# Short Communications

## Starfish saponins. Part 9. A novel 24-O-glycosidated steroid from the starfish *Hacelia attenuata*[1,2]

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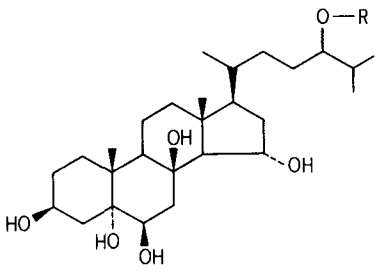
*Summary.* A novel steroidal glycoside has been isolated from the Mediterranean starfish *Hacelia attenuata.* Its structure includes a 5α-cholestane-3β,6α,8,15β,26ξ-pentol aglycone moiety and a sugar portion (2-O-methyl-β-D-xylopranosyl (1→2)-α-L-arabinofuranosyl), which is glycosidically attached at C-24 of the steroid.

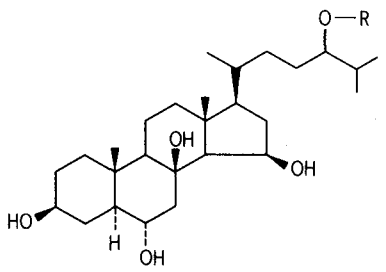
The occurrence of saponins in starfish has been known for a long time. From the chemical point of view they are steroidal glycosides, and up to now 3 different structural types have been encountered. The 1st type, which is of widespread occurrence, has a $\Delta^{9,11}$,3β,6α-dihydroxysteroidal moiety; the oligosaccharide moiety (4–6 sugar units, mostly fucose and quinovose) is attached at C-6 and a sulphate group is at C-3[5]. The 2nd structural type, recently discovered in 2 species of the genus *Echinaster,* has a $\Delta^{7}$,3β,6β-dihydroxysteroidal moiety, there is no sulphate group and, most remarkably, the carbohydrate chain, made up by 3 sugar units is cyclized between C-3 and C-6 of the aglycone[6]. The 3rd structural type is represented by nodososide, 24-O-[2-O-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]-5α-cholestane-3β,5,6β,8,15α,24ξ-hexol (**1**), very recently isolated from the Pacific starfish *Protoreaster nodosus*[2].

We now report the discovery of a further example of this novel group of 24-O-glycosidated steroidal glycosides in the Mediterranean starfish *Hacelia attenuata.* The lyophilized animals (1.2 kg, collected in the Bay of Naples) were extracted in a Soxhlet apparatus with light petroleum (b.p. 40–70 °C), then with methanol/chloroform (1:9), followed by methanol and water/methanol (1:1). The methanol/chloroform extract, which contained the steroidal glycoside **2**, gave 32 g of residue, which was chromatographed by preparative LC (Waters Associates LC/system 500 instrument on a prepak-500 $SiO_2$ column by using methanol/chloroform (1:9) and increasing methanol content to 20%). After TLC monitoring ($SiO_2$ with $CHCl_3$/MeOH/$H_2O$, 80:18:2) the fractions were combined to give 8 main fractions, A–H. Fraction D was rechromatographed on Sephadex LH-20 and 20 ml fractions were eluted using methanol as solvent. Fraction 17 gave 80 mg of the glycoside **2.** An additional quantity of **2** was obtained from the subsequent fractions 18–20 by HPLC on a μ-bondapack C-18 column (7.8 mm × 30 cm) using $H_2O$/methanol (3.5:6.5) to afford 42 mg of **2** (total yield 0.01%).

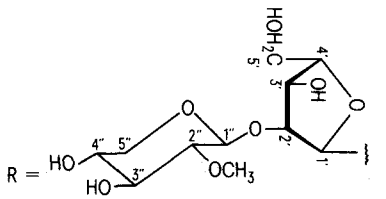
The glycoside **2**, named attenuatoside A-I, $[\alpha]_D^{20} = -20.6$ ($CH_3OH$; c = 1), did not crystallize. The FD mass spectrum (Hitachi M-80 double focus MS) showed a peak at m/e 753 $(M+Na)^+$, corresponding to the molecular formula $C_{38}H_{66}O_{13}$. On acid methanolysis **2** gave methyl arabinoside (GLC, OV-101, TMS-derivatives) and a 2nd methyl glycoside, while the aglycone was degraded to intractable material. The comparison of the $^1$H-NMR spectra of attenuatoside A-I (**2**) and nodososide (**1**) immediately indicated that the novel compound is related to **1** by having the same sugar moiety. We have assigned every sugar signal in the 500-MHz $^1$H-NMR spectrum ($CD_3OD$, Bruker Spectrospin) of **2** [2-O-methyl-β-D-xylopyranosyl: δ 4.408 (d, J = 7.8 Hz; 1-H″), 2.846 (dd, J = 9.1 and 7.8 Hz; 2-H″), circa 3.35 (under solvent signal, assignement confirmed by decoupling; 3-H″), 3.460 (ddd, J = 10.2, 9.1 and 5.6 Hz; 4-H″), 3.130 (dd, J = 11.3 and 10.2 Hz; 5-$H_{ax}$), 3.780 (dd, J = 11.3 and 5.6 Hz; 5-$H''_{eq}$). 3.524 (s; $OCH_3$); α-L-arabinofuranosyl residue: δ 5.706 (d, J = 1.1 Hz; 1-H′), 4.058 (dd, J = 4.0 and 1.1 Hz; 2-H′), 3.978 (dd, J = 7.8 and 4.0 Hz; 3-H′), 3.932 (ddd, J = 7.8, 5.1 and 2.9 Hz; 4-H′), 3.750 (dd, J = 12.5 and 2.9 Hz), 3.634 (dd, J = 12.5 and 5.1 Hz; 5-$H'_2$)] and they closely corresponded to those observed in the spectrum of **1**. Treatment of **2** with excess acetic anhydride in pyridine at room temperature produced a heptaacetate [7 $CH_3$–C=O at δ 2.03(×2), 2.05, 2.08(×3), 2.10] showing the 2-O-Me-xyl 2-H″ and arab 2-H′ signals essentially unshifted at δ 3.137 and δ 4.178, thus confirming the sequence and the interglycosidic linkgage of the sugar moiety as shown in **2** (and **1**). The comparison of the $^{13}$C-NMR of the novel **2** and **1** provided further corroborative evidence (table). The D-configuration of the 2-O-methyl-xylosyl and the L-configuration of the arabinosyl residues were established by using the same procedure used with nodososide (**1**)[2].




**1**




**2**




Acid methanolysis of attenuatoside A-I (**2**) followed by benzoylation with p-bromobenzoyl chloride and pyridine of the reaction mixture and TLC-$SiO_2$ separation in ethyl ether/light petroleum, b.p. 40–70 °C (3:7), gave methyl 2,3,4-tri-O-(p-bromobenzoyl)-β-L-arabinopyranoside (as major arabinoside component), $^1$H-NMR ($CDCl_3$, 270

MHz): 8.04–7.20 (m, 12-H, aromatic-H'S), 5.86 (dd; J = 10.2, 3.4 Hz, 3-H), 5.70 (br d; J = 3.4 Hz; 4-H), 5.61 (dd; J = 10.2, 3.4 Hz; 2-H), 5.17 (d; J = 3.4 Hz; 1-H), 4.12 (br d; J = 12.0 Hz; 5-$H_{eq}$), 3.90 (br d; J = 12.0 Hz; 5-$H_{ax}$), 3.34 (s, 3H; OMe); CD: 236/253, $\Delta\varepsilon$ −30/+90, A = +120 and both the anomeric methyl 2-O-methyl-3,4-di-O-(p-bromobenzoyl)-D-xylopyranosides, $\beta$-anomer, $^1$H-NMR ($CDCl_3$, 270 MHz): $\delta$ 7.88–7.48 (m, 8-H; aromatic-H's), 5.50 (dd, J = 8.8, 8.8 Hz; 3-H), 5.18 (br dt; J = 4.5, 8.8 Hz; 4-H), 4.40 (d, J = 6.2; 1-H), 4.25 (dd, J = 11.0, 4.5 Hz, 5-$H_{eq}$), 3.55 (s, OMe), 3.47 (s; OMe), 3.46 (br t; J = 11.0 Hz; 5-$H_{ax}$), 3.30 (dd; J = 8.8, 6.2; 2-H); CD: 236/253, $\Delta\varepsilon$ +14/−36, A = −50; $\alpha$-anomer, $^1$H-NMR ($CDCl_3$, 270 MHz): $\delta$ 7.97–7.47 (m, 8-H; aromatic-H's), 5.80 (br t; J = 8.6 Hz; 3-H), 5.20 (dt; 4.9, 8.8 Hz; 4-H), 4.94 (d, J = 3.8 Hz; 1-H), 3.95 (dd, J = 10.0, 4.9 Hz; 5-$H_{eq}$), 3.71 (t, J = 10.0 Hz, 5-$H_{ax}$), 3.52 (dd; J = 8.8, 3.8 Hz; 2-H), 3.48 (s; OMe), 3.42 (s; OMe).

The signs and amplitudes of the exciton-split CD curves accompanying the 2 structures established the D-configuration of the xylosides and the L-configuration of arabinoside[7].

The glycosyl residue accounts for $C_{11}H_{19}O_8$ out of the $C_{38}H_{66}O_{13}$ molecular formula, leaving $C_{27}H_{47}O_5$ for the aglycone moiety. $^{13}$C-NMR showed the absence of carbon-carbon double bonds. A saturated sterol with 5 hydroxyl groups (4 secondary and 1 tertiary indicated by the $^{13}$C-NMR single-frequency off-resonance decoupled spectrum) was thus a plausible candidate for a structure assignment. In agreement with a cholestane structure, the $^1$H-NMR of **2** showed 2 methyl singlets at $\delta$ 0.986 (19-H) and 1.266 (18-H) and 3 methyl doublets at $\delta$ 0.780 (J = 6.7 Hz), 0.912 (J = 6.7 Hz) and 0.910 (J = 6.9 Hz). The multiplet centered around 3.50 ppm has the complexity normally seen for the 3$\alpha$-proton of an A/B trans-3$\beta$-hydroxysteroid, while the shape (ddd, J = 11.0, 11.0 and 4.5 Hz) of the signal at $\delta$ 3.673 was suggestive of the axial proton associated with the 6$\alpha$-hydroxyl group[8]. Significant shifts were noted for both the angular methyl resonances of **2** when the spectrum was measured in pyridine-$d_5$ ($\delta$ 1.612 and 1.386; cf. 1.260 and 0.976 in $CD_3OD$), indicating that both the angular methyl groups were subjected to 1,3-diaxial interaction with hydroxyl groups. This suggested the location of the tertiary hydroxyl group at C-8. The 3$\beta$,6$\alpha$,8$\beta$-trihydroxy oxidation pattern in **2**, already encountered in a series of polyhydroxylated sterols from the starfish *Protoreaster nodosus*[9], was supported from the $^{13}$C-NMR chemical shifts of the carbons 1–12 and carbon 19 which are close to those of the corresponding atoms in 5$\alpha$-cholestane-3$\beta$,6$\alpha$,8,15$\alpha$,16$\beta$,26-hexol (table). The slight shift differences of carbons 7 and 8 for the 2 compounds can be attributed to the different stereochemistry at C-15. The 3rd secondary hydroxyl was located at C-15$\beta$ on the basis of a) the characteristics of the hydroxymethine signal in the $^1$H-NMR, 4.412 (ddd, J = 5.1, 5.1 and 1.7 Hz)[8], b) the downfield position of the 18-protons ($\delta$ 1.26)[10], c) the $^{13}$C-NMR data, which are consistent with a 15$\beta$-OH substitution[11] and d) the formation of a phenylboronate[12] when **2** was treated with phenyl boronic anhydride, which requires the sec-hydroxyl group be situated at 15$\beta$-position.

The position of the 4th secondary hydroxyl group, which must be the site of glycosidation, remained to be established. The comparison of the $^{13}$C-NMR spectra of the novel glycoside **2** and nodososide **1** (table) immediately indicated that the remaining secondary hydroxyl group is at C-24 and confirmed that it is the site of glycosidation.

$^{13}$C-NMR data

| Carbons | | 5$\alpha$-cholestane-3$\beta$,6$\alpha$,8,15$\alpha$,16$\beta$,26-hexol[9] | **2** | **1**[2] |
|---|---|---|---|---|
| 1 | | 39.1 | 39.2 | |
| 2 | | 32.0 | 32.2 | |
| 3 | | 71.2 | 71.4 | |
| 4 | | 33.2 | 33.2 | |
| 5 | | 53.7 | 54.0 | |
| 6 | | 66.5 | 66.5 | |
| 7 | | 50.9 | 50.0 | |
| 8 | | 75.9 | 76.7 | |
| 9 | | 56.8 | 56.8 | |
| 10 | | 37.2 | 37.4 | |
| 11 | | 19.0 | 19.3 | |
| 12 | | 42.6 | 42.6 | |
| 13 | | | 43.7 | |
| 14 | | | 61.8 | |
| 15 | | | 70.2 | |
| 16 | | | 42.2 | |
| 17 | | | 57.2 | |
| 18 | | | 16.6 | |
| 19 | | 14.4 | 14.3 | |
| 20 | | | 35.6 | 35.4 |
| 21 | | | 18.9 | 18.9 |
| 22 | | | 32.1 | 31.9 |
| 23 | | | 28.1 | 27.9 |
| 24 | | | 83.3 | 83.5 |
| 25 | | | 30.8 | 30.6 |
| 26 | | | 18.1 | 18.2 |
| 27 | | | 18.2 | 18.2 |
| arab | 1′ | | 107.5 | 107.6 |
| | 2′ | | 92.8 | 93.1 |
| | 3′ | | 77.6 | 77.6 |
| | 4′ | | 85.0 | 85.0 |
| | 5′ | | 62.7 | 62.5 |
| 2-O-Me-xyl | 1″ | | 105.1 | 105.2 |
| | 2″ | | 84.3 | 84.1 |
| | 3″ | | 77.8 | 77.8 |
| | 4″ | | 71.1 | 71.0 |
| | 5″ | | 67.1 | 67.1 |
| $OCH_3$ | | | 60.6 | 60.7 |

The spectra were recorded in pyridine-$d_5$ solution on a Bruker WX-270 or WX-300 spectrometer. Chemical shifts are given in ppm with respect to TMS used as an internal standard. Assignments were confirmed by using single frequency off-resonance decoupling technique.

1 This contribution is part of the Progetto Finalizzato 'Chimica fine e secondaria' del C.N.R., Roma.

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3 Acknowledgments. We thank Professor K. Nakanishi (Columbia University, New York) for FD-mass spectral analyses, the Bruker spectrospin (Karlsruhe) for 500 MHz NMR spectral analyses and Dr T. McCabe (Cornell University, Ithaca, USA) for some $^{13}$C-NMR spectra. We are also grateful to Miss R. Aquino for part of the experimental work.

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12 The monophenylboronate was characterized by $^1$H-NMR spectroscopy: $\delta$ 1.12 (3H, s, 18-H), 1.31 (3H, s, 19-H) and 4.68 (1H, dt, J = 5.5 Hz, 15$\alpha$-H); the remaining hydroxymethine signals as well as the sugar signals remained essentially unshifted; aromatics: $\delta$ 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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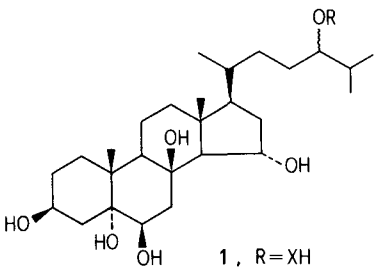
*Summary.* On the basis of comparative spectral data, the structures of 3 novel steroidal glycosides from the Mediterranean starfish *Hacelia attenuata* have been elucidated as **3, 4** and **5.** These are further examples of a novel group of 24-O-glycosidated steroids recently encountered in the same species and in the Pacific species *Protoreaster nodosus.*

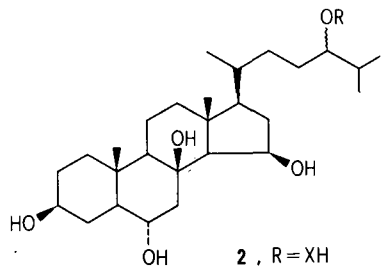
Recently we have isolated 2 novel steroidal glycosides, nodososide, 24-O-[2-O-methyl-$\beta$-D-xylopyranosyl-(1→2)-$\alpha$-L-arabinofuranosyl]-5$\alpha$-cholestane-3$\beta$,5,6$\beta$,8,15$\alpha$,24$\xi$-hexol (**1**), from the Pacific starfish *Protoreaster nodosus*[4], and attenuatoside A-I, 24-O-[2-O-methyl-$\beta$-D-xylopyranosyl)-(1→2)-$\alpha$-L-arabinopyranosyl]-5$\alpha$-cholestane-3$\beta$,6$\alpha$,8,15$\beta$,24$\xi$-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

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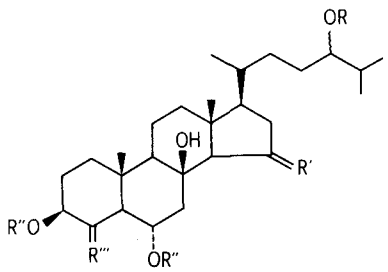


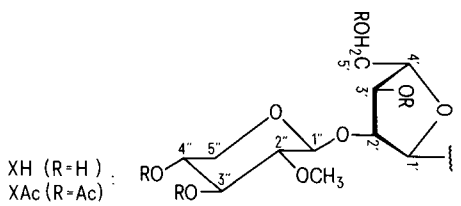


**3**, R = XH, R′ = R‴ = $\alpha$-OH, R″ = H

**3a**, R = XAc, R′ = $\alpha$-OAc, R″ = Ac, R‴ = $\alpha$-OH

**3b**, R = XAc, R′ = $\alpha$-OAc, R′ = Ac, R‴ = O

**4**, R = $\alpha$-L-arabinofuranosyl, R′ = R‴ = $\alpha$-OH, R″ = H

**5**, R = $\alpha$-L-arabinofuranosyl, R′ = $\beta$-OH, R″ = H, R‴ = $\alpha$-OH