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STUDIES OF SWEDISH MARINE ORGANISMS, IX.[1] POLYHYDROXYLATED STEROIDAL GLYCOSIDES FROM THE STARFISH *PORANIA PULVILLUS*

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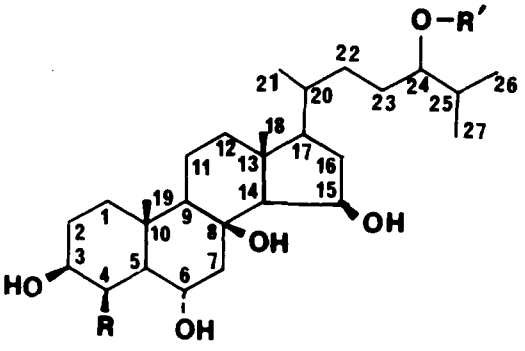
In our screening of Swedish marine organisms for biological activity, extracts from starfish tested showed activity in several of our bioassays. Pronounced activity was found in a guinea-pig ileum preparation, as well as hemagglutination activity and an inhibiting activity of malignant cells (2-4).

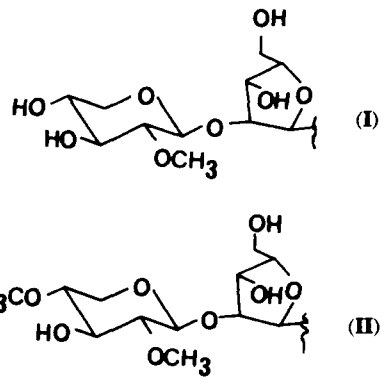
We have recently isolated a novel polyhydroxylated steroidal glycoside, crossasteroside A, from the starfish *Crossaster papposus* L. This compound inhibits electrically induced contractions in a guinea-pig ileum preparation in vitro (5). Crossasteroside A is the first representative of this class of compounds that has been isolated from cold-water starfish. Three other novel, chemically related, steroidal glycosides have recently been isolated (1) from further studies of this species.

This report deals with further examples of this class of substances isolated from the starfish *Porania pulvillus* O.F. Müller, (Poraniidae), which is also collected in cold waters off the Swedish west coast. *P. pulvillus* is a cushion-like starfish, living on rock and sand bottoms and feeding mostly on small organic particles (6).

The separation of the polyhydroxylated steroidal glycosides has been described previously by Minale *et al.* (7) for other starfish. Compounds **1-3** are known and were identified by comparison with reported spectral data (fabms, ^{1}H and ^{13}C nmr) (8-9) and by sugar analysis. The minor compound **4**, isolated from the MeOH extract of *P. pulvillus*, is



	R	R′	
1	H	**I**	
2	OH	**II**	
3	H	**II**	
4	H	**II**	Δ^{22E}



new and has been named poranoside A.

Acid hydrolysis of **4** and identification of the released sugars as their alditol acetates by gc/ms and determination of their absolute configurations by gc after reaction with (+)-2-butanol and tri-

[1]For the previous part in this series, see Andersson *et al.* (1).

methylsilylation (10) indicated the presence of 2,4-di-*O*-methyl-D-xylose and L-arabinose. Reference compounds were obtained by similar treatment of halityloside D [**2**] in which the absolute configuration of the sugars is known. Examination of spectral data (^{1}H and ^{13}C nmr) showed that **4** contains one β-D-xylopyranosyl and one α-L-arabinofuranosyl residue. The anomeric configurations were evident from the chemical shifts and the coupling constants of the anomeric protons (Table 1). The chemical shift observed in **4** for C-2 of the α-L-arabinofuranosyl residue (92.0 ppm) established the location of the terminal 2,4-di-*O*-methyl-β-D-xylopyranosyl group at the 2-position of the α-L-arabinofuranosyl moiety. Thus, poranoside A [**4**] contains a 2,4-di-*O*-methyl-β-D-xylopyranosyl (1↦2)-α-L-arabinofuranosyl moiety. Fabms showed a molecular ion at *m/z* 765 $(M+Na)^+$, corresponding to the molecular mass of 742 daltons. The glycosyl residue accounts for 293 m.u., leaving 449 m.u. for the aglycone. This corresponds to an unsaturated C_{27} sterol with five hydroxyl groups. The ^{1}H-nmr spectrum of **4** contained two four-line signals at δ 5.47 and 5.36, respectively, with $J=15$ Hz, which could be assigned to Δ^{22}-*trans* protons. The position of the double bond was also confirmed by the shift in the methyl region. The doublet at δ 0.98 in **3** was shifted to δ 1.04 in **4** which was expected for the C-21 methyl group (20*R*-configuration) (11).

From a 2D-COSY spin correlation spectrum, all protons were assigned (Table 2) which established the 3β, 6α, 8, 15β, 24ξ-hydroxylation pattern. This is in agreement with comparison with spectra of **3**, as the chemical shifts for protons on carbons 1-17 were virtually identical. The 2D-COSY spectrum also indicated that the sugar moiety was attached at C-24 of the steroid.

Due to the small amounts of material, only partial information could be obtained from ^{13}C-nmr studies. The carbon signals were found at the same

TABLE 1. Assignments of ^{1}H-nmr Signals to the 2,4-Di-*O*-methyl-β-D-xylopyranosyl-(1↦2)-α-L-arabinofuranoxyl moiety of **4**[a]

Compounds	Protons						
	H-1	H-2	H-3	H-4	H-5	H-5′	OCH_3
Arabinose	5.11 (1.1)	4.12 (3.8)	4.03 (7.2)	3.96 (3.4, 4.8)	3.76 (12.0)	3.64	
2,4-Di-*O*-Me-xylose . .	4.45 (7.6)	2.91 (9.3)	3.42 (8.6)	3.22 (10.0, 4.9)	3.16 (10.8)	4.03	3.49, 3.59

[a]400 MHz, CD_3OD, chemical shifts are given in ppm relative to internal TMS and coupling constants in Hz in parenthesis.

TABLE 2. ^{1}H-nmr Signals of the Aglycone of the Polyhydroxylated Steroid Glycoside Poranoside A [**4**]

H-1	0.99 (e), 0.86 (a)	H-10	—	H-19	1.02 (3-H)
H-2	1.75 (e), 1.53 (a)	H-11	1.87 (e), 1.56 (a)	H-20	2.25
H-3	3.51	H-12	2.01 (e), 1.24 (a)	H-21	1.07
H-4	2.22 (e), 1.25 (a)	H-13	—	H-22	5.47
H-5	1.07	H-14	1.07	H-23	5.36
H-6	3.74 (β)	H-15	4.42 (α)	H-24	3.71
H-7	2.41 (e), 1.31 (a)	H-16	2.30 (e), 1.44 (a)	H-25	1.79
H-8	—	H-17	1.07	H-26	0.96 (3-H)
H-9	0.86	H-18	1.33 (3-H)	H-27	0.87 (3-H)

[a]400 MHz, CD_3OD, in ppm relative to internal TMS.

chemical shifts (<0.2 ppm) as those of **3** for carbons 1-17, except for C-16. This signal was 0.7 ppm downfield which could be due to the proximity of the Δ^{22}-double bond (δ-gauche effect).

EXPERIMENTAL

INSTRUMENTAL.—The following instruments were used: nmr, JEOL GX-400; fabms, JEOL DX 303; hplc, Waters Model 6000 pump with an Altex injector, and a Model 401 differential refractometer detector; gc/ms, Hewlett-Packard 5890; dccc, a DCCC-A apparatus manufactured by Rikakikai, Tokyo, equipped with 250 tubes. Nmr spectra were recorded at 30° for solutions in CD_3OD, at 400 MHz (^1H nmr) and 100 MHz (^{13}C nmr) using internal TMS as reference. The fab mass spectra were obtained by dissolving the samples in a thioglycerol-glycerol (1:3 v/v) matrix and placing them on a copper tip prior to bombardment with a 6 keV Xe beam. A Perkin-Elmer 241 polarimeter was used.

EXTRACTION, FRACTIONATION, AND ISOLATION OF THE STEROIDAL GLYCOSIDES.—Specimens of *P. pulvillus* (1.3 kg) were collected by scuba diving at a depth of 15-25 m in May 1985, at the Tjärnö Marine Biology Laboratory (TMBL) at the Koster Fjord on the northern Swedish west coast and were identified by Dr. Lars Afzelius (TMBL). A voucher specimen is kept at TMBL. The starfish, which were frozen prior to storage, were thawed and homogenized in an ultra turrax using distilled H_2O (5 liters) and centrifuged at 4°. The supernatant was added to a column of Amberlite XAD-2 (1 kg) and eluted with H_2O (3 bed volumes) followed by MeOH (3 bed volumes). The MeOH was evaporated giving a crude MeOH extract (2.6 g). Part of the MeOH extract (1.6 g) was loaded on a column of Sephadex LH-60 and eluted with MeOH-H_2O (2:1). Fractions were monitored by tlc on SiO_2 in $CHCl_3$-MeOH-H_2O (80:18:2) and detected with ceric sulfate-H_2SO_4. A fraction which showed the presence of glycosides was further fractionated by dccc with $CHCl_3$-MeOH-H_2O (7:13:8) as solvent, using the organic phase as stationary phase (ascending mode). Fractions of 6 ml (flow rate 0.15 ml/min) were collected and monitored by tlc as above and combined into 12 fractions. Fractions 7, 10, and 11 were further fractionated by hplc using a C_{18}-μ-Bondapak column (30×1,8 cm i.d.) and eluting with MeOH-H_2O (7:3); flow rate 5.0 ml/min. This resulted in the isolation of four glycosides; attenuatoside A-1 [**1**] 3.2 mg from fraction 7, halityloside D [**2**] 7.1 mg from fraction 10, halityloside E [**3**] 16.5 mg from fraction 11 together with the novel poranoside A [**4**] 3.0 mg. Physical data of poranoside A [**4**]: $[\alpha]^{22}D-9°$ (*c* 0.1, MeOH); fabms *m/z* 765 (M+Na), 743 (M+H), 433 (M-sugar), 415 (M-sugar-H_2O), 397 (M-sugar-$2H_2O$), 379 (M-sugar-$3H_2O$); ^1H nmr see Tables 1 and 2; sugar analysis: 2,4-di-*O*-methyl-D-xylose and L-arabinose.

SUGAR ANALYSIS.—The glycosides (0.2 mg) were hydrolyzed with 2 M trifluoroacetic acid (2 ml) at 120° for 1 h. The solutions were evaporated to dryness by a stream of nitrogen. The products were reduced with sodium borodeuteride (5 mg) in H_2O (1 ml) for 2 h, then treated with Dowex 50 (H^+) to pH 4, filtered, evaporated to dryness. The H_3BO_3 was removed as the methyl ester by repeated addition of MeOH (2×1 ml) followed by evaporation to dryness. The samples were acetylated with Ac_2O in pyridine (1:1, 0.4 ml) at 100° for 30 min and then concentrated to dryness. The alditol acetates were analyzed by gc/ms using a glass capillary column coated with SP-1000 (180°).

ABSOLUTE CONFIGURATION.—The glycoside (0.2 mg) was treated with 1 M HCl in (+)-2-butanol (200 μl) at 100° for 8 h in a sealed tube. The mixture was then evaporated to dryness, silylated with BSTFA in pyridine at 90° for 30 min, concentrated to dryness, dissolved in $CHCl_3$ (0.3 ml), and analyzed by gc (fused silica capillary column, SE-54, 130°).

2D-COSY SPIN CORRELATION NMR.—A frequency width of 2080 Hz was used in both dimensions. A total of 128 pulses in a 1024×256 data matrix were acquired. Resolution enhancement using a sine-bell function in both dimensions was performed prior to Fourier transformation.

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