New Pentacyclic Compounds from the Okinawan Marine Sponge Xestospongia sapra

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NEW PENTACYCLIC COMPOUNDS FROM THE OKINAWAN MARINE SPONGE *XESTOSPONGIA SAPRA*

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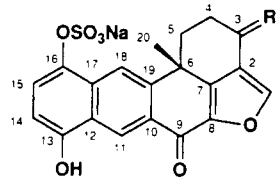
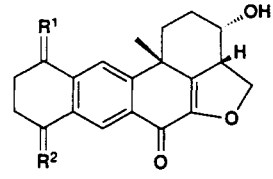
ABSTRACT.—Three new pentacyclic compounds, xestoquinol sulfate [**1**] and xestosaprols A [**2**] and B [**3**], have been isolated from the Okinawan marine sponge *Xestospongia sapra* and their structures elucidated on the basis of the spectroscopic data and a chemical conversion.

A variety of pentacyclic quinone and hydroquinone compounds has been isolated from marine sponges of the genera *Xestospongia* (1–3) and *Adocia* (4), and these unique secondary metabolites have exhibited useful biological activities such as antimicrobial, cardiotonic, and cytotoxic activities (1,2,4). During our studies on bioactive substances from Okinawan marine organisms (5–10), we examined extracts of the sponge *Xestospongia sapra* de Laubenfels [family Petrosiidae, order Petrosida (=Nephelio-spongida)] from a collection different from that used in the previous study (2). As a result we isolated a new hydroquinone sulfate, named xestoquinol sulfate [**1**], and two new pentacyclic alcohols, designated xestosaprols A [**2**] and B [**3**], from the sponge. In this paper we describe the isolation and structure elucidation of **1**, **2**, and **3**.

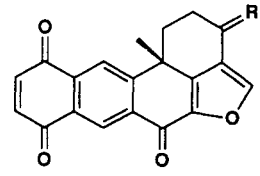
The sponge *X. sapra* was collected off the Kerama Islands, Okinawa, and kept frozen until used. The MeOH extract of the sponge was partitioned between EtOAc and H_2O, and the aqueous layer was subsequently extracted with *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to reversed-phase C_{18} cc and eluted with 40% MeOH containing 0.1% HOAc followed by gel permeation chromatography on Toyopearl HW-40F eluted with MeOH to give xestoquinol sulfate [**1**] (0.08% yield, wet wt) along with a known related compound, halenaquinol sulfate [**4**] (3). The EtOAc-soluble fraction was subjected repeatedly to Si gel cc with hexane/Me_2CO or $CHCl_3$/MeOH as eluent, followed by purification with hplc (Si gel



1 R=H_2
4 R=O



2 R^1=H,OH; R^2=O
3 R^1=R^2=H,OH
5 R^1=O; R^2=H,OH



6 R=H_2
7 R=O

and C_{18}) to give xestosaprols A [**2**] (0.0004%) and B [**3**] (0.0002%) together with a known related compound **5** (4).

Xestoquinol sulfate [**1**] was obtained as a yellow solid. The uv absorption spectrum (λ max 225, 274, 310, and 394 nm) of **1** was quite similar to that of halenaquinol sulfate [**4**], indicating the presence of closely related chromophores. The negative ion fabms of **1** showed an intense quasi-molecular ion peak at *m/z* 399 $[M-Na]^-$. The hrfabms analysis revealed the composition of this ion to be $C_{20}H_{15}O_7S$ (*m/z* 399.0554, Δ +1.6 mmu). This formula along with the chromatographic behavior suggested the presence of a sulfate group in **1**, which was supported by the ir absorption bands (ν max 1250 and 1225 cm^{-1}) (11). The ^{1}H-nmr spectrum of **1** showed three singlets and two doublets in the aromatic region, the latter being coupled to each other by 8.2 Hz. The ^{13}C-nmr spectrum of **1** showed signals due to fifteen sp^2 and five sp^3 carbons. These nmr results correlated well with those of halenaquinol sulfate [**4**] (3). The nmr data for both **1** and **4** were verifiably interpreted on the basis of the several types of 2D nmr spectra including ^{1}H-^{1}H COSY, HMQC (12), and HMBC (13) experiments, which enabled a complete assignment of the ^{13}C nmr signals. [It was found that in the literature (3) the ^{13}C-nmr signals due to C-7 and C-19 of compound **4** were assigned reversely, because HMBC connectivities for C-7/H-1 and C-19/H-11 were observed in our present study.] The structural difference between **1** and **4** was clarified as follows. In the ^{1}H-nmr spectrum of **1** the signal for H-1 (δ_H 7.90) was observed at higher field than that of **4** (δ_H 8.75), implying that the electron-withdrawing group around H-1 in **4** was lost in **1**. On the other hand, in the ^{13}C-nmr spectrum of **1** the carbonyl signal of the lowest resonance (δ_C 192.3, s; C-3 for **4**) was not observed, and was replaced by a signal due to an sp^3 methylene carbon (δ_C 18.0, t; C-3). In addition, the C-4 methylene carbon of **1** resonated at higher field (**1** δ_C 16.5, t; **2** δ_C 36.5, t). From these observations **1** was deduced to be a 3-deoxo form of **4**. This inference was substantiated by comparison with the spectral data of another related quinone compound, xestoquinone [**6**] (H-1 δ_H 7.62; C-3 δ_C 17.8, t; C-4 δ_C 16.2, t) (2). Xestoquinol sulfate [**1**] was converted into xestoquinone [**6**] through acid hydrolysis followed by air-oxidation.

Xestosaprol A [**2**] was obtained as a yellow solid. The molecular formula of **2** was determined by hreims to be $C_{20}H_{20}O_5$, which was the same as that of compound **5** (4). Uv and ir spectral data of **2** were quite similar to those of compound **5**. In the ^{1}H-nmr spectrum of **2**, however, the H-18 signal was shifted to higher field (**2** δ_H 8.28; **5** δ_H 8.45) and the H-11 signal to lower field (**2** δ_H 9.26; **5** δ_H 9.04). From this result xestosaprol A [**2**] was suggested to be the 16-hydroxy-13-keto form of **5**. In the ^{13}C-nmr spectrum of **2** the carbonyl signal at δ_C 196.4 was assigned to C-13 because of its HMBC long-range ^{1}H-^{13}C correlation with H-11. The relative configurations at C-2, C-3, and C-6 were confirmed by comparison of the ^{13}C- and ^{1}H-nmr data of **2** and **5**: nOe correlations were observed between H_3-20/H-2 and H-2/H-3 by difference nOe spectra, and the C-20 methyl signal resonated at higher field (δ_C 24.4) than that of C-1/C-2 unsaturated derivatives like halenaquinone [**7**] (δ_C 29–32) due to a steric compression effect from H-2. The hydroxy-bearing methine proton on C-3 of **2** was observed as a broad singlet with relatively small *J* values with H-2 and H_2-4, suggesting H-3 to be equatorial. The configuration at C-16 of **2** is under investigation.

Xestosaprol B [**3**] was also obtained as a yellow solid. The hreims analysis showed the molecular formula of **3** to be $C_{20}H_{22}O_5$, indicating that xestosaprol B [**3**] is a dihydro form of compound **2** (or **5**). Its uv absorption spectrum im-

plied that **3** possesses a conjugated system different from that of **2**. Its ^{1}H- and ^{13}C-nmr spectra were analogous to those of **2** or **5**, except for the observations that no signals due to protons adjacent to carbonyl group (H-14 for **2**; H-15 for **5**) were observed for **3**, suggesting that the carbonyl at C-13 of **2** (or C-16 of **5**) is reduced to a secondary alcohol for **3**. The ^{13}C-nmr of **3** showed the absence of a carbonyl signal around δ_C 197 and the presence of three hydroxyl groups (δ_C 66.2, 66.6, and 66.9 for C-3, C-13, and C-16, respectively). The configurations of C-2, C-3, and C-6 of **3** were deduced to be the same as those of **2** or **5** on the basis of ^{1}H- and ^{13}C-nmr data. The stereochemistry of the C-13 and C-16 positions of **3** remains to be defined.

Xestoquinol sulfate [**1**] and xestosaprols A [**2**] and B [**3**] exhibited inhibitory effect on DNA topoisomerase I activity with minimum inhibitory concentration (MIC) of 10, 12.5, and 12.5 μg/ml, respectively. Compound **5**, xestoquinone [**6**], and halenaquinone [**7**] were also found to inhibit topoisomerase I activity and to be more potent than **1–3**, with MIC values of 2.5, 2, and 0.4 μg/ml, respectively. Halenaquinol sulfate [**4**], however, proved to be inactive (>50 μg/ml) in this assay. Xestoquinone [**6**] and halenaquinone [**7**] were cytotoxic against murine lymphoma L1210 (IC_{50} 0.17 and 1.6 μg/ml, respectively) and human carcinoma KB (IC_{50} 1.7 and 3.1 μg/ml, respectively) cells in vitro, while xestoquinol sulfate [**1**] and halenaquinol sulfate [**4**] were less cytotoxic; **1** and **4** at 10 μg/ml exhibited 25.4% and 22.4% inhibition, respectively, against L1210 cells in vitro. Against KB cells compound **1** showed almost no effect and compound **4** was weakly cytotoxic (12.2% at 10 μg/ml). Xestosaprol B [**3**] was also cytotoxic against L1210 and KB cells in vitro with IC_{50} values of 2.7 and 8.6 μg/ml, respectively, whereas xestosaprol A [**2**] and compound **5** were less cytotoxic (L1210 34.9% and 14.5% at 10 μg/ml, respectively; KB 30.1% and 9.8% at 10 μg/ml, respectively).

EXPERIMENTAL

GENERAL METHODS.—Optical rotations were determined on a JASCO DIP-370 polarimeter. Uv and ir spectra were obtained on a Shimadzu uv-220 spectrometer and a JASCO ir Report-100 spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL EX-400 and GX-270 spectrometers. Fabms and eims were obtained on JEOL HX-110 and DX-303 spectrometers, respectively. Wako C-300 Si gel (Wako Pure Chemical) was used for glass cc, and tlc was carried out on Merck Si gel GF_{254}.

ISOLATION.—The sponge *X. sapra* (1 kg) was collected off Kerama Islands, Okinawa, by SCUBA, and a voucher specimen (SS-241) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University. The MeOH extract of the sponge (63 g) was partitioned between EtOAc (400 ml × 3) and H_2O (400 ml), and the aqueous layer was subsequently extracted with *n*-BuOH (400 ml × 3). The *n*-BuOH-soluble fraction (100 mg) was subjected to reversed-phase C_{18} cc (YMC-GEL ODS-A60-350/250; 2.4 × 36 cm) eluted with 40% MeOH containing 0.1% HOAc followed by gel permeation chromatography on Toyopearl HW-40F (1.8 × 80 cm) eluted with MeOH to give xestoquinol sulfate [**1**] (5.3 mg) and the known halenaquinol sulfate [**4**] (7.7 mg) (3). The EtOAc-soluble fraction (2.4 g) was subjected to Si gel cc (4.5 × 29 cm) eluted with 0–100% Me_2CO in hexane. The fraction eluting with 60% Me_2CO in hexane was separated by a Si gel column (2.2 × 12 cm) with 2–50% MeOH in $CHCl_3$ as eluent, to give a mixture of compounds **2**, **3**, and **5**, which was further purified by Si gel and C_{18} hplc [Senshu Pack Silica-4251-S, 10 × 250 mm, 3–4% MeOH in $CHCl_3$; Capcellpak C_{18}, 10 × 250 mm, MeCN-H_2O (7:3)] to give xestosaprols A [**2**] (2.1 mg) and B [**3**] (1.3 mg) and compound **5** (9.3 mg) (4).

Xestoquinol sulfate [**1**].—$[\alpha]^{25}D$ +27° (*c* = 0.56, MeOH); uv (MeOH) λ max 225 (ε 40000), 274 (2000), 310 (15000), 399 nm (5200); ir (KBr) ν max 3300, 3100, 1650, 1620, 1380, 1250, 1225, 1020, 800 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ_H 8.96 (1H, s, H-11), 8.25 (1H, s, H-18), 7.90 (1H, s, H-1), 7.34 (1H, d, *J* = 8.2 Hz, H-15), 6.83 (1H, d, *J* = 8.2 Hz, H-14), 1.45 (3H, s, H_3-20); ^{13}C nmr see Table 1; HMBC correlations (C/H) C-2/H-1, C-5/H_3-20, C-6/H-18, C-6/H_3-20, C-7/H-1, C-7/H_3-20, C-8/H-1, C-9/H-11, C-10/H-18, C-12/H-14, C-12/H-18, C-13/H-11, C-13/H-14, C-13/H-15, C-16/H-14, C-16/H-15, C-16/H-18, C-17/H-11, C-17/H-15, C-19/H-11, C-19/H_3-20; fabms (negative ion,

TABLE 1. ^{13}C-nmr Spectral Data of Compounds **1**, **2**, and **3** in DMSO-d_6.[a]

Carbon	Compound		
	1	**2**	**3**
C-1 . . .	144.9(1)	70.7(2)	70.5(2)
C-2 . . .	121.3(0)	46.6(1)	46.5(1)
C-3 . . .	18.0(2)	66.2(1)	66.2(1)
C-4 . . .	16.5(2)	28.2(2)	28.2(2)
C-5 . . .	31.7(2)	33.7(2)	34.0(2)
C-6 . . .	35.8(0)	—[b]	39.7(0)
C-7 . . .	146.9(0)	141.0(0)	140.3(0)
C-8 . . .	145.6(0)	146.5(0)	146.6(0)
C-9 . . .	171.6(0)	174.2(0)	175.0(0)
C-10 . . .	129.5(0)	129.4(0)	130.3(0)
C-11 . . .	123.2(1)	124.2(1)	124.9(1)
C-12 . . .	123.0(0)	130.9(0)	145.5(0)
C-13 . . .	150.8(0)	196.4(0)	66.6(1)
C-14 . . .	107.8(1)	35.5(2)	30.1(2)
C-15 . . .	120.6(1)	31.6(2)	29.9(2)
C-16 . . .	141.1(0)	66.1(1)	66.9(1)
C-17 . . .	129.9(0)	150.4(0)	145.5(0)
C-18 . . .	119.1(1)	125.1(1)	125.1(1)
C-19 . . .	144.9(1)	156.0(0)	149.8(0)
C-20 . . .	33.4(3)	24.4(3)	24.6(3)

[a]Numbers in parentheses denote the number of protons attached to each carbon.

[b]Overlapped with the signal of DMSO.

glycerol matrix) *m/z* $[M-Na]^-$ 399; hrfabms found *m/z* 399.0554, calcd for $C_{20}H_{15}O_7S$ $[M-Na]^-$ 399.0538.

Xestosaprol A [**2**].—$[\alpha]^{27}D$ −42° (*c* = 0.35, MeOH); uv (MeOH) λ max 248 (ε 22000), 326 nm (6300); ir (film) ν max 3450, 1670, 1640, 1600 cm^{-1}; ^{1}H nmr (pyridine-d_5) δ_H 9.26 (1H, s, H-11), 8.28 (1H, s, H-18), 5.22 (1H, dd, *J* = 4.0, 9.5 Hz, H-16), 4.92 (1H, dd, *J* = 6.2, 8.8 Hz, H-1), 4.63 (1H, dd, *J* = 8.8, 10.6 Hz, H-1′), 4.18 (1H, br s, H-3), 2.98 (1H, ddd, *J* = 4.4, 5.5, 17.0 Hz, H-14), 2.70 (1H, ddd, *J* = 4.9, 11.5, 17.0 Hz, H-14′), 2.46 (1H, m, H-15), 2.27 (1H, m, H-15′), 1.88–1.99, 1.99–2.10 (2H, m, each, H-4, 5), 1.49 (3H, s, H_3-20); ^{13}C nmr see Table 1; HMBC correlations (C/H) C-2/H-1, C-3/H-1, C-5/H-3, C-5/H_3-20, C-6/H-18, C-6/H_3-20, C-7/H-1, C-7/H-2, C-7/H-3, C-7/H_3-20, C-8/H-1, C-8/H-2, C-9/H-11, C-10/H-18, C-12/H-18, C-13/H-11, C-13/H-14, C-13/H-15, C-16/H-14, C-16/H-15, C-16/H-18, C-17/H-11, C-17/H-15, C-19/H-11, C-19/H_3-20; eims *m/z* $[M]^+$ 340, $[M-Me]^+$ 325, $[M-C_3H_5O]^+$ 283; hreims found *m/z* $[M]^+$ 340.1314, calcd for $C_{20}H_{20}O_5$ $[M]^+$ 340.1311.

Xestosaprol B [**3**].—$[\alpha]^{28}D$ +49° (*c* = 0.22, MeOH); uv (MeOH) λ max 214 (ε 14000), 268 (6500), 285 nm (5900); ir (film) ν max 3450, 1650, 1610, 1070, 1010 cm^{-1}; ^{1}H nmr (pyridine-d_5) δ_H 9.05 (1H, s, H-11), 8.20 (1H, s, H-18), 5.17 (2H, m, H-13, 16), 4.95 (1H, dd, *J* = 6.2, 8.8 Hz, H-1), 4.62 (1H, dd, *J* = 8.8, 10.6 Hz, H-1′), 4.16 (1H, br s, H-3), 3.42 (1H, ddd, *J* = 4.0, 6.2, 10.6, H-2), 2.54 (2H, m, H-14, -15), 2.09 (4H, m, H-14′, -15′, H_2-5), 1.97 (2H, m, H_2-4), 1.47 (3H, s, H_3-20); ^{13}C nmr see Table 1; eims *m/z* $[M]^+$ 342, $[M-Me]^+$ 327, $[M-2H_2O]^+$ 306, $[M-C_3H_5O]^+$ 285; hreims *m/z* $[M]^+$ 342.1464, calcd for $C_{20}H_{22}O_5$ $[M]^+$ 342.1467.

CONVERSION OF XESTOQUINOL SULFATE [**1**] INTO XESTOQUINONE [**6**].—Xestoquinol sulfate [**1**] (6.4 mg) was treated with 1 N HCl (2 ml) and MeOH (4 ml) at room temperature for 22 h. Tlc examination showed the formation of the desulfated product (xestoquinol) (14), the hydroquinone moiety of which was, however, subjected to autooxidation during evaporation of the solvent under reduced pressure to generate xestoquinone [**6**]. The residue was purified by Si gel tlc with hexane-EtOAc (1:1) to give xestoquinone [**6**] (0.8 mg).

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