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TWO NOVEL STEROIDAL GLYCOSIDE SULFATES FROM THE STARFISH *COSMASTERIAS LURIDA*

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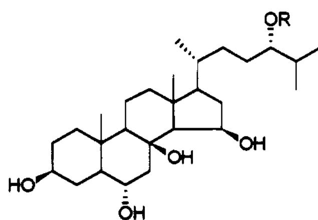
ABSTRACT.—Two novel steroidal monoglycoside sulfates, luridosides A [**1**] and B [**2**], have been isolated from the starfish *Cosmasterias lurida*. These compounds co-occur with one known steroidal monoglycoside, pycnopodioside C [**3**]. The structures of the new metabolites were determined from spectral data and comparison with those of related steroids.

Steroidal glycosides composed of a polyhydroxylated steroidal aglycone and a carbohydrate portion of one or two monosaccharide units have been reported from about fifty starfish species. Several examples include in their structure a 5α-cholestane-3β,6α,8,15β,24*S*-pentaol aglycone moiety and a sugar portion that is glycosidically attached at C-24 of the steroid. Attenuatoside A-I isolated from the Mediterranean starfish *Hacelia attenuata* Gray (1) was the first example of a compound containing this steroidal aglycone and a 2-*O*-methyl-β-D-xylopyranosyl-(1→2)-L-arabinofuranosyl residue. Further examples have been isolated from *Astropecten indicus* (2), *Halityle regularis* (3), *Porania pulvillus* (4), *Sphaerodiscus placenta* (5), *Pycnopodia helianthoides* (6), and *Pisaster giganteus* (7).

This report deals with two novel sulfated monoglycosides, luridosides A [**1**] and B [**2**], isolated from *Cosmasterias lurida* Philippi (family Asteriidae, order Forcipulatida), a very common starfish collected in cold waters off the Argentine Patagonian coast. The extract also contained pycnopodioside C [**3**] previously isolated from *Pyc. helianthoides* (6).

Luridoside A [**1**] exhibited a quasi molecular ion at *m/z* 709 $[M+Na]^+$ in the positive ion fabms. Examination of the spectral properties (^1H and ^{13}C nmr; Experimental and Table 1) and comparison of the ^{13}C resonances of the steroidal carbons with those of pycnopodioside A as reference compound (6) indicated that **1** contained a 5α-cholestane-3β,6α,8,15β,24-pentaol aglycone.

Significant shifts were produced for both angular methyl resonances when the ^1H-nmr spectrum was measured in pyridine-d_5 (δ 1.56 and 1.35; cf. 1.27 and 1.00 in CD_3OD), which indicated a 1,3-



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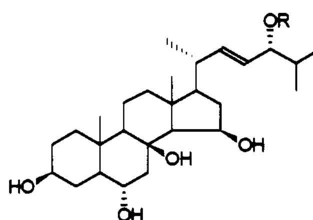
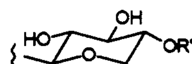
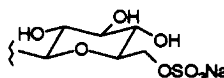
4 R=A, R′=H
3 R=B



2 R=A, R′=SO_3Na
5 R=A, R′=H



A



B

TABLE 1. ^{13}C-nmr Spectra of Luridosides A [**1**] and B [**2**] and Pycnopodioside A[a].

Carbon	Compound		
	Pycnopodioside A[b]	**1**	**2**
C-1	39.5	39.4	39.4
C-2	31.5	31.4	31.5
C-3	72.2	72.2	72.2
C-4	32.4	32.3	32.4
C-5	54.0	53.8	53.8
C-6	67.7	67.7	67.7
C-7	50.0	[c]	[c]
C-8	77.5	77.5	77.5
C-9	57.6	57.4	57.4
C-10	38.0	38.0	38.0
C-11	19.8	19.7	19.8
C-12	43.5	43.4	43.3
C-13	44.5	44.3	44.2
C-14	62.8	62.6	62.6
C-15	71.2	71.1	71.0
C-16	42.4	42.5	43.6
C-17	58.1	57.9	57.6
C-18	16.5	16.5	16.7
C-19	14.0	14.1	14.1
C-20	36.3	36.4	41.0
C-21	19.0	18.9	21.0
C-22	32.8	32.6	141.2
C-23	29.1	28.7	128.3
C-24	86.5	86.3	89.5
C-25	32.0	32.0	33.9
C-26	18.3	18.5	18.5
C-27	18.3	18.3	19.3
C-1′	104.9	104.7	104.3
C-2′	75.4	75.3	75.4
C-3′	78.0	76.0	76.1
C-4′	71.5	77.7	77.5
C-5′	66.7	64.6	64.9

[a]Spectra were run in CD_3OD; δ values relative to CD_3OD=49 ppm (central peak); multiplicities were determined by the delayed decoupling technique.

[b](24*S*)-24-*O*-(β-D-Xylopyranosyl)-5α-cholestane-3β,6α,8,15β,24-pentaol.

[c]Under solvent signal.

diaxial interaction between both angular methyl groups and the hydroxyl group at C-8. The spectral data (^{1}H and ^{13}C nmr; Experimental and Table 1) also showed the presence of a β-D-xylopyranosyl-4′-sulfate unit attached at C-24 of the steroid. The presence of xylose was confirmed by acid hydrolysis of **1** and analysis by gc of the peracetylated aldononitrile (8). The presence of a sulfate group was confirmed by solvolysis in a dioxane/pyridine mixture affording a desulfated derivative **4** (Experimental) identical with pycnopodioside A (6). An upfield shift of H-4′ from 4.19 in **1** to 3.50 ppm in the desulfated compound **4** (Experimental) showed that the sulfate was located at C-4′. This was confirmed by ^{13}C-nmr spectroscopy (Table 1). The C-4′ signal was shifted by 6.2 ppm to 77.7 ppm, whereas C-3′ and C-5′ were shifted upfield by 2.0 and 2.1 ppm, respectively, relative to pycnopodioside A. The D configuration of xylose is assumed by comparison with the many D-xylosides isolated from starfishes (9). The 24*S* configuration was suggested because all the signals assigned to the side chain are virtually identical with those assigned to the corresponding carbons of pycnopodioside A (6). Luridoside A [**1**] was isomeric with pycnopodioside B (6), the only difference being the location of the sulfate group at C-4′ of the xylosyl moiety of **1** instead of C-3 of the aglycone as in pycnopodioside B.

Luridoside B [**2**] was the Δ^{22} analogue of luridoside A [**1**]. Its fabms (positive ion mode) showed pseudomolecular ion species at *m/z* 707 $[M+Na]^+$ and 723 $[M+K]^+$ shifted two mass units relative to **1**.

The 200 MHz ^{1}H-nmr spectra of **1** and double resonance experiments allowed us to assign the multiplet at δ 5.35 to Δ^{22} protons. The ^{13}C-nmr data (Table 1) and comparison with those of luridoside A [**1**] and those corresponding to the side chain and sugar carbon atoms of amurensoside C [(24*S*)-24-*O*-(β-D-xylopyranosyl-4′-sulfate)-5α-cholest-(22*E*)-ene-3β,6α,15α,24-tetraol] (10) confirmed the presence of 5α-cholest-(22*E*)-ene-3β,6α,8,15β,24-pentaol as the steroid aglycone with the 4′-*O*-sulfated xylosyl residue glycosidically linked at C-24.

Acid hydrolysis of luridoside B [**2**] and analysis by gc of the peracetylated aldononitrile confirmed the presence of xylose. Upon solvolysis in dioxane/pyridine compound **2** was desulfated to afford compound **5**. The ^{1}H-nmr spectra of **5** (Experimental) showed the same dis-

placement for H-4′ as **4**, confirming the location of the sulfate group at C-4′.

EXPERIMENTAL

INSTRUMENTAL.—^{1}H- and ^{13}C-nmr spectra were recorded on Varian XL-100-15, Bruker ACE-200, and Varian 400 instruments. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Fabms were obtained on a Finnigan model 4500 mass spectrometer. Samples were dissolved in a glycerol matrix and placed on a probe prior to bombardment with Ar atoms. Glc was performed on a Hewlett Packard 5890 capillary column (SP-2330, 25 m) chromatograph. Hplc was performed on a Micromeritics liquid chromatograph equipped with a Model 760 solvent delivery system, a model 730 manual injector, and a refractive index detector using a Versapack C-18 column (30 cm×4.1 mm i.d.). Tlc was performed on precoated Si gel F254 and C-18 reversed-phase plates.

EXTRACTION AND ISOLATION.—Specimens of *C. lurida* (6.0 kg) were collected in 1989 off the Golfo Nuevo near Puerto Madryn at the Argentine Patagonian coast and were identified by Dr. Alejandro Tablado of the Museo de Ciencias Naturales "Dr. Bernardino Rivadavia" where a voucher specimen (N° 31231) is preserved. The animals, frozen prior to storage, were homogenized in EtOH (6 liters) and centrifuged. The EtOH was evaporated, and the aqueous extract thus obtained was purified through an Amberlite XAD-2 column (1 kg), eluted with distilled H_2O until negative reaction of chloride, followed by MeOH. The MeOH eluate was evaporated to give a glassy material (8.1 g) which was then chromatographed on a column of Sephadex LH 60 (80 cm×4 cm i.d.; 100 g) with MeOH-H_2O (2:1) as eluent. Fractions (10 ml) were collected and analyzed by tlc on SiO_2 in *n*-BuOH–HOAc–H_2O (4:1:5) (upper layer) and detected by spraying with H_2SO_4. A fraction which showed the presence of glycosides was further purified by Si gel chromatography [CH_2Cl_2-MeOH-H_2O (70:30:0.5)], obtaining 2 fractions. Each fraction was purified by Sephadex LH 20 (MeOH) and Davisil C-18 (35-75μ) [MeOH-H_2O (65:35)]. This resulted in the isolation of pycnopodioside C [**3**] (27 mg) from fraction 1 and luridosides A [**1**] (18 mg) and B [**2**] (10 mg) from fraction 2. Each compound showed one spot on C-18 reversed-phase tlc [MeOH-H_2O (65:35)] and one peak by hplc [MeOH-H_2O (60:40)], flow 0.7 ml/min, retention time of **1** 12.5 min and of **2** 15.0 min.

Luridoside A [**1**].—$[\alpha]^{25}D$ +3.7° (c=0.4, MeOH); positive ion fabms *m/z* $[M+K]^+$ 725, $[M+Na]^+$ 709, $[M+H-SO_3]^+$ 607; ^{1}H nmr δ (aglycone) 0.91 (3H, d, J=6.7 Hz, H_3-26 or H_3-27), 0.92 (3H, d, J=6.7 Hz, H_3-26 or H_3-27), 0.97 (3H, d, J=6.7 Hz, H_3-21), 1.00 (3H, s, H_3-19), 1.28 (3H, s, H_3-18), 2.40 (1H, dd, J=12.0, 3.5 Hz, H-7eq), 2.40 (1H, m, H-16eq), 3.54 (1H, m, H-3α), 3.73 (1H, ddd, J=10.5, 10.5, 4.0, H-6β), 4.45 (1H, m, H-15α); δ (sugar) 3.26 (1H, under solvent signal, H-2′), 3.30 (1H, under solvent signal, H-5′ ax), 3.50 (1H, t, J=9 Hz, H-3′), 4.15 (1H, dd, J=12, 5, 5 Hz, H-5′ eq), 4.19 (1H, m, H-4′), 4.26 (1H, d, J=7.5 Hz, H-1′); ^{13}C nmr see Table 1.

Luridoside B [**2**].—$[\alpha]^{25}D$ +1.5° (c=0.3, MeOH); positive ion fabms *m/z* $[M+K]^+$ 723, $[M+Na]^+$ 707, $[M+H-SO_3]^+$ 605; ^{1}H nmr δ (aglycone) 0.87 (3H, d, J=7.0 Hz, H_3-26), 0.94 (3H, d, J=7.0 Hz, H_3-27), 1.00 (3H, s, H_3-19), 1.02 (3H, d, J=6.5 Hz, H_3-21), 1.29 (3H, s, H_3-18), 2.40 (1H, dd, J=12.0, 3.5 Hz, H-7eq), 2.40 (1H, m, H-16eq), 3.54 (1H, m, H-3α), 3.73 (2H, m, H-6β and H-24), 4.41 (1H, m, H-15α), 5.35 (2H, m, H-22 and H-23); δ (sugar) 3.26 (1H, under solvent signal, H-2′), 3.30 (1H, under solvent signal, H-5′ ax), 3.50 (1H, t, J=9 Hz, H-3′), 4.14 (1H, dd, J=12.0, 5.5 Hz, H-5′ eq), 4.19 (1H, m, H-4′), 4.26 (1H, d, J=7.5 Hz, H-1′); ^{13}C nmr see Table 1.

HYDROLYSIS AND SUGAR ANALYSIS OF LURIDOSIDES A [**1**] AND B [**2**].—The glycosides (4–6 mg) were hydrolyzed with 2 N H_2SO_4 at 100° for 2 h. The mixture was then extracted with CH_2Cl_2. The aqueous phase of the hydrolyzate was neutralized with $Ba(OH)_2$ and centrifuged, and the supernatant was evaporated to dryness in vacuo. The dried sample in pyridine (0.5 ml) was treated with hydroxylamine hydrochloride (60% of sugar wt) and heated at 65° for 30 min. Ac_2O (0.5 ml) was added, and the solution was heated for 20 min, cooled, and extracted with CH_2Cl_2-H_2O (1:2) (3 ml) (8). The organic extract was washed with H_2O (3×2 ml), saturated aqueous $NaHCO_3$ solution (2×2 ml), and H_2O (2×2 ml) and dried over $MgSO_4$. The solution was evaporated to dryness to yield the per-*O*-acetylated aldononitriles, and these were analyzed by gc [Rt 3.59 min (xylose)].

SOLVOLYSIS OF LURIDOSIDES A [**1**] AND B [**2**].—A solution of each of the glycosides (2–6 mg) in dioxane (0.35 ml) and pyridine (0.55 ml) was heated at 120° for 2 h in a stoppered reaction vial. To the cooled solution H_2O (1 ml) was added and the solution was extracted with *n*-BuOH (3×0.5 ml). The combined organic extracts were washed with H_2O and evaporated to dryness under reduced pressure. The residues were analyzed to tlc and by 200 MHz ^{1}H-nmr (CD_3OD) measurement, without purification. Spectral data for desulfated compounds are given below.

Compound **4**.—^{1}H-nmr δ (aglycone) signals virtually identical with those of **1**; δ (sugar) 3.18 (1H, t, J=10.0 Hz, H-5′a), 3.20 (1H, dd, J=7.5,

9.5 Hz, H-2′), 3.35 (1H, under solvent signal, H-3′), 3.50 (1H, m, H-4′), 3.85 (1H, dd, J=10.0, 5.0 Hz, H-5′ eq), 4.29 (1H, d, J= 7 Hz, H-1′).

Compound **5**.—^{1}H-nmr δ (aglycone) signals virtually identical with those of **2** and δ (sugar) signals virtually identical with those of **4**.

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