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STRUCTURAL STUDIES ON THE POLYSACCHARIDE OF *Swietenia mahogany* GUM

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ABSTRACT

The acidic polysaccharide isolated from the gum exudate of *Swietenia mahogany*, and the degraded polysaccharide obtained by its mild hydrolysis with acid, are composed of residues of D-galactose, L-arabinose, L-rhamnose, and D-galacturonic acid. The products of methylation of these two polysaccharides and their carboxyl-reduced derivatives were characterized. The results of the methylation and periodate-oxidation experiments showed that these polysaccharides are extensively branched, and that their backbone is a (1→3)-linked D-galactan chain. The periodate consumption was in accord with the linkage distributions proposed. End-group analysis showed that the polysaccharide has a high value of average degree of polymerization.

INTRODUCTION

Swietenia mahogany (Meliaceae) is a large, deciduous, medicinally and economically important plant[1]. A number of terpenes, coumarins, steroids, and their glycosides have been isolated[2–5] from the various parts of the plant. Usually, it produces a small amount of gummy material from mechanically injured parts of its stem.

We now report structural studies on this gum. The original polysaccharide isolated from the purified gum contains residues of D-galactose, L-arabinose, L-rhamnose, and D-galacturonic acid in the molar ratios of 37:5:4:10. Mild hydrolysis with acid under controlled conditions produces a degraded polysaccharide consisting of the same four sugars in the ratios of 37:3:1:10. The results of complete acid hydrolysis, mild acid hydrolysis, carboxyl reduction, methylation analysis, periodate-oxidation studies, and Smith degradation, and the determination of the average degree of polymerization of the gum polysaccharide are herein reported.

RESULTS AND DISCUSSION

The natural gum was freed of soluble impurities by repeated washing with benzene–diethyl ether–ethanol, and the porous mass thus obtained was taken up in water, the suspension centrifuged, the polysaccharide precipitated from the supernatant liquor by ethanol–acetone, and the process repeated twice. The crude poly-

saccharide was freed of cationic impurities by passage through a column of Amberlite IR-120 (H^+) resin. The eluate was concentrated, and the product finally dried over P_2O_5. Homogeneity of the pure polysaccharide was checked by high-voltage, paper electrophoresis and gel filtration through Sephadex G-100. The carbohydrate content (estimated by the phenol–sulfuric acid method[6]) and the uronic acid content (by the carbazole[7] method) of the purified polysaccharide (OP) were found to be 99 and 16.26 %, respectively.

Suitable conditions for preparation of the degraded polysaccharide were established by trial experiments. Prolonged autohydrolysis (120 h) at 100° of the OP, with periodic examination of the hydrolyzate by paper chromatography, revealed that L-arabinose and L-rhamnose appeared during the earlier stages of hydrolysis. The labile cleavage of these two monosaccharides indicated that they might be at branch chains, and that there were some arabinose residues in the furanose form. Further degradation of the OP was then conducted by using 2.5mM sulfuric acid for 40 h at 60°. The solution was exhaustively dialyzed, and the degraded polysaccharide (DGP) isolated by precipitation with ethanol–acetone. That the DGP was electrophoretically homogeneous was ascertained by high-voltage, paper electrophoresis.

The relatively high content (~16.3 %) of uronic acid in the OP hindered complete hydrolysis by mineral acid. The carboxyl groups in the OP and DGP were, therefore, reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC) and sodium borohydride[8]. Twice-repeated application of this method gave reduced products (CMC-reduced OP and CMC-reduced DGP) that responded negligibly to the carbazole test.

Complete hydrolysis of OP, DGP, CMC-reduced OP, and CMC-reduced DGP with M sulfuric acid revealed the presence of D-galactose, L-arabinose, L-rhamnose, and D-galacturonic acid. The enhanced proportions of D-galactose in the carboxyl-reduced-OP and -DGP were in close agreement with the uronic acid contents in OP and DGP. The identities of the monosaccharides present were confirmed by measurement of the specific rotation, t.l.c.[9], and p.c., and by g.l.c. as their alditol acetates. Their compositions are presented in Table I.

TABLE I

CONTENT[a] OF MONOSACCHARIDES IN ORIGINAL (OP), DEGRADED (DGP), CARBOXYL-REDUCED ORIGINAL (CMC-REDUCED OP), AND CARBOXYL-REDUCED, DEGRADED (CMC-REDUCED DGP) POLYSACCHARIDES FROM *Swietenia mahogani*

Monosaccharide	*OP*	*DGP*	*CMC-reduced OP*	*CMC-reduced DGP*
D-Galactose	66	72	82	90
L-Arabinose	9	6	8	5
L-Rhamnose	7	2	7	2
D-Galacturonic acid	17	19	–	

[a]Contents are given in percent, to the nearest whole number.

TABLE II

METHYLATED PRODUCTS FROM OP, DGP, CMC-REDUCED OP, AND CMC-REDUCED DGP OF *Swietenia mahogany*

Methylated monosaccharides	*Mole proportions*				*Linkage pattern*
	OP	*DGP*	*CMC-reduced OP*	*CMC-reduced DGP*	
2,3,4,6-Tetra-*O*-methyl-D-galactose	10	12	20	22	Gal*p*-(1→
2,4,6-Tri-*O*-methyl-D-galactose	2	2	2	2	→3)-Gal*p*-(1→
2,3,4-Tri-*O*-methyl-D-galactose	9	7	9	7	→6)-Gal*p*-(1→
2,4-Di-*O*-methyl-D-galactose	1	1	1	1	→3,6)-Gal*p*-(1→
2,6-Di-*O*-methyl-D-galactose	6	6	6	6	→3,4)-Gal*p*-(1→
4,6-Di-*O*-methyl-D-galactose	1	1	1	1	→2,3)-Gal*p*-(1→
6-*O*-Methyl-D-galactose	6	6	6	6	→2,3,4)-Gal*p*-(1→
2-*O*-Methyl-D-galactose	2	2	2	2	→3,4,6)-Gal*p*-(1→
2,3,4-Tri-*O*-methyl-L-arabinopyranose	—	2	—	2	Ara*p*-(1→
2,3,5-Tri-*O*-methyl-L-arabinofuranose	2	1	2	1	Ara*f*-(1→
2,4-Di-*O*-methyl-L-arabinopyranose	2	—	2	—	→3)-Ara*p*-(1→
2,5-Di-*O*-methyl-L-arabinofuranose	1	—	1	—	→3)-Ara*f*-(1→
2,3,4-Tri-*O*-methyl-L-rhamnopyranose	3	—	3	—	Rha*p*-(1→
2-*O*-Methyl-L-rhamnopyranose	1	1	1	1	→3,4)-Rha*p*-(1→

The mode of linkage of the constituent monosaccharides and their distribution pattern were apparent from the methylation experiment. The OP, DGP, CMC-reduced OP, and CMC-reduced DGP were methylated first by the Hakomori method[10] and then by the Purdie method[11]; the product showed no free OH absorption in the infrared spectrum. The methylation products were hydrolyzed completely, the sugars converted into the methylated alditol acetates, and these characterized by g.l.c.[12] using column *2*. The data from the methylation analysis are presented in Table II.

The results of the methylation experiment could be interpreted by first considering the characterized products from methylated DGP and methylated, CMC-reduced DGP. The formation of 2,3,4,6-tetra-*O*-methyl-D-galactose (12 mol) indicated that 12 nonreducing termini are occupied by D-galactosyl groups. The CMC-reduced DGP gave 22 mol of 2,3,4,6-tetra-*O*-methyl-D-galactose per mol. This larger proportion could be explained as the result of carboxyl reduction of D-galactosyluronic acid groups (10 mol) occupying nonreducing, branch ends, together with the nonreducing D-galactosyl units (12 mol). The characterization of 2,4,6- and 2,3,4-tri-*O*-methyl-D-galactose (see Table II) from both DGP and CMC-reduced DGP indicated the presence of →3-Gal-1→ and →6-Gal-1→ units, respectively. The 2,6-di-*O*-methyl-D-galactose (6 mol), 6-*O*-methyl-D-galactose (6 mol), and 2-*O*-methyl-D-galactose (2 mol) might be formed from a (1→3)-linked D-galactan core (supported later by periodate-oxidation studies) in which 6 mol are branched at C-4, 6 mol at C-4 and C-2, and 2 mol at C-4 and C-6, respectively. One mol proportion each of

2,4- and 4,6-di-*O*-methyl-D-galactose could be explained either as afforded by D-galactosyl residues, linked contiguously to the D-galactan core, which are branched at C-6 and C-2, respectively, or by a (1→3)-linked D-galactosyl branch chain carrying subchains linked at C-6 and C-2, respectively. Formation of 2,3,4-tri-*O*-methyl-L-arabinopyranose (2 mol) and 2,3,5-tri-*O*-methyl-L-arabinofuranose (1 mol) indicated their presence as nonreducing termini. The formation of 2-*O*-methyl-L-rhamnose (1 mol) must be due to an L-rhamnose unit having linkages at its C-1, C-3, and C-4 atoms, and appearance of 2,3,4-tri-*O*-methyl-L-rhamnose (3 mol) suggested the presence of L-rhamnose at some nonreducing termini in the OP.

The results of methylation analysis of OP and CMC-reduced OP corroborated in most cases those of the methylated DGP and methylated, CMC-reduced DGP (see Table II). This suggested extensive branching, coupled with D-galactosyluronic acid groups occupying nonreducing, branch ends in the OP (see earlier), which help the DGP to retain the major, structural pattern of the OP. That is to say, when degrading the OP with 2.5mM sulfuric acid, there was no substantial cleavage of monosaccharide residues from the intact OP.

Periodate oxidation and kinetic study[13,14] of the OP and DGP showed that, in both cases, periodate uptake became constant within 28 h. The amount of periodate consumed was 1.14 and 1.23 mol per mol of hexose, respectively. The values of periodate consumption were in approximate agreement with the theoretical amounts for the linkages proposed. The OP was subjected to periodate oxidation followed by borohydride reduction. Complete hydrolysis of part of the borohydride-reduced, periodate-oxidized material revealed the presence of 86% of D-galactose, 12% of L-arabinose, and a trace of L-rhamnose. When the rest was subjected to a second Smith degradation, only D-galactose survived, showing that, in the OP, there was a (1→3)-linked D-galactan core.

The end-group analysis[15] showed a very high value (~300) for the average degree of polymerization ($\overline{d.p.}$). The $\overline{d.p.}$ value for the OP showed that it had the high molecular weight that is characteristic of acidic polysaccharides of gums.

It was also apparent that this gum polysaccharide was of the galacturonogalactan[16] type, where the D-galactosyluronic acid groups occupy the branch-chain ends.

EXPERIMENTAL

Source of gum specimen. — A sample of the gum of *Swietenia mahogany* (Meliaceae) was collected in West Bengal, India, in June, 1978, from mechanically injured parts of the stems.

Purification of the gum. After washing 4 times at 35° with 2:3:1 benzene–diethyl ether–ethanol (500 mL), the natural gum (60 g), light yellow in color (moisture content, 5%; ash content, 2%), was taken up in water (2 L), and the suspension was centrifuged for 0.5 h at 6000 r.p.m. at 5°. From the centrifugate, the crude polysaccharide was precipitated with 3:1 ethanol–acetone (2.5 L). The precipitation was repeated twice (yield 40 g). A solution of the gum polysaccharide thus obtained in

water (4 L) was passed through a column (100 × 2 cm) of Amberlite IR-120 (H^+) resin. The eluate (5 L) was concentrated, and the polysaccharide precipitated with ethanol–acetone (yield 35 g), $[\alpha]_D^{24}$ +10° (*c* 2, 0.1M NaOH). The homogeneity of this original polysaccharide (OP) was tested by high-voltage, paper electrophoresis in borate buffer (pH 10.2), and by gel filtration through Sephadex G-100 in pyridine–acetate buffer (pH 4.5).

General experimental conditions. — The estimations of sugars (by the phenol–sulfuric acid and carbazole methods) and the kinetic study of periodate uptake at 4° were conducted with a Beckman Model 26 spectrophotometer. Unless otherwise specified, all optical rotations were measured at 24° with a Perkin–Elmer Model 241 MC spectropolarimeter at 589.6 nm. Infrared spectra were recorded with an Acculab 10 Beckman instrument for KBr pellets. High-voltage, paper electrophoresis at 2° was conducted with a Shandon Model L-24 instrument, on Whatman No. 1 filter paper, using borate buffer (pH 10.2). Paper chromatography for detection, and for preparative isolation of sugars, was performed on Whatman No. 1 and Whatman No. 3 MM paper, respectively. The solvent systems (v/v) used for paper chromatography were (*A*) 8:3:1 ethyl acetate–pyridine–water, and the upper layers of (*B*) 4:1:5 1-butanol–acetic acid–water, and (*C*) 4:1:5 1-butanol–ethanol–water. Spraying agents were (*a*) alkaline silver nitrate solution in aqueous acetone, and (*b*) a saturated, aqueous solution of aniline oxalate. Thin-layer chromatography[9] with silica gel G (100–200 mesh) was performed by using solvent systems (*A*) and (*C*), and anisaldehyde–sulfuric acid as the spraying agent. Gel filtration was performed on a column (100 × 1.4 cm) of Sephadex G-100 with pyridine acetate buffer (pH 4.5). Gas–liquid chromatography of the monosaccharides as their alditol acetates and partially methylated alditol acetates was conducted in glass columns containing (*1*) 3% of OV-225 on Gas-Chrom Q (100–120 mesh), and (*2*) 3% of ECNSS-M on Gas-Chrom Q (100–120 mesh), with nitrogen as the carrier gas, in a Packard Model 419 Becker instrument fitted with a flame-ionization detector. Sugar solutions were always evaporated in a rotary evaporator at 50°, and the products dried by lyophilization.

Preparation of the degraded polysaccharide (DGP). — The original polysaccharide (OP, 4 g) was treated with 2.5mM sulfuric acid (100 mL) for 40 h at 60°, the duration of treatment being ascertained from the results of pilot experiments. The mixture was dialyzed for 120 h at 4°, and the DGP was precipitated from the dialyzate by means of 3:1 (v/v) ethanol–acetone (800 mL), and dried over P_2O_5; yield 1.5 g, $[\alpha]_D^{24}$ −9° (*c* 1, water). It migrated as a single, broad spot in electrophoresis (60 V/cm).

Carboxyl reduction of OP and DGP. — Both OP and DGP (150 mg each) were separately dissolved in water (40 mL). 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMC, 1.5 g) was added to each polysaccharide solution, with stirring, and the pH maintained at ~4.75 by dropwise addition of 0.01M hydrochloric acid during 4 h. Sodium borohydride solution (2.5M, 50 mL) was then added to each solution, the pH being maintained at 7 by simultaneous addition of 4M hydrochloric acid. The mixtures were stirred for a further 1 h, dialyzed,

and concentrated. The entire process of reduction was repeated twice. CMC-reduced OP and CMC-reduced DGP (70 and 80 mg, respectively) were thus obtained.

Characterization of monosaccharides in the polysaccharides (OP, DGP, CMC-reduced OP, and CMC-reduced DGP). — The OP, DGP, CMC-reduced OP, and CMC-reduced DGP (10 mg each) were hydrolyzed with M sulfuric acid (20 mL) for 10 h at 100°, using D-mannose as the internal standard. After neutralization of the acid with $BaCO_3$, and decationization with Amberlite IR-120 (H^+) resin, the monosaccharides were identified by paper chromatography in solvent systems *A*, *B*, and *C*, by matching against authentic samples, and also by thin-layer chromatography in solvent systems *A* and *C*. Their complete identification as D-galactose, L-arabinose, L-rhamnose, and D-galacturonic acid was then achieved by isolation through preparative paper-chromatography in solvent *B*, followed by specific rotation measurements. The composition of the neutral monosaccharides, estimated as their alditol acetates by g.l.c. in columns *1* and *2*, and of the uronic acid by the carbazole method, is presented in Table I.

Methylation analysis. — The OP, DGP, CMC-reduced OP, and CMC-reduced DGP (10 mg each) were placed in separate, air-tight vials. They were mixed with dry dimethyl sulfoxide (12 mL) followed by 2M methylsulfinyl sodium (10 mL) in an atmosphere of dry N_2, stirring being continued overnight, and then treated with methyl iodide (10 mL) by dropwise addition. The products were dialyzed exhaustively against distilled water, the solutions concentrated, and the concentrates extracted with hot chloroform. They were remethylated once by the Purdie method. The infrared spectra of these methylated products showed no absorption band near 3500 cm^{-1} (yield 5, 4, 6, and 7 mg, respectively).

The methylated products (4 mg each) were refluxed with 80% formic acid for 3 h, and, after removal of the formic acid as the aqueous azeotrope, were hydrolyzed with 0.5M sulfuric acid for 10 h at 100°. After the usual treatment, the products were converted into partially methylated alditol acetates, and characterized by g.l.c. in column *2*. The results are presented in Table II.

Periodate oxidation and Smith degradation of the OP and DGP. — Periodate oxidation of OP and DGP was performed with 0.04M sodium metaperiodate in the dark at 4°, the rate of oxidation of the polysaccharides, and the needed duration of treatment, being monitored spectrophotometrically. The periodate consumption became constant at 28 and 26 h, respectively; the materials were respectively found to consume 1.14 and 1.23 mol of periodate per mol of hexosyl residue.

In separate experiments, OP and DGP (22 mg each) were each treated with 0.04M sodium metaperiodate (25 mL) for 34 h at 4° in the dark. The excess of oxidant was decomposed with ethylene glycol (10 mL), and the solutions were dialyzed, and concentrated. The contents were reduced with sodium borohydride (1 g), and, after the usual treatment, the borohydride-reduced, periodate-oxidized polysaccharides were isolated by precipitation with ethanol (yield, 9 mg). One portion (3 mg) of this material was hydrolyzed with M sulfuric acid at 100°, and the products, after conversion into alditol acetates, analyzed by g.l.c. in column *1*. Another part (5 mg) was

treated with 0.5M sulfuric acid (8 mL) for 18 h at room temperature, the acid neutralized with $BaCO_3$, and the solution decationized, and dialyzed. The solution was concentrated, and the product subjected to a second Smith degradation. After the usual processing, the periodate-immune material was hydrolyzed with 0.5M sulfuric acid, and the monosaccharides were characterized by g.l.c. in column *1*.

End-group analysis. — To a solution of the polysaccharide (50 mg) in very dilute sodium hydroxide solution (5 mL) was added sodium borohydride (30 mg), and the mixture was kept for 48 h. The excess of borohydride was decomposed with dilute acetic acid, and the pH was adjusted to 6. The solution was cooled to 4°, 0.25M sodium metaperiodate solution (10 mL) was added, and the volume of the mixture was adjusted to 25 mL. The oxidation was allowed to proceed in the dark, and, periodically, aliquots (2 mL) of the mixture were transferred to test tubes, and the excess of periodate immediately precipitated by addition of a saturated solution of lead acetate (3 mL). A length of dialysis tubing containing distilled water (5 mL) was introduced into all of the test tubes. The concentration of formaldehyde in the dialysis tubings was determined by the chromotropic acid procedure[15], using D-mannitol as the internal standard.

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