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A Novel Sulphated Steroid with a 7-membered 5-oxalactone B-ring from an Antarctic Starfish of the family *Asteriidae*.

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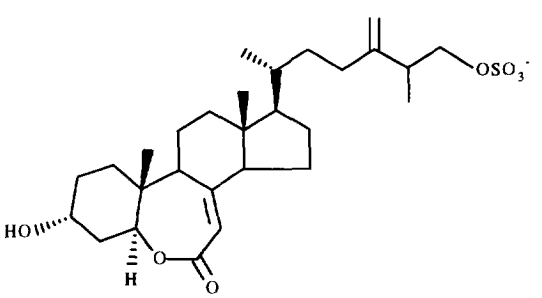
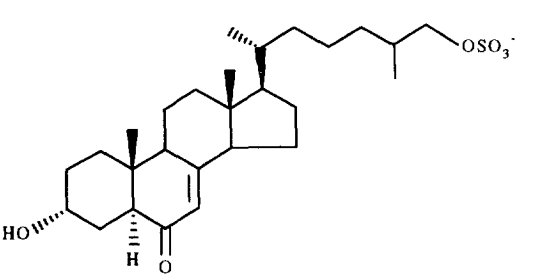
ABSTRACT. Three novel sulphated polyhydroxylated steroids, named asterasterols A-C (**1-3**), have been isolated from an Antarctic starfish of the family *Asteriidae* and their structures elucidated by spectroscopic studies. Steroid **1** has the seven-membered 5-oxalactone B-ring; **2** and **3** are the corresponding 6-oxo steroids. © 1997 Published by Elsevier Science Ltd.

In connection with our systematic investigation of steroid constituents from echinoderms, we examined some Antarctic starfish species.[1,2] These studies have led to the isolation of a series of sulfated polyhydroxysteroids and steroidal glycosides structurally very close, some identical, with those found in species collected in warm environments.[3] During the investigation of the polar extracts of an Antarctic starfish of the family *Asteriidae*, which is the subject of this report, we have isolated a structurally novel steroid exhibiting 7-membered 5-oxalactone B-ring, along with the corresponding 6-oxo steroids.

The starfish, *Asteriidae* family, was collected in Tethis Bay during the Italian-Antarctic expedition in January 1990 at a depth of 50-100 mt. The starfish (500 g fresh) was extracted with water and then with acetone; from the aqueous extract the sulphated steroids and steroidal glycosides were recovered by passing it through a column of Amberlite, washing out salts with distilled water, and eluting the absorbed material with methanol. The acetone extracts were solvent partitioned between water and *n*-butanol. The combined methanol eluate and *n*-butanol soluble material, were separated by sequential application of gel permeation on Sephadex LH-60, DCCC and HPLC, to give the sulphated steroids **1-3**, in small amounts, ranging from 7.2 mg of **1** (major) to 3.4 mg of **3** (minor).



1



2; **3** Δ^{22}

Asterasterol A (**1**), $[\alpha]_D$= +4.5°. It showed a molecular anion peak in the HRFAB mass spectrum (-ve ion) at m/z 523.240906 [MSO_3^-], corresponding to molecular formula $C_{28}H_{43}O_7S$ (calc. 523.272951). The ^1H NMR and COSY spectra readily implied that steroid **1** had a 24-methylene-26-sulphoxycholestane side chain, structural entity already encountered as 26-hydroxy analog in many polyhydroxylated steroids from starfishes.[3,4] The COSY spectrum showed that both the 26-oxymethylene dd at δ 4.06 (J=9.0, 5.8 Hz) and 3.83 dd (J=9.0, 6.5 Hz) were coupled to H-25, sextet downfield shifted to δ 2.51, which in turn was coupled to a methyl doublet again downfield shifted to δ 1.15 (J=7.0 Hz; Me27), because of the presence of the exomethylene at C24, δ 4.84 (2H, s). This structural unit was supported by HMBC correlations from Me27 protons to C24 (δ_C 153.1), C25 (δ_C 40.5) and C26 (δ_C 72.8 ppm) and confirmed by solvolysis using dioxane/pyridine affording a desulphated derivative, FABMS m/z 443 [M-H]$^-$, whose 26-methylene protons were now observed resonating highfield shifted to δ 3.60 dd-3.40 dd. The ^1H NMR of **1** also exhibited signals for two methyl singlets at δ 0.67 (Me18) and 0.94 (Me19), one methyl doublet [δ 1.03 (J=6.8 Hz, Me21)], two oxygenated methines at δ 4.16 (brt, J=2.5 Hz; equatorial), and δ 4.63 (dd, J=10.5, 5.2 Hz; axial) and one olefinic singlet at δ 5.60. The ^{13}C NMR signals at 171.4 s, 162.3 s and 113.1 d were indicative for the presence of an α,β-unsaturated ester function, as confirmed by UV [224 nm (ε 6800)]. Interpretation of COSY cross peaks identified the structural moiety from C-1 to C-5. The chemical shift of 5α-H (δ 4.63) suggested that C-5 was participating in the ester linkage. HMBC cross peaks from the olefinic proton at δ 5.60 to C9, C14 and to carbonyl carbon at δ 171.4 ppm implied a Δ^7-5-oxalactone structure. The structural deduction for **1** as 3α-hydroxy-24-methylene-26-sulphoxy-B-homo-5-oxa-5α-cholest-7-en-6-one was supported by analysis of the ^{13}C NMR spectrum, comparison with appropriate models [3] and HMBC data, which included cross peaks Me19/C1, C5, C9, C10; Me18/C12, C13, C14, C17; Me21/C17, C22; H5/C9, C19. Thus asterasterol A represents the second example of naturally occurring steroids with a seven-membered lactone B ring after the discovery of brassinosteroids, plant growth regulators, with a 7-oxalactone structure.[5] The biosynthesis of **1** should presumably commence from Δ^7-6-keto steroids, coocurring in the same organism.

Asterasterol B (**2**), $[\alpha]_D$= +12.5°, FABMS, m/z 495 [MSO_3^-]. The COSY spectrum of **2** showed the oxymethylene ($H_2$26) at δ 3.89 (1H, dd, J=10.5, 6.8 Hz) and 3.81 (1H, dd, J=10.5, 6.5 Hz) to be coupled with the 1H multiplet at δ 1.85, (H25), which in turn was coupled with a methyl doublet at δ 0.99 (J=7.0 Hz; H_3-27). This indicated a 26-sulphoxycholesterol side chain, already encountered in some steroids from the ophiuroid *Ophiorachna incrassata* [6] and as 26-hydroxy analogous in many polyhyroxysteroid from starfishes.[3] The ^1H NMR spectrum also contained signals for three more methyls [δ_H 0.69 s, 0.89 s and 1.02 d (J=6.8 Hz)], a narrow hydroxymethine signal at δ 4.13 ($W_{1/2}$=8.0 Hz) typical for an equatorial proton, and one olefinic singlet at δ 5.69. The ^{13}C NMR spectrum revealed signals for an α,β-unsaturated ketone (δ 166.9 s, 123.3 d and 204.1 s ppm), which was supported by UV [242 nm (ε 5800)]. Analysis of the COSY spectrum of **2** indicated the presence of the 3α-OH and 5α-H, this latter, downfield shifted δ 2.74 (J=12.4, 3.9 Hz; axial), because of the ketone at C6. The ^{13}C NMR spectrum of **2**, interpretation of HMBC data, which included cross peaks Me19/C1, C5, C9, C10; Me18/C12, C13, C14, C17; Me21/C20, C17, Me27/C25, C26, and comparison with appropriate models [3] allowed all the signals to be assigned, and confirmed the 3α-hydroxy-26-sulphoxy-5α-cholest-7-en-6-one structure **2** for asterasterol B. The ^1H NMR spectrum of asterasterol C (**3**), FAB mass m/z 493 [MSO_3^-], $[\alpha]_D$= +8.3°, also included two olefinic protons at δ 5.34 dd (J=15.0, 8.0 Hz) and 5.40 dt (J=15.0, 7.0 Hz) consistent with the presence of a 22(23)E double bond.

BIOLOGICAL ACTIVITY

The cytotoxic activity *in vitro* of the sulphated steroids **1-3** were tested against human broncopulmonary non-small-cell-lung-carcinoma cells (NSCLC-N6). Two of them, **1** and **2**, were inactive at the concentrations tested (3.3, 10 and 30 μg/ml), while compound **3**, showed a moderate activity with IC_{50} > 30μg/ml.

Table 1. ^{1}H and ^{13}C NMR chemical shifts and HMBC correlations of compounds **1** and **2**

	1			**2**		
position	δ_H[a]	δ_C	HMBC[b]	δ_H[a]	δ_C	HMBC[b]
1		34.1			32.6	
2	1.60	28.2			28.4	
3	4.16 brt (2.5)	66.8		4.13 brs ($W_{1/2}$=8)	65.9	
4	2.11 dd (11.0, 2.5)	36.5	C10	1.95, 1.63	28.3	
	1.84					
5	4.63 dd (10.5, 5.2)	79.6	C9, C19	2.74 dd (12.4, 3.9)	48.0	
6		171.4			204.1	
7	5.60 s	113.1	C6, C9, C14	5.69 s	123.3	
8		162.3			166.9	
9		59.3			51.2	
10		40.7			39.8	
11		26.7			22.4	
12		41.1			39.6	
13		48.5			45.6	
14	2.28	59.2	C8, C13, C15, C18		56.6	
15		23.7			23.4	
16		28.6			28.6	
17		57.6			57.3	
18	0.67 s	12.4	C12, C13, C14, C17	0.69 s	12.5	C12, C13, C14, C17
19	0.94 s	14.2	C1, C5, C9, C10	0.89 s	12.6	C1, C5, C9, C10
20	1.52	37.2			36.9	
21	1.03 d (7.0)	19.2	C17, C22	1.02 d (6.8)	19.1	C17, C20
22		35.5			37.1	
23		32.5			24.2	
24		153.1			34.5	
25	2.51 sext	40.5			34.2	
26	4.06 dd (9.0, 6.5)	72.8	C24, C25, C27	3.89 dd (9.5, 5.6)	73.8	
	3.83 t (9.0)		C27	3.81 dd (9.5, 6.4)		
27	1.15 d (6.8)	17.5	C24, C25, C26	0.99 d (7.0)	17.0	C25, C26
28	4.84 s	109.7	C23, C25			

[a]Coupling costants are in parentheses and given in Hz. ^{1}H assignments aided by COSY experiments.
[b]HMBC optimized for $J_{2,3}$=8.0 Hz.

EXPERIMENTAL

General Methods.

NMR measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package.

Two-dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional sequence.[7] The COSY spectra were obtained using a data set (t_1xt_2) of 1024x512 points for a spectral data width of 3125 Hz for **1** and 3758.4 Hz for **2** (relaxation delay 1 sec). The data matrix was processed using a sine bell window function following transformation to give a magnitude spectrum [digital resolution in both F2 and F1 dimensions were 3.05 Hz/pt (**1**) and 3.7 Hz/pt (**2**)].

^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to Bax and co-workers.[8,9] The spectral widths in the 1H dimensions were 3125 Hz for **1** and 3758.4 Hz for **2**; 256 experiments of 80 scans (**1**) and 256 experiments of 32 scans (**2**) in 1K data points each (relaxation delay 1 sec., low pass J-filter delay 3.5 ms, long range coupling evolution delay 60 ms) were collected. The resulting final digital resolution in F2 dimension was 1.52 Hz/pt (**1**) and 1.83 Hz/pt (**2**).

Mass spectra, VG Autospec instruments (Cs^+ ions bombardment) with fab source were recorded in a glycerol or glycerol-thioglycerol (3:1) matrix.

Animal Collection.

The animal (only one organism was collected), was collected at the Tethys Bay in January 1990, in the course of the Italian-Antarctic expedition, a voucher specimen is kept at Istituto per la Chimica di Molecole di Interesse Biologico del CNR, Arco Felice, Napoli under the ref. number MOR 24.

Extraction and Isolation.

The animal (0.5 Kg), was cut into small pieces and soaked in water for 5h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (700 g). The column was washed with distilled water (1 l) and then eluted with methanol (4 l). The methanol eluate was taken to dryness to give the glassy material (2.5 g). The remaining solid mass, after extraction with water, was then re-extracted with Me_2CO (2 l), and the Me_2CO extracts were combined, evaporated under vacuum and partitioned between H_2O and Et_2O. The aqueous residue was then extracted with *n*-BuOH. Evaporation of the *n*-BuOH extracts afforded 1.1 g of a glassy material which was combined with the above MeOH eluate from Amberlite XAD-2 column and chromatographted on a column of Sephadex LH-60 (4 × 80 cm) with MeOH-H_2O (2:1) as eluent. Fractions (9 ml) were collected and analysed by TLC on SiO_2 in *n*-BuOH-AcOH-H_2O (12:3:5) and $CHCl_3$-MeOH-H_2O (80:18:2). Fractions 40-57 (700 mg) mainly contained the asterosaponins. Fractions 58-71 (500 mg) contained steroidal glycoside sulphates and fractions 72-95 (800 mg) contained the sulphated steroids, which latter was further fractionated by DCCC using $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode (the lower phase was the stationary phase). Fractions (6 ml each) were collected and monitored by TLC on SiO_2 with $CHCl_3$-MeOH-H_2O (80:18:2).

Fractions 12-18 were purified by HPLC with MeOH-H_2O (60:40) to give pure **1** (4.1 mg), **2** (3.4 mg) and **3** (7.2 mg). Additional physical data:

Asterasterol C (**3**): ^1H and ^{13}C NMR of steroidal nucleus are identical to the data reported for **2**.

^1H NMR of side chain: 5.40 m (H23), 5.34 dd (J=15.0, 8.0 Hz, H22), 3.90 dd (J=9.5, 5.6 Hz, H23), 3.82 dd (J=9.5, 6.4 Hz, H26), 1.09 d (J=6.8 Hz, $H_3$21), 0.97 d (J=7.0 Hz, $H_3$27).

^{13}C NMR of side chain: 139.7 (C22), 126.8 (C23), 73.4 (C26), 41.7 (C20), 37.3 (C24), 34.8 (C25), 21.4 (C21), 16.9 (C27).

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