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POLYSACCHARIDE COMPONENT IN THE STIGMATIC EXUDATE FROM *LILIUM LONGIFLORUM*

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Abstract—The polysaccharide component of the stigmatic exudate from *Lilium longiflorum* has the composition, arabinose (26%), rhamnose (6%), galactose (57%) and glucuronic acid (11%). The highly branched polysaccharide bears a striking resemblance to the acidic polysaccharide exudate from *Araucaria bidwillii* in belonging to the galactan group and in carrying outer chains terminated by arabinofuranose, rhamnopyranose, galactopyranose and glucuronic acid residues. Both polysaccharides contain the sequence *O*-rhamnopyranosyl-(1→4)-glucopyranosyluronic acid-(1→6)-galactopyranose in some of the outer chains.

INTRODUCTION

Labarca *et al.* [1–3] have shown that the stigmatic exudate from *Lilium longiflorum* plays a nutritional role in pollen tube wall formation. The sugar composition of the exudate suggested a possible structural relationship to the acidic arabinogalactans frequently found as exudate gums [4], such as gum arabic. Through the kindness of Professor Frank Loewus a small quantity of the exudate was placed at our disposal for detailed structural investigation. The amount of available material did not permit fragmentation to give oligosaccharides by partial acid hydrolysis, but using GLC–MS [5] for the analysis of methylated sugars from the methylated polysaccharide and its carboxyl-reduced and base-degraded derivatives we have established the main structural features of the polysaccharide. Parallel experiments have been carried out on the acidic polysaccharide component of *Araucaria bidwillii* gum [6–8] to which the exudate shows a marked similarity.

RESULTS AND DISCUSSION

Samples of the stigmatic exudate from Ace and Croft varieties of *Lilium longiflorum* were chromatographed on DEAE-cellulose [9]. The Ace variety exudate gave two fractions of identical composition which were combined for further study. The Croft variety exudate gave a major fraction of the same composition and a minor fraction which contained arabinose as the sole sugar constituent.

Lilium longiflorum exudate was methylated with methyl iodide and sodium hydride in methyl sulfoxide [10]. Björndal *et al.* [5] have shown that a single treatment under these conditions does not lead to detectable base-catalyzed degradation of acidic polysaccharides, and subsequent analysis of the methylated polysaccharide showed this to be the case in this instance. The methylated polysaccharide was hydrolyzed and the neutral methylated sugars were converted into the corresponding partially methylated alditol acetates which were quantitatively analyzed and their identities confirmed by GC–MS (Table 1). A sample of the methylated polysaccharide was reduced with lithium aluminum deuteride and the methylated sugars formed on hydrolysis were similarly characterized and estimated. The formation of two additional sugars, namely, 2,3,4-tri- and 2,3-di-*O*-methylglucose, whose deuterium labelling indicated their origin in the corresponding uronic acid derivatives, showed that glucuronic acid was the sole detectable uronic acid component in the polysaccharide. Since deuterium could not be detected in any galactose methyl ethers we conclude that galacturonic acid, which was reported to be present to the extent of 2.5% in the whole exudate [1], occurs as a constituent of a minor polysaccharide component which was either not present in the sample under investigation or was not recovered during the fractionation. The methylated polysaccharide showed a qualitatively similar composition to that of the methylated acidic polysaccharide from *Araucaria bidwillii* gum [7], and a sample of the latter methylated polysaccharide was analytical in a parallel manner and the results are shown in Table 2. The results show that both polysaccharides contain interior chains of galactopyranose residues mutually joined by (1→3) and (1→6) linkages, and carry outer chains terminated by arabinofuranose, rhamnopyranose and glucuronic acid, in addition to some galactopyranose residues. The *Araucaria* polysaccharide contains, as features not detected in the *Lilium* exudate, some of its glucuronic acid units as the 4-methyl ether and a small proportion of non-terminal arabinofuranose residues.

The available quantities of the *Lilium* polysaccharide were not sufficient to permit fragmentation, e.g. by the Smith degradation [11], to show whether, as in the *Araucaria* gum [7], the (1→3) inter-galactose linkages were concentrated in the interior chains. However, analysis of the products of base-catalyzed degradations of methylated acidic polysaccharides provides information on sugar linkages in the vicinity of uronic acid residues [12, 13]. Thus, using a modification [14] of the procedure developed by Lindberg *et al.* [12], both methylated polysaccharides were treated with sodium

Table 1. Composition of *Lilium longiflorum* (var. Ace) exudate and neutral sugars from the methylated polysaccharide, and its carboxyl-reduced and base-degraded derivatives

Sugar		T*	Proportions (%) of alditol acetates† (a)	(b)	(c)	(d)	(e)		Composition of parent polysaccharide: Direct analysis	From methylation analysis (c)
2,3,4-Me_3	Rha‡	0.42	6	5	6			Rhamnose	6	6
2,3,5-Me_3	Ara‡	0.42	27	23	26	26	22	Arabinose	25	26
2,3,4,6-Me_4	Gal	1.19	11	9	8	15	12			
2,4,6-Me_3	Gal	2.03	10	8	8	19	16			
2,3,4-Me_3	Gal	2.89	5	4	6	4	3			
2,6-Me_2	Gal	3.14	2	2	2	2	2			
2,4-Me_2	Gal	5.1	34	29	28	29	24			
2-Me	Gal	6.4	5	4	5	5	4	Galactose	57	57
2,3,4-Me_3	Glc	2.22			5§			Glucuronic acid	12	11
2,3-Me_2	Glc	4.50			6§					

* Retention times of partially methylated alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on column b. † (a) Neutral sugar constituents only from methylated polysaccharide (the methylated polysaccharide from the Croft exudate had essentially the same composition); (b) calculated from (a) allowing for uronic acid constituents and assuming that only 50% of the neutral sugars from aldobiouronic acid units were liberated, (c) from carboxyl-reduced methylated polysaccharides; (d) from base-degraded methylated polysaccharide after trideuteriomethylation, (e) calculated from (d) as proportions of the parent methylated polysaccharide allowing for loss of uronic acid and rhamnose residues. ‡ The relative proportions of these two sugars were estimated separately by GLC of the methyl glycosides formed on methanolysis of the methylated polysaccharide. § Deuterium at C-6 (from MS). These constituents contained OCD_3 at C-6 (from MS).

methylsulfinylmethanide in methyl sulfoxide and then, without work-up, were directly alkylated with trideuteriomethyl iodide. The modified methylated polysaccharides were hydrolyzed and the resulting methylated sugars were analyzed by GC–MS as the alditol acetates. The results, for both methylated polysaccharides (Tables 1 and 2), showed that all the rhamnose residues were lost and that *O*-6 of some galactopyranose residues were the only positions carrying the trideuteriomethyl label. It may be concluded, therefore, that in both polysaccharides, as in gum arabic [14, 15], all the rhamnose end groups are linked in the partial structure,

L-Rha*p*-(1→4)-D-Glc*p*A-(1→6)-D-Gal*p*-(1-*.
3
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* The present paper provides no evidence for the enantiomeric configurations of the constituent sugars, but the correctness of the assignments is implied [1–3].

The galactose residues labelled at *O*-6 with trideuteriomethyl groups were non-uniformly substituted in both modified methylated polysaccharides, some appearing as end groups and others carrying base-stable substituents at *O*-3. Since the base treatment results in removal of glucuronic acid end groups in addition to units further substituted by rhamnose residues, it is not possible to indicate whether or not the outermost galactose residues in the rhamnose-terminated chains carried side-chains. The isolation of the modified polysaccharide as a methylated arabinogalactan necessarily implies that the arabinofuranose residues (as end groups) are linked to a core of galactopyranose residues.

Arabinogalactans with a branched core of (1→3)- and (1→6)-linked β-D-galactopyranose residues occur in plant cell walls [16] and are particularly abundant in coniferous woods [17]. Many exudate gums [4], especially those from *Acacia* species [18], belong to the same structural family, but are of even greater chemical complexity

Table 2. Composition of acidic component of *Araucaria bidwillii* gum and neutral sugars from the methylated polysaccharide, and its carboxyl-reduced and base-degraded derivatives

Sugar		T*	Proportions (%) of alditol acetates† (a)	(b)	(c)	(d)	(e)		Composition of parent polysaccharide: Previous work [7]	Analysis (c) from methylation
2,3,4-Me_3	Rha‡	0.42	7	5	6			Rhamnose	9	6
2,3,5-Me_3	Ara‡	0.42	13	10	11	12	10			
2,5-Me_2	Ara	0.88	5	4	3	5	4			
3,5-Me_2	Ara	0.75	2	2	1	2	2	Arabinose	17	15
2,3,4,6-Me_4	Gal	1.19	14	11	12	28	22			
2,4,6-Me_3	Gal	2.03	2	2	2	7	6			
2,3,4-Me_3	Gal	2.89	21	16	20	11	9			
2,4-Me_2	Gal	5.1	33	25	28	29	23			
2-Me	Gal	6.4	3	2	3	5	4	Galactose	56	65
2,3,4-Me_3	Glc	2.22			7§			Glucuronic acid (and its		
2,3-Me_2	Glc	4.50			8§			4-methyl ether)	18	15

* Footnotes as in Table 1.

in carrying highly ramified outer chains with terminal rhamnose and glucuronic acid, and frequently multiple units of arabinofuranose residues. The *Lilium* polysaccharide is intermediate in complexity between arabinogalactans, such as that from larch [17], and gum arabic, and has been shown to be structurally similar to the polysaccharide exudate of *Araucaria bidwillii* gum [6–8]. In contrast to the exudate gums for which only functional roles in wound-healing processes have been postulated, the *Lilium longiflorum* stigmatic exudate is of special interest as an arabinogalactan in performing a nutritional role in pollen tube wall formation [2, 3].

EXPERIMENTAL

General methods. Evapns were carried out under diminished pressure at temps of 40° or less. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ± 2°. GLC was performed isothermally with a Perkin–Elmer 990 chromatograph using teflon columns (190 × 0.15 cm) packed with Gas-Chrom Q (100–120 mesh) coated with (a) 3% of silicone-polyester copolymer ECNSS-M (at 190° for alditol acetates), (b) 3% of silicone gum OV-225 (at 170° for partially methylated alditol acetates), (c) 5% of neopentylglycol adipate polyester (at 160° for methyl glycosides of methylated sugars), and an OV-225 SCOT column (d). Retention times of methyl glycosides are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-*β*-D-glycopyranoside, and those of partially methylated alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. For GC–MS columns were attached to a Perkin–Elmer–Hitachi RMU-6 mass spectrometer, operated with an inlet temp. of 250°, an ionization potential of 70 eV, and an ion-source temp. of ~250°.

Examination of polysaccharides. Polysaccharide samples were dissolved in H_2O and the soln were shaken with Amberlite resin IR-120(H) to ensure complete removal of metal ions. The solns were filtered, concd and chromatographed on columns (30 × 4.5 cm) of diethylaminoethyl-cellulose [9]. The columns were eluted with increasing concns of Na dihydrogen phosphate buffer at pH 6, and fractions were analyzed with the PhOH–H_2SO_4 reagent [19]. Polysaccharide-containing fractions were appropriately combined and dialysed, and polysaccharides were recovered by pptn with EtOH (4 vol.), dissolution in H_2O and freeze-drying. The Ace exudate (300 mg) gave two fractions of identical sugar composition (see Table 1) and optical rotation ($[\alpha]_D$ −11° (*c* 0.6, H_2O)), which were combined (240 mg) for further examination. The Croft exudate (120 mg) furnished a major fraction (90 mg) of essentially similar composition to that of the Ace exudate, together with a minor fraction (10 mg), which gave arabinose only on hydrolysis but was not available in sufficient quantity for further study. Polysaccharide samples (5 mg) with D-xylose added as int. stand. were hydrolyzed in 0.25 M H_2SO_4 (3 ml) at 100° for 14 hr. The hydrolyzate was neutralized with $BaCO_3$, and the soln filtered and concd to dryness. When uronic acid was present the resulting mixture was trimethylsilylated [10], and the product in Et_2O (10 ml) was heated under reflux with $LiAlD_4$ (20 mg) for 4 hr. The reduction product was hydrolyzed with 0.25 M H_2SO_4 (3 ml) at 100° for 14 hr, and the hydrolyzate was neutralized with $BaCO_3$, filtered and concd to dryness. Sugar analyses of polysaccharide hydrolyzates were carried out by reduction with Na borohydride, followed by acetylation and GLC of the resulting alditol acetates [20] on column *a*. Analysis of the sugar constituents from the main polysaccharide fractions by GC–MS of the derived alditol acetates showed deuterium incorporation only in glucitol hexaacetate. It is concluded that glucuronic acid is the only acidic sugar constituent.

Preparation and analysis of methylated polysaccharides. Methylation of polysaccharides was performed by the Hakomori procedure [21] as described in ref. [10]. Methylated polysaccharides and their derivatives were purified by chromatography on columns (15 × 1 cm) of Sephadex LH20 using $CHCl_3$–Me_2CO (2:1) as eluant. The sugar components of methylated polysaccharides were analyzed as partially methylated alditol acetates formed on hydrolysis, followed by reduction with sodium borohydride and acetylation [5], by GLC alone or GC–MS on column *b* or *d*. The identities of all components of different retention times (see Tables 1 and 2) were confirmed by the presence of diagnostic fragment ions in their mass spectra. The sample of methylated polysaccharide from *Araucaria bidwillii* gum was that prepared in an earlier investigation [7]. Methyl glycosides of methylated sugars were prepared by heating methylated polysaccharide in methanolic 5% HCl under reflux for 16 hr, followed by neutralization with silver carbonate. Methyl glycosides of 2,3,4-tri-*O*-methylrhamnose (*T* 0.45 on column *c*) and 2,3,5-tri-*O*-methylarabinose (*T* 0.55 and 0.70) were analyzed quantitatively in such mixtures.

Modifications of methylated polysaccharides. (a) *Reduction.* Methylated polysaccharide (10 mg) in tetrahydrofuran (5 ml) containing $LiAlD_4$ (50 mg) was heated under reflux for 4 hr. Excess of reagent was destroyed by the addition of EtOAc, H_2O was added, and the mixture was acidified to pH 4, and reduced methylated polysaccharide was isolated by extraction into $CHCl_3$. Methylated sugar components formed on hydrolysis were analyzed by GC–MS of the derived alditol acetates (see Tables 1 and 2). (b) *Base-catalyzed degradation.* Methylated polysaccharide (5 mg) was kept in MNa methylsulfinylmethanide in methyl sulfoxide at room temp. for 16 hr, trideuteriomethyl iodide (1 ml) was added dropwise with cooling, and the mixture was stirred at room temp. for 0.5 hr. The degraded methylated polysaccharide was isolated in the usual way and the component sugars were analyzed by GC–MS of the derived alditol acetates (Tables 1 and 2).

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