Norpregnane Glycosides from a Thai Soft Coral, *Scleronephthya pallida*

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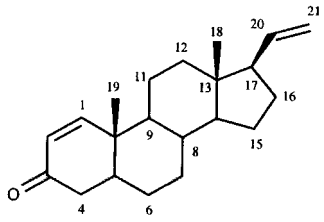
Two new norpregnane glycosides, 19-norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside (**3**) and 19-norpregna-1,3,5(10),20-tetraen-3-*O*-*β*-arabinopyranoside (**4**), were isolated from the soft coral *Scleronephthya pallida*, along with two known steroids, pregna-1,20-dien-3-one (**1**) and 19-norpregna-1,3,5(10),20-tetraen-3-ol (**2**). 19-Norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside (**3**) exhibits moderate antimalarial and cytotoxic activities. The chemical structures of **1**−**4** were elucidated from spectroscopic data.

Pregnane steroids are rare in the marine environment, and few have been reported to date.[1−5] Most marine organisms in Thailand have never been chemically investigated, and we report here our investigations of the steroid metabolites of the soft coral *Scleronephthya pallida* Whitelegge (family Neptheidae) collected by scuba from Phuket Island.

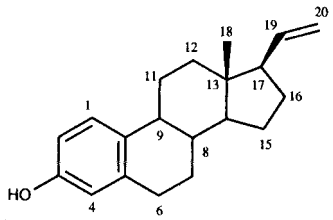
The chloroform extract of freeze-dried specimens of the soft coral *S. pallida* afforded four pregnane steroids, including the known pregna-1,20-dien-3-one (**1**) and 19-norpregna-1,3,5(10),20-tetraen-3-ol (**2**),[1,2] and two new glycosides **3** and **4** derived from **2**. The known pregnanes **1** and **2** were obtained from column chromatography of the chloroform extract on Si gel, while Si gel column chromatography followed by gel filtration on Sephadex LH-20 yielded **3** and **4**. The chemical structures of these compounds were assigned by spectral analysis.

The HREIMS of **1** and **2** showed molecular ion peaks at *m/z* 298.2293 and 282.1980, supporting molecular formulas of $C_{21}H_{30}O$ and $C_{20}H_{26}O$, respectively. The ^1H and ^{13}C NMR data of **1** and **2** were identical to those of the known pregnanes described previously,[1,2] confirming that **1** and **2** are pregna-1,20-dien-3-one (**1**) and 19-norpregna-1,3,5(10),20-tetraen-3-ol (**2**).

The ^1H NMR spectra of **3** and **4** in $CDCl_3/CD_3OD$ (2:1) were similar to those of **2**, except that there were additional signals at *δ* 4.1−6.1, suggesting the presence of a sugar moiety in the molecule. The ^{13}C NMR spectra of **3** and **4** were also similar to those of **2**, except for five additional oxymethine carbons between *δ* 65−100 (at *δ* 68.28, 69.44, 71.62, 73.22, 99.87 for **3** and at *δ* 65.06, 70.19, 70.37, 70.98, 100.03 for **4**). There was also an additional methyl carbon at *δ* 17.22 in the ^{13}C NMR spectrum of **3**. Comparison of the ^1H and ^{13}C NMR data of **2** with those of **3** and **4** revealed that **3** and **4** were different glycosides of the aglycon **2**. The ^1H NMR and ^{13}C NMR data of the sugar residues of **3** and **4** are shown in Table 1. The HREIMS of **3** and **4** exhibited molecular ion peaks at *m/z* 428.2560 and 414.2403, tentatively suggesting that **3** and **4** possessed molecular formulas of $C_{26}H_{36}O_5$ and $C_{25}H_{34}O_5$, respectively. The EIMS of both **3** and **4** also showed a fragment ion at *m/z* 282 characteristic of the aglycon **2**. HMQC, ^1H and ^{13}C NMR data as well as decoupling experiments of **3** and **4** were used to assign the signals of the sugar moiety.



1



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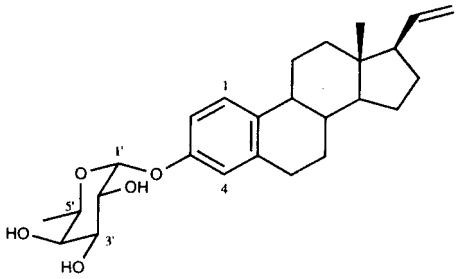
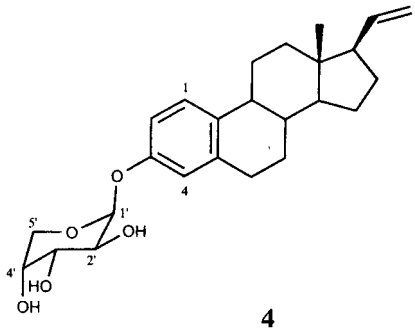


3



4

The $^1H-^1H$ COSY of **3** revealed contiguous coupling between H-1′ and H-2′; H-2′ and H-3′; H-3′ and H-4′; and H-5′ and 5′-CH_3. The coupling constants of the anomeric

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Table 1. ^{1}H and ^{13}C NMR Data of the Sugar Moiety of **3** and **4** in C_5D_5N[a]

	compound **3**		compound **4**	
carbon	δ_H, m (J in Hz)	δ_C	δ_H, m (J in Hz)	δ_C
C-1′	6.07, d (3.4)	99.87	6.13, d (3.4)	100.03
C-2′	4.78, dd (3.4, 10.2)	69.44	4.84, dd (3.4,9.7)	70.37
C-3′	4.72, dd (3.3, 10.2)	71.62	4.74, dd (3.4,9.7)	70.98
C-4′	4.25, br s	73.22	4.48, br s (1.8)	70.19
C-5′	4.46, q (6.7)	68.28	4.14, dd (2.4, 11.9)[b]	65.06
			4.30, dd (1.5, 11.9)[c]	
CH_3	1.52, d (6.7)	17.22		

[a] ^{1}H NMR and ^{13}C NMR data of the aglycon of compounds **3** and **4** in CD_3Cl/CD_3OD (2:1) are identical to that of compound **2**. [b] H-5′eq. [c] H-5′ax.

proton (δ 6.07, $J_{H1'-H2'} = 3.4$ Hz) and H-2′ (δ 4.78, $J_{H2'-H3'} = 10.2$ Hz) of **3** suggested an equatorial orientation for the anomeric proton, thus confirming the α or axial hemiacetal linkage to the aglycon and an axial orientation for both H-2′ (dd, 3.4, 10.2 Hz) and H-3′ (dd, 3.3, 10.2 Hz). The broad singlet signal of H-4′ suggested that H-4′ was equatorial relative to H-3′ and H-5′. Selected NOE difference experiments were used to confirm the configuration of the sugar residue in **3**; irradiation at H-5′ resulted in NOE enhancement at H-3′ (4.6%), confirming that both H-5′ and H-3′ were axial and that 5′-CH_3 was equatorial. Acetylation of **3** gave a triacetate and confirmed the presence of three hydroxyl groups in the sugar residue of **3**. Finally, on the basis of these spectral data, it was concluded that the sugar unit of **3** was α-fucopyranose. The presence of an α-fucopyranose moiety in **3** was confirmed by comparing the ^{13}C NMR chemical shifts of the sugar unit in **3** with those of known sugars.[8,9] However, the absolute stereochemistry of the fucose in **3** could not be conclusively assigned due to the limited amount of sample available for further studies. Compound **3** is therefore 19-norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside. The glycoside **3** inhibited growth of the malarial protozoa *Plasmodium falciparum* ($EC_{50} = 1.5$ μg/mL) and exhibited cytotoxicity ($ED_{50} = 10$ μg/mL) against a breast cancer cell line (BCA-1).

The anomeric proton of the pentose unit of **4** appeared as the most deshielded sugar methine proton (δ 6.13) in the ^{1}H NMR spectrum of this compound. The ^{1}H−^{1}H COSY was used to establish the coupling between H-1′ and H-2′; H-2′ and H-3′; H-4′ and H-5′ax and H-5′eq. The ^{1}H NMR spectrum of the triacetylated product of **4** suggested the presence of three hydroxyl groups in the sugar moiety of **4**. Similar to the ^{1}H NMR chemical shifts and coupling constants of the sugar moiety of **3**, the $J_{H1'-H2'} = 3.4$ Hz and $J_{H2'-H3'} = 9.7$ Hz of **4** implied that the anomeric proton was equatorial relative to H-2′ and that H-2′ and H-3′ were axial. The hemiacetal linkage was therefore axial. The signal of H-4′ in the ^{1}H NMR spectrum was also a broad singlet, suggesting that H-4′ was equatorial relative to H-3′ and H-5′. The 2D NOESY experiment of the acetylated sugar unit of **4** showed the expected correlation between the signals of H-3′ (δ 5.54, dd, $J = 10.8$, 3.3 Hz) and H-5′ax (δ 4.04, dd, $J = 12.2$, 1.5 Hz). On the basis of these spectral data, it was concluded that the sugar unit of the glycoside **4** was β-arabinopyranose. The comparison of the ^{13}C NMR chemical shifts of the sugar moiety of **4** with those of known sugars[8,9] conclusively confirmed the presence of the β-arabinopyranose unit in **4**. Again, the absolute configuration of the arabinose sugar in **4** could not be established because of the limited amount of material. Glycoside **4** is therefore 19-norpregna-1,3,5(10),20-tetraen-3-*O*-β-arabinopyranoside.

Experimental Section

General Experimental Procedures. The ^{1}H NMR, homonuclear COSY,[10] ^{13}C NMR, DEPT 90, DEPT 135,[11] and 2D NOESY experiments were carried out on a JEOL JNM-A500 spectrometer, operating at 500 MHz for protons and 125 MHz for carbon, and chemical shifts were referenced relative to the residual undeuterated solvent signal. The number of attached protons for the ^{13}C NMR signals was determined from DEPT experiments. Proton-detected heteronuclear correlations were measured using HMQC[12] (optimized for $^1J_{HC} = 145$ Hz) and HMBC[13] (optimized for $^nJ_{HC} = 8.0$ Hz) pulse sequences. The NOESY[14] was obtained with a 0.55 s mixing time. The EIMS spectra were recorded on a Micromass mass spectrometer (Platform II). Optical rotations were measured on a Perkin-Elmer 341 polarimeter using a sodium lamp operating at 589 nm. Infrared spectra and UV spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrometer and a Milton Roy UV-spectrometer (Genesys), respectively.

Animal Material. Samples of *S. pallida* were collected by scuba (−15 m) around Phuket Island in April 1995. Voucher specimens (PH594021) from this collection are maintained at the Marine Natural Product Research Unit, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand, and at the Department of Zoology, Faculty of Life Science, Tel-Aviv University. The soft coral was identified by Dr. Yehuda Benayahu, Faculty of Life Science, Tel-Aviv University.

Extraction and Isolation. Fresh samples were frozen immediately after collection and stored at −20 °C until processed. Samples were freeze-dried, yielding 262 g of dried material. The dried material was ground and extracted sequentially with $CHCl_3$ and MeOH. The $CHCl_3$ and MeOH fractions were evaporated at reduced pressure to give 8.2 and 15.5 g of extracts, respectively.

The $CHCl_3$ extract was subjected to a flash Si gel column using a gradient elution of hexane−$CHCl_3$−MeOH. Elution with 50% hexane in $CHCl_3$ gave a fraction (50 mg) that was rechromatographed on a Si column, yielding the known pregna-1,20-dien-3-one (**1**) (8 mg) and 19-norpregna-1,3,5(10),20-tetraen-3-ol (**2**) (12 mg). Later, elution with 30% $CHCl_3$ in MeOH gave a fraction (30 mg) that was subsequently subjected to gel filtration on a Sephadex LH-20 column, using 5% $CHCl_3$ in MeOH as eluent, to obtain 19-norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside (**3**) (9 mg) and 19-norpregna-1,3,5(10),20-tetraen-3-*O*-β-arabinopyranoside (**4**) (12 mg).

Bioassays. The antimalarial assay followed the method of Trager and Jensen[15] using continuous cultures (in vitro) of asexual erythrocytic stages of *P. falciparum* (K1, multidrug resistant strain). Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method described by Desjardins et al.[16] Effective concentration (EC_{50}) represents the concentration that causes 50% reduction in parasite growth as indicated by the in vitro uptake of [^{3}H]hypoxanthine by *P. falciparum*. An EC_{50} value of 1.60×10^{-7} g/mL (3.10×10^{-7} M) was observed for the standard sample, chloroquine diphosphate, in the same test system. Cytotoxicity was conducted on breast cancer cell line BCA-1 using the method previously described by Skehan et al.[17]

Pregna-1,20-dien-3-one (1): white amorphous solid; $[\alpha]^{25}_D$ +55° ($c = 0.57$, MeOH); EIMS m/z 298 (M^+, 27), 283 (32), 229 (21), 163 (23), 134 (35), 122 (100), 107 (60), 95 (65), 79 (78), 67 (64); HREIMS m/z 298.2293 (calcd for $C_{21}H_{30}O$, 298.2296).

19-Norpregna-1,3,5(10),20-tetraen-3-ol (2): white amorphous solid; $[\alpha]^{25}_D$ +23° ($c = 0.48$, MeOH); EIMS m/z 282 (M^+, 48), 213 (100), 160 (32), 133 (28), 91 (23), 77 (20); HREIMS m/z 282.1980 (calcd for $C_{20}H_{26}O$, 282.1983).

Compound 3: white amorphous solid; $[\alpha]^{25}_D$ +47° ($c = 0.26$, MeOH); UV (MeOH) λ_{max} (log ϵ) 281 (3.13), 274 (3.23), 202 (4.20) nm; FT-IR (KBr) ν_{max} 3339, 2931, 2870, 1496, 1450, 1381, 1250, 1173, 1023, 969, 819, 759 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 7.27 (1H, d, $J = 8.7$ Hz, H-1), 7.22 (1H, H-2), 7.10 (1H, d, $J = 2.8$ Hz, H-4), 6.07 (1H, d, $J = 3.6$ Hz, H-1′), 5.80

(1H, m, H-19), 5.05, 5.08 (each 1H, br s, H-20), 4.78 (1H, dd, J = 10.2, 3.4 Hz, H-2′), 4.72 (1H, dd, J = 10.1, 3.1 Hz, H-3′), 4.46 (1H, q, J = 6.7 Hz, H-5′), 4.25 (1H, br s, H-4′), 2.76 (2H, m, H-6), 2.21 (1H, qd, J = 13.4, 3.7 Hz, H-11a), 2.11 (1H, dt, J = 9.5, 3.4 Hz, H-9), 1.98 (1H, q, J = 9.4 Hz), 1.78 (1H), 1.74 (2H), 1.62 (1H, m), 1.54 (1H, d, J = 6.7 Hz), 1.52 (3H, d, J = 6.7 Hz), 1.38 (1H, dq, J = 13.2, 3.6 Hz), 1.26 (2H, m), 1.14 (1H, m), 1.04 (1H, m), 0.85 (1H), 0.58 (3H, s, H-18); ^{13}C NMR (C_5D_5N, 125 MHz) δ 156.38 (s, C-3), 140.12 (d, C-19), 138.26 (s, C-5), 134.43 (s, C-10), 126.85 (d, C-1), 117.67 (d, C-4), 114.95 (t, C-20), 114.94 (d, C-2), 99.87 (d, C-1′), 73.22 (d, C-4′), 71.62 (d, C-3′), 69.44 (d, C-2′), 68.28 (d, C-5′), 55.66 (d, C-17), 54.68 (d, C-14), 44.41 (d, C-9), 44.05 (s, C-13), 39.10 (d, C-8), 37.73 (t, C-12), 30.06 (t, C-6), 28.12 (t, C-7), 27.52 (t, C-16), 26.59 (t, C-11), 24.65 (t, C-15), 17.22 (q, C-6′), 13.00 (q, C-18); EIMS m/z 428 (M^+, 2), 282 (78), 213 (100), 146 (37), 75 (25); HREIMS m/z 428.2560 (calcd for $C_{26}H_{36}O_5$, 428.2562).

Compound 4: white amorphous solid; $[\alpha]^{25}_D$ +68° (c = 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (3.22), 274 (3.42), 207 (4.22) nm; FT-IR (KBr) ν_{max} 3340, 2929, 2870, 1492, 1451, 1379, 1252, 1170, 1080, 967, 915, 814, 760 cm^{-1}; ^{1}H NMR (C_5D_5N, 500 MHz) δ 7.27 (1H, d, J = 8.7 Hz, H-1), 7.22 (1H, dd, J = 8.7, 2.4 Hz, H-2), 7.10 (1H, d, J = 2.4 Hz, H-4), 6.13 (1H, d, J = 3.4 Hz, H-1′), 5.80 (1H, m, H-19), 5.05, 5.08 (each 1H, br s, H-20), 4.84 (1H, dd, J = 9.7, 3.4 Hz, H-2′), 4.74 (1H, dd, J = 9.5, 3.4 Hz, H-3′), 4.48 (1H, br s, H-4′), 4.30 (1H, dd, J = 11.9, 1.5 Hz, H-5′ax), 4.14 (1H, dd, J = 11.9, 2.4 Hz, H-5′eq), 2.76 (2H, m, H-6), 2.21 (1H, qd, J = 13.4, 3.7 Hz, H-11a), 2.11 (1H, dt, J = 9.5, 3.4 Hz, H-9), 1.98 (1H, q, J = 9.4 Hz), 1.78 (1H), 1.74 (2H), 1.62 (1H, m), 1.54 (1H, d, J = 6.7 Hz), 1.38 (1H, dq, J = 13.2, 3.6 Hz), 1.26 (2H, m), 1.14 (1H, m), 1.04 (1H, m), 0.85 (1H), 0.58 (3H, s, H-18); ^{13}C NMR (C_5D_5N, 125 MHz) δ 156.17 (s, C-3), 140.11 (d, C-19), 138.26 (s, C-5), 134.52 (s, C-10), 126.84 (d, C-1), 117.61 (d, C-4), 114.99 (t, C-20), 114.95 (d, C-2), 100.03 (d, C-1′), 70.98 (d, C-3′), 70.37 (d, C-2′), 70.19 (d, C-4′), 65.06 (t, C-5′), 55.65 (d, C-17), 54.66 (d, C-14), 44.41 (d, C-9), 44.03 (s, C-13), 39.10 (d, C-8), 37.71 (t, C-12), 30.05 (t, C-6), 28.11 (t, C-7), 27.52 (t, C-16), 26.59 (t, C-11), 24.64 (t, C-15), 13.00 (q, C-18); EIMS m/z 414 (M^+, 2), 282 (79), 213 (100), 160 (20), 73 (30); HREIMS m/z 414.2403 (calcd for $C_{25}H_{34}O_5$, 414.2406).

Acetylation of 3 and 4. Compounds **3** (3 mg) and **4** (4 mg) were acetylated with Ac_2O (50 μL) and anhydrous pyridine (60 μL) at room temperature for 20 h, followed by a standard workup: ^{1}H NMR ($CDCl_3$, 500 MHz) for acetylated product of **3** δ 7.21 (1H, d, J = 8.0 Hz), 6.85 (1H, d, J = 8.2 Hz), 6.71 (1H, s), 5.81 (1H, m), 5.71 (1H, d, J = 3.5 Hz), 5.50 (1H, dd, J = 10.9, 3.4 Hz), 5.31 (1H. br s), 5.20 (1H, dd, J = 10.9, 3.5 Hz), 4.99 (2H, br d, J = 12.1 Hz), 4.30 (1H, q, J = 6.7 Hz), 2.82 (2H, m), 2.25 (1H, qd, J = 13.4, 3.6 Hz), 2.18 (1H, dt, J = 10.1, 3.1 Hz), 2.04 (1H, q, J = 8.8 Hz), 1.86 (1H), 1.82 (1H), 1.80 (1H), 1.76 (1H, m), 1.60 (1H, dq, J = 10.3, 2.3 Hz), 1.50 (d, J = 6.7 Hz), 1.45 (1H, dq, J = 13.2, 3.6 Hz), 1.38 (1H), 1.36 (1H), 1.23−1.25 (3H), 0.6 (3H, s); EIMS m/z 554 (M^+, 4), 282 (70), 213 (100); ^{1}H NMR ($CDCl_3$, 500 MHz) for acetylated product of **4** δ 7.21 (1H, d, J = 8.0 Hz), 6.85 (1H, d, J = 8.2 Hz), 6.75 (1H, s), 5.81 (1H, m), 5.71 (1H, d, J = 3.5 Hz), 5.54 (1H, dd, J = 10.8, 3.3 Hz), 5.38 (1H. br s), 5.29 (1H, dd, J = 10.8, 3.5 Hz), 4.99 (2H, br d, J = 12.1 Hz), 4.04 (1H, dd, J = 12.2, 1.5 Hz), 3.72 (1H, dd, J = 12.2, 1.5 Hz), 2.82 (2H, m), 2.25 (1H, qd, J = 13.4, 3.6 Hz), 2.18 (1H, dt, J = 10.1, 3.1 Hz), 2.04 (1H, q, J = 8.8 Hz), 1.86 (1H), 1.82 (1H), 1.80 (1H), 1.76 (1H, m), 1.60 (1H, dq, J = 10.3, 2.3 Hz), 1.45 (1H, dq, J = 13.2, 3.6 Hz), 1.38 (1H), 1.36 (1H), 1.23−1.25 (3H), 0.6 (3H, s); EIMS m/z 540 (M^+, 5), 282 (64), 213 (100), 160 (45).

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