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

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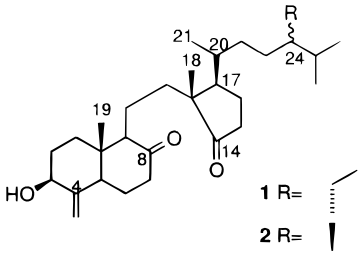
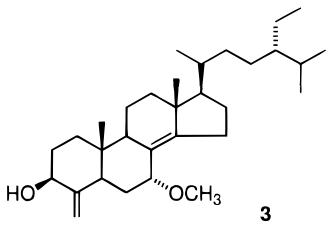
Three 4-methylene steroids, named swinhosterols A−C (**1**−**3**) were isolated from the Okinawan sponge *Theonella swinhoei* Gray. The structures of **1** and **2**, which combine rare 4-methylene and seco features, were determined as (24*S*)-3β-hydroxy-24-ethyl-4-methylene-5α-8,14-secocholestane-8,14-dione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





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An MeOH−CH_2Cl_2 (1:1) extract of the sponge was divided into EtOAc-, BuOH-, 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 three novel steroids, swinhosterols A−C (**1**−**3**).

Swinhosterol A (**1**) was obtained as a colorless oil. Its molecular formula was established as $C_{30}H_{50}O_3$ 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 ^{13}C-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 ^{1}H-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)]. ^{1}H−^{1}H COSY and ^{13}C−^{1}H COSY experiments revealed the partial structures a (CH_2CH_2CH: from C-1 to C-3), b ($CHCH_2CH_2$: from C-5 to C-7), c ($CHCH_2CH_2$: from C-9 to C-12), d {CH_2CH_2CHCH (CH_3) $CH_2CH_2CHCH_2CH_3$: from C-15 to C-29}, and e {CH (CH_3) CH_2: 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 H_2-12 protons were coupled to C-14, and the H_3-18 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*)-3β-hydroxy-24-ethyl-4-methylene-5α-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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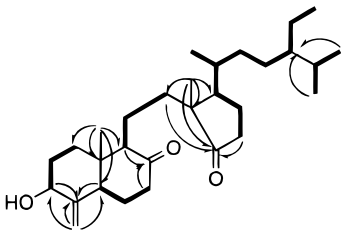

Figure 1. $^1H-^1H$ COSY (bold lines) and HMBC (arrows) correlations for swinhosterol A (**1**).

$C_{29}H_{48}O_3$ by HREIMS, differing from the molecular formula of **1** by loss of CH_2. Comparison of physicochemical 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*)-3β-hydroxy-24-methyl-4-methylene-5α-8,14-secocholestane-8,14-dione.

Swinhosterol C (**3**) was obtained as a white powder. The molecular formula of **3** was established as $C_{31}H_{52}O_2$ 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 Hz), 0.83 (d, J = 6.6 Hz), 0.82 (d, J = 6.6 Hz)], one methyl triplet [δ 0.86 (t, J = 6.6 Hz)], one methoxy group (δ 3.20), two methine protons [δ 4.13 (dd, J = 3.0, 3.0 Hz), 4.04 (dd, J = 11.7, 5.9 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 Hz). The stereochemistry of the 7α-OCH_3 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 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-7α-methoxy-4-methylene-5α-cholest-8(14)-en-3β-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 °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 MeOH−CH_2Cl_2 (1:1) (2 L × 4) 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 (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; $[\alpha]^{25}_D$ −50.0° (*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 Hz, Me-26), 0.85 (3H, d, J = 7.3 Hz, Me-27), 0.86 (3H, s, Me-18), 0.88 (3H, t, J = 7.3 Hz, Me-29), 0.97 (1H, m, H-24), 1.10 (3H, d, J = 6.6 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 Hz, H-5), 2.37 (1H, m, H-15), 2.42 (1H, m, H-7), 4.07 (1H, dd, J = 11.7, 5.9 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 $C_{30}H_{50}O_3$ 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; $[\alpha]^{25}_D$ −50.0° (*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 Hz, Me-28), 0.81 (1H, m, H-11), 0.82 (3H, d, J = 6.6 Hz, Me-26), 0.86 (3H, s, Me-18), 0.87 (3H, d, J = 6.6 Hz, Me-27), 1.08 (3H, d, J = 6.6 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 Hz, H-5), 2.34 (1H, m, H-15), 2.41 (1H, m, H-7), 4.06 (1H, dd, J = 11.7, 5.9 Hz, H-3), 4.67 (1H, s, H-29), 5.18 (1H, s, H-29); ^{13}C

NMR ($CDCl_3$) δ13.0 (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 $C_{29}H_{48}O_3$ 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; $[\alpha]^{25}_D$ +20.0° (*c* 0.71, $CHCl_3$); FT-IR (film) 3445 cm^{-1}; ^1H NMR ($CDCl_3$) 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, -OCH_3), 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); ^{13}C NMR ($CDCl_3$) 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 $C_{31}H_{52}O_2$ 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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