Journal of Natural Products
Vol. 50, No. 2, pp. 191-194, Mar-Apr 1987

ISOLATION AND STRUCTURE ELUCIDATION OF TWO NEW POLYHYDROXYLATED STEROLS FROM THE MEDITERRANEAN HYDROID *EUDENDRIUM GLOMERATUM*

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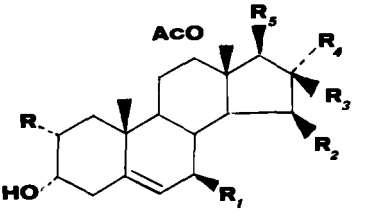
ABSTRACT.—The extract from the marine hydroid *Eudendrium glomeratum* yielded very small amounts of two new polyoxygenated sterols, cholest-5-ene-2α,3α,16β,18-tetrol-2,16,18-triacetate [**4**] and cholest-5-ene-3α,7β,15β,18-tetrol-7,15,18-triacetate [**5**]. Their stereostructures were established through extensive ^{1}H-nmr analysis.

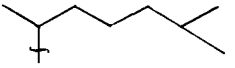
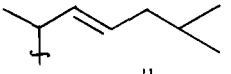
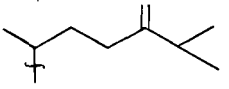
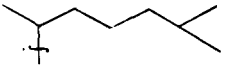
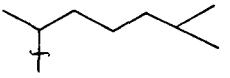
Marine hydroids are the simplest Cnidarians, generally characterized by an alternation of generation; they occur in most habitats from the shore to the deep sea. The sessile animals (polyps) grow as bush-like colonies on stones, shells, or seaweeds. Only a few reports (1-6) on their chemical constituents have appeared until now, due largely to the difficulties of collecting and identifying the biological material.

Our recent investigation on the sterol fractions of these organisms indicated that all the species examined have a similar composition, the only exception being *Eudendrium glomeratum* Picard (Eudendriadae), which, in addition to common C_{26}, C_{27}, C_{28}, and C_{29} mono-hydroxysterols, contains three new compounds with an unusual hydroxylation pattern on the nucleus [**1-3**] (4-5). In this paper we propose structures for two new polyhydroxysteroids, cholest-5-ene-2α,3α,16β,18-tetrol-2,16,18-triacetate [**4**] and cholest-5-ene-3α,7β,15β,18-tetrol-7,15,18-triacetate [**5**], which were isolated as minor constituents of this organism.

The methanolic extract from colonies of *E. glomeratum* collected in the Bay of Naples was chromatographed on a silica gel column. Selected fractions were further purified by reversed-phase hplc to obtain, in addition to **1-3**, the compounds **4** and **5**.

Compound **4**, obtained as an amorphous powder, analyzed for $C_{33}H_{52}O_7$ by hrms



	R	R_1	R_2	R_3	R_4	R_5
1	OAc	OAc	OAc	H	H	
2	OAc	H	H	H	OAc	
3	OAc	H	OAc	H	H	
4	OAc	H	H	OAc	H	
5	H	OAc	OAc	H	H	

data on the first fragment at *m/z* 500 (M^+-AcOH). In the ^{1}H-nmr spectrum methyl signals at δ 1.07 (3H, s), 1.06 (3H, d), and 0.85 (6H, d) are present; this last signal must be due to the methyls of an isopropyl group because it collapsed to a singlet by irradiation at δ 1.52. An hydroxyl group was required by the ir absorption at 3540 cm^{-1} ($CHCl_3$); this function is linked to a secondary carbon as indicated by the presence in the ^{1}H-nmr spectrum of a 1-H multiplet at δ 4.10. An intense absorption at 1740 cm^{-1} in the ir and two singlets in the ^{1}H nmr [δ 2.08 (6H) and 2.02 (3H)] indicated the presence of three acetate functions, one of which must be linked to a methylene and two to methine groups as indicated by the low-field signals at δ 4.28 and 4.34 (1H each; AB), 5.03 (1H, ddd), and 5.24 (1H, ddd).

All the above data indicate that compound **4** was a C_{27} triacetoxy-mono-hydroxysterol.

The location of an acetoxy group at C-18 was evidenced by the presence of AB doublets (δ 4.28 and 4.34) and by a major peak at *m/z* 427 in the mass spectrum corresponding to $M^+ -(AcOH+AcOCH_2)$; furthermore, the ^{1}H-nmr singlet due to the C-18 methyl group (usually present at δ 0.7) was missing.

The C_1-C_6 portion of **4** was assigned by a detailed interpretation of the 500 MHz ^{1}H-nmr spectrum that uncovered a series of signals very similar to those of the corresponding protons in **1** (4) and **2-3** (5). The assignments, reported in Table 1, were substantiated by spin-decoupling and spin-decoupling-difference experiments.

The remaining acetoxyl group was located at C-16 as indicated by the following spin-decoupling experiments, which allowed the establishment of the sequence C_{15}-C_{16}-C_{17}-C_{20}-C_{21}. Irradiation at δ 1.82 (H-20) collapsed the doublet at δ 1.06 (3H-21) into a singlet and simplified the double-doublet at δ 1.30 (H-17) into a doublet; conversely, irradiation at the H-16 frequency (δ 5.24) simplified, in addition to the double-doublet at δ 1.30, the multiplets at δ 2.45 and 1.09 due to H-15α and H-15β, respectively.

The stereochemistry at C-2 and C-3 was assumed to be as reported in **4**, due to the observed coupling constants of H-2 and H-3 (Table 1), which are consistent with their equatorial and axial nature, respectively.

Results from ^{1}H-nmr nOeds studies defined the stereochemistry at C-16. Enhancement of H-17 and H-15α signals was observed following irradiation of the C-16 proton at δ 5.24, while no significant enhancement of H-16 was observed on irradiation at δ 4.31 (2H-18).

The remaining sterol **5** isolated from *E. glomeratum* was an isomer of **4** (from hrms) and exhibited a similar ir spectrum (see Experimental). Its ^{1}H-nmr spectrum clearly indicated the presence of one CH-OH (δ 4.10, 1H, ddd) and three acetoxyl groups (δ 2.04, 2.05, 2.13; 3H each), two of which are linked to methine (δ 5.03 and 5.24, 1H, each, ddd) and one to a methylene group (2H, AB) centered at δ 4.31; a 1H broad singlet at δ 5.28 attributable to an olefinic hydrogen is also present.

For the location of the above functionalities on the sterol skeleton we performed extensive ^{1}H-nmr studies including spin-decoupling experiments that resulted in the complete assignments of all the proton resonances apart from the side-chain methylene hydrogens (Table 1).

The hydroxyl group at C-3 was clearly axial because the H-3 signal showed no large *J* value attributable to an axial-axial coupling; the stereochemistry at C-7 was deduced from the *J* values of H-7 (*J* 7-8=9 Hz; *J* 6-7=2 Hz) which are compatible with its β-position (4,7,8). Finally, the β-position for the acetoxyl group at C-15 was indicated by the small value of the coupling constants of H-15 identical to those of the corresponding signals of **1** and **3** and in good agreement with the literature data (4,5,9).

TABLE 1. Nmr Data (500 MHz, $CDCl_3$) of Compounds **4** and **5**; ^1H Chemical Shifts (δ)[a]

Assignment	Compounds	
	4[b]	**5**[c]
1α (ax)	1.58 (dd)	1.10[d]
1β (eq)	1.83 (dd)	1.30[d]
2	5.03 (ddd)	1.76 (bm) 1.44[d]
3 (eq)	4.10 (ddd)	4.10 (bs)
4α (eq)	2.25 (dd)	2.13 (dd)
4β (ax)	2.59[e] (bdd)	2.58[e] (bdd)
6	5.44 (m)	5.28 (bs)
7		5.02 (bd)
8 (ax)		1.85[d]
9 (ax)	1.33 (ddd)	1.40[d]
11α (eq)		1.65 (dddd)
	1.5-1.65[d]	
11β (ax)		1.49 (dddd)
12α (ax)	1.10 (ddd)	1.12[d]
12β (eq)	2.44 (ddd)	2.50 (ddd)
14		1.24 (ddd)
15	2.45 (α) 1.09 (β)	5.24 (α, ddd)
16α	5.24 (ddd)	2.31[d]
16β		1.30[d]
17	1.30 (dd)	2.12[d]
18	4.34 and 4.28	4.35 and 4.27
19	1.07 (s)	1.09 (s)
20	1.82[d]	1.83[d]
21	1.06 (d)	1.10 (d)
25	1.52 (m)	1.55 (m)
26	0.85 (d)	0.88 (d)
27	0.85 (d)	0.88 (d)
CH_3CO	2.02 (3H)	2.04 (3H)
	2.08 (6H)	2.05 (3H)
		2.13 (3H)

[a] ^1H-nmr spectra were recorded on a Bruker WM-500 spectrometer. The δ values are in ppm downfield from TMS.

[b] *J* (Hz) of **4**: 1α-1β=12.5; 1α-2=12.5; 1β-2=3.0; 2-3=2.5; 3-4β=3; 3-4α=2.5; 4α-4β=15; 15α-16α=5; 15β-16α=7.5; 16α-17=7; 17-20=11; 18-18=12.5; 20-21=7; 25-26=7; 25-27=7.

[c] *J* (Hz) of **5**: 1α-2β=10; 1α-2α=3; 2α-2β=12.5; 2β-3=very small; 2α-3=very small; 3-4β=3; 3-4α=3; 4α-4β=14; 6-7=2; 7-8=9; 11α-11β=14; 11α-12α=3.5; 11α-12β=3.5; 11β-12α=14; 11β-12β=3.5; 12α-12β=14; 14-15=3; 15-16α=6.5; 15-16β6.5; 18-18=12.5; 20-21=6.5; 25-26=7; 25-27=7.

[d] Submerged by other signals.

[e] Broadened by allylic and homoallylic couplings.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra ($CHCl_3$) were recorded on a Perkin-Elmer 157 spectrometer. Mass spectra were taken on an AEI MS-902 instrument. Optical rotations were measured on a Perkin-Elmer 191 polarimeter with a 10 cm microcell in $CHCl_3$. ^1H-nmr spectra were determined on a Bruker WM-500 spectrometer in $CDCl_3$, and the assignments were confirmed by decoupling and decoupling difference experiments. Determination of nOes were performed on a Bruker WM-250 spectrometer in $CDCl_3$ with the aid of Aspect 2000 microprograms, which allowed direct accumulations of difference fids. The sample used for nOe measurements was previously degassed by bubbling Ar through the solution for 40 min. Chemical shift values are in ppm downfield from TMS.

The chromatographic separations were performed on a Varian HPLC Model 5000 with a Hibar RP-

18 Lichrosorb 7 μm or a Hibar RP-18 Lichrospher super 100 column using a dual-cell refractometer detector.

ISOLATION OF **4** AND **5**.—Colonies of the hydroid *E. glomeratum*, identified by Dr. M. Pansini, University of Genoa, were collected in the Bay of Naples (January-February, 1985) and freed by hand from macroscopic epibionts. A voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali, University of Naples.

Freshly collected material (wet weight 600 g) was freeze-dried and extracted at room temperature three times with MeOH. The extract was concentrated in vacuo affording a brown oil (3.5 g). The residue was fractioned by flash chromatography on a silica gel column (Merck, 250 g) under pressure using as eluent C_6H_6 followed by C_6H_6-Et_2O (7:3) and then Et_2O.

Rechromatography of the more polar fractions (300 mg) on a column of silica gel (40 g) under pressure using Et_2O-C_6H_6 (7:3) as eluent, afforded 38 fractions of 50 ml. The fractions 20-26 (35 mg) containing **4** and **5** were further purified by hplc (Lichrosorb RP-18; eluent MeOH-H_2O, 95:5) to give a mixture of **4** and **5** (5 mg). Final separation was achieved by hplc on Lichrospher RP-18 using MeOH-H_2O (95:5) as eluent to give 2.8 mg of **4** $[\alpha]^{26}D+17.2$ (*c* 0.03, $CHCl_3$); ir ($CHCl_3$) ν max 3400, 1745, and 1235 cm^{-1}; ^1H-nmr data (500 MHz) are listed in Table 1; hrms (70 eV) *m/z* 500.3508 (M^+-AcOH; 500.3503 calcd. for $C_{33}H_{52}O_7$); 2.1 mg of **5** $[\alpha]^{26}D+23$ (*c* 0.03, $CHCl_3$); ir ($CHCl_3$) ν max 3400, 1745, 1235 cm^{-1}; ^1H-nmr data (500 MHz) are listed in Table 1; hrms (70 eV) *m/z* 500.3500 (M^+-AcOH; 500.3503 calcd. for $C_{33}H_{52}O_7$).

ACKNOWLEDGMENTS

This work was supported by CNR (Progetto Finalizzato Chimica Fine e Secondaria) and by Ministero della Pubblica Istruzione, Italy. 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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Received 23 June 1986