Pergamon

Tetrahedron Letters 39 (1998) 7645–7648

TETRAHEDRON LETTERS

New Polyhydroxydinostane Sterols from the Caribbean Gorgonian Octocoral *Pseudopterogorgia americana*

Abimael D. Rodríguez,* Jocelyn Rivera,[1] and Anna Boulanger[2]

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, Río Piedras, Puerto Rico 00931-3346

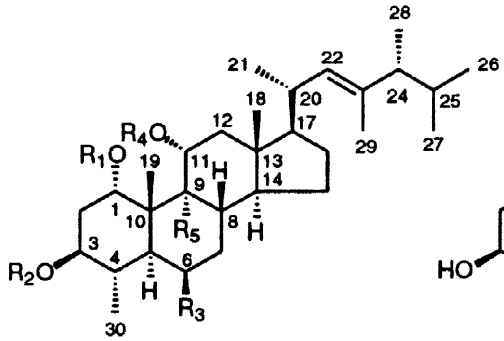
Received 21 July 1998; accepted 6 August 1998

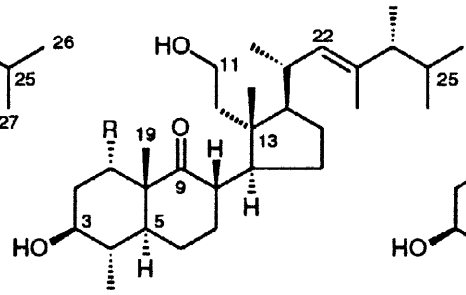
Abstract: A recent study of the hexane extracts of *Pseudopterogorgia americana* led to the isolation of two new polyhydroxydinostane sterols **1-2**, in addition to a known secogorgosterol **3**. The structures were established by spectroscopic and chemical derivatization studies.

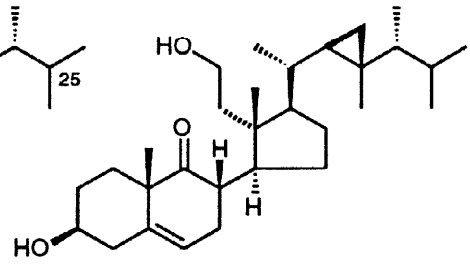
Keywords: coelenterates; marine metabolites; natural products; steroids

A number of unusual bioactive marine sterols have been isolated from several gorgonian corals of the genus *Pseudopterogorgia*.[3-7] For instance, the gorgonian *Pseudopterogorgia* sp. collected near Florida was found to produce 9(11)-secosteroids which exhibit moderate inhibitory activity against protein kinase C as well as potent anti-proliferative and anti-inflammatory activity.[6] As part of our ongoing screening program for novel compounds from marine invertebrates we have further investigated the hexane extract of the common sea plume *Pseudopterogorgia americana* Gmelin (phylum Cnidaria, order Gorgonaceae)[8] and report here the isolation and structure determination of two new polyhydroxydinostane steroids, designated 1α,9α,11α-trihydroxydinosterol (**1**) and 1α-hydroxy-9(11)-secodinosterol (**2**), and a known secogorgosterol **3**.

A lyophilized specimen of *P. americana* (2.3 kg) collected at La Parguera, Puerto Rico was extracted with 1:1 $CHCl_3$-MeOH, and the extract was partitioned between water and hexane. The hexane-soluble fraction was subjected to silica gel flash filtration (*n*-hexane/acetone/MeOH), followed by silica gel column chromatography and normal-phase HPLC to afford two new polyhydroxylated C_{30} sterols [**1** (20.4 mg) and **2** (15.3 mg)] and a known secogorgosterol **3** (2.5 g).[3,6]







1 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$
4 $R_1 = R_2 = R_4 = Ac$, $R_3 = H$, $R_5 = OH$
5 $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OH$

2 R = OH
6 R = H

3

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01695-5

The molecular formula of **1** [a white powder, $[\alpha]_D^{25}$ +0.88° (*c* 1.1, $CHCl_3$)], was established as $C_{30}H_{52}O_4$ by HREIMS (*m/z* 476.3850; calcd 476.3866) and showed a strong infrared absorption at 3387 cm^{-1} (OH). The ^1H NMR spectrum showed resonances ascribable to one vinylic, two tertiary, and five secondary methyl groups. The presence of a trisubstituted double bond was revealed by a resonance at δ 4.85 (d, 1H, *J*= 9.5 Hz) and ^{13}C signals at δ 131.7 (d) and 135.9 (s). The ^{13}C NMR spectrum also displayed signals due to a quaternary carbon resonance with an oxygen substituent attached at δ 79.8 (C9) and three oxymethine carbons at δ 76.2 (C1), 71.3 (C3), and 70.3 (C11), while a HMQC experiment revealed the corresponding ^1H NMR resonances at δ 4.63 (br s), 3.48 (t, *J*= 6.7 Hz), and 3.97 (dd, *J*= 5.0, 11.0 Hz). Upon acetylation with a mixture of Ac_2O and pyridine for 5h at 80°C, **1** afforded the triacetate **4**.[9] Although the HREIMS did not give a molecular ion, an M-CH_3CO_2H fragment ion was observed at *m/z* 542.4003 which indicated that **4** had the molecular formula $C_{36}H_{58}O_7$. These results suggested that **1** was a tetrahydroxy sterol.

The presence of a dinosterane skeleton was established unambiguously from the ^1H, ^{13}C, COSY, and HMBC data (Table 1).[10,11] In the COSY spectrum connectivities to the oxymethine protons at δ 4.63, 3.48, and 3.97 placed these protons at C1, C3, and C11 of the sterol skeleton, respectively. The locus of the oxymethines were further established by their long-range HMBC correlations. The C5 resonance at δ 38.4 in **1** showed a strong HMBC correlation to the oxymethine resonance at δ 4.63 (H1) in the ^1H NMR spectrum. In turn, H1 showed an additional correlation to the oxymethine carbon at δ 71.3, assigned to C3. The C3 resonance showed a correlation to a methyl resonance at δ 1.01 (d) which was assigned to Me-30, whereas those at δ 79.8 (C9) and 70.3 (C11) gave HMBC correlations to a pair of diastereotopic protons at δ 2.05 and 1.45 (H12). The structure of the side chain was identical to that of dinosterol[10,11] and acerosterol (**5**)[4] as implied by the chemical shifts of C20-C29. Several HMBC correlations (Figure 1) provided additional unambiguous evidence for the side chain substructure. The relative stereochemistry of **1** was determined by comparison of the ^{13}C NMR resonances with those of other dinosterols[4,6] and difference NOE spectra as detailed below. The strong NOEs between Me-19 and H1/H4/H8/H11 and those between Me-18 and H8/H11 indicated that they were *cis* to each other and oriented β. Likewise, the NOEs between H3 and Me-30 indicated that they were *cis* to each other and oriented α. The relative configurations at C9, C13, C14, C17, C20, and C24 were assumed to have the normal steroidal configurations. The nearly identical chemical shifts of C13, C14, C17, C20, and C24 when compared to the related acerosterol (**5**)[4] and the co-occurrence of **1** with known secogorgosterol **3**[3,6] support this assumption. The geometry of the C22 double bond was assumed to be *E* by analogy with dinosterol[11] and acerosterol (**5**).[4]

1α-Hydroxy-9(11)-secodinosterol (**2**) was isolated as a white powder, $[\alpha]_D^{25}$ +7.0° (*c* 1.42, $CHCl_3$), and had infrared absorptions due to hydroxy (3397 cm^{-1}) and ketone (1691 cm^{-1}) functionalities. The HREIMS established the molecular formula as $C_{30}H_{52}O_4$ (*m/z* 476.3867; calcd 476.3866). That the structure of new secosterol **2** possessed the secodinosterone skeleton and bore the same side chain as that of dinosterol, was apparent from the ^1H, ^{13}C NMR and HREIMS. The complete structure and the assignment of all proton and carbon resonances (Table 1) were achieved by a combination of 2D NMR experiments, which included COSY, HMQC, and HMBC. The ^1H NMR spectrum of **2** had signals due to eight methyls of which five were secondary, two were tertiary, and

one was olefinic. The 9(11)-seco skeleton was evident from the diagnostic resonances of an oxymethylene (δ 3.73/3.67, m; 59.1, t, C11) and a saturated ketone (δ 220.1, s, C9). The ketone functionality at C9 was further established by its long-range HMBC correlations to H5 (δ 1.84, m), H8 (δ 2.80, dt), and H14 (δ 2.47, m). Thus, the structure of **2** resembled that of known secosterol **6**,[6] except for the hydroxylation at C1 as implied by the chemical shifts of C1 (δ 4.09, t; 71.6). The relative stereochemistry of **2** was assigned using difference NOE spectra[12] and by comparison of the ^{13}C resonances with those of other 9(11)-secosterols.[5,6] The co-occurrence of 1α-hydroxy-9(11)-secodinosterol (**2**) with **1** (the latter is a plausible biosynthetic precursor of **2** upon further oxidative cleavage of the C9/C11 σ bond) also supports the relative stereochemistry depicted in structure **2**.

Inasmuch as cnidarians (coelenterates) appear incapable of sterol synthesis, the complex mixtures of sterols found in many gorgonian species probably reflect both a dietary accumulation and a contribution from their endosymbiotic dinoflagellates (zooxanthellae).[13] Thus, *P. americana*, which harbors zooxanthellae, produces an abundance of cyclopropylsterols and 4-methysterols, indicating dinoflagellate-derived sterols.[3-7] Critically, the isolation of steroids **1** and **2** represents the first example of polyhydroxydinostanes possessing, respectively, a 9α-hydroxydinostane and a 1α-hydroxy-9(11)-secodinostane nucleus.

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for 1 and 2 in $CDCl_3$.[14]

Position	1α,9α,11α-trihydroxydinosterol (1)		1α-hydroxy-9(11)-secodinosterol (2)	
	δ, mult (J)	^{13}C	δ, mult (J)	^{13}C
1	4.63, br s	76.2, d	4.09, t (2.7)	71.6, d
2	1.88, m; 1.77, m	39.1, t	2.03, m; 1.66, m	36.4, t
3	3.48, t (6.7)	71.3, d	3.53, ddd (5.2, 9.9, 11.7)	71.4, d
4	1.35, m	40.3, d	1.51, m	38.6, d
5	1.87, m	38.4, d	1.84, m	44.1, d
6	1.40, m; 1.33, m[a]	25.3, t[a]	1.71, m; 1.33, m[a]	24.6, t[a]
7	1.77, m; 1.06, m[a]	24.7, t[a]	2.06, m; 1.31, m	31.7, t
8	1.56, m	37.5, d	2.80, dt (5.1, 13.3)	44.5, d
9	-	79.8, s	-	220.1, s
10	-	43.7, s	-	53.5, s
11	3.97, dd (5.0,11.0)	70.3, d	3.73, m; 3.67, m	59.1, t
12	2.05, dd (4.9, 11.9); 1.45, m	47.1, t	1.91, m; 1.68, m	40.8, t
13	-	42.8, s	-	45.3, s
14	1.47, m	48.2, d	2.47, m	41.7, d
15	1.44, m; 0.92, m	24.2, t	1.46, m; 1.33, m	23.3, t
16	1.71, m; 1.34, m	28.3, t	1.90, m; 1.55, m[a]	24.0, t[a]
17	1.22, m	56.8, d	1.67, m	50.4, d
18	0.70, s	12.9, q	0.65, s	17.5, q
19	1.00, s	16.4, q	1.18, s	17.2, q
20	2.31, m	34.8, d	2.42, m	32.7, d
21	0.93, d (6.1)	20.9, q	0.94, d (6.7)	21.3, q
22	4.85, d (9.5)	131.7, d	5.00, d (9.7)	130.0, d
23	-	135.9, s	-	135.7, s
24	1.62, m	50.5, d	1.66, m	50.5, d
25	1.52, m	31.1, d	1.48, m	30.8, d
26	0.76, d (6.6)[b]	22.0, q[b]	0.76, d (6.6)[b]	21.7, q[b]
27	0.83, d (6.5)[b]	20.4, q[b]	0.84, d (6.5)[b]	20.2, q[b]
28	0.92, d (5.3)	17.3, q	0.91, d (6.9)	16.8, q
29	1.48, d (0.8)	13.5, q	1.48, d (0.8)	12.8, q
30	1.01, d (6.3)	15.7, q	1.02, d (6.3)	15.1, q

Signals labeled with identical superscripts within a column may be interchanged.

ACKNOWLEDGMENT

The gorgonian samples were collected and identified by Dr. Paul Yoshioka, University of Puerto Rico at Mayagüez, Department of Marine Sciences. We thank Mrs. Catherine Ramírez for recording the 2D NMR and HREIMS data and Miss Janet Figueroa for the infrared spectra. This study was supported by grants from the National Science Foundation MRCE (R11-8802961) and EPSCoR (R118610677) Programs and the National Institutes of Health MBRS Program (SO6RR08102-17).

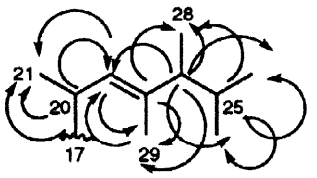


Figure 1. HMBC correlations ($^{13}C \rightarrow {}^{1}H$) of the side chain of **1**.

REFERENCES

1. Undergraduate student sponsored by the TRC, AMP, and MRCE Programs of the University of Puerto Rico.
2. Postdoctoral associate sponsored by the NSF-EPSCoR Tropical Marine Biotechnology Center.
3. Enwall, E.L.; Van Der Helm, D.; Hsu, I.N.; Pattabhiraman, T.; Schmitz, F.J.; Spraggins, R.L.; Weinheimer, A.J. *J. Chem. Soc. Chem. Commun.* **1972**, 215-216.
4. John, L.M.D.; Tinto, W.F.; McLean, S.; Reynolds, W.F. *J. Nat. Prod.* **1993**, *56*, 144-146.
5. Miller, S.L.; Tinto, W.F.; Yang, J-P.; McLean, S.; Reynolds, W.F. *Tetrahedron Lett.* **1995**, *36*, 1227-1228.
6. He, H.; Kulanthaivel, P.; Baker, B.J.; Kalter, K.; Darges, J.; Cofield, D.; Wolff, L.; Adams, L. *Tetrahedron* **1995**, *51*, 51-58.
7. Kerr, R.G.; Rodríguez, L.C.; Kellman, J. *Tetrahedron Lett.* **1996**, *37*, 8301-8304.
8. a) Rodríguez, A.D.; Boulanger, A. *J. Nat. Prod.* **1996**, *59*, 653-657. b) Rodríguez, A.D.; Boulanger, A. *ibid.* **1997**, *60*, 207-211. c) Rodríguez, A.D.; Boulanger, A.; Martínez, J.R.; Huang, S.D. *ibid.* **1998**, *61*, 451-455.
9. **4**: a colorless oil; IR (neat) 3571, 2955, 2869, 1738, 1456, 1373, 1238, 1028, 986 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.26 (dd, 1H, J= 4.6, 10.9 Hz), 4.91 (br s, 1H), 4.83 (d, 1H, J= 9.6 Hz), 4.61 (ddd, 1H, J= 4.8, 11.2, 11.5 Hz), 2.18 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.48 (s, 3H), 1.09 (s, 3H), 0.92 (d, 3H, J= 6.8 Hz), 0.90 (d, 3H, J= 6.2 Hz), 0.87 (d, 3H, J= 6.6 Hz), 0.83 (d, 3H, J= 6.5 Hz), 0.77 (s, 3H), 0.76 (d, 3H, J= 6.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 170.5, 169.0, 135.6, 131.2, 79.7, 78.0, 73.4, 73.0, 56.3, 50.2, 47.9, 43.6, 42.2, 42.0, 39.1, 37.9, 36.8, 34.4, 31.0, 30.7, 27.9, 24.6, 24.5, 23.7, 22.0, 21.8, 21.7, 21.2, 20.5, 20.1, 17.0, 15.9, 15.3, 13.1, 12.4; EIMS *m/z* [M-CH_3CO_2H]$^{+}$ 542 (1), 482 (13), 422 (4), 404 (4), 283 (6), 134 (24), 107 (27), 69 (100); HREIMS *m/z* [M-CH_3CO_2H]$^{+}$ 542.4003 (calcd for $C_{34}H_{54}O_5$, 542.3971).
10. Shimizu, Y.; Alam, M.; Kobayashi, A. *J. Am. Chem. Soc.* **1976**, *98*, 1059-1060.
11. Finer, J.; Clardy, J.; Kobayashi, A.; Alam, M.; Shimizu, Y. *J. Org. Chem.* **1978**, *43*, 1990-1992.
12. Selected NOEs for **2**: H1/Me-19, H4/Me-19, H8/Me-19, H8/Me-18, H20/Me-18, H5/Me-30.
13. Withers, N. In "Marine Natural Products Chemistry, Chemical and Biological Perspectives"; Scheuer, P.J., Ed.; Academic Press: New York, **1983**; Vol V, pp 87-130.
14. Assignments were aided by ^{1}H-^{1}H COSY, spin splitting patterns, comparison of J values, HMBC and HMQC experiments, numbers of attached protons as measured from DEPT spectra, and chemical shift values. The δ values are in ppm and are referenced to either the residual $CHCl_3$ signal (7.26 ppm) or $CDCl_3$ (77.0 ppm).