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A NOVEL GROUP OF HIGHLY HYDROXYLATED STEROIDS FROM THE STARFISH *PROTOREASTER NODOSUS*†

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Abstract—Three novel polyhydroxylated sterols, 5α-cholestane-3β,6α,8,15α,16β,26-hexol, 5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol and 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol, have been isolated from the Pacific Starfish *Protoreaster nodosus* and their structures have been established through extensive ^{1}H NMR and ^{13}C NMR studies, chemical transformations and related spectroscopic data.

A part of the specialized class of ecdysones produced by Crustaceans,[1] only a few polyhydroxylated steroids have been reported from marine sources and they have been isolated from Alcyonarians[2] and Asteroids.[3] Indeed, all the polyhydroxysterols found in Asteroids (starfish) have been aglycone constituents of saponins except one, 5α-cholesta-3β,6β,15α,16β,26-pentol **1**, which we have recently isolated from Mediterranean starfish *Hacelia attenuata*.[4] We now report the isolation and characterization of three more polyhydroxylated sterols, 5α-cholestane-3β,6α,8,15α,16β,26-hexol **2**, 5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol **3** and 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol **4**, from the Pacific starfish *Protoreaster nodosus*, collected at Nouméa, Nouvelle Caledonie.

The materials were obtained in 0.0035%, 0.002% and 0.0044% yield (dry weight basis), respectively, from the methanol extract of the lyophilized "starfish".

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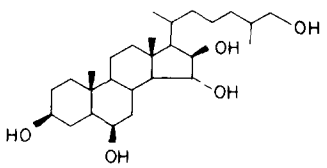

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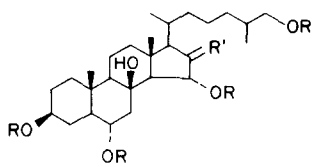

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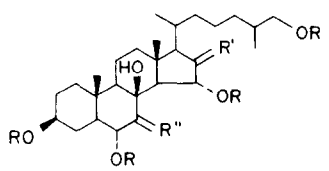
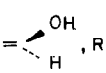
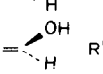
2a, R = Ac, R' =

2b, R = Ac, R' = O

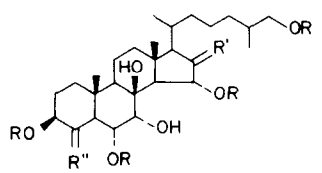


3, R = H, R' =  , R'' = 

3a, R = Ac, R' =  , R'' = 

3b, R = Ac, R' = O, R'' = 

3c, R = Ac, R' = R'' = O



4, R = H, R' = R'' =

4a, R = Ac, R' = R'' =

4b, R = Ac, R' = R'' = O

5α - *cholestane* - 3β,6α,8,15α,16β,26 - *hexol* **2**, *m.p.* 285°–287°; $[\alpha]_D$ + 13.8°

In the electron impact mass spectrum the highest molecular weight ion observed (*m/e* 450) corresponded to loss of water from the molecular formula $C_{27}H_{48}O_6$, which was determined by elemental analysis. Intense peaks at *m/e* 432, 414 and 396 for stepwise water loss and peaks at *m/e* 321, 303, 385 and 267 corresponding to the loss of a hydroxylated C_8 side chain with one, two, three and four molecules of water were also observed. Treatment with excess acetic anhydride in pyridine at room temperature produced a tetraacetate **2a** showing four acetate methyl singlets in the ^{1}H NMR. The protons α to the acetoxy groups were centered at δ 4.92 (1H), 4.69 (2H), a *ca* 1 ppm shift, and 3.82–3.95 (2H), a *ca* 0.5 ppm shift, consistent with three secondary and one primary acetates.[5] Oxidation with Jones reagent of the tetraacetate **2a** produced a monoketone **2b** (M^+/e: 634), which still contained one hydroxyl functionality (several fragmentation in the mass spectrum arising from the loss of a H_2O unit). The tertiary nature of the sixth hydroxyl group was readily apparent from the ^{13}C NMR off resonance singlet observed at 75.9 ppm in the spectrum of **2** and at 74.8 ppm in the spectrum of **2a**. These data established the presence of one tertiary, four secondary and one primary hydroxyl groups in **2**.

The ^{1}H NMR spectrum of the hexol **2** contained several features, two doublets of doublets at δ 4.04 (J = 11.5 and 2.5 Hz) and 3.98 (J = 8.5 and 2.5 Hz) and the A portion of an ABX system at δ 3.42 (J_{AB} = 10.5 Hz; J_{AX} = 6 Hz; the B portion resonated under the methanol signal), already observed in the spectrum of the pentol **1**, and assigned to 15β-H, 16α-H and 26-H, respectively. In agreement with the presence of an 26-hydroxycholestane side chain, the ^{1}H NMR spectrum contained only two three-protons methyl doublets at δ 0.91 and 0.93, one of which is shifted to δ 0.99 (21-H) in the spectrum of the 16-ketosteroid **2b**. Notably, in the spectrum of **2** the double doublet assigned to 15β-H is downfield shifted of 0.2 ppm relative to that of **1** and this was suggestive for the location of the new tertiary hydroxyl group at C-8. In agreement with this assignment are the ^{13}C NMR frequencies at δ 64.5 and 80.2 ppm, assigned to C-14 and C-15, respectively, when compared with the resonances of the corresponding carbon atoms in **1** (Table 1). As expected, the introduction of the axial hydroxyl group at C-8 produced a downfield shift (+3.4 ppm) of C-14 (β-carbon) and an upfield shift (−4.3 ppm) of C-15 (γ-carbon). The large upfield γ-gauche substituent effect experienced by C-15 requires the presence on it of an hydrogen atom able to interact with the 8β-hydroxyl,[7] i.e. 15β-H, thus supporting the 15α-OH stereochemistry in **2**. The remaining two secondary hydroxyl groups were located at 3β- and 6α-positions; in the ^{1}H NMR spectrum the 7-lines multiplet ($W_{\frac{1}{2}}$ = 20 Hz) at δ 3.56 is typical of 3α-proton of an A/B *trans*-3β-hydroxy steroid[6] and the double triplet (J = 3 and 10 Hz) at δ 3.63 is characteristic of the axial proton associated with the 6α-hydroxyl group.[6] The ^{1}H NMR spectrum also contained one-proton double doublet (J = 12 and 3 Hz) at δ 2.40. Decoupling proved that the 3 Hz coupling is due to interaction with the 6β-proton thus allowing the assignment of this peak to the equatorial proton at C-7. Again, the tertiary hydroxyl group should be placed at C-8. Further, using 5α-cholestane-3β,6α-diol as model compound the ^{13}C NMR signals for the carbon atoms in rings A and B as well as for the carbons 11 and 12 in **2** well corresponded to those expected upon introduction of an axial hydroxyl at C-8.[8,9] C-7 and C-9 (β-carbons) are downfield shifted 7.4 and 3.7 ppm, respectively, while C-6 and C-11 (γ-carbons) are upfield shifted 2.9 and 3.2 ppm, respectively. In 5α-steroids the introduction of an axial hydroxyl deshields the β-carbons by values ranging from 8.8 to 5.2 ppm for methylene carbons and ranging from 4.1 to 2.5 ppm for methine carbons, while shielding the γ-carbons by 6.5 (average value) for methylene carbons and 7.8 (average value) ppm for methine carbons except in compounds where the hydroxyl is 1,3-syn-diaxial to a methyl group, in which the γ-gauche hydroxyl effect is decreased to *ca* 4.5 ppm for both methylene and methine γ-carbons.[7] In compound **2**, where the 8β-hydroxyl group suffers from two 1,3-syn-diaxial interactions, the γ-gauche effect is further reduced to 3.2 and 2.9 ppm, probably because of the more severe deformations taking place to relieve the 1,3-syn-diaxial interaction. These deformations probably also accounts for the absence of the deshielding δ effect expected for C-19 and associated with the 1,3-diaxial OH–CH_3 interaction. As expected the resonance of the γ-gauche quaternary C-10 is essentially unshifted, and the resonances of the remaining carbon atoms 1–5 and 12, four or more bonds removed from the 8β-hydroxyl group, are only slightly shifted (less than 1.0 ppm) relative to the model 5α-cholestane-3β,6α-diol, except C-12 which has moved from 40.9 to 42.6 ppm. A similar large downfield δ effect for C-12 was also observed in compounds **3** and **4** and we think that this effect is caused by the 8β-hydroxyl group. The comparison of the spectrum of the pentol **1** with that of the model 5α-cholestane-3β, 6β-diol has shown that the 15α-, 16β-hydroxyls functionality has no effect at C-12.[4]

The spectral data of the derived ketone **2b** provided further corroborative evidence supporting the 5α-cholestane-3β,6α,8,15α,16β,26-hexol formulation for this novel steroid. The electron impact mass spectrum displayed two ions at *m/e* 464(a) and 449 (*b*, base peak), corresponding to the diagnostically most important 16-ketosteroid fragmentations, namely side chain loss with migration of one hydrogen and 18-methyl fission[10] (Fig. 1).

The ^{1}H NMR spectrum contained two clear doublets at δ 5.13 and 1.85 coupled each to the other by 13.5 Hz (decoupling) which had to be associated with the 15β and 14α-protons, respectively. Once again, the tertiary hydroxyl group should be placed at C-8. Further, the spectrum contained well separated signals for 6β-H at δ 4.92 (dt, J = 5 and 12 Hz), 3α-H at δ 4.68 (m, $W_{\frac{1}{2}}$ = 24 Hz) and 26-H at δ 3.95 (dd, J = 11.5 and 6.5 Hz) −3.84 (dd, J = 11.5 and 7.5 Hz). Perhaps the most significant feature of the ^{1}H NMR spectrum was the small change in

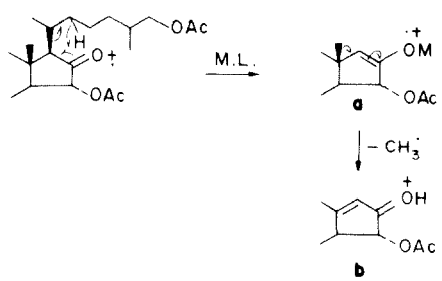


Fig. 1. Diagnostic fragments in the mass spectrum of 16-ketosteroids.[10]

the resonance frequency of the 18-protons on passing from the tetraacetate **2a** to the ketone **2b** (δ 1.19→1.15) which is only compatible with a 16β-oriented hydroxyl group in **2a**.[6,11]

Nakanishi *et al.*[12] showed that in the exciton chirality method the coupled Cotton effect is still observable for remote dibenzoates and they reported a quantitative treatment of coupled Cotton effects observed in a series of steroidal dibenzoates and found an excellent agreement between the calculated and observed CD curves. More recently Liu and Nakanishi[13] have shown that an additivity relation exists in the amplitudes of the exiton-split curves resulting from multiple interacting chromophores. We treated the hexol **2** with *p*-bromobenzoyl chloride in pyridine and obtained the 5α-cholestane-3β,6α,8,15α,16β,26-hexol 3,6,15,26-tetra(p-bromobenzoate). The CD curve, $\Delta\epsilon_{252} = +37.2$, $\Delta\epsilon_{243} = 0$, $\Delta\epsilon_{235} = -20.0$, displayed a strong positive 1st and negative 2nd Cotton effects in agreement with the clockwise twist (positive chilarity) of the three interactions, 3β/6α, 3α/15α and 6α/15α-dibenzoates in a cholestane skeleton with the absolute 5α-H configuration.

5α-cholestane - 3β,6α,7α,8,15α,16β,26 - heptol **3** *m.p.* 255–258°$[\alpha]_D + 33.8°$

The electron impact mass spectrum showed a small molecular ion at *m/e* 484 corresponding to a fully saturated cholestane–heptol. The fragmentation pattern, with ions for stepwise water loss and ions corresponding to the loss of an hydroxylated C_8 side chain together with two, three and four molecules of water, closely resembled that observed in the spectrum of the hexol **2**. The ^{1}H NMR also contained several features already observed in the spectrum of **2**, namely two doublets of doublets at δ 4.00 ($J = 8$ and 2.5 Hz) and 4.13 ($J = 11.5$ and 2.5 Hz), a 7-lines multiplet with $W_{\frac{1}{2}}$ of 20 Hz at δ 3.55 and the A portion of an ABX system at δ 3.42 ($J_{AB} = 10.5$ Hz, $J_{SX} = 6$ Hz; the B portion is under the methanol signal), assigned to 16α-H, 15β-H, 3α-H and 26-H, respectively. The chemical shift of the methyl signals, δ 0.91d, 0.93d, 1.00s and 1.12s are also close to the values for 21-H, 27-H, 19-H and 18-H, respectively, in the hexol **2**. In the ^{1}H NMR spectrum of the heptol **3** two hydroxymethine protons overlap at δ 3.78, but when we measured the spectrum of the derived tetraacetate **3a** (M^+/e –CH_3CO_2H: 592, 4 CH_3–C = 0 at δ 2.03, 2.05, 2.10 and 2.12) the resonance frequency of one of the two methine protons remained essentially unshifted, δ 3.71 (t, $J = 3$ Hz), while the other has moved donwfield to δ 5.16 (dd, $J = 12.5$ and 3 Hz). Decoupling proved that the two protons are coupled by 3 Hz and that the remaining 3 Hz coupling of the triplet at δ 3.71 is due to interaction with one hydroxyl proton resonating at δ 2.57 (d, $J = 3$ Hz), which disappeared on D_2O treatment. Hence we located the new secondary hydroxyl group at C-7α, which resisted to acetylation, and assigned the dd at δ 5.16 to the acethoxymethine 6β-proton. *Inter alia* we would note that the shape of the 7α-H signal is consistent with the presence of an hydroxyl group at C-8. The comparison of ^{1}H NMR spectra of the heptol **3** and its tetraacetate **3a** showed that the signal due to 16α-H remained unshifted, δ 4.02 (dd, $J = 8$ and 2.5 Hz), while the signals due to 26-H, 3α-H and 15β-H are shifted downfield in the acetate to δ 3.82 (dd, $J = 11.5$ and 8 Hz) –3.95 (dd, $J = 11.5$ and 6 Hz), 4.70 (broad m) and 4.73 (dd, $J = 13$ and 2.5 Hz), respectively, thus confirming that acetylation had occurred at 3β-, 6α-, 15α- and 26-hydroxyl groups. The location of the new hydroxyl group at C-7α received strong support by the ^{13}C NMR frequencies of C-5, C-9 and C-14 (γ-carbons) in the spectrum of the heptol **3**, which are upfield shifted by 9.2, 6.2 and 4.9 ppm, respectively, relative to the hexol **2** (Table 1).

The proposed formulation 5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol for the new steroid received additional confirmation by the following chemical transformations and related spectral properties. Jones's oxidation at room temperature of the tetraacetate **3a** produced a monoketone **3b**. The mass spectrum showed a very small parent ion at *m/e* 650 and fragments at *m/e* 480 (a) and 465 (b) indicative of a 16-ketosteroid (Fig. 1). This assignment was strengthened by the comparison of the ^{1}H NMR spectra of the parent acetate **3a** and the ketone **3b**, which showed that the methyl doublet at δ 0.91 (21-H) in **3a** has moved downfield to 0.99 in the ketone as well as the dd at δ 4.73 ($J = 13$ and 2.5 Hz, 15β-H) in **3a** is simplified to a sharp doublet ($J = 14$ Hz) and has moved to δ 4.97 in the ketone **3b**. Comparison of the ^{1}H NMR spectra of the ketone **2b** and **3b** showed that the one-proton doublet at δ 1.85 (14α-H) in **2b** has moved to δ 2.32 (d, $J = 14$ Hz) in **3b**, thus giving additional confirmation for the location of the new secondary hydroxyl group at C-7α in the sterol **3**. Decoupling experiments confirmed that the doublet at δ 2.32 (14α-H) is coupled with the acethoxymethine 15β-proton, δ 4.97 ($J = 14$ Hz). Treatment of the acetate **3a** with dimethylsulfoxide–trifluoroacetic anhydride[14] led to oxidation of both the sterically hindered 7α- and 16β-hydroxyl groups giving rise to the formation of the diketone **3c**. The mass spectrum showed a small parent ion at *m/e* 648 and the diagnostically important 16-ketosteroid fragment at *m/e* 463 (b) (see Fig. 1). The ^{1}H NMR spectrum of the diketone **3c** was similar to that of the monoketone **3b**, except that the dd at δ 5.18 due to 6β-H is replaced by a sharp doublet at δ 5.61 ($J = 12$ Hz), the hydroxymethine signal is absent and the methyl singlets have moved downfield to δ 1.27 (18-H) and 1.36 (19-H). Furthermore the oxidation of the 7α-hydroxyl function also resulted in a upfield shift of the 14α-H signal which in the spectrum of **3c** become confused in the region of the methylene and methine protons between δ 2 and 1.5.

Treatment of **3** with p-bromobenzoylchloride in pyridine afforded the 5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol 3,6,15,26-tetra(p-bromobenzoate) whose CD curve, $\Delta\epsilon_{251} = +35.0$, $\Delta\epsilon_{242} = 0$, $\Delta\epsilon_{233} = -16.3$, is almost identical to that of the previous 5α-cholestane-3β,6α,8,15α,16β,26-hexol 3,6,15,26-tetra(p-bromobenzoate).

5α - cholestane - 3β,4β,6α,7α,8,15α,16β,26 - *octol* **4**, *m.p.* 263–266° $[\alpha]_D + 10°$

The third polyhydroxylated sterol contains one more hydroxyl group relative to the heptol **3**. The field desorption mass spectrum gave peaks at *m/e* 501 $(M + H)^+$ and 523 $(M + Na)^+$ corresponding to the molecular formula $C_{27}H_{48}O_8$ and the ^{13}C NMR spectrum revealed that there were eight carbons bonded to oxygen (see Table 1). The comparison of ^{13}C NMR spectra of **3** and **4** immediately indicated that the novel sterol **4** was related to **3** by introduction of the eighth hydroxyl group at 4β-position. The resonances associated with the carbon atoms of ring A as well as with C-6 and C-19 showed the expected chemical shift differences, while the resonances of the remaining carbon atoms have chemical shift values essentially identical (±0.1 ppm) in both spectra (Table 1)

Table 1. ^{13}C NMR chemical shifts[a] for starfish-derived polyhydroxysterols (1–4) and acetate derivatives (1a–4a)

Carbon atoms	1[b]	1a[b]	2	2a	3	3a	4	4a
1	39.8	38.0	39.6	37.8	39.6	37.5	39.7	38.0
2	32.2	27.2	31.5	26.5	31.5	26.5	26.1	21.8
3	72.5	73.3	72.2	73.1	72.3	73.1	73.6	74.9
4	36.4	30.9	32.4	27.7	32.3	27.3	69.5	66.6
5	c	46.0	53.7	49.2	44.5	40.5	47.9	44.3
6	72.5	72.8	67.6	70.0	68.9	71.3	66.1	69.7
7	41.9	36.0	d	45.2	76.5	72.6	76.6	73.1
8	31.2	30.3	75.9	74.8	77.7	77.3	77.6	77.3
9	55.8	53.6	57.4	55.3	51.2	48.3	52.1	49.3
10	36.6	35.4	37.8	37.0	37.8	36.9	37.9	37.4
11	21.9	20.6	19.4	18.0	19.3	17.6	18.6	17.1
12	40.6	40.1	43.2	41.1	43.2	40.2	42.9	40.5
13	44.7	43.3	45.3	43.9	45.5+	43.9	45.4+	43.9
14	61.1	56.2	64.5	59.5	59.6+	53.7	59.5+	53.8
15	85.0	88.4	80.7	83.9	79.3	84.4	79.7	84.5
16	82.9	79.3	83.0	79.0	82.7+	78.6	82.6+	78.7
17	59.9	58.7	60.6	59.4	61.4+	59.4	61.3+	59.5+
18	15.0	14.5	16.9	16.2	16.9	16.0	16.8	16.4+
19	16.3	15.2	14.2	13.4	13.9	13.3	16.8	16.1+
20	30.9	29.4	30.6	29.0	30.6	29.0	30.5	29.0
21	18.6	17.9	18.4	17.7	18.4	17.6	18.3	17.7
22	37.4	35.8	37.2	35.5	37.1	35.4	36.9	35.6
23	24.8	23.6	24.9	23.5	24.9	23.4	24.8	23.6
24	34.9	33.7	35.0	33.7	35.0	33.7	34.9	33.7
25	37.0	32.4	37.1	32.4	37.1	32.5	37.0	32.6
26	68.4	69.5	68.5	69.4	68.5	69.4	68.5	69.6
27	17.3	16.9	17.4	16.9	17.4	16.9	17.3	17.0

a. The spectra were run at 67.88 MHz on a Brücker WX-270 spectrometer for solutions in CD_3OD (polyhydroxysterols) and in $CDCl_3$ (acetate derivatives). Chemical shifts are expressed in ppm relative to TMS. ^{13}C-NMR. signals were assigned using ^1H single-frequency off-resonance decoupling technique, hydroxyl substituent parameters found in simpler steroids[7] taking into account the deviations from additivity for proximate diols reported in ref.17, by acetylation shifts and by chemical shift comparison from compound to compound.

b. The chemical shift values of compounds 1 and 1a (5α-cholestane-3β,6β, 15α,16β,26-pentol 3,6,15,26-tetraacetate) are from ref.4.

c. Signal under solvent signal; in pyridine-d_5 solution it resonated at 48.3 ppm.

d. signal under solvent signal; in pyridine-d_5 solution it resonated at 50.9 ppm

+. Assignement may be reversed.

except C-9, C-11 and C-12 (see below). The most significant features of the ^{13}C NMR spectrum of the octol **4**, which suggested the location of the new hydroxyl group at C-4β, were the upfield shifts exhibited by C-2 (5.5 ppm) and C-6 (2.8 ppm) and the downfield shifts experienced by C-5 (3.4 ppm) and C-19 (2.9 ppm) relative to the heptol **3**. The hydroxyl β shift at C-5 as well as the hydroxyl γ shifts at C-2 and C-6 and the hydroxyl δ shift at C-19 are close to the shifts observed in 4β-hydroxysteroids (e.g. in 4β-cholestanol relative to the parent 5α-cholestane the β shift at C-5 is +2.9 ppm, the γ shifts at C-2 and C-6 are −5.3 and −3.2 ppm, respectively, and the δ shift at C-19 is +2.5 ppm).[7] It is noteworthy that carbons 9, 11 and 12 were affected by the presence of 4β-hydroxyl group, since their resonances are shifted slightly, but consistently, in **4** relative to **3** (the shift exhibited by C-9 is 0.9 ppm downfield, while the shifts exhibited by C-11 and C-12 are 0.7 and 0.3 ppm upfield, respectively). Significantly similar shifts were observed in 4β-cholestanol relative to 5α-cholestane, the shift exhibited by C-9 is 0.7 ppm downfield, while the shifts exhibited by C-11 and C-12 are 0.6 and 0.2 ppm upfield, respectively.[7]

The structure assignment for the *P. nodosus* highly hydroxylated sterol received confirmation by the analysis of the ^1H NMR spectra of **4** and its derivatives **4a** and **4b**. The ^1H NMR spectrum of the parent octol **4** contained two mutually coupled doublets of doublets at δ 4.01 (J = 8 and 2.5 Hz) and 4.16 (J = 11.5 and 2.5 Hz) due to 16α and 15β-protons, respectively, a well separated one-proton doublet (J = 3 Hz) at δ 3.86 due to 7β-H and three methyl signals at δ 0.92d, 0.94d and 1.13s, which are close to the values for 21-H, 27-H and 18-H, respectively, observed in the spectrum of the heptol **3**. The fourth methyl group (CH_3-19) gave rise to a signal at δ 1.19s downfield shifted by 0.19 ppm relative to the heptol **3** (Table 2), in agreement with the postulation of a 4β-OH in **4**.[15] Of the remaining hydroxymethine signals two overlap at δ 4.21 (6β-H and 4α-H) as well as the 3α-H signal overlaps with the 26-H signal at δ 4.43.

Table 2. Chemical shifts (δ; TMS = 0) of 19- and 18-protons in polyhydroxylated sterols (**2–4**) and acetate derivatives (**2a–4a**)[a]

	19-H		18-H	
Compound	observed	calculated[b]	observed	calculated[b]
2	1.02	1.05	1.11	1.12
2a	1.08	1.06	1.19	1.15
3	1.00	1.04	1.12	1.12
3a	1.08	1.05	1.19	1.17
4	1.19	1.28[c]	1.13	1.12
4a	1.30	1.29	1.19	1.16

a. The spectra of the polyhydroxylated sterols were taken for solutions in CD_3OD, while those of the derived acetates were taken for solutions in $CDCl_3$.

b. The chemical shifts were calculated based on the original data compiled by Zürcher,[11] except the effects of 4β-OH, 6α-OH, 6α-OAc and 16β-OH, which were taken from the ref.6.

c. The agreement between the calculated and observed values of the 19- and 18-protons resonances is satisfactory for all compounds listed in the Table, the deviations beings less than 0.04 ppm, except the value of the 19-protons resonance of 4, the discrepancy between the calculated and observed value being 0.09 ppm.[15]

Treatment with excess acetic anhydride in pyridine at room temperature gave a tetraacetate **4a** ($M^+ - CH_3CO_2H - H_2O$: 590; $4CH_3$–C=O at δ 2.06, 2.10, 2.14 and 2.17), whose ^1H NMR spectrum still contained overlapping hydroxy- and acethoxymethine signals, by significantly an isolated dd at δ 5.47 (J = 12 and 3 Hz) assigned to 6β-H. This peak is 0.3 ppm downfield shifted relative to that of the tetraacetate **3a**, thus providing further corroborative evidence for the presence in **4** of the 4β-hydroxyl moiety. Comparison of the ^1H NMR spectra of the parent **4** and the acetate **4a**, which showed that, in addition to 6β-H also the resonances associated with 3α-H, 15β-H and 26-H had moved downfield in the acetate, *ca* 1 ppm shift 3α-H and 15β-H and *ca* 0.5 ppm shift 26-H, established that acetylation had occurred at 3β-, 6α, 15β- and 26-hydroxyls. Oxidation of the tetraacetate **4a** with pyridine dichromate in dichloromethane gave one major product, **4b**. The diketone **4b**, M^+/e 664, by virtue of the C-4 and C-16 oxo functionalities, provided for an apparent first-order spectrum in the downfield region with eight resolved one-proton bands. In particular the oxidation of **4a** to the diketone **4b**, produced in the ^1H NMR (a) the downfield shift of the 3α-H signal from δ 4.75 to δ 5.20 (dd, J = 11 and 7 Hz), the disappearance of the 4α-H signal and the change of the chemical shift of the 19-protons from δ 1.30 to δ 1.01, in agreement with the oxo function at C-4, and (b) the disappearance of the 16α-H signal, the transformation of the dd (J = 13 and 3 Hz) at δ 4.75 into a sharp doublet at δ 4.96 (J = 14 Hz; 15β-H), which is coupled to a doublet at δ 2.37 (14α-H) and the changes of the chemical shifts of the 21- and 18-protons from δ 0.91 in **4a** to 1.00 in **4b** and from δ 1.19 in **4a** to 1.16 in **4b**, respectively, in agreement with the second oxo function at C-16. Further, decoupling experiments with **4b** illustrated that 7β-H 3.71 ppm (t) is coupled (J = 3 Hz) with 6β-H 5.55 ppm (dd), which is itself coupled (J = 12 Hz) to the 5α-H 3.07 ppm (d). Treatment of **4** with p-bromobenzoylchloride in pyridine afforded the 5α-cholestane-3β,4β,6α,7α,8,15α,16β,26-octol 3,6,15,26-tetra(p-bromobenzoate) whose CD curve, $\Delta\epsilon_{251} = +34.0$, $\Delta\epsilon_{242} = 0$, $\Delta\epsilon_{233} = -16.0$, is almost identical to those of the previous tetra(p-bromobenzoate).

The 15α,16β,26-triol pattern, already encountered in the hydroxylated sterol **1**, isolated from *Hacelia attenuata*,[4] seems a common feature of starfish hydroxylated sterols. The hydroxylation at C-8 is uncommon to steroids and has been encountered before only in one marine sterol isolated from the soft coral *Litophyton viridis*.[16] Finally we would note that the octol **4** constitutes, as far as we know, the more highly hydroxylated sterol isolated from a natural source.

EXPERIMENTAL

Melting points were measured on a kofler hot-stage apparatus and are uncorrected. Optical rotations were taken on a Perkin–Elmer 141 polarimeter. Spectral data were determined on the following instruments: ^1H NMR and ^{13}C NMR, Brüker WX-270 or Brüker WM-250; EI mass spectra, A.E.I. MS-30 or A.E.I. MS-50; FD mass spectrum, Hithaci M-80; CD, JASCO J-40 spectropolarimeter. The preparative LC separation was performed on Waters Associates Prep LC/system 500 instrument. The semipreparative HPLC was performed with a Waters Model 6000 pump equipped with U6K injector and a Model 401 differential refractometer detector.

Collections and extraction of Protoreaster nodosus. The starfishs *P. nodosus* were collected off Nouméa—Nouvelle

Caledonie in August 1980 and March 1981. Both collections contained the same compounds, but in different relative percentages; for example in the first collection the octol **4** was present only in trace amounts and could not be isolated in sufficient quantities for a structure analysis. Here we report the extraction of the samples collected in March 1981. The starfish were lyophilized (2 kg dry weight) and extracted in a Soxhlet apparatus with light petroleum (b.p. 40–70°), then with methanol followed by 50% H_2O in ethanol. The methanol extract (181 g), which contained the hydroxylated sterols, was washed with ethyl acetate (1 l.). The solid material was filtered off and extracted with 30% methanol/chloroform (four 8 h soakings with 1 l. portions). The chloroform/methanol extracts were clarified by centrifugation and evaporated at reduced pressure to give a brown residue (52.6 g).

Chromatography. This material was chromatographed using 500 g of silica gel starting with chloroform eluent to remove most unwanted natural products and increasing the methanol content to 40%. The 40% methanol/chloroform fractions gave 19 g of residue, which was then chromatographed by preparative LC on two prepak-500 SiO_2 columns. Elution with 20% methanol/chloroform afforded the polyhydroxylated sterols with partial resolution, while elution with 30% methanol/chloroform afforded a fourth more polar product. The earlier fractions eluted with 20% methanol/chloroform contained essentially **2**,**3** and **4**, while the later fractions still contained additional amounts of **2** and **4** plus three more steroids as minor constituents. Individual hydroxylated sterols were isolated by semipreparative HPLC on a μ-bondapack C-18 column (7.8 mm × 30 cm) using 30% H_2O in methanol. After several collections of the major peaks 1, 2 and 3 the methanol was removed under reduced pressure and the water was removed by lyophilization to give **2** (59 mg), **4** (84 mg) and **3** (34 mg). The hydroxylated sterols were crystallized from methanol in the presence of dichloromethane vapors.

5α - *cholestane* - 3β,6α,8,15α,16β,26-*hexol* **2**. M.p. 285–287°; $[\alpha]_D^{20}$ + 13.8° (c, 1.5, MeOH); EI mass spectrum (70 eV) *m/e* (%) 450 (M^+ –H_2O, 11), 432 (M^+ –2H_2O, 10), 417 (M^+ –2H_2O –Me, 7), 414 (M^+ –3H_2O, 10), 399 (M^+ –3H_2O –Me, 7), 396 (M^+ –4H_2O, 3), 331 (12), 321 (M^+-side chain –H_2O, 15), 303 (M^+ -side chain –2H_2O, 23), 285 (M^+ -side chain -3H_2O, 21), 267 (M^+ -side chain -4H_2O, 14), 225 (100), 207 (60); ^{1}H NMR (CD_3OD) δ 0.91 (d, J = 7 Hz, 21-H), 0.93 (d, J = 6.5 Hz, 27-H), 1.02 (s, 19-H), 1.11 (s, 18-H), 2.40 (dd, J = 12 and 3 Hz, 7β-H), 3.42 (dd, 1H, J = 10.5 and 6 Hz, 26-H), 3.56 (broad m, $W_{\frac{1}{2}}^{1}$ = 20 Hz, 3α-H), 3.63 (dt, J = 3 and 10 Hz, 6β-H), 3.98 (dd, J = 8.5 and 2.5 Hz, 16α-H), 4.04 (dd, J = 11.5 and 2.5 Hz, 15β-H); ^{13}C NMR in the Table 1. Found: C, 68.70; H, 10.72. Calc. for $C_{27}H_{48}O_6$: C, 69.19; H, 10.32%.

5α - *cholestane* - 3β,6α,7α,8,15α,16β,26 - *heptol* **3**. M.p. 255–258°; $[\alpha]_D^{20}$ + 33.8° (c, 1 MeOH); EI mass spectrum (70 eV) *m/e* (%) 484 (M^+ < 1%), 466 (M^+ –H_2O, 8), 448 (M^+ –2H_2O, 100), 430 (M^+ –3H_2O, 55), 412 (M^+ –4H_2O, 20), 319 (M^+ -side chain –2H_2O, 12), 301 (M^+ -side chain –3H_2O, 30), 293 (70), 283 (M^+ -side chain -4H_2O, 8); ^{1}H NMR (CD_3OD) δ 0.91 (d, J = 7 Hz, 21-H), 0.93 (d, J = 6.5 Hz, 27-H), 1.00 (s, 19-H), 1.12 (s, 18-H), 3.42 (dd, 1H, J = 10.5 and 6 Hz, 26-H), 3.55 (broad m, $W_{\frac{1}{2}}^{1}$ = 20 Hz, 3α-H), 3.78 (broad m, 2H, 6β and 7β-H), 4.00 (dd, J = 8 and 2.5 Hz, 16α-H), 4.13 (dd, J = 11.5 and 2.5 Hz, 15β-H); ^{13}C NMR in the Table 1; Found: C, 66.19; H, 9.81 Calc. for $C_{27}H_{48}O_7$: C, 66.91; H, 9.98%.

5α - *cholestane* - 3β,4β,6α,7α,8,15α,16β,26 - *octol* **4**. M.p. 263–266° $[\alpha]_D^{20}$ + 10° (c, 1 MeOH); FD mass spectrum 501, 523; ^{1}H NMR (CD_3OD) δ 0.92 (d, J = 7 Hz, 21-H), 0.94 (d, J = 6.5 Hz, 27-H), 1.13 (s, 18-H), 1.19 (s, 19-H), 3.43 (dd, 1H, J = 10.5 and 6 Hz, 26-H), 3.50 (broad m, partially overlapped to the 26-H, 3α-H), 3.86 (d, J = 3 Hz, 7β-H), 4.01 (dd, J = 8 and 2.5 Hz, 16α-H), 4.16 (dd, J = 11.5 and 2.5 Hz, 15β-H), 4.21 (broad m, 2H, 4α and 6β-H); ^{13}C NMR in Table 1. Found: C, 64.18; H, 9.32. Calc. for $C_{27}H_{48}O_8$: C, 64.77; H, 9.66%.

5α - *cholestane* - 3β,6α,8,15α,16β,26 - *hexol* 3,6,15,26 - tetraacetate **2a**. The mixture of 5α - cholestane - 3β,6α,8,15α,16β,26 - hexol **2** (25 mg) and excess of acetic anhydride in 0.5 ml of dry pyridine was kept at room temperature for 9 h. After removal of the excess reagents *in vacuo* the residue was purified by preparative TLC (silica gel—30% ethyl acetate in benzene) to give the tetraacetate **2a** (15 mg), m.p. 168–170°; $[\alpha]_D^{20}$ + 64° (c, 0.5 $CHCl_3$); EI mass spectrum (70 eV) *m/e* (%) 576 (M^+ –CH_3CO_2H, 20), 558 (M^+ –CH_3CO_2H–H_2O, 6), 516 (M^+ –2CH_3CO_2H, 60), 498 (M^+ –2CH_3CO_2H–H_2O, 100), 480 (M^+ –2CH_3CO_2H–2H_2O, 15), 456 (M^+ –3CH_3CO_2H, 50), 438 (M^+ –3CH_3CO_2H–H_2O, 55); ^{1}H NMR ($CDCl_3$) δ 0.90 (d, J = 6.5 Hz, 21-H), 0.92 (d, J = 7 Hz, 27-H), 1.08 (s, 19-H), 1.19 (s, 18-H), 2.03, 2.04 and 2.06 (three s, 12H, O=C–CH_3), 3.82 (dd, 1H, J = 11.5, 8 Hz, 26-H), 3.95 (broad m, 2H, 26 and 16α-H), 4.69 (2H, one dd, J = 12 and 2.5 Hz emerging from a broad m, 15β and 3α-H), 4.92 (dt, J = 4 and 12 Hz, 6β-H); ^{13}C NMR in Table 1.

The other steroid tetraacetates were prepared by similar methods. Each product was purified by preparative TLC.

5α - *cholestane* - 3β,6α,7α,8,15α,16β,26-*heptol* 3,6,15,26 - *tetraacetate* **3a**. M.p. 162–164°; $[\alpha]_D$ + 66° (c, 0.5 $CHCl_3$); EI mass spectrum (70 eV) *m/e* (%) 592 (M^+ -CH_3CO_2H, <1%), 574 (M^+ –CH_3CO_2H–H_2O, 20), 532 (M^+ –2CH_3CO_2H, 25), 514 (M^+ –2CH_3CO_2H–H_2O, 100), 496 (M^+ –2CH_3CO_2H–2H_2O, 10), 472 (M^+ –3CH_3CO_2H, 15), 454 (M^+ –3CH_3CO_2H–H_2O, 60), 439 (M^+ –3CH_3CO_2H–H_2O–CH_3, 10), 436 (M^+ –3CH_3CO_2H–2H_2O, 15); ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 6.5 Hz, 21-H), 0.92 (d, J = 7 Hz, 27-H), 1.08 (s, 19-H), 1.19 (s, 18-H), 2.03, 2.05, 2.10 and 2.12 (four s, 12H, O=C–CH_3), 2.57 (d, J = 3 Hz, 7-OH), 3.71 (t, J = 3 Hz, 7α-H), 3.82 (dd, 1H, J = 11.5 and 8 Hz) and 3.95 (dd, 1H, J = 11.5 and 6 Hz) (26-H), 4.02 (dd, J = 8 and 2.5 Hz, 16α-H), 4.70 (broad m, partially overlapped with 15β-H, 3α-H), 4.73 (dd, J = 13 and 2.5 Hz, 15β-H), 5.16 (dd, J = 12 and 3 Hz, 6β-H); ^{13}C NMR in Table 1.

5α-*cholestane* - 3β,4β,6α,7α,8,15α,16β,26 - *octol* 3,6,15,26 - *tetraacetate* **4a**. M.p. 178–180°; $[\alpha]_D^{20}$ + 3.2° (c, 0.3 $CHCl_3$) EI mass spectrum (70 eV) *m/e* (%) 590 (M^+ –CH_3CO_2H–H_2O, 10) 572 (M^+ –CH_3CO_2H–2H_2O, 9), 548 (M^+ –2CH_3CO_2H, 9), 530 (M^+ –2CH_3CO_2H–H_2O, 80), 512 (M^+ –2CH_3CO_2H–2H_2O, 38), 488 (M^+ –3CH_3CO_2H, 20), 470 (M^+ –3CH_3CO_2H–H_2O, 100), 452 (M^+ –3CH_3CO_2H–2H_2O, 63); ^{1}H NMR ($CDCl_3$) δ 0.91 and 0.92 (two d, 6H, J = 6.5 and 7 Hz, 21-H and 27-H *or vice versa*), 1.19 (s, 18-H), 1.30 (s, 19-H), 2.06, 2.10, 2.14 and 2.17 (four s, 12H, O=C–CH_3), 2.60 (d, J = 3 Hz, 7-OH), 3.75–3.88 (broad signal, 2H, 7β and 26-H), 3.92–4.06 (broad signal, 3H, 4α, 16α and 26-H), 4.75 (2H, one dd, J = 13 and 3 Hz emerging from a broad m, 15β and 3α-H), 5.47 (dd, J = 12 and 3 Hz, 6β-H); ^{13}C NMR in Table 1.

3β,6α,15α,26 - *tetra*(*acetyloxy*) - 8 - *hydroxy* - 5α - *cholestan* - 16 - *one* **2b**. Jones reagent[18] (one drop) was added to a cold soln of **2a** (5 mg) in acetone (0.5 ml) and the solution was stirred at room temp. for 3 min. Usual work up gave a residue which was purified by TLC (silica gel—20% ethylacetate in chloroform) to afford the ketone **2b**: mass spectrum (20 eV) *m/e* (%) 634 (M^+ < 1%), 574 (M^+ –CH_3CO_2H, 6), 559 (M^+ –CH_3CO_2H–Me, 3), 556 (M^+ –CH_3CO_2H–H_2O, 3), 514 (M^+ –2CH_3CO_2H, 12), 499 (M^+ –2CH_3CO_2H–Me, 8), 496 (M^+ –2CH_3CO_2H–H_2O, 20), 454 (M^+ –3CH_3CO_2H, 15), 439 (M^+ –3CH_3CO_2H–Me, 15), 436 (M^+ –3CH_3CO_2H–H_2O, 12), 464 (**a**, 1, Fig. 1), 404 (**a**, –CH_3CO_2H, 15), 344 (**a**, –2CH_3CO_2H, 28), 284 (**a**, –3CH_3CO_2H, 48), 449 (**b**, 100 Fig. 1), 389 (**b** –CH_3CO_2H, 30), 371 (**b** –CH_3CO_2H–H_2O, 15), 329 (**b** –2CH_3CO_2H, 30), 311 (**b** –2CH_3CO_2H–H_2O, 25), 269 (**b** –3CH_3CO_2H, 50), 251 (**b** –3CH_3CO_2H–H_2O, 30); ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 27-H), 0.99 (d, J = 6.5 Hz, 21-H), 1.11 (s, 19-H), 1.15 (s, 18-H), 1.85 (d, J = 13.5 Hz, 14α-H), 3.84 (dd, J = 11.5 and 8 Hz) and 3.95 (dd, J = 11.5 and 6.5 Hz) (26-H), 4.68 (broad m, $W_{\frac{1}{2}}^{1}$ = 24 Hz, 3α-H), 4.92 (dt, J = 5 and 12 Hz, 6β-H), 5.13 (d, J = 13.5 Hz, 15β-H).

3β,6α,26 - *tetra*(*acetyloxy*) - 7α,8 - *dihydroxy* - 5α - *cholestan* - 16 - *one* **3b**. Jones oxidation of **3a** (5 mg) in the same manner as described above gave, after purification by TLC (SiO_2—40% ethyl acetate in chloroform, 2 migrations), the monoketone **3b** and small amounts of the diketone **3c**, which was better obtained as described below. **3b**: mass spectrum (25 eV) *m/e* (%) 650 (M^+ < 1%), 590 (M^+ –CH_3CO_2H, 18), 572 (M^+ –CH_3CO_2H–H_2O, 9), 530 (M^+ –2CH_3CO_2H, 55), 515 (M^+ –2CH_3CO_2H–Me, 20), 512 (M^+ –2CH_3CO_2H–H_2O, 28), 497 (M^+ –2CH_3CO_2H–H_2O–Me, 12), 494 (M^+ –2CH_3CO_2H–2H_2O, 10), 470 (M^+ –3CH_3CO_2H, 55), 455 (M^+ –3CH_3CO_2H–Me, 30), 452 (M^+ –3CH_3CO_2H–H_2O, 45), 480 (**a**, 1, Fig. 1), 420 (**a** –CH_3CO_2H, 55), 360 (**a** –2CH_3CO_2H, 100),

300 (**a** $-3CH_3CO_2H$, 60), 465 (**b**, 100 Fig. 1), 405 (**b** $-CH_3CO_2H$, 63), 387 (**b** $-CH_3CO_2H-H_2O$, 45), 445 (**b** $-2CH_3CO_2H$, 40) and 285 (**b** $-3CH_3CO_2H$, 35); ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 7 Hz, 27-H), 0.99 (d, J = 6.5 Hz, 21-H), 1.11 (s, 19-H), 1.14 (s, 18-H), 2.32 (d, J = 14 Hz, 14α-H), 3.60 (d, J = 3 Hz, 7α-H), 3.84 (dd, J = 11.5 and 8 Hz) and 3.95 (dd, 1H, J = 11.5 and 6.5 Hz) (26-H), 4.70 (broad m, $W_{\frac{1}{2}}$ = 24 Hz, 3α-H), 4.97 (d, J = 14 Hz, 15β-H), 5.18 (dd, J = 12 and 3 Hz, 6β-H).

3β,6α,15α,26 - *tetra(acetyloxy)* - 8 - *hydroxy* - 5α - *cholestane* - 7,16 - *dione* **3c**. To a soln of dry dimethylsulphoxide (3 mg) in CH_2Cl_2 (0.1 ml) cooled below $-65°$ with a dry-ice acetone bath, trifluoroacetic anhydride (10 mg) in CH_2Cl_2 (0.1 ml) was added. After 10 min. below $-65°$ a soln of **3a** (10 mg) in CH_2Cl_2 (0.1 ml) was added. The mixture was left below $-65°$ for 30 min. and then triethylamine (0.1 ml) was added. The reaction mixture was then allowed to warm up to room temp., then washed with water and the aqueous layer back washed with CH_2Cl_2.[14] The organic layer was evaporated to dryness and the residue was purified by TLC (SiO_2—40% ethyl acetate/chloroform) to give the diketone **3c**: mass spectrum (20 eV) *m/e* (%) 648 (M^+ < 1%), 620 (12), 588 (M^+ $-CH_3CO_2H$, 4) 560 (100), 528 (M^+ $-2CH_3CO_2H$, 6), 500 (90), 463 (**b**, 10, Fig. 1), 440 (20), 418 (**a** $-CH_3CO_2H$, 12), 390 (55), 358 (**a** $-2CH_3CO_2H$, 25), 330 (48), 270 (15); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 7 Hz, 27-H), 1.01 (d, J = 6.5 Hz, 21-H), 1.27 (s, 18-H), 1.36 (s, 19-H), 2.00, 2.04, 2.06 and 2.12 (four s, 12H, O=C-CH_3), 3.84 (dd, 1H, J = 11.5 and 8 Hz) and 3.96 (dd, 1H, J = 11.5 and 6.5 Hz) (26-H), 4.66 (bm, $W_{\frac{1}{2}}$ = 24 Hz, 3α-H), 4.96 (d, J = 14 Hz, 15β-H), 5.61 (d, J = 12 Hz, 6β-H).

3β,6α,15α,26 - *tetra(acetyloxy)* - 7α,8 - *dihydroxy* - 5α - *cholestane* - 4,16 - *dione* **4c**. Excess of pyridine dichromate[19] was added to a soln of the tetrol **4a** (10 mg) in CH_2Cl_2 (1 ml) and the mixture was stirred at room temp. for 12 h. The ether was added and the supernatant was decanted from the black gum. Evaporation of the solvents yielded a organic product which was purified by TLC on SiO_2 in 40% ethylacetate/benzene to give the diketone **4b**: mass spectrum (70 eV) *m/e* (%) 664 (M^+, 20), 646 (M^+ $-H_2O$, 21), 604 (M^+ $-CH_3CO_2H$, 15), 586 (M^+ $-H_2O-CH_3CO_2H$, 40), 109 (100); ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 7 Hz, 27-H), 1.00 (d, J = 6.5 Hz, 21-H), 1.04 (s, 19-H), 1.16 (s, 18-H), 2.03, 2.06, 2.08 and 2.15 (four s, 12H, O=C-CH_3), 2.33 (d, J = 14 Hz, 14α-H), 3.07 (d, J = 12 Hz, 5α-H), 3.71 (t, J = 3 Hz, 7β-H), 3.84 (dd, 1H, J = 11.5 and 8 Hz) and 3.95 (dd, 1H, J = 11.5 and 6.5 Hz) (26-H), 4.96 (d, J = 14 Hz, 15β-H), 5.20 (dd, J = 11 and 7 Hz, 3α-H), 5.55 (dd, J = 12 and 3 Hz, 6β-H).

5α - *cholestane* - 3β,6α,8,15α,16β,26 - *hexol* 3,6,15,26 - *tetra*(p - *bromobenzoate*). The mixture of the hexol **2** (3 mg) and 1.5 equivalent p-bromobenzoyl chloride in 0.2 ml of dry pyridine was heated at 60° overnight. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 0.1N NaOH, water and dried. Evaporation afforded residue which was purified by silica gel chromatography in chloroform to give the *tetra*(p-*bromobenzoate*): ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 6.5 Hz, 21-H), 0.97 (d, J = 6.5 Hz, 27-H), 1.18 and 1.25 (two s, 18-H and 19-H *or vice versa*), 4.00–4.22 (overlapping signals, 3H, 16α and 26-H), 4.87 (dd, J = 11.5 and 2.5 Hz, 15β-H), 4.98 (broad m, 3α-H), 5.18 (dt, J = 4 Hz and 11 Hz, 6β-H); CD (MeOH), $\Delta\epsilon_{252}$ = + 37.2, $\Delta\epsilon_{243}$ = 0, $\Delta\epsilon_{235}$ = − 20.0. Sample concentration was estimated from the standard UVϵ value at 244 nm of 76,400.[13]

5α - *cholestane* - 3β,6α,7α,8,15α,16β,26-*heptol* 3,6,15,26 - *tetra*(p-*bromobenzoate*). This was prepared by similar method as above and gave the following spectral data: ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 6.5 Hz, 21-H), 0.98 (d, J = 6.5 Hz, 27-H), 1.19 and 1.26 (two s, 18-H and 19-H *or vice versa*), 3.95 (t, J = 3 Hz, 7β-H), 4.00–4.22 (overlapping signals, 3H, 16α and 26-H), 4.92 (dd, J = 11.5 and 2.5 Hz, 15β-H), 4.98 (broad m, 3α-H), 5.30 (dd, J = 12 and 3 Hz, 6β-H); CD (MeOH), $\Delta\epsilon_{251}$ = + 35.0, $\Delta\epsilon_{242}$ = 0, $\Delta\epsilon_{233}$ = − 16.3.

5α - *cholestane* - 3β,4β,6α,7α,8,15α,16β,26 - *heptol* 3,6,15,26 - *tetra*(p - *bromobenzoate*). This was prepared as above and gave the following spectral data: ^{1}H NMR ($CDCl_3$) δ 0.92 (d, J = 6.5 Hz, 21-H), 0.97 (d, J = 6.3 Hz, 27-H), 1.19 (s, 18-H), 1.32 (s, 19-H), 4.00–4.30 (overlapping signals, 4H, 4α, 16α and 26-H), 4.94 (dd, J = 11.5 and 2.5 Hz, 15β-H), 5.02 (bm, 3α-H), 5.68 (dd, J = 11 and 2 Hz, 6β-H); CD (MeOH), $\Delta\epsilon_{251}$ = + 34.0, $\Delta\epsilon_{242}$ = 0, $\Delta\epsilon_{233}$ = − 16.0.

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structural units. We would note that in the tetraacetate **4a** (3β-OAc, 4β-OH), the agreement between the calculated and observed value of 19-protons chemical shift is again excellent, the difference being 0.01 ppm.

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