Xestokerols A, B, and C, New C Steroids with a Cyclopropane Ring from the Okinawan Marine Sponge Xestospongia sp.

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XESTOKEROLS A, B, AND C, NEW C_{29} STEROIDS WITH A CYCLOPROPANE RING FROM THE OKINAWAN MARINE SPONGE *XESTOSPONGIA* SP.

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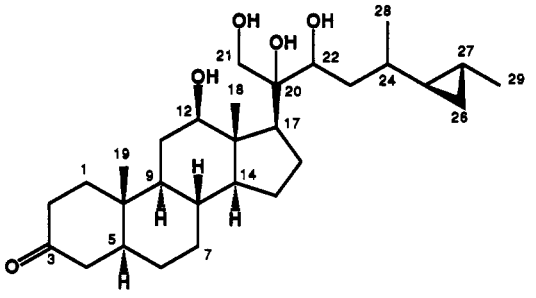
ABSTRACT.—Three new C_{29} steroids, xestokerols A [**1**], B [**2**], and C [**3**], including a cyclopropane ring in the side chain, have been isolated from the Okinawan marine sponge *Xestospongia* sp. and the structures elucidated on the basis of spectral data. Xestokerols A and B are very rare C-20-oxidized steroids from marine origin.

A number of sterols with unusual substitution patterns of the side chains have been isolated from marine sponges (1). In our search for bioactive substances from marine organisms (2–6), we have examined extracts of the Okinawan sponge *Xestospongia* sp. (order Petrosida, family Petrosiidae) and isolated three new C_{29} steroids, named xestokerols A [**1**], B [**2**], and C [**3**], with a cyclopropane ring in the side chain. Xestokerols A and B are rare C-20-oxidized steroids from marine origin. Here we describe the isolation and structure elucidation of compounds **1–3**.

The sponge *Xestospongia* sp. was collected off Ishigaki Island, Okinawa and kept frozen until used. The MeOH extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to Si gel cc followed by Si gel and reversed-phase hplc to afford xestokerols A [**1**] (0.0078% wet wt), B [**2**] (0.0056%), and C [**3**] (0.0011%).

Xestokerol A [**1**], a colorless oil, was shown to have the molecular formula $C_{29}H_{48}O_5$ by hrfabms (*m/z* 477.3560 $[M+H]^+$, $\Delta -2.0$ mmu), indicating six degrees of unsaturation. The ir absorptions at 3400 and 1710 cm^{-1} indicated the presence of hydroxyl and ketone carbonyl groups, respectively. The ^{1}H-nmr spectrum of **1** showed clearly the presence of a cyclopropane ring [0.17 (1H, dt, J=8.4 and 4.4 Hz), 0.30 (1H, dt, J=8.4 and 4.4 Hz), and 0.43 (1H, ddt, J=9.2, 8.4, and 4.4 Hz), 0.53 (1H, m)] and four deuterium-exchangeable protons [5.64 (1H, brd, J=6.2 Hz), 6.46 (1H, brs), 6.89 (1H brs), 7.04 (1H, brs)]. The ^{13}C-nmr (Table 1) spectrum of **1** showed signals due to four quaternary, four methyl, eleven methylene, and ten methine carbons. The nmr spectra of **1** were assigned on the basis of several types of 2D nmr data including ^{1}H-^{1}H COSY, HSQC (7), and HMBC (8) spectra. The ^{1}H-^{1}H COSY spectrum of **1** indicated the presence of four partial structures, C-1–C-2, C-4–C-7, C-9–C-14, and C-22–C-29. The ketone carbonyl carbon at δ_C 210.2 was assigned to C-3, since HMBC correlations for H-2/C-3 and H-4/C-3 were observed. The HMBC correlations of H-19/C-1 and C-5 indicated the presence of ring A in **1**. The cross peaks from H-18 to C-12, C-13, C-14, and C-17 in the HMBC spectrum revealed the connectivity between rings C and D. The connectivity of segment C-20–C-22 was established by HMBC correlations of H-21/C-20 and H-21/C-22. Furthermore, the linkage between C-17 and C-20 was confirmed by the H-C long-range correlation between H-21 and C-17. The partial stereochemistry of **1** was elucidated by NOESY experiments and analysis of ^{1}H-^{1}H coupling constants. The



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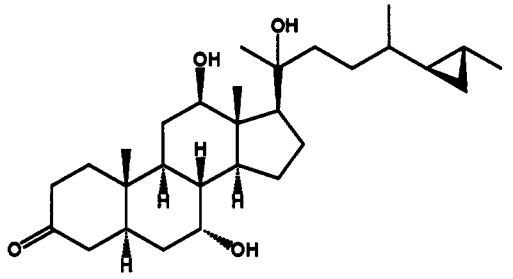

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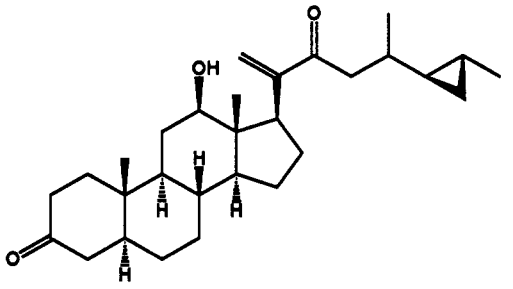

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NOESY spectrum showed the cross peaks of H_{ax}-2/H-19, H_{ax}-1/H-9, H_{ax}-5/H-9, H_{ax}-12/H-9, and H_{ax}-14/H-9, indicating a chair conformation for each of rings A–C and trans junction among the neighboring rings. The NOESY correlation between H-12 and H-17 revealed that the C-20–C-29 side chain was β-oriented on ring D. The hydroxyl group at C-12 was assigned to be β-oriented, judging from the coupling constants between H_2-11 and H-12 ($J_{11eq,12ax}$=4.3 Hz and $J_{11ax,12ax}$=10.7 Hz) (9). The *E* geometry of the cyclopropane ring (C-25–C-27) was deduced from NOESY correlations between H-25 and H-29. Thus the structure of xestokerol A was concluded to be **1** with the absolute configuration at C-20, C-24, C-25, and C-27 undetermined.

Xestokerol B [**2**] was obtained as a colorless amorphous powder. The hreims analysis showed the molecular formular to be $C_{29}H_{48}O_4$ (*m/z* 442.3445 $[M-H_2O]^+$, Δ −0.2 mmu). The ir absorptions at 3350 and 1705 cm^{-1} implied that **2** possessed hydroxyl and ketone groups. The ^{1}H-nmr spectrum of **2** showed the presence of a cyclopropane ring [0.13 (1H, m), 0.23 (2H, m), 0.55 (1H, m)] and three deuterium-exchangeable protons [5.58 (1H, d, J=3.7 Hz), 6.97 (2H, brs)]. The ^{1}H- and ^{13}C-nmr (Table 1) spectra were analogous to those of **1**. The structural differences between compounds **1** and **2** were found for ring B and the side chain. The HMBC correlations of H-7/C-5, H-7/C-9, and OH-7/C-6 suggested that a hydroxyl group (δ_H 5.58) was attached to C-7. The long-

TABLE 1. ^{13}C-nmr Data of Xestokerols A [**1**], B [**2**], and C [**3**].[a]

Carbon	Compound		
	1	**2**	**3**
C-1	38.5 t	38.5 t	38.5 t
C-2	38.6 t	38.6 t	38.3 t
C-3	210.2 s	210.4 s	210.2 s
C-4	44.8 t	44.6 t	44.8 t
C-5	46.6 d	44.7 d	46.6 d
C-6	29.1 t	37.9 t	29.0 t
C-7	31.4 t	66.2 d	31.5 t
C-8	34.1 d	38.8 d	35.0 d
C-9	52.7 d	39.5 d	52.9 d
C-10	35.7 s	36.1 s	35.8 s
C-11	30.3 t	30.0 t	32.0 t
C-12	78.2 d	78.0 d	77.3 d
C-13	49.6 s	49.0 s	48.9 s
C-14	54.6 d	49.6 d	54.9 d
C-15	24.1 t	23.4 t	24.2 t
C-16	23.7 t	25.5 t	27.5 t
C-17	57.0 d	65.9 d	48.4 d
C-18	9.7 q	10.1 q	7.9 q
C-19	11.3 q	10.4 q	11.3 q
C-20	77.3 s	74.4 s	151.2 s
C-21	65.9 t	28.5 q	121.1 t
C-22	72.8 d	35.1 t	203.6 s
C-23	38.2 t	32.5 t	46.4 t
C-24	35.3 d	39.7 d	35.2 d
C-25	28.7 d	27.6 d	27.6 d
C-26	12.9 t	11.8 t	12.3 t
C-27	12.5 d	13.3 d	12.6 d
C-28	19.0 q	20.8 q	20.2 q
C-29	19.3 q	19.3 q	19.2 q

[a]δ in ppm, multiplicity.

range correlations of H-17/C-21, H-21/C-20, H-21/C-17, and H-22/C-21 revealed that a methyl group (δ_H 1.36, s; δ_C 28.5, q) was attached to C-20. The relative stereochemistry was elucidated by NOESY experiments and ^{1}H-^{1}H coupling constants for **2**, indicating a chair conformation for each of rings A–C and trans junction between the adjacent rings. The β configuration of a hydroxyl group at C-12 was determined by the coupling constants between H_2-11 and H-12 ($J_{11eq,12ax}$=4.3 Hz and $J_{11ax,12ax}$=10.5 Hz). The NOESY correlations of H_{eq}-6/H-7 and H-7/H-8 revealed that the hydroxyl group at C-7 was α-oriented. The *E* geometry of the cyclopropane ring (C-25–C-27) was deduced from NOESY correlations between H-25 and H-29. Thus the structure of xestokerol B was assigned to be **2** with the absolute configuration at C-20, C-24, C-25, and C-27 undetermined.

Xestokerol C [**3**], a colorless amorphous powder, was shown to have a molecular formula, $C_{29}H_{44}O_3$, by the hrfabms (*m/z* 441.3343 $[M+H]^+$, Δ −2.6 mmu). The ir absorptions at 3500 and 1710 cm^{-1} indicated the presence of hydroxyl and ketone groups. The uv [226 nm (ε 6700)] and ir (1680 cm^{-1}) data of **3** suggested the presence of an α,β-unsaturated ketone group. The ^{1}H- and ^{13}C-nmr (Table 1) spectra implied that **3** possessed two ketone carbonyl groups [δ_C 210.2 (s) and 203.6 (s)] and a cyclopropane ring [δ_H 0.11, 0.23, 0.31, and 0.60]. Although these spectral data were similar to those of xestokerol A [**1**], the prominent difference was the presence of an α,β-unsaturated

ketone group [δ_H 5.74 (s) and 6.17 (s); δ_C 121.1 (t), 151.2 (s), and 203.6 (s)] for **3** in place of a triol moiety [δ_H 4.60 (H-21), 4.41 (H-21, -22), 5.64 (OH-21), 6.46 (OH-22), 6.89 (OH-20); δ_C 65.9 (C-21), 72.8 (C-22), and 77.3 (C-20)] for **1**. The connection from C-17 to C-23 through the α,β-unsaturated ketone was verified by the HMBC spectrum of **3** (cross peaks H-17/C-20, H-17/C-22, H-21/C-17, H-21/C-22, and H-23/C-22). The relative stereochemistry of **3** elucidated from the NOESY spectrum was the same as that of xestokerol A [**1**]. Thus the structure of xestokerol C was assigned to be **3** with the absolute configuration at C-24, C-25, and C-27 undetermined.

Xestokerols A [**1**], B [**2**], and C [**3**] are new C_{29} steroids with a cyclopropane ring in the side chain. Although there are no reports on steroids with a cyclopropane ring in the side chain from terrestrial sources (1), such steroids have been isolated from marine sponges of the genera *Petrosia* (10–14), *Calyx* (15), and *Cribrocalina* (16,17). However, xestokerols A [**1**], B [**2**], and C [**3**] are the first examples of steroids with a cyclopropane ring in the side chain from a sponge of the genus *Xestospongia*. Xestokerols A [**1**] and B [**2**] were very rare C-20-oxidized steroids from marine sources. Biogenetically, xestokerols A [**1**] and B [**2**] may have been generated through oxidation of C-20–C-21 double bond of xestokerol C [**3**]. Compounds **1**, **2**, and **3** exhibited cytotoxicity against L1210 murine leukemia cells with IC_{50} values of 2.1, 2.2, and 0.78 μg/ml and KB human epidermoid carcinoma cells with IC_{50} values of 4.4, 4.6 and >10 μg/ml in vitro, respectively. In addition, xestokerol A [**1**] showed antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus, Sarcina lutea,* and *Bacillus subtilis* with an MIC value of 16μg/ml. Xestokerol C [**3**] also showed antimicrobial activity against *Sar. lutea* [MIC 66 μg/ml], while xestokerol B [**2**] showed no antimicrobial activity at 260 μg/ml.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. Uv and ir spectra were taken on a Shimadzu UV-220 spectrometer and a JASCO Report-100 infrared spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JMN GX-270 and an EX-400 spectrometer in C_5D_5N and $CDCl_3$. The residual pyridine and $CHCl_3$ resonances at δ_H 7.19 and 7.26, δ_C 123.5 and 77.1 were used as internal references for ^{1}H- and ^{13}C-nmr spectra, respectively. The one-bond ^{1}H-^{13}C shift-correlation experiment was carried out with the standard JEOL HSQC pulse sequence. The two-and three-bond correlation experiment was carried out with the standard JEOL HMBC pulse sequence using delays optimized for J_{CH} of 8 Hz. Homonuclear ^{1}H nOe's were obtained by phase-sensitive NOESY experiments using 2.4 sec recycling delay and 700 msec mixing period. Eims spectra were obtained on a JEOL JMS DX-303 spectrometer operating at 70 eV.

SPONGE MATERIAL.—The sponge *Xestospongia* sp. was collected off Ishigaki Island, Okinawa and kept frozen until used. It is a soft compressible rather crumbly sponge; the texture is firmer at the surface. Dull orange in color when preserved, greenish at the surface. The surface has many small oscules. The skeleton is reticulate to round meshed and the reticulation occurs in bands parallel to the surface. Interstitial spicules are abundant. Spicules are strongyloxeas without differentiation into distinct size categories, 300×10 μm, range 270–341×9–11 μm. Thinner forms are also abundant. The voucher specimen (SS-217) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

COLLECTION, EXTRACTION, AND ISOLATION.—The sponge (1.4 kg wet wt) was extracted with MeOH (1 liter×2). The MeOH extract was partitioned between EtOAc (500 ml×3) and H_2O (500 ml). The EtOAc-soluble materials (5.78 g) were partly (1.11 g) subjected to a Si gel column (Wako gel C-300, Wako Pure Chemical, 2.3×45 cm) with $CHCl_3$-MeOH (96:4). The fraction eluting from 680 to 860 ml was subjected to a Si gel column (Wako gel C-300, 1.2×17 cm) with hexane-EtOAc (1:2). The fraction eluting from 80 to 300 ml was separated by reversed-phase hplc [ODS, YMC-Pack AM323, YMC Co., 1.0×25 cm; flow rate 2.0 ml/min; ri detection; eluent MeOH-H_2O (80:20)] to afford xestokerols A [**1**] (21.0 mg, Rt 47 min) and B [**2**] (15.9 mg, Rt 62 min). The fraction eluting from 290–390 ml of the first Si gel column was subjected to a Si gel column (Wako gel C-300, 1.4×50 cm) with hexane-$CHCl_3$-MeCN (7:2:1). The fraction eluting from 70–90 ml was separated by reversed-phase hplc [ODS, YMC-Pack AM323, 1.0×25 cm; flow rate 2.5 ml/min; uv detection at 254 nm; eluent MeOH-H_2O (95:5)] to give xestokerol C [**3**] (1.6 mg, Rt 37.2 min).

Xestokerol A [**1**].—A colorless oil: $[\alpha]_D^{21}$ +30.0° (c=1.1, MeOH); ir (KBr) ν max 3400 and 1710 cm^{-1}; ^1H nmr (C_5D_5N) δ_H 0.17 (1H, dt, J=8.4, and 4.4, H-26), 0.30 (1H, dt, J=8.4 and 4.4 Hz, H-26), 0.43 (1H, ddt, J=9.2, 8.4, and 4.4 Hz, H-25), 0.53 (1H, m, H-27), 0.90 (3H, s, H_3-19), 0.94 (3H, d, J=5.9 Hz, H_3-29), 1.20 (3H, d, J=6.6 Hz, H_3-28), 1.31 (3H, s, H_3-18), 3.65 (1H, dd, J=10.7 and 4.3 Hz, H-12), 4.41 (2H, m, H-21 and H-22), 4.60 (1H, dd, J=10.6 and 4.0 Hz, H-21), 5.64 (1H, brd, J=6.2 Hz, OH-21), 6.46 (1H, brs, OH-22), 6.89 (1H, brs, OH-20), and 7.04 (1H, brs, OH-12); ^{13}C nmr see Table 1; fabms m/z $[M+H]^+$ 477; hrfabms m/z $[M+H]^+$ 477.3560 (calcd for $C_{29}H_{49}O_5$, 477.3580); HMBC correlations (C_5D_5N, H/C) 2/1,2/3, 4/3, 4/10, 9/10, 9/19, 11/9, 11/10, 11/12, 12/18, 17/13, 17/15, 17/18, 17/20, 17/21, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 21/17, 21/20, 21/22, 23/22, 23/24, 23/25, 23/28, 25/29, 26/24, 26/25, 26/27, 26/29, 27/24, 28/23, 28/24, 28/25, 29/25, 29/26; NOESY correlations (C_5D_5N, H/H) 1_{ax}/9, 2_{ax}/19, 5_{ax}/9, 9/12, 9/14, 12/14, 12/17, 15_{ax}/18, 25/26, 25/29, 26/29.

Xestokerol B [**2**].—A colorless amorphous powder: mp 218–221°; $[\alpha]_D^{21}$ −5.2° (C=1.1, MeOH); ir (KBr) ν max 3350 and 1705 cm^{-1}; ^1H nmr (C_5D_5N) δ_H 0.13 (1H, m, H-26), 0.23 (2H, m, H-25, -26), 0.55 (1H, m, H-27), 0.71 (1H, m, H-24), 0.99 (3H, s, H_3-19), 1.03 (3H, d, J=7.0 Hz, H_3-28), 1.07 (3H, d, J=5.9 Hz, H_3-29), 1.23 (3H, s, H_3-18), 1.36 (3H, s, H_3-21), 3.76 (1H, dd, J=10.5 and 4.3 Hz, H-12), 4.08 (1H, brs, H-7), 5.58 (1H, d, J=3.7 Hz, OH-7), 6.97 (2H, s, OH-12 and OH-20); ^{13}C nmr see Table 1; eims m/z $[M-H_2O]^+$ 442; hreims m/z $[M-H_2O]^+$ 442.3445 (calcd for $C_{29}H_{48}O_4$, 442.3447); HMBC correlations (C_5D_5N, H/C) 2/3, 4/3, 5/3, 6/4, 7/5, 7/9, OH-7/6, 12/18, 9/5, 11/12, 14/9, 14/13, 14/18, 15/13, 16/13, 17/13, 17/18, 17/20, 17/21, 17/22, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 21/17, 21/20, 22/20, 22/21, 22/23, 23/24, 23/25, 23/28, 24/25, 24/28, 26/24, 26/25, 26/27, 26/29, 27/24, 28/23, 28/24, 28/25, 29/25, 29/26, 29/27; NOESY correlations (C_5D_5N, H/H) 2_{ax}/19, 6_{ax}/19, 6_{eq}/7_{eq}, 7_{eq}/8, 7_{eq}/15, 8/19, 8/18, 12/17, 25/29, 26/27, 26/29.

Xestokerol C [**3**].—A colorless amorphous powder: mp 137–140°;$[\alpha]_D^{27}$ −43.6° (c=0.6, MeOH); ir (neat) ν max 3500, 1710, and 1680 cm^{-1}; uv (MeOH) λ max 226 nm (ϵ 6700); ^1H nmr (C_5D_5N) δ_H 0.11 (1H, dt, J=8.8 and 4.4 Hz, H-26′), 0.23 (1H, dt, J=8.8 and 4.4 Hz, H-26), 0.31 (1H, tt, J=8.8 and 4.4 Hz, H-25), 0.60 (1H, m, H-27), 0.71 (1H, m, H-9), 0.77 (1H, m, H-7′), 0.83 (3H, s, H_3-18), 0.88 (3H, s, H_3-19), 0.95 (3H, d, J=5.8 Hz, H_3-29), 1.02 (3H, d, J=6.8 Hz, H_3-28), 1.10 (1H, m, H-14), 1.15 (1H, m, H-1′), 1.17 (2H, m, H_2-6), 1.32 (2H, m, H-8 and H-15′), 1.34 (1H, m, H-5), 1.49 (1H, m, H-11′), 1.60 (1H, m, H-7), 1.63 (1H, m, H-24), 1.65 (1H, m, H-15), 1.78 (2H, m, H-1 and H-11), 1.79 (2H, m, H_2-16), 2.08 (1H, m, H-4′), 2.25 (1H, m, H-4), 2.29 (2H, m, H_2-2), 2.72 (1H, dd, J=16.1 and 6.8 Hz, H-23′), 3.11 (1H, dd, J=16.1 and 6.3 Hz, H-23), 3.65 (1H, dt, J=10.7 and 4.4 Hz), 5.74 (1H, s, H-21′), 6.17 (1H, s, H-21); ^{13}C nmr see Table 1; fabms m/z $[M+H]^+$ 441, $[M-H_2O+H]^+$ 423; hrfabms m/z $[M+H]^+$ 441.3343 (calcd for $C_{29}H_{45}O_3$, 441.3369); HMBC correlations ($CDCl_3$, H/C) 1/2, 1/3, 1/5, 1/10, 1/19, 2/3, 4/3, 4/4, 4/10, 5/9, 9/10, 9/19, 11/8, 11/9, 11/12, 11/13, 12/11, 14/12, 14/15, 14/18, 15/13, 16/13, 17/13, 17/16, 17/18, 17/20, 17/22, 21/17, 21/20, 21/22, 23/22, 23/24, 23/25, 23/28, 25/24, 25/27, 25/28, 26/24, 26/25, 26/27, 26/29, 27/24; NOESY correlations (C_5D_5N, H/H) 1_{ax}/5, 1_{ax}/9, 2_{ax}/19, 5/9, 6_{ax}/19, 7_{ax}/9, 7_{eq}/15_{eq}, 9/12, 9/14, 11_{ax}/19, 12/14, 12/17, 14/17, 15_{ax}/18, 16_{ax}/17, 16_{eq}/21, 21/23, 23/28, 25/26, 26/27, 25/28, 25/29.

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