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NEW DIHYDROXYLATED STEROLS FROM THE MARINE SPONGE *SPONGIONELLA GRACILIS*

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ABSTRACT.—The sponge *Spongionella gracilis* contains three new 3β,6α-dihydroxylated sterols that were isolated and characterized as 5α-cholest-7-ene-3β,6α-diol (**1a**), 5α-cholest-7,24-diene-3β,6α-diol (**2a**), and 24-methylene-5α-cholest-7-ene-3β,6α-diol (**3a**).

In recent years, polyhydroxylated sterols have been isolated from marine invertebrates, particularly from gorgonians and alcyonarians (1-3). Recently, a tetrahydroxylated sterol has been isolated from a sponge, *Dysidea sp.* (4), while steroids having sulfated hydroxyl groups have been isolated from two sponges of the family Halichondriidae (5,6).

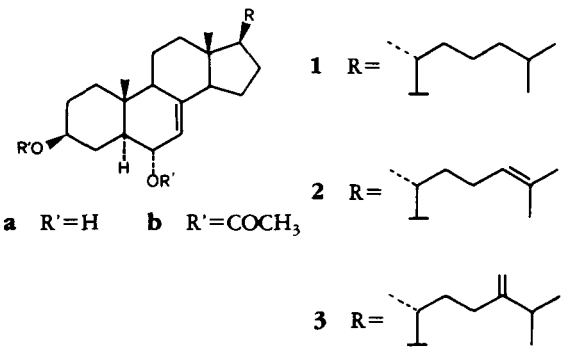
In the course of our studies on sterols from marine invertebrates we have examined *Spongionella gracilis* Vosmaer (order Dictyoceratida, family Dysioleidae), a sponge that contains $\Delta^{5,7}$-sterols in considerable amount (7). We report now that this organism also produces some new dihydroxylated sterols (**1a-3a**) which possess identical nuclei but differ in the side-chain composition.

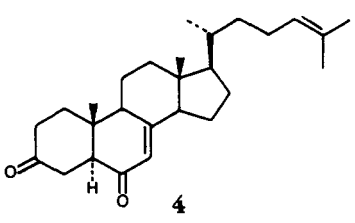
RESULTS AND DISCUSSION

Fresh tissue of *S. gracilis* was extracted with Me_2CO, the solvent was removed, and the resulting aqueous suspension was extracted with Et_2O. The crude extract was chromatographed on a silica gel column to give a fraction containing 5α-cholest-7-ene-3β,6α-diol (**1a**), 5α-cholest-7,24-diene-3β,6α-diol (**2a**), and 24-methylene-5α-cholest-7-ene-3β,6α-diol (**3a**). Pure samples of the three dihydroxylated sterols were obtained by hplc on a Partisil column using $MeOH/H_2O$ as eluent.

Compound **1a** had the molecular formula $C_{27}H_{46}O_2$, deduced by hrms. Its mass spectrum showed the molecular ion at *m/z* 402 and peaks due to fragment ions at *m/z* 384 (M^+-H_2O), 369 (M^+-H_2O and CH_3), and 351 (M^+-$2H_2O$ and CH_3) that suggested the presence of two hydroxyl groups. The presence of a conventional C_8H_{17} side chain and a nuclear double bond was indicated by the ion at *m/z* 271 (M^+-H_2O and C_8H_{17}).

Successive losses of 18 mass units from the molecular ions of **2a** and **3a** suggested the presence of two hydroxyl groups in these compounds, also. The presence of the *m/z* 271 (M^+-H_2O and side chain), 253 (M^+-$2H_2O$ and side chain), and 211 (M^+-$2H_2O$ and ring D fission) peaks in the mass spectra of all three sterols **1a-3a** suggested that





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they differed only in the side chain and located the nuclear double bond and the hydroxyl groups in rings A,B, and C.

Acetylation of **1a-3a** with Ac_2O and pyridine at room temperature yielded the corresponding diacetates **1b-3b**. The ^{1}H-nmr spectra of all the acetates exhibited two acetate methyl singlets, thus indicating the presence of two acetylatable hydroxyl groups.

The ^{1}H-nmr spectrum of **1a** contained an olefinic proton signal at δ 5.18 (1H, d, J=1.6 Hz, 7-H) and signals for five methyl groups of a cholestane structure at δ 0.54 (3H, s, 18-H_3), 0.85 (3H, s, 19-H_3), 0.859 (3H, d, J=6.5 Hz, 26-H_3 or 27-H_3), 0.860 (3H, d, J=6.5 Hz, 27-H_3 or 26-H_3), and 0.92 (3H, d, J=6.5 Hz, 21-H_3). The ^{1}H-nmr spectrum also showed signals at δ 3.60 (1H, m, 3α-H) and 3.81 (1H, br d, J=7.3 Hz, 6β-H), consistent with the presence of two secondary carbinol methines. A ^{13}C-nmr spectrum confirmed the presence of two carbons attached to oxygen (δ 69.8 and 71.1) and the presence of a carbon-carbon double bond (δ 122.2 and 141.3). The broad methine multiplet at δ 3.60 is typical of the 3α-proton of an A/B *trans* steroid (8), whereas the broad doublet at δ 3.81 showed coupling with the olefinic proton at δ 5.18. Comparison of the ^{1}H-nmr spectrum of **1a** with those of **2a** and **3a** showed almost identical chemical shift values for 3-H, 6-H, 7-H, 18-H_3, and 19-H_3, suggesting that they all possessed identical nuclei.

The presence of an allylic alcohol function in **1a-3a** was confirmed by Jones oxidation of the most abundant sterol, **2a**, to the corresponding α,β-unsaturated diketone **4** which displayed an intense uv absorption at λ max (MeOH) 243 nm (ε 12000) (9). The ^{1}H-nmr spectrum of **4** showed singlet signals at δ 0.64 and 1.08 that are in agreement with the calculated values for the C-18 and C-19 methyl groups (δ 0.675 and 1.078) of a Δ^7-cholestene-3,6-dione structure (10,11). These data and the small coupling constant for 6-H and 7-H in dihydroxylated sterols **1a-3a** are only compatible with the Δ^7-3β,6α-diol structure.

Compound **1a**, formulated as 5α-cholest-7-ene-3β,6α-diol, has not been found as a naturally occuring sterol but has been previously synthesized (12,13). An authentic specimen was identical in all respects with the naturally occuring sterol **1a**.

Compound **2a** had the molecular formula $C_{27}H_{44}O_2$. The mass spectrum contained the molecular ion at *m/z* 400 with fragmentation ion peaks at *m/z* 271 (M^+-H_2O and C_8H_{15}), 253, and 251, indicating the presence of a C_8H_{15} side chain containing one double bond. In the ^{1}H-nmr spectrum of **2a** the doublets at δ 0.859 and 0.860 due to the isopropyl group of **1a** disappeared, while signals at δ 1.60 (3H, br s, 26-H_3 or 27- H_3) and 1.68 (3H, br s, 27-H_3 or 26-H_3), corresponding to the presence of two vinylic methyl groups, were observed. In addition, an olefinic triplet signal at δ 5.09 (1H, br t, J=6.9 Hz, 24-H) broadened by long range coupling with 26-H_3 and 27-H_3 was present. These data suggested that the only difference between **1a** and **2a** was the presence of the Δ^{24} olefinic bond. The desmosterol type side chain for **2a** was confirmed by ^{13}C-nmr data (see Table 1). Consequently, the structure of **2a** was established as 5α-cholest-7,24-diene-3β,6α-diol.

The mass spectrum of compound **3a** contained the molecular ion at *m/z* 414 ($C_{28}H_{46}O_2$) and other fragments at *m/z* 271 (M^+-H_2O and C_9H_{17}), 253, and 251, indicating the presence of a C_9H_{17} unsaturated side chain. The ^{1}H-nmr spectrum included two signals at δ 4.66 (1H, br s, 28-H) and 4.72 (1H, br s, 28-H) and a proton signal at δ 2.24 (1H, septet, J=6.6 Hz, 25-H) which was coupled to the methyl doublet at δ 1.03 (6H, d, J=6.6 Hz, 26-H_3 and 27-H_3) indicating a methylene group attached at C-24, confirmed by its mass spectrum which exhibited the ion at *m/z* 330 derived from a McLafferty rearrangement in a $\Delta^{24(28)}$-unsaturated side chain. A detailed analysis of the ^{13}C-nmr spectrum of **3a** showed that the side chain of **3a** was of the 24-

TABLE 1. ^{13}C-nmr Data for Compounds **2a** and **3a**[a]

Compound	Carbon Atom													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2a($CDCl_3$)	37.3	31.2	70.9	33.9	49.1[b]	70.1	122.0	141.5	49.4[b]	35.4	21.5	39.4	43.7	54.9
3a($CDCl_3$)	37.3	31.3	70.9	34.0	49.1[c]	70.1	122.0	141.6	49.4[c]	35.4	21.5	39.5	43.8	54.9
	15	16	17	18	19	20	21	22	23	24	25	26	27	28
2a	22.9	27.9	56.2	11.9	13.9	35.5	18.8	36.1	24.8	125.4	130.3	17.6	25.7	
3a	22.9	27.9	56.2	11.9	13.9	36.1	18.9	34.8	31.3	156.8	36.1	21.9[d]	22.0[d]	106.1

[a]Side-chain carbon assignments are based on literature data (16,17) and nuclear carbon assignments are by analogy with **1a** (13). The assignments are confirmed by DEPT experiments and calculations (18).

[b-d]Assignments may be reversed.

methylenecholestane type. Thus, the structure of this dihydroxylated sterol is 24-methylene-5α-cholest-7-ene-3β,6α-diol (**3a**).

It seems probable that 3β,6α-dihydroxy-Δ^7-sterols **1a-3a** originate from the corresponding $\Delta^{5,7}$-sterols present in the sponge (14). These sterols have not previously been encountered among naturally occurring sterols.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded with a Perkin-Elmer Model 399 spectrophotometer. Uv spectra were taken on a Perkin-Elmer 550S spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were taken on a Bruker WM-250 spectrometer, 500-MHz ^{1}H-nmr spectra on a Bruker WM-500 spectrometer in $CDCl_3$ solution and TMS as internal reference. Low resolution mass spectra were recorded at 70 eV on an AEI 30 instrument. Hrms was obtained on an AEI MS 902 spectrometer. Reverse-phase hplc was carried out on a Waters instrument equipped with a differential refractometer. The column used was a Partisil M9 10/50 ODS-2 (9 mm i.d., 50 cm). Sponge identification was made by Prof. R. Pronzato, Instituto di Zoologia dell' Università di Genova.

ISOLATION OF **1a-3a** FROM S. GRACILIS.—Sponge *S. gracilis* was collected in the Bay of Naples and supplied by the Zoological Station of Naples. A voucher specimen is on file at our laboratories. Fresh sponge (25 g dry weight after extraction) was cut into small pieces and extracted three times with Me_2CO at room temperature for 3 days. Solvent was removed under reduced pressure and the aqueous residue was extracted with Et_2O. After evaporation, the oily residue (2.77 g) was chromatographed on a silica gel column (200 g) eluted with increasing amounts of MeOH in $CHCl_3$ (from 0 to 4%). The fractions eluted with $CHCl_3$-MeOH (96:4) yielded a mixture of dihydroxylated sterols homogeneous by tlc. The sterol mixture was fractionated by reverse phase hplc with a Partisil M9 10/50 ODS-2 column using MeOH-H_2O (95:5) as eluent to obtain **1a** (3 mg), **2a** (4 mg), and **3a** (3 mg).

Compound **1a**.—Ir ($CHCl_3$) 3420 cm^{-1}; ^{1}H nmr (250 MHz) δ 5.18 (1H, d, J=1.6 Hz, 7-H), 3.81 (1H, br d, J=7.3 Hz, 6β-H), 3.60 (1H, m, 3α-H), 0.860 (3H, d, J=6.5 Hz, 26-H_3 or 27-H_3), 0.859 (3H, d, J=6.5 Hz, 27-H_3 or 26-H_3), 0.92 (3H, d, J=6.5 Hz, 21-H_3), 0.85 (3H, s, 19-H_3), 0.54 (3H, s, 18-H_3); the ^{13}C-nmr data were in agreement with the literature (13);ms *m/z* (rel. int.) 402 (M^+, 36), 384 (M^+-H_2O, 46), 369 (M^+-H_2O and CH_3, 19), 351 (M^+-2H_2O and CH_3, 100), 325 (M^+-2H_2O and loss of C-1 to C-3, 31), 271 (M^+-side chain and H_2O, 69), 253 (M^+-side chain and 2H_2O, 41), 227 (30), 211 (M^+-H_2O and ring D fission, 44); the spectral properties of **1a** were identical to those of an authentic sample.

Compound **2a**.—Ir ($CHCl_3$) 3420 cm^{-1}; ^{1}H nmr (250 MHz) δ 5.18 (1H, d, J=1.6 Hz, 7-H), 5.09 (1H, br t, J=6.9 Hz, 24-H), 3.81 (1H, br d, J=7.3 Hz, 6β-H), 3.59 (1H, m, 3α-H), 1.68 (3H, br s, 26-H_3 or 27-H_3), 1.60 (3H, br s, 27-H_3 or 26-H_3), 0.94 (3H, d, J=6.6 Hz, 21-H_3), 0.84 (3H, s, 19-H_3), 0.54 (3H, s, 18-H_3); ^{13}C nmr see Table 1; ms *m/z* (rel. int.) 400 (M^+, 26), 382 (M^+-H_2O, 87), 367 (M^+-H_2O and CH_3, 22), 349 (M^+-2H_2O and CH_3, 100), 323 (M^+-2H_2O and loss of C-1 to C-3, 29), 271 (68), 253 (56), 251 (M^+-side chain-2H and H_2O, 26), 227 (51), 211 (61); hrms *m/z* 400.3330 ($C_{27}H_{44}O_2$ requires 400.3341).

Compound **3a**.—Ir ($CHCl_3$) 3420, 1640, 890 cm^{-1}; ^{1}H nmr (250 MHz) δ 5.19 (1H, d, J=1.6 Hz, 7-H), 4.72 (1H, br s, 28-H), 4.66 (1H, br s, 28-H), 3.82 (1H, br d, J=7.3 Hz, 6β-H), 3.60 (1H, m, 3α-H), 2.24 (1H, septet, J=6.6 Hz, 25-H), 1.03 (6H, d, J=6.6 Hz, 26-H_3 and 27-H_3), 0.96 (3H, d, J=6.5 Hz, 21-H_3), 0.85 (3H, s, 19-H_3), 0.55 (3H, s, 18-H_3); ^{13}C nmr see Table 1; ms *m/z* (rel. int.) 414 (M^+, 29), 396 (M^+-H_2O, 57), 381 (M^+-H_2O and CH_3, 17), 363 (M^+-2H_2O and CH_3, 100), 337 (M^+-2H_2O and loss of C-1 to C-3, 31), 330 (M^+-C_6H_{12}, 8), 271 (73), 253 (52), 251 (33), 227 (48), 211 (72); hrms *m/z* 414.3480 ($C_{28}H_{46}O_2$ requires 414. 3498).

ACETYLATION OF **1a-3a**.—The dihydroxylated sterol (3 mg) was acetylated with Ac_2O (0.4 ml) in pyridine (0.8 ml) at room temperature overnight. After addition of MeOH and solvent removal, the product was purified by hplc on LiChrosorb Si 60 eluting with *n*-hexane-Et_2O (8:2).

Compound **1b**.—^{1}H nmr (500 MHz) δ 5.08 (1H, d, J=8.0 Hz, 6β-H), 5.07 (1H, br s, 7-H), 4.69 (1H, m, 3α-H), 2.07 (3H, s, acetate), 2.04 (3H, s, acetate), 0.93 (3H, s, 19-H_3), 0.92 (3H, d, J=6.5 Hz, 21-H_3), 0.882 (3H, d, J=6.5 Hz, 26-H_3 or 27-H_3), 0.878 (3H, d, J=6.5 Hz, 27-H_3 or 26-H_3), 0.55 (3H, s, 18-H_3); ms *m/z* (rel. int.) 426 (M^+-AcOH, 13), 411 (M^+-AcOH and CH_3, 10), 384 (M^+-AcOH and CH_2CO, 4), 366 (M^+-2AcOH, 71), 351 (M^+-2AcOH and CH_3, 76), 325 (M^+-2AcOH and loss of C-1 to C-3, 9), 313 (M^+-side chain and AcOH, 34), 253 (M^+-side chain and 2AcOH, 100), 227 (38), 211 (M^+-side chain-2AcOH and ring D fission, 55).

Compound **2b**.—^{1}H nmr (500 MHz) δ 5.09 (1H, br t, J=6.9 Hz, 24-H), 5.08 (1H, d, J=8.0 Hz, 6β-H), 5.07 (1H, br s, 7-H), 4.69 (1H, m, 3α-H), 2.07 (3H, s, acetate), 2.04 (3H, s, acetate), 1.69 (3H, br s, 26-H_3 or 27-H_3), 1.61 (3H, br s, 27-H_3 or 26-H_3), 0.95 (3H, d, J=6.3 Hz, 21-H_3), 0.93 (3H, s, 19-H_3), 0.55 (3H, s, 18-H_3); ms *m/z* (rel. int.) 424 (M^+-AcOH, 18), 409 (M^+-AcOH and CH_3, 10), 382 (M^+-AcOH and CH_2CO, 6), 364 (M^+-2AcOH, 96), 349 (M^+-2AcOH and CH_3, 79), 323 (M^+-2AcOH and loss of C-1 to C-3, 10), 313 (33), 311 (M^+-side chain-2H and AcOH, 21), 253 (100), 251 (M^+-side chain-2H and 2AcOH, 50), 227 (29), 211 (43).

Compound **3b**.—^{1}H nmr (500 MHz) δ 5.08 (1H, d, J=8.0 Hz, 6β-H), 5.07 (1H, br s, 7-H), 4.72 (1H, br s, 28-H), 4.69 (1H, m, 3α-H), 4.66 (1H, br s, 28-H), 2.23 (1H, septet, J=7.0 Hz, 25-H), 2.07 (3H, s, acetate), 2.03 (3H, s, acetate), 1.037 (3H, d, J=7.0 Hz, 26-H_3 or 27-H_3), 1.032 (3H, d, J=7.0 Hz, 27-H_3 or 26-H_3), 0.96 (3H, d, J=7.0 Hz, 21-H_3), 0.93 (3H, s, 19-H_3), 0.55 (3H, s, 18-H_3); ms *m/z* (rel. int.) 438 (M^+-AcOH, 16), 423 (M^+-AcOH and CH_3, 9), 396 (M^+-AcOH and CH_2CO, 5), 378 (M^+-2AcOH, 80), 363 (M^+-2AcOH and CH_3, 72), 337 (M^+-2AcOH and loss of C-1 to C-3, 10), 313 (36), 311 (19), 253 (100), 251 (46), 227 (30), 211 (48).

JONES OXIDATION OF **2a**.—A solution of **2a** (2 mg) in 1 ml of Me_2CO was treated with Jones reagent (15) by dropwise addition until an orange color persisted. A few drops of MeOH were added to destroy excess reagent, and the mixture was partitioned between Et_2O and H_2O. The Et_2O extract was washed with H_2O, saturated with $NaHCO_3$, and dried. Evaporation gave the crude diketone **4** which was purified by hplc on LiChrosorb Si 60 with *n*-hexane-Et_2O (6:4) as mobile phase, to give pure **4** (1 mg); uv λ max (MeOH) 243 nm (ε 12000); ir ($CHCl_3$) 1710, 1665, 1615 cm^{-1}; ^{1}H nmr (500 MHz) δ 5.78 (1H, s, 7-H), 5.08 (1H, br t, J=6.9 Hz, 24-H), 1.69 (3H, br s, 26-H_3 or 27-H_3), 1.61 (3H, br s, 27-H_3 or 26-H_3), 1.08 (3H, s, 19-H_3), 0.97 (3H, d, J=6.2 Hz, 21-H_3), 0.64 (3H, s, 18-H_3); ms *m/z* (rel. int.) 396 (M^+, 95), 381 (M^+-CH_3, 38), 312 (M^+-C-22 to C-27 and H, 60), 285 (M^+-side chain, 35), 283 (M^+-side chain and 2H, 66), 259 (100), 245 (32).

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