Cholest-5-ene-2α,3α,7β,15β,18-pentol 2,7,15,18-Tetraacetate, a Novel Highly Hydroxylated Sterol from the Marine Hydroid *Eudendrium glomeratum*

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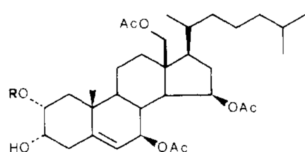
The novel polyhydroxylated sterol cholest-5-ene-2α,3α,7β,15β,18-pentol 2,7,15,18-tetraacetate (1) was isolated in very small amounts from the marine hydroid *Eudendrium glomeratum*. Its stereostructure was established through extensive ^{1}H NMR, ^{13}C NMR, and MS studies and chemical evidence.

Although in the last decade a large number of new sterol structures have been discovered from marine invertebrates,[1] relatively few data have been reported on the sterol composition of marine hydroids, probably owing to the difficulties deriving from their collection and identification. In 1980 Cimino et al.[2] isolated from an *Eudendrium* species an unusual polyhydroxylated steroid, (22*R*)-3-oxo-cholest-4-ene-4,16β,18,22-tetrol 16,18-diacetate. More recently[3] we reported the distribution of monohydroxylated sterols in four Mediterranean hydroids belonging to the suborder Tecata (*Aglaophoenia pluma* and *Sertularella crassicaulis*) and Atecata (*Halocordyle disthica* and *Eudendrium glomeratum*).

We now describe the isolation from the last organism of a novel polyhydroxylated sterol, cholest-5-ene-2α,3α,7β,15β,18-pentol 2,7,15,18-tetraacetate (1). This new



1 R = Ac
2 R = H

pentol adds to the steadily growing number of polyhydroxylated steroids isolated from marine animals, particularly Alcyonarians and starfish; the 2α,3α-diol pattern present in 1, however, is an uncommon feature in naturally occurring steroids.

Chromatography (silica gel) of the methanol extract of colonies of *E. glomeratum* gave a fraction containing monohydroxylated sterols together with a more polar fraction apparently homogeneous by TLC, which through high-pressure C_{18} reverse-phase chromatography yielded 1 in very small quantities.

Compound 1, $[\alpha]^{26}_{D}$ +96° (*c* 0.03, $CHCl_3$), colorless needles, mp 139–141 °C (from methanol), showed the molecular formula $C_{35}H_{54}O_9$ established by high-resolution mass spectral data on the first fragment at *m/z* 558 ($M^+ - 60$).

A substituted cholestene nucleus was inferred from the ^{1}H NMR spectrum in $CDCl_3$, which showed the signals for four of the five methyl groups typical of a sterol at δ 0.90 (6 H, d, H_3-26 and H_3-27), 1.13 (3 H, d, H_3-21), and 1.19 (3 H, s, H_3-19).

The absence of a methyl singlet assignable to the C-18 angular methyl, coupled with the presence of an AB quartet centered at δ 4.36 (J = 12.5 Hz), was ascribable to an acetoxylated C-18 group. The presence of this functionality, which recently was found in a sterol derivative from a marine hydroid,[2] was also deduced from an infrared absorption at 1745 cm^{-1}, a sharp three-proton singlet at δ 2.06 in the ^{1}H NMR spectrum, and a singlet at δ 171.2 and a triplet at δ 62.71 in the off-resonance ^{13}C NMR spectrum. Consistent with this assignment was the presence in the mass spectrum of a characteristic intense ion at *m/z* 485 due to the loss of 73 mass units from $M^+ - 60$.

In the mass spectrum the fragmentation illustrating the losses of acetate units at *m/z* 558 (M^+ – AcOH), 498 (M^+ – 2AcOH), 438 (M^+ – 3AcOH), and 365 (M^+ – 3AcOH – CH_2OAc) suggested the presence in 1 of three further acetoxy groups.

^{1}H NMR [1-H multiplets at δ 5.06, 5.08, and 5.25 and 3-H singlets at δ 2.12, 2.18, and 2.20] and ^{13}C NMR [singlets at δ 170.2, 170.4, and 170.9] spectra supported this hypothesis and indicated that these functionalities must be linked to methine groups.

Other ^{1}H NMR and ^{13}C NMR features showed compound 1 to possess a trisubstituted double bond [^{1}H NMR δ 5.36 (1 H, dd); off-resonance ^{13}C NMR δ 123.4 (d) and 141.0 (s)] and one secondary hydroxyl group [^{1}H NMR δ 4.12 (1 H, ddd); off-resonance ^{13}C NMR δ 67.83 (d)].

An accurate analysis of the ^{1}H NMR spectrum at 500 MHz of 1 and extensive decoupling and difference decoupling experiments allowed one to locate these functionalities on the sterol skeleton. The acetoxymethine group at C-2 resonates as a double double doublet at δ 5.08, coupled to the protons linked at C-1, resonating at δ 1.58 and 1.84, and to the hydroxymethine signal at C-3 (δ 4.12). Decoupling experiments established that the last signal was coupled to the protons linked to C-4 resonating at δ 2.32 (dd) and 2.63 (dddd).

The C-6 olefinic proton signal at δ 5.36 (1 H, dd) exhibited allylic coupling to the H-4β proton signal (J = 3 Hz) and vicinal coupling (J = 2 Hz) to the H-7 proton signal at δ 5.06 (1 H, ddd) that was in turn coupled to the H-8 proton signal at δ 1.84 (J = 9 Hz) and, through an homoallylic coupling, to H-4β (J = 3 Hz).

The proton at C-15 resonates as a double double doublet at δ 5.25 and by decoupling experiments resulted to be coupled with the H-14 proton, which appears at δ 1.31 as a double doublet by further coupling with H-8.

Further support for the location of the functionalities on the cholestene nucleus was provided by additional decoupling and decoupling-difference experiments, which established the assignment (Table I) of all protons of 1, except those of the side-chain methylene groups.

The stereochemistry at C-2 could be inferred from the magnitude of the appropriate coupling constants. Thus,

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Table I. Nuclear Magentic Resonance Data ($CDCl_3$) for 1[a]

assignment	^1H chemical shift, δ	^{13}C chemical shift, δ
1α	1.58 (dd)	
1β	1.84[b]	
2	5.08 (ddd)	72.20[c]
3	4.12 (ddd)	67.83
4α	2.32 (dd)	
4β	2.63 (dddd)	
5		141.0
6	5.36 (dd)	123.4
7	5.06 (ddd)	74.85[c]
8	1.84[b]	
9	1.40 (ddd)	
11α	1.69(dddd)	
11β	1.52 (dddd)	
12α	1.15 (ddd)	
12β	2.51 (ddd)	
14	1.31 (dd)	
15	5.25 (ddd)	75.06[c]
16α	2.32[b]	
16β	1.33[b]	
17	2.14[b]	60.06
18	4.36 (AB q)	62.71
19	1.19 (s)	
20	1.84[b]	
21	1.13 (d)	
25	1.48 (m)	
26	0.90 (d)	
27	0.90 (d)	

[a] The methyl protons of acetoxy groups resonate in the ^1H NMR spectrum at δ 2.06, 2.12, 2.18, and 2.20; in the ^{13}C NMR spectrum the carbon atoms of the carbonyls appear at δ 170.2, 170.4, 170.9, and 171.2. 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; 4β–6 = 3; 4β–7 = 3; 6–7 = 2; 7–8 = 9; 8–9 = 12; 8–14 = 12; 9–11α = 3.5; 9–11β = 12; 11α–11β = 13; 11α–12α = 3; 11α–12β = 3; 11β–12α = 12; 11β–12β = 3; 12α–12β = 12; 14–15 = 3; 15–16α = 6.5; 15–16β = 6.5; 18–18 = 12.5; 20–21 = 6.5; 25–26 = 7; 25–27 = 7. [b] Submerged by other signals. [c] Assignments may be reversed.

the observed $J_{1\beta-2}$ = 2.5 Hz, $J_{1\alpha-2}$ = 12.5 Hz, and J_{2-3} = 2.5 Hz imply that H-2 must be axial. Analogously the equatorial nature of H-3 was deduced from the small value of the coupling constants H-3/H-4α and H-3/H-4β (J = 2.5 and 3 Hz).

The stereochemistry at C-7 was evidenced by the chemical shift of H-6 (δ 5.36, 1 H, dd) and supported by the coupling constant between H-6 and H-7 (J = 2 Hz), which is consistent with a dihedral angle of about 80° deducible from an examination of the molecular models of the 7β-acetoxy-Δ^5-steroids, whereas the epimeric 7α-acetoxy isomers show larger coupling constant values (J = 5 Hz) due to a H-6/H-7 angle of about 25°.[4,5]

The β-position for the acetoxy group at C-15 was indicated by the small values of the coupling constants of H-15 (δ 5.25, J = 6.5, 6.5, and 3 Hz), which are in good agreement with the literature data.[6] Additional evidence was obtained by NOE difference experiments; irradiation at δ 5.25 led to the enhancements of the H-14 (δ 1.31) and H-16α (δ 2.32) signals, indicating a cis relationship for these three protons. In addition, when the H-18 signal was saturated, a NOE was registered for the H-20 signal, while no detectable effect was observed for the H-15 signal.

All the above spectral data allowed us to propose the stereostructure 1 to the compound under investigation. Additional support for this assignment and in particular data to confirm the position of the two oxygenated functions on ring A and their cis relationship were gained as follows.

Selective alkaline hydrolysis of the compound 1 afforded the diol 2, whose structure was based on the interpretation of ^1H NMR spectrum which showed in addition to three CH_3CO signals (δ 2.09, 2.00, and 1.99) significant resonances at δ 5.30 (1 H, m, H-6), 5.19 (1 H, m, H-15), 4.97 (1 H, m, H-7), 4.28 (2 H, AB q, H_2-18), 3.95 (1 H, m, H-3), 3.82 (1 H, m, H-2), 2.53 (1 H, m, H-4β), 2.27 (1 H, m, H-4α), and 1.07 (3 H, s, H_3-19).

This compound, by treatment with an excess of dry acetone in the presence of p-toluenesulfonic acid, gave its 2,3-acetonide, whose ^1H NMR spectrum was very similar to that of **2**, the most significant difference being the presence of two methyl singlets at δ 1.32 and 1.51 and the small upfield shift of H-2 and H-3, from δ 3.82 and 3.85 in **2** to δ 3.75 in the 2,3-acetonide.

Experimental Section

The infrared spectrum of 1 was recorded on a Perkin-Elmer 157 spectrophotometer.

^1H NMR and decoupling experiments were performed on a Bruker WM-500 spectrometer in $CDCl_3$ solution, with tetramethylsilane as internal reference. Determination of nuclear Overhauser effects and decoupling-difference experiments were performed on a Bruker WM-250 spectrometer in $CDCl_3$ solutions with the aid of Aspect 2000 microprograms which allowed direct accumulations of difference fid's. The sample used for NOE measurements was previously degassed by bubbling Ar through the solutions for 40 min.

^{13}C NMR spectra were determined at 62.9 MHz on a Bruker WM-250 spectrometer in $CDCl_3$. Multiplicities were determined from single-frequency off-resonance decoupling, and assignments were based upon selective decoupling experiments and/or a comparison to models.

Mass spectra were taken on a AEI 902 instrument. Optical rotation of 1 was measured on a Perkin-Elmer 191 polarimeter with a 10-cm microcell in $CHCl_3$ solution. The chromatographic adsorbent used was Merck silica gel (230–400 mesh). High-pressure liquid chromatographic separations were performed on a Varian HPLC Model 5000, with a Whatman Partisil M9 10/50 ODS-2 column, using a dual-cell refractometer detector.

Isolation of 1. Colonies of the hydroid *E. glomeratum* were collected in the Bay of Naples (Jan–Feb 1984) and freed by hand from macroscopic epibionts. Freshly collected material (wet weight 500 g) was freeze-dried and extracted at room temperature with methanol, first for 2 days and then for 1 week. Evaporation of the extracts in vacuo afforded residues weighing 1.8 and 0.7 g, respectively. The combined extracts were chromatographed on a column of silica gel (200 g) under pressure, using benzene followed by benzene with increasing amounts of diethyl ether. Rechromatography of the more polar fractions (350 mg) on a column of silica gel (40 g), under pressure, using diethyl ether–benzene (7:3) as the eluant, afforded 20 fractions. Crystallization of fraction 16 (32 mg) from methanol overnight in a refrigerator yielded 8 mg of white crystals. This crystalline material was purified further by high-pressure liquid chromatography using a reverse-phase ODS-2 column with a mobile phase of methanol–water (92:8).

Crystallization from methanol of the major HPLC fraction yielded 4.8 mg of white crystals: mp 139–141 °C; $[\alpha]^{26}_D$ +96° (c 0.03, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1745 and 1235 cm^{-1}; ^1H NMR (500 MHz) and ^{13}C NMR (62.9 MHz) are listed in Table I; high-resolution mass spectrum (70 eV), m/z 558.754 (M^+ – AcOH); low-resolution mass spectrum, m/z (relative intensity) 558 (8, M^+ – AcOH), 516 (28, M^+ – AcOH – 42), 498 (32, M^+ – 2 AcOH), 485 (5, M^+ – AcOH – $AcOCH_2$), 483 (7, M^+ – 2AcOH – CH_3), 480 (10, M^+ – 2AcOH – H_2O), 456 (60, M^+ – 2AcOH – 42), 438 (75, M^+ – 3AcOH), 425 (23, M^+ – 2AcOH – $AcOCH_2$), 423 (30, M^+ – 3AcOH – CH_3), 396 (100, M^+ – 3AcOH – 42), 378 (70, M^+ – 3AcOH – H_2O – 42), 365 (57, M^+ – 3AcOH – $AcOCH_2$), 363 (50, M^+ – 3AcOH – H_2O – 42 – CH_3).

Hydrolysis of 1 To Obtain 2. A solution of 1 (3.5 mg) in hydroalcoholic (1:1) 0.1 N KOH (2 mL) was kept at room tem-

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perature for 2 h. After acidification and removal of EtOH in vacuo, the solution was extracted with Et_2O. The residue (3 mg) obtained from the organic phase, after evaporation of the solvent, was purified by TLC (SiO_2, using benzene–Et_2O (1:1) as the eluant) to yield **2** (2.5 mg), which without further purification was used to obtain the acetonide of **2** as described below. **2**: ^1H NMR δ 5.30 (1 H, m, H-6), 5.19 (1 H, m, H-15), 4.97 (1 H, m, H-7), 4.28 (2 H, AB q, H_2-18), 3.95 (1 H, m, H-3), 3.82 (1 H, m, H-2), 2.53 (1 H, m, H-4β), 2.27 (1 H, m, H-4α), 2.09 (3 H, s, CH_3CO), 2.00 (3 H, s, CH_3CO), 1.99 (3 H, s, CH_3CO), 1.07 (3 H, s, H_3-19), 1.06 (3 H, d, J = 7 Hz, H_3-21), 0.84 (6 H, d, J = 7 Hz, H_3-26 and H_3-27); mass spectrum, m/z 558 ($M^+ - H_2O$), 516 (M^+ – AcOH), 498 (M^+ – AcOH – H_2O).

Acetonide Formation from Sterol 2. **2** (2.5 mg) in dry acetone (0.5 mL) containing p-TsOH (1 mg) was stirred overnight at room temperature. The mixture was neutralized with $BaCO_3$ and centrifuged and the supernatant evaporated to dryness. The residue was then purifed by TLC (SiO_2, benzene–Et_2O (1:1)) thus obtaining the 2,3-acetonide of **2**: ^1H NMR δ 5.30 (1 H, m, H-6), 5.22 (1 H, m, H-15), 5.02 (1 H, m, H-7), 4.28 (2 H, AB q, H_2-18), 3.75 (2 H, m, H-3 and H-2), 2.09 (3 H, s, CH_3CO), 2.02 (3 H, s, CH_3CO), 2.01 (3 H, s, CH_3CO), 1.06 (3 H, s, H_3-19), 1.05 (3 H, d, J = 7 Hz, H_3-21), 0.84 (6 H, d, J = 7 Hz, H_3-26 and H_3-27); mass spectrum, m/z 541 (M^+ – AcOH – CH_3), 499 (M^+ – AcOH – CH_3 – 42).

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Studies on Amphiprotic Compounds. 2. Experimental Determination of the Hydrogen Bond Acceptor Basicities of "Monomeric" Alcohols[1]

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Equilibrium constants have been determined for the hydrogen-bonding association between 3,4-dinitrophenol (ArOH) and "monomer" alcohols (ROH) in highly dilute cyclohexane solutions. These data have been anchored

to the empirical β scale of hydrogen bond acceptor basicities to yield the hitherto unavailable β_m parameters for the "monomeric" alcohols. These values have been compared with bulk solvent (mainly "oligomer") β values. The differential structural effects on the monomer acidities and basicities of the alcohols have been quantitatively analyzed in terms of field, resonance, and polarizability effects.

Alcohols, phenols, carboxylic acids, and amides are valuable solvents and reagents in organic chemistry, and these same functionalities are found in a wide variety of compounds of biological importance. A common feature of these species in their neat liquid forms or when acting as solvents is their extensive self-association through hydrogen bonding.[3] We have recently undertaken a program aimed at obtaining quantitative information on the hydrogen bond donor (HBD) acidities and hydrogen bond acceptor (HBA) basicities of these compounds in the "monomeric" forms they assume when acting as solutes. Such data should allow a more complete understanding of effects of structure and self-association on hydrogen-bonding interactions.

The empirical scales of solvent dipolarity/polarizability (π^*), HBD acidity (α), and HBA basicity (β) provide a good deal of information on non-self-associating compounds and on self-associating compounds in their "polymeric" forms, as well as a framework and methodology for the analysis of these data.[4] The recent extension of this methodology to the treatment of *solute* properties such as solubilities in water[5] and octanol/water partition coefficients[6] and the importance of including non-self-associated amphiprotic solutes in these linear solvation energy relationships further highlights the need to determine monomer HBA basicities (β_m) of such compounds.

Several methods, which lead to remarkably coincident values, are available for the determination of β values of HBA bases.[7] Certain of these techniques involve the use of solvatochromic indicators dissolved in the pure bases and, when applied to alcohols or other self-associated species, yield values measuring the average HBA basicities of the monomers and oligomers present in the bulk sol-

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