

Swinhosterols A–C, 4-Methylene Secosteroids from the Marine Sponge *Theonella swinhoei*

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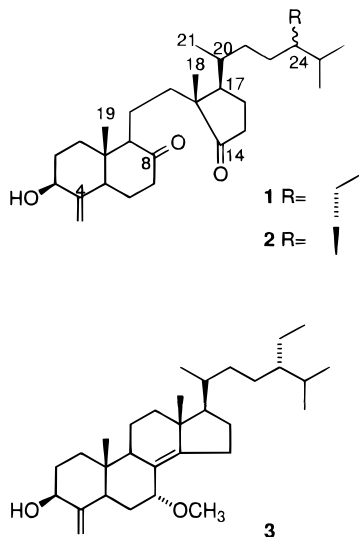
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Three 4-methylene steroids, named swinhosterols A–C (**1**–**3**) were isolated from the Okinawan sponge *Theonella swinhoei* Gray. The structures of **1** and **2**, which combine rare 4-methylene and seco features, were determined as (24*S*)-3 β -hydroxy-24-ethyl-4-methylene-5 α -8,14-secocholestane-8,14-dione and (24*R*)-3 β -hydroxy-24-methyl-4-methylene-5 α -8,14-secocholestane-8,14-dione, and the structure of **3** was determined as (24*S*)-24-ethyl-7-methoxy-4-methylene-5 α -cholest-8(14)-en-3 β -ol on the basis of spectroscopic investigations.

Marine sponges continue to be a rich source of unique steroids.^{1,2} Only two papers have been published on 4-methylene steroids from marine organisms up to the present. In 1981, Djerassi *et al.* reported two unprecedented sterols with a 4-methylene nucleus;³ and in 1992, Kobayashi *et al.* reported two 3-keto-4-methylene steroids from the same species, *Theonella swinhoei*.⁴ On the other hand, very few secosteroids have been reported from marine organisms.^{5–9} Comparatively recently, Minale *et al.* reported (24*R*)-3 β -methoxy-24-methyl-8,14-secocholestane-8,14-dione from the Pacific sponge *Jereicopsis graphidiophora* as the first 8,14-secosteroid.¹⁰

As part of an ongoing investigation of metabolites isolated from marine organisms collected off Okinawa Island, it was found that an extract of the sponge *Theonella swinhoei* Gray contained the three rare 4-methylene steroids swinhosterols A–C, of which the first two were also secosteroids. We now describe the isolation and structure elucidation of swinhosterols A–C (**1**–**3**).



An MeOH–CH₂Cl₂ (1:1) extract of the sponge was divided into EtOAc-, BuOH-, and H₂O-soluble portions. The EtOAc-soluble portion was chromatographed on

Sephadex LH-20 and Si gel columns. Final purification by reversed-phase HPLC afforded three novel steroids, swinhosterols A–C (**1**–**3**).

Swinhosterol A (**1**) was obtained as a colorless oil. Its molecular formula was established as C₃₀H₅₀O₃ on the basis of HREIMS and corresponds to six degrees of unsaturation. The IR spectrum suggested that **1** possessed a hydroxyl group (3450 cm⁻¹) and two carbonyl groups (1734, 1712 cm⁻¹). Because resonances in the ¹³C-NMR spectrum indicated the presence of one double bond [δ 150.8 (s), 104.3 (t)], two carbonyl groups [δ 225.0 (s), 211.3 (s)], and one carbon containing hydroxy group [δ 72.8 (d)], the carbon skeleton consists of three rings. The ¹H-NMR spectrum indicated a steroidal structure and contained two methyl singlets (δ 0.53, 0.86), three methyl doublets [δ 0.83 (d, *J* = 7.3 Hz), 0.85 (d, *J* = 7.3 Hz), 1.10 (d, *J* = 6.6 Hz)], one methyl triplet [δ 0.88 (t, *J* = 7.3 Hz)], one oxygenated methine proton [δ 4.07 (1H, dd, *J* = 11.7, 5.9 Hz)], and one terminal methylene group [δ 4.67 (br s), 5.18 (br s)]. ¹H–¹H COSY and ¹³C–¹H COSY experiments revealed the partial structures a (CH₂CH₂CH: from C-1 to C-3), b (CHCH₂CH₂: from C-5 to C-7), c (CHCH₂CH₂: from C-9 to C-12), d {CH₂CH₂CHCH (CH₃) CH₂CH₂CHCH₂CH₃: from C-15 to C-29}, and e {CH (CH₃) CH₂: from C-26 to C-27}. An HMBC experiment revealed that the H-30 methylene protons were coupled to C-3, C-4, and C-5, and the H-19 methyl protons to C-1, C-5, C-9, and C-10. This suggested a linkage among partial structures a, b, and c. Furthermore, the HMBC spectrum showed that the H₂-12 protons were coupled to C-14, and the H₃-18 methyl protons to C-12, C-13, C-14, and C-17. These data established the connectivity between the partial structures c and d. The HMBC spectrum also showed couplings between H-26 and C-24, H-27 and C-24. Thus, the planar structure of **1** was determined. The relative stereochemistry of swinhosterol A was established by NOESY experiments and coupling constants. The β -OH group at C-3 position could be assigned from the observed coupling constants for H-3 (*J* = 11.7, 5.9 Hz). The stereochemistry of the side chain for **1** was determined by comparison of NMR data with those of xeniasterol C, which was isolated from the soft coral *Xenia* sp.³ Swinhosterol A (**1**) could thus be assigned as (24*S*)-3 β -hydroxy-24-ethyl-4-methylene-5 α -8,14-secocholestane-8,14-dione.

Swinhosterol B (**2**) was obtained as a colorless oil. The molecular formula of **2** was determined to be

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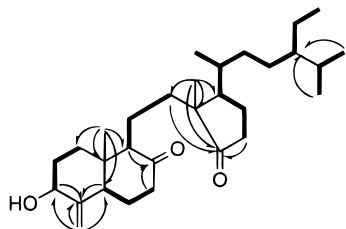


Figure 1. ^1H – ^1H COSY (bold lines) and HMBC (arrows) correlations for swinhosterol A (**1**).

$\text{C}_{29}\text{H}_{48}\text{O}_3$ by HREIMS, differing from the molecular formula of **1** by loss of CH_2 . Comparison of physico-chemical data of **2** with those of **1** revealed that the only difference was that **2** had a methyl group at C24 instead of an ethyl group. The connectivity of the COSY and HMBC experiments (see Experimental Section) supported the assumed structure of **2**. The stereochemistry at C24 position for **2** was determined to be *R* by comparison with chemical shifts value of jereisterol B.¹⁰ The other configurations of asymmetric carbons were determined by NOESY experiments and coupling constants and found to be the same as in **1**. Swinhosterol B (**2**) can be designated as (24*R*)-3 β -hydroxy-24-methyl-4-methylene-5 α -8,14-secocholestane-8,14-dione.

Swinhosterol C (**3**) was obtained as a white powder. The molecular formula of **3** was established as $\text{C}_{31}\text{H}_{52}\text{O}_2$ on the basis of HREIMS. A broad IR absorption at 3445 cm^{-1} was attributable to hydroxyl groups, and no bands near 1720 cm^{-1} were observed. The ^{13}C -NMR spectrum in CDCl_3 indicated the presence of two double bonds [δ 152.8 (s), 149.4 (s), 124.3 (s), 102.6 (t)] and two carbons bearing an oxygen function [δ 74.3 (d), 73.3 (d)] but no carbonyl groups. The ^1H -NMR spectrum contained two methyl singlets (δ 0.86, 0.58), three methyl doublets [δ 0.96 (d, $J = 6.6\text{ Hz}$), 0.83 (d, $J = 6.6\text{ Hz}$), 0.82 (d, $J = 6.6\text{ Hz}$)], one methyl triplet [δ 0.86 (t, $J = 6.6\text{ Hz}$)], one methoxy group (δ 3.20), two methine protons [δ 4.13 (dd, $J = 3.0, 3.0\text{ Hz}$), 4.04 (dd, $J = 11.7, 5.9\text{ Hz}$)], and one exomethylene [δ 5.07 (s), 4.59 (s)]. These data indicated **3** was a C30 steroid with an ethyl group and exomethylene group. The ^1H – ^1H COSY and ^{13}C – ^1H COSY experiments (see Experimental Section) enabled us to construct the structure of **3**. The relative stereochemistry of **3** was established by NOESY experiments and coupling constants. The β -OH group at the C-3 position could be assigned from the observed coupling constants for H-3 ($J = 11.7, 5.9\text{ Hz}$). The stereochemistry of the 7 α -OCH₃ group of **3** was proven by the shape of the H-7 proton signal in the ^1H -NMR spectrum (δ 4.13, dd, $J = 3.0, 3.0\text{ Hz}$). The *S* configuration of C-24 was determined by comparison of chemical shift values as in the case of compound **1**. The structure of **3** was determined as (24*S*)-24-ethyl-7 α -methoxy-4-methylene-5 α -cholest-8(14)-en-3 β -ol.

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT/IR-5300 (IR), JASCO DIP-360 polarimeter (optical rotation), JEOL JMS-HX-100 mass spectrometer (HRMS), JEOL JNM-GX-400FT NMR or Varian UNITY 600 spectrometer (^1H and ^{13}C NMR).

Sponge Material. A specimen of *T. swinhoei* Gray was collected by netting at a depth of 40–70 m off

Okinawa Island and was kept frozen ($-20\text{ }^\circ\text{C}$) until used. The voucher specimen (MS032) is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan.

Extraction and Isolation of Metabolites. The frozen sample of *T. swinhoei* (1.5 kg, wet wt) was lyophilized and exhaustively extracted with $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (1:1) (2 L \times 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL \times 3). The EtOAc-soluble portion (7.5 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in CHCl_3 as eluent), followed by Sephadex LH-20 column chromatography (CHCl_3 –MeOH, 1:1) and reversed-phase HPLC (80% MeOH) to give **1** (300 mg, 0.02% wet wt), **2** (58 mg, 0.0039%), and **3** (21 mg, 0.0014%).

Swinhosterol A (1): colorless oil; $[\alpha]_{\text{D}}^{25} -50.0^\circ$ (*c* 1.67, CHCl_3); FT-IR (film) 3450, 1735, 1710 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.53 (3H, s, Me-19), 0.80 (1H, m, H-11), 0.83 (3H, d, $J = 7.3\text{ Hz}$, Me-26), 0.85 (3H, d, $J = 7.3\text{ Hz}$, Me-27), 0.86 (3H, s, Me-18), 0.88 (3H, t, $J = 7.3\text{ Hz}$, Me-29), 0.97 (1H, m, H-24), 1.10 (3H, d, $J = 6.6\text{ Hz}$, Me-21), 1.10 (1H, m, H-22), 1.11 (1H, m, H-23), 1.17 (1H, m, H-28), 1.33 (1H, m, H-28), 1.34 (1H, m, H-2), 1.38 (1H, m, H-23), 1.41 (1H, m, H-12), 1.46 (1H, m, H-16), 1.47 (1H, m, H-22), 1.49 (1H, m, H-1), 1.50 (1H, m, H-20), 1.63 (1H, m, H-12), 1.70 (1H, m, H-15), 1.73 (1H, m, H-11), 1.76 (1H, m, H-1), 1.84 (1H, m, H-6), 1.95 (1H, m, H-6), 1.98 (1H, m, H-17), 2.00 (1H, m, H-2), 2.04 (1H, m, H-15), 2.12 (1H, m, H-9), 2.13 (1H, m, H-16), 2.29 (1H, dd, $J = 12.0, 3.0\text{ Hz}$, H-5), 2.37 (1H, m, H-15), 2.42 (1H, m, H-7), 4.07 (1H, dd, $J = 11.7, 5.9\text{ Hz}$, H-3), 4.67 (1H, s, H-30), 5.18 (1H, s, H-30); ^{13}C NMR (CDCl_3) δ 12.3 (q, C-29), 13.0 (q, C-19), 18.0 (t, C-11), 18.3 (q, C-21), 18.4 (q, C-18), 18.9 (q, C-26), 19.5 (q, C-27), 22.9 (t, C-28), 23.6 (t, C-16), 26.0 (t, C-6), 26.8 (t, C-23), 28.9 (d, C-25), 32.3 (t, C-2), 32.4 (t, C-22), 34.7 (d, C-20), 36.5 (t, C-1), 37.2 (t, C-12), 37.9 (t, C-15), 41.6 (t, C-7), 44.3 (s, C-10), 46.0 (d, C-24), 46.6 (d, C-17), 48.3 (d, C-5), 52.5 (s, C-13), 62.5 (d, C-9), 72.8 (d, C-3), 104.2 (t, C-30), 150.8 (s, C-4), 211.3 (s, C-8), 225.2 (s, C-14); HREIMS *m/z* 458.3773, calcd for $\text{C}_{30}\text{H}_{50}\text{O}_3$ 458.3760; COSY (H/H) 1/2, 2/3, 5/6, 6/7, 9/11, 11/12, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/28, 25/26, 25/27, 28/29; HMBC (H/C) 3/4, 5/4, 7/8, 9/8, 12/14, 15/14, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 26/24, 27/24, 30/3, 30/4, 30/5; NOESY 1/3, 1/11, 2/19, 3/5, 5/7, 5/9, 6/30, 6/19, 7/9, 18/20, 18/21.

Swinhosterol B (2): colorless oil; $[\alpha]_{\text{D}}^{25} -50.0^\circ$ (*c* 1.04, CHCl_3); FT-IR (film) 3460, 1735, 1710 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.52 (3H, s, Me-19), 0.81 (3H, d, $J = 5.1\text{ Hz}$, Me-28), 0.81 (1H, m, H-11), 0.82 (3H, d, $J = 6.6\text{ Hz}$, Me-26), 0.86 (3H, s, Me-18), 0.87 (3H, d, $J = 6.6\text{ Hz}$, Me-27), 1.08 (3H, d, $J = 6.6\text{ Hz}$, Me-21), 1.16 (1H, m, H-23), 1.21 (1H, m, H-22), 1.25 (1H, m, H-24), 1.30 (1H, m, H-23), 1.34 (1H, m, H-2), 1.41 (1H, m, H-12), 1.43 (1H, m, H-22), 1.46 (1H, m, H-16), 1.49 (1H, m, H-1), 1.52 (1H, m, H-20), 1.53 (1H, m, H-25), 1.63 (1H, m, H-12), 1.72 (1H, m, H-11), 1.76 (1H, m, H-1), 1.84 (1H, m, H-6), 1.95 (1H, m, H-6), 1.98 (1H, m, H-17), 2.00 (1H, m, H-2), 2.04 (1H, m, H-15), 2.12 (1H, m, H-9), 2.14 (1H, m, H-16), 2.29 (1H, dd, $J = 12.0, 3.0\text{ Hz}$, H-5), 2.34 (1H, m, H-15), 2.41 (1H, m, H-7), 4.06 (1H, dd, $J = 11.7, 5.9\text{ Hz}$, H-3), 4.67 (1H, s, H-29), 5.18 (1H, s, H-29); ^{13}C

NMR (CDCl₃) δ 13.0 (q, C-19), 15.4 (q, C-28), 18.0 (t, C-11), 18.1 (q, C-21), 18.2 (q, C-26), 18.4 (q, C-18), 20.2 (q, C-27), 23.6 (t, C-16), 26.0 (t, C-6), 30.6 (t, C-23), 32.2 (t, C-2), 32.2 (t, C-22), 32.3 (d, C-25), 34.3 (d, C-20), 36.5 (t, C-1), 37.2 (t, C-12), 37.9 (t, C-15), 38.8 (d, C-24), 41.6 (t, C-7), 44.3 (s, C-10), 46.6 (d, C-17), 48.3 (d, C-5), 52.5 (s, C-13), 62.5 (d, C-9), 72.7 (d, C-3), 104.3 (t, C-29), 150.8 (s, C-4), 211.4 (s, C-8), 225.2 (s, C-14); HREIMS m/z 444.3597, calcd for C₂₉H₄₈O₃ 444.3604; COSY (H/H) 1/2, 2/3, 5/6, 6/7, 9/11, 11/12, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/28, 25/26, 25/27; HMBC (H/C) 3/4, 5/4, 7/8, 9/8, 12/14, 15/14, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 26/24, 27/24, 30/3, 30/4, 30/5; NOESY 1/3, 1/11, 2/19, 3/5, 5/7, 5/9, 6/30, 6/19, 7/9, 18/20, 18/21.

Swinhosterol C (3): white powder; $[\alpha]_D^{25} +20.0^\circ$ (c 0.71, CHCl₃); FT-IR (film) 3445 cm⁻¹; ¹H NMR (CDCl₃) δ 0.58 (3H, s, Me-19), 0.81 (3H, d, $J = 6.6$ Hz, Me-26), 0.83 (3H, d, $J = 6.6$ Hz, Me-27), 0.86 (3H, s, Me-18), 0.87 (3H, t, $J = 6.6$ Hz, Me-29), 0.94 (1H, m, H-24), 0.96 (3H, d, $J = 6.6$ Hz, Me-21), 1.04 (1H, m, H-22), 1.06 (1H, m, H-23), 1.14 (1H, m, H-28), 1.17 (1H, m, H-12), 1.20 (1H, m, H-17), 1.32 (1H, m, H-23), 1.33 (1H, m, H-28), 1.37 (1H, m, H-2), 1.37 (1H, m, H-1), 1.44 (1H, m, H-22), 1.44 (1H, m, H-16), 1.45 (1H, m, H-20), 1.47 (1H, m, H-11), 1.52 (1H, m, H-6), 1.67 (1H, m, H-11), 1.68 (1H, m, H-25), 1.72 (1H, m, H-1), 1.84 (1H, m, H-6), 1.88 (1H, m, H-16), 1.97 (1H, m, H-12), 2.00 (1H, m, H-2), 2.14 (1H, m, H-9), 2.28 (1H, m, H-5), 2.30 (1H, m, H-15), 2.45 (1H, m, H-15), 3.18 (3H, s, -OCH₃), 4.04 (1H, dd, $J = 11.7, 5.9$ Hz, H-3), 4.13 (1H, dd, $J = 3.0, 3.0$ Hz, H-7), 4.59 (1H, s, H-30), 5.07 (1H, s, H-30); ¹³C NMR (CDCl₃) δ 12.4 (q, C-29), 12.5 (q, C-19), 17.7 (q, C-18), 19.0 (q, C-26), 19.3 (q, C-21), 19.6 (q, C-27), 19.9 (t, C-11), 23.0 (t, C-28), 25.6 (t, C-15), 26.6 (t, C-23), 26.9 (t, C-16), 29.0 (d, C-25), 30.1 (t, C-6), 33.0 (t, C-2), 33.8 (t, C-22), 35.0 (d, C-20), 36.4 (t, C-1), 36.9 (t, C-12), 40.0 (s, C-10), 42.8

(d, C-5), 43.4 (s, C-13), 46.1 (d, C-9), 46.1 (d, C-24), 57.1 (d, C-17), 73.3 (d, C-3), 74.3 (d, C-7), 102.6 (t, C-30), 152.8 (s, C-4), 124.3 (s, C-8), 149.4 (s, C-14); HREIMS m/z 456.3972, calcd for C₃₁H₅₂O₂ 456.3968; COSY (H/H) 1/2, 2/3, 5/6, 6/7, 9/11, 11/12, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/25, 24/28, 25/26, 25/27, 28/29; HMBC (H/C) 6/8, 7/14, 9/8, 9/14, 15/8, 15/14, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 21/22, 30/3, 30/4, 30/5; NOESY 1/3, 2/19, 3/5, 5/9, 6/19, 6/30, 7/15, 11/19, 12/17, 17/21.

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