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STRUCTURAL STUDIES OF AN ACIDIC POLYSACCHARIDE FROM THE MUCIN SECRETED BY *Drosera capensis*

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ABSTRACT

The polysaccharide of the mucin secreted by the leaves of *Drosera capensis* is composed of L-arabinose, D-xylose, D-galactose, D-mannose, and D-glucuronic acid in the molar ratio of 3.6 : 1.0 : 4.9 : 8.4 : 8.2. For structural elucidation, methylation analysis using g.l.c. and g.l.c.–m.s. was performed on the native, the carboxyl-reduced, and the degraded polysaccharides. Partial hydrolysis, periodate oxidation, chromium trioxide oxidation, and uronic acid degradation were also performed on the native and carboxyl-reduced polysaccharides. Partial hydrolysis of the native and carboxyl-reduced polysaccharides gave various oligosaccharides that were characterized and suggest a structure containing a D-glucurono-D-mannan backbone having a repeating unit →4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→. L-Arabinose and D-xylose are present as nonreducing furanosyl and pyranosyl end-groups, respectively, both attached to O-3 of D-glucuronic acid residues of the backbone. D-Galactose is present as non-reducing pyranosyl end-group linked to O-3 of D-mannose residues.

INTRODUCTION

Mucin droplets secreted by the leaves of the carnivorous plant *Drosera capensis* have been shown to be a 4% aqueous solution of an acidic polysaccharide[1]. This polysaccharide is the only macromolecule present in the mucin. Its homogeneity has been shown by gel filtration, ion-exchange chromatography, sedimentation analysis, and electrophoresis. It was reported to be composed of xylose, galactose, mannose, and glucuronic acid[1]. The present paper describes the structure of this polysaccharide.

EXPERIMENTAL

General. — The lyophilized polysaccharide sample previously isolated[1] was used for the present study.

Analytical procedures. — *Paper chromatography.* Descending paper chromatography (p.c.) was performed on Whatman No. 1 paper for analytical, and Whatman

3MM paper for preparative purpose, with the following solvent systems (v/v): (*A*) 5:1:3:3 (upper layer) 1-butanol–benzene–pyridine–water; (*B*) 8:2:1 ethyl acetate–pyridine–water; (*C*) 4:1:5 (upper layer) 1-butanol–acetic acid–water; (*D*) 18:8:3:9 ethyl acetate–acetic acid–formic acid–water; and (*E*) 5:5:1:3 ethyl acetate–pyridine–acetic acid–water. Unless otherwise stated, solvent systems *A* and *C* were used in parallel. Sugars were detected with the *p*-anisidine hydrochloride[2] and alkaline silver nitrate[3] reagents.

Gas–liquid chromatography. — G.l.c. was performed with a Packard 428 gas chromatograph, fitted with a flame-ionization detector, and (a) a glass column (200 × 0.2 cm) containing 3% of OV-225 on Gas Chrom Q (80–100 mesh) for partially methylated alditol acetates (oven temperature 185°), or (b) a glass column (200 × 0.4 cm) packed with 5% of OV-210 on Varaport 30 (100–120 mesh) for fully acetylated alditols (oven temperature programmed from 140° to 195°, at a rate of 3°/min), both systems with nitrogen as carrier gas (30 mL/min). The partially methylated sugars were identified as the alditol acetates by determining the retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, and by g.l.c.–m.s.

Gas-liquid chromatography–mass spectrometry. — G.l.c.–m.s. was performed with a Varian 3700 gas chromatograph (column material OV-225), coupled to a Varian MAT 44S mass spectrometer and a Varian Spectro Spin MAT 200 data-processing system. Mass spectra were recorded at 70 eV, ion-source temperature 220°, and ionization current 0.3 mA.

Other analytical techniques. — Optical rotations were measured for aqueous solutions of the sugars with a Perkin–Elmer 141 polarimeter at 20 ± 1°, after isolation by preparative p.c. I.r. spectra (using KBr discs) of permethylated polysaccharide samples were recorded with a Perkin–Elmer 257 infrared spectrophotometer.

Hydrolysis. — Unless otherwise stated, the polysaccharide samples were hydrolyzed with 0.5M sulfuric acid for 10–12 h at 100°. Each hydrolyzate was made neutral with barium carbonate and filtered. The clear filtrate was passed through Dowex 50 (H^+) and Dowex 2-X8 (HCO_2^-), eluted with water, concentrated by rotary evaporation <40°, and the residue examined by p.c. The neutral sugars in the eluate were converted into the alditol acetates[4], and analyzed by g.l.c. and g.l.c.–m.s. The Dowex 2-X8 resin was eluted with 2M formic acid, the effluent evaporated to dryness, and the residue examined by p.c. for acidic sugars.

Sugar composition of the native and the carboxyl-reduced polysaccharide. — A portion of the polysaccharide was hydrolyzed, and the resulting sugars were isolated by preparative p.c. (solvents *A* and *C*). Another portion (100 mg) was reduced twice according to Taylor and Conrad[5] to give a carboxyl-reduced polysaccharide (yield 60 mg). This material (10 mg) was hydrolyzed and analyzed by p.c. directly, or by g.l.c. as the alditol acetates[4].

Partial hydrolysis of the polysaccharide. — The polysaccharide (500 mg) was hydrolyzed with 0.25M sulfuric acid for 6 h at 100°. The hydrolyzate was made neutral with barium carbonate, filtered, treated with Dowex 50 (H^+), and concentrated. Ethanol (6 vol.) was added, and the precipitated, degraded polysaccharide

was recovered by centrifugation and dried (yield 88 mg). This degraded polysaccharide was further hydrolyzed and the hydrolyzate examined by p.c. The supernatant solution from the ethanol precipitation was concentrated, separated into a neutral and an acidic portion with Dowex 2-X8 (HCO_2^-) resin, and analyzed by p.c.

Characterization of the acidic oligosaccharides **1–3**. — From the acidic portion of the hydrolyzate, three oligosaccharides having respectively R_{GlcA} values of 0.57, 0.26, and 0.13 (solvent *C*), and R_{Gal} values of 0.75, 0.34, and 0.20 (solvent *D*) were isolated by preparative p.c. in solvent *C*. Their homogeneity was ascertained by p.c. in solvents *D* and *E*. The yields from two combined experiments were 13.6 mg, 7.0 mg, and 5.8 mg, respectively. For the determination of the constituent sugars, the acidic oligosaccharides were hydrolyzed with 0.5M sulfuric acid for 6 h at 100°.

A part of each oligosaccharide was reduced with sodium borohydride, converted into the methyl ester with 2% methanolic hydrogen chloride for 24 h at room temperature, and again reduced with sodium borohydride. The resulting oligosaccharide was hydrolyzed with 0.5M sulfuric acid for 4 h at 100°, and analyzed by p.c.

The remaining portion of each oligosaccharide was treated with 2% methanolic hydrogen chloride for 24 h at room temperature to give the methyl ester, methyl glycoside. A part of each methyl ester, methyl glycoside was subjected, in sequence, to periodate oxidation, sodium borohydride reduction, and acid hydrolysis (0.25M sulfuric acid for 5 h at 100°), and analyzed by p.c.

The remaining portion of each methyl ester, methyl glycoside was reduced with sodium borohydride to give a carboxyl-reduced oligosaccharide glycoside, a portion of which was hydrolyzed with 0.5M sulfuric acid for 4 h at 100°, and examined by p.c. The other portion was methylated according to the Hakomori procedure[6]. The permethylated oligosaccharide was hydrolyzed with 0.25M sulfuric acid for 5–6 h at 100°, and the resulting, partially methylated sugars, were converted into the alditol acetates, and analyzed by g.l.c. and g.l.c.–m.s.

Isolation and characterization of the neutral oligosaccharides **4–8** *from the carboxyl-reduced polysaccharide*. — To obtain a maximum yield of low M_r oligosaccharides, the carboxyl-reduced polysaccharide (250 mg) was hydrolyzed with 0.5M trifluoroacetic acid for 3 h at 100°, and the solution evaporated. P.c. in solvent *A* of the residue revealed the presence of four oligosaccharide fractions (R_{Gal} 0.84, 0.66, 0.42, and 0.26, respectively) in addition to L-arabinose, D-xylose, D-galactose, D-mannose, and D-glucose. The oligosaccharides were isolated by preparative p.c. in solvent *A*.

The neutral oligosaccharides were hydrolyzed with 0.5M sulfuric acid for 4–5 h at 100°, and the hydrolyzates examined by p.c. for their sugar composition. The neutral oligosaccharides were reduced with sodium borohydride and hydrolyzed under the conditions just given, and also methylated according to the procedure of Hakomori[6]. The permethylated oligosaccharide alditols were hydrolyzed with 0.25M sulfuric acid for 5–6 h at 100°, and the resulting, partially methylated sugars converted into the alditol acetates and analyzed by g.l.c. and g.l.c.–m.s.

Methylation analysis of the native polysaccharide. — The native polysaccharide (200 mg) was successively methylated, twice by the Haworth procedure[7], once by

the methods of Falconer and Adams[8], and of Kuhn *et al.*[9], respectively, and finally four times by the method of Purdie and Irvine[10]. The resulting product showed negligible i.r. absorption for hydroxyl groups.

A portion of the methylated polysaccharide (20 mg) was dissolved in dry oxolane and reduced with lithium aluminium hydride[11]. The resulting product was hydrolyzed[12] with 90% formic acid for 2 h at 100°, and, after evaporation of the formic acid, with 0.5M sulfuric acid for 12 h at 100°. The derived, partially methylated sugars were analyzed by g.l.c. and g.l.c.–m.s.[13,14] as the alditol acetates.

Methylation analysis of the carboxyl-reduced polysaccharide. — The carboxyl-reduced polysaccharide (10 mg) was methylated by the Hakomori method[6]. The product was hydrolyzed[12] with 90% formic acid for 2 h at 100°, and, after evaporation of the formic acid, with 0.5M sulfuric acid for 10 h at 100°. The resulting, partially methylated sugars were converted into the alditol acetates, and analyzed by g.l.c. and g.l.c.–m.s.[13,14].

Methylation analysis of the degraded polysaccharide. — The degraded polysaccharide (10 mg) was methylated once by the method of Hakomori[6] and four times by that of Purdie and Irvine[10]. It was reduced with lithium aluminium hydride in dry oxolane[11] and hydrolyzed as just described. The resulting, partially methylated sugars were converted into the alditol acetates and analyzed by g.l.c. and g.l.c.–m.s.[13,14].

Oxidation of the carboxyl-reduced polysaccharide with chromium trioxide[15,16]. — The carboxyl-reduced polysaccharide (10 mg) was acetylated twice by the method of Carson and Maclay[17]. A solution of the per-*O*-acetylated polysaccharide in glacial acetic acid (4 mL) was treated with chromium trioxide (400 mg) in an ultrasonic bath for 1.5 h at 50°. Water (10 mL) was added, and the cooled solution extracted with chloroform (5 × 10 mL). The combined extracts were washed three times with water and evaporated. The residue was hydrolyzed with 0.5M sulfuric acid for 16 h at 100°. The sugars were converted into the alditol acetates, and analyzed by g.l.c.

Periodate oxidation of the polysaccharide[18]. — The native polysaccharide (100 mg) was oxidized with 45mM sodium metaperiodate (100 mL) in the dark at room temperature. Periodate consumption was monitored by titration of aliquots with sodium thiosulfate. After 48 h, the remaining solution was treated with 1,2-ethanediol (0.5 mL), dialyzed, and reduced with sodium borohydride. The resulting material was hydrolyzed with 0.5M sulfuric acid for 8 h at 100°. The hydrolyzate was examined by p.c.

Periodate oxidation of the carboxyl-reduced polysaccharide. — The carboxyl-reduced polysaccharide (10 mg) was oxidized with 45mM sodium metaperiodate (10 mL) for 120 h at room temperature in the dark. The reaction mixture was treated with 1,2-ethanediol (0.1 mL), dialyzed, and reduced with sodium borohydride. The resulting material was hydrolyzed. A part of the hydrolyzate was examined by p.c. The remaining portion was analyzed by g.l.c. of the alditol acetates.

Uronic acid degradation according to Lindberg et al.[19]. — The permethylated, native polysaccharide (5 mg) was dissolved in 19:1 (v/v) dry dimethyl sulfoxide–2,3-dimethoxypropane (6 mL) containing *p*-toluenesulfonic acid (2 mg). The solution

was stirred for 3 h, and then 2M methylsulfinyl anion in dimethyl sulfoxide (4 mL) was added. After further stirring overnight, iodomethane was added at ice-bath temperature. The product was hydrolyzed with 90% formic acid for 1 h at 100° and, after evaporation of formic acid, with 0.25M sulfuric acid for 10 h at 100°. The resulting, partially methylated sugars were converted into the alditol acetates, and analyzed by g.l.c. and g.l.c.–m.s.

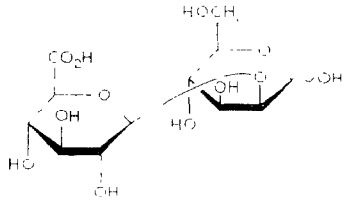
Uronic acid degradation according to Aspinall and Chaudhari[20]. — The permethylated native polysaccharide (15 mg) was dissolved in dry benzene (1.2 mL) containing 1,5-diazobicyclo[5.4.0]undec-5-ene (0.6 mL) and acetic anhydride (0.3 mL), and heated for 24 h at 100°. The cooled solution was washed successively with M hydrochloric acid and water, and then evaporated. The residue was hydrolyzed with 10% acetic acid for 1 h at 100°, *O*-deacetylated with sodium methoxide in methanol, and reduced with sodium borodeuteride. A part of the product was analyzed, after acetylation, by g.l.c. and g.l.c.–m.s. The remaining portion was methylated with iodo[$^2H_3$]methane–silver oxide in *N*,*N*-dimethylformamide, and analyzed by g.l.c. and g.l.c.–m.s.

RESULTS AND DISCUSSION

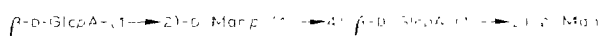
Sugar composition. — Acid hydrolysis of the polysaccharide indicated that it is composed of arabinose, xylose, galactose, mannose, and glucuronic acid. After isolation of these sugars by preparative p.c., optical rotation measurements showed that only arabinose has the L configuration, whereas the others have the D configuration. The native polysaccharide gave high amounts of the aldobiouronic acid, 2-*O*-β-D-glucopyranosyluronic acid-D-mannose (see later), even after prolonged acid hydrolysis (0.5M sulfuric acid for 12 h at 100°), indicating that at least a major portion of D-mannose and D-glucuronic acid residues are linked to eachother. Acid hydrolysis of the carboxyl-reduced polysaccharide and g.l.c. of the resulting sugars as the alditol acetates[4] gave L-arabinose, D-xylose, D-galactose, D-mannose, and D-glucose (derived from D-glucuronic acid) in the molar ratios of 3.6:1.0:4.9:8.4:8.2.

Partial hydrolysis of the native polysaccharide. — Partial hydrolysis of the polysaccharide gave, in addition to D-glucuronic acid and the neutral sugars, three acidic oligosaccharides **1**–**3**, and a degraded, acidic polysaccharide that is composed of D-mannose and D-glucuronic acid, together with small proportions of D-galactose and D-xylose, as estimated by p.c. Oligosaccharides **1**–**3** were isolated in pure form by preparative p.c. and characterized.

The first oligosaccharide (R_{Gal} 0.75, solvent *D*) showed $[\alpha]_D^{20}$ −30.5° (*c* 0.2, water). Acid hydrolysis gave D-glucuronic acid and D-mannose in an equal proportion. Reduction with sodium borohydride, followed by acid hydrolysis gave only D-glucuronic acid as the reducing sugar, but not D-mannose. Periodate oxidation, sodium borohydride reduction, and acid hydrolysis of the methyl ester, methyl glycoside of this oligosaccharide furnished glycerol but no undegraded sugar. Methylation analysis of the methyl glycoside of the carboxyl-reduced oligosaccharide yielded



1



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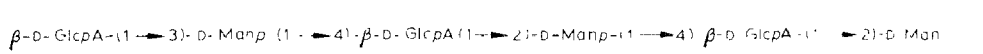
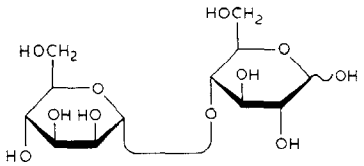


3

2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,6-tri-*O*-methyl-D-mannose in equal proportion. The fragmentation pattern of the methyl ester of the permethylated oligosaccharide alditol, obtained by sodium borodeuteride reduction, was in accordance with that reported for 2-*O*-D-glucopyranosyluronic acid-D-mannitol[21]. Further, the observed $[\alpha]_D$ value was in good agreement with the literature[22] value of −32°, indicating a β-D configuration for the glycosidic linkage. Hence, the aldobiouronic acid is 2-*O*-β-D-glucopyranosyluronic acid-D-mannose (**1**).

On acid hydrolysis, the second oligosaccharide (R_{Gal} 0.34, solvent *D*) gave D-glucuronic acid and D-mannose in equal proportion, together with **1**. A portion of the oligosaccharide was first reduced with sodium borohydride, and then converted into its methyl ester which, on sodium borohydride reduction and acid hydrolysis, gave D-glucose and D-mannose in the molar ratio of ~2:1, together with a small proportion of a disaccharide that was chromatographically identical with 2-*O*-D-glucopyranosyl-D-mannose (see later). On sodium borohydride reduction and acid hydrolysis, the methyl ester, methyl glycoside of the oligosaccharide gave D-glucose and D-mannose in approximately equal proportion, together with a small proportion of the just mentioned neutral disaccharide. On periodate oxidation, sodium borohydride reduction, and acid hydrolysis, the methyl ester, methyl glycoside of the oligosaccharide, gave no undegraded sugar. Methylation analysis of the methyl glycoside of carboxyl-reduced oligosaccharide yielded 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-glucose. These results and the chromatographic behavior (R_{Gal} 0.34, solvent *D*) similar to that reported by Aspinall *et al.*[23] (R_{Gal} 0.32, solvent *D*) suggest the linear tetrasaccharide structure **2**. On acid hydrolysis, the third oligosaccharide (R_{Gal} 0.20, solvent *D*) gave D-glucuronic acid and D-mannose in equal proportion together with **1**. A portion of the oligosaccharide was first reduced with sodium borohydride, and then converted into the methyl ester. On sodium borohydride reduction, followed by acid hydrolysis, the methyl ester gave D-glucose and D-mannose in the molar ratio of ~1.5:1, together with a small proportion of a neutral disaccharide that was chromatographically identical with 2-*O*-D-glucopyranosyl-D-mannose (see later). On sodium borohydride reduction and acid hydrolysis, the methyl ester, methyl glycoside of the oligosaccharide gave D-glucose

and D-mannose in approximately equal proportion, together with a small proportion of the neutral disaccharide just mentioned. On periodate oxidation, sodium borohydride reduction, and acid hydrolysis, the methyl ester, methyl glycoside of this oligosaccharide gave no undegraded sugar. Methylation analysis of the methyl glycoside of the carboxyl-reduced oligosaccharide yielded 2,3,4,6-tetra-*O*-methyl- and 2,3,6-tri-*O*-methyl-D-glucose, and 3,4,6-tri-*O*-methyl-D-mannose. These data and the p.c. behavior suggest a linear hexasaccharide structure **3**.



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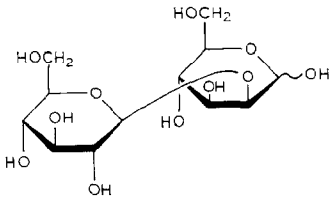


4

D-Glc*p*-(1→2)-D-Man*p*-(1→4)-D-Glc

6

D-Man*p*-(1→4)-D-Glc*p*-(1→2)-D-Man

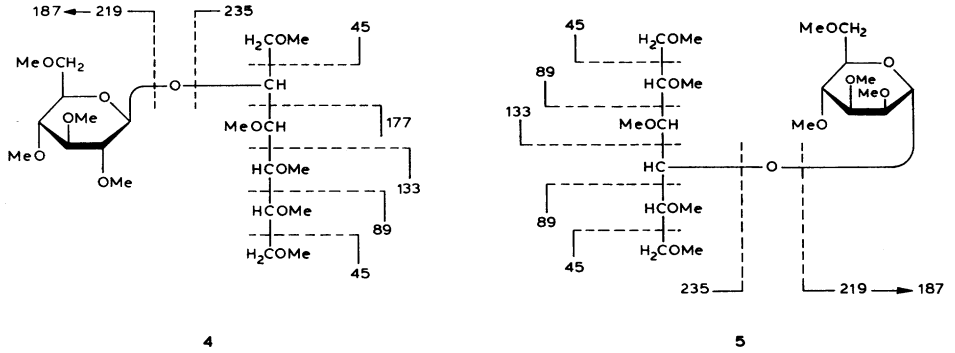
7

D-Glc*p*-(1→2)-D-Man*p*-(1→4)-D-Glc*p*-(1→2)-D-Man

8

Partial hydrolysis of the carboxyl-reduced polysaccharide. — Partial hydrolysis of the carboxyl-reduced polysaccharide yielded four additional oligosaccharide fractions. These were isolated by preparative p.c. and their structures determined. On acid hydrolysis, the fourth fraction (R_{Gal} 0.84, solvent *A*) gave D-glucose and D-mannose in equal proportion. Sodium borohydride reduction followed by acid hydrolysis furnished only D-glucose as the reducing sugar, but no D-mannose. On methylation analysis, the oligosaccharide alditol yielded 1,3,4,5,6-penta-*O*-methyl-D-mannitol and 2,3,4,6-tetra-*O*-methyl-D-glucose. Hence, the oligosaccharide is 2-*O*-D-glucopyranosyl-D-mannose (**4**), which was confirmed by the m.s. analysis of the permethylated alditol, the fragmentation pattern of which is shown in Scheme 1.

On acid hydrolysis of the fifth fraction (R_{Gal} 0.66, solvent *A*), D-glucose and D-mannose were obtained in equal proportion. Sodium borohydride reduction followed by acid hydrolysis gave only D-mannose as the reducing sugar, but no D-glucose. On methylation analysis, the oligosaccharide alditol yielded 1,2,3,5,6-penta-*O*-methyl-D-glucitol and 2,3,4,6-tetra-*O*-methyl-D-mannose. Thus, this oligosaccharide is 4-*O*-D-mannopyranosyl-D-glucose (**5**), which was further confirmed by m.s.



Scheme 1. Mass fragmentation patterns of the permethylated alditols of oligosaccharides **4** and **5**.

analysis of the permethylated alditol, the fragmentation pattern of which is shown in Scheme 1.

On acid hydrolysis, the sixth fraction (R_{Gal} 0.42, solvent *A*) gave D-glucose and D-mannose in a ratio of ~2:1. Sodium borohydride reduction, followed by acid hydrolysis resulted in the formation of D-glucose and D-mannose in the molar ratio of ~1:1. By methylation analysis, mainly 1,2,3,5,6-penta-*O*-methyl-D-glucitol, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-mannose, and two minor components (~5% each of 1,3,4,5,6-penta-*O*-methyl-D-mannitol and 2,3,6-tri-*O*-methyl-D-glucose) were obtained. Therefore, the oligosaccharide fraction is a mixture of **6** (major compound) and **7** (minor compound). The expected 2,3,4,6-tetra-*O*-methyl-D-mannose formed from the nonreducing end-group of **7** could not be distinguished from 2,3,4,6-tetra-*O*-methyl-D-glucose, as they have identical retention times.

On acid hydrolysis, the seventh fraction (R_{Gal} 0.26, solvent *A*) gave D-glucose and D-mannose in the molar ratio of 1:1. Sodium borohydride reduction, followed by acid hydrolysis yielded D-glucose and D-mannose in the molar ratio of 2:1. Methylation analysis of the oligosaccharide alditol indicated 1,3,4,5,6-penta-*O*-methyl-D-mannitol, 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4,6-tri-*O*-methyl-D-mannose, and 2,3,6-tri-*O*-methyl-D-glucose. These data suggest that this oligosaccharide is the linear tetrasaccharide **8**.

Formation of oligosaccharides **4** and **5** provides direct evidence for the alternate occurrence of 4-*O*-substituted D-glucuronic acid and 2-*O*-substituted D-mannose residues in the polysaccharide. Furthermore, formation of the acidic oligosaccharides **1–3** and of the neutral oligosaccharides **4–8** indicates that the polysaccharide contains a D-glucurono-D-mannan backbone with the repeating unit →4)-β-D-Glc*p*A-(1→2)-D-Man*p*-(1→.

Methylation analyses. — By methylation analysis of the native polysaccharide, the following results (see Table I) were obtained: L-Arabinose, D-xylose, and D-galactose each gave rise to only one methylated derivative, 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, respec-

TABLE I

METHYLATION ANALYSIS DATA

Alditol acetates of	*Molar proportion from*[a]		
	A	*B*	*C*
2,3,5-Tri-*O*-methyl-L-arabinose	2.4	7.5	
2,3,4-Tri-*O*-methyl-D-xylose	1.0	1.0	1.0
2,3,4,6-Tetra-*O*-methyl-D-mannose			[b]
2,3,4,6-Tetra-*O*-methyl-D-galactose	5.4	7.0	2.7
3,4,6-Tri-*O*-methyl-D-mannose	4.9	15.0	12.9
2,3,4-Tri-*O*-methyl-D-glucose[c]			0.8
2,3,6-Tri-*O*-methyl-D-glucose[c]		12.3	
4,6-Di-*O*-methyl-D-mannose	7.2	11.1	3.3
2,6-Di-*O*-methyl-D-glucose[c]		13.8	
2,3-Di-*O*-methyl-D-glucose[c]	6.2		12.9
2-*O*-Methyl-D-glucose[c]	3.0		[b]

[a]A, native polysaccharide; B, carboxyl-reduced polysaccharide; and C, degraded polysaccharide. [b]Compound identified by g.l.c. and g.l.c.–m.s. but not quantitatively determined because of low amount. [c]Derived from D-glucuronic acid residues.

tively, indicating the presence of nonreducing L-arabinofuranosyl, and D-xylo- and D-galactopyranosyl end-groups. Formation of 3,4,6-tri-*O*-methyl- and 4,6-di-*O*-methyl-D-mannose shows the occurrence of D-mannose only as nonterminal pyranose residues that are substituted at O-2 and, in part, additionally at O-3. 2,3-Di-*O*-methyl- and 2-*O*-methyl-D-glucose were derived from D-glucuronic acid, indicating that this sugar occurs in nonterminal position, and is substituted at O-4 and, in part, additionally at O-3.

The results of methylation analysis of the carboxyl-reduced polysaccharide (see Table I) indicate that the D-glucurono-D-mannan backbone contains 4-*O*-substituted D-glucuronic acid and 2-*O*-substituted D-mannose residues. The additional linkages at O-3 of D-glucuronic acid and O-3 of D-mannose are due to substitution by L-arabinofuranosyl, D-xylopyranosyl, and D-galactopyranosyl residues.

From methylation analysis of the degraded polysaccharide (see Table I), it can be inferred that the nonreducing L-arabinofuranosyl end-groups are linked to O-3 of D-glucuronic acid residues of the backbone, as removal of L-arabinose resulted in the formation of 2,3-di-*O*-methyl-D-glucose in high yield. Formation of 4,6-di-*O*-methyl-D-mannose and 2,3,4,6-tetra-*O*-methyl-D-galactose in almost equal proportion indicated that D-galactopyranosyl end-groups are linked to O-3 of D-mannose residues of the backbone. Formation of 2-*O*-methyl-D-glucose, even after the complete removal of L-arabinose, suggests that D-xylopyranosyl end-groups are linked to O-3 of D-glucuronic acid residues of the backbone. 2,3,4,6-Tetra-*O*-methyl-D-mannose and 2,3,4-tri-*O*-methyl-D-glucose were derived from the nonreducing end of the polysaccharide backbone.

Chromium trioxide oxidation. To determine the configuration of the glycosidic linkages, the carboxyl-reduced polysaccharide was subjected to chromium trioxide oxidation[15,16]. About 90% of the D-glucose and about 50% of the L-arabinose were oxidized, whereas D-xylose, D-galactose, and D-mannose were resistant to the oxidation. This suggests that D-xylose, D-mannose, and D-galactose are linked by γ, and D-glucuronic acid by β glycosidic bonds. From these studies, however, the nature of the glycosidic linkage of L-arabinose could not be determined, as this sugar is present in the furanose form.

Periodate oxidation. — On periodate oxidation, the native polysaccharide consumed 1.0 mol of periodate per hexosyl residue. This value is in close agreement with the theoretical value of 1.1, as calculated on the basis of the methylation data. On borohydride reduction and hydrolysis, followed by p.c., the periodate-oxidized material gave mainly glycerol, together with D-mannose and D-glucuronic acid.

After periodate oxidation, sodium borohydride reduction, hydrolysis, and g.l.c. of the resultant sugars as their alditol acetates, the carboxyl-reduced polysaccharide gave mainly glycerol, together with erythritol, D-mannose, and D-glucose. The resistance of a part of the D-glucose and D-mannose to periodate oxidation can be explained by the substitution of these residues by L-arabinose, D-galactose, and D-xylose.

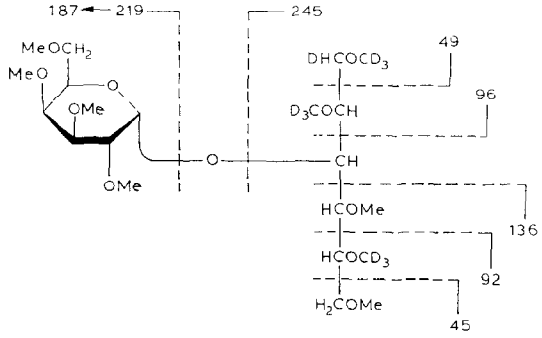
Uronic acid degradation. – Uronic acid degradation of the permethylated native polysaccharide according to Lindberg *et al.*[19] showed that only 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose residues were resistant towards this alkaline treatment. These derivatives were present in the molar ratio of 3.3:1.0.

The complete base-catalyzed degradation of D-mannose and D-galactose is in accordance with our suggestion that O-4 of D-glucuronic acid is substituted by D-mannose residues, to which D-galactopyranosyl end-groups can be linked at O-3. The resistance of L-arabinose and D-xylose residues may be explained by linkage to O-3 of D-glucuronic acid.

Partial hydrolysis of the acidic polysaccharide and the carboxyl-reduced polysaccharide did not give oligosaccharides having intact glycosidic bonds formed by L-arabinose, D-xylose, and D-galactose residues. To liberate such oligosaccharides, uronic acid degradation according to Aspinall and Chaudhari[20] was performed. A part of the resulting material was acetylated and analyzed by g.l.c. and g.l.c.–m.s., which gave mainly 3,4,6-tri-*O*-methyl-D-mannose besides 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, and a partially methylated hexosylhexitol (see later). During uronic acid degradation according to Aspinall and Chaudhari[20], the sugar residues released by β-elimination were protected, thus preventing further degradation. The survival of 3,4,6-tri-*O*-methyl-D-mannose during this degradation and its complete loss during the Lindberg *et al.*[19] degradation are in agreement with the proposal that a D-mannopyranosyl is linked to O-4 of a D-glucopyranosyluronic acid residue. The release of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose by mild acid hydrolysis confirmed the suggestion that both residues are directly linked to the D-glucopyranosyluronic acid residues of the polysaccharide backbone.

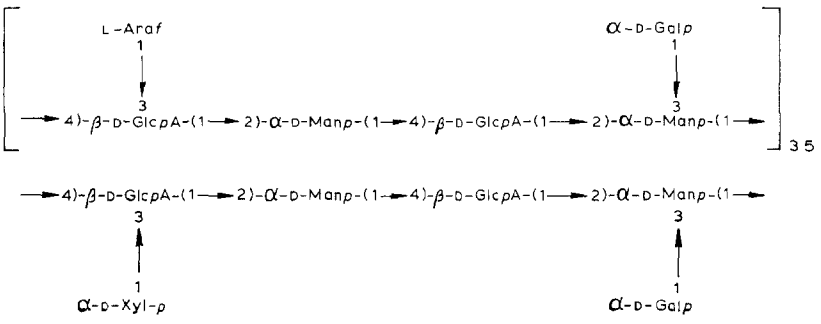
Further methylation of the remaining material using trideuteriomethyl iodide,

followed by g.l.c. and g.l.c.–m.s. gave a permethylated 3-*O*-hexosylhexitol. On the basis of all data obtained so far, this compound is per-*O*-methylated 3-*O*-D-galactopyranosyl-D-mannitol (see Scheme 2), thus confirming the site of attachment of the D-galactopyranosyl groups. Oligosaccharides containing intact glycosidic bonds formed by L-arabinose or D-xylose units could not be identified.



Scheme 2. Mass fragmentation pattern of permethylated 3-*O*-D-galactopyranosyl-D-mannitol.

Conclusion. — Based on the foregoing results, the following conclusions may be drawn: methylation analyses of the native and the carboxyl-reduced polysaccharides indicate the presence of L-arabinofuranosyl, D-xylopyranosyl, and D-galactopyranosyl end-groups, whereas D-mannopyranosyl and D-glucopyranosyluronic acid residues constitute the backbone. Furthermore, ~50% of the D-mannopyranosyl and ~50% of the D-glucopyranosyluronic acid residues of the backbone carry substituents at O-3. Formation of the acidic oligosaccharides **1**–**3** during partial hydrolysis of the native polysaccharide, and the neutral oligosaccharides **4**–**8** during partial hydrolysis of the carboxyl-reduced polysaccharide indicates that the polysaccharide contains a D-glucurono-D-mannan backbone having a repeating unit →4)-β-D-Glc*p*A-(1→2)-D-Man*p*-(1→. Methylation analysis of the degraded acidic polysaccharide indicated that the L-arabinofuranosyl and D-xylopyranosyl end-groups are directly linked to O-3 of the D-glucopyranosyluronic acid residues, and the D-galactopyranosyl end-groups also to O-3 of the D-mannopyranosyl residues of the backbone. The results of partial hydrolysis, periodate oxidation, and uronic acid degradation are in good agreement with these suggestions. Furthermore, the results



of chromium trioxide oxidation suggested the β configuration for the D-glucopyranosyluronic acid and the α configuration for the D-xylopyranosyl, D-galactopyranosyl, and D-mannopyranosyl residues. Based on these data, structure **9** is proposed for the polysaccharide.

Thus, the acidic polysaccharide from the mucin of *D. capensis* belongs to the family of D-glucurono-D-mannans. Polysaccharides containing a D-glucurono-D-mannan backbone consisting of alternate residues of 4-*O*-substituted D-glucuronic acid and 2-*O*-substituted D-mannose units have been reported from gum exudates of a variety of plants[24], as well as from an extracellular polysaccharide[25], and a solubilized polysaccharide[21] of suspension-cultured tobacco cells. In all these cases, there is a great variation in the nature and the length of the side chains. The acidic polysaccharide from the mucin of the related species, *Drosera binata*, has a structure similar to that reported here for *D. capensis*[26].

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