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Note

Structure of some sulfated sugars isolated after acid hydrolysis of the extracellular polysaccharide of *Porphyridium sp.*, a unicellular red alga

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Several polysaccharides isolated from red, brown, and green seaweeds[1], and from unicellular green and red algae[2], were found to be sulfated. In determining the structure of the extracellular sulfated polysaccharide produced by the unicellular marine red alga *Porphyridium sp.*, sulfated sugars were isolated in addition to the reported aldobiouronic acid[3–6] and neutral sugars. The aldobiouronic acid [3-*O*-(α-D-glucopyranosyluronic acid)-L-galactopyranose] is a basic building block of the polysaccharides of various unicellular red algae (the marine algae *P. sp.* and *P. cruentum*, the fresh-water alga *P. aerugineum*, and the brackish-water alga *Rhodella reticulata*)[3–6]. Evidence is now reported for the structure of three of the sulfated sugars.

Mild hydrolysis (0.1M trifluoroacetic acid, 3 h, 100°) of the polysaccharide followed by elution from resin DE-52 with 0.5M NH_4HCO_3 gave a charged fraction which was then subjected to gel filtration on Sephadex G-10. One of the fractions (*A*) showed (t.l.c., silica gel) components whose R_F values were commensurate with anionic monosaccharides, but were higher than those noted for oligo- and di-saccharides having carboxylate groups. Fraction *A* had i.r. absorbances typical of sulfate half-esters.

Desulfation of fraction *A* by acid hydrolysis, followed by borohydride reduction, and acetylation gave (g.l.c.) mainly acetylated xylitol, glucitol and galactitol in the ratios ~1.0:2.6:0.8.

Chromatography on Bio-gel P-2 of fraction *A* gave a later sub-fraction, which represented 1% of the total carbohydrates (phenol–sulfuric acid method)[7] of the native polysaccharide, and had i.r. bands at 820, 823, and 870 cm^{-1} (C–O–S bending), and 1238 and 1258 cm^{-1} (S=O stretching, broad). The molar ratio of carbohydrate (phenol–sulfuric acid method)[7] and sulfate (sodium rhodizonate method)[8] in this sub-

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TABLE I

^{13}C-N.m.r. data[a] (δ_C) for the fraction of the products of hydrolysis that contained a mixture of glucose 6-sulfate (**1**), galactose 6-sulfate (**2**), and galactose 3-sulfate (**3**)

Atom	*Glucose 6-sulfate*			*Galactose 6-sulfate*			*Galactose 3-sulfate*		
	Lit.[b]	*Found*[c]	*Intensity*	*Lit.*[b]	*Found*[c]	*Intensity*	*Lit.*[b]	*Found*[c]	*Intensity*
β-Anomer									
C-1	96.82	96.84	5.11	97.27	97.30	3.76	97.02	97.05	1.80
C-2	74.84	74.82	8.58	72.57	72.56	4.00	70.74	70.75	1.19
C-3	76.40	76.38	7.98	73.39	73.40	3.99	81.20	81.24	1.51
C-4	70.11	70.07	9.52	69.35	69.34	3.67	67.84	67.84	1.00
C-5	73.54	73.55	3.05	74.59	74.58	4.70	75.52	75.56	1.41
C-6	67.97	67.96	12.41[d]	68.14	68.12	3.85	61.58	61.70	1.58
α-Anomer									
C-1	92.99	93.02	5.14	93.18	93.21	1.45	93.09	[e]	[f]
C-2	72.19	72.17	6.84	69.06	69.05	1.94	67.19	67.21	1.19
C-3	73.47	73.46	6.68	69.75	69.76	2.30	78.52	78.57	1.17
C-4	70.18	70.12	5.38	69.93	69.91	2.19	68.46	68.46	[f]
C-5	69.14	69.15	2.63	70.34	70.30	6.91	71.02	71.04	[f]
C-6	67.97	67.96	12.41[d]	68.48	68.48	2.08	61.84	61.88	[f]

[a] Determined at 75 MHz for solutions in D_2O at 298 K; intensities measured relative to that for the resonance at δ 67.84. [b] P.p.m. downfield from the resonance for Me_4Si; lit. values taken from ref. 9. [c] P.p.m. downfield from the resonance for Me_4Si. [d] Resonances for C-6α,β not resolved in this spectrum nor in that reported in ref. 9. [e] Not resolved due to close proximity of the C-1 resonance of α-D-glucose 6-sulfate. [f] Low-intensity signal; value not reported due to poor signal-to-noise ratio.

fraction was ~0.92:1.08. Desulfation, followed by borohydride reduction, and acetylation gave (g.l.c.) galactitol and glucitol hexa-acetates in the ratio ~1.0:1.6.

The ^{1}H-n.m.r. spectrum (300 MHz) of this sub-fraction revealed three main species (**1**–**3**) in the ratios ~3:2:1, each with doublets for H-1α,β in the ratio ~1:2. ^{13}C-N.m.r. data for the isomeric D-glucose and D-galactose sulfates have been reported[9]. At 75 MHz, 34 out of the 36 ^{13}C resonances for **1**–**3** (there are two pairs of overlapping peaks) were observed, which allowed identification without recourse to additional fractionation. The twelve ^{13}C resonances for the largest component (**1**) accorded with those for glucose 6-sulfate[9] (see Table I) and an ~1:2 α,β-mixture. Likewise, the second (**2**) and third (**3**) largest components were correlated with galactose 6- and 3-sulfate, respectively. Characteristically, all C-6 signals afforded negative peaks in the DEPT[10] spectrum (pulse width 135°), and were absent when the pulse width was 90°. Archbald *et. al.*[9] also provided data for the H-1 doublets in α-D-glucose 6-sulfate, α-D-galactose 6-sulfate, and α-D-galactose 3-sulfate [5.17, 5.17, and 5.27 p.p.m., respectively; $J_{1,2}$ 2.5, 2.5, and 3.5 Hz, respectively]. Our sample gave doublets at 5.18, 5.22, and 5.27 p.p.m., respectively, with $J_{1,2}$ 3.6, 3.8, and 3.9 Hz, respectively.

The structure based on n.m.r. data was consistent with the i.r. bands at 820 cm^{-1} (*cf.* 818 for D-galactopyranose 6-sulfate and 823 cm^{-1} for D-glucopyranose 6-sulfate[9]) and 870 cm^{-1} (*cf.* 862 cm^{-1} for D-galactopyranose 3-sulfate[9]). Reaction[11] with D-galactose oxidase showed that 20% of the galactose in the desulfated fraction was D.

EXPERIMENTAL

The extracellular polysaccharide from *Porphyridium sp.* (UTEX-637) was grown and isolated as reported[6]. The polysaccharide (500 mg) was hydrolysed in 0.1M trifluoroacetic acid for 3 h at 100° in a glass tube fitted with a Teflon-lined screw cap. The hydrolysate was filtered and concentrated, and the residue was chromatographed on DE-52 ion-exchanger, then on Sephadex G10 according to Geresh *et al.*[6].

Positive fractions were subjected to t.l.c. on Silica Gel 60 (Merck), using ethanol–1-butanol–water–acetic acid–pyridine (100:10:30:3:10), with detection using 0.1% orcinol in aqueous 20% H_2SO_4 at 100–110°. The fraction (R_F 0.8–0.9) eluted between the aldobiouronic acid and the neutral monosaccharides was rechromatographed on a column (25 × 90 cm) of Bio-gel P-2 (Bio-Rad, 200–400 mesh) pre-equilibrated and eluted with distilled water. Fractions (2.5 mL) were analysed (phenol–sulfuric acid method). Two main fractions were collected, filtered, and lyophilised.

Sulfated sugars (200 μg) from the later fraction (R_F ~0.86) were desulfated by treatment with 2M HCl for 2 h at 100°, and the products were converted into alditol hexa-acetates[12] and analysed by g.l.c. [Varian Model 3300 gas chromatograph, f.i.d. detector, N_2 carrier gas, Supelco SP-2100 fused-silica capillary column (30 m × 0.25 mm i.d.), Spectra-Physics 4290 integrator, 130° initial oven temperature (15 min), 2°/min to 240°, then 240° for 10 min]. Oxidation of the desulfated sugars with D-galactose oxidase was done according to Geresh *et al.*[6].

I.r. spectra (KBr pellet) were recorded with a Nicolet ZDX F.t. spectrophoto-

meter. The ^{1}H- (300.1 MHz) and ^{13}C-n.m.r. (75.5 MHz) spectra were recorded with a Bruker AM-300 F.t. spectrometer. The D_2O solvent was used as the internal lock signal, and acetone as an internal secondary standard [δ 2.09 (^{1}H), δ 31.2 (^{13}C)] referenced to Me_4Si.

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