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ACEROSTEROL, A NOVEL POLYHYDROXYLATED STEROL FROM THE GORGONIAN OCTOCORAL *PSEUDOPTEROGORGIA ACEROSA*

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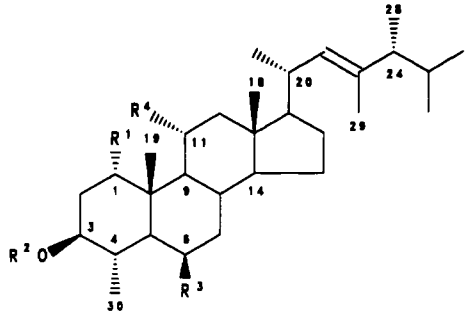
ABSTRACT.—A new tetrahydroxysterol, acerosterol [**2**], has been isolated from the gorgonian octocoral *Pseudopterogorgia acerosa*. The structure has been determined to be 4α,23,24(*R*)-trimethyl-5α-cholest-22*E*-ene-1α,3β,6β,11α-tetraol on the basis of 2D nmr spectroscopy.

Gorgonian corals of the genus *Pseudopterogorgia* are a rich source of biologically active compounds (1). We previously reported the isolation and structure elucidation of some novel pseudopterane diterpenoids from the Caribbean sea whip *Pseudopterogorgia acerosa* Pallas (Gorgonidae) (2–5). We have further investigated the extracts of this organism and report here the isolation and structure determination of a new tetrahydroxysterol, designated acerosterol [**2**], from the most polar fractions.

Acerosterol [**2**], $C_{30}H_{52}O_4$, was isolated as white needles, and had an ir absorption at 3440 cm^{-1} (OH). The ^{1}H-nmr spectrum had resonances due to one olefinic, two tertiary, and five secondary methyl groups. The presence of a trisubstituted double bond was revealed by a one-proton resonance at δ 4.87 (dd, J = 1.1 and 9.3 Hz) and ^{13}C signals at δ 131.32 (d) and 135.72 (s).



1 $R^1=R^2=R^3=R^4=H$
2 $R^1=R^3=R^4=OH$, $R^2=H$
3 $R^1=R^3=R^4=OAc$, $R^2=Ac$

The ^{13}C-nmr spectrum displayed signals due to four oxymethine carbons at δ 74.69, 71.48, 67.35, and 66.79, while a ^{13}C-^{1}H shift correlated experiment showed the corresponding ^{1}H-nmr resonances at δ 3.92 (dd, J = 3.0 and 3.2 Hz), 3.45 (ddd, J = 5.0, 10.0, and 11.5 Hz), 3.95 (ddd, J = 5.0, 10.0, and 10.0 Hz), and 4.20 (ddd, J = 2.8, 3.0, and 5.7 Hz). On acetylation, **2** afforded the tetraacetate **3**. These results suggested that acerosterol was a tetrahydroxy sterol, probably a dinostane derivative (6).

A ^{1}H-^{1}H 2D correlated spectrum revealed that the oxymethine protons at δ 3.92, 3.45, and 4.20 were attached to C-1, C-3, and C-6 of the sterol skeleton. These observations were confirmed by the use of a long-range ^{13}C-^{1}H shift correlated experiment using our FLOCK pulse sequence (7). In this regard, C-3 (δ 71.48) showed three-bond correlations with H-1 (δ 3.92) and H-30 (δ 1.04), while C-7 (δ 39.98) and C-8 (δ 29.01) showed correlations with H-6 (δ 4.20). The fourth hydroxyl group (δ 3.95) was placed at C-11 (δ 67.35) based on the observation that this carbon had long-range correlations with H-9 and H-12. The assignments for C-9 and C-12 are

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secure since they showed three-bond correlations to H-19 and H-18, respectively. The stereochemistry at the relevant positions in the nucleus followed from the coupling constants. The structure of the side chain was also revealed from the COSY and FLOCK experiments. The stereochemistry at C-20 and C-24 in **2** is assumed to be the same as in dinosterol [**1**] (6,8,9) and related compounds (10). These results are summarized in Table 1 and led to the structure **2** for acerosterol.

The tetraacetate **3**, $[\alpha]_D$ −22.0°, had the molecular formula $C_{38}H_{60}O_8$ on the basis of hreims. The assignment of the carbons and protons (Table 1) followed from the HETCOR and FLOCK experiments.

Dinosterol [**1**] and other derivatives bearing a 4α-methyl group are characteristic sterols of dinoflagellates (10). They have also been obtained from marine invertebrates containing symbiotic zooxanthellae (11). It seems likely therefore, that acerosterol [**2**] might have originated from symbiotic zooxanthellae present in *P. acerosa*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were taken on a Kofler hot stage apparatus and are uncorrected. Ir spectra were obtained on a Nicolet 5DX FTIR spectrometer using KBr discs. Optical rotations were measured on a Perkin-Elmer 243B polarimeter. The nmr spectra were recorded on a Varian XL-400 spectrometer operating at 400 MHz for protons and 100.6 MHz for carbons, with TMS as the internal stan-

TABLE 1. ^{13}C and ^{1}H Assignments for Acerosterol [**2**][a] and Tetraacetate **3**.[b]

Position	Compound **2**		Observed 2- or 3-bond Connectivity	Compound **3**[c]	
	δC	δ_H		δ_C	δ_H
1	74.69	3.92(3.0, 3.2)	1.14, 1.95	76.81	4.74(2.9, 3.2)
2	37.05	1.95, 1.76	3.92	30.42	2.23, 1.63
3	71.48	3.45(5.0, 10.0, 11.5)	3.92, 1.95, 1.76, 1.04	73.67	4.62(5.3, 11.5, 11.5)
4	36.18	1.68	1.95, 1.34, 1.04	33.37	1.88
5	46.68	1.34	3.92, 1.83, 1.14, 1.04	45.28	1.61
6	66.79	4.20(2.8, 3.0, 5.7)	1.83	69.24	5.25(3.0, 3.0, 5.5)
7	39.98	1.83, 1.12	4.20	35.38	1.92, 1.17
8	29.01	1.77	4.20, 1.19	29.06	2.00
9	53.27	1.19	2.28, 1.83, 1.14	49.10	1.64
10	41.39	—	4.20, 1.34, 1.14	40.20	—
11	67.35	3.95(5.0, 10.0, 10.0)	2.28, 1.19	71.46	5.20, (4.6, 9.3, 10.7)
12	50.70	2.28, 1.23	0.69	46.55	2.18, 1.17
13	42.86	—	0.69	42.60	—
14	54.95	1.11	0.69	55.22	1.18
15	24.35	1.56, 1.05		24.09	1.54, 1.08
16	28.07	1.72, 1.16	1.20	27.85	1.70, 1.18
17	56.76	1.20	0.94, 0.69	56.68	1.19
18	13.04	0.69	1.20	13.28	0.80
19	16.62	1.14	1.34, 1.19	16.31	1.18
20	34.56	2.33	0.94	34.38	2.31
21	20.60	0.94(6.1)		20.56	0.88(6.5)
22	131.32	4.87(1.1, 9.3)	1.49	131.02	4.83(1.2, 8.6)
23	135.72	—	1.49, 0.92	135.91	—
24	50.24	1.64	4.87, 1.49, 0.92, 0.76	50.20	1.63
25	30.80	1.51	0.82, 0.76	30.73	1.51
26	21.75	0.76(6.6)	0.82	22.03	0.77(6.6)
27	20.11	0.82(6.5)	0.76	20.11	0.83(6.4)
28	16.97	0.92(6.6)	1.64	16.99	0.92(7.1)
29	13.26	1.49(1.1)	4.87	13.20	1.49(1.2)
30	14.45	1.04(6.3)		14.73	0.83(6.4)

[a]Data are for solutions in $CDCl_3$ + 1 drop MeOH-d_4.

[b]Data are for solutions in $CDCl_3$. Coupling constants (in Hertz) are in parentheses.

[c]Acetates: δ 171.18 and 21.72 (2.00); 170.42 and 21.16 (2.01); 170.38 and 21.36 (2.07); 169.98 and 21.38 (2.10).

dard. Mass spectra were measured on a VG 70-25S mass spectrometer operating at 70 eV.

P. acerosa (dry wt 675 g) was collected in August 1987 at Lau's reef (−10 m), Tobago. The collection and identification were made by Mr. Richard Laydoo of the Institute of Marine Affairs, Trinidad and Tobago, where a voucher specimen has been kept. The sample was immediately stored in Me_2CO for transportation to the laboratory. In the laboratory it was blended with fresh Me_2CO (12 liters), and the solvent was evaporated to provide an aqueous suspension, which was thoroughly extracted with $CHCl_3$. The extract yielded a dark red viscous oil (91 g) which was chromatographed on Si gel, with hexane-Me_2CO (3:1) and $CHCl_3$-MeOH (95:5) as eluents, to give seven major fractions. The most polar seventh fraction was rechromatographed on Si gel with $CHCl_3$-MeOH (95:5) to give acerosterol [**2**] (50 mg).

ACEROSTEROL [**2**].—Mp 118–120°; $[\alpha]_D$ −3.3° (c=0.04, MeOH); ir 3440 cm^{-1}; eims *m/z* (% rel. int.) $[M]^+$ 476 (5), 458 (8), 440 (6), 422 (5), 346 (13), 335 (10), 142 (64), 69 (100); hreims 476.3852 (calcd for $C_{30}H_{52}O_4$, 476.3866).

Acerosterol **2** (20 mg) was acetylated with a mixture of Ac_2O (0.5 ml) and pyridine (1 ml) for 6 h at 80°. The solvent was removed in a stream of N_2 to give the tetraacetate **3** (23 mg) as a colorless gum: $[\alpha]_D$ −22.0° (c=0.15, $CHCl_3$); ir 1726 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 644 (1), 584 (7), 524 (5), 481 (7), 464 (6), 404 (8), 361 (23), 69 (100); hreims 644.4347 (calcd for $C_{38}H_{60}O_8$, 644.4288).

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