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STARFISH SAPONINS, PART 35.[1] TWO NOVEL STEROIDAL XYLOSIDE SULFATES FROM THE STARFISH *MARTHASTERIAS GLACIALIS*

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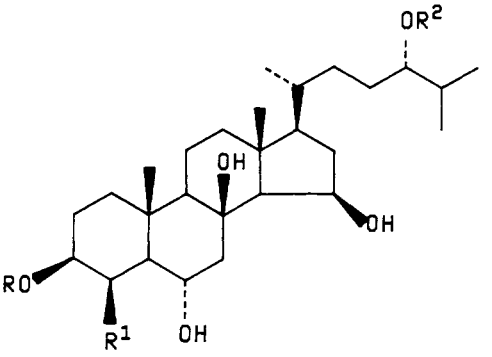
The chemical and biological studies of the saponins of *Marthasterias glacialis* L. (Forcipulata, Asteriidae), a very common starfish in the Mediterranean Sea, have continued for a long time. In 1970 Mackie and Turner (1) reported that the steroid glycosides of *M. glacialis* consist mainly of two components, M_1 and M_2, glycoside M_2 being the most active in eliciting the avoidance reaction of the mollusk *Buccinum undatum*. The same group determined the structures of the principal aglycones obtained by prolonged acid hydrolysis of the saponin mixture, marthasterone [3β,6α-dihydroxy-5α-cholesta-9(11),24-diene-23-one], and 24(25)-dihydromarthasterone (2). Partial hydrolysis gave marthasterone-6α-*O*-β-D-glucopyranoside (3). In a more recent investigation the asterosaponin mixture of *M. glacialis* was resolved into four major individual components, marthasterosides A_1, A_2, B, and C (4) and their structures completely determined (5). Marthasterosides A_1 and A_2 are hexaglycosides of the common thornasterol A 3-*O*-sulfate, and marthasterosides B and C are pentaglycosides of 3β-sulfomarthasterone and 3β-sulfodihydromarthasterone, respectively.

Continuing with our work on biologically active compounds from echinoderms (6), we have re-investigated the extractives from the whole bodies of *M. glacialis* and have now isolated, in small amounts, two new sulfated steroidal monoglycosides, **1** and **2**, named glacialosides A and B.

Fabms (negative-ion mode) of **1** showed a molecular anion peak at *m/z* 663. Upon solvolysis using dioxane/pyridine, **1** was desulfated to **1a**, which gave a quasi molecular ion at *m/z* 583 $[M-H]^-$. Elimination of 132 mass units (= pentose unit) from $[M]^-$ in the spectrum of **1**, *m/z* 531, indicated that the natural compound is a glycoside of a sulfated steroid aglycone. The mol wt of



1 R=β-xylopyranosyl, R^1=H, R^2=$SO_3^-Na^+$
1a R=β-xylopyranosyl, R^1=H, R^2=H
2 R=$SO_3^-Na^+$, R^1=OH, R^2=β-xylopyranosyl
2a R=H, R^1=OH, R^2=β-xylopyranosyl

[1]For part 34, see Riccio *et al.* (11).

the steroid is 452, corresponding to a molecular formula of $C_{27}H_{48}O_5$ (pentahydroxycholestane).

The ^1H nmr spectrum (250 MHz, CD_3OD) of **1**, with signals at δ 4.39 (1H, d, $J=7.0$ Hz, 1′-H), 3.17 (1H, dd, $J=7$, 9 Hz, 2′-H), 3.35 (1H, partially obscured by the solvent signal, 3′-H), 3.50 (1H, m, 4′-H), 3.20 (dd, $J=9$, 11 Hz, 5′-Hax) and 3.85 (dd, $J=5$, 11 Hz, 5′-Heq), and its ^{13}C nmr spectrum (Table 1) indicated that the molecule bears a β-xylopyranosyl moiety.

The ^1H nmr signals for the steroid aglycone at δ 0.95 (3H, d, $J=6.5$ Hz; 26- or 27-Me), 0.96 (3H, d, $J=6.5$ Hz; 27- or 26-Me), 0.98 (3H, d, $J=6.5$ Hz, 21-Me), 1.02 (3H, s, Me-19), 1.29 (3H, s, Me-18), 2.40 (1H, dd, $J=$ 12.5, 4 Hz, 7β-H), 2.40 (1H, m, overlapping with 7β-H, 16β-H), 3.55 (1H, m, 3α-H), 3.75 (1H, dd, $J=4$, 10 Hz, 6β-H), 4.14 (1H, q, $J=6.5$ Hz, 24-H), and 4.45 (1H, m, 15α-H) ppm, were suggestive of a 3β,6α,8,15β,24-pentahydroxycholestane structure (7), with the sulfate group located at C-24.

TABLE 1. ^{13}C-nmr Shifts (δ ppm) of Sulfated Steroidal Glycosides **1** and **2** and Reference Polyhydroxysteroids.[a] (Pertinent Shifts Discussed in the Text are in Italics).

Carbon	Compound			
	1	Reference steroid[b]	**2**	Reference steroid[c]
1	39.4	39.5	39.8	39.7
2	*29.8*	31.5	*24.0*	26.3
3	*79.7*	72.2	*81.9*	73.7
4	*28.6*	32.4	67.6	69.1
5	53.6	54.0	58.5	57.4
6	67.4	67.7	64.7	64.8
7	49.4	49.8	50.0	49.8
8	77.5	77.5	77.4	77.4
9	57.4	57.6	57.3	58.0
10	38.1	38.0	38.1	38.1
11	19.7	19.8	19.0	19.0
12	43.4	43.5	43.4	43.4
13	44.4	44.5	44.4	44.0
14	62.5	62.8	62.9	62.9
15	71.1	71.2	71.4	71.2
16	42.4	42.4	42.4	42.4
17	58.0	58.1	58.0	58.5
18	16.5	16.5	16.5	16.5
19	14.0	14.1	17.0	16.9
20	36.3	36.4	36.3	36.4
21	18.9	19.1	19.2	19.1
22	32.1	33.4	32.8	33.3
23	*28.0*	31.8	*28.9*	31.7
24	*85.9*	78.2	*86.4*	78.2
25	*31.8*	34.5	*32.0*	34.5
26	18.5	19.4	18.4	19.2
27	17.9	17.5	18.3	17.4
1′	103.1		105.0	
2′	75.0		75.4	
3′	77.9		78.0	
4′	71.3		71.2	
5′	66.8		66.8	

[a]Spectra were run in CD_3OD; δ-values relative to $CD_3OD = 49$ ppm (central peak).

[b](24*S*)-5α-cholestane-3β,6α,8,15β,24-pentol (7).

[c](24*S*)-5α-cholestane-3β,4β,6α,8,15β,24-hexol (7).

The upfield shift of the 24-H signal from 4.14 in **1** to 3.24 ppm in **1a** confirmed the location of the sulfate group. Analysis of the ^{13}C-nmr spectrum (Table 1) of **1** and comparison with that of 5α-cholestane-3β,6α,8,15β,24-pentol (7) established the location of the xylopyranosyl residue at C-3. Significantly, the signal for C-3 in **1** was shifted down-field by 7.5 ppm to 79.7, while those for C-2 and C-4 were shifted up-field by 1.7 and 3.5 ppm, to 29.8 and 28.9 ppm, respectively [cf. glycosidation shift (8–10)].

In order to determine the configuration at C-24, the (+)-methoxytrifluoromethylphenylacetate (MPTA ester) of **1a** was prepared. The shifts of the isopropyl methyl protons of the (+)-(*R*)-MTPA ester (two doublets at δ 0.85 and 0.87) match those found in the (+)-(*R*)-MTPA ester of (24*S* -6β-methoxy-3α,5-cyclo-5α-cholestan-24-ol (δ 0.83, 0.85) (11). In the spectrum of the (24*R*)-model epimeric (+)-(*R*)-MTPA ester the resonances of the isopropyl methyl protons were seen as a 6H doublet shifted down-field to δ 0.91 (11). Based on these data the 24*S* stereochemistry was assigned to glacialoside A [**1**].

Fabms (negative ion mode) of **2** showed a molecular anion peak at *m/z* 679, shifted 16 mass units relative to **1**. Upon solvolysis using dioxane/pyridine, **2** was desulfated to **2a**, which gave a quasi molecular ion at *m/z* 599 $[M-H]^-$.

An examination of its spectral data [^{1}H (sugar signals superimposable with those of **1**) and ^{13}C (Table 1) nmr] indicated that **2** contains the same β-xylopyranosyl unit as **1**. In addition to the sugar moiety, the ^{1}H-nmr spectrum of **2** in CD_3OD showed five methyl signals at δ 0.95 (3H, d, $J=6.5$ Hz, sec-Me), 0.97 (6H, d, $J=6.5$ Hz, sec-Me's), 1.20 (3H, s, Me-19) and 1.28 (3H, s, Me-18), and methine signals at δ 2.40 (1H, m, 16β-H), 2.46 (1H, dd, $J=12.5$, 4 Hz, 7β-H), 3.37 (partially obscured by the solvent signal, 24-H), 4.18 (1H, dt, $J=4$, 10.5 Hz, 6β-H), 4.20 (1H, m, $W½=20$ Hz, 3α-H), and 4.63 (1H, m, $W½=9$ Hz, 4α-H).

Based on these nmr spectral data and comparison with those of 5α-cholestane-3β,4β,6α,8,15β,24-hexol (7), the 3β,4β,6α,8,15β,24-hexahydroxycholestane structure with the sulfate group located at C-3 was suggested for the steroid aglycone of **2**.

The location of the sulfate at C-3 was indicated by the downfield shift exhibited by the signals for 3-H and 4-H, δ 4.20 and 4.63 vs. 3.50 and 4.30 in the model non-sulfated steroid (7), respectively. This was confirmed by the ^{13}C-nmr spectrum (Table 1), which also established the location of the β-xylopyranosyl residue at C-24. All the signals assigned to the side chain carbon atoms were virtually identical with those assigned to the same carbons in the spectrum of amurensoside A, (24*S*)-24-*O*-β-D-xylopyranosyl-5α-cholestane-3β,6α,15α,24-tetraol, isolated from the starfish *Asterias amurensis* (11). On this basis we also assign the 24*S*-stereochemistry to glacialoside B [**2**] from *M. glacialis*.

EXPERIMENTAL

INSTRUMENTAL.—For instruments used, see Riccio *et al.* (11).

EXTRACTION AND ISOLATION.—The animals (9 specimens, ca. 400 g each), *M. glacialis*, were collected in the Bay of Naples. A specimen is deposited at Dipartimento di Chimica delle Sostanze Naturali, University, Naples. Details of the extraction recovery of the polar materials from the aqueous extracts by chromatography of Amberlite XAD-2 and fractionation on a column of Sephadex LH-60 are reported in Dini *et al.* (4). The first eluted fractions from the column of Sephadex LH-60 contained a mixture of "asterosaponins" (3.07 g). The last eluted fractions containing a complex mixture including **1** and **2** were combined (2.4 g) and purified by dccc [*n*-BuOH-Me_2CO-H_2O (3:1:5)] in ascending mode; that is, the lower phase was used as the stationary phase. Fractions of 4 ml were collected and monitored by tlc in *n*-BuOH-HOAc-H_2O (60:15:25). Fractions 170–250 were then chromatographed by hplc on a C_{18} μ-Bondapak column (30 cm × 8 mm i.d.) two successive times to give glacialoside A [**1**] (7.6 mg), $[\alpha]_D$ +3° ($c=0.7$, MeOH), and glacialoside B [**2**] (1.4 mg), $[\alpha]_D$ +2° ($c=0.1$, MeOH). The results of fabms and ^{1}H-nmr spec-

troscopy are in the text; ^{13}C nmr data are in Table 1.

SOLVOLYSIS OF **1** AND **2**.—A solution of 7 mg of compound **1** in dioxane (0.5 ml) and pyridine (0.5 ml) was heated at 120° for 2 h in a stoppered reaction vial. After the solution had cooled, the solvents were removed under reduced pressure, and the residue was purified by hplc [C_{18} μ-Bondapak; MeOH-H_2O (75:25)] to give the desulfated compound **1a**, negative ion fabms, *m/z* $[M-H]^-$ 583 (100%), 451 (30); ^1H nmr (CD_3OD) virtually unshifted with respect to **1** except the signals for 24-H, 26-H_3, and 27-H_3 up-field shifted to δ 3.24 m, 0.92 d ($J=6.5$ Hz), and 0.94 d ($J=6.5$ Hz), respectively.

Compound **1a** (3 mg) was then treated with freshly distilled (+)-methoxytrifluoromethylphenylacetyl chloride (40 μl) in 0.1 ml dry pyridine at room temperature for 4 h. After solvent removal, the product was eluted through a Pasteur pipet filled (5 cm) with a slurry of Si gel in $CHCl_3$ to give a fully esterified derivative, ^1H nmr ($CDCl_3$) δ 7.57, 7.43, and 7.37 (Ph-H's), 4.95 (1H, m, 24-H), 0.90 (3H, d, $J=6$ Hz, 21-H_3), 0.87 and 0.85 (each 3H, d, $J=7$ and 6.5 Hz, 26-H_3 and 27-H_3).

A solution of 1 mg of compound **2** was treated with dioxane and pyridine as above. Removal of solvents gave a residue which, without further purification, was submitted to fabms, *m/z* $[M-H]^-$ 599 (100%), 467 (30).

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