*Carbohydrate Research*, 191 (1989) 321–332
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands



# STRUCTURE OF WATER-SOLUBLE ACIDIC POLYSACCHARIDES ISOLATED FROM THE BARK OF *Ceiba pentandra* var. indica

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(Received December 10th, 1988; accepted for publication, March 1st, 1989)

ABSTRACT

The water-soluble polysaccharide fraction, isolated from the bark of *C. pentandra* var. indica by delignification, contained L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, D-glucuronic acid, and D-galacturonic acid. Fractionation using Cetavlon and ethanol gave two acidic polysaccharides, each containing L-rhamnose, D-glucuronic acid, and D-galacturonic acid with mol. wts. of $1.318 \times 10^6$ and $1.445 \times 10^5$. On the basis of the results of methylation analysis of the native and carboxyl-reduced polysaccharides, partial hydrolysis, oxidation with periodate and chromium trioxide, and $^1$H-n.m.r. spectroscopy, the two acidic polysaccharides were concluded to have the following repeating unit.

$$\begin{array}{l} \rightarrow 4)\text{-}\alpha\text{-D-Gal}p\text{A-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow \\ \qquad\qquad\quad 3 \\ \qquad\qquad\quad \uparrow \\ \qquad\qquad\quad 1 \\ \qquad\quad \beta\text{-D-Glc}p\text{A} \end{array}$$

## INTRODUCTION

The Kapok tree, *Ceiba pentandra* var. indica, which is a South-East Asian variety of *C. pentandra* Linn., is widely distributed in India and has extensive industrial and medicinal applications[1]. The bark of the tree is a rich source of a hitherto unstudied mucilaginous substance which consists mainly of several polysaccharide components. Because of the economic importance of the Kapok tree and recent reports on the mucilaginous polysaccharides from commercially important plant barks[2], we have studied the polysaccharides present in the bark of *C. pentandra* var. indica and now report the isolation, purification, and structure of two water-soluble acidic polysaccharides.

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0008-6215/89/$ 03.50 

RESULTS AND DISCUSSION

*Isolation and purification.* — The extractive-free bark powder of *C. pentandra* var. indica, on delignification[3], gave an insoluble residue and an aqueous extract from which a mucilaginous fraction with a carbohydrate content of 82% was isolated in 24% yield. This polysaccharide fraction contained 9.12% of protein and was composed of L-rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, D-glucuronic acid, and D-galacturonic acid together with *O*-acetyl (0.02%), sulfate (0.02%), phosphate (0.021%), lignin (0.5%), and ash (4.5%).

Treatment[4] of the polysaccharide fraction with Cetavlon afforded an insoluble (WSCP) and a soluble (WSCNP) fraction in yields of 72% and 16%, respectively. WSCP contained L-rhamnose, D-glucuronic acid, D-galacturonic acid, minor proportions of D-xylose, L-arabinose, D-glucose, and D-galactose, and 0.25% of protein, whereas WSCNP contained D-xylose, L-arabinose, D-glucose, D-galactose, minor proportions of L-rhamnose, D-glucuronic acid, and D-galacturonic acid, and 8.5% of protein. Analysis of the neutral sugars, after hydrolysis, by g.l.c. of their alditol acetates, indicated L-arabinose, D-xylose, D-glucose, and D-galactose to be present in each fraction in the molar ratios 3:0.5:1:1.5, which indicated that the minor sugar components (L-arabinose, D-xylose, D-glucose, and D-galactose) of WSCP were co-precipitated impurities of WSCNP. Likewise, the minor sugar components (L-rhamnose, D-glucuronic acid, and D-galacturonic acid) of WSCNP were associated with impurities derived from WSCP.

Addition of 1 and 3 vol. of ethanol[5], respectively, to an aqueous 1% solution of WSCP gave fractions WSCP-I and WSCP-II, respectively, each of which contained L-rhamnose, D-glucuronic acid, D-galacturonic acid, and minor proportions of D-xylose, L-arabinose, D-glucose, and D-galactose.

Chromatography[6] of WSCP-I on DEAE-cellulose gave a neutral fraction eluted with water, which contained D-galactose, D-glucose, L-arabinose, and D-xylose in the molar ratios 1.5:0.5:1:1. Elution with a linear gradient of sodium chloride then gave major and minor acidic fractions, eluted with ~0.5M and ~0.2M sodium chloride, respectively (see Fig. 1), each of which contained L-rhamnose, D-glucuronic acid, and D-galacturonic acid. Similar chromatography of WSCP-II gave a neutral fraction which contained D-galactose, D-glucose, L-arabinose, and D-xylose in the molar ratios 1.5:0.5:3:1, and major and minor acidic fractions eluted with ~0.2M and ~0.5M sodium chloride, respectively (see Fig. 1), each of which contained L-rhamnose, D-glucuronic acid, and D-galacturonic acid.

Thus, the neutral fractions of WSCP-I and WSCP-II had compositions which were similar, and were most probably contaminants derived from WSCNP. The minor acidic fraction of WSCP-I and the major acidic fraction of WSCP-II were eluted with ~0.2M sodium chloride and had similar compositions, suggesting that the former was most probably a co-precipitated contaminant of the latter. This conclusion is not surprising since there is a greater possibility of co-precipitation of a polysaccharide during graded precipitation with ethanol. The minor acidic com-

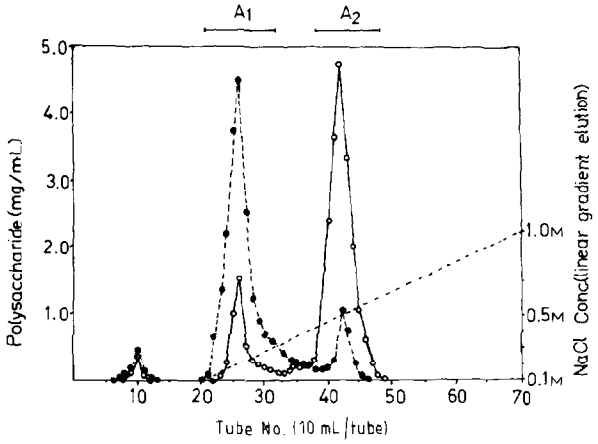



Fig. 1. Chromatography of WSCP-I (—○—) and WSCP-II (--●--) on DEAE-cellulose.

ponent of WSCP-II is most probably a contaminant derived from WSCP-I since they were eluted with ~0.5M sodium chloride and had similar compositions. Only the major acidic fractions of WSCP-I and WSCP-II were studied further.

WSCP-I was eluted as a single peak from Sephadex G-200 at the void volume, whereas WSCP-II was eluted as a single symmetrical peak at a slightly included volume, indicating different values of d.p. WSCP-I and WSCP-II were each eluted from Bio-GelA-15m, calibrated with standard dextrans[7], as a single symmetrical peak at elution volumes corresponding to mol. wts. of ~$1.318 \times 10^6$ and ~$1.445 \times 10^5$, thus confirming that they had different molecular sizes. The compositions of these polysaccharides were unchanged after elution from Sephadex G-200 and Bio-GelA-15m, indicating the absence of heterogeneity. The polysaccharides also gave single symmetrical peaks on sedimentation analysis[8]. They were sharply precipitated from aqueous solutions by barium hydroxide, calcium chloride, and Cetavlon, and their compositions remained unaltered even after several precipitations.

*Methylation analysis.* — Carboxyl reduction[9] of WSCP-I and WSCP-II gave products that contained L-rhamnose, D-glucose, and D-galactose in equimolar proportions.

Methylation analysis of the carboxyl-reduced polysaccharides afforded derivatives of 3,4-di-*O*-methyl-L-rhamnose, 2,3,4,6-tetra-*O*-methyl-D-glucose, and 2,6-di-*O*-methyl-D-galactose in almost equimolar proportions (see Table I). The formation of 3,4-di-*O*-methyl-L-rhamnose indicated that the L-rhamnosyl residues in WSCP-I and WSCP-II carried substituents at O-2. The formation of 2,6-di-*O*-methyl-D-galactose and 2,3,4,6-tetra-*O*-methyl-D-glucose indicated that the D-galactose residues were substituted at O-3 and O-4, and that all the D-glucose residues were present as non-reducing end groups.

WSCP-I and WSCP-II were methylated by the Hakomori[10], Haworth[11], Kuhn

TABLE I

METHYLATION ANALYSIS DATA OF CARBOXYL-REDUCED WSCP-I AND WSCP-II

| *Polysaccharide* | *Alditol acetate of* | T[a] *on* | | | *Average molar proportion* | *Characteristic mass fragments* (m/z) | *Mode of linkage* |
|---|---|---|---|---|---|---|---|
| | | *OV-225* | *SE-54* | *SP-2330* | | | |
| WSCP-I | 3,4-$Me_2$-Rha[b] | 0.97 | 0.84 | 0.90 | 1.23 | 189, 131, 129, 99, 89 | →2)-L-Rha*p*-(1→ |
| | 2,3,4,6-$Me_4$-Glc | 1.00 | 1.00 | 1.00 | 1.15 | 205, 173, 161, 145, 129, 117, 113, 101, 87 | D-Glc*p*-(1→ |
| | 2,6-$Me_2$-Gal | 1.53 | 1.79 | 2.53 | 1.14 | 305, 261, 245, 203, 189, 185, 129, 117, 97, 87 | →3,4)-D-Gal*p*-(1→ |
| WSCP-II | 3,4-$Me_3$-Rha | 0.97 | 0.83 | 0.90 | 1.13 | 189, 131, 129, 99, 89 | →2)-L-Rha*p*-(1→ |
| | 2,3,4,6-$Me_4$-Glc | 1.00 | 1.00 | 1.00 | 0.98 | 205, 173, 161, 145, 129, 117, 113, 101, 87 | D-Glc*p*-(1→ |
| | 2,6-$Me_2$-Gal | 1.52 | 1.78 | 2.51 | 1.07 | 305, 261, 245, 203, 189, 185, 129, 117, 97, 87 | →3,4)-D-Gal*p*-(1→ |

[a]Retention times relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]3,4-$Me_2$-Rha = 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol, *etc.*

and Roth[12], and Purdie and Irvine[13] methods in succession. Hydrolysis of the products, then removal of hexuronic acid derivatives with an anion-exchange resin, followed by g.l.c. and g.l.c.–m.s.[14] of the neutral sugars as the alditol acetate derivatives revealed only the derivative of 3,4-di-*O*-methyl-L-rhamnose. Hence L-rhamnose, was the only neutral sugar in WSCP-I and WSCP-II.

*Partial hydrolysis.* — Treatment of WSCP-I with 0.5M sulphuric acid at 100° for 30 min afforded, in addition to L-rhamnose, D-glucuronic acid, and D-galacturonic acid, higher acidic saccharides **1–3**, which were isolated by preparative p.c.

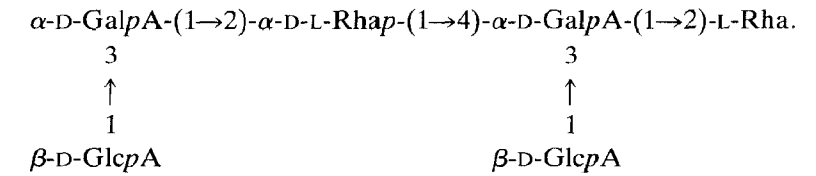
Saccharide **1** ($R_{GlcA}$ 0.81, solvent *G*), on acid hydrolysis, gave D-galacturonic acid and L-rhamnose. Reduction with sodium borohydride followed by acid hydrolysis gave D-galacturonic acid as the only reducing sugar. Treatment of **1** with methanolic 2% hydrogen chloride, followed by sodium borohydride, and acid hydrolysis of the product gave D-galactose and L-rhamnose in equimolar proportions, indicative of a disaccharide structure with L-rhamnose as the reducing unit. Methylation analysis of the methyl glycoside of the carboxyl-reduced oligosaccharide yielded derivatives of 2,3,4,6-tetra-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose in equal proportions. Further, the mass spectrum of the methylated alditol[15] derivative of **1** contained, *inter alia*, fragments with *m/z* 169 ($aA_3$), 201 ($aA_2$), 205 ($aldJ_2$), 233 ($aA_1$), and 265 ($aldJ_1$) confirming the disaccharide structure. The $[\alpha]_D$ value (+93.5°) of **1** was in good agreement with the literature value[16] (+93.2°), suggesting the linkage to be $\alpha$. Hence, **1** was α-D-Gal*p*A-(1→2)-L-Rha.

On acid hydrolysis, **2** ($R_{GlcA}$ 0.6, solvent *G*) gave D-galacturonic acid, D-glucuronic acid, and L-rhamnose together with **1**. When the methyl ester methyl glycoside of **2** was reduced with sodium borohydride, the product contained D-galactose, D-glucose, and L-rhamnose in almost equimolar proportions. When **2** was reduced with sodium borohydride, treated with methanolic 2% hydrogen chloride, and reduced again with sodium borohydride, acid hydrolysis of the product gave D-galactose and D-glucose in the molar ratio 1:1 as the only reducing sugars, indicating **2** to be a trisaccharide. Methylation analysis of the carboxyl-reduced methyl glycoside of **2** afforded derivatives of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-L-rhamnose in the molar ratios 1:1:1. The mass spectrum of the methylated alditol[15] of **2** contained, *inter alia*, fragments with *m/z* 169 ($aA_3$), 201 ($aA_2$), 205 ($aldJ_2$), 233 ($aA_1$), 265 ($aldJ_1$), 391 ($baldJ_3$), 419 ($abA_2$), 423 ($baldJ_2$), 451 ($abA_1$), and 483 ($baldJ_1$) confirming the trisaccharide structure. The $[\alpha]_D$ value (+84.5°) of **2** was in good agreement with the literature value[16] (+85.4°). Thus, **2** was

β-D-Glc*p*A-(1→3)-α-D-Gal*p*A-(1→2)-L-Rha.

Saccharide **3** ($R_{GlcA}$ 0.25, solvent *G*), on acid hydrolysis, gave D-galacturonic acid, D-glucuronic acid, and L-rhamnose together with an appreciable amount of **1**.

Treatment of **3** with methanolic 2% hydrogen chloride followed by reduction with sodium borohydride gave a product which contained D-galactose, D-glucose, and L-rhamnose in the molar ratios 1:1:1. Reduction of **3** with sodium borohydride, followed by esterification, further reduction with sodium borohydride, and acid hydrolysis gave D-galactose, D-glucose, and L-rhamnose in the molar ratios 1:1:0.5, indicating a hexasaccharide structure. The carboxyl-reduced methyl glycoside of **3**, on methylation analysis, gave derivatives of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-galactose, 2,6-di-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-L-rhamnose in the molar ratios 2:1:1:2 confirming the hexasaccharide structure. On the basis of the above results and the $[\alpha]_D$ value (+82°) of **3** (literature value[16], +82.1°), **3** is proposed to be




The formation of **1–3** suggested that WSCP-I contained the backbone →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→ with all of the α-D-Gal*p*A residues substituted at O-3 with D-Glc*p*A end-groups.

Partial hydrolysis of WSCP-II gave D-galacturonic acid, D-glucuronic acid, L-rhamnose, and **1–3**, which were isolated by preparative p.c. Thus, the structures of WSCP-I and WSCP-II were similar.

*Oxidation experiments.* — Carboxyl-reduced WSCP-I and WSCP-II were each oxidised with chromium trioxide[17,18]. The D-galactose residues were unaffected but ~90% of the D-glucose residues and ~15% of the L-rhamnose residues were oxidised. This suggested that, in WSCP-I and WSCP-II, the D-galacturonic acid and L-rhamnose residues were α and the D-glucuronic acid residues were β.

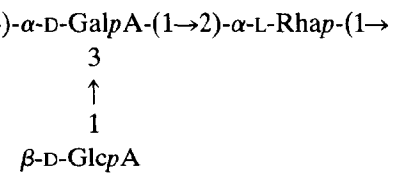
In accord with the above data, the $^1$H-n.m.r. spectrum of WSCP-I contained signals for anomeric protons at δ 4.59 (s), 5.0 (s), and 5.43 (bs), which were assigned to β-D-glucuronic acid, α-L-rhamnose, and α-D-galacturonic acid, respectively. A sharp singlet at δ 1.25 was assigned to Me-5 of L-rhamnose.

On treatment[19] with periodate, WSCP-I and WSCP-II each consumed 1.05 mol of oxidant per "anhydrohexuronosyl" residue, and carboxyl-reduced WSCP-I and WSCP-II each consumed 1.08 mol of oxidant and released 0.33 mol of formic acid per "anhydrohexose" residue. These values accord with the methylation analysis data.

The periodate-oxidised products of the carboxyl-reduced WSCP-I and WSCP-II, on reduction with sodium borohydride[20] followed by acid hydrolysis and g.l.c. of the resulting sugars as their alditol acetates, gave D-galactose as the only unoxidised sugar. The resistance of D-galactose to periodate oxidation is due to

substitution at O-4 and O-3 with L-rhamnose and D-glucose residues, respectively.

Based on the foregoing results, it is concluded that WSCP-I and WSCP-II can be represented by the following structure.



Polysaccharides containing a →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→ backbone have been isolated from the bark of the slippery elm (*Ulmus fulva*)[21], stems of *Opuntia ficus-indica*[22], and from the roots and leaves of some *Althaea*[23], *Abelmoschus*[24], and *Hibiscus*[16] species. However, in these polysaccharides, the types of substituents attached to the backbone are different. For example, in the slippery elm polysaccharide, all the L-rhamnosyl residues carry substituents at O-3 or O-4. Similarly, in the polysaccharides isolated from the *Opuntia ficus-indica* stem[22] and *Althaea officinalis* L. var. rhobusta roots[23], all the L-rhamnosyl residues of the backbone carry substituents at O-4. The structure proposed for WSCP-I and WSCP-II is similar to that reported for the "Hibiscus mucilage $M_0$" and "Abelmoschus mucilage M" isolated from the roots of *Hibiscus moscheutes* L.[16] and *Abelmoschus manihot* medicus[24], respectively.

## EXPERIMENTAL

*General.* —The bark was collected from locally available trees during May–June (in the Manasagangotri Campus, Mysore, India). The bark was dried at 26–27° for a week, then at 40–45° for 24 h, and powdered in a handmill.

Solutions were concentrated under diminished pressure at <40°. Analytical and preparative p.c. was performed by the descending mode on Whatman Nos. 1 and 3MM papers, respectively, using *A*, 1-butanol–benzene–pyridine–water (4:1:3:3, upper layer); *B*, ethyl acetate–pyridine–water (8:2:1); *C*, 1-butanol–pyridine–water (6:4:3); *D*, 1-butanol–ethanol–water (4:1:5, upper layer); *E*, 1-butanol–acetic acid–water (4:1:5, upper layer); *F*, ethyl acetate–pyridine–acetic acid–water (5:5:1:3); *G*, butanol–acetic acid–pyridine–water (4:1:3:3).

Solvents *A–D* were used for neutral sugars, and solvents *E–G* for acidic sugars. Sugars were detected with *p*-anisidine hydrochloride[25] for alkaline silver nitrate[26].

Carbohydrate[27], protein[28], *O*-acetyl[29], phosphate[30], sulfate[31], and ash[32] contents of the polysaccharides were determined by the standard methods. The general methods and analytical procedures employed have been reported[2].

*Isolation of the polysaccharide fraction.* — The bark powder (15 g) was extracted exhaustively, in succession, with light petroleum (b.p. 60–80°), 1:2

benzene–methanol, and acetone to give the extractive-free bark powder (13.2 g) and organic extractable matter (2.2 g).

The bark powder (10 g) was allowed to swell overnight with water (500 mL), stirred for 4 h, delignified[3] at 50°, and then filtered through a linen cloth. The insoluble residue was washed with water, followed by ethanol, and dried (5.5 g). This bark residue was used for further extraction of polysaccharides with alkali.

The filtrate and the washings of the insoluble residue were combined, concentrated, and dialysed, and the polysaccharide (3 g) was recovered from the dialysate by precipitation with ethanol.

*Precipitation with Cetavlon*[4]. — The above polysaccharide fraction (1 g) was suspended in 0.1M sodium hydroxide (100 mL), stirred at ~50° for 30 min, then dialysed, and centrifuged, and the clear solution was diluted with water to 500 mL. Solid sodium sulfate was added to 0.02M, and then aqueous 25% Cetavlon (12.5 mL) was added slowly with stirring. The precipitate was collected by brief centrifugation and dissolved in 4M sodium chloride (100 mL), ethanol (600 mL) was added with stirring, and the precipitate was collected by centrifugation. This step was repeated three times and finally the sodium chloride solution of the polysaccharide was dialysed. From the dialysate, the polysaccharide WSCP (720 mg) was recovered by precipitation with ethanol.

The supernatant solution was extracted with 1-pentanol to remove Cetavlon, concentrated to ~50 mL, dialysed, concentrated to ~20 mL, and lyophilised to give the polysaccharide WSCNP (200 mg).

*Graded precipitation with ethanol*[5]. — To an aqueous solution of WSCP (500 mg in 100 mL) was added 1 vol. of ethanol, and the precipitated polysaccharide WSCP-I (200 mg) was recovered.

The polysaccharide WSCP-II (250 mg) was recovered from the supernatant solution (~200 mL) by adding ethanol (~600 mL).

*Chromatography*[6] *on DEAE-cellulose.* — A solution of WSCP-I (250 mg) in water (7 mL) was applied to a column (2.5 × 20 cm) of DEAE-cellulose (neutral form). The column was washed with water (200 mL) to give unbound material (5 mg), then with a linear gradient of 0.1 → M sodium chloride (250 mL + 250 mL). Fractions (10 mL) were analysed by the phenol–sulfuric acid method[27]. The polysaccharide-containing fractions were combined as shown in Fig. 1 [*i.e.*, fractions 24–28 ($A_1$) and 38–47 ($A_2$) were combined separately], concentrated, and dialysed, and the polysaccharides were recovered by ethanol precipitation. The major (210 mg) and a minor (30 mg) fraction were eluted at ~0.5M and ~0.2M sodium chloride, respectively.

Elution of WSCP-II (500 mg) from a column (2.5 × 20 cm) of DEAE-cellulose, as for WSCP-I, gave a neutral fraction (10 mg), and major (200 mg) and minor (25 mg) acidic fractions, eluted at ~0.2M and ~0.5M sodium chloride, respectively. Further quantities of the polysaccharides were obtained by step-wise elution with water, and 0.2M and 0.5M sodium chloride.

*Gel-permeation chromatography. — (a) On Sephadex G-200.* Separate solu-

tions of WSCP-I and WSCP-II (10 mg of each) in 0.1M sodium chloride were applied to columns (2 × 82 cm) of Sephadex G-200, pre-equilibrated and then eluted with the same solvent. Fractions (10 mL) were monitored by the phenol–sulfuric acid method[27]. WSCP-I was eluted as a single peak at the void volume, whereas WSCP-II was eluted at a slightly included volume.

*(b) On Bio-GelA-15m*[7]. Solutions of WSCP-I and WSCP-II (10 mg of each) in 50mM sodium acetate–acetic acid buffer (pH 4.5) were applied to columns (2.0 × 82.5 cm) of Bio-GelA-15 m and eluted with the same buffer. Fractions (10 mL) were analysed for carbohydrate by the phenol–sulfuric acid method[27]. The column was calibrated with dextrans T-2000, T-500, T-40, and T-10 (Pharmacia).

*Sedimentation analysis*[8]. — The solutions (1%) of WSCP-I and WSCP-II in 0.1M sodium chloride were analysed in a Beckman analytical ultracentrifuge Model E at 25° at 59,780 r.p.m. The movement of the boundary was followed using Schlieren optics.

*Sugar composition.* — The water-soluble polysaccharide fraction (500 mg) was treated with aqueous 72% sulfuric acid at room temperature for 1 h. The solution was diluted with water to 0.5M acid, heated at 100° for 8–10 h, neutralised ($BaCO_3$), filtered, and deionised with Amberlite IR-120($H^+$) and IRA-400-($HCOO^-$) resins. P.c. of the hydrolysate revealed rhamnose, xylose, arabinose, glucose, and galactose. The anion-exchange resin was eluted with M formic acid and the eluate was concentrated. P.c. of the residue revealed glucuronic acid and galacturonic acid. The sugars were isolated by preparative p.c. and the $[\alpha]_D$ values indicated that only the arabinose and rhamnose were L. Purified WSCP-I and WSCP-II (250 mg of each) were each hydrolysed as described above and the resulting sugars were isolated by preparative p.c. Further, both the polysaccharides (100 mg of each) were reduced[9] thrice to give carboxyl-reduced polysaccharides (65 mg from WSCP-I and 70 mg from WSCP-II) which were hydrolysed with 0.25M sulfuric acid for 6–8 h at 100°, and the products were analysed by p.c. or by g.l.c. of their alditol acetates.

*Methylation analysis.* — The carboxyl-reduced products of both WSCP-I and WSCP-II (10 mg of each) were methylated separately (Hakomori procedure[10]), then hydrolysed with aqueous 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 resulting partially methylated sugars were analysed by g.l.c., and g.l.c.–m.s.[14], as their alditol acetates.

WSCP-I and WSCP-II (100 mg of each) were separately methylated, once by the Hakomori procedure[10], twice by the Haworth method[11], twice by the Kuhn and Roth method[12], and thrice by the Purdie and Irvine method[13]. The products (55 and 60 mg from WSCP-I and WSCP-II, respectively), which showed negligible i.r. absorption for hydroxyl groups, were hydrolysed as described above, and the resulting partially methylated neutral sugars were analysed by g.l.c., and g.l.c.–m.s.[14], as the alditol acetates.

*Partial hydrolysis.* — WSCP-I (500 mg) was hydrolysed with 0.5M sulfuric

acid for 30 min at 100°. The hydrolysate was neutralised ($BaCO_3$), filtered, and deionised with Amberlite IR-120($H^+$) and IRA-400($HCOO^-$) resins. P.c. then revealed L-rhamnose only. The Amberlite IRA-400($HCOO^-$) resin was eluted with 2M formic acid and the eluate was concentrated; p.c. (solvent *E*) of the residue revealed D-glucuronic acid, D-galacturonic acid, and higher acidic saccharides of which **1–3** ($R_{GlcA}$ 0.81, 0.6, and 0.25, respectively) were isolated by preparative p.c. (solvent *G*). Their homonogeneity was ascertained by p.c. in solvents *E* and *F*. The combined yields of **1–3** from two experiments were 18, 15, and 17 mg, respectively.

Similar hydrolysis of WSCP-II (500 mg) gave **1–3**, which were identical with the saccharides described above.

*Characterisation of the higher acidic saccharides* **1–3**. — Each saccharide **1–3** was hydrolysed with 0.5M sulfuric acid for 8–10 h at 100° and the products were analysed by p.c. Each saccharide **1–3** was reduced with sodium borohydride. Each alditol product was (*a*) hydrolysed with 0.5M sulfuric acid for 8 to 10 h at 100° and the products were analysed by p.c. for both neutral and acidic sugars; (*b*) converted into the methyl ester by treatment with methanolic 2% hydrogen chloride for 24 h at room temperature, then reduced with sodium borohydride, and hydrolysed with 0.25M sulfuric acid for 6 h at 100°, and the products were analysed by p.c. for neutral sugars; (*c*) methylated (Hakomori procedure[10]), and the product was purified by reversed phase chromatography, using a Sep-pak $C_{18}$ cartridge[33], and analysed by e.i.-m.s.[15].

Each saccharide **1–3** was treated separately with methanolic 2% hydrogen chloride at room temperature for 24 h and the resulting methyl ester methyl glycosides were carboxyl-reduced with sodium borohydride. The products were (*a*) hydrolysed with 0.25M sulfuric acid for 6 h at 100° and the products were analysed by p.c.; (*b*) methylated (Hakomori procedure[10]), and then hydrolysed with 0.25M sulfuric acid for 5–6 h at 100°, and the resulting partially methylated sugars were converted into the alditol acetates and analysed by g.l.c. and g.l.c.–m.s.[14].

*Oxidations.* — *(a) With chromium trioxide.* Carboxyl-reduced WSCP-I and WSCP-II (10 mg of each) were each acetylated[17] thrice. Solutions of the products in glacial acetic acid (4 mL) were stirred with chromium trioxide[18] (400 mg) at 50°. Aliquots were withdrawn at intervals, diluted with water (10 mL), and extracted with chloroform (5 × 5 mL). The combined extracts were washed with water (3 × 10 mL), treated with methanolic 0.2M sodium methoxide, deionised with Amberlite IR-120 ($H^+$) resin, and concentrated. The residues were hydrolysed, and the resulting sugars were converted into their alditol acetates and analysed by g.l.c.

*(b) With periodate.* WSCP-I and WSCP-II (100 mg of each) were oxidised separately with 50mM sodium periodate[19] (100 mL) at room temperature in the dark. Periodate consumption was monitored by titration of aliquots with sodium thiosulfate. After 72 h, the remaining solutions were treated with 1,2-ethanediol[20] (0.5 mL), dialysed, and reduced with sodium borohydride. The products were hydrolysed with 0.5M sulfuric acid for 8–10 h at 100° and the hydrolysates were examined by p.c.

Carboxyl-reduced WSCP-I and WSCP-II (50 mg of each) were oxidised separately with 50mM periodate (50 mL) for 90 h at room temperature in the dark. The periodate consumption, formic acid liberated, and sugar compositions of the oxidised products were determined.

ACKNOWLEDGMENTS

We thank Professors L. Kenne (Stockholm University, Sweden), P. Albersheim and A. G. Darvill (Georgia University, Athens, U.S.A.) for the g.l.c. and g.l.c.–m.s. analyses, Dr. V. Prakash (C.F.T.R.I., Mysore) for the sedimentation analysis, and the scientists of the Instrumentation Division, C.D.R.I. (Lucknow) for the e.i.-m.s. and g.l.c. analyses. One of us (T.S.R.) thanks the C.S.I.R. (New Delhi, India) for the award of a research fellowship.

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