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A NOVEL MARINE STEROIDAL SULFATE FROM THE STARFISH *LUIDIA LUDWIGI*

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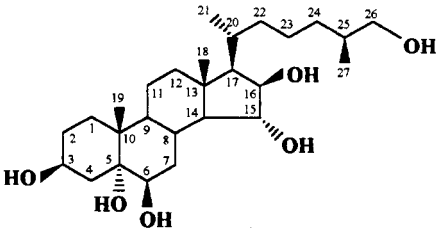
ABSTRACT.—A new steroid, 5α-cholestane-3β,5,6β,15α,26-pentol 26-sulfate [**3**], has been isolated from the starfish *Luidia ludwigi*. This compound co-occurs with two known asterosaponins, acanthaglycosides B and C, and two polyhydroxysteroids [**1** and **2**], isolated previously from the starfish *Myxoderma platyacanthum*. The structure of the new metabolite was determined from spectral data (^{1}H- and ^{13}C-nmr and fabms) and chemical transformations. The configuration of C-25 was determined by correlating ^{1}H-nmr data of chiral esters of **3** with those of related steroids.

Steroidal glycosides and polyhydroxysteroids are the predominant metabolites of starfish. Although polyhydroxysteroids have been isolated from only a limited number of species in other marine phyla, they are widespread in starfish, where they have been found, usually as complex mixtures, in almost all species examined (1).

In a continuation of our studies on marine organisms collected in cold waters off the Patagonian coast of Argentina (2,3), we have examined an EtOH extract of the starfish *Luidia ludwigi* Scotti Bell (Luidiidae) and have isolated two known asterosaponins, acanthaglycosides B and C (4), and three polyhydroxylated steroids, the known 5α-cholestane-3β,5,6β,15α,16β,26-hexol [**1**] (5) and 5α-cholestane-3β,5,6β,15α,26-pentol 15-sulfate [**2**] (6), and the novel 5α-cholestane-3β,5,6β,15α,26-pentol 26-sulfate [**3**].

Hydroxylation at C-26 is a common feature of the polyhydroxysteroids isolated from starfish, but the presence of a sulfate group at this position is unusual in these echinoderms. The only examples reported previously from starfish are the polyhydroxylated steroids isolated as minor compounds from the deep-water species *Styracaster caroli* (7). On the other hand, two minor compounds containing the same side-chain as polyhydroxysteroid **3**, but as a mixture of 25*R*- and 25*S*-isomers, have been isolated from the Pacific ophiuroid *Ophiarachna incrassata* (8).



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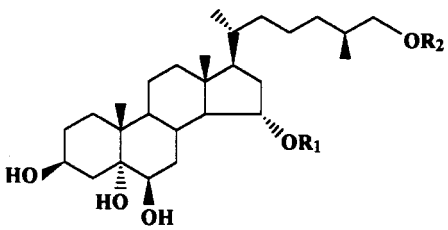


2 $R_1=SO_3^-Na^+$, $R_2=H$
3 $R_1=H$, $R_2=SO_3^-Na^+$
4 $R_1=R_2=H$

Steroid **3** showed a molecular anion peak at *m/z* 531 in its fabms (negative-ion mode) corresponding to a monosulfated derivative of a cholestane-pentol. The ^{13}C-nmr spectrum was consistent with the presence of twenty-seven carbon atoms (Table 1) and DEPT measurements revealed the presence of four methyl groups, ten methylene, six methine, and three quaternary carbons, three -OCHR- carbons, and one $-OCH_2-$ carbon. Comparison of the ^{13}C-nmr resonances with

TABLE 1. Comparison of the ^{13}C-Nmr Spectral Data[a] for the Side-chain Carbons of Compounds **3**, **5** and **6**.

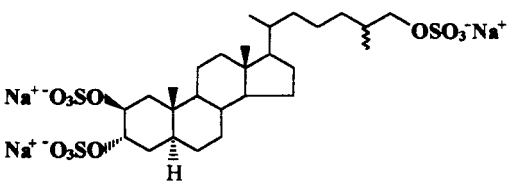
Carbon	Compounds		
	3	**5**[b]	**6**[b]
20	36.60	37.00 (37.10)	37.00 (37.10)
21	19.10	19.20	19.20
22	37.36	37.40 (37.30)	37.40 (37.30)
23	24.33	24.51 (24.45)	24.51 (24.45)
24	34.88	34.77 (34.88)	34.71 (34.88)
25	34.42	34.36 (34.40)	34.36 (34.41)
26	73.85	74.08 (73.96)	74.08 (73.96)
27	17.33	17.04 (17.25)	17.04 (17.25)

[a]Values relative to CD_3OD=49 ppm (central peak).

[b]Mixture of 25*R*- and 25*S*-isomers; the shifts of the minor isomer are in parentheses.

those of compound **2** and 5α-cholestane-3β,5,6β,15α,26-pentol [**4**] (6) as reference compounds indicated the presence of a 3β,5α,6β,15α,26-pentol moiety. The differences observed in the chemical shifts of C-25 (−2.5 ppm) and C-26 (+5.4 ppm) of **3** relative to pentol **4** and comparison of the ^{13}C-nmr data (Table 1) of the side-chain carbon atoms with those of compounds **5** and **6** isolated from *Ophiarachna incrassata* established clearly the location of the sulfoxy group at C-26. Thus, the ^{13}C-nmr signals of **3** and **4** were identical within 0.1 ppm except for the C-25 and C-26 signals already mentioned and the signal for C-7 (0.8-ppm difference). The structure of **3** was further confirmed by analysis of its ^{1}H-nmr spectrum and by comparison with those of **2** (6), **4** (Experimental), and 26-sulfoxy steroids (8). A significant shift of the C-10 methyl resonance was observed when the ^{1}H-nmr spectrum of **3** was measured in pyridine-d_5 (δ 1.74; δ 1.16 in CD_3OD). This indicated a 1,3-diaxial interaction between the C-10 methyl group and the hydroxyl group at C-6, confirming the β-configuration for this substituent (9). The presence of the sulfate group was confirmed by solvolysis in a dioxane/pyridine mixture, which afforded the desulfated derivative **4** in which the chemical shift of C-26 moved from 73.8 to 68.4 ppm.

The only feature needed to establish the complete structure of polyhydroxysteroid **3** was the stereochemistry at C-25. It has been shown (10,11) that (25*R*)- and (25*S*)-26-hydroxysteroids may be differentiated by the ^{1}H-nmr spectra of their (+)-(*R*)- and (−)-(*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid [MTPA, Mosher's reagent (12)] esters. Thus, we treated **4** with (+)- and (−)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride to afford the 3β,26-di-(+)-MTPA and the 3β,26-di-(−)-MTPA esters, respectively, and recorded their ^{1}H-nmr spectra. Assignment of the *S*-configuration at C-25 in **3** was supported



5 (25*R*+25*S*)
6 Δ^5 (25*R*+25*S*)

by the ^{1}H-nmr pattern of the 26-methylene proton signals in the 3β,26-di-(+)-MTPA ester [δ 4.15 (1H, dd, J=10 and 5.5 Hz) and δ 4.24 (1H, dd, J=10 and 5.5 Hz) ppm] and 3β,26-di-(−)-MTPA ester [δ 4.13 (1H, dd, J=10 and 5.5 Hz) and δ 4.26 (1H, dd, J=10 and 5.5 Hz) ppm)] in accordance with the values reported by Finamore *et al.* (6). The 25*S* configuration was similarly confirmed for the known polyhydroxysteroids **1** and **2**.

Comparison of the data in Table 1 allows assignment of the identity of each epimer in the mixture of 25*R*- and 25*S*-isomers of compounds **5** and **6** isolated from the ophiuroid *Ophiaracna incrassata*. Based on the chemical shifts recorded for C-24, C-25, C-26, and C-27, the major epimer is the 25*R*-isomer.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker ACE-200 instrument. Fabms were obtained on a VG-ZAB mass spectrometer. Prep. hplc was carried out on a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector, and a refractive index detector using a C_{18} Bondclone 10 μm column (30 cm×7.8 mm i.d.); flow rate 2 ml/min. Tlc was performed on precoated Si gel F_{254} and C_{18} reversed-phase plates.

ANIMAL MATERIAL.—Specimens of *L. ludwigi* (5.2 kg) were collected in 1993, off Bajo Oliveira near San Antonio Oeste in the Argentine Patagonian coast and were identified by Dr. Alejandro Tablado of the Museo de Ciencias Naturales "Dr. Bernardino Rivadavia" where a voucher specimen is preserved (MACN No. 31241).

EXTRACTION AND ISOLATION.—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 passed through an Amberlite XAD-2 column (1 kg) and eluted with distilled H_2O until a negative chloride reaction was obtained, followed by MeOH. The MeOH eluate was evaporated to give a glassy material (7.9 g) that was chromatographed on a Sephadex LH 60 column (80 cm×4 cm i.d.; 100 g) with MeOH-H_2O (2:1) as eluent. Fractions (10 ml) were analyzed by tlc on SiO_2 in *n*-BuOH-HOAc-H_2O (4:5:1) (upper layer) and detected by spraying with H_2SO_4. Fractions 33–44 contained the crude "asterosaponins" while fractions 45–55 contained the mixture of polyhydroxylated steroids. Each fraction was subjected to dry column flash chromatography on Davisil C_{18} (35–75 μm) using MeOH-H_2O (50:50 and 65:35); 50 ml fractions were collected and analyzed by C_{18} reversed-phase tlc [MeOH-H_2O (65:35)]. Final purification was accomplished by hplc on a C_{18} Bondclone column with MeOH-H_2O (60:40), to give acanthaglycosides B (10.5 mg) and C (24.0 mg) and the polyhydroxylated steroids **1** (18.9 mg), **2** (47.0 mg), and **3** (9.7) mg) as pure compounds. Acanthaglycosides B and C and compounds **1** and **2** were identified by ^{1}H- and ^{13}C-nmr spectroscopy and fabms.

5α-Cholestane-3β,5,6β,15α,26-pentol 26-sulfate [**3**].—$[\alpha]^{25}D$ +64.0° (*c*=0.1, MeOH); negative-ion fabms *m/z* 531; ^{1}H nmr (CD_3OD) δ 0.77 (3H, s, H_3-18), 0.94 (3H, d, J=6.7 Hz, H_3-27), 0.99 (3H, d, J=6.7 Hz, H_3-21), 1.20 (3H, s, H_3-19), 3.51 (1H, br s, H-6α), 3.75–3.90 (3H, m, H_2-26, H-15β), 4.04 (1H, m, H-3α); ^{13}C nmr (CD_3OD) δ 13.8 (C-18), 17.3 (C-27), 17.4 (C-19), 19.1 (C-21), 22.1 (C-11), 24.5 (C-23), 31.3 (C-8), 31.7 (C-1), 33.6 (C-2), 34.4 (C-25), 34.9 (C-24), 35.3 (C-7), 36.6 (C-20), 37.4 (C-22), 39.3 (C-10), 41.5 (C-16), 41.8 (C-4, C-12), 45.1 (C-13), 46.6 (C-9), 55.0 (C-17), 63.6 (C-14), 68.3 (C-3), 73.8 (C-26), 74.3 (C-15), 76.4 (C-5), 76.6 (C-6).

PRODUCTION OF **4** VIA SOLVOLYSIS OF **3**.—A solution of **3** (4.5 mg) in dioxane (0.1 ml) and pyridine (0.1 ml) was heated at 120° for 2 h in a stoppered reaction vial. After the solution had cooled, 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 residue (3.0 mg) was analyzed by tlc and by 200 MHz ^{1}H-nmr spectroscopy without purification: ^{1}H nmr (CD_3OD) δ 0.76 (3H, s, H_3-18), 0.94 (3H, d, J=6.7 Hz, H_3-27), 0.99 (3H, d, J=6.7 Hz, H_3-21), 1.20 (3H, s, H_3-19), 3.34 (1H, H-26, overlapped with solvent signal), 3.45 (1H, dd, J=10.5 and 5.0 Hz, H-26), 3.51 (1H, br s, H-6α), 3.90 (1H, dt, J=3.0 and 9.0 Hz, H-15β), 4.04 (1H, m, H-3α).

MTPA ESTERS OF STEROID **4**.—Steroid **4** (1.5 mg) was treated with commercial (+)-α-methoxy-α-(trifluoromethyl)-phenyl acetyl chloride (10 μl) in dry pyridine (0.3 ml) for 1 h at room temperature. After removal of the solvent the residue was purified by passage through a C_{18} cartridge (MeOH-H_2O, 50:50, MeOH) to obtain the 3β,26-di-(+)-MTPA ester; ^{1}H nmr (CD_3OD) δ 0.80 (3H, s, H_3-18), 0.97 (6H, d, J=6.7 Hz, H_3-21, H_3-27), 1.23 (3H, s, H_3-19), 3.50 (1H, br s, H-6α), 3.90 (1H, dd, J=3.0 and 9.0 Hz, H-15β), 4.15 (1H, dd, J=10.0 and 5.5 Hz) to 4.24 (1H, dd, J=10.0 and 5.5 Hz, H_2-26), 5.50 (1H, m, H-3α). The 3β,26-di-(−)-MTPA ester of **4** was prepared using commercial (−)-α-methoxy-α-(trifluoromethyl)-phe-

nyl acetyl chloride. The ^{1}H-nmr (CD_3OD) spectrum was identical with the values reported for the (+)-MTPA ester except for the signals of H_2-26 at δ 4.13 (1H, dd, J=10.0 and 5.5 Hz) and δ 4.26 (1H, dd, J=10.0 and 5.5 Hz).

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