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12 The monophenylboronate was characterized by ^{1}H-NMR spectroscopy: δ 1.12 (3H, s, 18-H), 1.31 (3H, s, 19-H) and 4.68 (1H, dt, J = 5.5 Hz, 15α-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: δ 7.35 (3H, m), 7.25 (2H, d, J = 7.5 Hz).

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Starfish saponins. Part 10[1]. Further 24-O-glycosidated steroids from the starfish *Hacelia attenuata*

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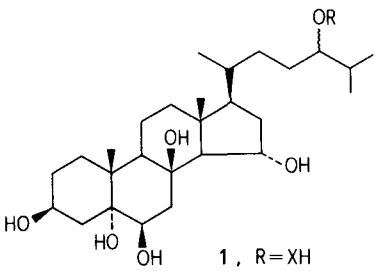
Summary. On the basis of comparative spectral data, the structures of 3 novel steroidal glycosides from the Mediterranean starfish *Hacelia attenuata* have been elucidated as **3, 4** and **5.** These are further examples of a novel group of 24-O-glycosidated steroids recently encountered in the same species and in the Pacific species *Protoreaster nodosus.*

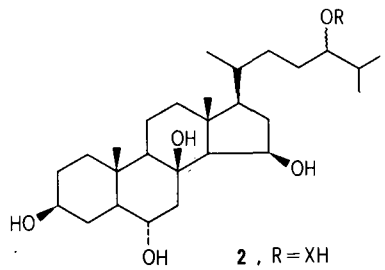
Recently we have isolated 2 novel steroidal glycosides, nodososide, 24-O-[2-O-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-5α-cholestane-3β,5,6β,8,15α,24ξ-hexol (**1**), from the Pacific starfish *Protoreaster nodosus*[4], and attenuatoside A-I, 24-O-[2-O-methyl-β-D-xylopyranosyl)-(1→2)-α-L-arabinopyranosyl]-5α-cholestane-3β,6α,8,15β,24ξ-pentol (**2**), from the Mediterranean starfish *Hacelia attenuata*[1].

In this paper we describe 3 further examples of this novel group of steroidal glycosides from the same starfish *Hacelia attenuata.* The extraction was described in the preceding paper[1]. The 10% MeOH/$CHCl_3$ extract of the lyophilized animals (1.2 kg, collected in the bay of Naples) was chromatographed by preparative LC (Waters Associates LC/system 500 instrument on prepak-500 SiO_2 using 10% MeOH/$CHCl_3$ and increasing MeOH content up to 20%) to give 8 main fractions, A-H. Fraction D was rechromatographed on Sephadex LH-20 and 20 ml fractions were eluted using MeOH as solvent. Fractions 18–20 were further chromatographed by HPLC on μ-bondapack C-18 (35% H_2O/MeOH) to give the previous attenuatoside A-I (**2**) and the novel monoglycoside **4**, attenuatoside B-II, which was further purified by preparative TLC on SiO_2 in $CHCl_3$/MeOH/H_2O, 80:18:2 (17 mg, yield 0.0014%). Fraction E was also rechromatographed on Sephadex LH-20 and 20 ml fractions were eluted using MeOH as solvent. From the fractions 22–28, the novel glycoside **3**, attenuatoside B-I (40 mg) crystallized out on standing. An additional quantity of this material was obtained from the subsequent fraction F, by chromatography on Sephadex LH-20 as before, followed by HPLC on μ-bondapack C-18 (35% H_2O/MeOH) of the fractions 12–14 (from Sephadex LH-20) to afford 24 mg of **3** (total yield 0.0053%) and smaller amount of the novel monoglycoside **5,** attenuatoside C (12 mg, yield 0.001%).

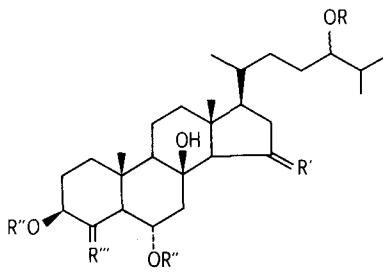
Attenuatoside B-I (**3**), m.p. 228–300 °C, $[\alpha]_D = -12°$ (c, 1 MeOH), contains 1 more hydroxyl group relative to the attenuatoside A-I (**2**). The field desorption mass spectrum gave a peak at 769 ($M^+ + Na$) corresponding to the molecular formula $C_{38}H_{66}O_{14}$. The comparison of the ^{13}C NMR-

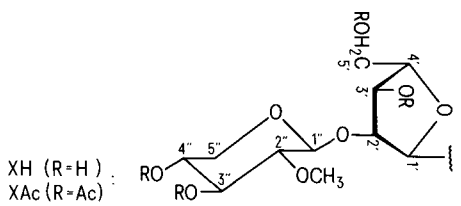


1, R = XH



2, R = XH





3, R = XH, R′ = R‴ = α-OH, R″ = H

3a, R = XAc, R′ = α-OAc, R″ = Ac, R‴ = α-OH

3b, R = XAc, R′ = α-OAc, R′ = Ac, R‴ = O

4, R = α-L-arabinofuranosyl, R′ = R‴ = α-OH, R″ = H

5, R = α-L-arabinofuranosyl, R′ = β-OH, R″ = H, R‴ = α-OH

spectra of **2** and **3** (table 1) indicated that the novel glycoside **3** was related to **2** by introduction of the new hydroxyl group at the 4β-position. The most significant features of the ^{13}C NMR-spectrum of **3**, which immediately suggested the location of the new hydroxyl group at C-4β, were the upfield shifts exhibited by C-2 (5.3 ppm) and C-6 (2.6 ppm) and the downfield shifts experienced by C-5 (3.4 ppm) and C-19 (3.0 ppm) relative to **2**, which are close to the shifts observed in 4β-hydroxysteroids (e.g. in 4β-cholestanol relative to 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)[5]. We noted that carbons 9 and 11 were also affected by the presence of the 4β-hydroxyl group since their resonances are shifted slightly in **3** relative to **2** (C-9: $+1.0$ ppm, C-11: -0.5 ppm). Significantly, similar shifts were observed in 4β-cholestanol relative to 5α-cholestane (C-9: $+0.9$ ppm, C-11: -0.6 ppm)[5]. Furthermore, taking **2** as starting structure the chemical shifts of C-3 and C-4 were calculated for the compound with an additional 4β-hydroxyl group, using the substituent effects and the deviations from additivity for proximate diols which have been published for hydroxylated steroids[5,6]. The calculated (C-3: 72.3, C-4: 69.8) and observed (C-3: 73.1, C-4: 68.9) values were significantly similar. Of the remaining signals in the spectrum of the novel glycoside **3**, two (C-1 and C-7, δ-carbons relative to the 4β-OH) were slightly downfield shifted (0.4 and 0.6 ppm, respectively) in the spectrum of **3** relative to **2** and the other were within 0.2 ppm from **2**. The most significant features of the ^{1}H NMR-spectrum of attenuatoside B-I (**3**), described in table 2, confirming the postulation of a 4β-OH in **3**, were the bs at δ 4.30 for 4α-H, the signal at δ 4.20 (ddd, J = 11.0, 11.0 and 4.5 Hz) assigned to 6β-H, 0.53 ppm downfield shifted relative to that of **2**, and the downfield position of the 19-protons signal relative to **2** (δ 1.19 vs 0.99 ppm). In the ^{1}H NMR-spectrum of **3**, the 3α-H and the 4″-H signals overlap at about δ 3.50, but when we measured the spectrum of the derived heptaacetate **3a**[7] the resonance frequency of the 3α-H appeared as separate signal at δ 4.66 (dt, J = 11.0 and 4.2 Hz) and decoupling proved its interac-

Table 1. ^{13}C-NMR-data*

Carbons		2	3	4	5
1		39.2	39.6	39.4	39.5
2		32.2	26.8	26.6	26.7
3		71.4	73.1	73.0	72.9
4		33.2	68.9	68.7	68.7
5		54.0	57.4	57.2	57.0
6		66.5	63.9	63.6	63.6
7		50.0	50.6	50.3	51.4
8		76.7	76.5	76.5	75.3
9		56.8	57.8	57.6	57.7
10		37.4	37.6	37.5	37.4
11		19.3	18.8	18.7	18.7
12		42.6[a]	42.6[a]	42.4[a]	42.1[a]
13		43.7	43.8	43.6	44.6
14		61.8	62.0	61.7	66.9
15		70.2	70.2	70.0	68.9
16		42.2[a]	42.2[a]	42.1[a]	41.7[a]
17		57.2	57.2	57.0	54.8
18		16.6	16.6	16.4	15.5
19		14.3	17.3	17.2	17.4
20		35.6	35.6	35.5	35.2
21		18.9	18.9	18.6	18.6
22		32.1	32.2	32.0	31.7
23		28.1	28.1	28.2	28.1
24		83.3	83.3	83.3	83.5
25		30.8	30.8	30.9	30.8
26		18.1[b]	18.1[b]	18.0[b]	18.0[b]
27		18.2[b]	18.2[b]	18.1[b]	18.2[b]
arab	1′	107.5	107.4	109.4	109.6
	2′	92.8	92.8	83.8	83.8
	3′	77.6	77.6	78.6	78.6
	4′	85.0	85.0	85.3	85.2
	5′	62.7	62.6	62.6	62.7
2-O-Me-xyl	1″	105.1	105.1		
	2″	84.3	84.3		
	3″	77.8	77.8		
	4″	77.1	77.1		
	5″	67.1	67.1		
OCH_3		60.6	60.6		

* Spectra were recorded in pyridine-d_5 solution on a Brüker WX-270 spectrometer. Chemical shifts are given in ppm with respect to TMS used as an internal standard. Chemical shifts of compound **2** are from Minale et al.[1]. Assignments for the major compound **3** were confirmed by using single-frequency off-resonance decoupling technique. [a,b]Signals within a column may be reversed.

Table 2. ^{1}H-NMR-data in δ (Hz), TMS = 0*

H at C	2	3	4	5
3	3.50m[a]	3.50m[a]	3.46ddd (12.0, 4.5, 4.5)	3.46ddd (12.0, 4.5, 4.5)
4	-	4.30b ($W_{\frac{1}{2}}$ = 8)	4.29b ($W_{\frac{1}{2}}$ = 8)	4.28b ($W_{\frac{1}{2}}$ = 7.5)
6	3.67ddd (11.0, 11.0, 4.5)	4.20ddd (11.0, 11.0, 4.5)	4.19ddd (11.5, 11.5, 4.5)	4.11ddd (11.4, 11.4, 4.5)
7β	2.48dd (13.5, 4.5)	2.48dd (13.5, 4.5)	2.48dd (13.5, 4.5)	2.47dd (14, 4.5)
15	4.46ddd (5.1, 5.1, 1.7)	4.48m[b]	4.45ddd (6.0, 6.0, 1.8)	4.24ddd (11.0, 11.0, 4.5)
18	1.27s	1.29s	1.29s	0.99s
19	0.99s	1.19s	1.18s	1.21s
1′	5.08d (1.1)	5.11bs	4.95d (1.0)	4.95d (1.0)
2′	4.06dd (4.0, 1.1)	4.09bd (4.0)	3.98-4.02m	3.98-4.02m
3′	3.98dd (7.8, 4.0)	4.00-3.92m	3.86dd (7.5, 4.5)	3.86dd (7.5, 4.5)
4′	3.93ddd (7.8, 5.1, 2.9)	4.00-3.92m	3.98-4.02m	3.98-4.02m
5′	3.75dd (12.5, 2.9) and 3.63dd (12.5, 5.1)	3.79dd (12.5, 3.0) and 3.64dd (12.5, 5.0)	3.78dd (13.0, 3.0) and 3.64dd (13.0, 5.0)	3.78dd (13.0, 3.0) and 3.64dd (13.0, 5.0)
1″	4.41d (7.8)	4.42d (8.0)	-	-
2″	2.85dd (9.1, 7.8)	2.88dd (9.5, 8.0)	-	-
3″	3.3[c]	3.3[c]	-	-
4″	3.46ddd (10.2, 9.1, 5.6)	3.46m[d]	-	-
5″S	3.13dd (11.3, 10.2)	3.14dd (11.5, 11.5)	-	-
5″R	3.78dd (11.3, 5.6)	3.83dd (11.5, 5.5)	-	-
OCH_3	3.52s	3.55s		

* Spectra were determined in CD_3OD solution at 270 MHz (Bruker WX-270 spectrometer) except that of **2** which was recorded at 500 MHz[1]. Each spectrum also contained methyl doublets (J ranging from 6.7 to 7.1 Hz) for 21, 26 and 27-H at δ 0.89, 0.91 and 0.93 (**2**), 0.92, 0.94, 0.96 (**3**), 0.93, 0.94, 0.96 (**4**), 0.94, 0.94, 0.94 (**5**); the 24-H signal in each spectrum was under methanol signal. [a]Signal partially overlapped with 4″-H signal; [b]signal under 1″-H signal; [c]signal under methanol signal; [d]signal partially overlapped with 15α-H signal.

tion (4.2 Hz) with the 4α-proton resonating at δ 3.92 (bs, $W_{\frac{1}{2}}^{1}=7$). Oxidation with pyridinium dichromate in CH_2Cl_2 of heptaacetate **3a** gave a monoketone **3b**, whose ^{1}H NMR-spectrum[8] was devoid of the 4α-H signal and showed the 19-H signal at upfield position, δ 1.00, relative to **3a**, δ 1.22, thus giving evidence for the removal of a 1,3-diaxial methyl-hydroxyl interaction in the conversion **3a** → **3b**, consistent with a 4β-OH assignment in **3a** (and **3**).

The D-configuration of the 2-OMe-xylosyl and the L-configuration of the arabinosyl residues in **3** were established by using the same procedure used with nodososide (**1**)[4] and attenuatoside A-I (**2**)[1].

Acid methanolysis of attenuatoside B-I (**3**) followed by benzoylation with p-bromobenzoyl chloride and pyridine of the reaction mixture and TLC-SiO_2 separation in 30% ethyl ether/light petroleum, b.p. 40–70 °C, gave a) methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-arabinopyranoside, characterized by ^{1}H NMR which is described in the preceding paper[1], whose large positively split CD curve, $\Delta\varepsilon_{253}+90$, $\Delta\varepsilon_{236}-29$, A = +119 indicated that arabinose belong to the L series[9] and b) both the anomeric methyl 2-O-methyl-3,4-di-O-(p-bromobenzoyl)-xylopyranosides, characterized by ^{1}H NMR which are described in the preceding paper[1], whose negatively split CD curves (β-anomer; CD: 236/253, $\Delta\varepsilon+14/-36$, A = −50) established that 2-OMe-xylose belong to the D series[9].

Attenuatoside B-II, $[\alpha]_D-9.0°$ (c, 0.5 MeOH), was assigned the related structure **4** by simply comparing the ^{13}C and ^{1}H NMR-spectra with those of the previous glycoside **3** (tables 1 and 2). Assignements of the sugar carbon atoms have been made by comparing its spectrum with that of methyl-α-L-arabinofuranoside (C-1: 109.2, C-2: 81.8, C-3: 77.5, C-4: 84.9, C-5: 62.4 ppm)[10].

Attenuatoside C (**5**), $[\alpha]_D+4.7°$ (c, 0.5 MeOH), is isomeric with the previous monoglycoside **4**. The ^{1}H NMR-spectrum of attenuatoside C (table 2) was very similar to that of attenuatoside B-II (**4**), the major difference being at C-15 [δ 4.24 ddd (J = 11.0, 11.0, 4.5 Hz) vs 4.45 ddd (J = 6.0, 6.0, 1.8 Hz) and C-18 (0.99s vs 1.29s)]. This clearly suggested the 2 compounds be epimeric at C-15. The ^{13}C NMR of **5** differed from that of **4** at C-7 (51.4 vs 50.3), C-8 (75.3 vs 76.5), C-13 (44.6 vs 43.6), C-14 (66.9 vs 61.7), C-15 (68.9 vs 70.0), C-17 (54.8 vs 57.0) and C-18 (15.5 vs 16.4). The differences observed in the 2 spectra are close to those expected for 2 epimeric 15-hydroxysteroids based on the substituent effects recently published for hydroxysteroids[5,6]. In confirmation, attenuatoside C (**5**, 8β-OH, 15α-OH), unlike attenuatoside B-I (**3**) and B-II (**4**), did not react with phenylboronic anhydride. Indeed both the glycosides **3** and **4** formed monophenylboronates[11] in agreement with their 8β-OH 15β-OH stereochemistries.

The arabinose unit in both **4** and **5** belongs to the L series as shown by the large positively split CD curves of the methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-arabinopyranoside (amplitude + 120, in both cases) obtained from the monoglycosides by using the procedure described above.

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7 The heptaacetate **3a** was prepared by using an excess of acetic anhydride in pyridine at room temperature, ^{1}H NMR (270 MHz, $CDCl_3$), δ 0.86 (d, J = 6.7 Hz, 26- and 27-H), 0.93 (d, J = 7.0 Hz, 21-H), 1.22 (s, 19-H), 1.29 (s, 18-H), 2.03, 2.09 and 2.10 (singlets, 21H, $CH_3C=O$), 3.31 (m, overlapping with 5″-Hax, 24-H), 3.92 (bs, $W_{\frac{1}{2}}=7$ Hz, 4α-H), 4.66 (dt, J = 11.0 and 4.2 Hz, 3α-H), 5.12 (m, overlapping with 3″-H, 15α-H), 5.40 (ddd, J = 10.5, 10.5 and 3 Hz, 6β-H).

8 **3b**, ^{1}H NMR (270 MHz, $CDCl_3$), δ 0.86 (d, J = 6.7 Hz, 26- and 27-H), 0.93 (d, J = 7.0 Hz, 21-H), 1.00 (s, 19-H), 1.21 (s, 18-H), 2.03, 2.10, 2.11, 2.14 (singlets, 21H, $CH_3C=O$), 2,47 (d, J = 10.5, H-5), 3.31 (m, overlapping with 5″-Hax, 24-H), 5.10–5.20 (m, overlapping with 3″-H and 5′-H, 3α-H and 15α-H), 5.52 (ddd, J = 10.5, 10.5 and 3.0 Hz, 6β-H).

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11 The monophenylboronates of **3** and **4** were characterized by ^{1}H NMR-spectroscopy: **3**, δ 1.11 (3H, s, 18-H), 1.48 (3H, s, 19-H), 4.27 (1H, ddd, J = 10.5, 10.5, 4.0 Hz, 6β-H), 4.68 (1H, brt, J = 5.5 Hz, 15α-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: δ 7.35 (3H, m), 7.75 (2H, d, J = 7.5 Hz); **4**, δ 1.11 (3H, s, 18-H), 1.48 (3H, s, 19-H), 4.27 (1H, ddd, J = 10.5, 10.5, 4.0 Hz, 6β-H), 4.68 (1H, brt, J = 5.5 Hz, 15α-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: δ 7.35 (3H, m), 7.75 (2H, d, J = 7.5 Hz).

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Formation of microspheres from simple molecules under simulated primitive earth conditions; ultrasound and light radiation

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Summary. We have synthesized microspheres from aqueous solutions of simple molecules submitted to sonolysis at 20 kHz and 800 kHz in an argon atmosphere. Photochemical reaction under room light or UV-irradiation yielded the same microstructures, whose formation has been studied with optical and electronic microscopes.

For a number of years, it has been known that microspheres or microspherules are formed when simple molecules are exposed to the action of heat, UV-radiation or spark discharge. Fox and Dose[2], Labadie et al.[3], Yanagawa and Egami[4] synthesized microspheres by means of exposure to heat. Using formaldehyde as the starting material,