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New anti-inflammatory sterols from a gorgonian *Pinnigorgia* sp.

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ABSTRACT

Chemical investigation on the EtOAc-soluble fraction from the MeOH/DCM extract of a gorgonian *Pinnigorgia* sp. afforded two new sterols, 11-acetoxy-24*S*-methyl-3β,5α,6α-trihydroxy-9,11-secocholest-7-en-9-one (**1**) and 5β,6β-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**2**). The structures of sterols **1** and **2** were elucidated on the basis of spectroscopic analysis and by comparison of their spectroscopic data with those of related analogues. Both **1** and **2** were shown to significantly inhibit the accumulation of the pro-inflammatory iNOS and COX-2 protein in LPS-stimulated RAW264.7 macrophage cells.

Studies on the chemical constituents of octocorals collected off the waters of Taiwan have led to the isolation of various 9,11-secosterols from *Cespitularia*,[1] *Cladiella*,[2] and *Sinularia* spp.[3–6] Sterols of this type were found to possess interesting bioactivities, such as cytotoxic,[1–6] anti-inflammatory,[3] and antiviral activities.[5,6] In our continuing investigation of bioactive natural substances from Formosan marine invertebrates, two new sterols, 11-acetoxy-24*S*-methyl-3β,5α,6α-trihydroxy-9,11-secocholest-7-en-9-one (**1**) and 5β,6β-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3β,7β-diol (**2**), were obtained from *Pinnigorgia* sp. (family Gorgoniidae) (Fig. 1). We report herein the isolation, structural elucidation, and anti-inflammatory activity of sterols **1** and **2**.

11-Acetoxy-24*S*-methyl-3β,5α,6α-trihydroxy-9,11-secocholest-7-en-9-one (**1**) was isolated as colorless oil. HRESIMS of **1** exhibited a pseudo molecular peak at *m*/*z* 529.35023 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6$ + Na, 529.34996) and established a molecular formula of $C_{30}H_{50}O_6$, indicating six degrees of unsaturation. The presence of hydroxy, ester, and α,β-unsaturated ketone groups were suggested by absorptions at 3394, 1740, and 1678 cm^{-1} in the IR spectrum. The ^{13}C NMR and DEPT spectroscopic data of **1** (Table 1) showed the presence of 30 carbon atoms, including seven methyls, nine sp^3 methylenes, seven sp^3 methines, one sp^2 methine, three sp^3 quaternary carbons, and three sp^2 quaternary carbons. The ketonic carbonyl signal at δ_C 202.1 combined with the chemical shifts of H_3-18 (δ_H 0.70, 3H, s), H_3-19 (δ_H 1.24, 3H, s), H_3-21 (δ_H 0.98, 3H, d, J = 6.8 Hz), H-3 (δ_H 3.99, 1H, m), H-6 (δ_H 4.45, 1H, br s), H-7 (δ_H 6.24, 1H, d, J = 1.6 Hz), and H_2-11 (δ_H 4.14, 2H, m) were found to be similar to those of 3,5,6-trihydroxy-9,11-seco-7-en-9-one sterol analogues.[7,8] Moreover, the signals appearing at δ_C 66.8 (CH), 79.4 (C), 70.8 (CH), 140.6 (CH), 137.7 (C), 202.1 (C), and 61.5 (CH_2) indicated the presence of functional groups of three hydroxy, one α,β-unsaturated ketone, and one acetoxymethylene in **1**. From the ^1H–^1H COSY spectrum of **1**, the proton sequences from H_2-1/H_2-2/H-3/H_2-4, H-6/H-7, H_2-11/H_2-12, H-14/H_2-15/H_2-16/H-17/ H-20/H_2-22/H_2-23/H-24/H-25/H_3-26, H-20/H_3-21, H-24/H_3-28, and H-25/H_3-27 (Fig. 2) were established. These data, together with the key HMBC correlations between H_2-1/C-10; H_2-4/C-5, -10; H-7/C-5, -9; H_2-11/C-13; H_2-12/C-13; H-14/C-7, -8, -9, -13; H_2-16/C-13; H_3-18/C-12, -13, -14, -17; and H_3-19/C-1, -5, -9, -10, permitted the elucidation of the main carbon skeleton of **1** (Fig. 2). An HMBC correlation

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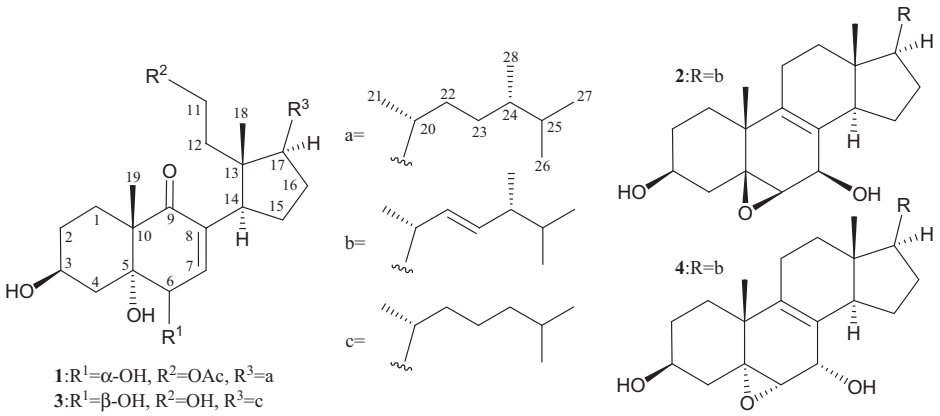

Figure 1. The structures of 11-acetoxy-24*S*-methyl-3*β*,5*α*,6*α*-trihydroxy-9,11-secocholest-7-en-9-one (**1**), 5*β*,6*β*-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3*β*,7*β*-diol (**2**), 3*β*,5*α*,6*β*,11-tetrahydroxy-9,11-secocholest-7-en-9-one (aplidiasterol B) (**3**), and 5*α*,6*α*-epoxy-(22*E*,24*R*)-ergosta-8,22-diene-3*β*,7*α*-diol (**4**).

between H_2-11 (δ_H 4.14) and the acetate carbonyl (δ_C 171.2) revealed the presence of an acetate ester at C-11. By comparison of NMR data with those of a known sterol, 3*β*,5*α*,6*β*,11-tetrahydroxy-9,11-secocholest-7-en-9-one (aplidiasterol B) (**3**),[7] the planar structure of **1** was established.

The stereochemistry of **1** was elucidated from the correlations observed in the NOESY experiment and by comparison of NMR data with those of sterol **3**. The configurations at C-3, C-5, C-10, C-13, C-14, C-17, and C-20 in **1** were found to be the same as those of **3**. Key NOE correlations for **1** showed interactions between H-3/H-4*α* (δ_H 2.22) and H-6/H_3-19. Thus, H-3 and H-6 should be positioned on the *α*- and *β*-face, respectively (Fig. 3). The configuration of stereogenic center at C-24 was assigned as *S* on the basis

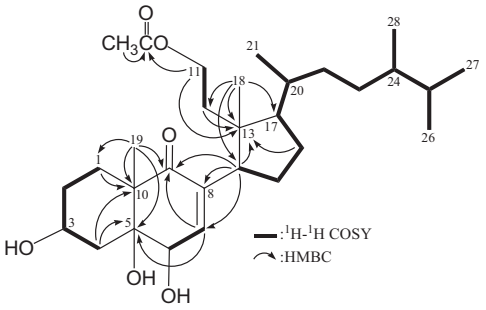

Figure 2. The ^1H–^1H COSY and key HMBC correlations of **1**.

Table 1
NMR data for sterols **1** and **2**

Position	1		2	
	δ_H (*J* in Hz)[a]	δ_C, Mult.[b]	δ_H (*J* in Hz)[a]	δ_C, Mult.[b]
1	2.05 m; 1.71 m	27.2, CH_2	1.80 m; 1.62 m	30.2, CH_2
2	1.96 m; 1.45 m	30.1, CH_2	2.01 m; 1.61 m	30.9, CH_2
3	3.99 m	66.8, CH	3.96 m	68.6, CH
4*α*/*β*	2.22 ddd (13.6, 4.8, 1.6); 1.60 m	38.5, CH_2	1.46 m; 2.19 dd (12.4, 11.6)	39.2, CH_2
5		79.4, C		65.6, C
6	4.45 br s	70.8, CH	3.31 d (2.4)	62.5, CH
7	6.24 d (1.6)	140.6, CH	4.22 br d (10.8)	67.1, CH
8		137.7, C		126.9, C
9		202.1, C		134.5, C
10		49.3, C		38.0, C
11	4.14 m	61.5, CH_2	2.08 m	23.8, CH_2
12	1.63 m; 1.27 m	37.1, CH_2	1.96 m; 1.42 m	35.7, CH_2
13		46.1, C		42.1, C
14	3.22 dd (10.0, 10.0)	43.5, CH	2.19 dd (12.4, 11.6)	49.6, CH
15	1.66 m	26.7, CH_2	1.83 m; 1.30 m	29.0, CH_2
16	1.84 m; 1.41 m	26.0, CH_2	1.26 m; 1.14 m	23.4, CH_2
17	1.67 m	50.4, CH	1.19 m	53.7, CH
18	0.70 s	17.2, CH_3	0.59 s	11.3, CH_3
19	1.24 s	19.7, CH_3	1.14 s	22.8, CH_3
20	1.40 m	35.1, CH	2.04 m	40.4, CH
21	0.98 d (6.8)	19.2, CH_3	1.02 d (6.4)	20.9, CH_3
22	1.43 m; 0.95 m	33.0, CH_2	5.13 dd (15.2, 5.2)	135.5, CH
23	1.51 m; 0.95 m	31.4, CH_2	5.22 dd (15.2, 8.0)	132.0, CH
24	1.22 m	39.1, CH	1.84 m	42.8, CH
25	1.57 m	31.5, CH	1.48 m	33.1, CH
26	0.79 d (6.8)	17.6, CH_3	0.84 d (6.4)	19.9, CH_3
27	0.86 d (6.8)	20.5, CH_3	0.82 d (6.8)	19.6, CH_3
28	0.78 d (6.8)	15.4, CH_3	0.91 d (6.8)	17.6, CH_3
11-OAc		171.2, C		
	2.01 s	21.2, CH_3		
7-OH			1.76 d (10.8)	

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.