Notes

Steroidal Compounds from the Caribbean Octocoral *Eunicea laciniata*

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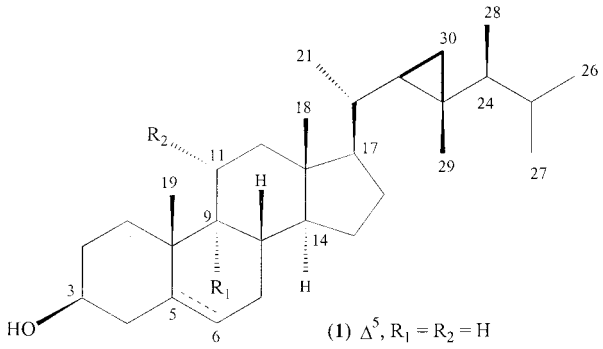
A previously isolated trihydroxy-gorgosterol derivative (**2**), a new gorgosterol (**3**), and the known sterol cholestane-3β,5α,6β-triol are reported from the Caribbean octocoral *Eunicea laciniata* collected off northeast Venezuela. Using high-resolution 2D NMR techniques, the total structural assignment and detailed stereochemistry of **2** is presented for the first time and the full structure of **3** advanced.

Marine organisms have yielded many steroid metabolites with unusual side chain structures.[1−3] Gorgosterol (**1**)[4,5] was the first sterol reported bearing a cyclopropane ring on the side chain. Since then several additional sterols with this feature as well as sterols with polyoxygenated functional groups have been isolated.[1,3] Furthermore, there is a growing interest in highly functionalized sterols because of their biological and pharmacological activities.[3]



(**1**) Δ^5, $R_1 = R_2 = H$
(**2**) Δ^5, $R_1 = R_2 = OH$
(**3**) 5α,6β - dihydroxy; $R_1 = R_2 = OH$

In our continuing search for biologically and chemically interesting compounds from Caribbean gorgonian corals, we have isolated three such sterols from the octocoral *Eunicea laciniata* Duchassaing and Michelotti (order Gorgonacea, subclass Octocorallia, phylum Coelenterata) collected at Mochima Bay, Sucre State, Venezuela. The EtOAc-soluble fraction of the MeOH extract of this organism, on Si gel column chromatography followed by preparative TLC, afforded a trihydroxy-gorgostene, previously obtained from a gorgonian,[6] whose structure was reported but with undetermined stereochemistry at C-9 and C-11. We report the complete structure including detailed stereochemistry for this sterol, gorgost-5-ene-3β,9α,11α-triol (**2**), using high-resolution 2D NMR techniques (^{1}H−^{1}H COSY, HSQC, T-ROESY, and HMBC). The other two isolated sterols are a new pentahydroxy-gorgostane identified as gorgostane-3β,5α,6β,9α,11α-pentol (**3**) and the known compound cholestane-3β,5α,6β-triol. The latter was isolated earlier from an octocoral (*Pteroeides esperi*) belonging to the order Pennatulacea.[7] It was characterized by comparison of its spectral data and that of its acetylated derivative with those previously reported.[7−9]

Gorgost-5-ene-3β,9α,11α-triol (**2**) was obtained as crystalline needles. HREIMS confirmed the molecular formula $C_{30}H_{50}O_3$. Fragment ions in the EIMS at *m*/*z* 440 [M − H_2O]$^+$, 422 [M − 2H_2O]$^+$, and 404 [M − 3H_2O]$^+$ showed the loss of three successive H_2O molecules, characteristic of hydroxyl groups. The IR spectrum showed a broad hydroxyl absorption at 3450 cm^{-1}. The ^{1}H NMR spectrum showed a proton signal at δ 5.47 (J = 4.9 Hz, br d) characteristic of H-6 of the trisubstituted 5,6 double bond, singlets at δ 0.68, δ 1.24, and δ 0.89 assignable to three tertiary methyl groups, and doublets at δ 0.85, δ 0.95, and δ 0.93 due to three secondary methyl groups. A seventh methyl signal appeared as a broad singlet at δ 1.02,[10] which overlapped with a methine multiplet, and protons at δ_H 3.53 (m) and δ_H 4.11 (m) were obviously bonded to carbons bearing hydroxyl groups. The ^{13}C NMR spectrum confirmed the presence of three hydroxyl groups (δ_C 70.92, δ_C 75.50, and δ_C 69.28), and the trisustituted double bond (δ_C 139.03 and δ_C 121.66). In addition, the ^{1}H NMR spectrum of **2** exhibited signals for four high-field protons characteristic of a cyclopropane containing a gorgosterol-type side chain at δ −0.12 (dd; 6.0, 4.4), δ 0.47 (dd; 4.3, 9.6), δ 0.16 (m), and δ 0.24 (m). A combination of ^{1}H−^{1}H COSY, HSQC, and HMBC experiments enabled us to deduce the structure of **2** (see Experimental Section). The similarity of the ^{1}H NMR signals around the cyclopropyl ring suggested that the stereochemistry around this moiety was equivalent to that in gorgosterol.[4,5] T-ROESY data (see Experimental Section) confirmed this stereochemistry as well as that of the hydroxyl groups at C-3 and C-11. Slow exchange of two OH peaks with H_2O (EXSY) suggested that the hydroxyl groups at C-9 and C-11 must be H-bonded to each other, confirming their cis relationship and thus the α-orientation of the C_9−OH.

Gorgostane-3β,5α,6β,9α,11α-pentol (**3**) was isolated as a white powder. This compound has the molecular formula $C_{30}H_{52}O_5$ as established by HREIMS. The IR spectrum exhibited a broad absorption due to hydroxyl groups (3300−

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3500 cm^{-1}). The ^1H NMR spectrum was consistent with a gorgostane derivative. It showed three methyl doublets (δ 0.88, δ 0.98, and δ 0.98), three methyl singlets (δ 0.74, δ 1.31, and δ 0.93), and a seventh methyl signal which, as in **2**, appeared as a broad singlet at δ 1.04[10] overlapping with a methine multiplet at δ 1.05. The ^{13}C NMR spectrum displayed signals for five hydroxyl-bearing carbon atoms (δ_C 67.83, 78.83, 76.28, 70.47, and 80.80), of which only three were protonated [δ 4.02 (m), δ 3.45 (br s), and δ 3.99 (m)], indicating the presence of two tertiary hydroxyl groups. In addition, prominent fragment ions were observed in the EIMS at 474 $[M - H_2O]^+$, 456 $[M - 2H_2O]^+$, 438 $[M - 3H_2O]^+$, and 420 $[M - 4H_2O]^+$ corresponding to the loss of four successive H_2O molecules. Four protons characteristic of a cyclopropane ring on a gorgosterol (**1**) type side chain appeared at δ −0.10 (dd; 5.6, 4.4), δ 0.48 (dd; 9.1, 4.5), δ 0.19 (m), and δ 0.26 (m). Comparison of the ^1H and ^{13}C NMR data of this compound with those of **2** revealed that the main difference between both sterols was that the latter contained two additional hydroxyl groups on a saturated steroid nucleus.

A combination of $^1H-^1H$ COSY, HSQC, and HMBC (see Experimental Section) were used to establish the connectivities and to locate the hydroxyl groups at C-3, C-5, C-6, C-9, and C-11. The HMBC spectrum of **3** showed the expected correlations in support of its structure. The relative stereochemistry of the side chain was determined by comparison of the NMR data of **3** with those of **2** and was confirmed by NOESY experiments. The NOESY spectra (see Experimental Section) also confirmed the β-orientation of the hydroxyl groups at C-3 and C-6 and the α-orientation of the hydroxyl groups at C-5 and C-11. The orientation of the hydroxyl group at C-9 (α) was assumed to be the same as that of **2**, thus defining the structure of **3** as the 5α,6β-dihydroxy analogue of **2**. Compound **3** is unique in that it combines the relatively uncommon pentahydroxylated sterol skeleton with a gorgosterol side chain.

Experimental Section

General Experimental Procedures. Melting points were determined on a Reichert melting point apparatus and were uncorrected. IR spectra were recorded on a Pye Unicam SP3-200 IR spectrophotometer. Mass spectral experiments were performed on a Kratos/AEI MS-200 spectrometer. The 1D and 2D NMR spectra were recorded on either a Varian Unity 500 or a Bruker Avance DRX-400 spectrometer using either $CDCl_3$ or CD_3OD as solvent and TMS as internal reference. Chemical shifts are given in δ (ppm) and coupling constants expressed in hertz. Optical rotation were measured on a POLARTRONIC D polarimeter. Si gel 60 (70−230 mesh) was used for column chromatography, and precoated Si gel plates (Kieselgel 60 $F_{254+338}$) were used for preparative TLC.

Animal Material. A sample of *Eunicea laciniata* was collected at a depth of −11 m from the Punta Aguirre coast in Mochima Bay, Sucre State, Venezuela, during August 1997 and identified by the Institute of Marine Affairs, Trinidad and Tobago (specimen no. IMA-1481).

Extraction and Isolation. The freshly collected organism was cut into pieces and immersed in methanol (≈2.0 L) at the collection site. After standing (2 days), the suspension was filtered and the residue further extracted with methanol (1.5 L) for an additional 2 days. The total filtrate was concentrated to give an aqueous suspension, which was extracted with ethyl acetate (3 × 250 mL), dried over anhydrous sodium sulfate, and evaporated to give a brown gum (2.5 g). This extract was subjected to Si gel (70−230 mesh) column chromatography, eluting with light petroleum and increasing concentrations of ethyl acetate. A fraction eluting with 35% EtOAc in light petroleum on purification by preparative TLC (light petroleum−EtOAc, 2:1, × 2), decolorization with charcoal, and successive recrystallizations from MeOH yielded compound **2** (3.4 mg). Another fraction eluting with 50% EtOAc in light petroleum on further purification by column chromatography and preparative TLC followed by repeated crystallization from MeOH gave cholestane-3β,5α,6β-triol (11.7 mg). The latter was acetylated with Ac_2O−pyridine at room temperature for 24 h to give the expected diacetate. A later fraction eluting with 100% EtOAc afforded compound **3** (3.7 mg) after preparative TLC (light petroleum−EtOAc, 1:1, × 2) followed by column chromatography on Si gel ($CHCl_3$−MeOH mixture as eluting solvent) and recrystallization from MeOH−H_2O (9:1).

Gorgost-5-ene-3β,9α,11α-triol (**2**): needles; mp 188−190 °C; IR ($CHCl_3$) ν_{max} 3450 cm^{-1}; ^1H NMR ($CDCl_3$) δ −0.12 (1H, dd, J = 6.0, 4.4 Hz, H-30), 0.16 (1H, m, H-22α), 0.24 (1H, m, H-24α), 0.47 (1H, dd, J = 9.1, 4.3 Hz, H-30), 0.68 (3H, s, H_3-18), 0.85 (3H, d, J = 6.5 Hz, H_3-26), 0.89 (3H, s, H_3-29), 0.93 (3H, d, J = 6.4 Hz, H_3-28), 0.95 (3H, d, J = 6.6, Hz, H_3-27), 1.02 (1H, m, H-20), 1.02 (1H, br s, H_3-21), 1.03 (1H, m, H-16β), 1.24 (3H, s, H_3-19), 1.35 (1H, m, H-15β), 1.35 (1H, m, H-17), 1.44 (d, 3-OH), 1.47 (1H, m, H-12α), 1.52 (1H, m, H-14), 1.53 (1H, m, H-2β), 1.54 (1H, m, H-16α), 1.56 (1H, m, H-25), 1.71 (d, 11-OH), 1.72 (1H, m, H-8), 1.74 (1H, m, H-1α), 1.81 (1H, m, H-7β), 1.83 (s, 9-OH), 1.88 (1H, m, H-2α), 1.93 (1H, m, H-7α), 2.07 (1H, m, H-15α), 2.14 (1H, m, H-12β), 2.15 (1H, m, H-1β), 2.24 (1H, m, H-4β), 2.37 (1H, m, H-4α), 3.53 (1H, m, H-3α), 4.11 (1H, m, H-11β), 5.47 (1H, br d, J = 4.9 Hz); ^{13}C NMR ($CDCl_3$) δ 12.1 (C-18), 14.3 (C-29), 15.5 (C-28), 21.1 (C.21), 21.3 (C-30), 21.4 (C-19), 21.6 (C-26), 22.2 (C-27), 24.2 (C-16), 25.8 (C-23), 26.9 (C-7), 28.2 (C-15), 31.0 (C-1), 31.5 (C-2), 31.9 (C-22), 31.9 (C-25), 34.4 (C-8), 35.2 (C-20), 42.7 (C-4), 42.9 (C-13), 43.3 (C-10), 46.8 (C-12), 49.3 (C-14), 50.7 (C-24), 57.4 (C-17), 69.3 (C-11), 70.9 (C-3), 75.5 (C-9), 121.7 (C-6), 139.0 (C-5); COSY H-1/H-2; H-3/H-2, H-4; H-6/H-4, H-7; H-7/H-8; H-8/H-14; H-11/H-12; H-14/H-8, H-15; H-17/H-16, H-20; H-20/H-22; H-24/H-25, H-28; H-25/H-26, H-27; H-30/H-22; HMBC correlations H-4/C-5,C-3; H-6/C-8, C-10; H-7/C-8, C-9; H-8/C-9, C-11; H-12/C-9, C-11, C-13, C-14; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-24/C-22, C-23, C-28, C-29, C-30; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-23, C-24, C-25; H-29/C-22, C-24, C-30; H-30/C-20, C-22, C-23, C-24, C-29; T-ROESY correlations H-1β/H-19; H-2α/H-3, H-4α; H-4α/H-6; H-6/H-7α; H-7α/H-14; H-8/H-18; H-11/H-12β, H-18, H-19; H-12α/H-14; H-14/H-15α; H-15α/H-17; H-17/H-21, H-22; H-18/H-12β, H-20; H-19/H-4β; H-21/H-22; H-22/H-24, H-30α; H-24/H-30α; H-29/H-30β; EIMS m/z 458 $[M^+]$ (0), 440 $[M - H_2O]^+$ (30), 422 $[M - 2H_2O]^+$ (24), 404 $[M - 3H_2O]^+$ (10), 120 (100); HREIMS m/z $[M - H_2O]^+$ 440.3660 (calcd for $C_{30}H_{48}O_2$, 440.3654).

Gorgostane-3β,5α,6β,9α,11α-pentol (**3**): white powder; mp 243−247 °C; $[\alpha]_D$ +15.5° (*c* 0.51, MeOH); IR (Nujol) ν_{max} 3300−3500 cm^{-1}; ^1H NMR (CD_3OD) δ − 0.10 (1H, dd, J = 5.6, 4.4 Hz, H-30β), 0.19 (1H, m, H-22α), 0.26 (1H, m, H-24α), 0.48 (1H, dd, J = 9.1, 4.5 Hz, H-30α), 0.74 (3H, s, H_3-18), 0.88 (3H, d, J = 6.2, H_3-26), 0.93 (3H, s, H_3-29), 0.98 (3H, d, J = 6.9, H_3-27), 0.98 (3H, d, J = 7.0, H_3-28), 1.04 (3H, br s, H_3-21), 1.05 (1H, m, H-20), 1.12 (1H, m, H-16β), 1.31 (3H, s, H_3-19), 1.36 (1H, m, H-17), 1.40 (1H, m, H-7β), 1.42 (1H, m, H-15β), 1.49 (1H, m, H-12α), 1.51 (1H, m, H-2β), 1.53 (1H, m, H-16α), 1.58 (1H, m, H-25), 1.69 (1H, m, H-14), 1.78 (1H, m, H-2α), 1.96 (1H, m, H-7α), 1.99 (1H, m, H-4β), 2.04 (1H, m, H-1α), 2.06 (1H, m, H-4α), 2.08 (1H, m, H-15α), 2.11 (1H, m, H-8), 3.45 (1H, br s, H-6), 3.99 (1H, m, H-11β), 4.02 (1H, m, H-3α); ^{13}C NMR (CD_3OD) δ 12.81 (C-18), 14.7 (C-29), 15.9 (C-28), 20.0 (C-19), 21.5 (C-21), 21.9 (C-26), 22.1 (C-30), 22.6 (C-27), 25.0 (C-16), 26.7 (C-23), 29.5 (C-15), 29.7 (C-7), 30.4 (C-1), 31.4 (C-2), 32.9 (C-22), 33.2 (C-8), 33.3 (C-25), 36.4 (C-20), 41.8 (C-4), 43.5 (C-10), 44.2 (C-13), 47.3 (C-12), 49.0 (C-14), 52.2 (C-24), 59.1 (C-17), 67.9 (C-3), 70.5 (C-11), 76.8 (C-6), 78.8 (C-5), 80.8 (C-9); COSY H-1/H-2; H-6/H-7; H-7/H-8; H-11/H-12; H-15/H-16; H-16/H-15, H-17; H-22/H-30; H-24/H-28; H-25/H-26, H-27; HMBC correlations H-4/C-2, C-3, C-5, C-6, C-10; H-6/C-5, C-8, C-10; H-8/C-9, C-11; H-12/C-9, C-11, C-13, C-17, C-18; H-14/C-9, C-13, C-18; H-18/C-12, C-13, C-14, C-17; H-19/C-1, C-5, C-9, C-10; H-21/C-17, C-20, C-22; H-24/C-22, C-23, C-28, C-29,

C-30; H-26/C-24, C-25, C-27; H-27/C-24, C-25, C-26; H-28/C-23, C-24, C-25; H-29/C-22, C-23, C-24, C-30; H-30/C-22, C-24; NOESY correlations H-1α/H-3; H-1β/H-19; H-2α/H-3, H-4α; H-2β/H-19; H-3/H-4α; H-4α/H-6α; H-6α/H-7α; H-7β/H-8; H-8/H-11, H-18; H-11/H-12β, H-18, H-19; H-12α/H-14; H-14/H-16α; H-15α/H-16α, H-17; H-17/H-21, H-22; H-18/H-20; H-20/H-30β; H-21/H-22, H-30α; H-22/H-30α; H-24/H-30α; H-25/H-29; H-29/H-30β; EIMS *m/z* 492 $[M^+]$ (3), 474 $[M - H_2O]^+$ (14), 456 $[M - 2H_2O]^+$ (100), 438 $[M - 3H_2O]$ (45), 420 $[M - 4H_2O]^+$ (13); HREIMS *m/z* $[M^+]$ 492.3819 (calcd for $C_{30}H_{52}O_5$, 492.3815).

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(10) The deceptive appearance of H_3-21 as a singlet on each of **2** and **3** was due to the coincidence of chemical shifts for H-20 and H_3-21 in each case, so that the coupling between H_3-21 and H-20 was not observed.

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