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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-