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STRUCTURAL STUDIES ON A POLYSACCHARIDE OF MAHUA (*Madhuca indica*) FLOWERS*

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ABSTRACT

On hydrolysis, the purified polysaccharide, PS-AII, of Mahua flowers yielded D-galactose, D-glucose, L-arabinose, and D-glucuronic acid in the mole ratios of 3.9:2.3:1.3:1.0. A degraded polysaccharide was prepared by autohydrolysis of an aqueous solution of PS-AII. Methylation studies were conducted on the degraded polysaccharide, the whole polysaccharide, and their carboxyl-reduced derivatives. The results were corroborated by those from periodate oxidation followed by Smith degradation. The anomeric configurations of the different sugar residues in the whole polysaccharide and carboxyl-reduced, degraded polysaccharide were determined by chromium trioxide oxidation. Finally, the oligosaccharides obtained by autohydrolysis of the whole polysaccharide and by graded hydrolysis of the degraded polysaccharide were characterized. From these results, a tentative structure was assigned to the average repeating-unit in polysaccharide PS-AII.

INTRODUCTION

The characteristics and versatile uses of Mahua flowers have been described in a previous communication[2]. Cold-water-soluble polysaccharide from Mahua flowers contains a mixture of two polysaccharide fractions. The structure of one of them (PS-AI) was reported earlier[1,2]. In the present communication, the structures of the other polysaccharide fraction (PS-AII) is discussed.

EXPERIMENTAL

General methods. — Paper partition-chromatography was performed for preparative purposes on Whatman Nos. 1 and 3 MM papers with the following solvent systems (v/v): (*A*) 8:2:1 ethyl acetate–pyridine–water, (*B*) 4:1:5 1-butanol–acetic

*Part III. For Part II, see ref. 1.

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acid–water (upper layer), (*C*) 9:2:2 ethyl acetate–acetic acid–water, and (*D*) 3:1:1 ethyl acetate–acetic acid–water. The spray reagents used were (*a*) aniline oxalate, (*b*) alkaline silver nitrate, and (*c*) benzidine–periodate. Gas–liquid chromatography (g.l.c.) was performed with a Hewlett–Packard model 5730A gas chromatograph fitted with a flame-ionization detector and glass columns (1.83 m × 6 mm) packed with (*i*) 3% of ECNSS-M on Gas-Chrom Q (100–120 mesh) and (*ii*) 3% of OV-225 on Gas-Chrom Q (100–120 mesh). All g.l.c. analyses were conducted (at 185° for unmethylated sugars, and at 165° for methylated sugars) by first converting the sugars into their alditol acetates. All other methods and instruments used were as described previously[1,2].

Isolation and purification of the Mahua polysaccharide, PS-AII. — The procedure for the isolation and purification of the Mahua polysaccharide PS-AII was given previously[2].

Hydrolysis and sugar analysis of polysaccharide[2] *PS-AII.* — Polysaccharide PS-AII (2 mg) was mixed with *myo*-inositol (0.2 mg) as the internal standard, and the mixture was hydrolyzed for 20 h in a boiling-water bath with 0.5M sulfuric acid. Alditol acetates were prepared from the hydrolyzate in the usual way, and the proportions of component sugars present were estimated by g.l.c. using column (*i*). The uronic acid in PS-AII was estimated by the carbazole–sulfuric acid method[3], with D-glucuronic acid as the standard.

Preparation of the carboxyl-reduced PS-AII. — Polysaccharide PS-AII (30 mg) in water (30 mL) was reduced with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate (CMCS) (750 mg) by the method of Taylor and Conrad[4] as described previously[2]. The yield was 25 mg. The carboxyl-reduced PS-AII (2 mg) was hydrolyzed with 0.5M sulfuric acid as previously described. The alditol acetates of sugars were estimated by g.l.c. using column (*i*), with *myo*-inositol (0.2 mg) as the internal standard.

The carboxyl-reduced PS-AII was prepared in several batches, and hydrolyzed as described earlier. The sugars were resolved, and isolated, by preparative paper-chromatography using solvent *A*. The specific rotations of these sugars were determined: galactose, +78° (lit.[5] +83.3° for D-galactose); glucose, +50° (lit.[5] +52.5° for D-glucose); and arabinose, +104° (lit.[5] +108° for L-arabinose).

Preparation of the degraded polysaccharide. — In a pilot experiment, a solution of polysaccharide PS-AII (20 mg) in water (8 mL) was heated in a boiling-water bath and the progress of hydrolysis was monitored by paper-chromatographic examination of the hydrolyzate at regular intervals. Arabinose was detected first, and, after 50 h, a trace of galactose appeared. Guided by this pilot experiment, polysaccharide PS-AII (500 mg) in water (200 mL) was subjected to autohydrolysis for 50 h. The resulting solution was cooled, concentrated to ~50 mL, and dialyzed exhaustively against distilled water until no sugar was detected in the dialyzate. The contents of the dialysis bag were lyophilized; yield, 300 mg. The dialyzates were collected, concentrated to a small volume, and lyophilized. On paper-chromatographic examination, the dialyzate showed spots corresponding to

arabinose, galactose, and a few oligosaccharides. The oligosaccharides were isolated by preparative paper-chromatography using solvent *D*.

Purification of the degraded polysaccharide PS-AII. — A solution of the degraded polysaccharide PS-AII (80 mg) in water (4 mL) was applied to a column (95 × 1.8 cm) of Sephadex G-100. The column was eluted with water, 5-mL fractions being collected. The fractions were automatically monitored with a Waters Associates Differential Refractometer Model 403 fitted with a recorder. The material was eluted as a single peak; yield 75 mg; $[\alpha]_D^{23}$ +8.4° (*c* 0.3, water).

When electrophoresed (37 V/cm) for 90 min in phosphate buffer, pH 6.9, the purified material (sprayed with reagent *c*) moved as a single component.

The degraded polysaccharide PS-AII was converted into its carboxyl-reduced product by the method of Taylor and Conrad[4]. The degraded polysaccharide (3 mg) and its carboxyl-reduced product (2 mg) were hydrolyzed with 0.5M sulfuric acid for 20 h at 100°. After the usual treatment, the hydrolyzate was examined by paper chromatography, using solvents *A*, *B*, *C*, and *D* and spray reagent *b*; spots corresponding to galactose, glucose, arabinose, glucuronic acid, and an oligosaccharide (faint) were obtained. Neutral sugars, as their alditol acetates, were estimated by g.l.c. using *myo*-inositol as the internal standard. Uronic acid was estimated by the carbazole–sulfuric acid method[3], with D-glucuronic acid as the standard.

Methylation analysis. — Polysaccharide PS-AII (8.0 mg) and its carboxyl-reduced product (4.0 mg), and the degraded polysaccharide (6 mg) and its carboxyl-

TABLE I

METHYLATION ANALYSIS OF THE POLYSACCHARIDE (PS-AII) FROM MAHUA FLOWERS, ITS CARBOXYL-REDUCED PRODUCT, AND THE DEGRADED POLYSACCHARIDE AND ITS CARBOXYL-REDUCED PRODUCT

Methylated sugars[a]	T^b		*Approximate mol. proportions*				
	i	*ii*	*Original PS-AII*	*Carboxyl-reduced PS-AII*	*Degraded PS-AII*	*Carboxyl-reduced, degraded PS-AII*	*Mode of linkage*
2,3,5-Ara	0.47	0.43	7	6	4	3	Ara*f*-(1→
2,5-Ara	1.08	0.86	3	2	—	—	→3)-Ara*f*-(1→
2,3,4,6-Glc	**1.00**	**1.00**	1	7	1	6	Glc*p*-(1→
2,3,4-Glc	2.50	2.23	9	9	7	7	→6)-Glc*p*-(1→
2,4,6-Glc	1.98	1.83	2	1	2	1	→3)-Glc*p*-(1→
2,3-Glc	5.38	4.51	3	3	3	3	→4,6)-Glc*p*-(1→
2,3,4,6-Gal	1.24	1.19	5	4	3	3	Gal*p*-(1→
2,3,4-Gal	3.38	2.90	4	3	8	7	→6)-Gal*p*-(1→
2,4,6-Gal	2.25	2.03	4	2	3	2	→3)-Gal*p*-(1→
2,4-Gal	6.40	5.10	14	14	9	9	→3,6)-Gal*p*-(1→

[a]2,3,5-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, etc. [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as unity, on (*i*) a 3% ECNSS-M column at 165°, and (*ii*) a 3% OV-225 column at 165°.

reduced product (3 mg), were methylated by the Hakomori method[6] followed by the Kuhn procedure[7], as described in earlier papers. Portions of the methylated materials were separately hydrolyzed, first with 90% formic acid for 1 h in a boiling-water bath and then with 0.5M sulfuric acid for 16 h at 100°. The methylated sugars were converted into their alditol acetates by the usual procedure, and identified, and estimated, by g.l.c. using columns (*i*) and (*ii*). The results are given in Table I.

Periodate oxidation and Smith degradation of PS-AII and degraded PS-AII. — Polysaccharide PS-AII and degraded polysaccharide PS-AII were subjected to periodate oxidation, Smith degradation[8], and sequential, periodate oxidation as described previously[2]. The kinetics of the periodate oxidation were measured spectrophotometrically[9].

In another set of experiments, the Smith-degraded product of degraded PS-AII (4 mg) was methylated by the Kuhn method[7]. The partially methylated sugars obtained by hydrolysis of the fully methylated product were identified, and estimated, by g.l.c., using columns (*i*) and (*ii*).

Graded hydrolysis of degraded polysaccharide PS-AII. — Guided by the results of pilot experiments, the degraded polysaccharide PS-AII (160 mg) in 25% formic acid (50 mL) was heated for 6 h on a boiling-water bath. Formic acid was removed by co-distillation with water under diminished pressure, and the hydrolyzate was passed through a column of Dowex-1 X-4 (HCO_2^-) ion-exchange resin. The eluate and the washings (with distilled water) were concentrated to a small volume, to give the neutral oligosaccharides. The column was next eluted with 20% formic acid, and the eluate was evaporated to dryness, yielding the acidic oligosaccharides. Both the neutral and the acidic oligosaccharides were resolved on Whatman No. 3 MM papers, using solvent *C*, and the individual oligosaccharides were isolated by eluting the corresponding strips with water. The homogeneity of each oligosaccharide was examined by p.c.

These oligosaccharides, and those obtained from the dialyzate during dialysis of the autohydrolytic product of polysaccharide PS-AII, were hydrolyzed with 0.5M sulfuric acid for 16 h at 100°, and the hydrolyzates, after the usual treatments, were analyzed both by paper chromatography and by g.l.c. using column (*i*). The reducing-end units in the oligosaccharide were determined by sodium borohydride reduction followed by hydrolysis. The sugars in the hydrolyzate were identified by p.c., using spray reagent (*a*), and by g.l.c. in column (*i*). The oligosaccharides were methylated by the Kuhn method[7]. The methylated, acidic oligosaccharides were reduced[10] with $LiAlH_4$. The methylated oligosaccharides were hydrolyzed as described earlier, and the partially methylated sugars were identified, and estimated, as their alditol acetates, by g.l.c. in columns (*i*) and (*ii*).

Oxidation of polysaccharide PS-AII and carboxyl-reduced, degraded polysaccharide PS-AII with chromium trioxide. — Polysaccharide PS-AII (6 mg) and carboxyl-reduced, degraded PS-AII (5 mg), in their acetylated form, were subjected to oxidation with chromium trioxide[11], using *myo*-inositol (1 mg) as the internal standard. The results are summarized in Table II.

TABLE II

OXIDATION OF PERACETYLATED POLYSACCHARIDE PS-AII AND PERACETYLATED, CARBOXYL-REDUCED, DEGRADED POLYSACCHARIDE PS-AII WITH CrO_3

Material	*Time of oxidation (h)*	*Galactose*	*Glucose*	*Arabinose*	myo-*Inositol*
PS-AII	0	15.70	7.84	5.80	10
	1	0.42	0.55	3.11	10
	2	0.34	0.54	2.13	10
Carboxyl reduced, degraded PS-AII	0	15.30	11.00	3.10	10
	1	0.38	0.54	1.10	10
	2	0.13	0.52	0.55	10

RESULTS AND DISCUSSION

On gel filtration through a column of Sephadex G-150, the cold-water-soluble polysaccharide PS-A gave two homogeneous fractions. The second fraction, designated PS-AII, had $[\alpha]_D^{23}$ −19.5°. On hydrolysis of polysaccharide PS-AII with 0.5M sulfuric acid, paper-chromatographic examination using different solvent systems gave spots corresponding to galactose, glucose, arabinose, glucuronic acid, and oligosaccharide (and faint spots of rhamnose and xylose). Polysaccharide PS-AII was purified by passing it through a column (95 × 1.8 cm) of Sephadex G-100. The homogeneity of PS-AII was tested by high-voltage electrophoresis in phosphate buffer (pH 6.9). On hydrolysis, it gave galactose (40.0), glucose (26.4), arabinose (15.3), and glucuronic acid (14.0%). The uronic acid units were reduced by the method of Taylor and Conrad[4], and, on hydrolysis, the reduction product gave galactose (45.0), glucose (38.4), and arabinose (15.7%), as shown by g.l.c., thereby confirming the presence of glucuronic acid in polysaccharide PS-AII. From the specific rotations of the sugars isolated, the configurations of these sugar residues were determined to be D-galactose, D-glucose, L-arabinose, and D-glucuronic acid.

Aided by the results of a pilot experiment, the degraded polysaccharide PS-AII was prepared by autohydrolysis of an aqueous solution of PS-AII for 50 h on a boiling-water bath. The solution was then dialyzed exhaustively against distilled water until the dialyzate showed no test for any sugar. The degraded PS-AII was obtained by lyophilization of the dialyzed solution. The material was purified by gel-filtration through a column of Sephadex G-100, being eluted as a single peak. In high-voltage electrophoresis in 0.1M phosphate buffer, the degraded PS-AII moved as a single substance, indicating its homogeneity. It had $[\alpha]_D^{23}$ +8.4°. On hydrolysis with 0.5M sulfuric acid, the purified, degraded PS-AII yielded galactose (45.0), glucose (26.4), arabinose (7.0), and glucuronic acid (14.4%). The carboxyl groups in the degraded PS-AII were reduced by using the method of Taylor and

Conrad[4], and, on hydrolysis of the product, and g.l.c. analysis of the alditol acetates, the hydrolyzate showed galactose (51.0), glucose (40.0), and arabinose (7.3%).

Polysaccharide PS-AII, degraded PS-AII, and their carboxyl-reduced derivatives were methylated by the Hakomori method[6] followed by the Kuhn method[7], to yield fully methylated products. The permethylated products were hydrolyzed with 90% formic acid, and then with 0.5M sulfuric acid for 16 h at 100°. The alditol acetates of the resulting methylated sugars in each hydrolyzate were identified, and their relative mole proportions determined, by g.l.c. From the results, summarized in Table I, some structural features of PS-AII and degraded PS-AII could be ascertained.

All of the L-arabinosyl units in methylated, carboxyl-reduced PS-AII gave either 2,3,5-tri-*O*-methyl- (6 mol) or 2,5-di-*O*-methyl-L-arabinose (2 mol). This definitely indicated the presence of arabinosyl groups as nonreducing ends, as well as (1→3)-linked furanosyl residues in the interior of the molecule. Interestingly, 2,5-di-*O*-methyl-L-arabinose was absent from the hydrolyzate of the methylated, carboxyl-reduced, degraded PS-AII, and the proportion of 2,3,5-tri-*O*-methyl-L-arabinose was lessened, showing that these L-arabinosyl groups had been removed during autohydrolysis of the PS-AII.

In the hydrolyzate of methylated PS-AII and degraded PS-AII, the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose (1 mol) indicated that this unit is present as a nonreducing end, whereas, in their carboxyl-reduced products, this unit had been increased to 6 and 7 mol, respectively, indicating that all of the D-glucuronic acid residues had been reduced, and that they occupy nonreducing ends. Both the PS-AII and degraded PS-AII in their carboxyl-reduced form contain (1→3)-linked D-glucose, as evidenced by the 2,4,6-tri-*O*-methyl-D-glucose (1 mol). Both polysaccharide PS-AII and degraded PS-AII, and their carboxyl-reduced products, gave 2,3,4-tri-*O*-methyl-D-glucose (9 mol and 7 mol, respectively), indicating that a (1→6)-linked D-glucose unit had been removed from the former during the preparation of the latter.

All of the D-galactose units in PS-AII and its degradation product are present in the pyranose form. Some of the D-galactose units occupy the nonreducing ends, as shown by the presence of 2,3,4,6-tetra-*O*-methyl-D-galactose in the hydrolyzates of both the methylated, reduced and nonreduced PS-AII and degraded PS-AII. From Table I, it is evident that the reduced, degraded PS-AII contains one less unit of D-galactose than the reduced PS-AII, owing to the release of D-galactose-containing oligosaccharide during the preparation of degraded PS-AII. There are two units of (1→3)-linked D-galactose in the carboxyl-reduced PS-AII and its degraded product, as evidenced by the formation of 2,4,6-tri-*O*-methyl-D-galactose. Characterization of 2,3,4-tri-*O*-methyl-D-galactose in the hydrolyzates of methylated, carboxyl-reduced PS-AII and its degraded product showed that these D-galactose residues, present in the repeating unit of the polysaccharide, have (1→6) linkages. Interestingly, there were three units of 2,3,4-tri-*O*-methyl-D-galactose from car-

boxyl-reduced PS-AII, and the number increased to seven units for carboxyl-reduced degraded PS-AII. The increase of such units in degraded, reduced PS-AII is due to release of oligosaccharides from PS-AII during autohydrolysis. Characterization of large proportions of 2,4-di-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-D-glucose in PS-AII and degraded PS-AII indicated that the molecule is highly branched, and that the branch points are at O-1, O-3, and O-6 of the D-galactosyl, and O-1, O-4, and O-6 of the D-glucosyl, residues.

Both polysaccharides PS-AII and degraded PS-AII were subjected to oxidation with sodium metaperiodate in the dark at 4°, and the consumption of periodate was monitored spectrophotometrically[9]. The consumption of periodate in PS-AII became constant in 21 h, and corresponded to 1.10 mol of oxidant per mol of hexosyl residue, whereas, for degraded PS-AII, it became constant in 30 h, and corresponded to 1.24 mol of periodate per mol of hexosyl unit. The observed values of periodate uptake were in good agreement with the theoretical values (1.08 and 1.26 mol) required for the linkages proposed. On complete hydrolysis, the periodate-oxidized and reduced PS-AII gave galactose (29.7), glucose (2.2), and arabinose (3.3%), besides glycerol and other polyhydric alcohols, and aldehydes. The proportion of sugars resistant to periodate oxidation was in good agreement with the theoretical values (31.3, 2.0, and 3.9%, respectively) expected from the methylation studies. On complete hydrolysis of the periodate-oxidized, reduced, degraded PS-AII, g.l.c. examination revealed galactose (22.5) and glucose (2.2%), besides lower alcohols and aldehydes. The calculated values of sugars resistant to periodate for the proposed structure are 26.8 and 2.4%, respectively.

On mild hydrolysis of periodate-oxidized, reduced, degraded PS-AII and whole PS-AII with 0.5M sulfuric acid for 24 h at room temperature, and the usual treatment, p.c. gave spots of galactose and glucose as the monosaccharides, and a spot near the base line (besides spots of lower alcohols and aldehydes); in addition to these spots, whole PS-AII gave a spot of arabinose. The Smith-degraded product of degraded PS-AII was methylated by the Kuhn method[7], and the resulting methyl sugars obtained on hydrolysis of the permethylated product were examined by g.l.c. using columns (*i*) and (*ii*). The methylated sugars identified, and estimated, were 2,3,4,6-tetra-*O*-methylgalactose (2.9 mol), 2,3,4-tri-*O*-methylgalactose (7.8 mol), and 2,3,4,6-tetra-*O*-methylglucose (1 mol). The large proportion of 2,3,4-tri-*O*-methylgalactose might have arisen from the main backbone of the degraded PS-AII molecule.

When the Smith-degraded materials from degraded PS-AII and whole PS-AII were subjected to a second periodate oxidation, no monosaccharide spot was obtained from degraded PS-AII, whereas a faint spot of galactose appeared in the case of whole PS-AII. From the results of methylation and second-periodate-oxidation studies on Smith-degraded material from degraded PS-AII, it is evident that there is a sequence of (1→6)-linked galactose residues in the polysaccharide.

To ascertain the sequence of the different sugar residues, the degraded polysaccharide PS-AII was subjected to graded hydrolysis. Guided by the results of

pilot experiments for maximal release of oligosaccharides, the degraded PS-AII was hydrolyzed with 25% formic acid for 6 h at 100°. On p.c. examination, the neutral hydrolyzate showed spots for monosaccharides and spots in the region of oligosaccharides. The mixture was separated into five neutral and two acidic oligosaccharides by using an ion-exchange column, followed by p.c. Each fraction was found by paper chromatography to be homogeneous, and was characterized as follows.

Neutral oligosaccharides. — On hydrolysis, oligosaccharide **5** {3.3 mg; R_{Gal} 0.08 (solvent *C*); $[\alpha]_D^{23}$ +20° (*c* 0.3, water)} gave galactose and glucose in the mol ratio of 1.4:1. On reduction with $NaBH_4$ followed by hydrolysis, the oligosaccharide showed an alditol peak corresponding to galactitol only. The material was fully methylated by the Kuhn method[7], and the product was hydrolyzed. The methylated sugars were identified, and estimated, by g.l.c. using columns (*i*) and (*ii*); they were 2,3,4,6-tetra- (1.0 mol), 2,4,6-tri- (0.8 mol), and 2,3,4-tri-*O*-methylgalactose (0.92 mol), and 2,4,6-tri- (0.85 mol) and 2,3,4-tri-*O*-methylglucose (0.87 mol). Oligosaccharide **5** was therefore assigned the following structure.

Gal-(1→3)-Gal-(1→6)-Glc-(1→3)-Glc-(1→6)-Gal
5

On hydrolysis, oligosaccharide **4** {4.2 mg; R_{Gal} 0.096 (solvent *C*); $[\alpha]_D^{23}$ +6.30° (*c* 0.35, water)} yielded galactose only. On methylation of the oligosaccharide by the Kuhn method[7], followed by hydrolysis in the usual way, g.l.c. of the hydrolyzate showed 2,3,4,6-tetra- (1.0 mol) and 2,3,4-tri-*O*-methylgalactose (2.7 mol). Hence, oligosaccharide **4** has the following structure.

Gal-(1→6)-Gal-(1→6)-Gal-(1→6)-Gal
4

On hydrolysis, oligosaccharide **3** {2.7 mg; R_{Gal} 0.14 (solvent *C*); $[\alpha]_D^{23}$ −12° (*c* 0.25, water)} showed a spot corresponding to glucose in t.l.c. Methylation of oligosaccharide **3**, hydrolysis, and g.l.c. showed peaks corresponding to 2,3,4,6-tetra- (1 mol) and 2,3,4-tri-*O*-methylglucose (1.9 mol). Thus, the structure of oligosaccharide **3** is as follows.

Glc-(1→6)-Glc-(1→6)-Glc
3

On hydrolysis, oligosaccharide **2** {4.5 mg; R_{Gal} 0.28 (solvent *C*); $[\alpha]_D^{23}$ +11° (*c* 0.4, water)} gave galactose and glucose in the molar ratio of 1.9:1. On reduction of **2** with $NaBH_4$, and hydrolysis, g.l.c. examination gave an alditol peak corresponding to glucitol only, showing that the glucose occupied the reducing end. Completely methylated material, on hydrolysis, and analysis by g.l.c. in the usual way,

yielded peaks corresponding to 2,3,4-tri-*O*-methylglucose (1 mol), and 2,3,4,6-tetra- (1 mol) and 2,3,4-tri-*O*-methylgalactose (0.98 mol). Thus, oligosaccharide **2** has the structure depicted.

Gal-(1→6)-Gal-(1→6)-Glc
2

On hydrolysis, oligosaccharide **1** {5.0 mg; R_{Gal} 0.37 (solvent *C*); $[\alpha]_D^{23}$ +9.3° (*c* 0.45, water)} gave galactose and glucose in the molar ratio of 1.8:1. On reduction of the oligosaccharide with $NaBH_4$, followed by hydrolysis and g.l.c. examination, the material gave a glucitol peak corresponding to glucose as the reducing end-unit. The material was fully methylated by the Kuhn method[7], the product was hydrolyzed, and the alditol acetates of the resulting methylated sugars were identified by g.l.c. as 2,3,4-tri-*O*-methylglucose (0.95 mol) and 2,3,4,6-tetra- (1.0 mol) and 2,4,6-tri-*O*-methylgalactose (0.9 mol). Hence, the structure of oligosaccharide **1** is as follows.

Gal-(1→3)-Gal-(1→6)-Glc
1

Acidic oligosaccharides. — On hydrolysis, oligosaccharide **6** {9.0 mg; R_{GlcA} 0.22 (solvent *C*); $[\alpha]_D^{23}$ +15° (*c* 0.6, water)} gave galactose, glucose, and glucuronic acid (p.c.), estimated to be in the mole ratios of 1:0.95:1.0. On reduction with $NaBH_4$, followed by hydrolysis, glucose was found to constitute the reducing end-unit. The oligosaccharide was methylated by the Kuhn method, and the product hydrolyzed; g.l.c. then indicated 2,3,4-tri-*O*-methylglucose (1.0 mol) and 2,3,4-tri-*O*-methylgalactose (0.98 mol). The methylated oligosaccharide was reduced with $LiAlH_4$, and the product hydrolyzed. The hydrolyzate was found to contain 2,3,4-tri-*O*-methylglucose (1.9 mol) and 2,3,4-tri-*O*-methylgalactose (1.0 mol). All of these results indicated that the structure of oligosaccharide **6** is as follows.

GlcA-(1→6)-Gal-(1→6)-Glc
6

Oligosaccharide **7** {8.4 mg; R_{GlcA} 0.07 (solvent *C*); $[\alpha]_D^{23}$ +5° (*c* 0.7, water)} contained galactose, glucose, and glucuronic acid in the mole ratios of 3.7:1:0.98. On methylation and hydrolysis, g.l.c. examination indicated 2,3,4,6-tetra- (1.0 mol), 2,3,4-tri- (1.9 mol), and 2,4-di-*O*-methylgalactose (0.8 mol), and 2,3,4-tri-*O*-methylglucose (1.0 mol). The methylation product was reduced with $LiAlH_4$, hydrolyzed, and the sugars analyzed, as their alditol acetates, by g.l.c. The mol proportion of 2,3,4-tri-*O*-methylglucose (1.8 mol) increased, while that of the other methylated sugars remained almost the same, indicating that the increase was due to carboxyl-reduction of glucuronic acid. On mild hydrolysis, p.c. examination of

the material gave spots for galactose, glucose, glucuronic acid (trace), and an oligosaccharide whose mobility was almost the same as that of the original oligosaccharide on a paper chromatogram. Based on these data, the structure assigned to this oligosaccharide is as follows.

Gal-(1→6)-Gal-(1→6)-Gal
3
↑
1
Glc
6
↑
1
Gal
6
↑
1
GlcA
7

The structures of oligosaccharides **4** and **7** indicate that the main chain of the degraded polysaccharide PS-AII is composed of (1→6)-linked galactose units to which glucose residues are glycosidically linked at O-3. To the main chain, instead of a glucose unit, one galactose unit is also glycosidically joined at O-3, as is evidenced by the structure of oligosaccharide **1**. The structures of oligosaccharides **2** and **3** show that nonreducing ends of degraded PS-AII are occupied by galactose and glucose, and are obviously present as branching from the main chain. The occurrence of galactose as a nonreducing end is also evidenced by the structures of oligosaccharides **1** and **5**. The three nonreducing arabinofuranose residues are present as side chains attached glycosidically to O-4 of some of the glucose units.

The structures of oligosaccharides **6** and **7** show that an aldobiouronic acid (although not isolated) is glycosidically linked to O-6 of a glucopyranosyl unit in the molecule thereby forming the aldotriouronic acid **6**. The structure of the latter further indicates that the aldotriouronic acid **6** is glycosidically linked to O-3 of a galactose residue in the main chain. From these results and those of methylation, periodate-oxidation, and Smith-degradation studies, one of the possible structures that can be assigned to the average repeating-unit of the degraded, Mahua polysaccharide PS-AII is given in Fig. 1.

The material obtained by lyophilization of the dialyzate, collected during the dialysis of the autohydrolytic product of the whole PS-AII, showed, in p.c., spots of arabinose, a trace of galactose, and a few oligosaccharides. The oligosaccharides were separated, and isolated, by preparative paper chromatography into three homogeneous fractions. These were characterized as follows.

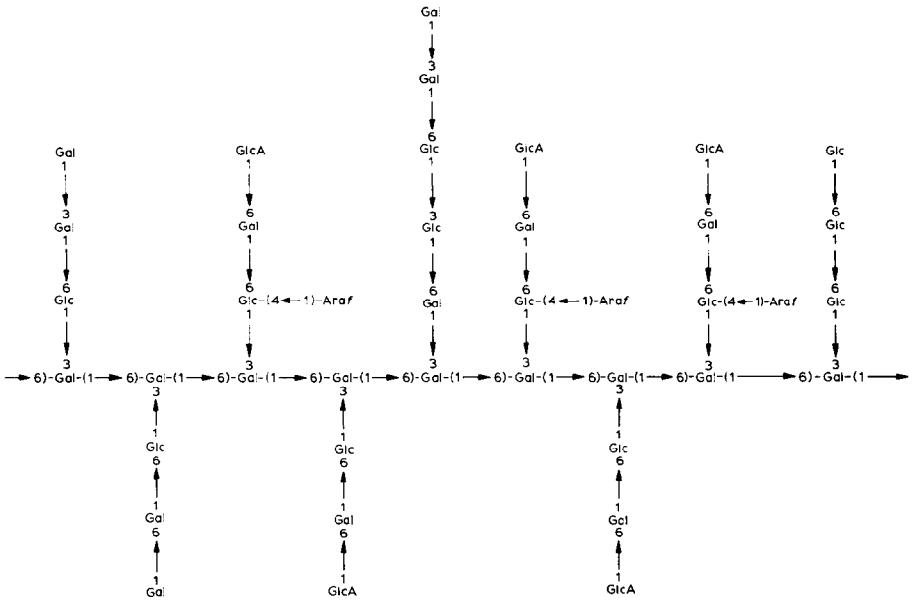

Fig. 1. Structure of the degraded polysaccharide PS-AII of Mahua flowers.

Fraction 1 {4.0 mg; R_{Gal} 0.61 (solvent *D*); $[\alpha]_D^{23}$ +48° (*c* 0.37, water); lit.[12] +53°} contained galactose and arabinose in the mol ratio of 1:1, as estimated by g.l.c. On reduction with $NaBH_4$ followed by hydrolysis and g.l.c. examination, arabinose was detected as the reducing end. Fully methylated product, on hydrolysis and g.l.c. analysis, gave peaks corresponding to 2,3,4,6-tetra-*O*-methylgalactose and 2,5-di-*O*-methylarabinose in almost equal proportions. Hence, Fraction 1 has the structure Gal-(1→3)-Ara*f*.

Fraction 2 {3.3 mg; R_{Gal} 0.42 (solvent *D*); $[\alpha]_D^{23}$ +10° (*c* 0.3, water)} contained arabinose and glucose as constituent sugars in the mol ratio of 1.8:1. After reduction of the material with $NaBH_4$, and the usual treatment, glucose was detected as the reducing unit. On complete methylation, followed by hydrolysis, g.l.c. analysis indicated 2,3,5-tri- (1 mol) and 2,5-di-*O*-methylarabinose (0.8 mol), and 2,3,4-tri-*O*-methylglucose (1.0 mol). Thus, Fraction 2 has the structure Ara*f*-(1→3)-Ara*f*-(1→6)-Glc.

Fraction 3 {6.0 mg; R_{GlcA} 0.36 (solvent *D*); $[\alpha]_D^{23}$ +18° (*c* 0.5, water)} contained galactose, glucose, and glucuronic acid in almost equal proportions. Glucose occupied the reducing end of the material, as determined by reduction ($NaBH_4$) and hydrolysis as usual. Complete methylation followed by the usual treatment indicated 2,3,4-tri-*O*-methylglucose (1 mol), and 2,3,4-tri-*O*-methylgalactose (0.97 mol). On reduction with $LiAlH_4$ and subsequent analysis, the methylated material gave 2,3,4-tri-*O*-methylglucose (1.9 mol) and 2,3,4-tri-*O*-methylgalactose (1.0 mol). Based on these results, Fraction 3 has been assigned the structure GlcA-(1→6)-Gal-(1→6)-Glc.

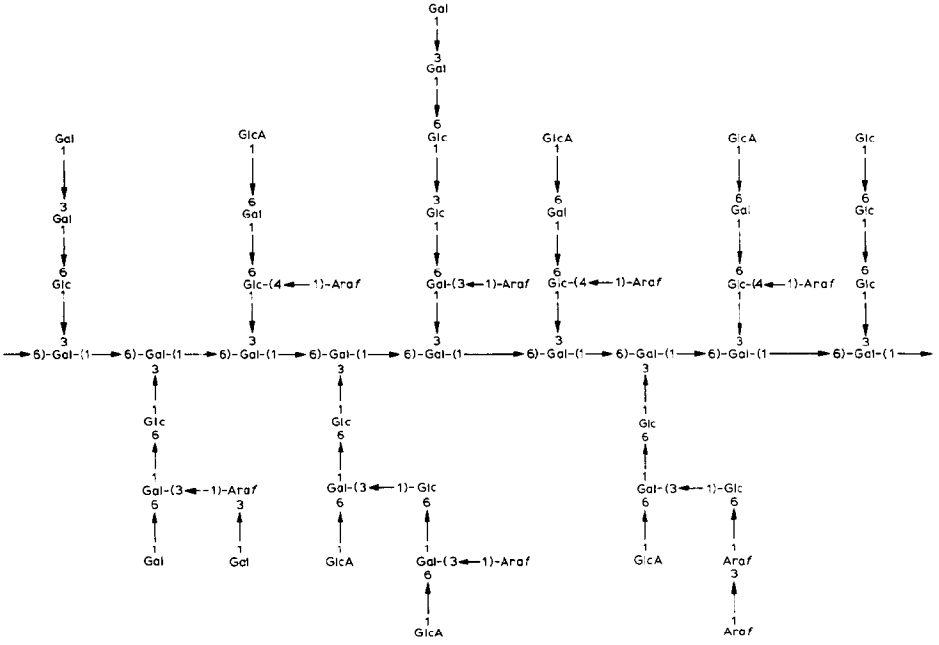

Fig. 2. Structure of the polysaccharide PS-AII of Mahua flowers.

In order to ascertain the anomeric configurations of the different sugar residues, the acetylated derivatives of carboxyl-reduced, degraded PS-AII and of whole PS-AII were subjected to chromium trioxide oxidation[11]. The results are shown in Table II. The rapid disappearance of galactose and glucose, both in carboxyl-reduced, degraded PS-AII and whole PS-AII, indicates that galactose, glucose, and glucuronic acid in the polysaccharide are β-linked. However, the rate at which the arabinose was autohydrolyzed suggested that it possibly had the α configuration in the macromolecules.

From the structure of the degraded PS-AII, depicted in Fig. 1, coupled with the structure of the oligosaccharides obtained during the preparation of the degraded polysaccharide (together with the other analytical results mentioned), one of the plausible structures that can be assigned to the repeating unit of the whole polysaccharide is that given in Fig. 2. This structure merely represents the general nature of the linkages of the different fragmentation products obtained, and it does not necessarily indicate the true sequence of the branches present in the Mahua polysaccharide PS-AII.

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