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New 4-methylated and 19-oxygenated steroids from the Formosan soft coral *Nephthea erecta*

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ABSTRACT

Two new 4-methylated steroids, erectasteroids A and B (**1** and **2**), six new 19-oxygenated steroids, erectasteroids C–H (**3**–**8**) and two known 19-oxygenated steroids (**9** and **10**) were isolated from the acetone solubles of the Formosan soft coral *Nephthea erecta*. The structures were elucidated by extensive NMR spectroscopic analysis and their cytotoxicity against selected cancer cells was measured in vitro.

1. Introduction

Soft corals of the genus *Nephthea* are rich in terpenoids and steroids [1–14]. As part of our search for bioactive substances from marine organisms, the Formosan soft coral *Nephthea erecta* Kükenthal (Nephtheidae) was studied because the acetone extracts showed significant cytotoxicity to HT-29 (human colon adenocarcinoma) and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures [15,16]. Bioassay-guided fractionation resulted in the isolation of two new cytotoxic 4-methylated steroids, erectasteroids A and B (**1** and **2**), six new 19-oxygenated steroids, erectasteroids C–H (**3**–**8**), and two known 19-oxygenated steroids (**9** and **10**) [19].

2. Experimental

2.1. General

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. NMR spectra were recorded on a Varian Inova 500 or a Bruker Avance 300 spectrometer. 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.

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2.2. Animal material

The soft coral *N. erecta* was collected at Green Island, off Taiwan, in September 2005, at a depth of 3–4 m and was stored for 4 weeks in a freezer until extraction. A voucher specimen, NSUGN-080, was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Taiwan.

2.3. Extraction and isolation

The bodies of the soft coral *N. erecta* were freeze dried to give 1.8 kg of a solid, which was extracted with acetone (20 L × 3). The acetone solubles was evaporated to give a dark brown residue (35.0 g), which was chromatographed on a silica gel column using eluents of increasing polarity from *n*-hexane to EtOAc to obtain fractions 1–27. Fraction 11 was subjected to RP-18 HPLC column chromatography (95% MeOH in H_2O) to afford compounds **1** (6 mg) and **2** (1 mg). Compounds **3** (5 mg), **4** (4 mg), **5** (5 mg), and **6** (1 mg) were obtained from fraction 20 by RP-18 HPLC column chromatography (90% MeOH in H_2O). Repeated chromatography of fraction 22 over RP-18 HPLC column (84% MeOH in H_2O) led to the isolation of compounds **7** (1 mg), **8** (3 mg), **9** (4 mg), and **10** (7 mg).

2.3.1. Erectasteroid A (1)

Colorless syrup. $[\alpha]_D^{24}$ +3.2 (c 0.6, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 221 (3.63) nm; IR (KBr) ν_{max}: 3337, 2926, 1682, 1634, 1437, 1385, 1239, 1031, 921, 708 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 451.3555 (calcd. for $C_{29}H_{48}O_2Na$, 451.3552).

2.3.2. Erectasteroid B (2)

Colorless syrup. $[\alpha]_D^{24}$ −62.0 (c 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 222 (3.62) nm; IR (KBr) ν_{max}: 3343, 2925, 1707, 1686, 1638, 1557, 1454, 1381, 1036, 927, 736 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 465.3346 (calcd. for $C_{29}H_{46}O_3Na$, 465.3344).

2.3.3. Erectasteroid C (3)

Limpid molasses. $[\alpha]_D^{24}$ +13.6 (c 0.5, $CHCl_3$); IR (KBr) ν_{max}: 3390, 2952, 1734, 1457, 1369, 1239, 1046, 1020, 968, 739 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 493.3292 (calcd. for $C_{30}H_{46}O_4Na$, 493.3294).

2.3.4. Erectasteroid D (4)

Limpid molasses. $[\alpha]_D^{24}$ +29.0 (c 0.4, $CHCl_3$); IR (KBr) ν_{max}: 3405, 2948, 1723, 1671, 1463, 1374, 1254, 1046, 754 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 481.3292 (calcd. for $C_{29}H_{46}O_4Na$, 481.3294).

2.3.5. Erectasteroid E (5)

Limpid molasses. $[\alpha]_D^{24}$ +9.6 (c 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 231 (3.67) nm; IR (KBr) ν_{max}: 3405, 2943, 1734, 1458, 1369, 1244, 1046, 1020, 739 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 483.3453 (calcd. for $C_{29}H_{48}O_4Na$, 483.3450).

Table 1 – ^1H NMR spectral data[a] (500 MHz) of 1 and 2 in $CDCl_3$

	1	2
1α	1.02 m	1.32 m
1β	1.72 m	2.05 m
2α	1.81 m	2.31 ddd (15.0, 6.5, 3.5)
2β	1.48 m	2.49 td (15.0, 6.5)
3	3.08 td (10.0, 5.0)[b]	
4	1.29 m	2.35 m
5	0.72 m	1.18 m
6α	1.28 m	0.92 m
6β	1.52 m	1.53 m
7α	0.78 m	1.24 m
7β	1.69 m	1.68 m
8	1.30 m	
9	0.61 td (11.5, 4.3)	0.60 m
11α	1.80 m	1.03 m
11β	1.29 m	1.55 m
12α	1.12 m	1.18 m
12β	1.97 dt (12.5, 3.0)	2.06 m
14	0.98 m	1.25 m
15α	1.58 m	1.02 m
15β	1.00 m	0.86 m
16α	1.66 m	1.86 m
16β	1.06 m	1.36 m
17	1.15 m	1.12 m
18	0.70 s	0.99 s
19	0.83 s	1.23 s
20	2.03 m	2.04 m
21	0.89 d (6.5)	0.88 d (6.5)
22	2.68 dd (15.5, 3.0), 2.40 dd (15.5,10.0)	2.67 dd (15.5, 5.3), 2.40 dd (15.5,10.0)
25	2.92 heptet (7.0)	2.92 heptet (6.5)
26	1.01 d (7.0)	1.10 d (6.5)
27	1.03 d (7.0)	1.03 d (6.5)
28	5.91 s, 5.67 s	5.91 s, 5.67 s
29	0.95 d (7.0)	1.00 d (6.0)

[a] Assigned by COSY, HSQC, NOESY, and HMBC experiments.
[b] *J* values (in Hz) in parentheses.

2.3.6. Erectasteroid F (6)

Limpid molasses. $[\alpha]_D^{24}$ −70.0 (c 0.1, $CHCl_3$); IR (KBr) ν_{max}: 3380, 2932, 1738, 1452, 1374, 1233, 1036, 890, 739 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 495.3477 (calcd. for $C_{30}H_{48}O_4Na$, 495.3450).

2.3.7. Erectasteroid F (7)

Limpid molasses. $[\alpha]_D^{24}$ −44.0 (c 0.1, $CHCl_3$); IR (KBr) ν_{max}: 3318, 2948, 1677, 1640, 1525, 1447, 1385, 1187, 1031, 927, 734, 634 cm^{-1}; ^1H NMR, see Table 4; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 453.3345 (calcd. for $C_{28}H_{46}O_3Na$, 453.3344).

2.3.8. Erectasteroid H (8)

White amorphous powder. $[\alpha]_D^{24}$ −16.0 (c 0.3, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 232 (3.65) nm; IR (KBr) ν_{max}: 3333, 2937, 1651, 1458, 1380, 1031, 962, 884 cm^{-1}; ^1H NMR, see Table 4; ^{13}C NMR, see Table 2; HRESIMS, *m*/*z* 451.3186 (calcd. for $C_{28}H_{44}O_3Na$, 451.3188).

2.4. Cytotoxicity testing

P-388 cells were kindly supplied by Prof. J.M. Pezzuto, formerly of the Department of Medicinal Chemistry and Pharmacog-

Table 2 – ^{13}C NMR data of 1–10

C	1[a]	2[a]	3[b]	4[b]	5[b]	6[a]	7[c]	8[d]	9[c]	10[d]
1	36.8	39.8	33.2	33.2	33.2	34.0	34.5	33.1	34.5	33.1
2	31.0	39.4	28.4	31.7	31.7	31.5	32.4	25.9	32.8	25.9
3	76.6	213.7	70.9	70.9	70.9	71.2	72.1	70.7	72.2	70.7
4	39.2	44.6	41.6	41.2	41.6	42.1	43.1	41.4	42.6	41.4
5	50.9	53.6	140.0	140.0	140.0	141.1	142.5	138.7	140.2	138.7
6	21.1	18.5	126.7	126.9	126.7	127.7	128.6	129.7	131.2	129.7
7	32.2	37.5	75.1	75.1	75.1	64.9	65.9	72.0	73.5	72.0
8	34.8	73.5	37.8	37.8	37.8	38.7	40.1	41.1	42.5	41.0
9	54.5	55.8	48.5	48.5	48.4	42.5	44.0	49.2	50.7	49.2
10	36.0	36.6	41.4	41.6	41.4	40.3	43.1	41.1	43.3	41.0
11	28.5	21.3	21.7	21.7	21.6	21.4	22.6	21.6	23.1	21.6
12	40.0	40.8	39.7	39.6	39.6	39.3	41.1	40.0	41.5	40.2
13	42.7	43.2	43.0	42.9	43.1	42.3	43.5	42.9	44.3	42.9
14	56.5	59.1	56.5	56.6	56.5	50.2	51.7	57.2	58.8	57.2
15	24.1	18.9	23.8	25.0	25.0	24.2	25.0	28.4	27.4	23.6
16	24.1	27.8	24.9	28.8	28.6	28.2	29.4	31.3	30.2	28.3
17	56.6	57.0	55.4	55.2	55.2	55.6	57.2	55.6	57.0	55.7
18	12.1	13.5	12.1	12.3	12.4	11.7	12.4	11.6	13.0	11.3
19	13.3	13.0	62.8	62.9	62.9	64.7	63.6	62.1	63.6	62.1
20	33.5	32.8	35.7	39.6	40.2	35.7	37.0	40.4	41.7	35.8
21	19.6	19.4	18.7	20.9	20.5	18.7	19.2	19.9	21.7	18.0
22	45.3	45.3	36.1	137.9	135.8	34.6	36.0	135.8	139.7	36.1
23	202.9	202.6	31.7	126.4	129.3	30.8	32.0	129.2	127.6	31.3
24	156.0	155.9	39.4	41.9	153.1	156.8	157.8	153.1	42.9	39.4
25	27.8	27.8	28.0	28.5	29.3	33.8	34.9	29.3	29.9	27.8
26	21.9	21.9	22.5	22.2	22.4	21.8	22.3	21.5	22.9	21.8
27	22.0	22.0	22.8	22.3	22.0	22.0	22.4	21.2	22.8	21.6
28	120.7	120.8			109.7	106.0	106.8	108.8		
29	15.1	11.6								
OAc			171.4 21.7	171.4 21.7	171.4 21.6	170.6 21.0				

[a] Spectrum recorded at 125 MHz in $CDCl_3$.
[b] Spectrum recorded at 75 MHz in $CDCl_3$.
[c] Spectrum recorded at 125 MHz in CD_3OD. The values are in ppm downfield from TMS and assignments were made by DEPT, COSY, HMQC, and HMBC experiments.
[d] Spectrum recorded at 75 MHz in CD_3OD.

nosy, University of Illinois at Chicago; HT-29 was purchased from the American Type Culture Collection. Cytotoxic assays were carried out according to the procedure described previously [15,16].

3. Results and discussion

Compound **1** had a molecular formula of $C_{29}H_{48}O_2$ as established by HRESIMS and NMR spectroscopic data. The ^{1}H NMR spectrum revealed the presence of two tertiary methyls (δ_H 0.70 and 0.83), four secondary methyls (δ_H 0.95, 0.89, 1.01, and 1.03), and an oxymethine [δ_H 3.08 (1H, td, J = 10.0, 5.0 Hz)]. The presence of a vinylic methylene conjugated to a carbonyl group was revealed by NMR signals [(δ_H 5.91 and 5.67); δ_C 120.7 (CH_2), 156.0 (qC), and 202.9 (qC)] (Tables 1 and 2), as well as from IR absorption at 1682 and 1634 cm^{-1}. ^{13}C NMR and DEPT spectra of **1** also exhibited nine sp^3 methylenes, nine sp^3 methines, and two sp^3 quaternary carbons. The NMR and HRMS data could account for 2 of the 6 degrees of unsaturation, suggesting the tetracyclic nature of **1**. Twenty-nine carbons including six methyls and comparison of NMR chemical shift values of **1** with those of 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one reported from the soft coral *Litophyton viridis* suggested that **1** may be a 8-deoxy analogue of 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one [17]. Interpretation of the ^{1}H–^{1}H COSY spectrum led to partial structures I and II (Fig. 1). Partial structures I and II were connected through the conjugated enone by HMBC correlations between H_3-26, H_3-27/C-24, C-25, between H_2-28/C-23, C-24, C-25, and between H_2-22/C-23. Rings A and B were elucidated on the basis of HMBC cross-peaks between H_3-19/C-1, C-5, C-9, C-10 and H-4/C-29, whereas rings C and D were completed based on HMBC correlations between H_3-18/C-12, C-13, C-14, C-17. The NOESY correlations (Fig. 2) observed between H-11β and H-8, H-11β and H_3-18, H-11β and H_3-19, H-9 and H-14, H_3-18 and H-8, H_3-19 and H-8, H_3-18 and H-20, H-3 and H-1α, H-3 and H-5, and H_3-19 and H-4β in **1** confirmed the relative configurations for each ring junction and chiral center.

Compound **2** had a molecular formula of $C_{29}H_{46}O_3$ as determined by HRESIMS. The spectral data (Tables 1 and 2) resembled those of **1** except for NMR signals due to a ketone at C-3 (δ_C 213.7) and a tertiary hydroxyl at C-8 (δ_C 73.5). Comparison of NMR chemical shift values of

Table 3 – ^{1}H NMR spectral data[a] of 3–5 (300 MHz), and 6 (500 MHz) in $CDCl_3$

	3	4	5	6
1α	1.06 m	1.08 m	1.11 m	1.14 m
1β	1.92 m	1.97 m	1.97 m	2.06 m
2α	1.84 m	1.85 m	1.86 m	1.90 m
2β	1.38 m	1.43 m	1.39 m	1.43 m
3	3.60 m	3.60 m	3.61 m	3.63 dddd (10.0, 8.3, 5.5, 4.5)
4α	2.38 m	2.39 m	2.40 dd (13.2, 2.7)	2.40 m
4β	2.21 m	2.21 m	2.24 m	2.34 m
6	5.58 br s	5.58 br s	5.58 br s	5.86 d (4.5)
7	4.96 br d (8.6)[b]	4.96 br d (8.5)	4.96 br d (8.7)	3.91 dd (4.5, 4.0)
8	2.14 m	2.18 m	2.17 m	1.78 td (12.0, 4.0)
9	1.08 m	1.08 m	1.06 m	1.28 m
11α	1.66 m	1.67 m	1.70 m	1.62 m
11β	1.58 m	1.60 m	1.64 m	1.54 m
12α	1.27 m	1.12 m	1.19 m	1.18 m
12β	2.07 m	2.02 m	2.06 m	2.04 m
14	1.02 m	1.07 m	1.08 m	1.36 m
15α	1.31 m	1.38 m	1.42 m	1.71 m
15β	1.09 m	1.25 m	1.30 m	1.14 m
16α	1.40 m	1.60 m	1.66 m	1.93 m
16β	1.26 m	1.25 m	1.27 m	1.31 m
17	1.03 m	1.07 m	1.17 m	1.22 m
18	0.74 s	0.76 s	0.78 s	0.71 s
19	3.89 d (11.5), 3.66 d (11.5)	3.88 d (11.5), 3.65 d (11.5)	3.89 d (11.5), 3.66 d (11.5)	4.53 d (12.0), 3.97 d (12.0)
20	1.34 m	2.02 m	2.14 m	1.44 m
21	0.91 d (6.4)	1.00 d (6.5)	1.05 d (6.8)	0.96 d (7.0)
22	1.03 m	5.21 dd (15.0, 7.8)	5.58 dd (15.8, 8.8)	1.54 m, 1.18 m
23	1.23 m, 1.79 m	5.23 dt (15.0, 7.8)	5.93 d (15.8)	2.10 m
24	1.13 m	1.81 m		
25	1.50 m	1.56 m	2.54 heptet (6.8)	2.23 heptet (7.0)
26	0.86 d (6.6)	0.85 d (6.1)	1.07 d (6.8)	1.03 d (7.0)
27	0.86 d (6.6)	0.85 d (6.1)	1.05 d (6.8)	1.03 d (7.0)
28			4.82 s, 4.84 s	4.72 s, 4.66 s
OAc	2.03 s	2.02 s	2.01 s	2.03 s

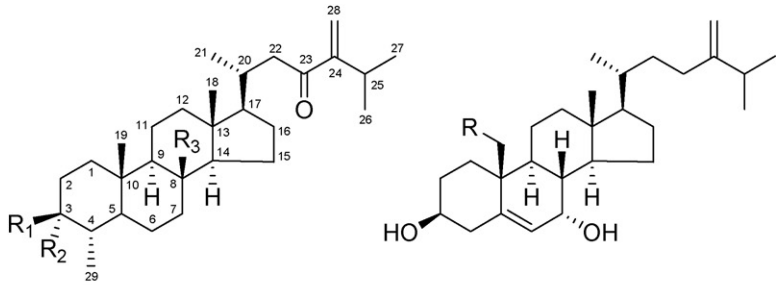
[a] Assigned by COSY, HSQC, NOESY, and HMBC experiments.
[b] *J* values (in Hz) in parentheses.

2 with those of Jone's oxidation products of 4α-methyl-3β,8β-dihydroxy-5α-ergost-24(28)-en-23-one suggested that **2** may be 4α-methyl-8β-hydro-xy-5α-ergost-24(28)-en-3,23-dione [17]. HMBC correlations (Fig. 3) from H_2-2/H-4/H_3-29 to C-3 and from H-7/H-9/H-14 to C-8 helped positioning the ketone and the tertiary hydroxyl at C-3 and C-8, respectively. ^{13}C NMR spectral data of **2** were not reported and ^{1}H spectral data were not completely assigned in the literature [17].

Compound **3** had a molecular formula of $C_{29}H_{48}O_4$ as determined by HRESIMS. The IR spectrum showed the presence of hydroxyl group(s) (3390 cm^{-1}) and an ester group (1734 cm^{-1}). The presence of two oxymethines and one oxymethylene was shown by the ^{1}H NMR [δ_H 3.60 (1H, m), 4.96 (1H, br d, J = 8.6 Hz), 3.89 (1H, d, J = 11.5 Hz), 3.66 (1H, d, J = 11.5 Hz)] and ^{13}C NMR [δ_C 70.9 (CH), 75.1 (CH), 62.8 (CH_2)] spectra. The ^{1}H and ^{13}C NMR spectra also showed signals due to four methyl groups [δ_H 0.74 (3H, s), 0.86 (6H, d, J = 6.6 Hz), 0.91 (3H, d, J = 6.4 Hz)], and a trisubstituted double bond [δ_H 5.58 (1H, br s), δ_C 126.7 (CH), 140.0 (qC)]. The ^{13}C NMR and DEPT spectra of **3** also exhibited 10 sp^3 methylenes, 5 sp^3 methines, and 2 sp^3 quaternary carbons. This evidence suggested that **3** contained the same steroid nucleus (A, B, C, and D rings) as of 24-methylenecholest-5-en-3β,7β,19-triol 7-acetate [18] except for the side chain. Comparison of the ^{13}C NMR chemical shifts with those of cholesterol indicate unambiguously that compound **3** is cholest-5-en-3β,7β,19-triol 7-acetate. The COSY, HMBC (Fig. 4), and NOESY spectra (Fig. 5) of **3** confirmed this, showing the expected correlations.

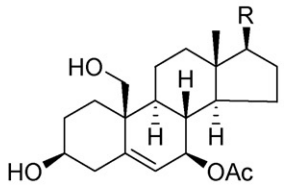
The spectral data of **4** and **5** were analogous to those of **3**, except for NMR signals due to the side chain. As shown in Tables 2 and 3, signals due to a 22-E disubstituted double bond [δ_H 5.21 (1H, dd, J = 15.0, 7.8 Hz) and 5.23 (1H, dt, J = 15.0, 7.8 Hz); δ_C 137.9 (CH) and 126.4 (CH)] were observed in **4**, and those due to a 22*E*,24(28)-diene [δ_H 5.58 (1H, dd, J = 15.8, 8.8 Hz) and 5.93 (1H, d, J = 15.8 Hz); 4.82 (1H, s) and 4.84 (1H, s); δ_C 135.8 (CH), 129.3 (CH), 153.1 (qC), and 109.7 (CH_2)] appeared in the side chain of **5**.

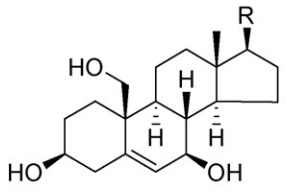
Compound **6** had a molecular formula of $C_{30}H_{48}O_4$ as determined by HRESIMS. The spectral data (Tables 2 and 3) showed some similarity to those of 24-methylenecholesta-5-en-3β,7β,19-triol [18] except for the 19-hydroxyl was replaced by an 19-acetoxyl [δ_H 4.53 and 3.97 (2H, each d, J = 12.0 Hz) and 2.03 (3H, s); δ_C 64.9 (CH_2), 21.0(CH_3), and 170.6 (qC)] in **6**. COSY correlations from H-20 to H_2-22 and from H_2-22 to H_2-23 as well as HMBC correlations from H_2-19 to C-5/C-10/C-1 and from H_2-28 to C-23 helped ascertain this assignment. The



1 R_1 = OH, R_2 = R_3 = H
2 R_1 = R_2 = O, R_3 = OH

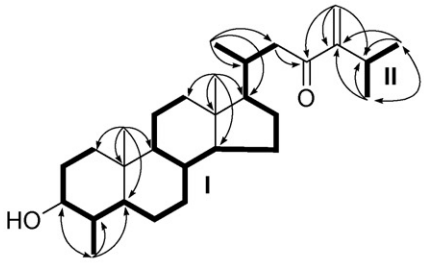
6 R = OAc
7 R = OH





3 R =
4 R =
5 R =

8 R =
9 R =
10 R =



: ^{1}H-^{1}H COSY correlations
: HMBC correlations

Fig. 1 – ^{1}H–^{1}H COSY and key HMBC correlations of 1.

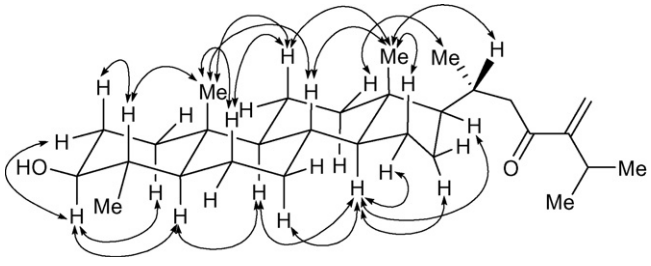
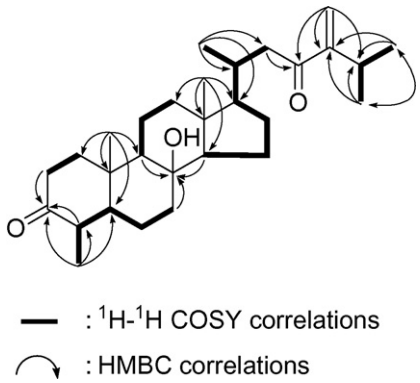


Fig. 2 – Selected NOESY correlations of 1.



: ^{1}H-^{1}H COSY correlations
: HMBC correlations

Fig. 3 – ^{1}H–^{1}H COSY and key HMBC correlations of 2.

Table 4 – ^{1}H NMR spectral data[a] of 7, 9 (500 MHz), and 8, 10 (500 MHz) in CD_3OD

	7	8	9	10
1α	1.11 m	0.98 m	0.98 m	0.99 m
1β	1.96 m	2.09 m	2.05 m	2.08 m
2α	1.77 m	1.81 m	1.78 m	1.84 m
2β	1.42 m	1.45 m	1.40 m	1.44 m
3	3.53 m	3.47 m	3.46 m	3.45 m
4α	2.33 m	2.30 m	2.31 m	2.31 m
4β	2.30 m	2.24 m	2.25 m	2.28 m
6	5.79 d (4.5)[b]	5.49 br s	5.49 br s	5.49 br s
7	3.79 t (4.5)	3.67 d (8.0)	3.66 d (8.0)	3.67 d (8.1)
8	1.94 m	1.82 m	1.80 m	1.77 m
9	1.26 m	1.02 m	0.99 m	1.02 m
11α	1.76 m	1.73 m	1.74 m	1.72 m
11β	1.58 m	1.64 m	1.60 m	1.62 m
12α	1.14 m	1.18 m	1.14 m	1.14 m
12β	2.04 m	2.03 m	2.04 m	2.08 m
14	1.37 m	1.10 m	1.06 m	1.06 m
15α	1.77 m	1.67 m	1.78 m	1.37 m
15β	1.10 m	1.30 m	1.40 m	1.18 m
16α	1.88 m	1.78 m	1.67 m	1.89 m
16β	1.30 m	1.50 m	1.28 m	1.29 m
17	1.18 m	1.21 m	1.12 m	1.10 m
18	0.77 s	0.80 s	0.78 s	0.77 s
19	3.87 d (11.5), 3.58 d (11.5)	3.86 d (11.7), 3.60 d (11.7)	3.85 d (11.5), 3.60 d (11.5)	3.86 d (11.6), 3.60 d (11.6)
20	1.45 m	2.14 m	2.05 m	1.40 m
21	0.98 d (6.5)	1.06 d (6.6)	1.02 d (6.5)	0.95 d (6.4)
22	1.57 m, 1.17 m	5.58 dd (15.8, 8.8)	5.22 dd (15.0, 8.0)	1.02 m
23	2.09 m, 1.92 m	5.93 d (15.8)	5.30 ddd (15.0, 7.5, 7.0)	1.45 m, 1.81 m
24			1.82 dd (13.0, 6.5)	1.29 m
25	2.23 heptet (6.5)	2.55 heptet (6.6)	1.57 m	1.51 m
26	1.02 d (6.5)	1.07 d (6.6)	0.87 d (7.0)	0.88 d (6.6)
27	1.03 d (6.5)	1.06 d (6.6)	0.87 d (7.0)	0.88 d (6.6)
28	4.72 s, 4.65 s	4.82 s, 4.84 s		

[a] Assigned by COSY, HSQC, NOESY, and HMBC experiments.
[b] *J* values (in Hz) in parentheses.

NOESY correlations (Fig. 6) between H-8 with all protons of H-7, H-11β, H_2-19, and H_3-18 positioned the above protons on the same side of the molecule, and revealed the α-orientation of the 7-OH. Compound **6** is thus 24-methylenecholest-5-en-3β,7α,19-triol 19-acetate.

Compound **7** possessed a molecular formula of $C_{28}H_{46}O_3$ as derived from its HRESIMS. The spectral data of compound **7** (Tables 2 and 4) differed from compound **6** only in the 19-hydroxyl functionality [δ_H 3.87 (1H, d, J = 11.5 Hz) and 3.58 (1H, d, J = 11.5 Hz); δ_C 63.6 (CH_2)].

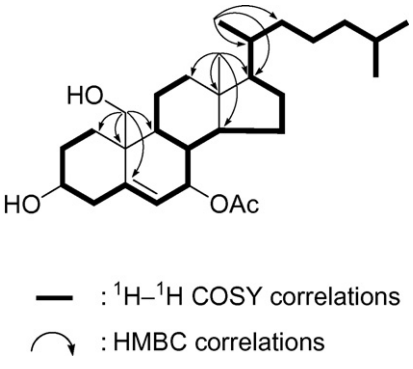


Fig. 4 – ^{1}H–^{1}H COSY and key HMBC correlations of 3.

The spectral data of **8** were quite similar to those of **9** isolated fron the black coral *Antipathes subpinnata* [19], except for NMR signals due to the side chain. As shown in Tables 2 and 4, signals due to a 22-E disubstituted double bond [δ_H 5.22 (1H, dd, J = 15.0, 8.0 Hz) and 5.30 (1H, ddd, J = 15.0, 7.5, 7.0 Hz); δ_C 139.7 (CH) and 127.6 (CH)] appeared in the side chain of **9**, and those due to a 22*E*,24(28)-diene [δ_H 5.58 (1H, dd, J = 15.8, 8.8 Hz) and 5.93 (1H, d, J = 15.8 Hz); 4.82 (1H, s) and 4.84 (1H, s); δ_C 135.8 (CH), 129.2 (CH), 153.1 (qC), and 108.8 (CH_2)] were observed in **8**. ^{13}C NMR spectral data of **9** and **10** were completely assigned, but ^{1}H spectral data were not completely assigned in the literature [19].

Compounds **8** and **10** exhibited cytotoxicity against P-388 with ED_{50} of 3.8 and 3.6 μg/ml, respectively. Compounds **8** and

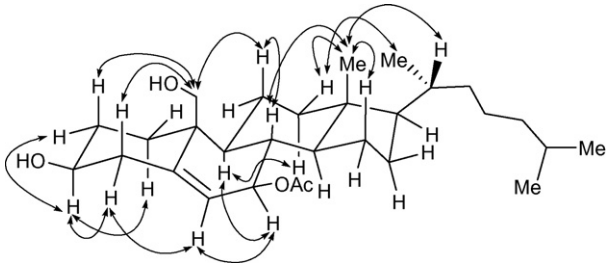


Fig. 5 – Selected NOESY correlations of 3.

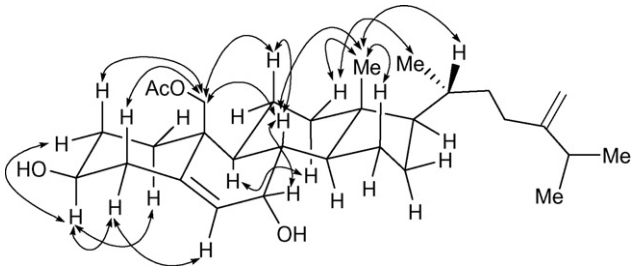


Fig. 6 – Selected NOESY correlations of 6.

10 exhibited cytotoxicity against HT-29 with ED_{50} of 4.7 and 4.3 μg/ml, respectively. Nevertheless, compounds **1–6** and **9** were not cytotoxic to P-388 and HT-29 cell lines.

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