Two New 26,27-Cyclosterols from the Marine Sponge *Strongylophora corticata*

Akemi Umeyama,* Seiichi Ito, Ayuko Yoshigaki, and Shigenobu Arihara

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

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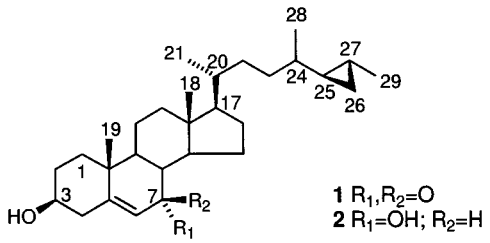
Two novel steroids with a cyclopropane ring at C-25 and C-26, 7-oxopetrosterol (**1**) and 7α-hydroxypetrosterol (**2**), along with two known compounds, petrosterol (**3**) and 23,24-dihydrocalysterol (**4**), have been isolated from the Japanese marine sponge *Strongylophora corticata*. The structures of **1** and **2** were determined as 26,27-cyclo-24,27-dimethyl-3*β*-hydroxycholest-5-en-7-one and 26,27-cyclo-24,27-dimethylcholest-5-en-3*β*,7α-diol on the basis of spectroscopic investigations.

In the past quarter century, a number of steroids with unusual side chains have been isolated from marine invertebrates.[1,2] Steroids with a cyclopropane ring at C-25 and C-27 are relatively rare and were first reported in 1978 from sponges, a *Petrosia ficiformis*[3] and a *Halichondria* sp.[4] As part of an ongoing investigation of metabolites isolated from marine organisms, it was found that extracts of the sponge *Strongylophora corticata* Wilson (Petrosiidae) contained two new steroids with a cyclopropane ring at C-25 and C-27, 7-oxopetrosterol (**1**) and 7α-hydroxypetrosterol (**2**), along with two known compounds, petrosterol (**3**)[3,4] and 23,24-dihydrocalysterol (**4**).[5] We now describe the isolation and structure elucidation of 7-oxopetrosterol (**1**) and 7α-hydroxypetrosterol (**2**).



The sponge *S. corticata* was extracted with MeOH and MeOH/CH_2Cl_2 (3:1). The combined extract was divided into EtOAc- and H_2O-soluble portions. The EtOAc-soluble portion was chromatographed on Sephadex LH-20 and Si gel columns. Final purification by reversed-phase HPLC afforded **1**−**4**.

7-Oxopetrosterol (**1**) was obtained as an amorphous powder. The molecular formula of **1** was established as $C_{29}H_{46}O_2$ on the basis of HRCIMS and corresponds to seven degrees of unsaturation. The IR absorptions at 3350 and 1670 cm^{-1} and ^{13}C NMR signals at δ 70.5 (d) and at δ 126.1 (d), 165.1 (s), and 202.3 (s) suggested that **1** possessed a hydroxyl group and an α,*β*-unsaturated ketone. The ^1H NMR spectrum indicated the presence of a disubstituted cyclopropane ring from signals at δ 0.09, 0.14, 0.15, and 0.46 and also contained two methyl singlets at δ 0.69 and 1.20, three methyl doublets at δ 0.90 (J = 6.9 Hz), 0.93 (J = 6.6 Hz), and 1.01 (J = 6.0 Hz), one oxygenated methine proton at δ 3.68 (dddd, J = 11.3, 11.3, 4.4, 4.4 Hz), and an olefinic proton at δ 5.70 (d, J = 1.6 Hz). A DEPT experiment indicated five methyl, 10 methylene, 10 methine, and four quaternary carbons. The ^{13}C NMR spectrum indicated the presence of a trisubstituted double bond {δ 165.1 (s) and 126.1 (d)}, a carbonyl group {δ at 202.3 (s)}, and a carbon bearing a hydroxy group {δ 70.5 (d)}. These data indicated that **1** was a C-29 steroid with a cyclopropane ring in the side chain. Further interpretation of the COSY, HMQC, and HMBC data allowed all signals in both the ^1H and ^{13}C NMR spectra to be assigned (Table 1).

Results of selected HMBC correlations are summarized in Figure 1. The HMBC spectrum showed that the H-3 methine proton was coupled to C-1 and C-5, the H-4 methylene protons to C-6, and the H-9 methine proton to the α,*β*-unsaturated carbonyl C-7. The cyclopropane ring was located at C-25 based on HMBC correlations which showed that the H-24 methine proton was coupled to C-26, the H-26 methylene protons to C-24, the H-27 methine proton to C-25, and the H-29 methyl proton to C-25. Thus, the planar structure of **1** was determined.

The relative stereochemistry of **1** was established in part by NOESY experiments and coupling constants. The NOE between H-18/H-8, -15, and -20 and between H-19/H-2 *β*, -4 *β*, -8, and -11*β* suggested a common sterol nucleus. The *β*-OH group at the C-3 position could be assigned from the observed coupling constants for H-3 (J = 11.3, 11.3, 4.4, 4.4 Hz). The 17*β*-orientation of the side chain was disclosed by NOESY cross-peaks H-12/H-21 and H-18/H-20. The geometry of the cyclopropane ring was deduced to be *E* by NOESY cross-peaks H-25/H-29, but the relative stereochemistry of the cyclopropane ring could not be determined definitively by NOESY experiments. The CD spectrum [Δϵ −1.11 (237 nm)] of **1** showed a negative Cotton effect, ensuring the absolute stereochemistry of a sterol nucleus.[6] Finally, 7-oxopetrosterol (**1**) can be designated as 26,27-cyclo-24,27-dimethyl-3*β*-hydroxycholest-5-en-7-one.

7α-Hydroxypetrosterol (**2**) was assigned the molecular formula $C_{29}H_{48}O_2$ on the basis of EIMS (M^+ at m/z 428), HREIMS {m/z ($M - 2H_2O$)$^+$}, and NMR data, differing from the molecular formula of **1** by H_2. Comparison of the NMR data of **2** with those of **1** revealed that the only difference was that **2** had a hydroxyl group instead of a carbonyl group at C-7. The IR absorptions at 3300 cm^{-1} and ^{13}C NMR signals at δ 65.4 (d) and at δ 71.4 (d) suggested that **2** possessed two hydroxyl groups. The ^1H and ^{13}C NMR data (Table 1) of **2** were assigned fully by COSY, HMQC, and HMBC experiments.

The relative stereochemistry of **2** was established by the same types of NOEs as observed for **1** (Experimental Section). The *β*-OH group at C-3 and the α-OH group at C-7 could be assigned from the observed coupling constants for H-3 (J = 11.3, 11.3, 4.4, 4.4 Hz) and the coupling pattern for H-7 (br s), respectively. Thus, the structure of 7α-

* To whom correspondence should be addressed. Tel.: 088-622-9611. Fax: 088-655-3051. E-mail: umeyama@ph.bunri-u.ac.jp.

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Table 1. NMR Data for **1** and **2** in $CDCl_3$

	1		2	
C no.	δ_H mult, *J* (Hz)	δ_C[a]	δ_H mult, *J* (Hz)	δ_C[a]
1	1.94 m	36.3 (t)	1.86 m	37.0 (t)
	1.22 m		1.13 m	
2	1.95 m	31.2 (t)	1.85 m	31.4 (t)
	1.62 m		1.53 m	
3	3.68 dddd, 11.3, 11.3, 4.4, 4.4	70.5 (d)	3.59 dddd, 11.3, 11.3, 4.4, 4.4	71.4 (d)
4	2.51 ddd, 14.0, 4.4, 2.5	41.8 (t)	2.29 m	42.0 (t)
	2.40 ddd, 14.0, 11.3, 1.6		2.34 m	
5		165.1 (s)		146.2 (s)
6	5.70 d, 1.6	126.1 (d)	5.61 dd, 5.2, 1.6	123.9 (d)
7		202.3 (s)	3.86 br s	65.4 (d)
8	2.25 dd, 12.6, 11.0	45.4 (d)	1.46 m	37.5 (d)
9	1.34 m	49.9 (d)	1.22 m	42.3 (d)
10		38.3 (s)		37.4 (s)
11	1.58 m	21.2 (t)	1.56 m	20.7 (t)
12	2.04 ddd, 12.9, 3.6, 3.6	38.7 (t)	2.02 dddd, 12.9, 12.9, 3.3, 3.3	39.2 (t)
13		43.1 (s)		42.1 (s)
14	1.50 m	50.0 (d)	1.43 m	49.4 (d)
15	2.42 m	26.3 (t)	1.72 m	24.3 (t)
	1.26 m		1.15 m	
16	1.92 m	28.5 (t)	1.92 m	28.3 (t)
	1.26 m		1.32 m	
17	1.10 m	54.8 (d)	1.17 m	55.8 (d)
18	0.69 s	12.0 (q)	0.69 s	11.7 (q)
19	1.20 s	17.3 (q)	1.00 s	18.2 (q)
20	1.36 m	35.9 (d)	1.36 m	35.9 (d)
21	0.93 d, 6.6	18.9 (q)	0.93 d, 6.6	18.7 (q)
22	1.48 m	33.5 (t)	1.48 m	33.5 (t)
	1.01 m		1.03 m	
23	1.32 m	33.9 (t)	1.32 m	33.8 (t)
	1.25 m		1.26 m	
24	0.61 m	38.7 (d)	0.60 m	38.7 (d)
25	0.14 m	27.4 (d)	0.12 m	27.4 (d)
26	0.15 m	11.6 (t)	0.13 m	11.6 (t)
	0.09 m		0.07 m	
27	0.46 m	12.8 (d)	0.45 m	12.8 (d)
28	0.90 d, 6.9	19.8 (q)	0.89 d, 6.9	19.9 (q)
29	1.01 d, 6.0	19.1 (q)	1.01 d, 5.8	19.1 (q)

[a] Multiplicity inferred from a DEPT experiment.

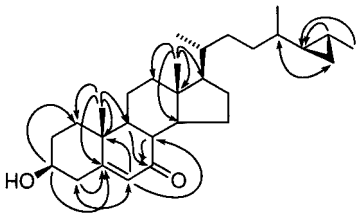

Figure 1. Selected HMBC correlations for **1**.

hydroxypetrosterol (**2**) was concluded to be 26,27-cyclo-24,27-dimethylcholest-5-en-3β,7α-diol.

Experimental Section

General Experimental Procedures. The following instruments were used: a JASCO FT/IR-5300 (IR), a JASCO DIP-360 polarimeter (optical rotation), a JASCO J-500 (CD), a JEOL JMS-HX-100 mass spectrometer (HRMS), and a Varian UNITY 600 NMR spectrometer (^{1}H and ^{13}C NMR).

Animal Material. The marine sponge *Strongylophora corticata* Wilson (460 g, wet weight) was collected off the coast of Tokushima prefecture {(134°30′E: 33°35′N) by netting at a depth between 10 × 70 m} and was kept frozen (−20 °C) until used; it was identified by Professor P. R. Bergquist of Auckland University. The voucher sample (TS877) of the organism under consideration is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University.

Extraction and Isolation of Metabolites. The frozen sample was exhaustively extracted with MeOH (1 L × 1) and MeOH/CH_2Cl_2 (3:1) (1 L × 2) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL × 3). The EtOAc-soluble portion (6.5 g) was repeatedly subjected to Sephadex LH-20 column chromatography (MeOH/CH_2Cl_2) and Si gel flash column chromatography (using increasing concentrations of CH_2Cl_2 in hexane and MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (90−95% MeOH) to give **1** (0.00272% wet weight), **2** (0.00048%), **3** (0.09348%), and **4** (0.00130%).

7-Oxopetrosterol (1): white amorphous powder; mp 107−110 °C; $[\alpha]^{21}_D$ −65.4° (*c* 0.62, MeOH); UV λ_{max} (MeOH) 238 nm (log ϵ 3.93); CD $\Delta\epsilon$ −1.11 (237 nm) (*c* 3.3 × 10^{-5} M, MeOH); FT-IR (film) 3350, 1670 cm^{-1}; COSY (H/H) 1/2, 2/3, 3/4, 4/6 (^4J), 8/9, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/25, 24/28, 25/26, 26/27, 27/29; HRCIMS *m*/*z* $[M + 1]^+$ 427.3581 (calcd for $C_{29}H_{47}O_2$, 427.3576).

7α-Hydroxypetrosterol (2): white amorphous powder; mp 124−127 °C; $[\alpha]^{21}_D$ −55.3° (*c* 0.11, MeOH); FT-IR (film) 3300 cm^{-1}; COSY (H/H) 1/2, 2/3, 3/4, 4/6 (^4J), 6/7, 7/8, 8/9, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/25, 24/28, 25/26, 26/27, 27/29; selected HMBC (H/C) 3/1, 3/5, 4/5, 4/6, 6/4, 6/8, 7/10, 8/7, 9/7, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 24/26, 26/24, 27/25, 29/25; EIMS *m*/*z* 428, 410, 392; NOESY 1α/3α, 1α /9, 2β/19, 4β/19, 7β/8, 8/18, 8/19, 9/12α, 11β/19, 12α/14, 12β/21, 15β/18, 18/20, 24/27, 25/28, 25/29; EIMS *m*/*z* 428 $[M]^+$, 410, 392; HREIMS *m*/*z* $[M - 2H_2O]^+$ 392.3425 (calcd for $C_{29}H_{44}$, 392.3443).

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