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STRUCTURAL FEATURES OF AN ACIDIC POLYSACCHARIDE FROM THE MUCIN OF *DROSERA BINATA*

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Key Word Index—*Drosera binata*; Droseraceae; mucin; acidic polysaccharide; polysaccharide structure; GC and GC/MS.

Abstract—The polysaccharide from the mucin secreted by the leaves of *Drosera binata* is composed of L-arabinose, D-xylose, D-galactose, D-mannose and D-glucuronic acid in the molar ratio of 8.4:1.0:9.6:18.3:17.1. By partial hydrolysis of the polysaccharide three acidic oligosaccharides were obtained, each comprising an equal proportion of D-glucuronic acid and D-mannose. These were probably a di-, tetra- and hexasaccharide, respectively, representing the repeating unit $\cdots->4)$-β-D-GlcpA-$(1->2)$-α-D-Manp $(1->\cdots$ of the backbone of the polysaccharide. Anomeric configurations and types of linkages were established by chromium trioxide oxidation and methylation analysis using GC and GC/MS. These methods also revealed that both D-xylose and D-galactose form end-groups which are α-glycosidically linked to the backbone, while L-arabinose occurs as terminal furanosidic sugar. Molar ratios of the different sugar derivatives obtained by methylation analysis of the polysaccharide after carboxyl reduction, and after mild acid hydrolysis followed by carboxyl reduction, suggest that *ca* 50% of the D-glucuronic acid is substituted by either L-arabinofuranosyl or D-xylopyranosyl residues in position 3 and that 50% of the D-mannose carries D-galactopyranosyl residues also in position 3. These structural features were further supported by periodate oxidation and uronic acid degradation.

INTRODUCTION

The mucin secreted by the leaves of *Drosera binata* has been reported to contain an acidic polysaccharide as the only high MW compound [1]. This polysaccharide has been isolated and its homogeneity ascertained by various methods [1]. It has been shown to be composed of xylose, galactose, mannose and glucuronic acid. Interest in the structure of this polysaccharide has arisen, as hitherto no detailed chemical information is available about polysaccharides from carnivorous plants, apart from our studies on the structure of the polysaccharide from the related species *Drosera capensis* [2].

RESULTS AND DISCUSSION

Isolation and identification of the constituent monosaccharides from the hydrolysate of the native polysaccharide by prep. PC, and sugar analysis of the carboxyl-reduced polysaccharide by GC, indicated that the polysaccharide is composed of L-arabinose, D-xylose, D-galactose, D-mannose and D-glucuronic acid in the molar ratio of 8.4:1.0:9.6:18.3:17.2.

Mild acid hydrolysis of the acidic polysaccharide released only L-arabinose indicating that it is probably present in the furanosidic form. Prolonged mild acid hydrolysis liberated all L-arabinose together with *ca* 10% of the D-galactose, leaving a degraded polysaccharide. Carboxyl-reduction of this degraded polysaccharide, followed by sugar analysis with GC, revealed the presence of D-xylose, D-galactose, D-mannose and D-glucose (derived from D-glucuronic acid) in the molar ratio of 1.0:7.3:15.8:15.0.

Partial hydrolysis of the native polysaccharide gave, in addition to the constituent sugars, three acidic oligosaccharides which were found to be chromatographically identical to those isolated from the related species *D. capensis* [2], thus most probably representing the following structures (the R_{GlcA} values in system b are given in brackets): (1) β-D-GlcpA-$(1->2)$-D-Man (R_{GlcA} 0.57); (2) β-D-GlcpA-$(1->2)$-α-D-Manp-$(1->4)$-β-D-GlcpA-$(1->2)$-D-Man (R_{GlcA} 0.26); and (3) β-D-GlcpA-$(1->2)$-α-D-Manp-$(1->4)$-β-D-GlcpA-$(1->2)$-α-D-Manp-$(1->4)$-β-D-GlcpA-$(1->2)$-D-Man (R_{GlcA} 0.13).

These findings suggest that the polysaccharide contains a glucuronomannan backbone. In the case of *D. capensis* these oligosaccharides have been characterized by: (a) acid hydrolysis and PC; (b) acid hydrolysis and PC after sodium borohydride reduction; (c) periodate oxidation, sodium borohydride reduction, acid hydrolysis and PC of the methyl ester, methyl glycoside; (d) sodium borohydride reduction, acid hydrolysis and PC of the methyl ester, methyl glycoside; and (e) Hakomori methylation, acid hydrolysis and analysis by GC and GC/MS of the alditol acetates of the methyl ester, methyl glycoside.

The positions of the various glycosidic linkages were determined by subjecting the carboxyl-reduced and the degraded carboxyl-reduced polysaccharide to

methylation analysis, the results of which are given in Table 1. The occurrence of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4,6-tetra-*O*-methyl-D-galactose as the only derivatives of these sugars indicates that L-arabinose is present as non-reducing furanosidic end-group, D-xylose and D-galactose as non-reducing pyranosidic end-groups. D-Mannose and D-glucose, the latter derived from D-glucuronic acid after carboxyl-reduction, each form two different derivatives, i.e. 3,4,6-tri-*O*-methyl- and 4,6-di-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl- and 2,6-di-*O*-methyl-D-glucose. The two tri-*O*-methylated species are derived from the glucuronomannan backbone. These data together with the finding of the three acidic oligosaccharides (1)–(3) mentioned above lead to the suggestion that in the polysaccharide 4-*O*-substituted D-glucuronic acid and 2-*O*-substituted D-mannose occur alternately. The di-*O*-methylated derivatives are formed when the backbone sugars D-mannose and D-glucuronic acid are further substituted at O-3 by the non-reducing monosaccharides L-arabinose, D-xylose or D-galactose. The ratio of di- and tri-*O*-methylated derivatives is *ca* 1:1 for D-mannose and D-glucose, indicating that *ca* 50% of each of these backbone sugars carry one of the non-reducing end-group monosaccharides.

The site of attachment of these end-groups can be recognized by comparison of the methylation data of the carboxyl-reduced polysaccharide and the degraded carboxyl-reduced polysaccharide. The complete removal of L-arabinose in the latter results in a substantial decrease of 2,6-di-*O*-methyl-D-glucose, whereas the di-*O*-substituted D-mannose derivative is almost unaffected. This indicates that L-arabinose is linked to O-3 of the D-glucuronic acid residues of the backbone. The occurrence of 2,3,4,6-tetra-*O*-methyl-D-galactose and 4,6-di-*O*-methyl-D-mannose in equal amounts suggests that D-galactose is linked to D-mannose in position 3. The small amount of 2,6-di-*O*-methyl-D-glucose left after removal of L-arabinose can readily be explained by the linkage of D-xylose to the 3-position of D-glucuronic acid.

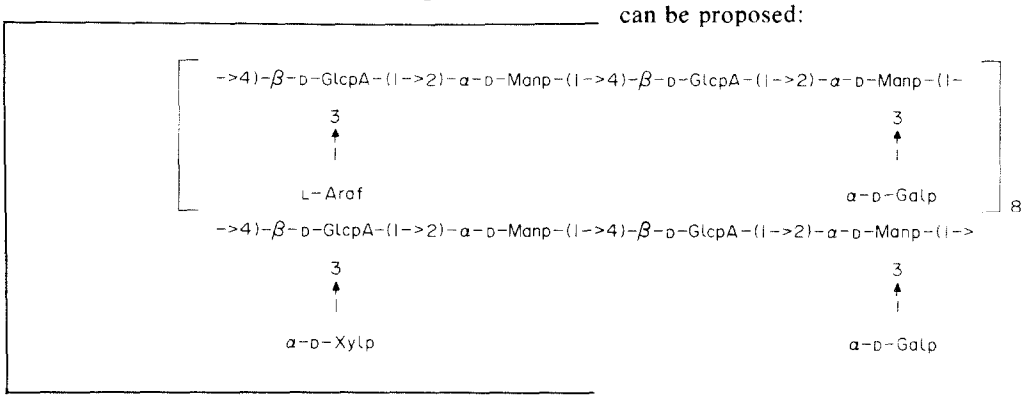
To determine the anomeric configurations of the constituent sugars, the carboxyl-reduced polysaccharide was subjected to chromium trioxide oxidation [3]. These experiments resulted in an oxidation of 95% D-glucose (derived from D-glucuronic acid) and of 80% L-arabinose residues, while D-xylose, D-galactose and D-mannose were almost resistant. Thus, the latter three sugars are probably linked by α-glycosidic bonds, whereas D-glucuronic acid is β-glycosidically connected. The anomeric configuration of L-arabinose cannot be recognized by these experiments because it is present as a furanosidic sugar.

On periodate oxidation [4], the native polysaccharide consumed 1.0 mol periodate per hexosyl residue. After sodium borohydride reduction and acid hydrolysis, analysis by PC revealed the presence of glycerol and other degradation products together with D-mannose and D-glucuronic acid. Treatment of the carboxyl-reduced polysaccharide in a similar way and analysis by PC and GC indicated glycerol and erythritol, together with D-mannose and D-glucose. This suggests that a part of both D-mannose and D-glucuronic acid is further substituted, thus becoming resistant to periodate oxidation.

Uronic acid degradation of the permethylated native polysaccharide according to ref. [5] followed by acetylation gave mainly 3,4,6-tri-*O*-methyl-D-mannose, besides 2,3,5-tri-*O*-methyl-L-arabinose and a partially methylated 3-*O*-(hexopyranosyl)-hexitol, as analysed by GC and GC/MS. In addition, there were indications of the presence of 2,3,4-tri-*O*-methyl-D-xylose.

To confirm the nature of the disaccharide alditol, a part of the degraded material was methylated using trideuteriomethyl iodide and again analysed by GC/MS (Fig. 1). The results from this analysis are in agreement with a 3-*O*-(galactopyranosyl)-mannopyranose unit in which the mannose residue was glycosidically linked and carried a further substituent at O-2.

Summarizing all the data obtained, the following tentative structure of the *D. binata* polysaccharide can be proposed:



Thus, this polysaccharide is very similar to the corresponding acidic polysaccharide from *Drosera capensis* [2]. The only difference is that the D-xylose content in this case is one-half of that in *D. capensis*.

Besides these two examples there are reports in the literature about polysaccharides with a glucuronomannan core structure having a repeating unit of $\cdots->)$-β-D-GlcpA-(1->2)-α-D-Manp-(1->$\cdots$. They were obtained from gum exudates of a variety of plants [6], and from suspension-cultured tobacco cells [7, 8]. However, in these cases the structures are far more complex and different in the nature and the length of the side-chains attached to the common backbone.

Table 1. Methylation analysis data for the carboxyl-reduced polysaccharide (A) and the degraded carboxyl-reduced polysaccharide (B) from *Drosera binata*

Sugar	Molar ratio A	Molar ratio B
2,3,5-tri-*O*-methyl-L-arabinose	9.9	—
2,3,4-tri-*O*-methyl-D-xylose	1.0	1.0
2,3,4,6-tetra-*O*-methyl-D-galactose	8.3	7.0
3,4,6-tri-*O*-methyl-D-mannose	13.3	20.6
2,3,6-tri-*O*-methyl-D-glucose	10.7	23.9
4,6-di-*O*-methyl-D-mannose	10.2	10.4
2,6-di-*O*-methyl-D-glucose	13.5	*

*Compound identified by GC and GC/MS but not quantified, due to low amount.

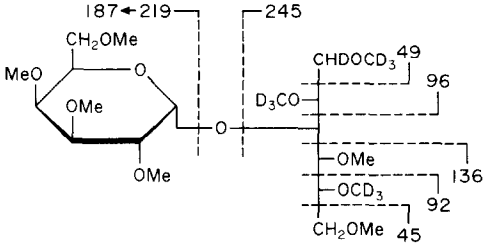


Fig. 1. Mass fragmentation pattern of permethylated 3-*O*-(D-galactopyranosyl)-D-mannitol.

EXPERIMENTAL

Analytical methods. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. IR spectra were recorded on a Perkin-Elmer 457 spectrophotometer. Evaporations were carried out under red. pres. below 40°.

Descending PC was performed on Whatman No. 1 and 3 MM papers with the solvent systems (v/v): (a) *n*-BuOH–C_6H_6–C_5H_5N–H_2O (5:1:3:3, upper layer); (b) *n*-BuOH–HOAc–H_2O (4:1:5, upper layer); (c) *n*-PrOH–EtOH–H_2O (7:1:2); (d) EtOAc–C_5H_5N–HOAc–H_2O (5:5:1:3); and (e) EtOAc–C_5H_5N–H_2O (8:2:1). Sugars were detected by spraying with *p*-anisidine hydrochloride [9] and alkaline $AgNO_3$ [10]. GC was performed on a Packard 428 gas chromatograph [FID; (a) glass column 200 × 0.2 cm, packed with 3% OV-225 on Gas Chrom Q, 80–100 mesh, oven temp. 185° and (b) glass column 200 × 0.4 cm, packed with 5% OV-210 on Varaport 30, 100–120 mesh, oven temp. programmed from 140° to 195° at a rate of 3°/min; gas flow rate for N_2 in both systems 30 ml/min]. GC/MS was carried out on a Varian 3700 gas chromatograph with OV-225 as column material coupled to a Varian MAT 44S mass spectrometer and a Varian Spectro Spin MAT 200 data processing system. 70 eV MS were recorded at 0.3 mA ionization current and 220° ion source temp. Before GC and GC/MS analyses, sugars were converted into their alditol acetates and identified according to lit. data [11–13].

Sugar composition and enantiomeric configuration. The lyophilized polysaccharide sample previously isolated [1] was used for the present study. Polysaccharide samples were hydrolysed with 0.5 M H_2SO_4 at 100° for 10–12 hr. (Unless otherwise stated, polysaccharides were hydrolysed under these conditions.) The hydrolysates were neutralized with $BaCO_3$, filtered, and the clear filtrates deionized on Dowex 50 (H^+) and Dowex 2 × 8 ($HCOO^-$) resins. The neutral sugars in this effluent were examined by PC and GC, and the acidic sugars were eluted from the anion exchange resin with 1 M HCOOH and analysed by PC. The enantiomeric configurations of the individual sugars were determined by measurement of the optical rotations after isolation by prep. PC.

Mild acid hydrolysis of the polysaccharide and isolation of the degraded polysaccharide. The polysaccharide was hydrolysed in 0.125 M H_2SO_4 at 100°. At intervals the hydrolysate was tested by PC for the release of sugars. According to these results, the polysaccharide (250 mg) was hydrolysed with 20 ml 0.125 M H_2SO_4 for 3 hr at 100°. EtOH (6 vol.) was added to the cooled soln to ppt the degraded polysaccharide, which was recovered after centrifugation, washed with EtOH and dried; yield 135 mg and $[\alpha]_D$ + 17° (H_2O; *c* 0.4).

Isolation and characterization of acidic oligosaccharides. The acidic polysaccharide (400 mg) was hydrolysed with 0.25 M H_2SO_4 for 6 hr at 100°. The acidic portion of the hydrolysate obtained by anion exchange chromatography as described above contained, in addition to glucuronic acid, three oligosaccharides with R_{GlcA} 0.57, 0.26 and 0.13 (PC, solvent system b).

Carboxyl-reduction of the native and the degraded polysaccharide. Polysaccharide samples (100 and 90 mg, respectively) were reduced twice according to ref. [14]; yield 45 and 40 mg, respectively. The resulting carboxyl-reduced polysaccharides were hydrolysed and the released sugars analysed by PC and GC.

Methylation analysis. The carboxyl-reduced and the degraded carboxyl-reduced polysaccharides (10 mg each) were methylated according to the method of ref. [15]. The methylated samples were hydrolysed with 90% HCOOH for 2 hr at 100° and, after evaporation of the HCOOH, with 0.5 M H_2SO_4 for 8–10 hr at 100°. The resulting, partially methylated sugars were analysed by GC and GC/MS [12, 13].

Chromium trioxide oxidation. The carboxyl-reduced polysaccharide (10 mg) was acetylated twice according to ref. [16]. The product was dissolved in 17 M HOAc (4 ml) and treated with CrO_3 (400 mg) in an ultrasonic bath at 50° for

1.5 hr. After the addition of H_2O the cooled soln was extracted with $CHCl_3$ (5 × 10 ml). The combined extracts were washed with H_2O and evaporated. The residue was hydrolysed with 0.5 M H_2SO_4 for 16 hr at 100°. The resulting sugars were analysed by GC.

Periodate oxidation. The native polysaccharide (100 mg) was oxidized with 45 mM $NaIO_4$ (100 ml) in the dark at room temp. The IO_4^- consumption was monitored by titration of aliquots with $Na_2S_2O_3$. After 48 hr the excess IO_4^- was destroyed with ethylene glycol (0.5 ml); the soln was dialysed, reduced with $NaBH_4$, dialysed again and then lyophilized.* A portion (10 mg) of this material was hydrolysed and examined by PC. The carboxyl-reduced polysaccharide (10 mg) was oxidized for 120 hr under similar conditions and analysed by PC and GC.

Uronic acid degradation [5]. The native polysaccharide (100 mg) was successively methylated twice by the Haworth [17], once by the Falconer [18], twice by the Kuhn [19] and finally × 4 by the Purdie [20] procedures. The permethylated polysaccharide (15 mg) was dissolved in dry C_6H_6 (1.2 ml) containing 1,5-diazobicyclo (5,4,0) undec-5-ene (0.6 ml) and AcOAc (0.3 ml) and heated at 100° for 24 hr. The cooled soln was successively washed with 1 M HCl and H_2O and evaporated. The residue was hydrolysed with 10% HOAc (5 ml) for 1 hr at 100°, deacetylated with NaOMe in MeOH and reduced with $NaBD_4$. After acetylation a part of the product was analysed by GC and GC/MS. The remaining portion was methylated with CD_3I and Ag_2O in DMF and examined by GC.

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