STARFISH SAPONINS. XXVI*. STEROIDAL GLYCOSIDES FROM THE STARFISH *Poraster superbus* (*)

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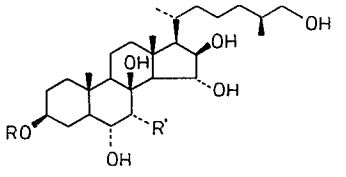
Summary — Three novel steroidal 2-*O*-methylxylosides, **2**, **4**, and **5**, have been isolated from the starfish *Poraster superbus*. They co-occur with major amounts of nodososide, **8**, a common steroidal glycoside from starfishes, the known xyloside **6**, two known polyhydroxysteroids, **1** and **3**, and one new sulphated polyhydroxysteroid, **7**. The structures of the new metabolites have been determined from spectral data.

In connection with our investigations of biologically active metabolites from starfishes we have examined the polar extracts of the Pacific starfish *Poraster superbus*, collected at North of New Caledonia.

Recently, we described the structures of a number of polyhydroxysteroids and glycosidated polyhydroxysteroids from starfishes[1]. In particular, we reported the structures of (25*S*)-5α-cholestane-3β,6α,8,15α,16β,26-hexol, **1**, and (25*S*)-5α-cholestane-3β,6α,7α,8,15α,16β,26-heptol, **3**, from the starfish *Protoreaster nodosus*[2]. In this paper we report the structures of the corresponding 3-*O*-2′-



1, R=R'=H
2, R=2-O-methyl-β-D-xylopyranosyl ; R'=H
3, R=H ; R'=OH
4, R=2-O-methyl-β-D-xylopyranosyl ; R'=OH

O-methyl-β-D-xylopyranosides, **2** and **4**, along with those of the new xyloside **5** and the sulphated steroid **7**, which were isolated as minor constituents of *Poraster superbus*. These compounds co-occur with the genins **1** and **3** and major amounts of nodososide **8**, which was firstly isolated from *Protoreaster nodosus*[3]. The polar extracts of *Poraster superbus* also contain the xyloside **6**, which was isolated as 15-sulphated derivative from *Echinaster sepositus*[4].

EXPERIMENTAL

EXTRACTION AND ISOLATION

The fresh animals (7 kg) collected at North of New Caledonia in September 1984, were chopped and soaked in water for 4 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (1 kg). This column was washed with water and then with methanol. The methanol extract was taken to dryness to give 1.5 g of glassy material, which was chromatographed on a column of Sephadex LH-20 (2.5×90 cm) using MeOH as eluant. Fractions of 4 ml were collected and checked by TLC on SiO_2 with 80:18:2 $CHCl_3$/MeOH/H_2O. Fractions 37-54 (768 mg) contained the mixture of polyhydroxysteroids and glycosides, which was submitted to droplet counter-current chromatography (DCCC; 7:13:8 $CHCl_3$/MeOH/H_2O in the ascending mode at a flow of 10 ml/h); fractions of 3.5 ml were collected to give the following fractions:

Fractions, No.	Amounts, mg	Compounds
7-22	90	sulphated material
23-40	115	nodososide
41-50	33	*1* and *3*
51-58	35	*2*
59-66	30	*2* and *4*
86-96	13	*5*
97-120	20	*6*

Each of the above groups of fractions was then submitted to HPLC on a C_{18} μ-bondpak column (30 cm × 7.8 mm i.d.) with 7:3 MeOH/H_2O (55:45 MeOH/H_2O for the more polar fractions 7-22) (flow rate 5 ml/min) to give single compounds. The results are summarized in table 1.

Rotations were taken for solutions in methanol (*c* ranging from 0.3 to 1.0).

SOLVOLYSIS OF 7 GIVING 7a

A solution of **7** (3 mg) in dioxane (0.15 ml) and pyridine (0.15 ml) was heated in a stoppered reaction vial at 120 °C for 4 h. The residue, which was obtained by evaporation of the solvents to dryness under reduced pressure, was purified by HPLC on a C_{18} μ-bondpak column (30 cm × 3.8 mm, i.d.) with 7:3 MeOH/H_2O, to give the desulphated **7a** (R_f, TLC on SiO_2 in 60:15:25 *n*-BuOH/AcOH/H_2O, 0.87; **7** had R_f 0.65), FAB-MS: *m/z* 519 (M+Na).

(*) Work supported by the National Research Council of Italy (CNR) under Progetto Finalizzato «Chimica Fine e Secondaria».

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(*) Part XXV: R. Riccio, M. V. D'Auria, M. Iorizzi, L. Minale, D. Laurent, D. Duhet, *Gazz. Chim. Ital.*, *115*, 405 (1985).

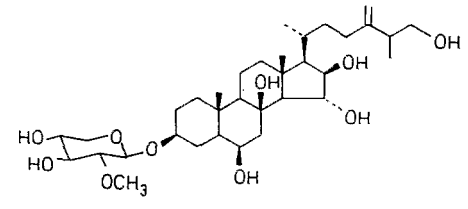
TABLE 1 - POLAR STEROIDS FROM *P. superbus* (7 kg fresh material)

Compound	Amount (mg)	Retention time in HPLC[a] (min)	$[\alpha]_D$	FAB-MS M+H,	FAB-MS M+Na
1	8	7.2	+14.0	—	
2 (new)	6	8.8	+10.4	615	637
3	30	8.0	+32.0	—	
4 (new)	6	13.6	+ 3.4	631	653
5 (new)	9	15.2	−67.1	627	649
6	3.9	18.8	—	625	647
7 (new)	8.7	10.0[b]	+ 4.9	599	621
Nodososide, **8**	88	6.4	−20.0	—	769

([a]) Eluant 7:3 MeOH/H_2O unless otherwise noted. ([b]) Eluant 55:45 MeOH/H_2O.

METHANOLYSIS OF GLYCOSIDES: SUGAR ANALYSIS

A solution of each glycosides **2**, **4**, and **5** (*ca.* 0.5 mg) in anhydrous methanolic 2 *M* HCl (0.1 ml) was heated at 80 °C in a stoppered reaction vial for 8 h. After being cooled, the



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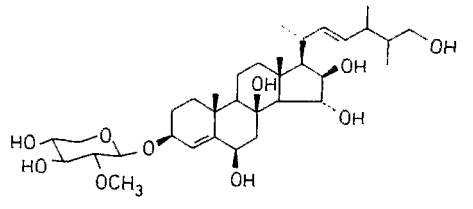

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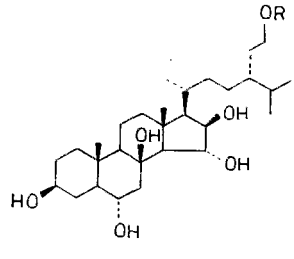
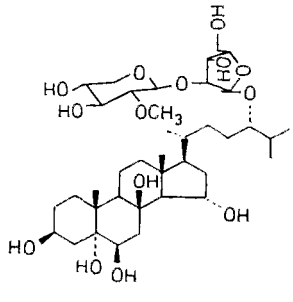
reaction mixture was neutralized with Ag_2CO_3, centrifuged and the supernatant evaporated to dryness under N_2. The residue was dissolved in TRISIL Z (0.03 ml; *N*-trimethylsilylimidazole in pyridine, Pierce Chemical Co.), left at room



7, R=SO_3^- Na^+

7a, R=H



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temperature for 15 min and analyzed by GLC (25 m capillary column SE-30, 140 °C, hydrogen carrier, flow of 10ml/min; Carlo Erba Fractovap 2900). The GLC peaks co-eluted with those of a silylated standard of methyl 2-*O*-methylxyloside.

INSTRUMENTAL

See Part XXV.

RESULTS AND DISCUSSION

Fractionation of the aqueous extracts from *Poraster superbus* was carried out by using the usual procedure (*Experimental*) to give three new glycosides, **2**, **4**, and **5**, the new sulphated polyhydroxysteroid **7**, the known polyhydroxysteroids **1** and **3**[2], and the glycosides, nodososide, **8**[3], and 15-desulphated echinasteroside A, **6**[4]. Nodososide was the major component of the mixture. The known compounds were identified by comparison with authentic samples.

An examination of the spectral data (^1H and ^{13}C NMR) of **2**, **4**, and **5** indicated that they contained the same 2-*O*-methyl-β-xylopyranosyl unit, a common sugar component among the steroidal glycosides isolated from starfishes[1] (tables 3 and 4). Acid methanolysis of **2**, **4**, and **5** afforded the same methyl 2-*O*-methylxylosides. The results of the FAB-mass spectroscopy (FAB-MS) are shown in table 2. All spectra contained molecular ion species in the form of (M+H) and (M+Na) and major fragments corresponding to the loss of the sugar residue (146 m.u.) from M+H and sequential loss of water. Further analysis of the ^1H NMR spectra of **2** and **4** indicated that the structures of their steroid aglycones were **1** and **3**, respectively. Finally, the comparison of the ^{13}C NMR spectra of **2** and **4** with those of their corresponding aglycones **1** and **3**, identified the site of glycosidation to be at C(3) in both compounds. C(3) is deshielded by 7.9 ppm in both **2** and **4** relative to **1** and **3**, respectively, and C(2) and C(4) are shielded (1.5-1.4 and 3.1-3.2 ppm), as expected upon glycosidation at C(3)[5]. All the remaining ^{13}C signals are virtually

TABLE 2 - FAB-MS DATA OF COMPOUNDS **2**, **4**, AND **5**[a]

2	**4**	**5**	Species
637 (3)	653 (10)	649 (5)	[M+Na]
615 (2)	631 (8)	627 (21)	[M+H]
597 (4)		609 (2)	[M+H]−H_2O
579 (2)		591 (5)	[M+H]−2 H_2O
561 (6)		573 (30)	[M+H]−3 H_2O
543 (6)		555 (10)	[M+H]−4 H_2O
	485 (20)		[M+H]−146
451 (20)	467 (20)	463 (2)	[M+H]−146−H_2O
433 (30)	449 (100)	445 (7)	[M+H]−146−2 H_2O
415 (100)	431 (50)	427 (30)	[M+H]−146−3 H_2O
397 (80)	413 (80)	409 (100)	[M+H]−146−4 H_2O
379 (20)	395 (25)		[M+H]−146−5 H_2O

([a]) Relative intensity in brackets. The 146 mass unit corresponds to the 2-*O*-methylxylose fragment.

identical in the pairs **1-3** and **2-4**.

Continuing now the analysis of the data for **5**, the FAB-mass spectrum and the DEPT (Distortless Enhancement by Polarization Transfer) ^{13}C NMR spectrum established the molecular formula $C_{34}H_{58}O_{10}$. The sugar residue accounts for $C_6H_{11}O_4$, leaving $C_{28}H_{47}O_6$ for the aglycone moiety. ^{13}C NMR showed the presence of one carbon-carbon double bond (exo-methylene; one quaternary carbon signal at 154.0 and one CH_2 signal at 109.2 ppm), confirmed by ^1H NMR olefinic signals at δ 4.78 bs and 4.87 bs. A 24-methylenecholestane with six hydroxyl groups (one primary, four secondary and one tertiary; DEPT ^{13}C NMR) was thus a reasonable candidate for a structural assignment. The ^1H NMR included two methyl singlets at δ 1.15 and 1.20 and only two methyl doublets at δ 0.99 (H_3-21) and 1.09 (H_3-27). The absence of the resonance of one methyl group, together with the presence of a 2 H *AB* system at δ 3.65 (dd, J_{AB}=12 Hz, J_{AX}=5 Hz) and 3.58 (dd, J_{BX}=6.5 Hz), suggested the presence of a primary hydroxy group at C(26). The proton at C(25) in an allylic position appeared shifted to δ 2.32 (m). Irradiation of this multiplet did indeed collapse the doublet at δ 1.09 (H_3-27) to a singlet and transformed the signals at δ 3.65 and 3.58 in two doublets. Thus, the 24-methylene-26-hydroxy side chain structure was established. The spectrum displayed four isolated signals for the hydroxymethine protons of the aglycone: a multiplet at δ 3.70 with the complexity normally seen for a 3β-hydroxy group, an apparent quartet at δ 3.91 with J=3 Hz, characteristic for an equatorial proton coupled with three other protons (6β- or 11β-hydroxy group), and two doublets of doublets at δ 4.02 (J=8 and 2.5 Hz) and 4.17 (J=11.0 and 2.5 Hz), already seen in the spectra of **1-4** and assigned to 16α,15β-dihydroxymethine protons. The signal at 19.6 ppm (CH_2 by DEPT pulse sequence) in the ^{13}C NMR spectrum was assigned to C(11), ruling out the possibility of an hydroxy group located there. The tertiary hydroxy group has been placed at C(8), which is a common element in starfish hydroxylated steroids. Two doublets of doublets at δ 1.62 (J=15 and 2.5 Hz) and 2.46 (J=15 and 2.5 Hz) coupled to each other by 15 Hz and coupled by 2.5 Hz with the 6α-proton were assigned to H_2-7. The lack of additional coupling to the protons with δ 1.62 and 2.46 [C(7)] suggested an adjacent quaternary centre [C(8)]. The 3β,6β,8-trihydroxy pattern has been already found in 5-deoxyisonodososide, (24*S*)-3-*O*-(2-*O*-methyl-β-D-xylopyranosyl)-24-*O*-α-L-arabinofuranosyl-5-α-cholestane-3β,6β,8,15α,24-pentol, minor constituents of *Achanthaster planci*[6], in halityloside F from *Halityle regularis*[7] and in attenuatoside S-I from

TABLE 3 - ^1H NMR DATA (CHEMICAL SHIFT, MULTIPLICITY, COUPLING CONSTANTS IN Hz) IN CD_3OD

H at C	**1**	**2**[a]	**3**	**4**[a]	**5**[a]	**7**	**7a**
3	3.51, m	3.63, m	3.51, m	3.62, m	3.70, m	3.51, m	3.51, m
6	3.66, dt, 4.5, 11	3.66, dt, 4.5, 11	3.82, m	3.82, m	3.91, q, 3	3.66, dt, 4.5, 11	3.66, dt, 4.5, 11
7	2.43[b], dd, 4.2, 13.5	2.44[b], dd, 4.2, 13.5	3.82, m	3.82, m	2.46, dd, 2.5, 15, 1.62, dd, 2.5, 15	2.44[b], dd, 4.2 13.5	2.44[b], dd, 4.2, 13.5
15	4.08, dd, 2.5, 11	4.08, dd, 2.5, 11	4.17, dd, 2.5, 11	4.17, dd, 2.5, 11	4.17, dd, 2.5, 11	4.06[c], m	4.08, dd, 2.5, 11
16	4.01, dd, 2.5, 8	4.01, dd, 2.5, 8	4.03, dd, 2.5, 8	4.03, dd, 2.5, 8	4.02, dd, 2.5, 8	4.06[c], m	4.02, dd, 2.5, 8
18	1.15, s	1.15, s	1.15, s	1.15, s	1.15, s	1.14, s	1.15, s
19	1.05, s	1.05, s	1.04, s	1.04, s	1.20, s	1.05, s	1.05, s
21	0.96, d, 6.3	0.96, d, 6.3	0.96, d, 6.3	0.96, d, 6.3	0.99, d, 7	0.96, d, 6.7	0.96, d, 6.7
26	3.46, dd, 6, 10.5 3.32, dd, 5, 10.5	3.46, dd, 6, 10.5 3.32, dd, 5, 10.5	3.46, dd, 6, 10.5 3.32, dd, 5, 10.5	3.46, dd, 6, 10.5 3.32, dd, 5, 10.5	3.65, dd, 5, 12 3.58, dd, 6.5, 12	0.89, d, 6.2	0.89, d, 6.2
27	0.94, d, 6.3	0.94, d, 6.3	0.94, d, 6.3	0.94, d, 6.3	1.09, d, 7,	0.92, d, 6.2	0.92, d, 6.2
28	—	—			4.78 bs - 4.87 bs		
29	—	—				4.06[c], m	3.60, m

([a]) Sugar proton signals; H-1: 4.44, d, J=7.5 Hz; H-2: 2.84, dd, J=9, 7.5 Hz; H-3: 3.35, t, J=9 Hz; H-4: 3.5 m; H_2-5: 3.16, dd, J=11, 12 Hz - 3.83, dd, J=12,5 Hz; in the spectrum of **4** the H_2-5 overlaps with the multiplet for H-6. ([b]) The remaining 7-proton signal is confused in the δ 2.0-1.5 region. ([c]) Overlapping signals.

TABLE 4 - ^{13}C NMR DATA FOR COMPOUNDS **1-5** AND **7** (AT 62.9 MHz, δ/ppm) in CD_3OD[a]. The glycosidation shifts are shown in brackets: **2** VS **1** AND **4** VS **3**

C	1[b]	2[b]	3[a]	4[c]	5[c]	7
1	39.6	39.6	39.6	39.4	41.4	39.6
2	31.5	30.1 (−1.4)	31.5	30.0 (−1.5)	30.3	31.5
3	72.2	80.1 (+7.9)	72.3	80.2 (+7.9)	80.4	72.2
4	32.4	29.3 (−3.1)	32.3	29.1 (−3.2)	33.3	32.4
5	53.7	53.6	44.5	44.5	[d]	53.7
6	67.6	67.6	68.9	68.9	74.2	67.7
7	[d]	50.2	76.5	76.3	45.5	50.1
8	75.9	76.0	77.7	77.8	76.9	76.0
9	57.4	57.4	51.2	51.2	57.3	57.4
10	37.8	37.9	37.8	37.9	36.8	37.9
11	19.4	19.4	19.3	19.2	19.6	19.4
12	43.2	43.3	43.2	43.1	43.2	43.2
13	45.3	45.4	45.5	45.5	45.4	45.3
14	64.5	64.5	59.6	59.6	64.0	64.5
15	80.7	80.8	79.3	79.8	81.0	80.8
16	83.0	83.1	82.7	82.7	83.1	82.9
17	60.6	60.7	61.4	61.4	60.6	60.6
18	16.9	16.9	16.9	16.8	16.8	16.9
19	14.2	14.1	13.9	13.8	15.7	14.2
20	30.6	30.6	30.6	30.6	30.6	31.1
21	18.4	18.4	18.4	18.3	18.4	18.4
22	37.2	37.2	37.1	37.1	35.6	35.1
23	24.9	24.8	24.9	24.8	32.9	28.9
24	35.0	35.0	35.0	35.0	154.0	42.4
25	37.1	37.0	37.1	37.0	43.5	31.1
26	68.5	68.6	68.5	68.5	67.6	19.3
27	17.4	17.2	17.4	17.3	17.2	19.9
28	—	—	—	—	109.2	31.8
29	—	—	—	—	—	68.3

[a] Assignments were aided by DEPT (Distortless Enhancement by Polarization Transfer) technique using polarization pulse of 90 and 135°, obtaining in the first case only CH groups and in the other case positive signals for CH and CH_3 and negative ones for CH_2 groups. [b] ^{13}C NMR data of **1** and **3** were taken from ref. 2. [c] δC of 2-*O*-methylxylose moiety: C(1): 103.5; C(2): 84.9; C(3): 77.5; C(4): 71.4; C(5): 66.7; OMe: 60.9 ppm. [d] Signal under solvent signal.

Hacelia attenuata[8]. In the spectrum of **5** the shift values for carbons 1-12 and C(19) were within ±0.1 ppm with those reported for 5-deoxyisonodososide[6]. This established the location of the xylosyl residue at C(3) in **5**. Assignments of the remaining ^{13}C NMR signals in **5** have been made by using the DEPT technique and the assignments reported for compounds **1-4**. The common 20*R* configuration proposed is based on the chemical shift of 21-methyl protons[9]. The stereochemistry at C(25) in **5** remains to be established. The absolute configuration of the xylosyl units in **2**, **4**, **5**, and **6** has not been proved. We prefer the common D configuration, because of the many D xylosides isolated from starfishes[7].

Considering now the data for **7**, the FAB-mass spectrum, which gave molecular ion species at *m/z* 621 (M+Na) and 599 (M+H) and one major fragment at *m/z* 519 corresponding to loss of SO_3 from M+H, suggested it to be a sulphated molecule. M is the molecular weight of the sodium salt. The presence of a sulphate group was also indicated by its polarity and confirmed by solvolysis in dioxan-pyridine, affording the desulphated derivative **7a**, FAB-MS, *m/z* 519 (M+Na). ^{13}C NMR of **7** showed the absence of carbon-carbon double bonds and indicated six carbons bonded to oxygen. Thus the MW 496 determined for **7a** corresponded to a saturated C_{29} sterol with six hydroxyl groups (one primary, four secondary and one tertiary; DEPT ^{13}C NMR). Comparison of ^1H- and ^{13}C NMR spectral data of **7a** (table 3 and 4) with those of **1** established the presence of a 3β,6α,8,15α,16β-pentahydroxytetracyclic nucleus in the new ste-

roid. A 24-(β-hydroxyethyl)side chain, already found in several steroidal glycosides from *Halityle regularis*[7] and *Echinaster sepositus*[4] accounts for the remaining data. The ^{13}C NMR spectrum of **7** and comparison with those of synthetic (24*R*)- and (24*S*)-24-ethyl-5α-cholest-7-ene-3β,29-diol[10] established the location of the sulphate residue at C(29) and suggested the 24*R* configuration. In the spectra of model 29-hydroxy compounds, C(28) and C(29) appeared at 34.3 and 62.1 ppm, respectively. The location of the sulphate group at C(29) received confirmation from the comparison of the ^1H NMR spectra of native **7** and its desulphated derivative **7a** (table 3). Further, in the spectrum of the 24*S* model compound the 26- and 27-carbons appear as very close signals at 19.2 and 19.3 ppm, while in the 24*R* isomer they are split by *ca.* 1 ppm (18.6-19.7 ppm). Our values (19.3 and 19.9 ppm) compared better with those of the 24*R* isomer. The proton shifts for the C(26)/C(27) methyl groups are also sensible to the stereochemistry at C(24); in the 220 MHz spectrum ($CDCl_3$) of 29-hydroxyclionasterol (*i.e.* 24*R*) the 26- and 27-methyl protons appear as a triplet because of the coincidental overlap of the low-field arm of one doublet (*δ 0.83*) with the high field arm of the other (*δ 0.86*), while they appear as overlapping doublets at δ 0.84 and 0.85 in the spectrum of 29-hydroxysitosterol (*i.e.* 24*S*)[11]. In our samples, **7** and **7a**, the 26- and 27-methyl protons appear as doublets separated by 0.03 ppm as do the 26- and 27-methyl signals in the 24*R* model compound.

We thank the divers J. L. Menou, G. Bargibant, and P. Tirard of the ORSTOM, Centre de Nouméa, for collecting the animals.

FAB mass spectra were provided by the «Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli». The assistance of the staff is gratefully acknowledged.

Received May 29th 1985.

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