

Polyoxygenated Steroids from a Formosan Soft Coral *Sinularia facile*

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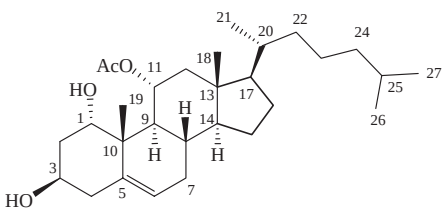
Five new polyoxygenated steroids **1**–**5** have been isolated from a Formosan soft coral *Sinularia facile*. The structures of new metabolites were elucidated on the basis of extensive spectroscopic analysis and cytotoxic activity of **1**–**5** against the proliferation of a limited panel of cancer cell lines was measured. Metabolite **4** has been shown to exhibit weak cytotoxicity against Hep G2, Hep G3B, MDA-MB-231, and Ca9-22 cancer cell lines.

Previous chemical investigations of the Formosan soft corals of the genus *Sinularia* have afforded several polyoxygenated steroids.[1] In a previous study, two cembranes have been isolated from the soft coral *Sinularia facile* (Durivault).[2] Our current chemical investigation on *S. facile* has also led to the isolation of five new polyoxygenated sterols **1**–**5** (Chart 1) from its EtOH extract. The structures of the new metabolites were determined on the basis of extensive spectroscopic analysis, including 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY) spectroscopy. Cytotoxicity of metabolites **1**–**5** against a limited panel of human tumor cell lines including liver (Hep G2 and Hep G3B), breast (MDA-MB-23), and gingival (Ca9-22) carcinoma cells are also reported.

The sliced bodies of the soft coral *S. facile* were extracted exhaustively with EtOH, and then the concentrated EtOH extract was partitioned between EtOAc and H_2O. The combined EtOAc-soluble fraction was concentrated under reduced pressure and the residue was repeatedly chromatographed to yield metabolites **1**–**5**.

Compound **1** was isolated as white power. Its molecular formula, $C_{29}H_{48}O_4$, was established by HR-ESI-MS (m/z 483.3448 $[M + Na]^+$) and ^{13}C NMR data, implying six degrees of unsaturation. IR absorptions were observed at 3422 and 1731 cm^{-1}, suggesting the presence of hydroxy and carbonyl groups. The structure of this compound was deduced from its ^{13}C NMR and DEPT spectra, which showed that the compound has 29 carbons, including six methyls, nine sp^3 methylenes, one sp^2 methine, nine sp^3 methines (including three oxymethines), and two sp^2 and two sp^3 quaternary carbons. From ^{1}H and ^{13}C NMR spectra (Tables 1 and 2), **1** was found to possess one acetoxy group [δ_H 2.05, s; δ_C 169.8 (C), 21.8 (CH_3)], in addition to one trisubstituted olefin [δ_H 5.62, (br d, $J = 5.5$ Hz), δ_C 137.7 (C), 125.1 (CH)]. Detailed analysis of the ^{1}H–^{1}H COSY and HMBC correlations (Figure 1) further established the planar structure of **1** as a cholesterol derivative bearing two hydroxy groups at C-1 and C-3, one acetoxy group at C-11, and one 5,6-trisubstituted double bond. In the NOESY spectrum of **1** (Figure 2), the



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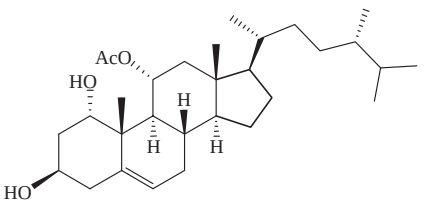

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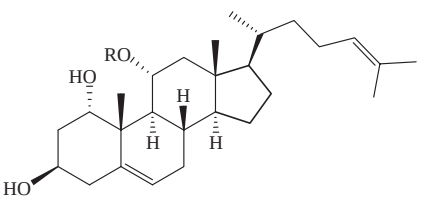

3 : R = Ac
4 : R = H

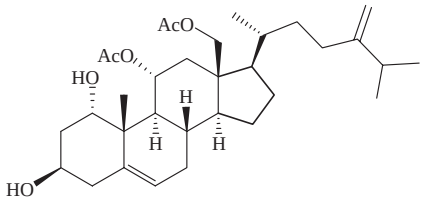

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Chart 1.

Published on the web October 10, 2008; doi:10.1246/bcsj.81.1304

Table 1. ^{1}H NMR Data for Sterols **1**–**5**

No.	**1**[a)]	**2**[b)]	**3**[a)]	**4**[b)]	**5**[a)]
1	3.70 t (3.0)[c)]	3.70 br s	3.70 br s	4.21 br s	3.74 br s
2	α: 2.10 m; β: 1.72 m	α: 2.12 m; β: 1.72 m	α: 2.10 m; β: 1.72 m	α: 2.14 m; β: 1.72 m	α: 2.10 m; β: 1.72 m
3	3.96 tt (11.5, 5.0)	3.97 m	3.95 tt (11.5, 5.0)	3.98 m	3.96 tt (11.5, 5.0)
4	α: 2.40 m; β: 2.26 m	α: 2.40 m; β: 2.33 m	α: 2.40 m; β: 2.26 m	α: 2.38 m; β: 2.27 m	α: 2.38 m; β: 2.26 m
6	5.62 br d (5.5)	5.62 br d (5.3)	5.62 br d (5.5)	5.56 br d (5.5)	5.61 br d (5.5)
7	2.00 m; 1.67 m	1.99 m; 1.68 m	2.01 m; 1.67 m	2.02 m; 1.67 m	2.03 m; 1.70 m
8	1.54 m	1.53 m	1.55 m	1.53 m	1.69 m
9	1.87 m	1.87 m	1.88 m	1.65 m	1.96 m
11	5.29 dt (5.5, 11.0)	5.30 dt (5.4, 10.8)	5.29 dt (5.5, 11.0)	4.08 m	5.16 dt (5.0, 11.0)
12	α: 1.18 m;	α: 1.18 m;	α: 1.18 m;	α: 1.25 m;	α: 1.16 m;
	β: 2.38 dd (12.5, 5.0)	β: 2.39 dd (12.0, 5.1)	β: 2.38 dd (11.5, 5.0)	β: 2.34 m	β: 2.71 dd (12.5, 5.0)
14	1.16 m	1.17 m	1.16 m	1.15 m	1.38 m
15	1.62 m; 1.07 m	1.63 m; 1.08 m	1.62 m; 1.07 m	1.68 m; 1.07 m	1.69 m; 1.07 m
16	1.88 m; 1.31 m	1.89 m; 1.32 m	1.90 m; 1.30 m	1.88 m; 1.32 m	2.01 m; 1.40 m
17	1.16 m	1.17 m	1.16 m	1.15 m	1.34 m
18	0.74 s	0.74 s	0.74 s	0.67 s	4.18 d (12.0); 3.84 d (12.0)
19	1.12 s	1.12 s	1.12 s	1.12 s	1.11 s
20	1.35 m	1.33 m	1.37 m	1.37 m	1.47 m
21	0.90 d (6.5)	0.90 d (6.4)	0.92 d (6.5)	0.95 d (6.4)	1.03 d (6.5)
22	1.30 m; 0.97 m	1.38 m; 0.94 m	1.38 m; 1.02 m	1.37 m; 1.03 m	1.50 m; 1.17 m
23	1.32 m; 1.12 m	1.35 m; 0.96 m	2.00 m; 1.38 m	2.03 m; 1.38 m	2.08 m; 1.88 m
24	1.11 m	1.18 m	5.07 t (7.0)	5.08 t (7.0)	
25	1.51 m	1.58 m			2.22 m
26	0.87 d (6.0)	0.85 d (6.8)	1.68 s	1.68 s	1.02 d (6.5)
27	0.86 d (6.0)	0.78 d (6.7)	1.60 s	1.60 s	1.01 d (6.5)
28		0.77 d (6.7)			4.72 s; 4.65 s
11-OAc	2.05 s	2.05 s	2.05 s		2.05 s
18-OAc					2.14 s

a) Spectra recorded at 500 MHz in $CDCl_3$. b) 300 MHz in $CDCl_3$. c) J values (in Hz) parentheses.

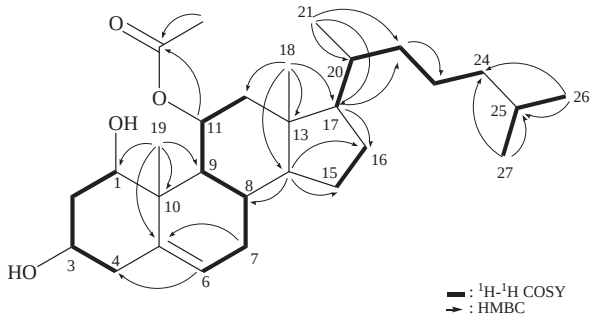

Figure 1. Selective ^{1}H–^{1}H COSY and HMBC correlations of **1**.

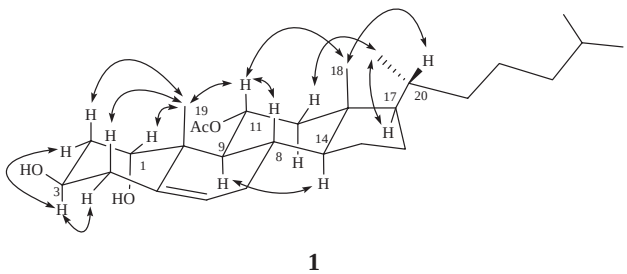

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Figure 2. Selective NOESY correlations of **1**.

NOE correlations between H-11 and H-8, H_3-18, and H_3-19; H_3-19 and H-1, H-2β (δ 1.72), and H-4β (δ 2.26) as well as between H_3-18 and H-20 indicated that these protons adapt a β-orientation.[3] This was further supported by comparison of these NOE correlations with those of the corresponding NOE interactions displayed between the protons of a known compound 24-methylenecholest-5-ene-1α,3β,11α-triol 11α-acetate.[4] The above data fully established the structure of compound **1** as cholest-5-ene-1α,3β,11α-triol 11-acetate (**1**).

The HR-ESI-MS of compound **2** showed the pseudomolecular ion at m/z 497.3605 ([M + Na]$^+$), which indicated the molecular formula $C_{30}H_{50}O_4$. Thus, six degrees of unsaturation were determined for the molecule **2**. It was shown that the NMR spectral data of **2** (Tables 1 and 2) is almost identical with those of **1**, except that the ^{1}H and ^{13}C NMR spectra of **2** exhibited an additional methyl group [δ_H 0.77 (3H, d, J = 6.7 Hz, H-28), δ_C 15.5 (CH_3, C-28)]. The ^{1}H–^{1}H COSY correlations between H_2-23 and H-24; H-24 and H_3-28 and HMBC correlations from both H_3-26 and H_3-27 to C-24 and C-25 confirmed that this methyl group should be positioned at C-24. The stereochemistry of compound **2** was established by comparing the very similar NOESY correlations to those of **1**. Furthermore, the 24S configuration of **2** was determined by comparison of NMR data with those of yonarasterol B which was isolated from the soft coral *Clavularia viridis*.[5] The proton shift of H_3-28, δ_H 0.77, was found to be identical with that of yonarasterol B. Also, the carbon shifts of C24–C28 are in excellent agreement with those of yonarasterol B and (24*S*)-24-methylcholestanol (vs. those of (24*R*)-24-methylcholestanol).[6] The structure of compound **2** was thus established as (24*S*)-24-methylcholest-5-ene-1α,3β,11α-triol 11-acetate.

Table 2. ^{13}C NMR Data for Sterols **1–5**

No.	**1**[a]		**2**[b]		**3**[a]		**4**[b]		**5**[a]	
1	74.4	(CH)[c]	74.5	(CH)	74.4	(CH)	75.0	(CH)	74.4	(CH)
2	37.9	(CH_2)	38.0	(CH_2)	37.9	(CH_2)	38.3	(CH_2)	38.0	(CH_2)
3	66.2	(CH)	66.3	(CH)	66.2	(CH)	66.5	(CH)	66.2	(CH)
4	42.0	(CH_2)	42.7	(CH_2)	42.0	(CH_2)	42.2	(CH_2)	42.1	(CH_2)
5	137.7	(C)	137.7	(C)	137.7	(C)	138.7	(C)	137.8	(C)
6	125.1	(CH)	125.1	(CH)	125.1	(CH)	124.8	(CH)	125.1	(CH)
7	32.1	(CH_2)	32.2	(CH_2)	32.1	(CH_2)	32.5	(CH_2)	32.2	(CH_2)
8	31.9	(CH)	32.0	(CH)	31.9	(CH)	31.9	(CH)	32.1	(CH)
9	45.3	(CH)	45.3	(CH)	45.3	(CH)	45.3	(CH)	45.2	(CH)
10	43.0	(C)	43.1	(C)	43.0	(C)	43.1	(C)	43.0	(C)
11	72.3	(CH)	72.3	(CH)	72.3	(CH)	68.3	(CH)	71.7	(CH)
12	45.6	(CH_2)	45.6	(CH_2)	45.5	(CH_2)	50.8	(CH_2)	41.1	(CH_2)
13	42.6	(C)	42.9	(C)	42.7	(C)	42.7	(C)	45.8	(C)
14	55.4	(CH)	55.4	(CH)	55.3	(CH)	55.6	(CH)	54.9	(CH)
15	24.2	(CH_2)	24.3	(CH_2)	24.2	(CH_2)	24.3	(CH_2)	24.0	(CH_2)
16	28.3	(CH_2)	28.3	(CH_2)	28.3	(CH_2)	28.4	(CH_2)	28.0	(CH_2)
17	55.8	(CH)	55.8	(CH)	55.8	(CH)	55.8	(CH)	56.0	(CH)
18	12.4	(CH_3)	12.5	(CH_3)	12.4	(CH_3)	12.6	(CH_3)	62.2	(CH_2)
19	19.1	(CH_3)	19.2	(CH_3)	19.1	(CH_3)	19.3	(CH_3)	19.1	(CH_3)
20	35.5	(CH)	36.0	(CH)	35.3	(CH)	35.5	(CH)	35.5	(CH)
21	18.7	(CH_3)	18.9	(CH_3)	18.6	(CH_3)	18.7	(CH_3)	19.0	(CH_3)
22	36.0	(CH_2)	33.6	(CH_2)	35.9	(CH_2)	36.0	(CH_2)	34.4	(CH_2)
23	23.7	(CH_2)	30.6	(CH_2)	24.6	(CH_2)	24.7	(CH_2)	30.6	(CH_2)
24	39.4	(CH_2)	39.1	(CH)	125.0	(CH)	125.1	(CH)	156.5	(C)
25	28.0	(CH)	31.6	(CH)	131.1	(C)	131.1	(C)	33.8	(CH)
26	22.5	(CH_3)	20.5	(CH_3)	25.7	(CH_3)	25.8	(CH_3)	22.0	(CH_3)
27	22.8	(CH_3)	17.7	(CH_3)	17.6	(CH_3)	17.7	(CH_3)	21.8	(CH_3)
28			15.5	(CH_3)					106.1	(CH_2)
11-OAc	21.8	(CH_3)	21.9	(CH_3)	21.8	(CH_3)			21.8	(CH_3)
	169.8	(C)	169.9	(C)	169.8	(C)			169.5	(C)
18-OAc									21.0	(CH_3)
									171.4	(C)

a) Spectra recorded at 125 MHz in $CDCl_3$. b) 75 MHz in $CDCl_3$. c) Attached protons were deduced by DEPT experiments.

Compound **3** has the molecular formula $C_{29}H_{46}O_4$, as determined by HR-ESI-MS and NMR spectral data. Both the ^{1}H and ^{13}C NMR signals of **3** were found to be very closely related to those of compound **1**, suggesting a very similar steroid skeleton. The only difference observed is that the side chain methyls of the isopropyl group (δ 0.87, d, $J = 6.0$ Hz, H_3-26; δ 0.86, d, $J = 6.0$ Hz, H_3-27) of **1** were replaced by two vinyl methyls (δ 1.60 and 1.68, each 3H, s) of **3**. The above observation and the signal of an additional olefinic proton (δ 5.07, t, $J = 7.0$ Hz) showed the presence of a 24,25-trisubstituted double bond in **3**. These results revealed that the structure of **3** should be established as cholesta-5,24-diene-1α,3β,11α-triol 11-acetate. A structurally similar metabolite **4**, was further isolated as a white solid. It's molecular formula, $C_{27}H_{44}O_3$ was established by HR-ESI-MS. The ^{1}H and ^{13}C NMR of **4** was very similar to that of **3** except that an acetyl group in **3** was lost and the chemical shift of H-11 of **4** was shifted to upper field by 1.21 ppm, in comparison with that of **3**. It was thus suggested that **4** is the 11-deacetyl derivative of **3**.

The molecular formula of metabolite **5** was assigned as $C_{32}H_{50}O_6$ from the HR-ESI-MS and NMR data (Tables 1 and 2). The ^{1}H and ^{13}C NMR spectral data of A–D rings in **5** were nearly identical with those of **1** except for the replacement of a methyl substitution at C-13 in **1** by an acetoxymethyl group [δ_H 4.18 (d, $J = 12.0$ Hz), 3.84 (d, $J = 12.0$ Hz); δ_C 62.2 (CH_2)] in **5**. This was further confirmed by the HMBC correlations from both H_2-18 and the acetoxyl methyl to the ester carbonyl carbon appeared at 171.4 (C). Furthermore, the structure of side chain (C-20 to C-28) was fully established by the ^{1}H–^{1}H COSY correlations from H-20 to H_3-21; H_2-22 to H_2-23; H-25 to H_3-26 and H_3-27, and HMBC correlations from H_3-21 to C-17, C-20, C-22; H_2-23 to C-24; H_3-26 and H_3-27 to C-24, C-25; and H_2-28 to C-23, C-25 and by comparing the NMR data to those of known compounds.[7] Thus, the structure of steroid **5** was established as 24-methylenecholest-5-ene-1α,3β,11α,18-tetraol 11,18-diacetate.

The cytotoxicity of compounds **1–5** against the proliferation of a limited panel of cancer cell lines, including human liver (Hep G2 and Hep G3B), breast (MDA-MB-23) and gingival (Ca9-22) carcinoma cells, was evaluated. The results showed that compound **4**, the more potent one of compounds **1–5**, exhibited cytotoxicity towards Hep G2, Hep G3B, MDA-MB-23, and Ca9-22 cancer cell lines with IC_{50}'s 12.8, 12.0, 9.6, and 10.8 μg mL^{-1}, respectively. Metabolites **1** and **5** also were

Table 3. Cytotoxicity Data of Compounds **1–5**

Compound	Cell lines $IC_{50}/\mu g\,mL^{-1}$			
	Hep G2	Hep G3B	MDA-MB-231	Ca9-22
1	—[a)]	16.7	17.3	18.6
2	—	—	—	—
3	—	—	—	—
4	12.8	12.0	9.6	10.8
5	17.9	16.4	—	19.5
Doxorubicin	1.6	0.2	0.2	0.1

a) $IC_{50} > 20\,\mu g\,mL^{-1}$.

found to show weak cytotoxicity toward some of the above four cancer cells (Table 3).

Experimental

General Experimental Procedures. Melting points were determined using a Fisher–Johns melting point apparatus. IR spectra were recorded on a Jasco FT/IR-4100 infrared spectrophotometer. Optical rotations were measured on a Jasco P-1020 polarimeter. NMR spectra were recorded on a Bruker AMX-300 FT-NMR at 300 MHz for ^1H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^1H and 125 MHz for ^{13}C, in $CDCl_3$. LRMS and HRMS were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel 60 (Merck, 230–400 mesh) was used for column chromatography. Precoated Silica gel plates (Merck Kieselgel 60 F_{254} 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT_{VP} apparatus equipped with a Shimadzu SPD-10A_{VP} UV detector. The columns used in HPLC separation are YMC-Pack Pro C18 (reverse-phase column, 250 × 10 mm, 5 μm) and Varian Dynamax, Si-60 (normal-phase column, 250 × 21.4 mm, 100 Å, 5 μm).

Animal Material. The soft coral *S. facile* was collected by hand using SCUBA off the coast of Pingtung County, located in southern Taiwan, in July 2001, at depths of 2–5 m and stored in a freezer until extraction. A voucher sample (20010719-1) was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. The frozen bodies of *S. facile* (1.05 kg, wet wt) were sliced and exhaustively extracted with EtOH (1 L × 4). The combined organic layer was filtered and concentrated by a rotorary evaporator, and the residue of the resulting aqueous suspension was partitioned between EtOAc and H_2O. The EtOAc extract was dried with anhydrous Na_2SO_4. After removal of solvent in vacuo, the residue (15 g) was subjected to column chromatography on Si gel and eluted with EtOAc in *n*-hexane (0–100% of EtOAc, gradient) to yield 26 fractions. Fraction 23, eluted with EtOAc–MeOH (3:1), was rechromatographed over a Sephadex LH-20 column, using acetone as the mobile phase to afford five subfractions (F1–F5). Subfraction F2 was separated by reverse-phase HPLC (CH_3CN–H_2O, 6:1 to 9:1) to afford compounds **1** (3.0 mg), **2** (5.0 mg), **3** (2.2 mg), **4** (4.1 mg), and **5** (2.3 mg), respectively.

Cholest-5-ene-1α,3β,11α-triol 11-Acetate (1). White powder; mp 128–130 °C; $[\alpha]_D^{25} = -23$ (*c* 0.3, $CHCl_3$); IR (neat) ν_{max} 3422, 1731 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; ESIMS *m/z* 483 $(M + Na)^+$; HRESIMS *m/z* 483.3448 (calcd for $C_{29}H_{48}O_4Na$, 483.3450).

24(*S*)-24-Methylcholest-5-ene-1α,3β,11α-triol 11-Acetate (2). White powder; mp 130–133 °C; $[\alpha]_D^{25} = -58$ (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3437, 1733 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2; ESIMS *m/z* 497 $(M + Na)^+$; HRESIMS *m/z* 497.3605 (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

Cholesta-5,24-diene-1α,3β,11α-triol 11-Acetate (3). White powder; mp 147–150 °C; $[\alpha]_D^{25} = -20$ (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3422, 1728 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; ESIMS *m/z* 481 $(M + Na)^+$; HRESIMS *m/z* 481.3292 (calcd for $C_{29}H_{46}O_4Na$, 481.3294).

Cholesta-5,24-diene-1α,3β-11α-triol (4). White powder; mp 120–123 °C; $[\alpha]_D^{25} = -60$ (*c* 0.4, $CHCl_3$); IR (neat) ν_{max} 3370 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz), see Tables 1 and 2; ESIMS *m/z* 439 $(M + Na)^+$; HRESIMS *m/z* 439.3185 (calcd for $C_{27}H_{44}O_3Na$, 439.3188).

24-Methylenecholest-5-ene-1α,3β,11α,18-tetraol 11,18-Diacetate (5). White powder; mp 137–140 °C; $[\alpha]_D^{25} = -42$ (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3411, 1738 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; ESIMS *m/z* 553 $(M + Na)^+$; HRESIMS *m/z* 553.3502 (calcd for $C_{32}H_{50}O_6Na$, 553.3505).

Cytotoxicity Testing. Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds **1–5** were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[8,9]

Financial support was provided by the Ministry of Education (No. 96C031702) and the National Science Council of Taiwan (NSC 95-2113-M-110-011-MY3) awarded to J.-H. Sheu.

References

1 a) A. F. Ahmed, C.-F. Dai, Y.-H. Kuo, J.-H. Sheu, *Steroids* **2003**, *68*, 377. b) A. F. Ahmed, Y.-T. Hsieh, Z.-H. Wen, Y.-C. Wu, J.-H. Sheu, *J. Nat. Prod.* **2006**, *69*, 1275. c) J.-H. Su, Y.-J. Tseng, H.-H. Huang, A. F. Ahmed, C.-K. Lu, Y.-C. Wu, J.-H. Sheu, *J. Nat. Prod.* **2006**, *69*, 850. d) A. F. Ahmed, S.-H. Tai, Y.-C. Wu, J.-H. Sheu, *Steroids* **2007**, *72*, 368. e) J.-H. Sheu, K.-C. Chang, C.-Y. Duh, *J. Nat. Prod.* **2000**, *63*, 149.

2 B. F. Bowden, J. C. Coll, S. J. Mitchell, R. Kazlauskas, *Aust. J. Chem.* **1981**, *34*, 1551.

3 W. Zhang, Y.-W. Guo, E. Mollo, A. Fontana, G. Cimino, *J. Nat. Prod.* **2004**, *67*, 2083.

4 B. M. Jagodzinska, J. S. Trimmer, W. Fenical, C. Djerassi, *J. Org. Chem.* **1985**, *50*, 1435.

5 M. Iwashima, K. Nara, K. Iguchi, *Steroids* **2000**, *65*, 130.

6 N. Koizumi, Y. Fujimoto, T. Takeshita, N. Ikekawa, *Chem. Pharm. Bull.* **1979**, *27*, 38.

7 B. M. Jagodzinska, J. S. Trimmer, W. Fenical, C. Djerassi, *J. Org. Chem.* **1985**, *50*, 2988.

8 M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1988**, *48*, 589.

9 D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827.