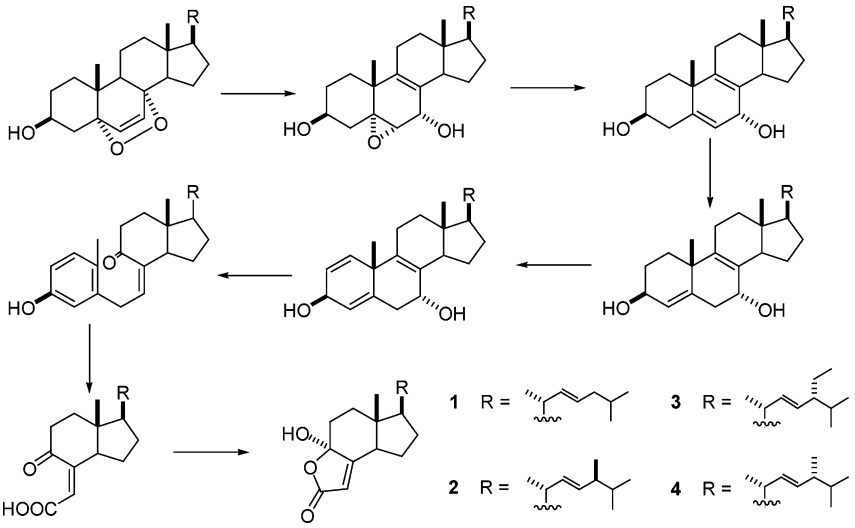

Figure 4. Plausible biosynthetic pathway of **1**−**4** from 5α,8α-epidioxy sterols.

correlation was observed between the H_3-18 methyl proton and H-20, showing that they are oriented on same side of the molecule. The chemical shift of H_3-21 (δ 1.03) also supported the 20*R* configuration.[24,25] The geometry of Δ^{22} was assigned as *E* from the coupling constant (15.0 Hz). Accordingly, the structure of homaxisterol A_1 (**6**) was established as (22*E*)-6-*O*-butylcholesta-7,22-diene-3β,5α,6β-triol.

Homaxisterol A_2 (**7**) was isolated as a light yellow oil. Its molecular formula was established as $C_{32}H_{54}O_3$ on the basis of FABMS and ^{13}C NMR spectral data. The exact mass of the $[M + Na]^+$ ion (*m/z* 509.3957) matched well with the expected molecular formula of $C_{32}H_{54}O_3Na$ (Δ −1.4 mmu). The NMR data of **7** were almost the same as those of **6** for the steroidal nucleus. The only difference was in the side chain. The ^{1}H NMR spectrum showed an additional doublet methyl proton signal at δ 0.93 (d, 7.0 Hz), indicating the presence of a H_3-24^1 methyl group. The fragment ion at *m/z* 435 supported the fact that the steroidal side chain of **7** contained an additional methyl group as compared to **6**.

Homaxisterol A_3 (**8**) was isolated as a light yellow oil. Its molecular formula was established as $C_{32}H_{54}O_3$ on the basis of FABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 509.3982) matched well with the expected molecular formula of $C_{32}H_{54}O_3Na$ (Δ +1.1 mmu). The ^{13}C NMR and MS data of **8** were almost identical to those of **7**. The comparison of the ^{1}H NMR spectrum of **7** with that of **8** revealed that they are 24 *S*/*R* isomers. In the case of the 24*S* isomer, the H_3-21 signal was reported to appear 0.01 ppm upfield-shifted as compared to that of the 24*R* epimer.[3,28] The H_3-21 signal of **7** appeared at δ 1.02, while that of **8** appeared at δ 1.03, suggesting that **7** and **8** are 24*S*/*R* isomers. Thus, the structures of **7** and **8** were established as (22*E*,24*S*)-6-*O*-butyl-24-methylcholesta-7,22-diene-3β,5α,6β-triol and (22*E*,24*R*)-6-*O*-butyl-24-methylcholesta-7,22-diene-3β,5α,6β-triol, respectively.

Homaxisterol A_4 (**9**) was also isolated as a light yellow oil. Its molecular formula was established as $C_{33}H_{56}O_3$ on the basis of HRFABMS and ^{13}C NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 523.4143) matched well with the expected molecular formula of $C_{33}H_{56}O_3Na$ (Δ +1.6 mmu). The NMR data of the steroidal nucleus were almost the same as those of **7** and **8**, with the only difference in the side chain. A triplet at δ 0.84 (t, 7.5 Hz) in the ^{1}H NMR spectrum indicated that it has an ethyl branch at C-24, which was confirmed by COSY and HMBC correlations and MS fragmentation pattern. The fragment ion at *m/z* 449 showed that the side chain contained an additional methylene group as compared to **7** and **8**. As in the case of **3**, the *R* configuration at this carbon atom was tentatively assigned on the basis of chemical shift differences between H_3-26/27 and H_3-29 ($\Delta\delta$ 0.03 and 0.02).[27] Therefore, its structure was established as (22*E*,24*R*)-6-*O*-butyl-24-ethylcholesta-7,22-diene-3β,5α,6β-triol.

Several 3β,5α,6β-trihydroxy sterols were previously reported from the sponges,[4−8] but the presence of 6-*O*-butyl derivatives is unusual. Previously reported 3β,5α,6β-trihydroxy sterol (**10**)[4] was also isolated along with new butylated derivatives (**6**−**9**). The chance of artifact formation with BuOH during the process of extraction and isolation is very low, as the process was conducted near or below room temperature. Compound **10** was treated with BuOH at 50 °C for 7 days, and changes in its chemical nature were monitored by ^{1}H NMR. A major portion of it (80%) gradually decomposed, but its ^{1}H NMR spectrum did not show any sign of formation of the butyl derivative.

In the present study, we have isolated three side-chain epimeric pairs **2/4**, **7/8**, and **12/13**. While comparison of the chemical shift values of H_3-14/21 is a more consistent parameter to define the absolute configuration at C-17/24,[3,28] the HPLC retention time may be another useful criterion in future analyses of naturally occurring sterol mixtures, especially in the event of very small quantities. The difference in HPLC retention times of C-17/24 epimers is probably due to the rigidity of the side chain, imposed by the unsaturation at C-15/22. In all of the three epimeric pairs, *S* isomers have shorter retention times as compared to their *R* isomers. This apparent difference in retention times of the 17/24 *R*/*S* isomers may be useful to assign the stereochemistry solely on the basis of chromatographic mobility. The optical rotation values were irrelevant to the epimerism at C-17/24 for epimeric pairs **2/4** and **7/8**,[4] as they showed the same sign of optical rotation, $[\alpha]_D$ +28°/+74° and $[\alpha]_D$ −9°/−3°, respectively. However, epimeric pair **12** and **13** showed the opposite sign of optical rotation, $[\alpha]_D$ +5° and $[\alpha]_D$ −2°, respectively. The same sign of optical rotation but weak activity of **13** as compared to its synthetic form {$[\alpha]_D$ −25°}[10] may be because of its impure nature.

The occurrence of a wide range of symbiotic microbes have been reported in marine sponges, including the genus *Homaxinella*.[33,34] Various studies have shown the involvement of microbes in the degradation of sterols.[35−37] The plausible biogenesis of the degraded sterols (**1**−**5**) might

Table 1. ^{1}H NMR Data of Compounds **1**−**5** (CD_3OD)[a]

position	**1**[b]−**5**	position	**1**[b]	**2**[c]	**3**[c]	**4**[c]	**5**[c]
2	5.67 (d, 1.8)	13	2.08 (m)	2.08 (m)	2.08 (m)	2.08 (m)	2.08 (m)
5	2.23 (ddd, 14.0, 4.0, 2.5)	14	1.05 (d, 7.0)	1.04 (d, 7.0)	1.07 (d, 7.0)	1.05 (d, 7.0)	1.05 (d, 7.0)
	1.84 (m)	15	5.24 (dd, 15.0, 9.5)	5.21 (m)	5.22 (dd, 15.0, 8.5)	5.21 (dd, 15.0, 8.0)	5.24 (dd, 15.0, 9.5)
6	1.98 (ddd, 13.0, 5.0, 2.5)	16	5.36 (dt, 15.0, 8.5)	5.26 (m)	5.11 (dd, 15.0, 8.5)	5.28 (dd, 15.0, 8.0)	5.36 (m)
	1.62 (m)	17	1.85 (m)	1.85 (m)	1.85 (m)	1.85 (m)	1.85 (m)
8	2.66 (ddd, 12.0, 7.0, 1.8)	18	1.57 (m)	1.57 (m)	1.57 (m)	1.57 (m)	1.57 (m)
9	1.73 (m)	19	0.84 (d, 7.0)	0.85 (d, 7.0)	0.82 (d, 6.0)	0.84 (d, 7.0)	0.84 (d, 7.0)
	1.62 (m)	20	0.88 (d, 7.0)	0.86 (d, 7.0)	0.87 (d, 6.0)	0.86 (d, 7.0)	0.88 (d, 7.0)
10	1.92 (m)	21		0.94 (d, 6.5)	1.45 (m)	0.94 (d, 6.5)	
	1.49 (m)				1.22 (m)		
11	1.50 (m)	22			0.84 (t, 7.5)		
12	0.68 (s)	OMe					3.21 (m)

[a] Multiplicities and coupling constants are in parentheses. [b] Spectrum was measured at 600 MHz. [c] Spectra were measured at 500 MHz.

Table 2. ^{13}C NMR Data of Compounds **1**−**3** and **5** (CD_3OD)

position	**1**[a]−**5**	position	**1**[a]	**2**[b]	**3**[b]	**5**[b]
1	173.6	13	41.5	41.5	41.5	41.5
2	112.6	14	21.4	21.4	21.4	21.4
3	172.9	15	138.6	137.5	138.9	138.2
4	104.1	16	128.3	133.2	132.2	129.1
5	36.2	17	43.1	43.1	53.2	43.1
6	36.5	18	29.7	29.7	29.7	29.7
7	49.0	19	22.7	22.0	21.5	22.4
8	51.7	20	22.7	21.5	20.0	22.6
9	22.3	21		20.0	27.2	
10	30.2	22			14.0	
11	56.7	OMe				51.1
12	12.1					

[a] Spectrum was measured at 50 MHz. [b] Signals were recorded by HMBC and HSQC experiments (500 MHz).

involve enzymatic degradation of sterols by symbiotic microbes, as reported a few decades ago, and the co-occurring 5α,8α-epidioxy sterols (**11**−**14**)[13] might serve as biogenetic precursors (Figure 4).

The isolated compounds (**1**−**4** and **6**−**9**) were assayed for cytotoxicity against a panel of five human tumor cell lines and displayed significant cytotoxicity (Table 5). Previously, the synthetic 4-hydroxy-17*R*-methylincisterol (**4**) was reported as an in vitro inhibitor of mammalian DNA polymerase.[22] Demethylincisterol A_3 (4-hydroxy-17*R*-methylincisterol, **4**) isolated from the *Homaxinella* sp. showed a significant cytotoxic profile to the solid tumor cell lines tested. Compound **3** was most cytotoxic to all of the cancer cell lines tested.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. The UV spectrum was obtained using a Shimadzu mini 1240 UV−vis spectrophotometer. IR spectra were measured using a JASCO FT/IR-410 spectrometer. CD spectra were measured using a JASCO J-715 spectropolarimeter (sensitivity 50 mdeg, resolution 0.2 nm). ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Varian Unity Plus 300, Varian INOVA 500, and Bruker DMX 600 spectrometers. Chemical shifts were reported with reference to the respective residual solvent or deutreated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A. HR-FABMS data were obtained on JEOL JMS SX-101A. FAB-CID tandem mass data were obtained using a JEOL JMS HX110/110A. HPLC was performed with an YMC packed ODS column (250 × 10 mm, 5 μm, 120 Å) and a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) using a Gilson 133-RI detector.

Table 3. ^{1}H NMR Data of Compounds **6**−**9** (CD_3OD, 500 MHz)[a]

position	**6**−**9**	position	**6**	**7**	position	**8**	**9**
	1.50 (m)	20	2.0 (m)	2.0 (m)	20	2.0 (m)	2.0 (m)
2	1.75 (m)	21	1.03 (d, 6.0)	1.02 (d, 7.0)	21	1.03 (d, 7.0)	1.05 (d, 7.0)
	1.42 (m)	22	5.22 (dd, 15.0, 8.5)	5.21 (m)	22	5.21 (m)	5.19 (dd, 15.0, 8.5)
3	3.95 (m)	23	5.31 (dd, 15.0, 8.5)	5.21 (m)	23	5.21 (m)	5.06 (dd, 15.0, 8.5)
4	2.14 (dd, 13.0, 12.0)	24	1.54 (m)	1.55 (m)	24	1.55 (m)	1.55 (m)
	1.66 (dd, 5.0, 4.5)	25	1.60 (m)	1.60 (m)	25	1.60 (m)	1.60 (m)
6	3.22 (m)	26	0.87 (d, 7.0)[b]	0.84 (d, 7.0)	26	0.84 (d, 7.0)	0.81 (d, 7.0)
7	5.36 (bd, 5.0)	27	0.87 (d, 7.0)[b]	0.86 (d, 7.0)	27	0.86 (d, 6.5)	0.86 (d, 7.0)
9	2.06 (m)	28		0.93 (d, 7.0)	28	0.93 (d, 7.0)	1.45 (m)
11	1.55 (m)						1.22 (m)
12	2.05 (m)				29		0.84 (t, 7.5)
14	1.94 (m)						
15	1.54 (m)						
16	1.28 (m)						
17	1.33 (m)						
18	0.62 (s)						
19	1.0 (s)						
1′	3.61 (dd, 9.0, 6.5)						
	3.39 (dd, 9.0, 6.50						
2′	1.5 (m)						
3′	1.4 (m)						
4′	0.91 (t, 7.5)						

[a] Multiplicities and coupling constants are in parentheses. [b] Assignments with the same superscript in the same column may be interchanged.

Table 4. ^{13}C NMR Data of Compounds **6**−**9** (CD_3OD)

position	**6**[a]−**9**	position	**6**[a]	**7**[b]	position	**8**[a]	**9**[d]
1	34.0	20	43.0	43.0	20	43.1	43.0
2	32.1	21	20.9	21.6	21	21.7	22.8
3	68.1	22	138.9	137.0	22	137.3	139.6
4	41.5	23	126.0	132.9	23	133.0	131.2
5	77.6	24	44.3	43.8	24	44.3	53.5
6	83.0	25	34.3	33.4	25	33.6	34.2
7	118.0	26	20.0[c]	19.6	26	20.1	21.5
8	144.0	27	20.0[c]	20.5	27	20.6	20.0
9	45.5	28		18.0	28	18.1	27.2
10	38.9				29		14.0
11	24.1						
12	41.1						
13	45.1						
14	56.8						
15	24.2						
16	31.2						
17	58.0						
18	13.3						
19	19.4						
1′	71.3						
2′	34.0						
3′	21.0						
4′	14.0						

[a] Spectra were measured at 50 MHz. [b] Spectrum was measured at 75 MHz. [c] Assignments with the same superscript in the same column may be interchanged. [d] Signals were assigned by HMBC and HSQC assignments.

Table 5. Cytotoxicity Data of Compounds **1**−**4** and **6**−**9**[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	2.79	1.70	1.94	1.08	1.42
2	1.51	1.49	1.21	0.93	1.28
3	0.92	0.79	0.55	0.68	0.65
4	1.62	1.97	1.30	1.14	1.39
6	8.54	7.57	6.88	6.33	7.62
7	6.23	4.43	3.69	4.17	4.40
8	8.42	7.85	6.10	5.03	7.10
9	>30.0	>30.0	>30.0	34.1	>30.0
doxorubicin	0.11	0.09	0.04	0.07	0.17

[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; HCT 15, human colon cancer.

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.[23]

Extraction and Isolation Procedure. 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. Aqueous MeOH fraction was subjected to step gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 400/500 mesh) with a solvent system of 60−100% MeOH/H_2O 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 step gradient solvent system of 60−100% MeOH/H_2O to afford 10 fractions. Compound **1** (1.9 mg) was obtained by separation of subfraction 5 (52.2 mg) on a reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 84% MeOH. The subfraction 6 of fraction 11 was subjected to reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 90% MeOH followed by another reversed-phase HPLC (YMC-Pack ODS, 250 × 10 mm, 5 μm, 120 Å) eluting with 81% MeOH to afford compounds **2** (0.8 mg), **3** (1.8 mg), and **4** (2.0 mg). Fraction 12 (1.2 g), one of the bioactive fractions (LD_{50} 27 μg/mL), was again subjected to reversed-phase flash column chromatography (YMC ODS-A, 120 Å, 30/50 μm) eluting with a step gradient solvent system of 65−100% MeOH/H_2O to afford 10 fractions. Compounds **4** (1.9 mg), **5** (1.0 mg), and **10** (4.5 mg) were obtained by separation of subfraction 4 (52.2 mg) on a reversed-phase HPLC (C18-5E Shodex packed, 250 × 10 mm, 5 μm, 100 Å) eluting with 84% MeOH. Fraction 13 was subjected to successive reversed-phase HPLC (C18-5E Shodex packed, 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 **6** (2.0 mg), **7** (4.0 mg), **8** (4.0 mg), **9** (2.5 mg), **11** (2.0 mg), **12** (14.7 mg), **13** (76.2 mg), and **14** (120.0 mg).

Demethylincisterol A_1 (1): colorless oil; UV (MeOH) λ_{max} nm (log ϵ) 220 (3.98); CD (c 1 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) +0.39 (229); IR (film) ν_{max} (cm^{-1}) 1751, 3359; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 318 $[M + H]^+$, *m/z* 341 $[M + Na]^+$, *m/z* 363 $[M + 2Na - H]^+$, HRFABMS *m/z* 341.2142 (calcd for $C_{20}H_{30}O_3Na$, 341.2093).

Demethylincisterol A_2 (2): colorless oil; $[\alpha]^{21}_D$ +28° (c 0.08, MeOH); CD (c 1 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) +0.22 (235); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 333 $[M + H]^+$, *m/z* 355 $[M + Na]^+$, *m/z* 377 $[M + 2Na - H]^+$, HRFABMS *m/z* 355.2292 (calcd for $C_{21}H_{32}O_3Na$, 355.2249), *m/z* 377.2060 (calcd for $C_{21}H_{31}O_3Na_2$, 377.2069).

Demethylincisterol A_4 (3): colorless oil; CD (c 1 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) +0.18 (235); ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; FABMS *m/z* 347 $[M + H]^+$, *m/z* 369 $[M + Na]^+$, *m/z* 391 $[M + 2Na - H]^+$; HRFABMS *m/z* 369.2340 (calcd for $C_{22}H_{34}O_3Na$, 369.2406), *m/z* 391.2231 (calcd for $C_{22}H_{33}O_3Na_2$, 391.2225).

Demethylincisterol A_3 (4): colorless oil; $[\alpha]^{21}_D$ +74° (c 0.1, MeOH); IR 1737 cm^{-1}; CD (c 1 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) +0.17 (234); ^1H NMR data, see Table 1; FABMS *m/z* 333 $[M + H]^+$, *m/z* 355 $[M + Na]^+$, *m/z* 377 $[M + 2Na - H]^+$; HRFABMS *m/z* 377.2036 (calcd for $C_{21}H_{31}O_3Na_2$, 377.2069).

Homaxisterol A_1 (6): light yellow oil; IR (film) ν_{max} (cm^{-1}) 3350; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS *m/z* 495 $[M + Na]^+$; HRFABMS *m/z* 495.3800 (calcd for $C_{31}H_{52}O_3Na$, 495.3814).

Homaxisterol A_2 (7): light yellow oil; $[\alpha]^{21}_D$ −9° (c 0.44, MeOH); ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS *m/z* 509 $[M + Na]^+$; HRFABMS *m/z* 509.3957 (calcd for $C_{32}H_{54}O_3Na$, 509.3971).

Homaxisterol A_3 (8): light yellow oil; $[\alpha]^{21}_D$ −3° (c 0.12, MeOH); ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS *m/z* 509 $[M + Na]^+$; HRFABMS *m/z* 509.3982 (calcd for $C_{32}H_{54}O_3Na$, 509.3971).

Homaxisterol A_4 (9): light yellow oil; ^1H NMR data, see Table 3; ^{13}C NMR data, see Table 4; FABMS *m/z* 523 $[M + Na]^+$; HRFABMS *m/z* 523.4143 (calcd for $C_{33}H_{56}O_3Na$, 523.4127).

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