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THE POLYSACCHARIDES OF *Phyllymenia hieroglyphica* (≡ *P. belangeri*) AND *Pachymenia hymantophora**

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ABSTRACT

The polysaccharide of *P. hymantophora* has been shown to be composed of (1→4)-linked galactopyranosyl, (1→3)-linked galactopyranosyl, (1→3)-linked galactopyranosyl 2- and 4-sulphate and 2,6-disulphate residues. The (1→3)- and (1→4)-linked units are present in approximately equal amounts. The polysaccharide of *P. hieroglyphica* has been shown to possess (1→4)-linked galactopyranosyl, (1→3)-linked galactopyranosyl, and (1→3)-linked galactopyranosyl 2- and 4-sulphate residues. The (1→3)- and (1→4)-linked units are present in a 4:1 ratio. Both polysaccharides contain small proportions of non-reducing xylosyl end-groups.

INTRODUCTION

Polysaccharides (aeodans)[1] obtained from seaweeds belonging to the Grateloupiaceae family (order Cryptonemiales)[1–8] differ significantly in their properties and structure from those of the carrageenan family[9–11]. Differences from the κ-carrageenans include behaviour in potassium chloride solution, extremely low content of 3,6-anhydrogalactose, and distribution of ester sulphate. The aeodans[1] exhibit solubility properties similar to those of the carrageenans of the λ-fraction (λ, μ, ν, and ε) but, with the exception of the polysaccharides of *Aeodes orbitosa*[2] and *Pachymenia hymantophora*[10,11], differ in that all of the sulphate residues reside on 3-linked units and hence are stable to alkali. The λ-fraction polysaccharides have both 3- and 4-linked sulphated units, and hence a feature of these polysaccharides is their ability to release sulphate, with concomitant formation of 3,6-anhydrogalactose residues, when treated with alkali.

Most of the aeodans thus far studied have approximately equal amounts of (1→3) and (1→4) linkages. The aeodan of *Pachymenia carnosa*[1], which has a preponderance (70%) of (1→3) linkages, is a notable exception. All of the Grateloupiaceae polysaccharides studied to date, with the exception of that from *P. hymantophora*[10], contain various amounts of mono-*O*-methylgalactoses. We now report on the polysaccharides of *Phyllymenia hieroglyphica* and *Pachymenia hymantophora.*

*Sulphated Polysaccharides of the Grateloupiaceae Family, Part XI. For Part X, see ref. 1.

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RESULTS AND DISCUSSION

The polysaccharide from *P. hymantophora*[10] (New Zealand) was a gift from Professor D. A. Rees, and the polysaccharide from *P. hieroglyphica* (South Africa) was extracted with hot water as previously described[2,5] for other Grateloupiaceae polysaccharides. Each polysaccharide was shown by hydrolysis, followed by paper chromatography and g.l.c. analysis of the derived alditol acetates, to contain galactose and a trace of xylose. The polysaccharide of *P. hieroglyphica* is only the second polymer obtained from the Grateloupiaceae which is devoid of methylated galactoses. Purification of the polysaccharides *via* their cetyltrimethylammonium salts failed to remove the xylose, which suggests that xylose is either an integral part of each macromolecule or present as a contaminating, sulphated xylan. The xylose associated with both polysaccharides was cleaved when the polymers were oxidised with periodate. The n.m.r. spectrum of each polysaccharide in D_2O revealed the absence of pyruvate and confirmed the absence of mono-*O*-methylgalactoses. The presence of pyruvate has been demonstrated in the sulphated polysaccharide of *Phyllemynia cornea*[12] and there are indications of its presence in other Grateloupiaceae polysaccharides, *viz. Aeodes orbitosa*[13] and *A. ulvoidea*[13].

The infrared spectrum of each polysaccharide exhibits the characteristic, broad absorption band for ester sulphate at 1240 cm^{-1}, and a broad band at 800–850 cm^{-1}, indicating the presence of more than one type of ester sulphate. In addition, the infrared spectrum of the *P. hieroglyphica* polysaccharide showed a weak peak at 930 cm^{-1} which has previously been assigned[9] to the presence of 3,6-anhydrogalactosyl residues in algal polysaccharides. However, the *P. hieroglyphica* polysaccharide was found to be devoid of 3,6-anhydrogalactose. The peak at 930 cm^{-1} is absent from the infrared spectrum of *P. hymantophora*[9] and all other Grateloupiaceae polysaccharides examined to date; nevertheless, the *P. hymantophora* polysaccharide has been shown[10] to contain ~3.5% of 3,6-anhydrogalactose. Rees and co-workers have shown[10] that the 3,6-anhydrogalactose content of the *P. hymantophora* polysaccharide increases by 50% on treatment with alkali. When *P. hieroglyphica* polysaccharide was similarly treated, no 3,6-anhydrogalactose was formed, nor was there any loss of sulphate. The infrared spectra and specific rotations of native and alkali-treated *P. hieroglyphica* polysaccharides were virtually identical.

An earlier study of the polysaccharide of *P. hymantophora*[10,11] showed the galactose plus 3,6-anhydrogalactose:sulphate ratio to be 1.06:0.77, whereas the methylation results revealed[11] a ratio of hexose–sulphate of 0.99:0.52. Clearly, the methylation results have only accounted for ~70% of the ester sulphate present. The mono-*O*-methylgalactose(s) and possibly the unmethylated galactose previously considered as undermethylation products must have structural significance. A sample[11] of the methylated polysaccharide used in the earlier study was, therefore, re-methylated by the Hakomori method[14]. After two successive treatments, the galactose concentration was drastically diminished. The small amount of unmethylated galactose that remained is probably an undermethylation product. Hydrolysis of methylated *P.*

TABLE I

G.L.C. DATA FOR THE METHYLATED ALDITOL ACETATES FROM METHYLATED *Pachymenia hymantophora*

	Column 2	
	Retention time	*Molar ratio*
2,3,4-Tri-*O*-methylxylose	0.57	0.58
2,3,4,6-Tetra-*O*-methylgalactose	1.00	0.47
2,3,6-Tri-*O*-methylgalactose	1.17	14.26
2,4,6-Tri-*O*-methylgalactose	1.34	0.32
2,6-Di-*O*-methylgalactose	1.44	0.49
4,6-Di-*O*-methylgalactose	1.54	7.12
2,3-Di-*O*-methylgalactose	1.83	0.33
4-*O*-Methylgalactose	2.51	8.86
Galactose	2.67	0.33

hymantophora polysaccharide followed by g.l.c. analysis of the derived alditol acetates gave the results shown in Table I. The identities of the methylated alditol acetates were confirmed by g.l.c.–m.s.

If the unmethylated galactose is regarded as an undermethylation product and is considered to represent (1→3)-linked, 2,6-disulphated units, the present methylation results (taken in conjunction with the previously determined 3,6-anhydrogalactose concentration) reveal a hexose-to-sulphate ratio of 1.06:0.80, which is in excellent agreement with the reported[10] ratio of 1.06:0.77.

The present methylation results show that the polysaccharide is composed of approximately equal amounts of (1→4)- and (1→3)-linked units. This is in agreement with the methylation results previously reported[11]. However, the present results show several differences from the earlier results. Firstly, xylose, which was reported[11] to have been lost during the methylation procedure, has been shown to be present as non-reducing end-group. In the previous study[11], the methylated polysaccharide was examined by g.l.c. of the derived methyl glycosides. It is almost certain that the methyl xylosides, which are extremely volatile and present in only very small amounts, were lost during the concentration step in the preparation of the methyl glycosides. Secondly, only a minor amount of 2,6-di-*O*-methylgalactose, indicative of (1→3)-

TABLE II

REVISED STRUCTURAL UNITS (AND THEIR PROPORTIONS) IN *P. hymantophora* POLYSACCHARIDE

3-linked residues		*4-linked residues*	
β-D-galactopyranose 2-sulphate	(21)	α-D-galactopyranose	(41)
β-D-galactopyranose 2,6-disulphate	(27)	3,6-anhydro-α-D-galactopyranose	(4)
β-D-galactopyranose 4-sulphate	(1)	α-D-galactopyranose 6-sulphate	(1)
β-D-galactopyranose	(1)		

TABLE III

G.L.C. DATA FOR THE METHYLATED ALDITOL ACETATES FROM METHYLATED *Phyllymenia hieroglyphica*

	Column 4	
	Retention time	*Molar ratio*
2,3,4-Tri-*O*-methylxylose	0.56	0.85
2,3,4,6-Tetra-*O*-methylgalactose	1.00	1.00
2,3,6-Tri-*O*-methylgalactose	1.15	5.06
2,4,6-Tri-*O*-methylgalactose	1.33	11.97
2,6-Di-*O*-methylgalactose	1.40	3.09
4,6-Di-*O*-methylgalactose	1.52	9.62
2,3-Di-*O*-methylgalactose	1.77	1.54
2-*O*-Methylgalactose	2.13	0.41

linked galactosyl 4-sulphate residues, has been shown to be present. Thirdly, 4-*O*-methylgalactose, which was not previously identified, has been shown to have structural significance.

The sulphate in *P. hymantophora* polysaccharide is present mainly as 3-linked galactose 2-sulphate and 3-linked galactose 2,6-disulphate. In the light of the present results, the structural units and their proportions shown in Table II represent a more accurate description of the polysaccharide of *P. hymantophora.*

Separation of the partial, acid hydrolysate of the polysaccharide of *P. hieroglyphica* afforded D-galactose and 4-*O*-β-D-galactopyranosyl-D-galactose as crystalline products. No other oligosaccharides were detected by paper chromatography.

Methylation of the polysaccharide of *P. hieroglyphica* was accomplished by the Haworth[15] and Hakomori[14] procedures. The fully methylated polysaccharide was hydrolysed and examined by paper chromatography, and the hydrolysate was then converted into the alditol acetates and examined by g.l.c. and g.l.c.–m.s. (Table III).

The structure of the polysaccharide of *P. hieroglyphica* differs significantly from that of *P. hymantophora.* While it appears that the main polysaccharide chain is composed entirely of (1→3) and (1→4) linkages, these are not present in equimolar amounts, but in a 4:1 ratio. This implies that only 40% of the polysaccharide structure can be accounted for in terms of a classical alternating-structure of (1→3)- and (1→4)-linked units. In this respect, the polysaccharide shows a marked resemblance to the polysaccharide of *Pachymenia carnosa*[1], where only 60% of the polysaccharide structure could be accounted for in terms of an alternating structure. If the polysaccharide structure is considered in terms of units X and Y (Fig. 1), X must account for 40%, and Y for 60%, of the polysaccharide structure.

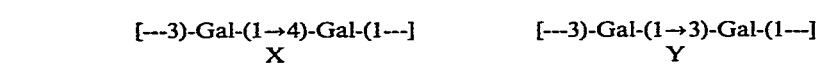

Fig. 1. Structural units (X and Y) for the polysaccharides.

TABLE IV

STRUCTURAL UNITS (AND THEIR PROPORTIONS) IN *P. hieroglyphica* POLYSACCHARIDE

3-linked residues		*4-linked residues*	
β-D-galactopyranose	(36)	α-D-galactopyranose	(20)
β-D-galactopyranose 4-sulphate	(9)		
β-D-galactopyranose 2-sulphate	(29)		

The polysaccharide of *P. hymantophora*, which possesses approximately equal amounts of (1→3)- and (1→4)-linked units is probably composed entirely of X units. It should be noted that conclusive proof for the presence of alternating (1→3)- and (1→4)-linkages has not been obtained for either *P. hymantophora* or *P. hieroglyphica* polysaccharides. It is possible, although in terms of previous findings[3,7,8] unlikely, that contiguous (1→4)- and (1→3)-linkages may occur in both polysaccharides. The structural units present in the polysaccharide of *P. hieroglyphica*, together with their proportions, are shown in Table IV.

The polysaccharide of *P. hieroglyphica*, unlike the polysaccharide of either *P. carnosa* or *P. hymantophora*, possesses a large proportion of unsulphated (1→3)-linked units and is devoid of (1→3)-linked 2,6-disulphate units. Approximately half of the (1→3)-linked units in the polysaccharide are sulphate-free. This accounts for the low sulphate content (20.5%) of the polysaccharide compared with most of the other Grateloupiaceae polysaccharides studied thus far.

The small amount of xylose present in *P. hieroglyphica* polysaccharide is present as non-reducing end-group and is probably linked to position 6 of (1→4)-linked galactopyranosyl residues. The somewhat larger proportion of 2,3,4,6-tetra-*O*-methylgalactose present in methylated *P. hieroglyphica* compared with methylated *P. hymantophora* suggests that some galactose residues occur as end-groups. These units are probably also attached to position 6 of (1→4)-linked galactopyranosyl residues. It is noteworthy that 4-*O*-methyl-L-galactosyl groups have been shown to occur as single branch-units attached to position 6 of 4-linked galactose residues in the polysaccharides of *Aeodes ulvoidea*[8] and *Pachymenia carnosa*[1].

The structure of *P. hieroglyphica* and the major structural features of *P. hymantophora* polysaccharides clearly establish these two polysaccharides as further examples of aeodans[1] and not carrageenans.

EXPERIMENTAL

The analytical methods were described in Parts IV[3] and X[1]. In addition, g.l.c. of alditol acetates was also performed on 20% of Apiezon M on Gas Chrom Q (100–120 mesh)[16] (column *4*) at 180°.

Extraction and purification of polysaccharides. — The extraction of the polysaccharide of *P. hymantophora* has been described[10]. The extraction of *P. hieroglyphica*

was performed as described[2,5] for other Grateloupiaceae seaweeds, to afford a 10% yield (based on wet weight) of polysaccharide. The polymer was purified *via* its cetyltrimethylammonium bromide complex and had $[\alpha]_D$ +44° (*c* 1.85, water); it gave galactose and a trace of xylose on hydrolysis, as shown by p.c. (solvents *1* and *2*) and by g.l.c. of the derived alditol acetates (column *4*) (Found: SO_3Na, 20.5%; 3,6-anhydrogalactose, <1%; OMe, 0.0%; N, 0.0%).

Partial hydrolysis of P. hieroglyphica *polysaccharide.* — The polysaccharide (0.5 g) was heated on a boiling water-bath with 0.5M sulphuric acid (15 ml) for 4 h, and a portion of the neutralised ($BaCO_3$) hydrolysate was subjected to p.c., to afford (*i*) D-galactose, m.p. 161–163°, mixture m.p. 163–165°, $[\alpha]_D$ +70° (*c* 0.69, water); and (*ii*) 4-*O*-*β*-D-galactopyranosyl-D-galactose, m.p. 195–198°, mixture m.p. 196–199°, $[\alpha]_D$ +63° (*c* 0.5, water). The i.r. spectrum was identical with that of an authentic sample[17].

Alkali treatment of P. hieroglyphica *polysaccharide.* — The polysaccharide (1.0 g) in water (75 ml) was treated with sodium borohydride and sodium hydroxide, as previously described[6]. The alkali-treated polysaccharide (910 mg) had $[\alpha]_D$ +43.5° (*c* 1.15, water) (Found: SO_3Na, 19.9%; 3,6-anhydrogalactose, <1%).

Methylation of polysaccharides. — (*a*) P. hymantophora. A sample (30 mg) of partially methylated polysaccharide[11] was subjected to two successive Hakomori methylations, as described previously[1], to afford the methylated polysaccharide (20 mg).

(*b*) P. hieroglyphica. The polysaccharide (1.0 g) was exhaustively methylated by the Haworth procedure[15], to afford partially methylated polysaccharide (1.0 g). A portion (200 mg) of this product was subjected to two successive Hakomori methylations[14], as previously described[1], to afford fully methylated polysaccharide (180 mg).

A portion of each polysaccharide was converted into the methyl glycosides and examined by g.l.c. on column *3*, while another portion of each polysaccharide was converted into the alditol acetates and examined on columns *1* and *4*.

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