

STARFISH SAPONINS, 29.¹ A NOVEL STEROIDAL GLYCOSIDE FROM THE STARFISH *PENTACERASTER ALVEOLATUS*²

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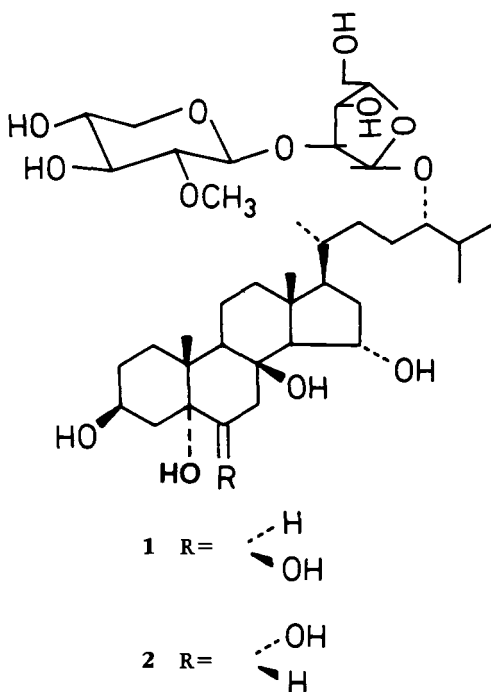
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We have reported previously the occurrence of a novel sulfated asterosaponin, protoreasteroside, 6 α -O-{ β -D-fucopyranosyl (1 \rightarrow 2)- β -D-quinovopyranosyl (1 \rightarrow 4)[- β -D-quinovopyranosyl (1 \rightarrow 2)] - β -D-xylopyranosyl (1 \rightarrow 3)- β -D-quinovopyranosyl-}20 R)-(22 S)-20,22-dihydroxy-cholesta-9(11),24(25)-diene-3 β -yl sodium sulfate, along with major amounts of the known thorna-

steroside A from the Pacific starfish *Pentacaster alveolatus* Perrier (1).

We have now examined the less polar components and have isolated the known nodoside (**1**) (2,3), and some polyhydroxysteroids: (25 S)-5 α -cholestane-3 β ,6 α ,8,15 α ,16 β ,26-hexol, (25 S)-5 α -cholestane-3 β ,6 α ,7 α ,8,15 α ,16 β ,26-heptol, (25 S)-5 α -cholestane-3 β ,4 β ,6 α ,8,15 α ,16 β ,26-heptol, and (25



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S)-5 α -cholestane-3 β ,4 β ,6 α ,7 α ,8,15 α ,16 β ,26-octol, previously isolated from *Protoreaster nodosus* (4,5), and one new steroidal glycoside, 6-*epi*-nodososide (**2**).

Fabms of **2** showed molecular ion species at m/z 769 ($M+Na$). The ¹H-nmr spectrum of **2** at 500 MHz was very

similar to that of nodosodide (**1**, except the signals due to the 6-H and 19-H₃). The 6β-H in **2** was seen at δ 3.88 as a dd with *J* of 11.2 and 4.5 Hz (indicative of an axial proton) (in **1** 6α-H appears at δ 3.68 as a dd with *J* of 3.1 and 2.8 Hz, equatorial proton). According to the change of stereochemistry at C-6 in **2**, 19-H₃ signal was seen at δ 1.20 (s), highfield shifted with respect to the corresponding signal in **1** (δ 1.34 s). This is consistent with the 6-*epi*-nodososide structure for the new compound. The ¹³C-nmr spectrum (Table 1) and com-

signals by using the substituent effects reported for hydroxy steroids (6-8).

EXPERIMENTAL

INSTRUMENTAL.—For instruments used, see Iorizzi *et al.* (9).

EXTRACTION AND ISOLATION.—The animals (6 kg) were collected in November 1983, off Nouméa and identified by Mr. Michel Jongoux of the Université Libre de Bruxelles. Details of the extraction, recovery of the polar materials from the aqueous extracts by chromatography on Amberlite XAD-2 and fractionation on a column of Sephadex LH-60 are reported in Riccio *et al.* (1). The less polar fractions containing polyhydroxy-

TABLE 1. Comparison of ¹³C-nmr Data for the Aglycone Carbons of Nodososide (**1**) and 6-*epi*-Nodososide (**2**) in CD₃OD^a

Atom	Compound		Atom	Compound	
	1	2		1	2
C-1	34.4	32.7	C-15	70.1	69.9
C-2	30.9	30.8	C-16	40.4	40.5
C-3	68.2	68.1	C-17	55.9	55.9
C-4	41.6	41.8	C-18	15.4	15.5
C-5	76.5	78.4	C-19	18.0	17.3
C-6	77.9	69.5	C-20	36.3	36.3
C-7	41.1	43.6	C-21	19.0	19.0
C-8	77.2	75.7	C-22	32.8	32.9
C-9	48.7	b	C-23	28.6	28.5
C-10	39.2	38.1	C-24	84.5	84.4
C-11	19.7	19.4	C-25	31.5	31.5
C-12	42.9	43.0	C-26	18.2	18.1
C-13	45.5	45.5	C-27	18.3	18.4
C-14	66.5	66.9			

^aAssignments are based on comparison and use of substituent effects in hydroxy steroids (6-8); the ¹³C-nmr spectrum of the aglycone carbons of nodosodide in CD₃OD is reported in Iorizzi *et al.* (9); Riccio *et al.* (2) reports the complete spectrum run in pyridine *d*₅; the signals for the sugar carbons were coincident in the two spectra; arabinose, C-1: 107.8, C-2: 92.8, C-3: 77.8, C-4: 83.7, C-5: 62.5; 2-*O*-methylxylose, C-1: 105.4, C-2: 84.8, C-3: 77.4, C-4: 71.1, C-5: 66.9, C-6: 61.1 ppm.

^bUnder solvent signals.

parison with reference nodososide added support to this assignment. The most significant differences in the ¹³C-nmr spectra of the two epimers, which indicated 6α-hydroxy stereochemistry in **2**, were the upfield shift exhibited by C-6 (69.5 vs 77.9 ppm), and C-19 (17.3 vs 18.0 ppm), and the downfield shifts exhibited by C-5 (78.4 vs 76.5 ppm) and C-7 (43.6 vs 41.1 ppm). Indeed, the observed shifts were consistent with those expected for the mentioned carbon

steroids and non-sulfated steroidal glycosides (1.07 g) arising from the chromatography on Sephadex LH-60, were submitted to a further column chromatography on Sephadex LH-20 (2×60 cm; eluent, MeOH; flow rate 30 ml/h; fractions of 8 ml were collected) to give, in the fractions 35-49, 360 mg of partially purified glycosides and steroids, which were submitted to droplet counter-current chromatography [dccc; CHCl₃-MeOH-H₂O (7:13:8) in ascending mode at a flow of 20 ml/h; fractions of 7 ml were collected and monitored by tlc on silica in CHCl₃-MeOH-H₂O (80:18:2)]. Fractions 19-24 contained nodososide (**1**); the successive fractions (25-34)

contained a mixture of nodososide (1) and 6-*epi*-nodososide (2); then the fractions 32-37 contained 5 α -cholestane-3 β ,4 β ,6 α ,8,15 α ,16 β ,26-heptol (5), and finally a mixture of polyhydroxysteroids was eluted in the fractions 41-53. Hplc of the above fractions on a μ -Bondapak C₁₈ column with 35% H₂O in MeOH gave pure compounds: nodososide (1), 26 mg; 6-*epi*-nodososide (2), 5.7 mg; 5 α -cholestane-3 β ,4 β ,6 α ,8,15 α ,16 β ,26-heptol, 14 mg; 5 α -cholestane-3 β ,6 α ,8,15 α ,16 β ,26-hexol, 12 mg; 5 α -cholestane-3 β ,5 α ,7 α ,8,15 α ,16 β ,26-heptol, 12.5 mg; 5 α -cholestane-3 β ,4 β ,6 α ,7 α ,8,15 α ,16 β ,26-octol, 17 mg.

6-EPI-NODOSOSIDE.—[α]_D \pm 0; fabms *m/z* 769 (M+Na, 100%), 751 (10), and aglycone fragments at *m/z* 433 (M+H-278-2H₂O, 25), 415 (M+H-278-3H₂O, 60), 397 (M+H-278-4H₂O, 90), 379 (M+H-278-5H₂O, 30); 500 MHz nmr (CD₃OD), aglycone, δ _H 0.92 and 0.94 (9H, two doublets with *J*=6.5, 6.4 Hz, 21-, 26-, 27-H₃), 1.00 (3H, s, 18-H₃), 1.20 (3H, s, 19-H₃), 3.88 (1H, dd, *J*=11.2, 4.5 Hz, 6 β -H), 4.00 (m, overlapping with 3-H of arabinose, 3 α -H), 4.24 (1H, dt, *J*=9.8, 3.0 Hz, 15 β -H), sugars, δ _H of xylose: 2.89 (1H, dd, *J*=9.0, 7.5 Hz, 2-H), 3.16 (1H, t, *J*=11.3 Hz, 5-H ax), 3.37 (partially overlapped with solvent signal, 3-H), 3.5 (1H, m, 4-H), 3.86 (1H, dd, *J*=11.3, 4.5 Hz, 5-H' eq), δ _H of arabinose: 3.68 (1H, dd, *J*=12.0, 5.0 Hz, 5-H), 3.83 (1H, dd, *J*=12.0, 3.0 Hz, 5-H), 3.97 (1H, m, 4-H), 4.02 (1H, t, *J*=3.7, 3-H), 4.07 (1H, dd, *J*=3.7, 1.0 Hz, 2-H), 5.11 (1H, d, *J*=1.0 Hz, 1-H).

The known compounds were identified by 250 MHz ¹H nmr and authentic sample comparison by hplc and SiO₂-tlc. Full details of the identifi-

cations of these compounds are available on request to the senior author.

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