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NEW POLYHYDROXYLATED STEROLS FROM THE STARFISH *LUIDIA MACULATA*[1,2]

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ABSTRACT.—Three new polyhydroxylated sterols, 5α-cholestane-3β,5,6β,15α,16β,26-hexol (**1**), 5α-cholestane-3β,6β,7α,15α,16β,26-hexol (**2**), and 5α-cholestane-3β,5,6β,7α,15α,16β,26-heptol (**3**), have been isolated from the Pacific starfish *Luidia maculata*.

The number of known polyhydroxylated sterols from marine animals is steadily growing. They have been isolated from soft corals (1-9), gorgonians (10), nudibranchs (11,12), and starfishes (13-15). Recently, we have described the occurrence of a group of highly hydroxylated sterols with moderate cytotoxicity from the starfish *Protoreaster nodosus* (13) and *Hacelia attenuata* (14,15). All of these compounds, except for one from *H. attenuata* (14), contain one hydroxyl group at the uncommon C-8 position. Another feature of these compounds is 3β,6α(or β),15α,16β,26-pentahydroxy substitution. We now wish to report the isolation of three more polyhydroxylated sterols, 5α-cholestane-3β,5,6β,15α,16β,26-hexol (**1**), 5α-cholestane-3β,6β,7α,15α,16β,26-hexol (**2**), and 5α-cholestane-3β,5,6β,7α,15α,16β,26-heptol (**3**), which lack the 8-hydroxyl function, from the Pacific starfish *Luidia maculata* M. Tr.

EXPERIMENTAL

EXTRACTION AND ISOLATION OF POLYHYDROXYLATED STEROLS.—The animals (*L. maculata*, 5 kg fresh animals), collected in August 1982, off Nouméa, were identified by P. Tirard, Centre ORSTOM de Nouméa, and chopped and extracted (3 h) with H_2O at room temperature, and the extracts were lyophilized to give 465 g of material. The lyophilized extract (220 g) was dissolved in H_2O (1 liter), clarified by centrifugation, and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with H_2O (2 bed volumes) and then MeOH. The MeOH eluates were dried on a rotary evaporator to give 4.03 g of a glassy material that was then chromatographed on a column of Sephadex LH-60 (4×60 cm) using MeOH-H_2O (2:1) as the eluant. Fractions of 10 ml each were collected and analyzed by tlc on SiO_2 in *n*-BuOH-HOAc-H_2O (60:15:25).

Fractions 41-83 contained the asterosaponins (1.28 g). Fractions 84-122, containing the mixture of polyhydroxylated steroids (1.43 g), were chromatographed on a Sephadex LH-20 column (80 g; 2.5×80 cm; 10 ml fractions were collected) with MeOH. Fraction 23, containing **1**, was subjected to preparative hplc on a C-18 μ-bondapack column with MeOH-H_2O (65:35) as the eluent to give a pure sample of **1**.

Fractions 24-25 and 26-29 were still mixtures: fractions 24-25 contained **1** and **2**, fractions 26-29 contained **2** and **3**. Unresolved fractions were separated by preparative hplc. The total yield of each compound was 20 mg (**1**), 70 mg (**2**), and 37 mg (**3**).

Compound **1**: This compound did not crystallize; $[\alpha]_D$ +12.1° (c, 0.5 MeOH); pmr and cmr are reported in Tables 1 and 2, respectively; eims (100°), *m/z* (%) 468 (M^+, <1), 450 (50), 435 (10), 432 (60), 417 (10), 414 (100), 399 (20), 396 (50), 381 (10), 378 (5), 349 (15), 322 (50), 321 (45), 303 (25), 285 (50), 267 (25).

Compound **2**: Compound **2** was crystallized from MeOH, mp 238-241°; $[\alpha]_D$ +3.8° (c, 0.5 MeOH);

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[2]Written with the technical collaboration of M. Pusset, Laboratoire des Plantes Médicinales, CNRS, Nouméa, New Caledonia.

pmr and cmr are reported in Tables 1 and 2, respectively; eims '(100°), *m/z* (%) 450 (M^+ -H_2O, 50), 432 (60), 414 (100), 399 (20), 396 (5), 349 (25), 338 (20), 321 (30), 320 (20), 303 (50), 285 (70), 261 (90).

Compound **3**: This compound was crystallized from MeOH, mp 243-246°; $[\alpha]_D$ −4.7° (c, 0.5 MeOH); pmr and cmr are reported in Tables 1 and 2, respectively; eims (170°), *m/z* (%) 484 (M^+, <1), 466 (10), 448 (100), 430 (60), 412 (100), 301 (100), 283 (100).

TABLE 1. 250 MHz Pmr Data in CD_3OD δ (Hz)

Compound	CH_3-18[a]	CH_3-19[a]	CH_3-21	CH_3-27	H-3
1	0.94	1.21	0.98 (d 7)	0.94 (d 7)	4.04[b] (m)
2	0.96	1.04	0.99 (d 7)	0.94 (d 7)	3.60[d] (m)
3	0.96	1.17	0.99 (d 7)	0.94 (d 7)	4.05[f] (m)

Compound	H-6	H-7	H-15	H-16	H_2-26
1	3.50 (br s)	—	3.76 (dd 10.5, 3)	4.00 (dd 7.5, 3)	3.45[c] (dd 11, 5.5)
2	3.62[d] (t 3.5)	3.91[e] (t 3.5	3.89[e] (dd 10.5, 3)	4.04 (dd 7.5, 3)	3.46[c] (dd 11, 5.5)
3	3.55 (d 3)	4.02[f] (t 3.0)	3.88 (dd 10.5, 3)	4.04[f] (dd, 7.5, 3)	3.46[c] (dd 11, 5.5)

[a]Calculated according to Zürcher (18) and Bridgemon *et al.* (19): **1**, CH_3-18: 0.973, CH_3-19: 1.240; **2**, CH_3-18: 0.973, CH_3-19: 1.059; **3**, CH_3-18: 0.983, CH_3-19: 1.230.

[b]Partially overlapped with H-16.

[c]The upfield part of the AB portion of the >CH-CH_2OH ABX system is under the MeOH signal.

[d]H-3 and H-6 partially overlap.

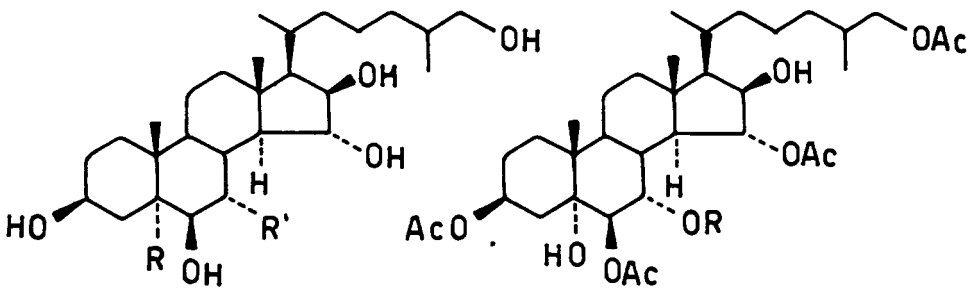
[e]H-7 and H-15 partially overlap.

[f]H-3, H-7 and H-16 partially overlap.

Acetylation of **3** *giving* **3a** *and* **3b**.—A mixture of 5α-cholestane-3β,5,6β,7α,15α,16β,26-heptol (**3**) (5 mg) and an excess of Ac_2O in 0.5 ml of dry pyridine was kept at room temperature overnight. After removal of the excess reagents in vacuo, the residue was purified by chromatography on a silica gel column with $CHCl_3$ (0.5 ml fractions were collected).

Fractions 16-22 contained the pentaacetate **3b** (1 mg), eims, *m/z* (%) 652 (M^+ -CH_2=C=O, <1), 634 (M^+ -AcOH, <1), 616 (<1), 574 (30), 514 (100), 496 (50), 454 (100), 436 (80); pmr, δ ($CDCl_3$) 0.945 (d, *J*=7 Hz, CH_3-27), 0.975 (d, *J*=7 Hz, CH_3-21), 1.03 (s, CH_3-18), 1.19 (s, CH_3-19), 2.01, 2.02, 2.06 and 2.14 (five s, 15 H, CH_3-C=O), 3.83 (dd, *J*=10.5 and 7 Hz, 26-H), 3.92 (dd, *J*=8.5 and 3 Hz, 16α-H), 3.96 (dd, *J*=10.5 and 5.5 Hz, 26-H), 4.47 (dd, *J*=10.5 and 3 Hz, 15β-H), 4.65 (t, *J*=3 Hz, 7β-H), 4.97 (d, *J*=3 Hz, 6α-H), 5.21 (7-lines m, W½=20 Hz, 3α-H).

Fractions 31-61 contained the tetraacetate **3a** (2 mg), eims, *m/z* (%) 634 (M^+-H_2O, <1), 616 (<1), 592 (M^+ -AcOH, <1), 574 (15), 556 (10), 532 (10), 514 (50), 496 (30), 454 (90), 436 (100); pmr, δ



1 R=OH, R'=H
2 R=H, R'=OH
3 R=R'=OH
4 R=R'=H

3a R=H
3b R=Ac

TABLE 2. Cmr Chemical Shifts of Polyhydroxysterols[a]

Carbon	5α-cholestane-3β,5,6β-triol (17)	1	4	2	3
1	32.5	31.7	39.4	39.9	31.7
2	33.4	33.5	32.2	32.3	33.6
3	67.6	68.4	72.5	72.5	67.7
4	42.1	41.6	36.4	35.9	41.5
5	76.0	76.7	49.0	42.9	78.2
6	76.3	76.6	72.5	76.4	77.3
7	35.8	35.4	40.6[b]	73.1	74.8
8	31.3	31.2	31.2	35.7	36.1
9	46.0	46.7	55.8	49.0[b]	41.3
10	39.2	39.5	36.6	36.6	39.9
11	22.0	22.0	21.9	22.0	22.0
12	40.8	42.1	41.9[b]	42.0	42.1
13		44.9	44.7	44.8	44.8
14		61.2	61.1	56.8	56.7
15		85.0	85.0	84.2	84.2
16		83.2	82.9	82.6	82.6
17		60.1	59.9	60.9	60.9
18		15.2	15.0	14.9	15.0
19		17.2	16.3	16.0	17.8
20		31.0	30.9	31.0	31.0
21		18.6	18.6	18.5	18.5
22		37.5	37.4	37.4	37.4
23		24.8	24.8	24.8	24.8
24		35.0	34.9	35.0	35.0
25		37.0	37.0	37.0	37.0
26		68.6	68.4	68.6	68.6
27		17.3	17.3	17.3	17.3

[a]The δ values are in parts per million in CD_3OD solution except 5α-cholestane-3β,5,6β-triol, which is in d_5-pyridine.

[b]In the original paper (14) the assignments were reversed; the comparison of the chemical shifts with those of structurally related compounds **1, 2, 3** assign now the 40.6 ppm signal to C-7 and the 41.9 ppm signal to C-12, which must be sensitive to 15α-OH, 16β-OH substituents.

($CDCl_3$) 0.945 (d, J=7 Hz, CH_3-27), 0.970 (d, J=7 Hz, CH_3-21), 1.03 (s, CH_3-18), 1.15 (s, CH_3-19), 2.02, 2.06, 2.15 (four s, 12 H, CH_3-C=O), 3.72 (t, J=3 Hz, 7β-H), 3.83 (dd, J=10.5 and 7 Hz, 26-H), 3.96 (dd, J=10.5 and 5.5 Hz, 26-H), 3.98 (dd, J=8.5 and 3 Hz, 16α-H), 4.53 (dd, J=10.5 and 3 Hz, 15β-H), 4.90 (d, 3 Hz, 6α-H), 5.27 (7-lines m, W½=20 Hz, 3α-H).

INSTRUMENTAL.—Pmr and cmr spectra were recorded on a Bruker WM-250 instrument. Ei mass spectra were obtained from an AEI MS-30 mass spectometer (70 eV). Hplc separation was made on a μ-Bondapak C-18 column (7.8 mm×30 cm) using a differential refractometer detector, model 401, a U6K injector and a solvent delivering system, M6000A, all from Waters Associates.

RESULTS AND DISCUSSION

5α-CHOLESTANE-3β,5,6β,15α,16β,26-HEXOL (**1**).—The eims showed a small molecular ion at *m/z* 468 corresponding to molecular formula $C_{27}H_{48}O_6$. The fragmentation pattern showed ions for stepwise H_2O losses (*m/z* 450, 432, 414, 396, 378) and ions corresponding to the loss of an hydroxylated C_8 side-chain together with the loss of H_2O (*m/z* 321, 303 and 285). Cmr showed the absence of carbon-carbon double bonds. A saturated sterol with six hydroxyl groups (one primary, four secondary, and one tertiary, cmr) was thus a plausible candidate for a structure assignment. The pmr spectrum (Table 1) contained several features, namely two doublets of doublets at δ 3.76 (J=10.5 and 3 Hz) and 4.00 (J=7.5 and 3 Hz) and the A portion of an ABX system at δ

3.45 (J_{AB}=11 Hz; J_{AX}=5.5 Hz; the B-portion resonated under the MeOH signal), already observed in the spectrum of 5α-cholestane-3β,6β,15α,16β,26-pentol (**4**) isolated from *Hacelia attenuata* (14), and assigned to 15β-H, 16α-H, and 26-H, respectively.

In the spectra of the many starfish-derived polyhydroxy sterols containing the 8, 15α, 16β-trihydroxy moiety (13,15), the double doublet assigned to 15β-H is shifted downfield by 0.2 ppm relative to **1**. In agreement with the presence of a 26-hydroxycholestane side-chain, the pmr spectrum contained only two three-proton methyl doublets at δ 0.94 and 0.98. A multiplet centered around 4.04 ppm had the complexity normally seen for a 3β-hydroxyl group and its downfield position, ~0.45 ppm shifted relative to 5α-cholestan-3β-ol, along with the broad singlet at δ 3.50 ppm characteristic of an equatorial proton, leads us to postulate the presence of a 3β,5α,6β-trihydroxy moiety, a common element in marine polyhydroxy sterols (1-9), which is also encountered in the starfish-derived steroidal glycoside nodososide (16). The cmr chemical shifts for 5α-cholestane-3β,5,6β-triol have been published (17). Taking this information and using 5α-cholestane-3β.6β,15α,16β,26-pentol (**4** (14) as a model structure, the cmr chemical shifts of compound **1**, reported in Table 2, were easily assigned and found to be fully consistent with the proposed 5α-cholestane-3β,5,6β,15α,16β,26-hexol (**1**) structure.

5α-CHOLESTANE-3β,6β,7α,15α,16β,26-hexol (**2**).—The second polyhydroxysterol is isomeric with **1**. In the eims the highest molecular weight observed (*m/z* 450) corresponded to loss of H_2O from the molecular formula $C_{27}H_{48}O_6$. The fragmentation pattern, with ions for stepwise H_2O losses (*m/z* 432, 414, and 396) and ions corresponding to the loss of an hydroxylated C_8 side-chain together with one, two, three, and four molecules of H_2O (*m/z* 321, 303, 285, and 267) closely resembled that observed in the spectrum of the hexol **1**.

Cmr spectrum revealed that there were six carbons bonded to oxygen (Table 2). The pmr spectrum showed two doublets of doublets at δ 3.89 (J=10 and 3 Hz) and 4.04 (J=7.5 and 3 Hz) coupled each to the other by 3 Hz, and one double doublet at δ 3.46 (J=11 and 5.5 Hz). These signals were very similar to the signals that we had previously seen for **1** and for other starfish-derived polyhydroxy sterols (13-15) corresponding to 15β-H, 16α-H, and 26-H. A multiplet with the complexity normally seen for 3β-hydroxyl group was also present at the "normal" value of 3.60 ppm. Two triplets at δ 3.91 (J=3 Hz) and 3.62 (J=3 Hz) completed the downfield region of the spectrum. Irradiation of the triplet at δ 3.91 simplified the triplet at δ 3.62 into a doublet and also transformed a double triplet (J=3 and 10.5 Hz) at δ 1.99 into a sharp triplet (J=10.5 Hz).

This nmr data indicates a structural element with vicinal, secondary hydroxy groups (axial) both adjacent to a carbon bearing one proton. There is only one way such a fragment can be put into a steroid skeleton, i.e., the 6β,7α-dihydroxy moiety. The double triplet at δ 1.99 must be due to 8-H, and consequently, the signals at δ 3.91 and 3.62 are assignable to 7β-H and 6α-H, respectively. The chemical shifts of the methyl singlets at δ 0.96 and δ 1.04, due to CH_3-18 and CH_3-19 protons, respectively, are in agreement with the 5α-cholestane-3β,6β,7α,15α,16β,26-hexol (**2**) formulation for this sterol (see Table 1). A comparison of the cmr spectrum of **2** with that of 5α-cholestane-3β,6β,15α,16β,26-pentol (**4**) (14) definitively confirmed that the novel sterol was related to **4** by the introduction of the sixth hydroxyl group at the 7α-position. Using **4** as the parent structure, the cmr spectrum was calculated for the compound with an additional 7α-hydroxy group, using the substituent effects that have been published for hydroxysteroids (17,20) and the deviations from additivity published for vicinal dihydroxy steroids (21). The calculated and experimental spectra were

significantly similar (calculated values in parentheses): C-4: 35.9 (35.9), C-5: 42.9 (42.5), C-6: 76.4 (76.5), C-7: 73.1 (72.9), C-8: 35.7 (35.3), C-9: *ca.* 49 (47.3), C-14: 56.8 (55.2), C-15: 84.2 (84.5). The remaining signals were within 0.2 ppm of those published for **4** except for C-17 (60.9 vs. 59.9 for **4**). The slight differences between the predicted and experimental values for C-14 and C-17 could be due to the interaction between the 7α-OH and 15α-OH groups and the greater flexibility in the 5-membered ring system.

5α-CHOLESTANE-3β,5,6β,7α,15α,16β,26-HEPTOL (**3**).—The eims (temperature, 170°) showed a small molecular ion at *m/z* 484 corresponding to a fully saturated cholestane-heptol and fragment ions for stepwise H_2O losses *m/z* 448, 430, and 412. The pmr spectrum (Table 1) contained several features already observed in the spectrum of **1**, namely two doublets of doublets at 3.88 (J=10.5 and 3 Hz) and 4.04 (J=7.5 and 3 Hz), the latter superimposed on the signals for the 3α- and 7β-protons with the same chemical shift, and the A portion of an ABX system at δ 3.46 (J_{AB}=11 Hz, J_{AX}=5.5 Hz; the B-portion is under the MeOH signal), assigned to 15β-H, 16α-H, and 26-H, respectively. The chemical shifts of the methyl signals, δ 0.94 d, 0.96 s, 0.99 d, and 1.17 s, are close to the values for CH_3-27, CH_3-18, CH_3-21, and CH_3-19, respectively, in the hexol **1**. In the pmr spectrum of the heptol **3** one hydroxymethine signal was observed as an isolated doublet (J=2.5 Hz) at δ 3.85. Irradiation of the overlapping signal around 4.00 ppm in a double resonance experiment collapsed the doublet at δ 3.85 into a singlet. This nmr data indicates a structural element with vicinal, secondary hydroxy groups, one adjacent to a carbon without protons. The 3β,5α,6β,7α-tetrahydroxy moiety seems the most likely structural element, which accounts for this nmr data, and 3β,5α,6β,7α,15α16β,26-heptol should be regarded as the most likely structure for **3**. Treatment of **3** with excess Ac_2O in pyridine at room temperature gave a tetraacetate **3a** (M^+-H_2O, *m/z* 634) showing four acetate methyl singlets in its pmr spectrum at δ 2.02, 2.06, 2.11, and 2.15, and a pentaacetate **3b** (M^+-CH_2=C=O, *m/z* 652; M^+-AcOH, *m/z* 634) showing five methyl singlets in its pmr spectrum, at δ 2.01, 2.02, 2.06, and 2.14 (x 2). Both derivatives provided apparent first-order spectra in the downfield region, with seven resolved one-proton bands. In the pmr spectrum of **3b**, the resonances associated with 3α-H, 15β-H, and 26-H_2 had moved to δ 5.21 (m, W½=20 Hz), 4.53 (dd, J=10.5 and 3 Hz) and 3.96 (dd, J=10.5 and 5.5 Hz) - 3.83 (dd, J=10.5 and 7 Hz), respectively. Two isolated acetoxymethine signals resonating at δ 4.97 (d, J=3 Hz) and 4.65 (t, J=3 Hz) coupled each to the other could be assigned to 6α-H and 7β-H, respectively. The 16α-H signal remained essentially unchanged at δ 3.92 relative to the parent heptol. In the spectrum of the tetraacetate **3a** also the triplet (J=3 Hz) due to 7β-H, remained essentially unshifted relative to the parent heptol. The comparison of the cmr spectrum of **3** with that of **1** and with that of **2** fully confirms that the new sterol was related to **1** by the introduction of the seventh hydroxy group at the 7α-position as well as related to **2** by the introduction of the seventh hydroxyl group at the 5α-position.

As the substituent parameters predict (20) the cmr chemical shifts of C-9 and C-14 (γ-carbons) in the spectrum of the heptol **3** are shifted upfield by 5.4 and 4.5 ppm, respectively, and the frequency of C-8 (β-carbon) is shifted downfield by 4.9 ppm relative to hexol **1**.

It is difficult to predict the chemical shifts of C-5 and C-6 for the compound **1** with an additional 7α-hydroxy group. The fact that the frequencies of C-5 and C-6 in **3** are slightly downfield relative to the model compound **1** is consistent with the deviations from additivity at the hydroxy-bearing carbons found in 1,3-syn-diaxial and 1,2-trans-diaxial dihydroxy steroids (21). Likewise, the frequencies of C-1, C-3, and C-9 (γ-carbons) in the spectrum of the heptol **3** are upfield shifted by 8.2, 4.2, and ~ 8.0 ppm,

respectively, and those of C-4 and C-10 (β-carbons) are downfield shifted by 5.6 and 3.3 ppm, respectively, relative to the hexol **2**. The chemical shifts of carbons 11 to carbons 27 in **3** are within 0.1 ppm with those of the hexol **2** (Table 2).

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