Sesquiterpenoids and Artificial 19-Oxygenated Steroids from the Formosan Soft Coral *Nephthea erecta*

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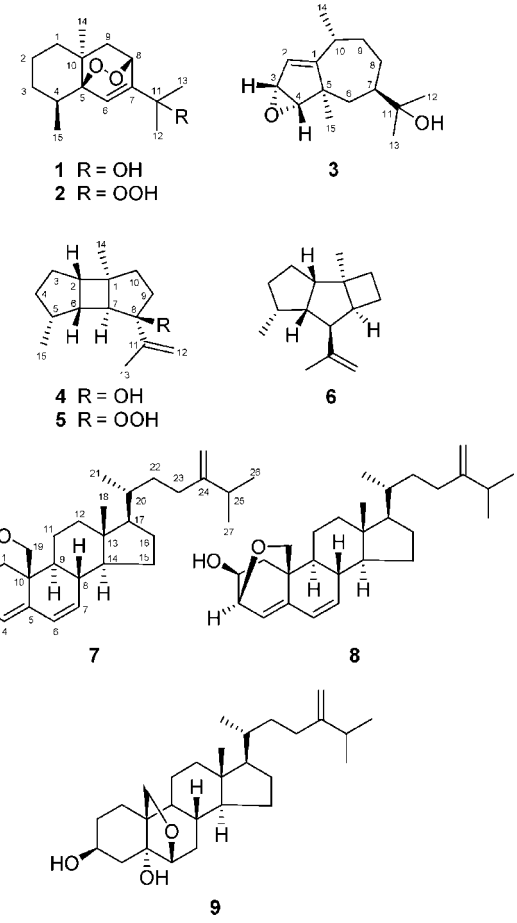
Chemical investigations on the acetone and MeOH solubles of the soft coral *Nephthea erecta* have afforded five new sesquiterpenoids (**1**–**5**), one known sesquiterpene, kelsoene (**6**), and two known 19-oxygenated steroids (**10** and **11**). In addition, three unexpected artificial 19-oxygenated steroids (**7**–**9**) were obtained by letting **10** and **11** stand in $CDCl_3$ for prolonged periods of time. The structures of **1**–**9** were elucidated by extensive spectroscopic analyses, and their cytotoxicity against selected cancer cells was measured *in vitro*.

The family Nephtheidae has been proved to be a rich source of bioactive compounds.[1–9] The ongoing search for bioactive constituents prompted us to reinvestigate the secondary metabolites of the soft coral *Nephthea erecta* Kükenthal (Nephtheidae).[9] Compounds **1**–**6**,[10,11,14–17] **10**,[12] and **11**[13] were isolated from the soft coral *N. erecta* while **7**–**9** are artifacts obtained by allowing **10** and **11** to stand in $CDCl_3$ for prolonged periods of time. Compound **7** was obtained from **10** by letting the latter stand in $CDCl_3$ overnight. Compound **7** was subsequently converted to **8** after 7 days. In the same conditions, **11** was transformed into **9** in $CDCl_3$ after a week through epoxylation (Scheme 1). However, no reactions occurred when **10** and **11** were treated in pyridine-d_5 for 2 months, implying **10** and **11** were stable under slightly basic solvent. In this article, we report the structure elucidation and the cytotoxicity of these metabolites.



Results and Discussion

Compound **1** was isolated as a colorless, viscous oil. HRESIMS of **1** exhibited a $[M + Na]^+$ peak at *m*/*z* 275.1625 and established a molecular formula of $C_{15}H_{24}O_3$, implying four degrees of unsaturation. The ^1H NMR spectrum of **1** (Table 1) showed signals corresponding to an oxygenated methine proton [δ_H 4.77 (1H, ddd, J = 3.5, 2.0, 1.5 Hz)], an olefinic proton [δ_H 6.21 (1H, d, J = 1.5 Hz)], a secondary methyl [δ_H 1.00 (3H, d, J = 7.0 Hz)], and three tertiary methyls [δ_H 1.44 (3H, s); δ_H 1.47 (3H, s); δ_H 0.89 (3H, s)], respectively. The ^{13}C NMR displayed 15 carbon resonances, and the DEPT spectrum (Table 1) was consistent with the presence of a methine [δ_C 71.4 (CH)], a quaternary carbon [δ_C 81.5 (qC)] bearing a peroxide ring, a quaternary carbon [δ_C 70.7 (qC)] bearing a hydroxyl, and trisubstituted olefinic carbons [δ_C 124.2 (CH) and 149.5 (qC)], as well as four methyls, four methylenes, three methines, and four quaternary carbons. The above data of **1** were similar to those of 5α,8α-epidioxy-6-eudesmene,[14] except for the presence of a tertiary hydroxyl at C-11. This was supported by the HMBC spectrum, which shows correlations from H_3-12 and H_3-13 to C-11 (Figure 1). On the basis of the above evidence, the planar structure of **1** was unambiguously established. The computer-modeled structure of **1** was generated by CS Chem 3D version 9.0 using MM2 force field calculations for energy minimization (Figure 2). The results were consistent with the stereochemistry of **1** as established by the NOESY experiments. The NOESY correlations between H_3-14 and all protons of H-1α, H-4, H-8, and H-9α positioned all these protons on the same side of the molecule and revealed the β-orientation of H_3-15. Therefore, the structure of 5β,8β-epidioxy-11-hydroxy-6-eudesmene was characterized as **1**.

5β,8β-Epidioxy-11-hydroperoxy-6-eudesmene (**2**) was isolated as a colorless, viscous oil, and its molecular formula was determined to be $C_{15}H_{24}O_4$, as deduced from HRESIMS spectroscopic data. The ^1H NMR spectrum of **2** showed a signal at δ_H 7.72 (1H, br s) that suggested the presence of a hydroperoxy group, while the hydroperoxyl could be located at C-11, as a result of the HMBC correlations (Figure 1). The carbon signals of Me-12 and Me-13 of **1** were at a lower field when compared to **2**, and the signal of C-11 was shifted downfield ($\Delta\delta_C$ 11.1 ppm). The ^{13}C NMR

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Scheme 1. Suggested Pathway for Conversion of **7** to **8** and **11** to **9**

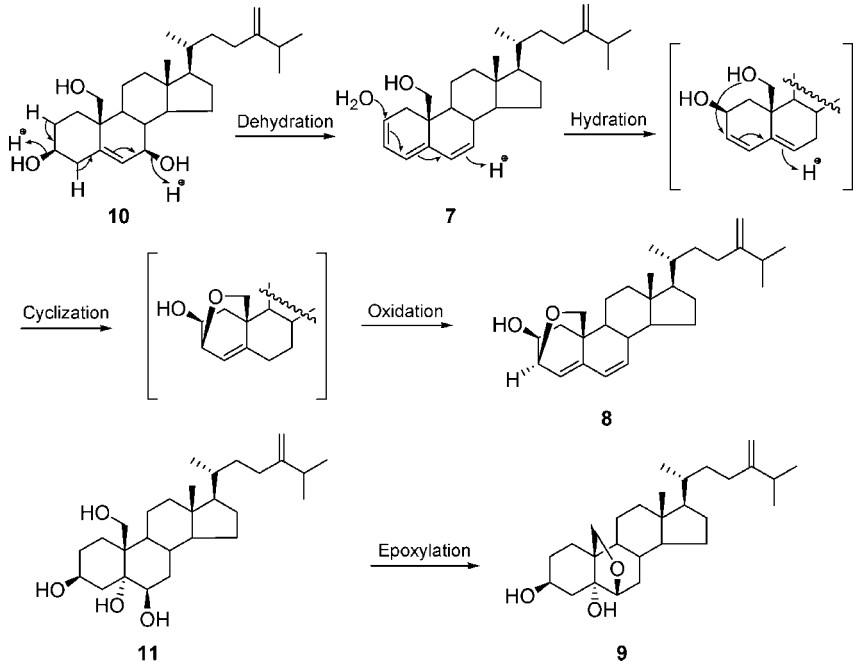

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **1**–**3**

	1[a]			**2**[a]			**3**[b]		
C/H	^{13}C		^{1}H	^{13}C		^{1}H	^{13}C		^{1}H
1	35.6 (CH_2)[c]	α	1.50 m	35.6 (CH_2)[c]	α	1.50 m	145.3 (qC)[c]		
		β	2.00 m		*β*	2.00 m			
2	21.0 (CH_2)		1.55 m	21.0 (CH_2)		1.55 m	116.3 (CH)		5.27 t (2.5)[d]
3	29.3 (CH_2)		1.48 m	29.7 (CH_2)		1.48 m	54.0 (CH)		3.46 dd (3.7, 2.5)
4	32.7 (CH)		2.07 m	32.7 (CH)		2.08 m	62.1 (CH)		2.77 d (3.7)
5	81.5 (qC)			81.6 (qC)			34.6 (qC)		
6	124.2 (CH)		6.21 d (1.5)[d]	128.9 (CH)		6.30 d (1.5)[d]	37.6 (CH_2)	α	1.62 m
								β	1.32 m
7	149.5 (qC)			145.7 (qC)			46.8 (CH)		2.54 m
8	71.4 (CH)		4.77 ddd (3.5, 2.0, 1.5)	71.0 (CH)		4.79 ddd (3.5, 2.0, 1.5)	17.6 (CH_2)	α	1.91 m
								β	1.52 m
9	41.6 (CH_2)	α	1.28 dd (13.5, 2.0)	41.0 (CH_2)	α	1.37 dd (13.5, 2.0)	34.5 (CH_2)	α	1.62 m
		β	1.95 dd (13.5, 3.5)		*β*	1.93 dd (13.5, 3.5)		*β*	1.50 m
10	35.0 (qC)			34.9 (qC)			40.3 (CH)		2.54 m
11	70.7 (qC)			81.8 (qC)			72.8 (qC)		
12	28.0 (CH_3)		1.44 s	22.7 (CH_3)		1.36 s	26.9 (CH_3)		1.24 s
13	28.1 (CH_3)		1.47 s	21.0 (CH_3)		1.49 s	28.6 (CH_3)		1.31 s
14	25.5 (CH_3)		0.89 s	25.4 (CH_3)		0.90 s	21.0 (CH_3)		1.16 d (7.6)
15	16.1 (CH_3)		1.00 d (7.0)	16.1 (CH_3)		1.00 d (7.0)	23.5 (CH_3)		1.31 s
OOH						7.72 br s			

[a] Spectra were measured in $CDCl_3$ (^{1}H, 300 MHz and ^{13}C, 75 MHz). [b] Spectra were measured in $CDCl_3$ (^{1}H, 500 MHz and ^{13}C, 125 MHz). [c] Multiplicities are deduced by HSQC and DEPT experiments. [d] *J* values (in Hz) are in parentheses.

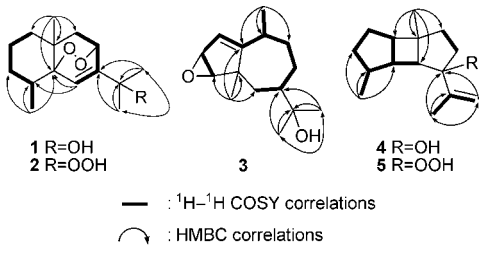

Figure 1. ^{1}H–^{1}H COSY and key HMBC correlation of **1**–**5**.

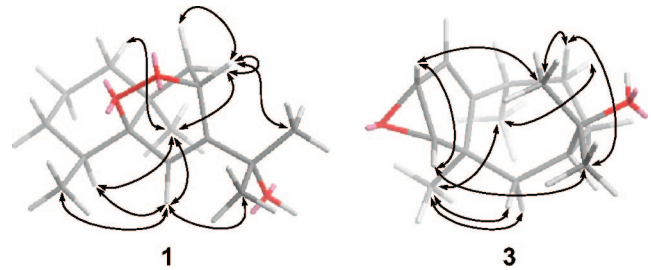

Figure 2. Selected NOSEY correlations of **1**–**3**.

spectroscopic data of **1** and **2** were in good accordance with those of compounds with a similar side chain with a hydroxyl or a hydroperoxyl.[16,17] Consequently, the structure of **2** was deduced unambiguously.

Compound **3** was obtained as a colorless, viscous oil, which analyzed for the molecular formula $C_{15}H_{24}O_2$ by HRESIMS coupled with the DEPT and ^{13}C NMR spectroscopic data (Table 1). A broad IR spectrum absorption at 3321 cm^{-1} indicated the presence of a hydroxy group. The ^{1}H and ^{13}C NMR spectra of **3** contained resonances for a trisubstituted double bond at C-1 and C-2 [δ_H 5.27 (ddd, J = 3.5, 2.0, 1.5 Hz, 1H); δ_C 145.3 (qC) and 116.3 (CH)]

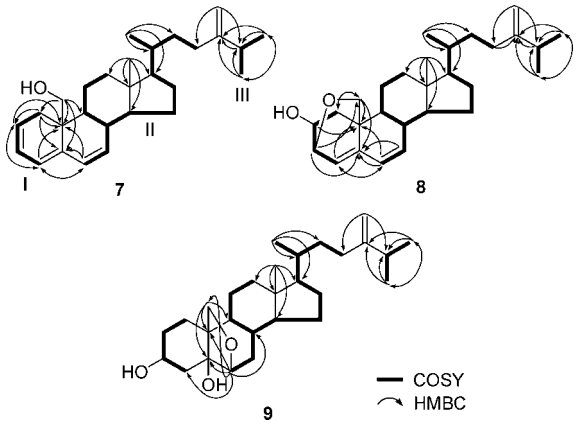

Figure 3. ^{1}H–^{1}H COSY and key HMBC correlations of **7**–**9**.

and a disubstituted epoxide [δ_H 3.46 (dd, J = 3.7, 2.5 Hz, 1H) and 2.77 (d, J = 3.7 Hz, 1H); δ_C 50.4 (CH) and 62.1 (CH)] at C-3 and C-4. From the above evidences, **3** was suggested to be a tricyclic sesquiterpenoid. From the COSY spectrum of **3** (Figure 1), it was possible to establish that the proton sequence connects from H-2 to H-4 and from H_2-6 to H_3-14. The ^{1}H–^{1}H COSY correlations further observed between H-2 and H-10 showed further the allylic coupling of the above protons. The connectivities between C-1/C-5, C-4/C-5, and C-5/C-6 were confirmed by the HMBC correlations of H_3-15 with C-1, C-4, C-5, and C-6. In addition, the HMBC correlations (Figure 1) from H_3-12 and H_3-13 to C-7 and C-11 proved the attachment of the isopropyl at C-7. On the basis of this evidence, the planar structure of **3**, possessing a pseudoguaiane[15] skeleton, was unambiguously established. The relative configuration of **3** was determined through inspection of the NOESY spectrum as well as a computer-generated lower energy conformation using MM2 force field calculations (Figure 2). From the NOESY spectrum of **3**, H-9β was found to show NOE correlations with both H_3-12 and H_3-13, and H-3 exhibited NOE correlations with H-4 and H_3-12, indicating the β-orientations of H-3 and H-4 and the β-orientation of the isopropanol group attached at C-11. Furthermore, NOE correlations could be observed between H-9α and H_3-14, H_3-14, and H_3-15. Therefore, H_3-14 and H_3-15 should be placed on the α-face. From the aforementioned observations, **3** was formulated as (3*R**,4*S**,5*R**,7*R**,10*R**)-3,4-epoxy-11-hydroxy-1-pseudoguaiene.

The molecular formula of **4** was assigned as $C_{15}H_{24}O$, as derived from its HRESIMS and in agreement with the NMR data. By comparison of the ^{13}C NMR spectroscopic data of **4** with those of the known sesquiterpene prespatane,[10,11] it was found that C-8 (δ_C 50.3 d) in prespatane was converted to a tertiary hydroxyl (δ_C 87.8 s) in **4**, as also confirmed by the HMBC correlations (H_3-13/C-8, C-11, and C-12). Thus, the structure of **4** was established and named 8β-hydroxyprespatane. From the NOESY spectrum of **4**, cross-peaks for the signals with H-2, H-6, and H-10β (δ 1.60) fixed the three rings in a stair or chair conformation, while the NOE interactions between H_3-15 and all protons of H-7, H_3-13, and H_3-14, which in turn showed correlation with H-10α, positioned the above protons on the same side of the molecule and revealed the β-orientation of the 8-OH.

HRESIMS of 8β-hydroperoxyprespatane (**5**), a colorless, viscous oil, established a molecular formula of $C_{15}H_{24}O_2$. By comparison of the NMR spectroscopic data of **5** with those of **4**, it was found that the ^{1}H and ^{13}C spectroscopic data of both compounds were nearly the same, except that the carbon shift of the tertiary hydroperoxyl at C-8 (δ_C 100.2, s) of **5** was shifted downfield relative to the signal of C-8 (δ_C 87.8, s) of **4**.[16,17] Thus, the structure of **5** was established unambiguously.

Compound **7** was obtained from **10** by letting the latter stand in $CDCl_3$ overnight. The ^{1}H and ^{13}C NMR spectroscopic data of **7** revealed the presence of a conjugated triene (δ_C 126.4 CH and 124.2 CH; δ_H 5.77 m and 5.92 m; δ_C 121.7 CH and 137.8 qC; δ_H 5.77 d, J = 5.5 Hz; δ_C 128.3 CH and 132.5 CH; δ_H 6.05 dd, J = 9.6, 2.4 Hz and 5.68 d, J = 9.6 Hz) in rings A and B. This was confirmed by 2D NMR spectroscopic analyses. Interpretation of the ^{1}H–^{1}H COSY spectrum led to partial structures **I**, **II**, and **III** (Figure 3). Partial structures **I**–**III** were connected by HMBC correlations.

24-Methylenecholesta-4,6-dien-3β,19-epoxy-2β-ol (**8**) was obtained from **7** by letting the latter stand in $CDCl_3$ for 1 week. The ^{13}C NMR and DEPT spectroscopic data of **8** showed signals for six olefin carbons, four methyl carbons, eight methylene carbons, eight methine carbons, and two quaternary carbons. The above functionalities account for three of the eight degrees of unsaturation, suggesting that **8** is a tetracyclic compound with a 3β,19-epoxy moiety. The ^{1}H–^{1}H COSY spectrum correlations of **8** were similar

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **4** and **5**

	4[a]			**5**[a]		
C/H	^{13}C		^{1}H	^{13}C		^{1}H
1	42.3 (qC)[b]			42.1 (qC)[b]		
2	44.6 (CH)		2.13 t (7.0)[c]	45.1 (CH)		2.18 m
3	27.9 (CH_2)	α	1.70 m	27.9 (CH_2)	α	1.70 m
		β	1.43 m		β	1.45 m
4	34.6 (CH_2)	α	1.71 m	34.6 (CH_2)	α	1.72 m
		β	1.30 m		β	1.29 m
5	37.1 (CH)		1.82 m	37.0 (CH)		1.82 m
6	41.0 (CH)		1.84 m	41.4 (CH)		1.95 m
7	50.4 (CH)		1.80 m	47.1 (CH)		1.72 m
8	87.8 (qC)			100.2 (qC)		
9	35.4 (CH_2)	α	1.65 m	29.2 (CH_2)		2.26 m
		β	2.40 td (12.5, 7.4)			
10	39.9 (CH_2)	α	1.85 td (12.5, 7.4)	39.3 (CH_2)	α	1.75 m
		β	1.60 td (12.5, 7.4)		β	1.58 m
11	146.4 (qC)			143.3 (qC)		
12	111.3 (CH_2)		4.87 br s	114.7 (CH_2)		5.04 br s
			4.94 br s			5.09 br s
13	19.3 (CH_3)		1.77 br s	19.5 (CH_3)		1.75 br s
14	21.0 (CH_3)		1.04 s	20.4 (CH_3)		0.97 s
15	14.5 (CH_3)		0.92 d (6.0)	14.5 (CH_3)		0.90 d (6.3)[c]
OOH						7.71 br s

[a] Spectra were measured in $CDCl_3$ (^{1}H, 300 MHz and ^{13}C, 75 MHz). [b] Multiplicities are deduced by HSQC and DEPT experiments. [c] J values (in Hz) are in parentheses.

Table 3. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds **7–9**

	7[a]			**8**[b]			**9**[c]		
C/H	^{13}C		^{1}H	^{13}C		^{1}H	^{13}C		^{1}H
1	32.3 (CH_2)[d]	α	2.18 m	38.4 (CH_2)[d]	α	1.58 m	32.4 (CH_2)[d]	α	1.64 m
		β	2.54 dd (18.0, 6.3)[e]		*β*	1.62 m		*β*	1.46 m
2	126.4 (CH)		5.77 m	69.6 (CH)		3.85 dt (9.0, 1.8)[e]	32.1 (CH_2)	α	1.83 m
								β	1.38 m
3	124.2 (CH)		5.92 m	71.7 (CH)		4.26 dd (5.8, 1.8)	67.3 (CH)		3.84 m
4	121.7 (CH)		5.77 d (5.5)	121.2 (CH)		6.05 d (5.8)	43.4 (CH_2)	α	1.67 m
								β	1.85 m
5	137.8 (qC)			144.4 (qC)			77.8 (qC)		
6	128.3 (CH)		6.05 dd (9.6, 2.4)	124.9 (CH)		6.19 dd (9.8, 2.5)	83.6 (CH)		3.66 d (4.5)[e]
7	132.5 (CH)		5.68 brd (9.6)	134.6 (CH)		5.86 brd (9.8)	32.3 (CH_2)	α	1.47 m
								β	1.84 m
8	37.6 (CH)		2.35 m	36.2 (CH)		1.92 m	35.0 (CH)		1.60 m
9	51.4 (CH)		1.42 m	45.5 (CH)		1.25 m	45.7 (CH)		1.58 m
10	40.3 (qC)			39.3 (qC)			45.5 (qC)		
11	21.7 (CH_2)	α	1.68 m	22.2 (CH_2)	α	1.68 m	23.5 (CH_2)	α	1.33 m
		β	1.56 m		*β*	1.33 m		*β*	1.36 m
12	40.3 (CH_2)	α	1.15 m	39.7 (CH_2)	α	1.20 m	41.6 (CH_2)	α	1.20 m
		β	2.04 m		*β*	2.06 dt (13.0, 4.5)		*β*	2.03 m
13	43.2 (qC)			43.0 (qC)			44.6 (qC)		
14	55.0 (CH)		1.18 m	54.3 (CH)		1.20 m	56.3 (CH)		1.22 m
15	23.7 (CH_2)	α	1.80 m	23.9 (CH_2)	α	1.82 m	24.8 (CH_2)	α	1.60 m
		β	1.18 m		*β*	1.20 m		*β*	1.54 m
16	28.1 (CH_2)	α	1.90 m	28.0 (CH_2)	α	1.92 m	29.6 (CH_2)	α	1.86 m
		β	1.28 m		*β*	1.33 m		*β*	1.29 m
17	55.8 (CH)		1.18 m	55.9 (CH)		1.19 m	57.6 (CH)		1.17 m
18	11.9 (CH_3)		0.74 s	11.7 (CH_3)		0.69 s	12.9 (CH_3)		0.74 s
19	64.6 (CH_2)		3.79 d (11.1)	65.7 (CH_2)	a	3.88 d (7.3)	70.2 (CH_2)	a	3.85 d (9.0)
			3.64 d (11.1)		b	3.28 dd (7.3, 3.5)		b	3.78 d (9.0)
20	35.7 (CH)		1.41 m	35.7 (CH)		1.44 m	37.1 (CH)		1.43 m
21	18.6 (CH_3)		0.95 d (6.4)	18.7 (CH_3)		0.95 d (6.5)	19.2 (CH_3)		0.95 d (6.5)
22	34.6 (CH_2)		1.58 m	34.6 (CH_2)		1.56 m	36.1 (CH_2)		1.56 m
			1.15 m			1.16 m			1.12 m
23	31.0 (CH_2)		2.10 m	31.0 (CH_2)		2.10 m	32.3 (CH_2)		2.12 m
			1.90 m			1.90 m			1.90 m
24	156.8 (qC)			156.8 (qC)			157.9 (qC)		
25	33.8 (CH)		2.22 m	33.8 (CH)		2.23 heptet (7.0)	34.8 (CH)		2.23 heptet (7.0)
26	21.8 (CH_3)		1.03 d (6.6)	21.8 (CH_3)		1.03 d (7.0)	22.4 (CH_3)		1.03 d (7.0)
27	22.0 (CH_3)		1.03 d (6.6)	22.0 (CH_3)		1.03 d (7.0)	22.6 (CH_3)		1.02 d (7.0)
28	106.0 (CH_2)		4.72 s	106.0 (CH_2)		4.72 s	107.0 (CH_2)		4.72 s
			4.66 s			4.66 s			4.65 s

[a] Spectra were measured in $CDCl_3$ (^{1}H, 300 MHz and ^{13}C, 75 MHz). [b] Spectra were measured in $CDCl_3$ (^{1}H, 500 MHz and ^{13}C, 125 MHz). [c] Spectra were measured in CD_3OD (^{1}H, 500 MHz and ^{13}C, 125 MHz). [d] Multiplicities are deduced by HSQC and DEPT experiments. [e] *J* values (in Hz) are in parentheses.

to those of **7**. These data, together with the HMBC correlations (Figure 3), established the structure of **8**.

HRESIMS of 24-methylenecholesta-6*β*,19-epoxy-3*β*,5α-diol (**9**) was obtained from **11** by letting the latter stand in $CDCl_3$ for 1 week. By comparison of the NMR spectroscopic data (Table 3) of **9** with those of **11**,[13] it was found that hydroxy groups attached to C-6 and C-19 in **11** were converted to a 6*β*,19-oxide ring in **9**. The position of the oxide group at C-6/C-19 was confirmed by the HMBC correlation (Figure 3) from H-6 to C-19.

Preliminary cytotoxicity screening revealed that **2**, **5**, and **6** exhibited significant cytotoxicty against P-388 (mouse lymphocytic leukemia) and HT-29 (human colon adenocarcinoma) cells (Table 4). The other tested compounds were not cytotoxic to P-388 and HT-29 cells. The results of further biological activity screening will be reported elsewhere in the future.

Table 4. Cytotoxicity[a] of Compounds **1–9**

	cell lines ED_{50} (*μ*g/mL)	
compound	HT-29	P-388
1	>10	>10
2	0.4	0.2
3	>10	>10
4	>10	>10
5	0.5	0.3
6	3.2	2.8
7	>10	>10
8	>10	>10
9	>10	>10

[a] For significant activity of pure compounds, an ED_{50} of ≤4.0 *μ*g/mL is required.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P1020 polarimeter. UV spectra were obtained on a Hitachi U-3210 spectrophotometer, and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. The NMR spectra were recorded on a Bruker Avance 300 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Unity INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, respectively, using TMS as internal standard. Chemical shifts are given in *δ* (ppm) and coupling constants in Hz. ESIMS were recorded by ESI FT-MS on a Bruker APEX II mass spectrometer. Si gel 60 (Merck, 230–400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

Animal Material. The soft coral *N. erecta* was collected by hand using scuba at Green Island located on the southeast coast of Taiwan, in July 2005, at a depth of 10 m, and was stored in a freezer for 5 weeks until extraction. A voucher specimen (GN-80) was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University.

Extraction and Isolation. A specimen of *N. erecta* was extracted sequentially with acetone and MeOH. After removal of solvent *in vacuo*, the acetone-soluble residue was partitioned between H_2O and EtOAc.

The dried EtOAc extract (35.0 g) was chromatographed over a Si column using *n*-hexane, *n*-hexane/EtOAc, and EtOAc/MeOH mixtures of increasing polarity. Elution with *n*-hexane gave fractions containing compound **6**, that with *n*-hexane/EtOAc (90:10) gave fractions containing compounds **4** and **5**, that with *n*-hexane/EtOAc (85:15) gave fractions containing compound **3**, that with EtOAc/MeOH (20:1) gave fractions containing compound **10**, and that with EtOAc/MeOH (10:1) gave fractions containing compound **11**. Compound **6** (3 mg) was further purified by HPLC (Si) by eluting with *n*-hexane. Compounds **4** (2 mg) and **5** (5 mg) were further purified by HPLC (Si) by eluting with *n*-hexane/EtOAc (90:10) and *n*-hexane/CH_2Cl_2 (50:50), respectively. Compound **3** (2 mg) was purified by repeated HPLC (Si) by eluting with *n*-hexane/EtOAc (85:15). Compound **10** (7.0 mg) was further purified by RP-HPLC by eluting with MeOH/H_2O (85:15). Compound **11** (5.0 mg) was further purified by RP-HPLC by eluting with MeOH/H_2O (85:15).

Compound **10** was fully transformed into compound **7** during NMR experiments in $CDCl_3$. Compound **7** was then converted to a mixture containing compounds **7** and **8**. Compound **8** (2 mg) was further purified by RP-18 HPLC by eluting with MeOH/H_2O (90:10). Under the same conditions, compound **11** was transformed into a mixture containing compounds **11** and **9** in $CDCl_3$ in 7 days. Compound **9** (2 mg) was further purified by RP-18 HPLC by eluting with MeOH/H_2O (85:15).

The MeOH-soluble residue (320 mg) was partitioned between H_2O and EtOAc. The dried EtOAc layer was then subjected to column chromatography on silica gel using CH_2Cl_2 and CH_2Cl_2/MeOH mixtures of increasing polarity. Elution with CH_2Cl_2/MeOH (80:1) gave fractions containing compounds **1** and **2**. Compounds **1** (7 mg) and **2** (5 mg) were purified by RP-HPLC by eluting with MeOH/H_2O (60:40).

5*β*,8*β*-Epidioxy-11-hydroxy-6-eudesmene (1): colorless, viscous oil; $[\alpha]^{23}_D$ +10 (*c* 0.7, CH_2Cl_2); IR (KBr) ν_{max} 3322, 2947, 1640, 1457, 1384, 1239, 1040, 927, 739 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 275 [M + Na]$^+$; HRESIMS *m/z* 275.1625 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1623).

5*β*,8*β*-Epidioxy-11-hydroperoxy-6-eudesmene (2): colorless, viscous oil; $[\alpha]^{23}_D$ +4 (*c* 0.5, CH_2Cl_2); IR (KBr) ν_{max} 3318, 2952, 1640, 1536, 1458, 1385, 1239, 1036, 927, 734 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 291 [M + Na]$^+$; HRESIMS *m/z* 291.1573 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_4Na$, 291.1572).

(3*R,4*S**,5*R**,7*R**,10*R**)-3,4-Epoxy-11-hydroxy-1-pseudoguaiene (3):** colorless, viscous oil; $[\alpha]^{23}_D$ +13 (*c* 0.3, CH_2Cl_2); IR (KBr) ν_{max} 3321, 2948, 1645, 1536, 1463, 1384, 1239, 1041, 931, 734 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 259 [M + Na]$^+$; HRESIMS *m/z* 259.1676 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

8*β*-Hydroxyprespatane (4): colorless, viscous oil; $[\alpha]^{23}_D$ +9 (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3307, 2947, 1640, 1457, 1384, 1239, 1035, 931, 738 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 243 [M + Na]$^+$; HRESIMS *m/z* 243.1726 [M + Na]$^+$ (calcd for $C_{15}H_{24}ONa$, 243.1725).

8*β*-Hydroperoxyprespatane (5): colorless, viscous oil; $[\alpha]^{23}_D$ +8 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3384, 2951, 1640, 1459, 1442, 1374, 1252, 1129, 1048, 945, 892 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; ESIMS *m/z* 433 [M + Na]$^+$; HRESIMS *m/z* 259.1675 [M + Na]$^+$ (calcd for $C_{15}H_{24}O_2Na$, 259.1674).

24-Methylenecholesta-2,4,6-trien-19-ol (7): colorless, viscous oil; $[\alpha]^{23}_D$ +43 (*c* 0.7, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 247 (3.97), 279 (3.18); IR (KBr) ν_{max} 3385, 2958, 1645, 1463, 1374, 1051, 884, 734 cm; ^{1}H NMR and ^{13}C NMR data, see Table 3; EIMS *m/z* 394 [M]$^+$; HREIMS *m/z* 394.3230 [M]$^+$ (calcd for $C_{28}H_{42}O$, 394.3235).

24-Methylenecholesta-4,6-dien-3*β*,19-epoxy-2*β*-ol (8): colorless, viscous oil; $[\alpha]^{23}_D$ +166 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 240 (3.63); IR (KBr) ν_{max} 3406, 2932, 1640, 1463, 1374, 1265, 1040, 884, 749 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; ESIMS *m/z* 433 [M + Na]$^+$; HRESIMS *m/z* 433.3084 [M + Na]$^+$ (calcd for $C_{28}H_{42}O_2Na$, 433.3082).

24-Methylenecholesta-6*β*,19-epoxy-3*β*,5α-diol (9): colorless, viscous oil; $[\alpha]^{23}_D$ +176 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3396, 2963, 1462, 1380, 1259, 1098, 1030, 858, 795 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; ESIMS *m/z* 453 [M + Na]$^+$; HRESIMS *m/z* 453.3343 [M + Na]$^+$ (calcd. for $C_{28}H_{46}O_3Na$, 453.3344).

Cytotoxicity Testing. Cytotoxicity was determined against P-388 (mouse lymphocytic leukemia) and HT-29 (human colon adenocarcinoma) tumor cells using the MTT assay method. The experimental details of this assay were carried out according to a previously described procedure.[18]

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