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3β,5α-DIHYDROXY-6β-METHOXYCHOLEST-7-ENES FROM THE MARINE SPONGE *SPONGIA AGARICINA*

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The discovery of new sterols from marine invertebrates characterized by novel side-chain alkylation patterns, nuclear modification, and/or polyhydroxylation is proceeding at a remarkable rate, and a number of Porifera (sponges) have considerably contributed to accumulation of these marine natural products.

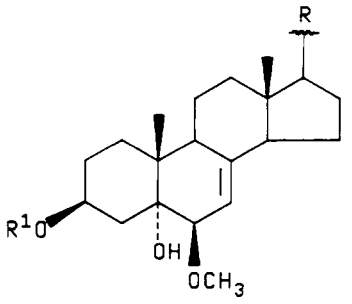
Recently, 3β, 5α,6β-trihydroxycholest-7-enes have been isolated from two primitive marine organisms, a coral-like colonial Bryozoa (*Myriapora truncata*) and an unidentified *Dysidea* sp., a sponge collected in the Pacific Ocean near the Guam Islands (1,2). We wish to report here that new sterols having the same oxidation pattern are also present in a demospongia, *Spongia agaricina* Pallas, commonly named "elephant ear," widely distributed in the Bay of Naples and generally living at about −30 m.

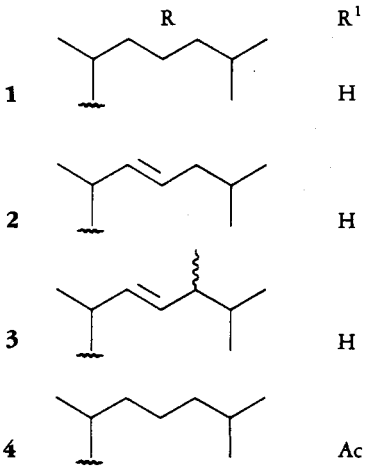
The MeOH extract from freeze-dried *S. agaricina* specimens was chromatographed on a Si gel column using solvent systems of increasing polarity from C_6H_6-Et_2O (7:3) to Et_2O. Selected fractions were further purified by reversed-phase hplc to obtain compounds **1–3**.

The molecular formula of **1** ($C_{28}H_{48}O_3$) was established by hrms. The ^{13}C-nmr spectrum supported the presence of 28 carbon atoms, including four oxygen-bearing [1 Me (δ 44.34), 2 -ĊH (δ 67.75 and 82.94), 1 -Ċ-(δ 76.20)] and two sp^2 carbons [1 $_H$>C= (δ 115.39) and 1 >C= (δ 143.70)].

The above functionalities were confirmed by the ^1H-nmr spectrum ($CDCl_3$) [-OMe (δ 3.38), 2 H−Ċ−O− (δ 4.07 and 3.18), >C=CH− (δ 5.45)], which also contained signals due to the five methyl groups typical of a sterol [H_3-18 (s, δ 0.59), H_3-19 (s, δ 1.00), H_3-21 (d, δ 0.92, J = 6.5 Hz), H_3-26 and H_3-27 (6H, d, δ 0.87, J = 7 Hz)]. The rather high-field position (δ 0.59) of the C-18 methyl signal is in good agreement with that expected for a Δ^7 sterol.

Further structural information on compound **1** was obtained from spin-spin decoupling experiments, per-





formed in C_6D_6 solution, chosen to minimize overlapping of ^{1}H-nmr resonances. The multiplet centered around 4.10 ppm had the complexity normally seen for a 3β-hydroxy group and was coupled to the triplet resonating at δ 2.25 attributable to an axial H-4; the multiplicity of this latter signal suggests that C-5 must be non-protonated. The downfield position (ca. 0.5 ppm) of the H-3 signal relative to that of 5α-cholestan-3β-ol led to the postulate that an α-oriented oxygenated function must be located at position 5 (3–6). This was further supported by the downfield shift of the H-3 signal to 4.5 ppm in pyridine-d_5, indicating an 1–3 diaxial interaction with an OH (or OR) group (2).

The interrelation of H-6, H-7, and H-14, which defined the oxidation pattern of ring B, was established as follows: The mutually coupled methine protons at C-6 and C-7 resonate as broad doublets at δ 3.03 and 5.41, respectively; on the basis of spin decoupling experiments, both were proven to be long-range coupled with the multiplet at δ 1.66 due to H-14.

The above data defined the gross structure of compound **1**, the only uncertainty being the location of the OMe function and one of the two OH groups, which could be linked at C-6 and C-5 or vice versa. Data that made it possible to discard the second possibility were obtained by treatment of **1** with pyridine/Ac_2O at room temperature for 12 h. Under these conditions the tertiary OH group was unaffected, while the hydroxyl group at C-3 was esterified as indicated by the ^{1}H-nmr spectrum of the resulting mono-acetyl derivative, which showed a significant downfield shift (ca. 1.4 ppm) of the H-3 signal relative to that of the parent compound.

Hence, **1** was concluded to be a 3β,5α-dihydroxycholest-7-ene having a methoxy group at C-6. The configuration at this carbon atom was established by the following observations.

The 4-Hz coupling constant of H-6 to H-7 is compatible with the near-zero dihedral angle for these protons in the 6β-methoxy-Δ^7 moiety, as assigned in **1**, whereas the epimeric 6α-methoxy isomer would be expected to have a near-zero coupling constant for H-6/H-7 (7–9). Furthermore, the lower than normal resonances of H_3-19 (δ 1.12 in C_6D_6 and δ 1.00 in $CDCl_3$) and H_{ax}-4 (δ 2.25 in C_6D_6 and δ 2.14 $CDCl_3$) are consistent with the deshielding from a quasi-axial 6β-methoxy group. According to Fujimoto *et al.* (10), the influence of the 6β-OR group through a 1,3-diaxial interaction is intensified in pyridine-d_5 solution, where H_3-19 resonates at δ 1.33 and H_{ax}-4 at δ 2.79.

A minor metabolite assigned as **2** was isolated from *S. agaricina*. This new compound was assigned a composition $C_{28}H_{46}O_3$ by hrms. Comparison of the most significant data of the ^{1}H-nmr spectrum of **2** reported in Table 1 and assigned on the basis of spin-decoupling and spin-decoupling-difference experiments with those of **1** revealed a strong structural analogy between these two sterols. Particularly, the protons of the two angular methyls and those linked to the sequence from C-3 to C-7 display ^{1}H-nmr signals almost identical to those of the corresponding protons of **1**, thus defining the identity of their nuclear moieties. The presence in **2** of a double bond in the side chain at C-22 was indicated by its mass spectrum (intense ion at *m/z* 387 $[M-C_3H_7]^+$) and ^{1}H-nmr spectrum aided by spin-decoupling experiments: irradiation of the methine signal at δ 1.99 (m, H-20) converted the methyl doublet at δ 1.07 (H_3-21, $J=6.5$ Hz) into a singlet and simplified the signal at δ 5.33 (H-22) into an A part of an AB system. Finally, the *E* configuration of this double bond was deduced from the large coupling constant between H-22 and H-23 ($J=17.5$ Hz).

Analogously, the third sterol isolated from *S. agaricina*, compound **3**, ($C_{29}H_{48}O_3$ from hrms) was found to be closely related to compounds **1** and **2** on

TABLE 1. ^{1}H-nmr Data for Compounds **1–3**.

Proton	Compound			
	1		**2**	**3**
	ppm (C_6D_6)[a]	ppm ($CDCl_3$)	ppm (C_6D_6)	ppm (C_6D_6)
1α (ax) . . .	1.16 ddd	1.28		
1β (eq) . . .	1.99 ddd	2.09		
2α (eq) . . .	1.78[b]	1.85		
2β (ax) . . .	1.44[b]	1.45		
3	4.10 m	4.07	4.08	4.11
4α (eq) . . .	1.72 dd	1.76		
4β (ax) . . .	2.25 dd	2.14	2.25	2.21
6	3.03 bd	3.18	3.01	3.01
7	5.41 bd	5.45	5.41	5.41
14	1.66			
18	0.58 s	0.59	0.58	0.58
19	1.12 s	1.00	1.12	1.13
20			1.99	2.00
21	0.98 d	0.92	1.07	1.08
22			5.33	5.25
23				
24				1.94
25	1.56 m	1.50		1.30–1.33
26	0.94 d	0.87	0.92	0.92
27	0.94 d	0.87	0.92	0.92
28				1.01
OMe	3.19 s	3.38	3.17	3.17

[a]J (Hz) of **1**: $J_{1\alpha,1\beta} = 12.5$, $J_{1\alpha,2\beta} = 12.5$, $J_{1\alpha,2\alpha} = 3.0$, $J_{1\beta,2\alpha} = 3.0$, $J_{1\beta,2\beta} = 2.5$, $J_{3,4\alpha} = 3.0$, $J_{3,4\beta} = 12.0$, $J_{4\alpha,4\beta} = 13.0$, $J_{6,7} = 5.0$, $J_{20,21} = 6.5$, $J_{25,26} = 7.0$, $J_{26,27} = 7.0$.
[b]Submerged in other signals.

the basis of detailed interpretation of its 500 MHz ^{1}H-nmr spectrum aided by spin-decoupling and spin-decoupling-difference experiments that uncovered a series of signals very similar to those of the above sterols (Table 1). The only significant difference between its ^{1}H-nmr spectrum and that of **2** was the presence of a 3H doublet at δ 1.01 (C_6D_6) due to a methyl group linked at C-24 as indicated by irradiation at δ 1.94 (tentatively the H-24 frequency), which caused the doublet at δ 1.01 to collapse into a singlet, and, at the same time, simplified the multiplet at δ 5.25 due to H-22 and H-23.

The possibility that compounds **1–3** were formed by methanolysis of an allylic alcohol during extraction and workup was excluded by repeating the extraction with EtOH. After partial purification of the EtOH extract by flash chromatography (C_6H_6/Et_2O), the presence of compounds **1–3** was proved by ^{1}H-nmr spectroscopy and by hplc.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—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 $CHCl_3$. ^{1}H-nmr spectra were determined on a Bruker WM-500 spectrometer in $CDCl_3$, C_6D_6, and pyridine-d_5, and the assignments were confirmed by decoupling and decoupling-difference experiments. ^{13}C-nmr spectra were taken on a Bruker WM-250 spectrometer in C_6D_6. Chemical shift values are in ppm downfield from TMS.

Chromatographic separations were performed on a Varian HPLC Model 5000 with a Hibar RP-18 LiChrosorb 7 μm or a Hibar RP-18 Li Chrospher super 100 column using a dual-cell refractomer detector.

ISOLATION OF COMPOUNDS **1–3**.—*S. agari-*

cina, identified by Dr. M. Pansini, University of Genova, was collected by hand using scuba in the Bay of Naples near Punta Campanella during the spring of 1986. A voucher specimen is deposited in the Dipartimento di Chimica delle Sostanze Naturali, University of Naples. Freshly collected material (wet wt 300 g) was freeze-dried and extracted at room temperature three times with MeOH. The extract was concentrated in vacuo affording a brown oil (18 g). The residue was fractionated by flash chromatography on a Si gel column (Merck, 750 g) under pressure using eluent C_6H_6-Et_2O (7:3) followed by C_6H_6-Et_2O (3:7) and then Et_2O.

Fractions eluted with Et_2O (500 mg) afforded a mixture containing compounds **1–3**, which were rechromatographed by hplc [LiChrosorb RP-18; eluent MeOH-H_2O (92:8)] to give a mixture of **1–3** (15 mg). Final separation was achieved by hplc on LiChrospher RP-18 using MeOH-H_2O (92:8) as eluent. Compound **1** (4 mg): $[\alpha]_D$ −83 (c = 0.02, $CHCl_3$); ^{1}H-nmr see Table 1; hrms (70 eV) *m/z* $[M]^+$ 432.6927, calcd for $C_{28}H_{48}O_3$, 432.6930. Compound **2** (2 mg): $[\delta]_D$ −159 (c = 0.02, $CHCl_3$); ^{1}H-nmr see Table 1; hrms (70 eV) *m/z* $[M]^+$ 430.6774, calcd for $C_{28}H_{48}O_3$, 430.6770. Compound **3** (2.5 mg): $[\alpha]_D$ −160 (c = 0.02, $CHCl_3$); ^{1}H-nmr see Table 1; hrms (70 eV) *m/z* $[M]^+$ 444.7048, calcd for $C_{29}H_{48}O_3$, 444.7041.

ACETYLATION OF **1**.—Compound **1** (2 mg) was dissolved in a mixture of Ac_2O (0.05 ml) and dry pyridine (0.15 ml) and kept at room temperature overnight (12 h). The usual workup afforded the mono-acetyl derivative **4**: ^{1}H nmr (C_6D_6) δ 5.50 (H-3, m), 2.50 (H-4ax, dd, J = 12.5 and 12.0 Hz), 3.00 (H-6, bd, J = 5 Hz), 3.17 (OMe, s), 1.78 (OAc, s).

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