

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

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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].

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.

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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 F₂₅₄, 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 $(20 L \times 3)$. 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 H₂O) 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 H₂O). Repeated chromatography of fraction 22 over RP-18 HPLC column (84% MeOH in H₂O) 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, CHCl₃); 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; ¹³C NMR, see Table 2; HRESIMS, *m*/z 451.3555 (calcd. for C₂₉H₄₈O₂Na, 451.3552).

2.3.2. Erectasteroid B (2)

Colorless syrup. $[\alpha]_D^{24}$ –62.0 (c 0.1, CHCl₃); 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; ¹³C NMR, see Table 2; HRESIMS, *m*/z 465.3346 (calcd. for C₂₉H₄₆O₃Na, 465.3344).

2.3.3. Erectasteroid C (3)

Limpid molasses. $[\alpha]_D^{24}$ +13.6 (c 0.5, CHCl₃); IR (KBr) ν_{max} : 3390, 2952, 1734, 1457, 1369, 1239, 1046, 1020, 968, 739 cm⁻¹; ¹H NMR, see Table 3; ¹³C NMR, see Table 2; HRESIMS, *m*/*z* 493.3292 (calcd. for C₃₀H₄₆O₄Na, 493.3294).

2.3.4. Erectasteroid D (4)

Limpid molasses. $[\alpha]_D^{24}$ +29.0 (c 0.4, CHCl₃); IR (KBr) ν_{max} : 3405, 2948, 1723, 1671, 1463, 1374, 1254, 1046, 754 cm⁻¹; ¹H NMR, see Table 3; ¹³C NMR, see Table 2; HRESIMS, *m*/z 481.3292 (calcd. for C₂₉H₄₆O₄Na, 481.3294).

2.3.5. Erectasteroid E (5)

Limpid molasses. $[\alpha]_D^{24}$ +9.6 (c 0.5, CHCl₃); 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; ¹³C NMR, see Table 2; HRESIMS, *m*/z 483.3453 (calcd. for C₂₉H₄₈O₄Na, 483.3450).

	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),	2.67 dd (15.5, 5.3),
	2.40 dd (15.5,10.0)	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)

^b J values (in Hz) in parentheses.

2.3.6. Erectasteroid F (6)

Limpid molasses. $[\alpha]_D^{24}$ –70.0 (c 0.1, CHCl₃); IR (KBr) ν_{max} : 3380, 2932, 1738, 1452, 1374, 1233, 1036, 890, 739 cm⁻¹; ¹H NMR, see Table 3; ¹³C NMR, see Table 2; HRESIMS, *m*/z 495.3477 (calcd. for C₃₀H₄₈O₄Na, 495.3450).

2.3.7. Erectasteroid F (7)

Limpid molasses. $[\alpha]_D^{24}$ –44.0 (c 0.1, CHCl₃); IR (KBr) ν_{max} : 3318, 2948, 1677, 1640, 1525, 1447, 1385, 1187, 1031, 927, 734, 634 cm⁻¹; ¹H NMR, see Table 4; ¹³C NMR, see Table 2; HRESIMS, *m*/z 453.3345 (calcd. for C₂₈H₄₆O₃Na, 453.3344).

2.3.8. Erectasteroid H (8)

White amorphous powder. $[\alpha]_D^{24}$ –16.0 (c 0.3, CHCl₃); 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; ¹³C NMR, see Table 2; HRESIMS, *m*/z 451.3186 (calcd. for C₂₈H₄₄O₃Na, 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										
С	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								
044			171.4	171.4	171.4	170.6				
UAC			21.7	21.7	21.6	21.0				

^a Spectrum recorded at 125 MHz in CDCl₃.

 $^{\rm b}\,$ Spectrum recorded at 75 MHz in CDCl_3.

 $^{\rm c}$ Spectrum recorded at 125 MHz in CD₃OD. The values are in ppm downfield from TMS and assignments were made by DEPT, COSY, HMQC, and HMBC experiments.

 $^{\rm d}$ Spectrum recorded at 75 MHz in CD₃OD.

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 ¹H 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, 5.0Hz)]. 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 (CH₂), 156.0 (qC), and 202.9 (qC)] (Tables 1 and 2), as well as from IR absorption at 1682 and 1634 cm⁻¹. ¹³C NMR and DEPT spectra of 1 also exhibited nine sp³ methylenes, nine sp³ methines, and two sp³ quaternary carbons. The NMR and HRMS data could account for 2 of the 6 degrees of unsaturation, suggesting the tetracyclic nature of 1. Twentynine carbons including six methyls and comparison of NMR

chemical shift values of 1 with those of 4α -methyl-3 β ,8 β 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 ¹H–¹H 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 H₃-26, H₃-27/C-24, C-25, between H₂-28/C-23, C-24, C-25, and between H₂-22/C-23. Rings A and B were elucidated on the basis of HMBC cross-peaks between H₃-19/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 H₃-18/C-12, C-13, C-14, C-17. The NOESY correlations (Fig. 2) observed between H-11 β and H-8, H-11 β and H₃-18, H-11 β and H₃-19, H-9 and H-14, H₃-18 and H-8, H₃-19 and H-8, H₃-18 and H-20, H-3 and H-1 α , H-3 and H-5, and H_3-19 and H-4 β 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 Assigne ^b J values	 ^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-3 β ,8 β -dihydroxy-5 α -ergost-24(28)-en-23-one suggested that 2 may be 4α -methyl-8 β -hydro-xy-5 α -ergost-24(28)-en-3,23dione [17]. HMBC correlations (Fig. 3) from H₂-2/H-4/H₃-29 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. ¹³C NMR spectral data of **2** were not reported and ¹H 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 ¹H NMR [$\delta_{\rm H}$ 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 ¹³C NMR [$\delta_{\rm C}$ 70.9 (CH), 75.1 (CH), 62.8 (CH₂)] spectra. The ¹H and ¹³C NMR spectra also showed signals due to four methyl groups [$\delta_{\rm H}$ 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 [$\delta_{\rm H}$ 5.58 (1H, br s), $\delta_{\rm C}$ 126.7 (CH), 140.0 (qC)]. The ¹³C NMR and DEPT spectra of **3** also exhibited 10 sp³ methylenes, 5 sp³ methines, and 2 sp³ quaternary carbons. This evidence suggested that **3** contained the same steroid nucleus (A, B, C, and D rings) as of 24-methylenecholest-5-en-3 β ,7 β ,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-3 β ,7 β ,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 $[\delta_{\rm H}$ 5.21 (1H, dd, J = 15.0, 7.8 Hz) and 5.23 (1H, dt, J = 15.0, 7.8 Hz); $\delta_{\rm C}$ 137.9 (CH) and 126.4 (CH)] were observed in **4**, and those due to a 22E,24(28)-diene [$\delta_{\rm H}$ 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); $\delta_{\rm C}$ 135.8 (CH), 129.3 (CH), 153.1 (qC), and 109.7 (CH₂)] 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-3 β ,7 β ,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 (CH₂), 21.0(CH₃), and 170.6 (qC)] in **6**. COSY correlations from H-20 to H₂-22 and from H₂-22 to H₂-23 as well as HMBC correlations from H₂-19 to C-5/C-10/C-1 and from H₂-28 to C-23 helped ascertain this assignment. The





: ¹H-¹H COSY correlations

: HMBC correlations 1 7









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 ₃ OD					
	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-11 β , H₂-19, and H₃-18 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 C₂₈H₄₆O₃ as derived from its HRESIMS. The spectral data of compound 7 (Tables 2 and 4) differed from compound 6 only in the 19hydroxyl functionality [δ_H 3.87 (1H, d, J = 11.5 Hz) and 3.58 (1H, d, J = 11.5 Hz; $\delta_C 63.6 (CH_2)$].



Fig. 4 – ¹H–¹H 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 [$\delta_{\rm H}$ 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 22E,24(28)-diene [$\delta_{\rm H}$ 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); $\delta_{\rm C}$ 135.8 (CH), 129.2 (CH), 153.1 (qC), and 108.8 (CH₂)] were observed in 8. ¹³C NMR spectral data of 9 and 10 were completely assigned, but ¹H 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 μ g/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 μ g/ml, respectively. Nevertheless, compounds 1–6 and 9 were not cytotoxic to P-388 and HT-29 cell lines.

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