# Hyrtiosenolides A and B, two new sesquiterpene gamma-methoxybutenolides and a new sterol from a Red Sea sponge Hyrtios species

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# Hyrtiosenolides A and B, Two New Sesquiterpene γ-Methoxybutenolides and a New Sterol from a Red Sea Sponge *Hyrtios* Species

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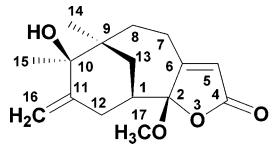
Two new sesquiterpene γ-methoxybutenolides, hyrtiosenolides A (**1**) and B (**2**), together with a new 4α-methyl polyoxygenated steroid, hyrtiosterol (**3**), were isolated from a Red Sea sponge, *Hyrtios* species. Their structures were elucidated by analysis of their 1D and 2D NMR spectra and HRFABMS. Compounds **1** and **2** showed weak antibacterial activity against *Escherichia coli*.

Marine sponges of the genus *Hyrtios* have proven to be a rich source of secondary metabolites including sesterterpenes,[1,2] sesquiterpene-quinones,[3,4] macrolides,[5] tryptamine-derived alkaloids,[6,7] and β-carboline alkaloids,[8] many of which show significant biological activities.[1,4−6,9−14] In the course of our study on bioactive metabolites in Red Sea organisms, a marine sponge *Hyrtios* species showed weak antimicrobial activity. This paper deals with the isolation and structural determination of two new sesquiterpenes and a new sterol from this sponge.

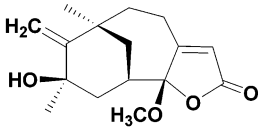
The frozen sponge was extracted with MeOH−$CH_2Cl_2$ (1:1). The combined extracts were dried, and the residue was partitioned between 90% aqueous MeOH and hexane, and 60% MeOH and $CH_2Cl_2$. The $CH_2Cl_2$ extract was successively subjected to size exclusion chromatography on Sephadex LH-20, flash ODS column, and final reversed-phase HPLC purification to afford hyrtiosenolides A (**1**) and B (**2**).

To substantiate the nonartifactual nature of **1** and **2**, the sponge was collected again but extracted with acetone. The fractionation and separation of the crude extract followed the same procedures for both collections (Experimental Section). Final purification of the extract afforded, in addition to **1** and **2**, a new polyhydroxylated sterol, hyrtiosterol (**3**). The existence of compounds **1** and **2** in both acetone (collection of July 2003) and MeOH−$CH_2Cl_2$ (collection of November 2001) extracts of the sponge supports the natural origin of these compounds. Therefore, both **1** and **2** are metabolites of the sponge and not artifacts that formed during the extraction of the sponge with MeOH.

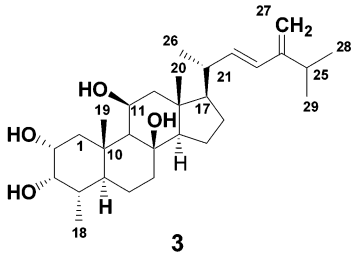
Hyrtiosenolide A (**1**) was purified as a white solid with a molecular formula of $C_{16}H_{22}O_4$ as deduced by HRFABMS. Its $^1$H NMR spectrum displayed signals for 22 protons including three methyl singlets, four methylenes, one methine, an exomethylene, an olefinic proton singlet for a trisubstituted olefin, and an exchangeable singlet for an O*H* functionality. In addition, the $^{13}$C NMR spectrum of **1** showed resonances for 16 carbons including three methyl groups, five methylenes, two methines, and six quaternary carbons.




**1**




**2**




**3**

Interpretation of 2D NMR data (COSY, HOHAHA, and HMQC) of **1** led to the assembly of two substructures, A and B (Figure 1). The COSY spectrum contained two spin systems including the fragments C-12/C-1/C-13 and C-7/C-8. The first spin system showed geminal coupling between the protons at δ 1.80 (H-12α) and 2.66 (H-12β). The latter was further coupled to the methine proton at δ 2.24 (H-1), which was in turn correlated with the proton at δ 2.01 (H-13β), which showed geminal coupling to the signal at δ 1.51 (H-13α), thereby completing the first spin-coupling system (C-13/C-1/C-12). The spin system was interrupted at this point, suggesting the quaternary nature of the adjacent carbons, C-2, C-9, and C-11, respectively. The placement of the methyl and exomethylene moieties at C-9 and C-11, respectively, was supported by long-range correlations in the HOHAHA spectrum. Correlations between the signal resonating at δ 1.50 (H-8α) and the three-proton singlet at δ 0.88 ($H_3$-14) as well as between H-12α

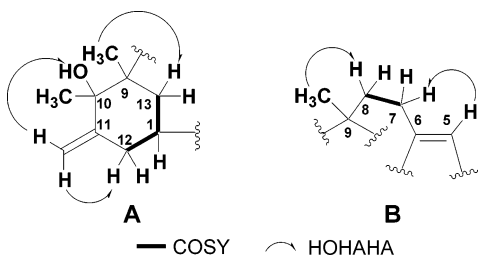



**Figure 1.** Subunits of **1**.

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**Table 1.** $^1$H (600 MHz) and $^{13}$C (150 MHz) Chemical Shift Data of Compounds **1** and **2** (DMSO-$d_6$)

| | **1** | | | **2** | | |
|---|---|---|---|---|---|---|
| no. | $\delta_C$ (mult.) | $\delta_H$ [mult., *J* (Hz)] | HMBC (H→C#) | $\delta_C$ (mult.) | $\delta_H$ [mult., *J* (Hz)] | HMBC (H→C#) |
| 1 | 37.9 (CH) | 2.24 t (6.0) | 2, 6, 11, 12, 13 | 34.3 (CH) | 2.47 m[b] | 2, 6, 9, 11, 12, 13 |
| 2 | 111.3 (C) | | | 113.2 (C) | | |
| 4 | 170.1 (C)[a] | | | 170.0 (C) | | |
| 5 | 119.1 (CH) | 5.84 s | 2, 4, 6, 7 | 120.3 (CH) | 6.10 s | 2, 4, 6, 7 |
| 6 | 170.1 (C)[a] | | | 168.5 (C) | | |
| 7α | 24.6 ($CH_2$) | 2.36 dt (17.5, 5.8) | 5, 6, 8 | 23.8 ($CH_2$) | 2.35 m | 5, 6 |
| 7*β* | | 2.58 ddd (17.5, 10.0, 5.0) | | | 2.68 ddd (12.3, 8.0, 4.3) | |
| 8α | 33.9 ($CH_2$) | 1.50 m[a] | 7, 9, 13, 14 | 39.5 ($CH_2$)[b] | 1.52 ddd (14.5, 7.7, 4.3) | 6, 7, 13 |
| 8*β* | | 1.41 ddd (15.0, 5.8, 5.0) | | | 1.72 ddd (14.5, 8.0, 4.3) | |
| 9 | 40.8 (C) | | | 37.0 (C) | | |
| 10 | 73.0 (C) | | | 157.7 (C) | | |
| 11 | 149.6 (C) | | | 69.5 (C) | | |
| 12α | 30.6 ($CH_2$) | 1.80 d (14.6) | 1, 10, 11, 13 | 35.1 ($CH_2$) | 0.85 dd (15.0, 6.0) | 1, 2, 10, 11, 13 |
| 12*β* | | 2.66 dd (14.6, 7.3) | | | 1.76 dd (15.0, 10.0) | |
| 13α | 32.4 ($CH_2$) | 1.51 br d (14.6)[a] | 1, 8, 9, 12, 14 | 31.4 ($CH_2$) | 1.67 br d (14.0) | 1, 2, 8, 9, 10, 12, 14 |
| 13*β* | | 2.01 dd (14.6, 6.0) | | | 2.24 dd (14.0, 7.5) | |
| 14 | 26.3 ($CH_3$) | 0.88 s | 8, 9, 10, 13 | 29.2 ($CH_3$) | 1.10 s | 8, 9, 10, 13 |
| 15 | 19.7 ($CH_3$) | 1.15 s | 9, 10, 11, | 107.9 ($CH_2$) | 5.11 s | 9, 10, 11 |
| | | | | | 4.86 s | |
| 16 | 111.4 ($CH_2$) | 4.83 s | 10, 11, 12 | 32.5 ($CH_3$) | 1.20 s | 10, 11, 12 |
| | | 4.47 s | | | | |
| 17 | 49.7 ($CH_3$) | 3.01 s | 2 | 50.5 ($CH_3$) | 3.07 s | 2 |
| O*H* | | 4.40 s | 9, 10, 15 | | 4.47 s | 10, 11, 12, 16 |

[a] In each column signals are partially or completely overlapped. [b] Signals are overlapped with solvent peak.

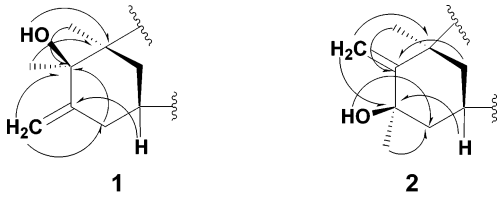


**Figure 2.** Partial key $^3J_{CH}$ HMBC correlations for **1** and **2**.

(*δ* 1.80) and one of the exomethylene protons at *δ* 4.47 supported the assignment of these moieties. Further long-range correlations were observed between the exchangeable singlet at *δ* 4.40 (O*H*) and the second exomethylene proton at *δ* 4.83, as well as between the three-proton singlet at *δ* 1.15 ($H_3$-15) and H-13*β* (*δ* 2.01), thus completing the subunit **A**. The second spin-coupling system showed geminal and vicinal correlations between the protons of the ethylenic moiety at C-7/C-8. The absence of any other correlation suggested the quaternary nature of the carbons (C-6 and C-9) adjacent to this moiety. However, long-range correlations between the olefinic singlet at *δ* 5.84 (H-5) and the proton at *δ* 2.58 (H-7*β*) confirmed the placement and the assignment of the trisubstituted olefin, thus completing the subunit **B**.

Unequivocal assignment of all protonated carbons could be done by HMQC experiments, while $^3J_{CH}$ and $^2J_{CH}$ correlations in HMBC experiments secured the assignment of all quaternary carbons as well as the fusion of the subunits of **1** (Figure 2 and Table 1). For example, $^3J_{CH}$ HMBC cross-peaks of H-1/C-6, H-1/C-9, H-1/C-11, $H_3$-17/C-2, and H-5/C-2 as well as $^2J_{CH}$ HMBC cross-peaks of H-1/C-2, H-1/C-12, and H-5/C-4 confirmed the assignment of these carbons and the fusion of the five-membered lactone moiety at C-2 and C-6. The assignment of the overlapped resonances of C-5 and C-6 at *δ* 170.1 was secured from HMBC correlations of H-1/C-6 ($^3J_{CH}$), H-5/C-4 ($^2J_{CH}$), H-5/C-6 ($^2J_{CH}$), $H_2$-7/C-6 ($^2J_{CH}$), and $H_2$-8/C-6 ($^3J_{CH}$). Similarly, $^3J_{CH}$ HMBC cross-peaks of $H_3$-14/C-10, $H_3$-15/C-9, O*H*/C-9, O*H*/C-11, $H_2$-16/C-12, and $H_2$-16/C-10 supported the assignment of all quaternary carbons as well as the substitution pattern on the carbons C-9, C-10, and C-11.

The assignment of the relative stereochemistry of **1**

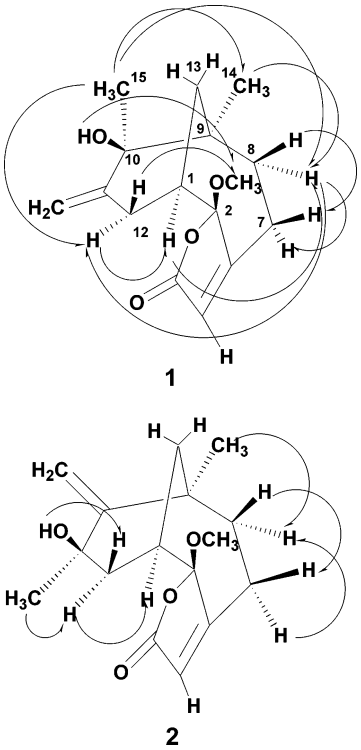


**Figure 3.** Key 2D ROESY correlations for **1** and **2**.

(Figure 3) was performed by 2D ROESY experiments; cross-peaks of H-12α/$H_3$-15, $H_3$-14/$H_3$-15, $H_3$-14/H-8α, H-8α/H-1, H-8α/H-7α, and H-12α/H-1 confirmed that these moieties are α to the bicyclo[4.3.1]decane ring. NOEs were observed between *H*O-10 and OC*H*$_3$-2 as well as between H-12*β* and OC*H*$_3$-2. Moreover, the absence of NOE correlation between H-1 and OC*H*$_3$-2 supported the *β*-configuration of the methoxyl moiety.

Hyrtiosenolide B (**2**) was an isomer of **1**, which was evident from HRFABMS and $^1$H and $^{13}$C NMR spectra. Careful studies of the 1D and 2D (COSY, HMQC, HMBC, ROESY) NMR spectra of **2** indicated that the only difference between both compounds was found in the position

**Table 2.** $^1$H (500 MHz) and $^{13}$C (125 MHz) Chemical Shift Data of Compound **3** ($CDCl_3$)

| no. | $\delta_C$ (mult.) | $\delta_H$ [mult., $J$(Hz)] | HMBC (H→C#) |
|---|---|---|---|
| 1 | 43.8 ($CH_2$) | 2.30 dd (13.7, 2.7) | C-2, C-3, C-10 |
| | | 1.25 m | |
| 2 | 69.9 (CH) | 4.06 q (2.7) | |
| 3 | 77.4 (CH) | 3.09 dd (7.1, 2.7) | |
| 4 | 32.6 (CH) | 1.75 dd (11.7, 7.1)[a] | C-2, C-3 |
| 5 | 52.7 (CH) | 0.74 ddd (11.7, 11.7, 2.9) | |
| 6 | 19.7 ($CH_2$) | 1.55 m | C-8 |
| 7 | 39.9 ($CH_2$) | 1.73 m,[a] 1.23 m | C-8 |
| 8 | 75.6 (C) | | |
| 9 | 58.0 (CH) | 0.86 brd (2.8) | C-8, C-19 |
| 10 | 36.9 (C) | | |
| 11 | 69.8 (CH) | 4.46 q (2.8) | C-8 |
| 12 | 49.1 ($CH_2$) | 2.32 dd (14.0, 2.8) | C-9, C-11, C-13, C-14, C-20 |
| | | 1.45 dd (14.0, 2.8) | |
| 13 | 42.0 (C) | | |
| 14 | 60.5 (CH) | 1.21 m | |
| 15 | 19.2 ($CH_2$) | 1.45 m | |
| 16 | 27.8 ($CH_2$) | 1.65 m, 1.31 m | |
| 17 | 58.1 (CH) | 1.10 m | |
| 18 | 14.8 ($CH_3$) | 1.01 d (6.7) | C-3, C-4, C-5 |
| 19 | 18.3 ($CH_3$) | 1.55 s | C-1, C-5, C-9, C-10 |
| 20 | 15.6 ($CH_3$) | 1.15 s | C-12, C-13, C-14, C-17 |
| 21 | 39.7 (CH) | 2.13 m | |
| 22 | 135.4 (CH) | 5.52 dd (15.8, 8.8) | C-21, C-24 |
| 23 | 129.5 (CH) | 5.92 d (15.8) | C-21, C-24, C-27 |
| 24 | 152.9 (C) | | |
| 25 | 29.4 (CH) | 2.53 m | C-23, C-24, C-27 |
| 26 | 20.1 ($CH_3$) | 1.04 d (6.6) | C-17, C-21, C-22, C-24 |
| 27 | 109.7 ($CH_2$) | 4.85 s, 4.82 s | C-23, C-24, C-25 |
| 28 | 22.4 ($CH_3$) | 1.05 d (7.0) | C-24, C-25 |
| 29 | 22.0 ($CH_3$) | 1.06 d (6.8) | C-24, C-25 |

[a] Partially overlapped signals.

of the substituents at C-10 ($CH_3$ and OH) and C-11 (exomethylene moiety). This was unequivocally confirmed by $^3J_{CH}$ and $^4J_{CH}$ HMBC correlations (Figure 2 and Table 1) of H-1/C-10, H-8/C-10, $H_3$-14/C-10, $H_2$-15/C-9, $H_2$-15/C-11, *H*O/C-10, *H*O/C-12, *H*O/C-16, $H_3$-16/C-10, and $H_3$-16/C-12. Similarly, the relative stereochemistry at C-1, C-2, C-9, and C-11 was assigned from 2D ROESY experiments (Figure 3). The ROESY cross-peaks of $H_3$-14/H-7α ($\delta$ 2.35), $H_3$-14/H-8α ($\delta$ 1.52), $H_3$-16/H-12α ($\delta$ 1.76), and H-12α/H-1 ($\delta$ 2.47) supported the α-configuration of these substituents. In addition, the correlation between O*H* ($\delta$ 4.47) and H-12$\beta$ ($\delta$ 1.76), as well as between H-7$\beta$ ($\delta$ 2.68) and H-8$\beta$ ($\delta$ 1.72), supported the $\beta$-configuration of these substituents.

The stereochemistry of the skeleton of hyrtiosenolides A and B is in good agreement with the stereochemistry of $\Delta^{7,14}$-isonakafuran-9-hydroperoxide, a sesquiterpene metabolite of the Great Barrier Reef sponge *Dysidea* sp., whose stereochemistry was confirmed by single-crystal X-ray analysis.[15]

Sesquiterpenes of the nakafuran family are characteristic of sponges of the genus *Dysidea* (family Dysideidae).[15−17] This is the first report of such metabolites (**1** and **2**) in a sponge of the genus *Hyrtios*, and this is noteworthy in view of the chemotaxonomy of the genus *Hyrtios* and the family Thorectidae.

Hyrtiosterol (**3**) was isolated as a colorless solid with a molecular formula of $C_{29}H_{48}O_4$, as established by HR-FABMS and NMR data, indicating six degrees of unsaturation. Its $^1$H NMR spectrum (Table 2) revealed resonances for 44 protons including two tertiary methyl signals at $\delta$ 1.55 ($H_3$-19) and 1.15 ($H_3$-20) and four secondary methyl resonances at $\delta$ 1.01 ($H_3$-18), 1.04 ($H_3$-26), 1.05 ($H_3$-28), and 1.06 ($H_3$-29). Additionally, signals for two olefinic methine protons at $\delta$ 5.52 and 5.92 (each d, $J$ = 15.8), two exomethylene protons at $\delta$ 4.82 and 4.85, together with three oxymethine protons at $\delta$ 4.06 (H-2), 3.09 (H-3), and 4.46 (H-11) were observed. The value of the coupling constant between H-22 and H-23 (15.8 Hz) supported the *E*-configuration of the olefinic moiety. The $^{13}$C NMR spectrum (Table 2) displayed 29 signals including six methyls, seven methylenes, 12 methines, and four quaternary carbons. The four signals between 69.8 and 77.4 ppm were indicative of oxygenated carbons. Three of the oxygenated carbons were assigned to methines and one to a quaternary carbon on the basis of COSY and HMQC data. Five signals for methyl groups ($\delta$ 18.3, 15.6, 20.1, 22.4, and 22.0) were typical for a sterol having a cholestane structure. The sixth methyl at $\delta$ 14.8 was indicative for a 4α-methyl group. This group is well known in zooxanthellae sterols.[18] Sterols with a 4α-methyl moiety have been found in many marine invertebrates including sponges and soft corals.[19,20]

The $^1$H−$^1$H COSY spectrum together with HMQC data revealed that **3** has four distinct $^1$H−$^1$H spin systems including the structural fragments C-1/C-7, C-9/C-12, C-14/C-23, and C-25/C-28,C-29. Interpretation of the COSY and HMQC spectra led to placement of three hydroxyl groups at C-2 ($\delta$ 69.9), C-3 ($\delta$ 77.4), and C-11 ($\delta$ 69.8). This assignment was also supported by HMBC cross-peaks of $H_2$-1/C-2, $H_2$-1/C-3, $H_3$-18/C-3, $H_3$-18/C-4, and $H_2$-12/C-11. The existence of the fourth OH moiety at C-8 ($\delta$ 75.6) was implied by HMBC cross-peaks of $H_2$-6/C-8, $H_2$-7/C-8, H-9/C-8, and H-11/C-8. In addition, the chemical shift of C-8 is in agreement with literature data for C-8 hydroxylated sterols.[21]

The 4α-methyl configuration was deduced from a coupling constant of 11.7 between H-4$\beta$ ($\delta$ 1.75, dd, $J$ = 11.7, 7.1 Hz) and H-5 ($\delta$ 0.74, ddd, $J$ = 11.7, 11.7, 2.9 Hz). Additional coupling of 7.1 Hz between 4$\beta$ and H-3 ($\delta$ 3.09, dd, $J$ = 7.1, 2.7 Hz) suggested the α-configuration of the OH moiety at C-3. The small coupling constant of 2.7 Hz between H-3 and H-2 supported the α-orientation of both OH moieties at C-2 and C-3, respectively. The quartet nature of H-11 ($J$ = 2.8 Hz) supported the $\beta$-configuration of the OH moiety at C-11. The placement of all functional moieties and the fusion of the four-ring system of **3** were supported by significant HMBC correlations (Table 2).

The 2D ROESY experiment indicated the relative stereochemistry of **3** as presented. Specifically, NOE interactions between H-2 and H-3, H-3, and H-4 unambiguously indicated that the OH groups attached to C-2 and C-3, as well as the methyl group at C-4, are α-oriented. Similarly, NOE correlations between H-11 and H-9 supported the $\beta$-configuration of the OH moiety at C-11. The $\beta$-configuration of the OH moiety at C-8 was assigned by comparison of its chemical shift value at C-8 with literature data.[21] C-8 hydroxylated sterols are common metabolites of starfishes.[21−25] The relative stereochemistry of the chiral centers C-13, C-14, C-17, and C-21 was established by comparison of the $^{13}$C chemical shift values of these carbons with literature data.[26−30]

Hyrtiosenolides A and B showed weak antibacterial activity against *Escherichia coli*. An inhibitory zone of 7 mm was observed when a 100 $\mu$g sample of **1** or **2** was applied to a 6 mm diameter paper disk on an agar plate inoculated with *E. coli*. At a concentration of 50 $\mu$g/disk, hyrtiosenolides A and B showed no inhibition.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-1000 or JASCO DIP-700

digital polarimeter. UV spectra were recorded on a Hitachi 300 spectrometer. NMR spectra were recorded on a JEOL α-600 spectrometer or a Varian Unity 500. Positive FAB mass spectral data were recorded on a JEOL JMS-700T or a Finnigan MAT-312 mass spectrometer using 3-NBA/NaCl as a matrix.

**Animal Material.** The sponge was collected in November 2001 and July 2003 by hand using scuba at depths between 8 and 20 m off Hurghada, Egypt, in the Red Sea. The sponge materials were frozen immediately and kept frozen at −20 °C until processed. The sponge forms pinkish-gray tubes, diameter 4.5−5.0 cm, walls 0.5−1.0 cm thick. The inner lumen measures about 3.0 cm in diameter, with a smaller side tube of 2.0 cm diameter and lumen of 1.0 cm. The larger tube is about 8.0 cm high. The surface of the sponge is densely conulose, with blunt conules 1.0 mm high and 1.0−2.0 mm apart. The consistency is firm, crumbly, and sandy. The skeleton is dense, anisotropic, and consisting of sand-filled primary and secondary fibers, near the surface forming fascicles. Individual primary fibers measure 220−385 μm in diameter, and secondary fibers have a diameter of 100−130 μm. Meshes are polygonal or rounded with a 250−1000 μm diameter. Between the fibers the mesophyl is lightly charged with debris and sand grains. The surface aspect and skeletal characters conform to the genus *Hyrtios* (class Demospongiae, order Dictyoceratida, family Thorectidae), but there are no matching descriptions at the species level for this sponge. The voucher is kept in the collections of the Zoological Museum of the University of Amsterdam, under registration No. ZMA POR. 17249.

**Extraction and Isolation.** Frozen specimens (230 g) of the sponge were extracted with MeOH−$CH_2Cl_2$ (1:1) (3 × 700 mL) at room temperature. The combined extracts were evaporated in vacuo, and the resulting brown residue was dissolved in 300 mL of MeOH−$H_2O$ (9:1) and extracted with hexane (3 × 400 mL). The methanolic layer was diluted with $H_2O$ to MeOH−$H_2O$ (3:2) and then extracted with $CH_2Cl_2$ (3 × 400 mL) to afford 850 mg of $CH_2Cl_2$ extract, which was fractionated on a Sephadex-LH-20 column eluted with MeOH to give 12 fractions. Fractions 10−12 were combined, and the resulting residue (640 mg) was flash chromatographed on an ODS column starting with 50% MeOH in $H_2O$ through pure MeOH. The fraction eluted with 60% MeOH was finally purified on a HPLC column (COSMOSIL AR-II, 25 × 20 mm, 5 μm ODS 18, Waters) using 40% MeOH isocratically as eluting solvent to yield **1** (2.0 mg, 8.6 × $10^{-4}$%) and **2** (3.5 mg, 1.5 × $10^{-3}$%) (based on wet weight).

A second specimen (185 g) of the sponge was collected from the same locality and extracted directly after collection with acetone. The crude extract was partitioned and fractionated as previously to give **1** (1.7 mg) and **2** (3.0 mg). In addition, a less polar fraction (21 mg) of the flash ODS column was subjected to final purification on a HPLC column (Ultracarb, 25 × 10 mm, 5 μm ODS 18, Phenomenex) using 80% MeCN in $H_2O$ isocratically to afford **3** (1.8 mg, 9.7 × $10^{-4}$%) (based on wet weight).

**Hyrtiosenolide A (1):** colorless solid; $[\alpha]_D$ −57.2° [*c* 0.1, MeOH−$H_2O$ (4:1)]; UV (MeOH) $\lambda_{max}$ 205 nm (log $\epsilon$ 3.47); NMR data, see Table 1; positive HRFABMS *m*/*z* 279.1590 (calcd for $C_{16}H_{23}O_4$, $[M + H]^+$, 279.1596).

**Hyrtiosenolide B (2):** colorless solid; $[\alpha]_D$ −132.4° [*c* 0.16, MeOH−$H_2O$ (4:1)]; UV (MeOH) $\lambda_{max}$ 207 nm (log $\epsilon$ 3.48); NMR data, see Table 1; positive HRFABMS *m*/*z* 279.1591 (calcd for $C_{16}H_{23}O_4$, $[M + H]^+$, 279.1596).

**Hyrtiosterol (3):** colorless solid; $[\alpha]_D$ −22.5° (*c* 0.10, $CH_2Cl_2$); NMR data, see Table 2; positive HRFABMS *m*/*z* 461.3637 (calcd for $C_{29}H_{49}O_4$, $[M + H]^+$, 461.3631).

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## References and Notes

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