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Hapaioside: A 19-Norpregnane Glycoside from the Sponge *Cribrochalina olemda*

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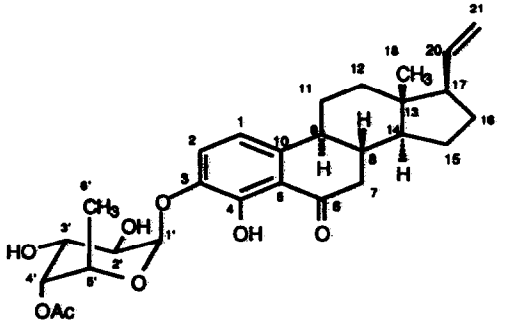
Abstract: A 4-hydroxy-6-oxopregnane-3-glycoside with an aromatic ring A was isolated from a Pohnpei sponge, *Cribrochalina olemda*. The sugar is a 6'-deoxy-L-β-altropyranose-4'-acetate.

Steroidal glycosides (saponins) are common constituents of many terrestrial plants.[4] They are also characteristic secondary metabolites of sea stars.[5] Conversely, pregnane derivatives and their glycosides are relatively rare constituents of marine invertebrates. Some years ago we[6] and others[7-11] reported a few simple pregnanes from coelenterates and sponges. More recently, Kashman *et al.*[12] isolated a C-4 glycosidic pregnane from a Mediterranean gorgonian. An underivatized pregnane with an aromatic ring A was described by Blackman *et al.*[13] from Tasmanian soft corals, *Capnella spp.* In this paper we report the structure of hapaioside,[14] which is a C-3 glycoside of a 6-oxonorpregnane with an aromatic ring A, isolated from a Pohnpei sponge, *Cribrochalina olemda*.[15] The sugar is 6'-deoxy-L-β-altropyranose-4'-acetate.

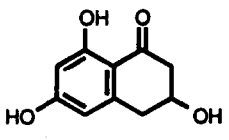


1

Hapaioside (1) has a molecular formula of $C_{28}H_{36}O_8$ by HRFABMS (M+H m/z 501.2503 Da, Δ -1.5 mmu). It was not immediately apparent that the compound was steroidal because of the high degree of unsaturation (u=11) and the aromaticity of ring A, which eliminated many obvious clues. A more thorough examination of the NMR data revealed features that suggested an aromatic pregnane. Two *ortho* protons at 6.8 (H-1, dd, *J*=8.4, 1.3 Hz) and 7.3 ppm (H-2, d, *J*=8.4 Hz) were characteristic of a tetrasubstituted benzene ring. The 1.3 Hz coupling of H-1 is attributed to benzylic coupling with H-9. Another diagnostic feature of the proton spectrum was a signal at 5.74 ppm (H-20, ddd), characteristic of a terminal vinyl group, confirmed by COSY data. The ^{13}C-NMR spectrum showed carbonyl resonances at 172.4 (C-7') and 206.5 ppm (C-6). The carbonyl at 172.4 ppm was characteristic of an acetyl group while the 206.5 ppm carbonyl was indicative of a ketone.

The ^{1}H-^{1}H COSY, HMQC, and HMBC data allowed expansion of the presumed steroidal protons H_2-7 (2.72, 2.36 ppm). Methylene protons at C-7 and H-8 show two- and three-bond HMBC correlations to the carbonyl carbon at 206.5 ppm. Since the signal at 2.45 ppm (H-9) does not show correlation to the carbonyl, it must be farther away than three bonds. Hence the carbonyl had to be at C-6. These NMR correlations place the carbonyl in conjugation with the aromatic ring; however, chemical shift, IR, and UV data do not readily support this. The carbonyl resonates at 206.5 ppm, which is about 5-10 ppm farther downfield than expected.[16] The IR spectrum has two carbonyl bands at 1735 and 1634 cm^{-1}. The band at 1735 cm^{-1} can be attributed to an acetate carbonyl but the ketone would be expected to absorb at higher frequency than the observed 1634 cm^{-1}.[16]

More complex analogs, scytalone (2)[17] and grandinol (3)[18] contain similar substructures and chromophores. The ^{13}C carbonyl chemical shifts (202 and 207 ppm), IR absorptions of the ketone (1637 and 1630 cm^{-1}) and UV data (λ_{max} (EtOH) 284nm (ε=12882) 322nm (ε=6760), and λ_{max} (EtOH) 278nm (ε=27700), 345nm (ε=3800)) of **2** and **3** respectively, support the proposed structure of **1**.



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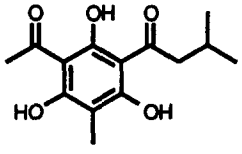

3

The *trans-anti-trans* fusion of the B-C-D rings and the stereochemistry of the C-17 substituent were determined from a ROESY experiment. The H-9 (2.45 ppm) showed correlations between H-14 (1.39) and H-12α (1.39 ppm). Since H-14 (1.39 ppm) and H-17 (2.05 ppm) show similar correlations, H-17 must also have α-configuration. The ROESY spectrum also showed correlations between the CH_3-18 protons (0.63 ppm) and H-8 (1.91 ppm). Therefore both B/C and C/D must be *trans* and the C-17 vinyl substituent is β-oriented.

Table I. NMR Data for 1(500 MHz, $CDCl_3/CD_3OD$ 1:1)

#	^{13}C	^{1}H (*J* in Hz)	HMBC	COSY
1	116.2	6.84 (H, 8.4, 1.3, dd)	H_α-9	H-2
2	125.1	7.31 (H, 8.4, d)		H-1
3	144.1		H_e-1', H-1, H-2	
4	154.3		H-1, H-2	
5	118.0		H_β-7, H-1, H-2	
6	206.5		H_α-7, H_β-7	
7	45.1	2.36 (H_α, 17.3, 13.3, dd);	H_α-9	H_β-8
		2.72 (H_β,17.3, 3.6, dd)		
8	40.6	1.91 (H_β,m)	H_β-15, H_α-15, H_α-9,	H_2-7, H_α-9, H-12
			H_α-7, H_β-7	
9	44.0	2.45 (H_α, 4.5, 11.6, dt)	H-11, H_α-7, H_β-7, H-1,	H_β-8, H_2-11
			H_α-12, H_β-12	
10	143.2		H-2, H_α-9	
11	25.9	1.61 (H_β, m); 2.30 (H_α, m)	H_α-12, H_α-9	H_α-9, H_2-12
12	37.5	1.37 (H_α, m); 1.85 (H_β, m)	H_3-18, H-11, H_α-17	H-11
13	44.2		H_α-17, H_β-16, H_α-16, H_3-18	
14	55.2	1.39 (H_α,m)	H_3-18, H_β-15, H_α-7	H_β-8, H_2-15
15	24.7	1.27 (H_β, m);	H_β-8	H_α-14, H_2-16
		1.77 (H_α, 6.8, 2.4, bdquint)		
16	27.8	1.62 (H_β, m); 1.83 (H_α, m)	H_β-15, H_α-17, H-20	H_2-15, H_α-17
17	55.9	2.05 (H_α, m)	H_α-16, H-20, 2H-21	H_2-16, H-20
18	13.0	0.63 ($3H_\beta$, s)	H_α-12, H_α-14, H-13	
20	139.7	5.74 (H, 17, 12.9, 6, ddd)	H_α-17, H_2-21	H_α-17, H_2-21
21	115.5	4.98 (H_2, 12.9, bd)	H_α-17	H-20
1'	100.5	5.45 (H, 3.7, d)	H_e-5', H_a-3', H_a-2'	H_a-2'
2'	69.8	3.86 (H, 10.2, 3.7, dd)	H_a-3', H_e-4', H_e-1'	H_e-1', H_a-3'
3'	69.1	4.18 (H, 10.2, 3.5, dd)	H_e-4', H_e-1', H_a-2'	H_a-2', H-4'
4'	74.7	5.22 (H, 3.5, dd*)	H_a-3', H_e-5', H_3-6'	H_a-3'
5'	67.1	4.32 (H, 6.6, dq*)	H_e-1', H_e-4', H_3-6'	H_e-4', H_3-6'
6'	16.5	1.06 (H_3, 6.6, d)	H_e-5'	H_e-5'
7'	172.4		H_e-4', H_3-8'	
8'	20.8	2.1 (H_3, s)		

*Second coupling constant too small to measure accurately.

Five methine carbon resonances with chemical shifts between 67 to 100 ppm plus a high oxygen content suggested the presence of a sugar, which was supported by a methine carbon resonating at 74.7 (C-4', acetyl) and one at 100.5 (C-1', acetal) ppm. The ^1H-^1H COSY, HMQC, and HMBC data provided the other three methine connectivities leading to a monoacetylated pyranose, which was compatible with the chemical shifts of the other methine carbons, (67.1(C-5'), 69.1(C-3'), and 69.8 (C-2') ppm).

Relative stereochemistry of the saccharide was determined by NOE experiments and coupling constants (Fig. 1.). H-2' (3.86 ppm) was split into a doublet of doublets J=10.2 and 3.6 Hz, which indicated one axial and one equatorial vicinal hydrogen. H-3' showed the same coupling constants (J=10.2 and 3.6 Hz). From this we concluded that H-2' was *trans axial* to H-3' and equatorial, (*cis*) to H-1' (J=3.6 Hz); furthermore, H-4' (J=3.6 Hz) had to be equatorial, (*cis*) to H-3'. H-4' also showed a very small vicinal coupling (<1 Hz) to H-5' and could not be measured accurately. The small J value is a result of the dihedral angle between H-4' and H-5' being nearly 90°. If one builds a Dreiding model of the sugar, the 1,3 diaxial interactions between the anomeric oxygen and the CH_3-6' group can flatten the pyran ring and as a result, cause the dihedral between H-4'-H-5' to be nearly 90°. A ROESY correlation from H_3-6' to H-3' confirmed the axial conformation of the CH_3-6', as was suggested by the coupling constants. Since the anomeric oxygen is *cis* to CH_3-6', the monosaccharide belongs to the β-series.

The C-1' substituent, 4'-acetoxyl and CH_3-6' are all axial, while the 2' and 3' hydroxyls are equatorial, from this we were able to initially identify the sugar as altrose. This was confirmed by acid hydrolysis of **1** and subsequent deacetylation of the 4'-acetoxyl group of the sugar. Comparison of the chemical shifts of the glycoside to the liberated sugar confirmed the isolation of the free 6-deoxyaltrose. The optical rotation of the hydrolyzed sugar was determined to be -19°, which corresponds to the reported $[\alpha]_D$ of -18° for L-6-deoxyaltrose.[19] This sugar was reported as a constituent of a lipopolysaccharide isolated from mammalian intestinal microorganisms.

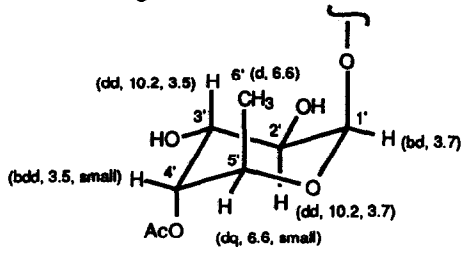

Fig. 1. Coupling constants of 6'-deoxy-L-β-altropyranosyl-4'-acetate.

An HMBC correlation between the anomeric proton at 5.45 ppm (H-1') to a carbon at 144.1 ppm (C-3) connected the monosaccharide to the aromatic ring system (Fig. 2) and yielded the final structure, **1**.

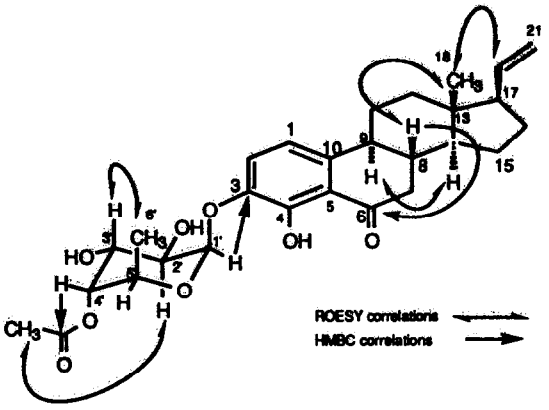

Fig. 2. HMBC and ROESY correlations.

Experimental Section

General Remarks. The IR spectrum was obtained using a Perkin-Elmer 1600 series FT-IR spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. ^{1}H-NMR and ^{13}C NMR spectra were measured on a General Electric GN-Omega-500 spectrometer. FABMS spectra was measured with a VG-70SE mass spectrometer. UV was obtained from an Hewlett Packard 8452A diode array spectrophotometer.

Sponge Collection and Taxomomy. The sponge, collected in Pohnpei, Federated States of Micronesia, August 1992, from a depth of 40 m, is *Cribrochalina olemda* (Niphatidae, Haplosclerida). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 003-891).

Isolation. The 387 g sample of freeze-dried sponge was extracted with 4x 2 L of ethanol followed by two successive 2 L extractions with acetone. The combined extracts were evaporated *in vacuo* to yield 54.8 g of a tan colored solid. The entire residue was subjected to reversed-phase silica gel C_{18} flash chromatography using a gradient of water to methanol, followed by EtOAc. The fractions were monitored by ^{1}H-NMR. The spectrum of the fraction eluted with MeOH/H_2O (80:20) contained signals in the aromatic region. The MeOH/H_2O (80:20) fraction (4.36 g) was subjected to HPLC using a semi-preparative reversed-phase C_{18} (Phenomenex Ultracarb 10 µm ODS 30, 250 X 22.5-mm) column. The solvent system was a gradient of MeOH/water from 75:25 to 85:15 with addition of 0.1% trifluoroacetic acid. The fraction containing hapaioside was eluted last. It was further purified by reversed-phase HPLC (CH_3CN/H_2O, 95:5, Phenomenex Ultracarb 5 µm ODS 30, 250 X 10-mm) to afford 7 mg of **1**.

Hydrolysis and Absolute stereochemistry of 6'-deoxy-L-β-altropyranose. Hapaioside (**1**, 5 mg) was hydrolyzed with glacial acetic acid/H_2O (2:1, 3 mL) by refluxing for one hour at 90°C. The residue was extracted thrice with 3 mL methylene chloride each to yield 2 mg of 6'-deoxyaltrose-4'-acetate. Deacetylation of the altrose was carried out by adding 10 mL of 5N NH_4OH to the sugar dissolved in MeOH, at 0°C for 40 minutes, when the solvent was removed under vacuum. Optical rotation of the sugar was $[\alpha]_D$ = -19° (c = 1.3, H_2O, 20°C). This matched the reported value of $[\alpha]_D$ = -18° for 6-deoxyaltrose.[19] The sugar therefore is 6-deoxy-β-L-altrose.

Hapaioside (1): white amorphous solid, $[\alpha]_D$ = - 34.8° (*c* = 0.028, MeOH, 20°C); IR (neat): ν_{max} 3416, 2941, 1735, 1634, 1446, 1245, 1087, 1026 cm^{-1}; UV (MeOH): λ_{max} 218 nm (3321), 268 nm (1894), 346 nm (793); FABMS: *m/z* 501 $(M+H)^+$; HRFABMS: *m/z* 501.2503 $(M+H)^+$, $C_{28}H_{37}O_8$ (Δ -1.5 mmu); LRFABMS *m/z* 189.1 (M^+ -$C_{20}H_{25}O_3$, 90%). ^1H and ^{13}C NMR ($CD_3OD/CDCl_3$ 1:1), see Table 1.

Acknowledgments

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