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INVESTIGATION OF THE ACETOLYSIS PRODUCTS OF THE SULPHATED POLYSACCHARIDE OF *Aeodes ulvoidea*

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ABSTRACT

Investigation of the acetolysis products of the sulphated polysaccharide of the seaweed *Aeodes ulvoidea* led to the isolation and characterization of the following oligosaccharides: 3-*O*-α-D-galactopyranosyl-D-galactose (**1**), 3-*O*-(2-*O*-methyl-α-D-galactopyranosyl)-D-galactose (**2**), 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-L-galactose (**3**), 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-D-galactose (**4**), *O*-β-D-galactopyranosyl-(1→4)-*O*-α-D-galactopyranosyl-(1→3)-D-galactose (**5**), *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-galactose (**6**), *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-*O*-methyl-D-galactose (**7**), *O*-(2-*O*-methyl-α-D-galactopyranosyl)-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-2-*O*-methyl-D-galactose (**10**), and *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-*O*-α-D-galactopyranosyl-(1→3)-D-galactose. In addition, the isolation of a tetrasaccharide possessing alternating D- and L-galactose residues demonstrates the hitherto unexpected presence of L-galactose in the polysaccharide. The structure of the polysaccharide is discussed.

INTRODUCTION

The polysaccharide of *Aeodes ulvoidea*[2] contains D-galactose, 2-*O*-methyl-D-galactose, 4-*O*-methyl-L-galactose, 6-*O*-methyl-D-galactose (trace), and sulphate. Methylation analysis[2] of the desulphated polysaccharide and a partial hydrolysis study[1] of the native polymer established that the polymer is composed of a (1→3)-α-D- and (1→4)-β-D-linked chain, and that the 4-*O*-methyl-L-galactose occurs as single branch units attached to position 6 of the 4-linked galactose residues in the polysaccharide chain. Only one oligosaccharide possessing a (1→3) linkage was isolated from the partial hydrolysate. In addition, no oligosaccharide was obtained to suggest that the (1→3) and (1→4) linkages occur in an alternating sequence. We now report the results of an acetolysis study carried out in order to obtain more information concerning the (1→3)-linked units in the polymer.

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*Sulphated Polysaccharides of the *Grateloupiaceae* Family: Part IX. For Part VIII, see Ref. 1.

RESULTS AND DISCUSSION

Acetolysis of the polysaccharide of *Aeodes ulvoidea*, followed by deacetylation and fractionation of the neutral oligosaccharides by a combination of gradient elution from a charcoal–Celite column and paper chromatography, resulted in the isolation of ten oligosaccharides. The saccharides isolated, together with the data essential for the determination of their structures, are listed in Table I.

Oligosaccharide **6** was the only saccharide obtained crystalline. Oligosaccharides **5** and **8** were previously obtained crystalline from the acetolysate of the polymer of *Pachymenia carnosa*[3] (Grateloupiaceae).

The isolation and characterization of oligosaccharide **3** establishes the hitherto unexpected presence of 2-*O*-methyl-L-galactose in the polysaccharide and demonstrates that it is linked in the same way as the D enantiomer. 2-*O*-Methyl-L-galactose was previously isolated from the polysaccharide of *Grateloupia elliptica*[4]. Oligosaccharide **3** appears to have been previously isolated from the acetolysate of *Pachymenia carnosa*[3], but insufficient material was available to establish which of the component sugars was present as the L isomer. The specific rotation (−33°) found for **3** suggests that the saccharide ($[\alpha]_D$ +7°) isolated from *P. carnosa*[3] polymer was a mixture of **3** and **4**.

The partial hydrolysis study[1] on the polymer furnished chromatographic evidence for the presence of 4-*O*-β-D-galactopyranosyl-L-galactose and hence L-galactose residues in the polymer. The isolation of the tetrasaccharide **9** confirms the presence of L-galactose in the polymer, since an acid hydrolysate of **9** gave galactose only and had a specific rotation of +2°. Insufficient of **9** was isolated for it to be completely characterized. However, chromatographic examination of a partial hydrolysate of **9** revealed the presence of two trisaccharides, one of which was chromatographically indistinguishable from authentic *O*-β-D-galactopyranosyl-(1→4)-*O*-α-L-galactopyranosyl-(1→3)-D-galactose[5], and a single disaccharide spot, which was chromatographically identical with 4-*O*-β-D-galactopyranosyl-L-galactose[5] and 3-*O*-α-L-galactopyranosyl-D-galactose[5], but distinguishable from 4-*O*-β-D-galactopyranosyl-D-galactose and **1** in the solvent system used. These results suggest that **9** has alternating D and L galactose residues. An oligosaccharide, *O*-β-D-galactopyranosyl-(1→4)-*O*-α-L-galactopyranosyl-(1→3)-*O*-D-galactopyranosyl-(1→4)-L-galactose with $[\alpha]_D$ −66° (*cf.*, $[\alpha]_D$ −60° for **9**), was isolated from the highly sulphated polysaccharide of *Anatheca dentata*[5].

The isolation of the trisaccharide **10** in much higher yield than **7** suggests that most of the 2-*O*-methyl-D-galactose residues occur in 2-*O*-methylgalactose-rich regions rather than every 5 to 6 (1→4)-linked units in the polysaccharide chain.

Significantly, disaccharides having (1→3) linkages were obtained in higher yield than those having (1→4) linkages, whereas no saccharide possessing a (1→6) linkage was obtained (*cf.* partial hydrolysis[1]). All the trisaccharides were found to possess both (1→3)-α-D and (1→4)-β-D linkages, and that these occur in an alternating sequence is supported by the isolation of the tetrasaccharide **8**.

TABLE I

OLIGOSACCHARIDES OBTAINED BY ACETOLYSIS

Oligosaccharide[a]	*Total hydrolysis products*	*Partial hydrolysis products*	*Partial hydrolysis products after reduction*	*Hydrolysis or methanolysis product of methylated oligosaccharide*[b]	*Reaction with triphenyl-tetrazolium chloride*
1 α-D-Gal-(1→3)-D-Gal (syrup)	Gal	Gal		*A*,*B*	
2 α-D-2Gal-(1→3)-D-Gal (syrup)	2Gal,Gal	2Gal,Gal	2Gal	*A*,*B*	+
3 β-D-Gal-(1→4)-L-2Gal (syrup)	2Gal,Gal		Gal	*A*,*C*	—
4 β-D-Gal-(1→4)-D-2Gal (syrup)	2Gal,Gal		Gal		—
5 β-D-Gal-(1→4)-α-D-Gal-(1→3)-D-Gal (syrup)	Gal	Gal,1,11[c]	Gal,11	*A*,*B*,*C*	
6 α-D-Gal-(1→3)-β-D-Gal-(1→4)-D-Gal (cryst.)	Gal	Gal,1,11[c]	Gal,1	*A*,*B*,*C*	
7 α-D-Gal-(1→3)-β-D-Gal-(1→4)-D-2Gal	Gal,2Gal	2Gal,Gal,1,4		*A*,*B*,*C*	—
8 α-D-Gal-(1→3)-β-D-Gal-(1→4)-α-D-Gal-(1→3)-D-Gal (syrup)	Gal	Gal,1,5,6,11[c]	Gal,1,6,11[c]		
9 Gal-Gal-Gal-Gal	Gal(D:L; 1:1)	See discussion			
10 α-D-2Gal-(1→3)-β-D-Gal-(1→4)-D-2Gal (syrup)	Gal,2Gal	Gal,2Gal,2,4	Gal,2Gal,2	*A*,*B*,*C*	—

[a] 2Gal, 2-*O*-methylgalactose; 6Gal, 6-*O*-methylgalactose. [b] *A*, 2,3,4,6-tetra-*O*-methylgalactose; *B*, 2,4,6-tri-*O*-methylgalactose; *C*, 2,3,6-tri-*O*-methylgalactose. [c] 4-*O*-β-D-galactopyranosyl-D-galactose (11).

It is not possible, at present, to propose a repeating unit for the polymer because of its complexity. However, the following description of the polysaccharide is consistent with the accumulated evidence: (*a*) The polymer is composed mainly of an alternating chain of (1→3)-α- and (1→4)-β-linked D-galactose (and derivatives) residues. (*b*) A few of the (1→3)-linked D-galactose residues occur as the 6-methyl ether. (*c*) 2-*O*-Methylgalactose residues constitute ~19% of the (1→4)-β-D-linked units. (*d*) Approximately 21% of the (1→4)-β-linked D-galactose residues carry 4-*O*-methyl-α-L-galactopyranosyl units on position 6. (*e*) Most of the 6-*O*-methyl-D-galactose residues are linked to 2-*O*-methyl-D-galactose residues. (*f*) The majority of the 2-*O*-methyl-D-galactose residues appear to be concentrated in certain regions of the polymer. (*g*) Some of the (1→4)-linked D-galactose and 2-*O*-methyl-D-galactose residues are replaced by their respective L enantiomers in the polysaccharide chain. (*h*) The polysaccharide contains a small amount of pyruvic acid[6]. (*i*) Most of the sulphate ester groups occur on (1→3)-linked D-galactose residues.

EXPERIMENTAL

The analytical methods used were described in Part VIII[1]. In addition, the following chromatographic solvent (*4*) was used: butanone–ethanol–water (9:1:2) saturated with boric acid. R_{GG} values refer to chromatographic mobilities relative to 4-*O*-β-D-galactopyranosyl-D-galactose in solvent *4*. The configurations of glycosidic linkages in oligosaccharides were inferred from specific rotations, unless otherwise stated.

Acetolysis of the polysaccharide. — Freeze-dried polysaccharide (30 g) was added to a mixture of acetic anhydride (160 ml), acetic acid (120 ml), and conc. sulphuric acid (16 ml) with continuous stirring over a period of 10 min. The mixture was shaken for 100 h at room temperature, after which the clear solution was decanted from the remaining solid, mixed with ice-cold water (1.6 l), and neutralized to Congo Red with sodium hydrogen carbonate. The precipitated oligosaccharide acetates were removed by filtration and the solution was extracted with chloroform. The extracts were combined with a solution of the precipitate in chloroform, dried (sodium sulphate), filtered, and evaporated to a syrup, which was dissolved in water and freeze-dried (19.4 g).

Deacetylation of the oligosaccharides was carried out as previously described. The oligosaccharide mixture (9.2 g), isolated by freeze-drying, was applied to a charcoal–Celite column (51 × 730 mm; 1:1 w/w) and eluted with water (15 l) and aqueous ethanol (74 l of 0–17% ethanol in water), using the gradient technique. Fractions were collected and, on the basis of paper chromatography, were recombined into 11 major fractions. The column was finally washed with 40% ethanol followed by 10% butanone.

Fraction 1. The white solid (3.73 g), eluted with water and 1.5% aqueous ethanol, was shown by paper chromatography (p.c.) to contain mainly galactose with small proportions of 2-*O*- and 4-*O*-methylgalactose.

Fraction 2. The syrup (67 mg), eluted with 1.5% aqueous ethanol, was shown by p.c. to be a mixture of 2-*O*- and 4-*O*-methylgalactose, together with traces of galactose, 6-*O*-methylgalactose, and an oligosaccharide having R_{GAL} 0.3.

Fraction 3. The syrup (1.45 g), eluted with 7% aqueous ethanol, was shown by p.c. to be a mixture of mono- and oligo-saccharides. Half of this fraction was fractionated on Whatman No. 1 paper, using solvent *3*, to give oligosaccharide **1** (440 mg) as a syrup. It had $[\alpha]_D^{25}$ +124°, R_{GAL} 0.35, and was contaminated (p.c., solvents *1*, *3*, and *4*) with a trace of probably 4-*O*-β-D-galactopyranosyl-D-galactose. Complete hydrolysis of **1** gave galactose only, while partial hydrolysis gave galactose and the original oligosaccharide. Examination of the hydrolysate of methylated **1** (Hakomori method[7]) by t.l.c. and p.c. (spray *a*) revealed spots having the mobilities of 2,3,4,6-tetra-*O*- and 2,4,6-tri-*O*-methylgalactose, while g.l.c. of the methanolysate of the hydrolysed, methylated oligosaccharide showed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides (*T* 1.60), methyl 2,4,6-tri-*O*-methylgalactosides (*T* 3.55, 3.92), and a peak at *T* 3.72 considered to be due to an alkali-degradation product. Oligosaccharide **1** is thus 3-*O*-α-D-galactopyranosyl-D-galactose.

Fraction 4. The syrup (175 mg), eluted with 8% aqueous ethanol, was shown by p.c. to consist mainly of a mixture of oligosaccharides having R_{GAL} 0.35, 0.61, and 0.75. Fractionation of the syrup on Whatman No. 1 paper with solvent *3* afforded the following oligosaccharides.

Oligosaccharide **2**. The syrup (31 mg) was chromatographically homogeneous in solvents *1* and *3*, and had R_{GAL} 0.61, $[\alpha]_D^{25}$ +130°. The oligosaccharide gave a pink spot with spray *b*, afforded galactose and 2-*O*-methylgalactose on hydrolysis, and gave the above two sugars and starting material on partial hydrolysis. Hydrolysis of reduced (sodium borohydride) **2** afforded 2-*O*-methylgalactose as the only reducing sugar. Methylation of **2** by the Hakomori procedure[7] and by the method of Haq and Percival[8] followed by g.l.c. examination of the derived methyl glycosides revealed, in each case, peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides (*T* 1.60) and methyl 2,4,6-tri-*O*-methylgalactosides (*T* 3.55, 3.92), and a peak at *T* 3.75. This oligosaccharide is assigned the structure 3-*O*-(2-*O*-methyl-α-D-galactopyranosyl)-D-galactose.

Oligosaccharide **3**. The syrup (24 mg) was chromatographically homogeneous in solvents *1* and *3*, and had R_{GAL} 0.74 and $[\alpha]_D^{25}$ −33°. The oligosaccharide failed to react with spray *b*, gave galactose and 2-*O*-methylgalactose on hydrolysis, and only galactose after reduction and hydrolysis. Separation of the hydrolysis products of the oligosaccharide by p.c. (solvent *1*) gave D-galactose, $[\alpha]_D^{20}$ +47° (*c* 0.131), and 2-*O*-methyl-L-galactose, $[\alpha]_D^{20}$ −44° (*c* 0.133). Hydrolysis of the methylated oligosaccharide afforded (t.l.c. and p.c.) 2,3,4,6-tetra-*O*-methylgalactose and 2,3,6-tri-*O*-methylgalactose, while g.l.c. of the derived methyl glycosides revealed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides (*T* 1.61) and methyl 2,3,6-tri-*O*-methylgalactosides (*T* 2.79, 3.38, 3.67, and 3.96). Oligosaccharide 3 is thus 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-L-galactose.

Fraction 5. The syrup (182 mg), eluted with 9% aqueous ethanol, was a mixture of several oligosaccharides. Fractionation of the mixture on Whatman No. 1 paper (solvent *3*) afforded oligosaccharide **4** as a syrup (45 mg). It had $[\alpha]_D^{25}$ +57°, R_{GAL} 0.81, was chromatographically identical with 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-D-galactose in solvents *1–3*, and failed to react with spray *b*. Hydrolysis of the syrup afforded galactose and 2-*O*-methylgalactose, while the reduced oligosaccharide afforded galactose only on hydrolysis. These facts indicate that **4** is identical with the 4-*O*-β-D-galactopyranosyl-2-*O*-methyl-D-galactose isolated from the partial hydrolysate of the polysaccharide of *Aeodes ulvoidea*[1].

Fraction 6. The syrup (718 mg), eluted with 11% aqueous ethanol, was shown by p.c. to be a mixture of oligosaccharides having R_{GG} 0.44, 0.51, and 0.64. The mixture was separated on Whatman No. 1 paper, using solvent *4*, to give the following oligosaccharides.

Oligosaccharide **5**. The syrup (133 mg) was chromatographically homogeneous in solvents *1* and *4*, and had $[\alpha]_D^{25}$ +103° and R_{GG} 0.51. Complete hydrolysis of **5** gave galactose only, while partial hydrolysis afforded galactose, 4-*O*-β-D-galactopyranosyl-D-galactose, **1**, and starting material. Partial hydrolysis of reduced **5** afforded galactose and 4-*O*-β-D-galactopyranosyl-D-galactose. Methylation of **5** by the Hakomori[7] procedure, followed by hydrolysis and p.c., showed spots corresponding to 2,3,4,6-tetra-*O*-methylgalactose, and 2,3,6-tri- and 2,4,6-tri-*O*-methylgalactose. G.l.c. of the methyl glycosides derived from the above hydrolysate showed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides (*T* 1.63), methyl 2,3,6-tri-*O*-methylgalactosides (*T* 2.78, 3.42, 3.66, and 3.95), and methyl 2,4,6-tri-*O*-methylgalactosides (*T* 3.55 and 3.93), and a peak at *T* 3.74 (degradation product). Oligosaccharide **5** is thus *O*-β-D-galactopyranosyl-(1→4)-*O*-α-D-galactopyranosyl-(1→3)-D-galactose.

Oligosaccharide **6**. The syrup (290 mg) crystallized readily and, on recrystallisation from aqueous ethanol, had m.p. 229–232°, $[\alpha]_D^{25}$ +125°, and R_{GG} 0.64. Hydrolysis of **6** afforded galactose, while partial hydrolysis afforded galactose, **1**, 4-*O*-β-D-galactopyranosyl-D-galactose, and starting material. Partial hydrolysis of reduced **6** gave galactose and **1**. Methylation analysis of **6**, as described for oligosaccharide **5**, gave 2,3,4,6-tetra-*O*-methylgalactose, and 2,3,6-tri- and 2,4,6-tri-*O*-methylgalactose or their methyl glycosides. Oligosaccharide **6** is thus *O*-α-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-galactose.

Fraction 7. The syrup (98 mg), eluted with 12% aqueous ethanol, was shown (p.c.) to be a mixture of oligosaccharides having R_{GG} 0.44, 0.51, 0.64, and 0.88 (trace). This fraction was not further investigated.

Fraction 8. The syrup (426 mg), eluted with 14% aqueous ethanol, was shown (p.c.) to be a mixture of the oligosaccharides found in fraction **7** and an oligosaccharide having R_{GG} 1.06. The mixture was fractionated on Whatman No. 1 paper, using solvent *4*, to give oligosaccharide **7**.

Oligosaccharide **7**. The syrup (17 mg) was chromatographically homogeneous in solvents 3 and 4, and had $[\alpha]_D^{25}$ +126°, R_{GG} 1.06. Hydrolysis of **7** afforded galactose and 2-*O*-methylgalactose, while partial hydrolysis gave galactose, 2-*O*-methylgalactose, **1**, **4**, and starting material. Reduction of the oligosaccharide followed by hydrolysis afforded galactose as the only reducing sugar. Methylation of the oligosaccharide followed by g.l.c. of the derived methyl glycosides showed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides, methyl 2,4,6-tri-*O*-methylgalactosides, and methyl 2,3,6-tri-*O*-methylgalactosides. Oligosaccharide **7** is thus *O*-*α*-D-galactopyranosyl-(1→3)-*O*-*β*-D-galactopyranosyl-(1→4)-2-*O*-methyl-D-galactose.

Fraction 9. The syrup (445 mg), eluted with 15% aqueous ethanol, was shown (p.c.) to be a mixture of oligosaccharides having R_{GG} 0.19, 0.25, 0.29, and 0.41. Fractionation of the mixture on Whatman No. 1 paper, using solvent *4*, gave the following chromatographically homogeneous products.

Oligosaccharide **8**. The syrup (190 mg) had $[\alpha]_D^{25}$ +153° and R_{GG} 0.29. Hydrolysis of **8** afforded galactose, while partial hydrolysis afforded galactose, 4-*O*-*β*-D-galactopyranosyl-D-galactose, **1**, **5**, **6**, and the original saccharide. Reduction of **8** followed by hydrolysis and p.c. revealed galactose, 4-*O*-*β*-D-galactopyranosyl-D-galactose, **1**, and **6**, but no trace of **5**. Oligosaccharide **8** is thus *O*-*α*-D-galactopyranosyl-(1→3)-*O*-*β*-D-galactopyranosyl-(1→4)-*O*-*α*-D-galactopyranosyl-(1→3)-D-galactose.

Oligosaccharide **9**. The syrup (8 mg) had $[\alpha]_D^{25}$ −60° and R_{GG} 0.19. Hydrolysis of **9** afforded galactose only, $[\alpha]_D^{25}$ $+5 \pm 3°$ (*c* 0.057). Partial hydrolysis of **9** followed by p.c. (solvent *4*) showed the presence of two trisaccharides and a single disaccharide. One of the trisaccharides was chromatographically identical with *O*-*β*-D-galactopyranosyl-(1→4)-*O*-*α*-L-galactopyranosyl-(1→3)-D-galactose, while the disaccharide spot had the same chromatographic mobility as 4-*O*-*β*-D-galactopyranosyl-L-galactose and 3-*O*-*α*-L-galactopyranosyl-D-galactose (solvent *4*).

Fraction 10. The syrup (227 mg), eluted with 17% aqueous ethanol, was shown (p.c.) to be a mixture. Fractionation of this mixture on Whatman No. 1 (solvent *3*) afforded oligosaccharide **10** (38 mg) as a syrup, $[\alpha]_D^{25}$ +109°, R_{GG} 1.49, which failed to react with spray *6*, and gave galactose and 2-*O*-methylgalactose on acid hydrolysis. Partial hydrolysis of **10** afforded galactose, 2-*O*-methylgalactose, **2**, **4**, and starting material, while partial hydrolysis of the reduced oligosaccharide afforded galactose, 2-*O*-methylgalactose, and **2**. Methylation of **10** followed by g.l.c. of a methanolysate revealed peaks due to the presence of methyl 2,3,4,6-tetra-*O*-methylgalactosides, and methyl 2,4,6-tri-*O*- and methyl 2,3,6-tri-*O*-methylgalactosides, while g.l.c. of the methanolysate of the methylated, reduced oligosaccharide showed peaks corresponding to methyl 2,3,4,6-tetra-*O*-methylgalactosides, methyl 2,4,6-tri-*O* methylgalactosides, and a penta-*O*-methylgalactitol (*T* 2.38). Oligosaccharide **10** is thus *O*-(2-*O*-methyl-*α*-D-galactopyranosyl)-(1→3)-*O*-*β*-D-galactopyranosyl-(1→4)-2-*O*-methyl-D-galactose.

Fraction 11. The syrup (1.56 g), eluted with 17–40% aqueous ethanol followed by 10% butanone in water, contained material of high molecular weight and was not further investigated.

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