Pregnane-Based Steroids from a Formosan Gorgonian *Subergorgia mollis*

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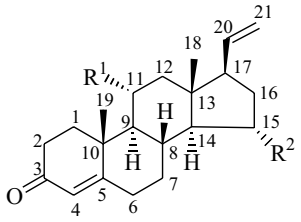
A new steroid, 11α,15α-diacetoxy-17α-pregna-4,20-dien-3-one (**1**), and a known one, 17α-pregna-4,20-dien-3-one (**2**), have been isolated from a Formosan gorgonian *Subergorgia mollis*. The structures of both compounds were determined on the basis of extensive NMR experiments, including HMQC, HMBC, ^{1}H-^{1}H COSY and NOESY. Metabolite **2** has not been isolated from a natural source before. The detailed ^{1}H and ^{13}C NMR spectral data of **2** are reported for the first time.

Keywords: Gorganian; *Subergorgia mollis*; Pregnane-Based Steroids.

INTRODUCTION

Previous investigations on secondary metabolites from Gorgonian corals of the genus *Subergorgia* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Gorgonacea, family Subergorgiidae) have shown the presence of a variety of sesquiterpenes[1-8] and steroids.[9] The Gorgonian coral *Subergorgia mollis* (Nutting, 1910) occurs widely in Taiwanese tropical waters, and its chemical constituents have not been reported. In this paper, we wish to describe the isolation and structural elucidation of a new steroid, 11α,15α-diacetoxy-17α-pregna-4,20-dien-3-one (**1**), and a known one, 17α-pregna-4,20-dien-3-one (**2**), from a Formosan gorgonian, *S. mollis*. The structures of the two pregnane-based steroids were determined on the basis of extensive NMR experiments (HMQC, HMBC, ^{1}H-^{1}H COSY and NOESY). Metabolite **2** was isolated as a natural product for the first time.



1: $R^1 = R^2 = OAc$
2: $R^1 = R^2 = H$

RESULTS AND DISCUSSION

The new metabolite **1** was obtained as a white solid. Its HRFABMS showed a pseudomolecular ion peak, $[M + H]^+$ at *m/z* 415.2474, appropriate for the molecular formula $C_{25}H_{34}O_5$ and thus requiring nine degrees of unsaturation. The ^{13}C NMR spectrum displayed signals for 25 carbons, which were assigned by the DEPT spectrum into four methyls, seven methylenes, and eight methines (Table 1). The remaining six signals appearing in the broad-band spectrum were attributed to the quaternary carbon atoms. An IR absorption band at 1726 cm^{-1} and the significant FABMS peaks at *m/z* 415 $[M + H]^+$, 355 $[M + H - HOAc]^+$, and 295 $[M + H - 2\ HOAc]^+$, revealed the presence of two acetoxy groups in **1**. The presence of two acetoxy moieties was further confirmed by ^{13}C NMR spectrum, with signals at δ 170.0 (s) and 170.9 (s), and by ^{1}H NMR spectrum (Table 2) with two acetyl methyl singlets at δ 2.02 and 2.03. An IR absorption band at 1667 cm^{-1} indicated the existence of a conjugate enone structural unit, which was further confirmed by a UV absorption at 248 nm. The signal at δ 5.76 (1H, s) was assigned for the proton attached to the α-carbon of the enone moiety. The presence of the enone unit could also be proved by the ^{13}C NMR spectrum, which showed signals at δ 199.3 (s), 124.7 (d), and 169.4 (s). The presence of a vinyl group was deduced from ^{1}H NMR signals at δ 5.65 (1H, ddd, J = 17.1, 10.2, 7.0 Hz), 5.05 (1H, d, J = 10.2 Hz), and 5.01 (1H, d, J = 17.1 Hz), and car-

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Table 1. ^{13}C NMR Data of Compounds **1** and **2**

Position	**1**[a]	**2**[a]
1	36.5 (t)[b]	35.7 (t)
2	34.1 (t)	33.9 (t)
3	199.3 (s)	199.7 (s)
4	124.7 (d)	123.8 (d)
5	169.4 (s)	171.6 (s)
6	33.2 (t)	32.9 (t)
7	31.2 (t)	32.0 (t)
8	34.7 (d)	35.7 (d)
9	55.6 (d)	54.0 (d)
10	39.8 (s)	38.7 (s)
11	70.5 (d)	20.6 (t)
12	44.1 (t)	37.2 (t)
13	43.6 (s)	43.4 (s)
14	57.4 (d)	55.0 (d)
15	75.5 (d)	24.7 (t)
16	36.8 (t)	27.1 (t)
17	52.6 (d)	55.2 (d)
18	14.6 (q)	12.8 (q)
19	18.5 (q)	17.4 (q)
20	136.7 (d)	139.5 (d)
21	116.6 (t)	114.8 (t)
acetate	21.3 (q)	
methyls	21.8 (q)	
carbonyls	170.0 (s)	
	170.9 (s)	

[a] Spectra recorded at 125 MHz in $CDCl_3$ at 25 °C.
[b] Multiplicity deduced by DEPT and indicated by usual symbols. The values are in ppm downfield from TMS.

Table 2. ^{1}H NMR Data of Compounds **1** and **2**

Position	**1**[a]	**2**[a]
1α	1.95 m	1.70 m
β	1.99 m	2.02 m
2α	2.40 m	2.42 m
β	2.38 m	2.35 m
4	5.76 s	5.73 s
6α	2.30 m	2.29 m
β		2.39 m
7α	1.25 m	1.04 m
β	1.82 m	1.86 m
8	1.84 m	1.68 m
9	1.51 m	0.95 ddd
		(11.5, 11.5, 4.5)
11α		1.40 m
β	5.22 ddd	1.55 m
	(10.5, 10.5, 5.5)[b]	
12α	1.21 m	1.05 m
β	2.08 m	1.72 m
14	1.51 m	1.02 m
15α		1.22 m
β	4.92 ddd	1.71 m
	(8.5, 3.5, 3.5)	
16α	1.68 m	1.80 m
β	2.31 m	1.57 m
17	2.28 m	1.97 m
18	0.78 s	0.64 s
19	1.28 s	1.25 s
20	5.65 ddd	5.75 ddd
	(17.1, 10.2, 7.0)	(17.5, 10.4, 7.5)
21	5.05 d (10.2)	4.98 d (10.4)
	5.01 d (17.1)	4.97 d (17.5)
acetate	2.02 s	
methyls	2.03 s	

[a] Spectra recorded at 500 MHz in $CDCl_3$ at 25 °C.
[b] *J* values (in Hz) in parentheses.

bons at δ 136.7 (d), and 116.6 (t). The molecular framework was established by ^{1}H-^{1}H COSY and HMBC spectra (Fig. 1). From the ^{1}H-^{1}H COSY spectrum of **1**, it was possible to establish the sequential proton sets from H_2-1 to H_2-2; H_2-6 to H-9; H-8 to H-14; H-9 to H-11; H-11 to H_2-12; H-14 to H-17; H-17 to H-20; and H-20 to H_2-21. The HMBC spectrum of **1** showed ^{1}H-^{13}C long range correlations of H_3-19 with C-1, C-5, C-9 and C-10, and H_3-18 with C-12, C-13, C-14 and

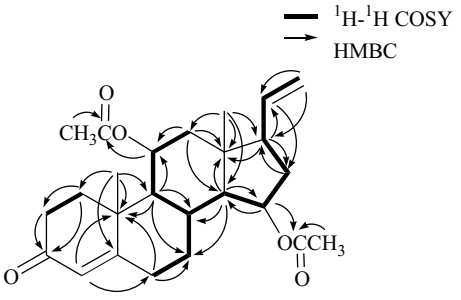

Fig. 1. ^{1}H-^{1}H COSY and HMBC Correlations for **1**.

C-17, suggesting that the ring-junctured C-18 and C-19 methyl groups should be positioned at C-13 and C-10, respectively. Comparison of the ^{1}H NMR spectral data of compound **1** with those of compound **2**, which was also isolated from the present study (discussed below), revealed that **1** is the diacetoxy derivative of **2**. The placement of acetoxy groups at C-11 and C-15 was confirmed from the HMBC connectivities of H-11 (δ 5.22) and H-15 (δ 4.92) with the carbonyl carbons of esters resonating at δ 170.0 (s) and 170.9 (s), respectively. Furthermore, both H_2-1 and H_2-2 have been shown to exhibit HMBC correlations with the enone carbonyl carbon at δ 199.3, revealing the ketonic functionality of C-3. On the basis of the above observations, metabolite **1** was determined to be the 11,15-diacetoxy derivative of pregna-

4,20-dien-3-one.

The relative stereochemistry of **1** was determined by the NOE correlations observed in the NOESY spectrum of **1** (Fig. 2). It was found that H-8 had NOE interactions with H_3-18 and H_3-19, but not with H-14 and H-9, suggesting the β-orientations of H-8, H_3-19, and H_3-18, and the α-orientations of H-9 and H-14. The H_3-19 showed NOE interaction with H-11, and H_3-18 showed NOE interactions with H-11, H-15, and H-20, indicating that H-11, H-15, and the vinyl group attached at C-17 should be in the β face. Finally, it was found that H-14 exhibited NOE crosspeaks with both H-9 and H-17, confirming the α-orientations of these three protons. On the basis of the above analyses, the structure of **1** was established unambiguously as 11α,15α-diacetoxy-17α-pregna-4,20-dien-3-one.

The other isolated compound, 17α-pregna-4,20-dien-3-one (**2**), has been obtained by chemical transformations.[10-14] To the best of our knowledge, **2** has not been isolated previously from natural sources. The ^{13}C NMR data and the detailed assignments of ^{1}H NMR spectral data for compound **2** are disclosed (Tables 1 and 2) for the first time by extensive analyses of a series of 2D NMR (HMQC, HMBC, ^{1}H-^{1}H COSY and NOESY) spectral data.

EXPERIMENTAL SECTION

General Methods

Melting points were determined using a Fisher-Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. UV spectra were taken in MeOH on a Perkin Elmer UV/VIS Lambda EZ 201 spectrometer. LRMS were obtained with a VG Quattro GC/MS spectrometer. HRMS were recorded on a JEOL JMS-SX/SX 102A mass spectrometer. The NMR spectra were recorded on a Varian UNITY INOVA 500 FT-NMR at 500 MHz for ^{1}H and 125 MHz for ^{13}C, in $CDCl_3$ using TMS as an internal standard. Silica gel (Merck, 230-400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F_{254}, 0.2 mm) were used for analytical TLC. High performance liquid chromatography (HPLC) was achieved on a Hitachi L-7100 apparatus equipped with a Hitachi L-7400 UV detector, by using normal phase column (250 × 4.6 mm 5 μm Hypersil HS silica).

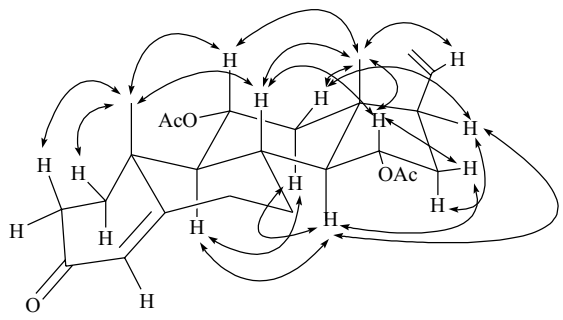

Fig. 2. Key NOESY Correlations for **1**.

Organism

The gorgonian *S. mollis* was collected by hand using scuba along the coast of southern Taiwan, in February 2001, at a depth of 18 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Resources, National Sun Yat-Sen University (specimen no. SC020704).

Extraction and Isolation

The gorgonian *S. mollis* (776 g fresh wt) was freeze-dried, minced and subsequently extracted with EtOAc (3 L × 6). The organic extract was evaporated under reduced pressure to give a residue (8.11 g). The residue was separated by Si gel column chromatography using *n*-hexane and *n*-hexane-EtOAc mixtures of increasing polarity to yield 32 fractions. Fraction 8 (52 mg) eluted with hexanes/EtOAc (18:1) was rechromatographed to yield compound **2** (20.5 mg). Fraction 22 (75 mg) eluted with *n*-hexane/EtOAc (5:1) was further chromatographed on Si gel by HPLC using *n*-hexanes-EtOAc (7:3) to yield **1** (1.3 mg).

11α,15α-diacetoxy-17α-pregna-4,20-dien-3-one (1)

White solid; mp: 100-102 °C; $[\alpha]_D^{27}$: +61.4° (*c* 0.44, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 243 (4.08) nm; IR ($CHCl_3$) ν_{max} 1726, 1667 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2; FABMS *m/z* (rel. int %) 415 [0.2, $(M + H)^+$], 355 [0.2, $(M + H - HOAc)^+$], 295 [0.3, $(M + H - 2\ HOAc)^+$] ; HRFABMS *m/z* 415.2474 (calcd. for $C_{25}H_{34}O_5$ + H, 415.2486).

17α-Pregna-4,20-dien-3-one (2)

White solid; mp: 72-73 °C; $[\alpha]_D^{27}$: +57.0° (*c* 2.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 241 (4.09) nm; IR ($CHCl_3$) ν_{max} 1663 cm^{-1}; ^{1}H and ^{13}C NMR spectral data, see Tables 1 and 2; EIMS *m/z* (rel. int %) 298 [28, M^+], 283 (21), 229 (22), 121 (31), 91 (100); HREIMS *m/z* 298.2306 (calcd. for $C_{21}H_{30}O$, 298.2298).

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