Journal of Natural Products
Vol. 48, No. 5, pp. 784-787, Sep-Oct 1985

TWO NEW POLYOXYGENATED STEROLS FROM THE MARINE HYDROID *EUDENDRIUM GLOMERATUM*

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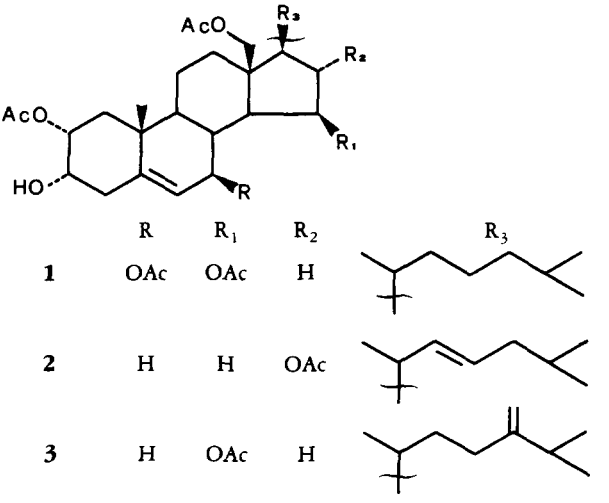
ABSTRACT.—Two new polyoxygenated sterols, (22*E*)-cholest-5, 22-dien-2α,3α,16α,18-tetrol-2,16,18-triacetate (**2**) and 24-methylcholest-5,24(28)-dien-2α,3α15β,18-tetrol-2,15, 18-triacetate (**3**) have been isolated by reverse-phase hplc from the marine hydroid *Eudendrium glomeratum* and their structures were elucidated by ^{1}H- and ^{13}C-nmr and mass spectrometric analyses.

Very recently marine hydroids belonging to the genus *Eudendrium* (family: Eudendridae) have been shown to elaborate complex polyhydroxysteroids, characterized by a C-18 oxygenated functionality. In particular, Cimino *et al.* (1) isolated (22*R*)-cholest-4-en-4,16β,18,22-tetrol-3-one-16,18-diacetate from an *Eudendrium* species in 1980, while in the extractives of *Eudendrium glomeratum* Picard we have found (2) cholest-5-en-2α,3α,7β,15β,18-pentol-2,7,15,18-tetraacetate (**1**).

We report here that the extract of the latter organism also contains two new steroids, (22*E*)-cholest-5,22-dien-2α,3α,16α,18-tetrol-2,16,18-triacetate (**2**) and 24-methylcholest-5,24(28)-dien-2α,3α,15β,18-tetrol-2,15,18-triacetate (**3**) which indicates that the polyhydroxysterols of this organism are characterized by a very similar oxidative pattern where positions 2,3, and 18 are always involved.



	R	R_1	R_2	R_3
1	OAc	OAc	H	
2	H	H	OAc	
3	H	OAc	H	

EXPERIMENTAL

Colonies of *E. glomeratum*, collected in the Bay of Naples (January-February 1984) near Pozzuoli and identified by Dr. M. Pansini (University of Genova), were freed by hand from macroscopic epibionts. A voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali. Fresh material (wet weight 700 g) was freeze-dried and exhaustively extracted at room temperature with MeOH. The extract was concentrated in vacuo affording a residue (3.5 g), which was fractionated by flash chromatography on a silica gel column (Merck, 250 g), using as eluent C_6H_6-Et_2O (7:3) and then Et_2O. The more polar fraction (480 mg) was rechromatographed on a column of silica gel (50 g) under pressure and eluted with Et_2O-C_6H_6 (7:3); 25 fractions of 40 ml were collected. The fractions 10-12 (40 mg), containing crude **2** and **3**,

were subjected to reverse-phase hplc (Varian-Model 5000) on a Whatman Partisil M9 10/50 ODS-2 column, using a dual cell refractometer detector, with MeOH-H_2O (92:8) as mobile phase to give 8 mg of a mixture of **2** and **3**. Final separation was achieved by silica gel tlc impregnated with $AgNO_3$ (15%) using Et_2O-C_6H_6 (9:1) as eluent. The bands at Rf 0.3 and 0.5, visualized by heating a thin strip of the plate sprayed with a 5% ceric sulfate in 10% aqueous H_2SO_4, gave crystalline **3** (3.6 mg, calcd. for $C_{34}H_{52}O_7$ C, 71.30; H, 9.15. Found C, 71.55; H, 9.07) and **2** (4.2 mg, calcd. for $C_{33}H_{50}O_7$: C, 70.94; H, 9.02. Found C, 70.85; H, 9.05), respectively.

RESULTS AND DISCUSSION

Compound **2**, mp 194-196° (from MeOH), $[\alpha]^{26}D$ +57° (c 0.04, $CHCl_3$) had the molecular formula $C_{33}H_{50}O_7$ from elemental analysis.

TABLE 1. Main Low-Resolution Mass Spectral Fragments

Fragments	*m/z* (relative intensity)[a]		
M^+-AcOH	558 (8)	498 (10)	512 (90)
M^+-AcOH-42	516 (28)	456 (4)	470 (97)
M^+-2AcOH	498 (32)	438 (30)	452 (52)
M^+-AcOH-$AcOCH_2$	485 (5)	425 (100)	439 (100)
M^+-2AcOH-CH_3	483 (7)	423 (5)	437 (9)
M^+-2AcOH-H_2O	480 (10)	420 (6)	434 (23)
M^+-2AcOH-42	456(60)	396(5)	410 (18)
M^+-2AcOH-$AcOCH_2$	425 (23)	365 (98)	379 (65)

[a]Mass spectra were taken on AEI MS-902 instrument.

The presence in **2** of hydroxyl and acetoxyl groups was suggested by ir absorptions at 3400, 1745, and 1235 cm^{-1} ($CHCl_3$) and by the mass spectrum (see Table 1) which contains fragmentation peaks due to the loss of HOAc and H_2O molecules. This was confirmed by ^{1}H-nmr (500 MHz) and ^{13}C-nmr (62.9 MHz) spectra, which indicated that the compound contains two secondary and one primary acetoxyl groups [^{1}H nmr: 3H singlets at δ 2.00, 2.09, and 2.12 (CH_3-CO), 1H multiplets at δ 5.05 and 5.08 (*CH*-OAc), and a 2H AB quartet (δ 4.34 and 4.38, J=12.5 Hz, CH_2-OAc); ^{13}C nmr: δ 167.3, 170.1, and 170.9 (singlets, >C=O), δ 72.5 and 75.3 (doublets, >*CH*-OAc) and δ 63.4 (t, CH_2-OAc)] and a secondary hydroxyl group [^{1}H nmr: δ 4.08 (1H, ddd, >CH-OH); ^{13}C nmr: δ 68.08 (d, >CH-OH)]. Consequently, compound **2** must be a C_{27} polyhydroxysteroid.

The most significant features of the ^{1}H-nmr spectrum of **2**, which suggested a strict relation between **2** and **1**, were the absence of the 3H singlet due to the C-18 methyl group substituted by an AB quartet (δ 4.38 and 4.34) and the signals assigned to the protons of the C_1 to C_6 part structure (see Table 2) on the basis of double resonance and double resonance difference experiments.

The ^{1}H-nmr spectrum also displayed two methyl signals at δ 0.85 (6H, d, J=7 Hz) and 1.17 (3H, d, J=6.5 Hz) and two signals at δ 5.21 (1H, dd, 22-H) and 5.30 (1H, ddd, 23-H) due to the side chain olefinic protons. Spin decoupling experiments indicated that this double bond was located between C-22 and C-23; irradiation at δ 2.46 (tentatively the C-20 proton) simplified the two doublets of doublets at δ 5.21 (HC-22) and 1.34 (HC-17) into two doublets, while the 3H doublet at δ 1.17 (H_3 C-21) collapsed into a singlet.

The location of the remaining secondary acetoxyl group, which resonates in the ^{1}H-nmr spectrum as a multiplet at δ 5.08, was indicated to be at C-16 by the chemical shift of C-17 (δ 60.47) in the ^{13}C-nmr spectrum (3), and this was confirmed by irradiation at δ 1.34 (HC-17), which simplified the acetoxymethine signal at δ 5.08.

Analysis of ^{1}H-nmr features for C-2 and C-3 protons (see Table 2) showed that the

TABLE 2. Selected Spectroscopic Data of **1-3** with Selected ^{1}H-nmr (500 MHz, $CDCl_3$) Chemical Shifts[a]

	1 (2)	**2**[b]	**3**[c]
1α	1.58 (dd)	1.57 (dd)	1.58 (dd)
1β	1.84[d]	1.84 (dd)	1.84[d]
2	5.08 (ddd)	5.05 (ddd)	5.08 (ddd)
3	4.12 (ddd)	4.08 (ddd)	4.09 (ddd)
4α	2.32 (dd)	2.29 (dd)	2.30 (dd)
4β	2.63 (bdd)[e]	2.61 (bdd)[e]	2.63 (bdd)[e]
6	5.36 (dd)	5.46 (m)	5.46 (m)
15α	5.25 (ddd)		5.25 (ddd)
16α	2.32[e]		2.43[d]
16β	1.33[d]	5.08 (ddd)	1.33[d]
17	2.14[d]	1.34 (dd)	2.14[d]
18	4.40 and 4.32 (AB system)	4.38 and 4.34 (AB system)	4.37 and 4.32 (AB system)
19	1.19 (s)	1.09 (s)	1.13 (s)
20	1.84[d]	2.46[d]	1.84[d]
21	1.13 (d)	1.17 (d)	1.15 (d)
22		5.21 (dd)	
23		5.30 (ddd)	
24		1.80[d]	
25	1.48 (m)	1.55 (m)	2.18[d]
26	0.90 (d)	0.85 (d)	1.06 (d)
27	0.90 (d)	0.85 (d)	1.05 (d)
28a			4.71 (bs)
28b			4.63 (bs)

[a]^{1}H-nmr spectra were recorded on a Bruker WM-500 spectrometer in $CDCl_3$ solution and the assignments were confirmed by decoupling and decoupling difference experiments. Determination of nOe's were performed on a Bruker WM-250 spectrometer in $CDCl_3$ with the aid of Aspect 2000 microprograms which allowed direct accumulations of difference fid's. The sample used for nOe measures was previously degassed by bubbling Ar through the solns for 40 min. The δ values are in ppm downfield from TMS.

[b]J (Hz) 1α-1β=12.5, 1α-2=12.5, 1β-2=2.5, 2-3=2.5, 3-4β=3, 3-4α=2.5, 4α-4β=15, 16-17=6.5, 17-20=11.5, 18-18=12.5, 20-21=6.5, 20-22=7, 22-23=14, 23-24=7, 25-26=7, 25-27=7.

[c]J (Hz) 1α-1β=12.5, 1α-2=12.5, 1β-2=2.5, 2-3=2.5, 3-4β=3, 3-4α=2.5, 4α-4β=15, 14-15=3, 15-16α=6.5, 15-16β=6.5, 18-18=12.5, 20-21=6.5.

[d]Submerged by other signals.

[e]Broadened by allylic and homoallylic couplings.

oxygenated functions linked at these positions had an identical stereochemistry as in **1**, while the α-orientation of the acetoxyl group at C-16 was assigned on the basis of ^{1}H-nOe experiments involving spectral subtraction techniques (difference nOe). Enhancements of the H-20 and H-16 signals were observed when the C-18 methylene protons were irradiated, whereas irradiation of H-16 produced enhancements of the H-20 and the H_2-18 signals, thus indicating a *cis*-relationship for these three protonated substituents. Finally, the stereochemistry of the side-chain double bond was assigned as *E* on the basis of the observed vicinal coupling (H-22/H-23) of 14 Hz.

The sterol **3**, isolated in smaller amounts as a crystalline solid (mp 185-187°, from MeOH), showed $[\alpha]^{26}D$ +21° (c 0.04, $CHCl_3$); it analyzed for $C_{34}H_{52}O_7$ by elemental analysis and was shown to contain three acetoxyl groups and one >CH-OH group by its ir (absorptions at 3400, 1745, and 1235 cm^{-1}), ^{1}H-nmr features [3H singlet at δ 2.02 and 6H singlet at δ 2.09 due to 3 CH_3-CO, 1H multiplets at δ 5.08 and 5.25 (*CH*-OAc), and 2H AB quartet (δ 4.37 and 4.32, J=12.5 Hz, CH_2-OAc)] and from the mass spectrum in which intense fragmentation peaks deriving from losses of H_2O and

HOAc molecules are present (Table 1). These data suggested that **3** must be a C_{28} di-unsaturated steroid.

Comparison of the most significant data of the ^{1}H-nmr spectrum of **3**, reported in the Table 2 and assigned on the basis of spin decoupling and spin decoupling difference experiments, with those of **1** and **2** revealed a strong structural analogy between these three steroids. In particular the protons linked to C_1 to C_6 display ^{1}H-nmr signals very similar to those of the relevant protons of **2** (see Table 2). Moreover, the absence in the ^{1}H-nmr spectrum of the methyl singlet typical for the C-18 protons and the presence of an AB quartet centered at δ 4.35 was again ascribed to an acetoxylated C-18 group. Consistent with this assignment was the prominent loss of CH_2OAc in the mass spectrum (see Table 1).

Spin decoupling and spin decoupling difference experiments clearly indicated (see Table 2) that the protons at C-21 (δ 1.15), C-20 (δ 1.84), C-17 (δ 2.14), C-16α (δ 2.43), C-16β (δ 1.33), and C-15α (δ 5.25) were inter-related, thus defining the presence of the remaining acetoxyl group on the cyclopentane ring at position 15.

Finally, the location of the remaining double bond on the side-chain between C-24 and C-28 was established on the basis of ^{1}H-nmr spectroscopic analysis; by irradiation at δ 2.18 the two 6H doublet at δ 1.06 collapsed into a singlet, and the two broad singlets at δ 4.71 and 4.63, attributable to the olefinic hydrogens at C-28, sharpened.

The orientations of the acetoxyl at C-2 and hydroxyl at C-3 were both assigned as α on the basis of ^{1}H-nmr features, which were identical with those of **1** and **2**.

In an analogous way, the small values of the coupling constants of H-16, identical to those of **1** and in good agreement with the literature data (4), suggested the β-position for the acetoxyl group at C-15.

ACKNOWLEDGMENTS

This work is a result of research sponsored by Consiglio Nazionale delle Ricerche (Rome) in the frame of the "Progetto Finalizzato Chimica Fine e Secondaria". We thank Dr. M. Pansini (Università di Genova, Italy) for identifying the hydroid. Mass spectral data were provided by "Servizio di Spettrometria di massa del CNR e dell'Università di Napoli." The assistance of the staff is gratefully appreciated.

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