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7α-Hydroxytheonellasterol, a Cytotoxic 4-Methylene Sterol from the Philippines Sponge *Theonella swinhoei*

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A new 4-methylene sterol, 7α-hydroxytheonellasterol (**3**), was isolated from the Philippines sponge *Theonella swinhoei*, along with the known compounds theonellasterol (**1**) and bistheonellide A. The structure of **3** was elucidated by interpretation of its spectroscopic data, which were compared with those of **1**.

Marine sponges of the genus *Theonella* are distinctive in producing biosynthetically unique 4-methylene sterols. To date, there are only four papers in the literature describing these steroids. In 1981, Djerassi et al. described the isolation and structure elucidation of conicasterol and theonellasterol (**1**) from *Theonella* spp. from the Red Sea.[1] Subsequently, Kitagawa et al.[2] reported two 3-keto-4-methylene sterols, Inouye et al.[3] isolated nine new oxygenated 4-methylene sterols, and Umeyama et al.[4] described three additional 4-methylene sterols, swinhosterols A, B, and C (**2**), all from Japanese *Theonella* spp. We describe herein the isolation of 7α-hydroxytheonellasterol (**3**) from *T. swinhoei* Gray, 1868 (Lithistida) collected in the Philippines. The EtOAc-soluble material from a methanolic extract of the massive henna-colored sponge *T. swinhoei* displayed activity in an eight cell-line cytotoxicity panel. In addition to isolating the known compounds theonellasterol (**1**)[1] and bistheonellide A,[5] we now report the isolation and identification of 7α-hydroxytheonellasterol (**3**).

Specimens of the sponge *T. swinhoei* were collected by hand in the Philippines in May 1996, and kept frozen until extracted with methanol. A concentrated methanolic extract, which was obtained after soaking the diced sponge tissue (400 g wet wt), was partitioned between ethyl acetate and water. The ethyl acetate extract was found to be active in the eight cell-line panel. The EtOAc-soluble material was further partitioned between hexane and methanol, and the hexane-soluble material concentrated under vacuum and then chromatographed on Si gel, followed by normal-phase HPLC, to obtain theonellasterol (**1**, 38 mg, 9.5×10^{-3} % wet wt) and 7α-hydroxytheonellasterol (**3**, 12 mg, 3×10^{-3}% wet wt).

7α-Hydroxytheonellasterol (**3**) was isolated as an optically active colorless oil. The molecular formula, $C_{30}H_{50}O_2$, which was established by HRFABMS and NMR data (Table 1), requires six degrees of unsaturation. The IR spectrum contained a hydroxyl band at 3340 cm^{-1}. The ^{13}C NMR spectrum of **3** (Table 1, $CDCl_3$) revealed the presence of 30 carbon atoms, two of which were oxygen-bearing methine carbons resonating at δ 79.8 and 73.2. Other characteristic signals observed, together with the results of the ^1H NMR, DEPT, and gHMQC experiments, strongly suggested the presence of two double bonds (153.6, 152.3, 122.4, 103.1). The ^1H NMR spectrum (Table 1, $CDCl_3$) contained two methyl singlet resonances at δ 0.87 (Me-18) and 0.60 (Me-19); three methyl doublet resonances at 0.97 ($J = 6.5$ Hz, Me-21), 0.83 ($J = 6.6$ Hz, Me-27), and 0.81 ($J = 6.6$ Hz, Me-26), and one methyl triplet resonance at 0.86 ($J = 6.6$ Hz, Me-29). The low-field portion of the ^1H NMR spectrum contained four one-proton signals, two of which (at δ 4.03 and 4.84) were assigned to protons attached to carbons bearing oxygen atoms, and two broad singlets at 4.64 and 5.11, which, from the COSY experiment, were coupled to each other. The HMQC experiment indicated that these two protons were correlated to a single carbon atom at δ 103.1. The relatively large difference in the chemical shifts between these two protons, together with the ^{13}C NMR resonance, is characteristic of an exomethylene group with a hydroxyl group on an adjacent carbon. These data

Table 1. ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Assignments for 7α-Hydroxytheonellasterol (**3**)

C no.	δ_C	δ_H	mult, J (Hz)	HMBC
1	36.3	1.36	m, 1 H	C-3, C-19
		1.74	m, 1 H	
2	32.9	1.39	m, 1 H	C-3
		2.00	m, 1 H	
3	73.2	4.03	m, 1 H	C-1, C-30
4	152.3			
5	42.6	2.20	m, 1 H	C-7, C-19, C-30
6	27.3	1.59	m, 1 H	C-8
		2.07	m, 1 H	
7	79.8	4.84	t, 1 H, 2.8, 2.8	C-5, C-8, C-9, C-14
8	122.4			C-6
9	44.6	2.13	m, 1 H	C-19
10	39.6			C-9, C-19
11	19.9	1.44	m, 1 H	C-8
		1.63	m, 1 H	
12	36.8	1.22	m, 1 H	C-14, C-18
		1.99	m, 1 H	
13	43.5			C-18
14	153.6			C-7, C-18
15	25.6	2.59	m, 2 H	
16	26.8	1.38	m, 1 H	
		1.87	m, 1 H	
17	56.5	1.23	m, 1 H	C-18, C-21
Me-18	17.9	0.87	s, 3 H	C-12, C-14, C-17
Me-19	12.4	0.60	s, 3 H	C-1, C-5, C-9, C-10
20	34.8	1.45	m, 1 H	C-21
Me-21	19.2	0.97	d, 3 H, 6.5	C-17, C-20, C-22
22	33.6	1.06	m, 1 H	C-21
		1.37	m, 1 H	
23	26.2	1.06	m, 1 H	
		1.38	m, 1 H	
24	46.0	0.94	m, 1 H	C-23, C-28
25	28.9	1.67	m, 1 H	C-28
Me-26	19.0	0.81	d, 3 H, 6.6	
Me-27	19.5	0.83	d, 3 H, 6.6	C-24, C-25, C-26
28	23.0	1.34	m, 2 H	C-24, C-25, C-29
Me-29	12.3	0.86	t, 3 H, 6.6	C-28
30	103.1	4.64	br s, 1 H	C-3, C-4, C-5
		5.11	br s, 1 H	
OH		6.75	br s, 1 H	
		7.65	br s, 1 H	

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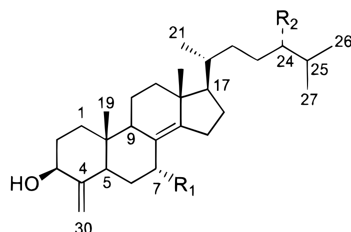
indicated that **3** was a C_{30} sterol with an ethyl group, two hydroxy groups, and an exocyclic methylene. Comparison of the ^1H and ^{13}C NMR spectra with those of the known compounds theonellasterol (**1**), the diol **4**,[3] and swinhosterol C (**2**)[4] provided further evidence for a 4-methylene sterol with an extra hydroxyl group at C-7 and an ethyl group at C-24. The structure of **3** was confirmed on the basis of COSY and HMBC correlations. COSY correlations between H-28 (δ 1.34) and both Me-29 (0.86) and H-24 (0.94) provided evidence of the ethyl group at C-24. The location of the two hydroxyl groups and two double bonds was established by a series of HMBC correlations observed from C-3 to H-30 to C-5 to H-7 to C-14 to Me-18.



1 R_1 = H, R_2 = Et
2 R_1 = OMe, R_2 = Et
3 R_1 = OH, R_2 = Et
4 R_1 = OH, R_2 = Me

The relative stereochemistry at C-7 was deduced from the small vicinal coupling constants of H-7, which are consistent with an equatorial conformation for this proton, thereby placing the hydroxyl group in an axial α-orientation. This was confirmed by the relatively low-field shifts of H-5 and H-9 caused by the 1,3-diaxial relationship of these protons to the hydroxy group at C-7, an effect that was also observed in compound **4**.[3]

The methanol-soluble material was chromatographed on Amberlite XAD-2 resin followed by Sephadex LH-20 to obtain bistheonellide A (35 mg, 8.75×10^{-3}% wet wt), which had identical spectral data to those described in the literature.[5]

Bistheonellide A, 7α-hydroxytheonellasterol (**3**), and theonellasterol (**1**) were tested for in vitro anticancer activity against an eight cell-line panel. They exhibited inhibitory activity with mean IC_{50} values for the eight cell lines of 0.15, 29.5, and >100 μM, respectively. Although the cytotoxicity of 7α-hydroxytheonellasterol is moderate compared with that of bistheonellide A, the increase in cytotoxicity caused by the addition of a 7α-hydroxyl group to theonellasterol appears to be significant.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. IR and UV spectra were recorded on Perkin-Elmer 1600 FT-IR and Lambda 3B instruments, respectively. The ^1H, g(radient)COSY, gHMQC, and gHMBC spectra were recorded on a Varian Inova 300 MHz spectrometer, and the ^{13}C and DEPT experiments were performed on a Varian Gemini 400 MHz spectrometer. HRMS data were obtained from the UC Riverside Regional Mass Spectrometry Facility. All solvents were distilled prior to use.

Animal Material. Specimens of *T. swinhoei* (Theonellidae, Lithistida) were collected by hand using scuba at Panglao Island, Bohol, Philippines, in 1996, immediately frozen after collection and kept frozen until used. The sponge has a smooth outer surface, a henna-colored exterior, and a cream-colored interior. The physical description and spicule measurements are in general agreement with those described in the literature.[6]

Extraction and Isolation. The frozen sponge (400 g wet wt) was diced and extracted with MeOH (3 × 300 mL). The combined MeOH extracts were concentrated and partitioned between equal volumes of EtOAc and H_2O (2 × 300 mL). The combined EtOAc extracts were concentrated under vacuum and further partitioned between hexane and methanol. The hexane-soluble material was concentrated and chromatographed on Si gel using a gradient of 100% hexane to 40% hexane in EtOAc as eluent, followed by HPLC on a Si gel column (Microsorb, 5 μ, 10 mm × 250 mm) with 85% hexane in EtOAc to yield theonellasterol (**1**, 38 mg, 9.5×10^{-3}% wet wt) and with 75% hexane in EtOAc to yield 7α-hydroxytheonellasterol (**3**, 12 mg, 3×10^{-3}% wet wt). The MeOH-soluble material was concentrated under vacuum and chromatographed on Amberlite XAD-2 resin followed by Sephadex LH-20 using MeOH as eluent to obtain bistheonellide A (35 mg, 8.75×10^{-3} % wet wt).

7α-Hydroxytheonellasterol (3): colorless oil; $[\alpha]_D$ +19.4° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} 248 nm (ϵ 1560); IR (film) ν_{max} 3340 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m/z* (rel int) 442 $[M]^+$ (7.6); HRFABMS *m/z* 441.3718 $[M - H]^+$ (calcd for $C_{30}H_{49}O_2$, 441.3733).

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