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STARFISH SAPONINS, PART 47.[1] STEROIDAL GLYCOSIDE SULFATES AND POLYHYDROXYSTEROIDS FROM *APHELASTERIAS JAPONICA*

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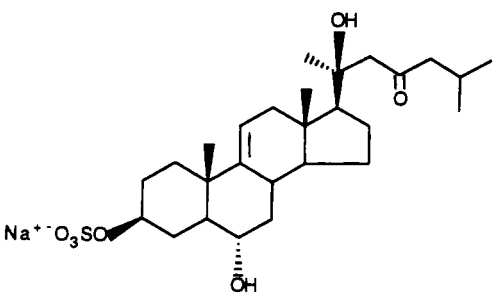
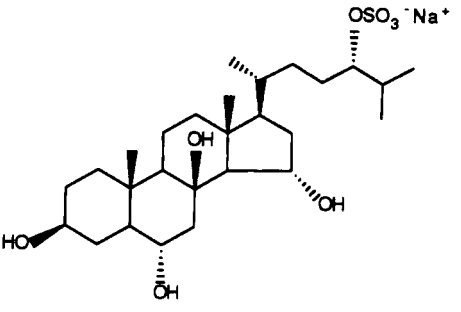
ABSTRACT.—Two novel steroidal xyloside sulfates, aphelasterosides A [**3**] and B [**5**], and two novel polyhydroxysteroids **2** and **7** have been isolated from the starfish *Aphelasterias japonica*. Their structures were elucidated mainly by spectroscopic methods.

Continuing our investigation of biologically active steroidal compounds from echinoderms, we have isolated two novel steroidal xyloside sulfates, aphelasterosides A [**3**] and B [**5**], and two novel polyhydroxysteroids **2** and **7**, along with the known sulfate **1**, from the MeOH extracts of the starfish *Aphelasterias japonica* Bell (family Asteriidae).

The negative ion fabms of compound **1** showed a molecular anion peak at *m/z* 511, and the ^{1}H-nmr spectrum revealed signals identical with those of the aglycone protons of many asterosaponins containing 3-*O*-sulfothornasterol A aglycone. So the structure



1



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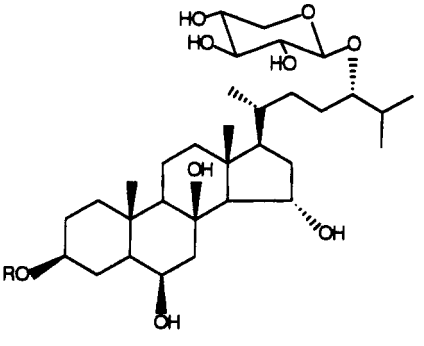


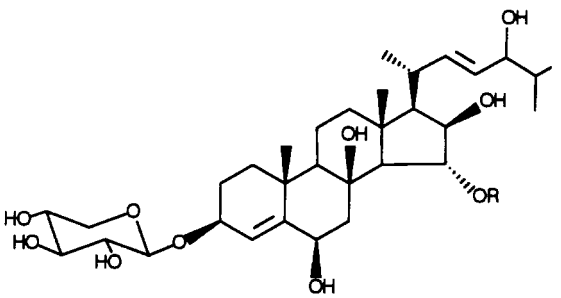
3 R=SO$_3$ $^-$Na$^+$
4 R=H

[1]For Part 46, see M. Iorizzi, L. Minale, R. Riccio, T. Higa, and J. Tanaka, *J. Nat. Prod.*, **54**, 1254 (1991).

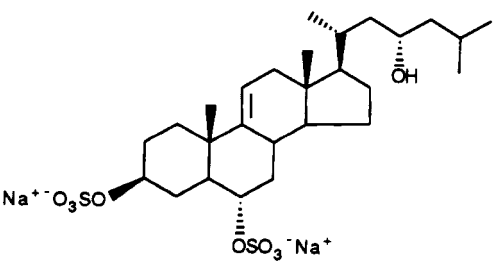
of the compound **1** is 3β,6α,20-trihydroxy-5α-cholest-9(11)-en-23-one-3-sulfate, also found as a non-glycosidal constituent of *Asterias amurensis* (1).

The negative ion fabms of compound **2** showed a molecular anion peak at *m/z* 531. Comparison of ^{1}H-nmr data with those reported for (24*S*)-5′-*O*-sulfate 24-*O*-(3-*O*-methyl-L-arabinofuranosyl)-5α-cholesta-3β,6α,8,15α,24-pentaol (asterosaponin P_1), previously isolated from *Patiria pectinifera* (2) and *Oreaster reticulatus* (3), clearly indicated a 3β,6α,8,15α,24-pentahydroxycholestane structure for the new compound. The downfield shift of the H-24 proton to δ 4.14 in **2**, when compared with the signal at δ 3.30 in 24-hydroxysteroids, established the presence of a sulfate group there. Thus compound **2** was identified as the new compound 5α-cholesta-3β,6α,8,15α,24-pentaol-24-sulfate, although the non-sulfated steroid has previously been described from *Asterina pectinifera* (4). The 24*S* stereochemistry is suggested by analogy with the many (24*S*)-24-hydroxy steroids isolated from starfishes.

The negative ion fabms of compound **3** showed a molecular anion peak at *m/z* 663. A detailed comparison of the ^{1}H- and ^{13}C-nmr data of **3** (see Experimental) with those of coscinasteroside B (5) [i.e., (24*S*)-24-*O*-(β-D-xylopyranosyl)-5α-cholesta-3β,6β,8,15α,24-pentaol-15-sulfate] indicated compound **3** differed from coscinasteroside B only in the location of the sulfate group. The downfield shift of the H-3 signal from δ 3.50 (m) in coscinasteroside B to 4.22 in **3** and the upfield shift of the H-15 signal from δ 4.90 (td 10, 3 Hz) to δ 4.30 in **3** established the location of the sulfate in **3** at C-3. Acid methanolysis of **3** confirmed the presence of a xylosyl moiety, and analysis of the ^{13}C-nmr spectrum of **3** confirmed the location of a xylopyranosyl residue at C-24 and of a sulfate group at C-3. On solvolysis in dioxane/pyridine the steroid afforded a desulfated derivative **4**, which gave a quasi molecular ion at *m/z* 583 $[M-H]^-$ and a major fragment at *m/z* 451 $[M-H-132]^-$ (loss of pentasaccharide unit). Compound **4** was identical (^{1}H nmr, hplc, tlc) with desulfated coscinasteroside B. Once again the 24*S* configuration is suggested by analogy with the many (24*S*)-24-hydroxylosides from starfishes.



5 R=$SO_3^-Na^+$
6 R=H



7

The negative ion fabms of compound **5** showed a molecular ion at *m/z* 675 $[M-H]^-$. On solvolysis using dioxane/pyridine, the steroid afforded a desulfated derivative with a quasi molecular ion at *m/z* 595 $[M-H]^-$ and a major fragment at *m/z* 463 $[M-H-132]^-$. These data indicated that the natural compound is a glycoside of a sulfated steroidal aglycone. Analysis of spectral data suggested the presence of a β-xylopyranosyl unit, and this was confirmed by acid methanolysis and glc analysis of the resulting methyl xylosides. The ^{1}H-nmr spectrum assisted by the decoupling results indicated a steroidal nucleus with a Δ^4,3β,6β,8,15α,16β pentahydroxylation pattern and a sulfate at the common 15α position previously observed in echinasteroside A (6). The same data also indicated the presence of a 24-hydroxy Δ^{22E} side chain (7).

The negative ion fabms of compound **7** exhibited molecular ion species at *m/z* 577 and 599 (major) corresponding to $[M(SO_3H)SO_3]^-$ and $[M(SO_3Na)SO_3]^-$, respectively. Intense fragmentation peaks at *m/z* 497 and 479 were interpreted as losses of $NaSO_3$ (+H) and $NaHSO_4$, respectively, from *m/z* 599. The ^{13}C-nmr spectrum measured in CD_3OD at 62.9 MHz (DEPT measurement) indicated the presence of 27 carbon atoms and revealed the presence of three methyl groups, nine methylenes, eight methines, two quaternary carbons, three-OCH<, and one -C=CH. Taken together, these data indicated a disulfated trihydroxycholestene structure. The presence in the 250-MHz ^{1}H-nmr spectrum of **7** of two singlets at δ 0.70 (Me-18) and 1.07 (Me-19), two multiplets at 3.75 and 4.23, one double triplet (J = 3, 12 Hz) at 4.35, and a broad olefinic doublet at 5.40 (J = 5 Hz) was suggestive of a 5α-cholesta-9(11)-3β,6α,diol-3,6-disulfate with one hydroxyl group located in the side chain. This structure was supported by ^{13}C-nmr signals at δ 79.6, 78.5, and 67.8 assigned to C-3, C-6, and C-23, respectively (8). COSY and H-C correlation experiments (Table 1) definitively located the hydroxyl group in the side chain at the C-23 position. The 23*S* configuration has been suggested by the upfield shift of the Me-18 signal in the ^{1}H-nmr of the benzoate derivative (9).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were measured on a Perkin-Elmer Model 243 B polarimeter. The fab mass spectra were recorded on a Kratos MS 50 mass spectrometer by dissolving the samples in a glycerol matrix and placing them on a copper probe tip prior to bombardment with argon atoms of energy 2–6 Kv. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WM-250 spectrometer. Hplc separation was made on a μ-Bondapak C_{18} column (30 cm × 7.8 mm i.d.) using a differential refractometer detector, model 401, a U6K injector, and a solvent delivery system, Model 6000A, all from Waters Associates. COSY HETCOR were obtained on a Bruker AMX-500 spectrometer by employing the conventional pulse sequences. The COSY spectra were obtained using a data set ($t_1 \times t_2$) of 512 × 2048 points for a spectral width of 2890 Hz; the HETCOR spectra were carried out with quadrature detection in F_1 using a data matrix of 128 × 2048 points and spectral widths of 19,230 and 2793 Hz for the ^{13}C (F_2) and ^{1}H (F_1) chemical shift domains, respectively.

EXTRACTION AND ISOLATION.—The animals, *Aph. japonica* (whole body, 340 g) were collected in Mutsu Bay, Aomori Prefecture, Japan, in March 1987; a voucher specimen is preserved at Dipartimento di Chimica delle Sostanze Naturali. The MeOH eluate (1.5 g) from the Amberlite XAD-2 column was chromatographed on a column of Sephadex LH-60 (4 × 80 cm, 100 g) using MeOH-H_2O (2:1) as eluent. The flow rate was 30 ml/h. The eluents were collected in 10-ml fractions and monitored by tlc on silica precoated glass sheets (Merck) with *n*-BuOH–HOAc–H_2O (12:3:5) and detection with ceric sulfate/H_2SO_4.

Fractions 99–117 (288 mg) contained the crude polyhydroxylated sterol sulfates and glycoside sulfates. Fractionation was continued by dccc with *n*-BuOH–Me_2CO–H_2O (3:1:5) (ascending mode, the lower phase was used as stationary phase, flow 24 ml/h); 6-ml fractions were collected and checked by tlc on silica with *n*-BuOH–HOAc–H_2O (12:3:5) to give five main fractions.

Fractions 86–134 (27 mg) contained compound **1**; fractions 135–193 (50 mg) contained compounds **2** and **3**; fractions 194–222 (55 mg) contained compound **3**; fractions 223–318 (76 mg) contained compound **5**; fractions 319–370 (100 mg) contained compound **7**. All fractions were finally separated by hplc on C_{18} μ-Bondapak column (30 cm × 7.8 mm i.d.) using MeOH-H_2O (1:1). The mixtures were dissolved

TABLE 1. ^{1}H- and ^{13}C-nmr Data (CD_3OD) of Compounds **3** and **7**.

Position	Compound		
	3	**7**[a]	
	δC	δC	δH ppm (H,H′)
1	41.4 t	36.9 t	1.80, 1.55
2	29.3 t	29.3 t	1.97, 1.45
3	80.1 d	79.6 d	4.23
4	32.1 t	31.3 t	2.55, 1.50
5	49.0 d	48.0 d	1.35
6	74.0 d	78.5 d	4.38
7	45.4 t	40.9 t	2.62, 1.05
8	76.9 s	37.0 d	2.20
9	57.1 d	146.6 s	
10	36.7 s	39.6 s	
11	19.8 t	118.1 d	5.40
12	42.8 t	43.2 t	2.25, 2.05
13	45.4 s	42.5 s	
14	66.6 d	55.1 d	1.40
15	70.2 d	26.3 t	1.80, 1.20
16	41.6 t	29.6 t	2.20, 1.65
17	55.8 d	58.5 d	1.15
18	15.4 q	12.1 q	0.70
19	15.7 q	19.7 q	1.07
20	36.2 d	33.4 d	1.72
21	18.9 q	18.9 q	1.00
22	33.9 t	45.7 t	1.50, 1.00
23	27.8 t	67.8 d	3.75
24	85.4 d	47.8 t	1.38, 1.15
25	32.5 d	25.8 d	1.76
26	18.6 q	23.6 q	0.94
27	18.7 q	22.7 q	0.94
Xylose			
1	104.3 d		
2	75.2 d		
3	78.0 d		
4	71.4 d		
5	66.8 t		

[a]Assignments made by COSY and H-C correlation experiments.

in MeOH (ca. 0.5 ml/100 mg), and the solution was added to an equal volume of saturated NaCl. This solution was applied to the column (ca. 20 mg mixture for each injection).

The total amounts of polyhydroxylated sterols were: **1**, 0.5 mg; **2**, 0.5 mg; **7**, 4.9 mg.

The total amounts of glycosides sulfates were: **3**, 1.4 mg; **5**, 3.6 mg.

Compound **1**.—[α]D −2.2° (*c* = 0.5, MeOH); negative ion fabms *m/z* $[M]^-$ 511 (100%); ^{1}H nmr (CD_3OD) δ 0.81 (3H, s, H_3-18), 0.94, 0.95 (6H, two d, *J* = 6.5 Hz, H_3-26, -27), 1.02 (3H, s, H_3-19), 1.37 (3H, s, H_3-21), 2.41 (2H, d, *J* = 7.5 Hz, H-24), 2.60 (2H, ABq, *J* = 15 Hz, H_2-22), 3.54 (1H, dt, *J* = 12, 3 Hz, H-6β), 4.22 (1H, m, H-3α), 5.37 (1H, bd, *J* = 5 Hz, H-11). Hplc retention time (5.0 ml/min) 7.2 min.

Compound **2**.—[α]D +23.5° (*c* = 0.5, MeOH); negative ion fabms *m/z* $[M]^-$ 531 (100%); ^{1}H nmr (CD_3OD) δ 0.95 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.96 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 0.99 (3H, d, *J* = 6.5 Hz, H_3-21), 1.00 (3H, s, H_3-18), 1.05 (3H, s, H_3-19), 2.41 (1H, td, *J* = 3, 12 Hz, H-6β), 3.53 (1H, m, H-3α), 3.65 (1H, td, *J* = 3, 2 Hz, H-6β), 4.14 (1H, q, *J* = 6.5 Hz, H-24), 4.23 (1H, td, *J* = 10, 3 Hz, H-15β). Hplc retention time (3.0 ml/min) 18.0 min.

Compound **3**.—[α]D −6.0° (*c* = 0.5, MeOH); negative ion fabms *m/z* $[M]^-$ 663 (100%); ^{1}H nmr (CD_3OD) δ 0.92 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.93 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 0.99 (3H, s, H_3-18), 1.20 (3H, s, H_3-19), 2.43 (1H, dd, *J* = 5, 12 Hz, H-7), 3.90 (1H, bs, H-6α), 4.22 (1H, m, H-3α), 4.30 (1H, td, *J* = 10, 3 Hz, H-15β); sugar 3.17 (1H, dd, *J* = 7, 9 Hz, H-2′), 3.20 (1H, dd, *J* = 9, 11 Hz, H_{ax}-5′), 3.35 (1H, under solvent signal, H-3′), 3.50 (1H, m, H-4′), 3.84 (1H, dd, *J* = 5, 11 Hz, H_{eq}-5′), 4.27 (1H, d, *J* = 7 Hz, H-1). Hplc retention time (3.0 ml/min) 25.2 min.

Compound **5**.—[α]D −16.8° (*c* = 1, MeOH); negative ion fabms *m/z* $[M]^-$ 675 (100%); ^{1}H nmr (CD_3OD) δ 0.88 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.94 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 1.08 (3H, d, 6.5 Hz, H_3-21), 1.25 (3H, s, H_3-18), 1.39 (3H, s, H_3-19), 2.69 (1H, dd, *J* = 5, 12 Hz, H-7), 3.70 (1H, t, *J* = 8 Hz, H-24), 4.33 (1H, bs, H-6α), 4.82 (1H, dd, *J* = 12, 2.5 Hz, H-15β), 5.47 (1H, dd, *J* = 15, 8 Hz, H-22); sugar 3.17 (1H, dd, *J* = 7.9 Hz, H-2′), 3.20 (1H, dd, *J* = 9, 11 Hz, H_{ax}-5′), 3.35 (1H, under solvent signal H-3′), 3.50 (1H, m, H-4′), 3.86 (1H, dd, *J* = 5, 11 Hz, H_{eq}-5′), 4.40 (1H, d, *J* = 7.0 Hz, H-1′). Hplc retention time (3.0 ml/min) 26.4 min.

Compound **7**.—[α]D +24.2° (*c* = 1, MeOH); negative ion fabms *m/z* $[M(SO_3Na)SO_3]^-$ 599 (80%), $[M(SO_3H)SO_3]^-$ 577 (50%), $[599 - NaSO_3 + H]^-$ 497 (100%), $[599 - NaHSO_4]^-$ 479 (30%); ^{1}H nmr δ see Table 1. Hplc retention time (3.0 ml/min) 8.8 min.

Compound **7** (1 mg) was treated with benzoyl chloride (30 μl) and dry pyridine (20 μl) at room temperature for 3 h. After solvent removal, the product was eluted through a Pasteur pipette filled with a slurry of Si gel in $CHCl_3$ to give the 23-benzoate derivative: ^{1}H nmr δ 0.56 (3H, s, H_3-18), 0.97 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.99 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 1.03 (3H, s, H_3-19), 1.05 (3H, d, *J* = 6.5 Hz, H_3-21), 2.60 (1H, m, H-7), 4.22 (1H, m, H-3α), 4.33 (1H, td, *J* = 3, 12 Hz, H-6β), 5.36 (1H, bd, 5 Hz, H-11), 5.43 (1H, m, H-23β).

SOLVOLYSIS OF SULFATED COMPOUNDS **3** AND **5**.—A solution of each of the compounds **3** and **5** in dioxane (0.25 ml) and pyridine (0.25 ml) was heated at 130° for 2 h in a stoppered reaction vial. After the solution had cooled, the solvents were removed under reduced pressure, and the residue was partitioned between H_2O (1 ml) and *n*-BuOH (1 ml). The extraction was repeated three times, and the combined extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residues were submitted to fabms and 250 MHz 1H-nmr (CD_3OD) measurements without purification. Spectral data for desulfated compounds are given below.

Compound **4**.—Negative ion fabms *m/z* $[M - H]^-$ 583 (100%), $[M - H - 132]^-$ 451 (70%); ^{1}H nmr (CD_3OD) δ (aglycone) 0.92 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.93 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 0.97 (3H, d, *J* = 6.5 Hz, H_3-21), 0.99 (3H, s, H_3-18), 1.20 (3H, s, H_3-19), 2.43 (1H, dd, *J* = 5, 12 Hz, H-7), 3.50 (1H, m, H-3α), 3.90 (1H, bs, H-6α), 4.30 (1H, td, *J* = 10, 3 Hz, H-15β), δ (sugar) signals virtually identical with those of **3**.

Compound **6**.—Negative ion fabms *m/z* $[M - H]^-$ 595 (100%), $[M - H - 132]^-$ 463 (60%). ^{1}H nmr (CD_3OD) δ (aglycone) 0.88 (3H, d, *J* = 6.5 Hz, H_3-26 or H_3-27), 0.94 (3H, d, *J* = 6.5 Hz, H_3-27 or H_3-26), 1.08 (3H, d, *J* = 6.5 Hz, H_3-21), 1.25 (3H, s, H_3-18), 1.39 (3H, s, H_3-19), 2.69 (1H, dd, *J* = 5, 12 Hz, H-7), 3.70 (1H, t, *J* = 8 Hz, H-24), 4.25 (1H, m, H-3α), 4.28 (1H, dd, *J* = 7.5, 2.5 Hz, H-16α), 4.33 (1H, bs, H-6α), 4.16 (1H, dd, 12, 2.5 Hz, H-15β), 5.47 (1H, dd, *J* = 15, 8 Hz, H-22); (sugar) 3.17 (1H, dd, *J* = 7.9 Hz, H-2′), 3.20 (1H, dd, *J* = 9, 11 Hz, H_{ax}-5′), 3.35 (1H, under solvent signal H-3′), 3.50 (1H, m, H-4′), 3.86 (1H, dd, *J* = 5, 11 Hz, H_{eq}-5′), 4.40 (1H, d, *J* = 7.0 Hz, H-1′).

METHANOLYSIS OF GLYCOSIDES AND SUGAR ANALYSIS.—A solution of each glycoside **3** and **5** (1 mg) in anhydrous 2 M HCl was heated at 80° in a stoppered reaction vial for 8 h. After being cooled, the reaction mixture was neutralized with Ag_2CO_3 and centrifuged, and the supernatant was evaporated to dryness under N_2. The residue was dissolved in TRISIL Z [0.1 ml, *N*-(trimethylsilyl)-imidazole in pyridine, Pierce Chemical], left at room temperature for 15 min, and analyzed by glc (40°, SE-30, 25 m). Glc peaks in the silylated hydrolysate co-eluted with those in silylated standards (methylxylosides).

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