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STRUCTURAL INVESTIGATIONS ON THE MUCILAGINOUS POLYSACCHARIDES ISOLATED FROM BARK OF THE AVOCADO TREE (*Persea americana* MILL)

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ABSTRACT

Two arabinoxylans, a water-extractable (molecular weight ~600,000) composed of L-arabinose and D-xylose in the molar ratio of 3:1, and an alkali-extractable (molecular weight ~1,300,000) composed of D-xylose (95%) and L-arabinose (5%), have been isolated from the bark of *Persea americana* Mill. For structural elucidation, the purified polysaccharides were subjected to methylation analysis, partial hydrolysis, periodate oxidation, and Smith degradation. The results suggested that the water-extractable arabinoxylan has a highly branched structure consisting of a (1→4)-linked, β-D-xylan backbone in which each of the D-xylopyranosyl residues is substituted at O-2 and O-3 with L-arabinofuranosyl-(1→3)-L-arabinofuranosyl and L-arabinofuranosyl groups, respectively, whereas the alkali-extractable arabinoxylan is essentially a linear, (1→4)-linked β-D-xylan, with limited branches at O-2 or O-3 consisting of L-arabinofuranosyl groups.

INTRODUCTION

The avocado pear tree, *Persea americana* Mill, is native to Central America, the Canary Islands, and South-East Asia. It is cultivated in South India[1] for its fruit, "the avocado". We have observed that the bark, as well as the primary wood of the tree, contains mucilaginous substances consisting mainly of arabinoxylans. Because of the economic importance of the avocado pear tree, and recent reports on novel, highly branched arabinoxylans from the barks of various trees[2–4], we have undertaken a detailed chemical characterization of polysaccharides present in the bark of *P. americana* Mill. Here, we report structural characterization of two arabinoxylans isolated from the bark of *P. americana*.

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RESULTS AND DISCUSSION

The mucilaginous polysaccharide (2.5% by dry weight) isolated from the aqueous extract of the bark of *P. americana* contained 80% of carbohydrate (see Table I) which was composed of arabinose, xylose, and traces of glucose and galactose; uronic acids were absent. The alkali-extractable polysaccharide obtained from the aqueous sodium hydroxide extract of the bark residue also contained 80% of carbohydrate (see Table I) which was composed of xylose (major) and arabinose; uronic acids were absent. The arabinose and xylose present in these polysaccharide fractions were found to be L and D, respectively.

In gel-permeation chromatography on a column of Bio-Gel A-15m calibrated with standard dextrans, the water-extracted polysaccharide was eluted as a broad asymmetrical peak, with a gradual rise in the leading edge, at an elution volume corresponding to a molecular weight of ~600,000. On similar chromatography, the alkali-extracted polysaccharide was eluted as a major symmetrical peak corresponding to a molecular weight of ~1,300,000, with a minor peak at ~600,000 which probably corresponded to contaminated water-extractable polysaccharide. The chemical compositions of the polysaccharides after elution from the Bio-Gel A-15m column were indistinguishable from those (see Table I) before gel chromatography, suggesting that the protein and the lignin associated with these polysaccharides were covalently bound. Mild treatment with sodium chlorite[5] resulted in the complete removal of lignin, and partial removal of protein, in both of the polysaccharides (see Table I).

Fractionation of the water-extracted and delignified polysaccharide with Fehling solution[6] gave an insoluble, polysaccharide complex (7.5%) which contained D-xylose (major), L-arabinose, and glucose, and a soluble polysaccharide (85%) composed of L-arabinose and D-xylose in the molar ratio of 3:1. The latter polysaccharide (designated water-extractable arabinoxylan) gave a single peak in

TABLE I

CHEMICAL COMPOSITION OF POLYSACCHARIDES ISOLATED FROM THE BARK OF *Persea americana* (PERCENT BY WEIGHT)

Component	*Water-extracted polysaccharide*		*Alkali-extracted polysaccharide*	
	Native	*After delignification*	*Native*	*After delignification*
Carbohydrate	79.83	94.92	80.26	95.26
Protein	9.33	4.21	6.21	5.20
Lignin	8.23		11.23	
O-Acetyl	0.03	0.02		
Phosphate				
Sulfate				
Ash	5.02	3.13	4.23	4.26

sedimentation analysis[7]; further fractionation with Fehling solution[6] or barium hydroxide[8] failed to give any insoluble complex, and the sugar compositions of fractions obtained by graded precipitation with ethanol were similar. Furthermore, the purified polysaccharide was eluted as a single, symmetrical peak from Bio-Gel A-15m. These data indicated the absence of heterogeneity in the polysaccharide.

Similar fractionation of the alkali-extracted polysaccharide, using Fehling solution, gave, as an insoluble, copper complex, a major portion of the polysaccharide, which was composed of D-xylose (95%) and L-arabinose (5%). The purified polysaccharide (designated alkali-extractable arabinoxylan) gave a single peak in sedimentation analysis, and was eluted as a single, symmetrical peak from Bio-Gel A-15m, and its sugar composition was unchanged on reprecipitation with Fehling solution or on graded precipitation with ethanol, suggesting that it was a single species.

Structure of the water-extractable arabinoxylan. — The positions of various glycosidic bonds in the purified, water-extractable arabinoxylan were established by methylation analysis[9,10] (see Table II). D-Xylose was obtained as the unmethylated sugar, indicating that it constitutes a heavily substituted, D-xylan backbone of the polysaccharide. L-Arabinose was converted into 2,3,5-tri-*O*-methyl and 2,5-di-*O*-methyl derivatives, suggesting that it was present exclusively in the furanoid form. The relative proportions of these methylated sugars indicated that ~66% of the L-arabinofuranosyl units were present as nonreducing terminal groups, 50% of which were directly attached to the D-xylan backbone at O-3 (see Smith degradation) and the remaining 50% linked to O-3 of the inner L-arabinofuranosyl residues which in turn were attached to O-2 of the D-xylan backbone.

Mild hydrolysis of the water-extractable arabinoxylan with acid released a major portion of the L-arabinofuranosyl residues as the free sugar, and gave a degraded polysaccharide (30%) which had $[\alpha]_D$ −101° and was composed of L-arabinose and D-xylose in the molar ratio of 1:4. Partial acid hydrolysis[2] of the degraded polysaccharide, and p.c. analysis of the hydrolyzate showed, in addition to free L-arabinose and D-xylose, the presence of oligosaccharides with R_{Glc} 0.72,

TABLE II

METHYLATION ANALYSIS DATA FOR THE WATER-EXTRACTABLE ARABINOXYLAN (*A*) AND ITS SMITH-DEGRADATION PRODUCT (*B*), AND THE ALKALI-EXTRACTABLE ARABINOXYLAN (*C*)

Alditol acetates from	*Molar proportion (%)*			*Mode of linkage*
	A	*B*	*C*	
2,3,5-Tri-*O*-methylarabinose	49	32	3.9	L-Ara*f*-(1→
2,5-Di-*O*-methylarabinose	27			→3)-L-Ara*f*-(1→
2,3-Di-*O*-methylxylose		31	92.5	→4)-D-Xyl*p*-(1→
3-*O*-Methylxylose		37	3.6	→2,4)-D-Xyl*p*-(1→
2-*O*-Methylxylose				→3,4)-D-Xyl*p*-(1→
Xylose	24			→2,3,4-D-Xyl*p*-(1→

0.31, 0.11, and 0.06 which were isolated homogeneous by preparative p.c. Based on their specific rotations[11], sugar compositions before and after borohydride reduction, methylation analysis, and e.i.-m.s. of their permethylated alditols, the oligosaccharides were shown to be β-(1→4)-linked xylobiose, xylotriose, xylotetraose, and xylopentaose. These results confirmed the presence of a (1→4)-linked β-D-xylan backbone in the water-extractable arabinoxylan.

On periodate oxidation[12], the water-extractable arabinoxylan consumed 0.53 mole of the oxidant per mole of pentosyl residue. Borohydride reduction and acid hydrolysis of the oxidized product gave glycerol, L-arabinose, and D-xylose in the molar ratios of ~2:1:1. These results were in agreement with the methylation analysis data for the polysaccharide.

To elucidate the nature of side chains and their mode of linkage to the D-xylan backbone in the water-extractable arabinoxylan, it was subjected to Smith degradation[13], and the degraded polymer was analyzed for its structure. This procedure[13] resulted in the release of glycerol and a significant proportion of L-arabinose, and yielded a Smith-degraded polysaccharide (39%) consisting of D-xylose and L-arabinose in the molar ratio of 1.8:1.0.

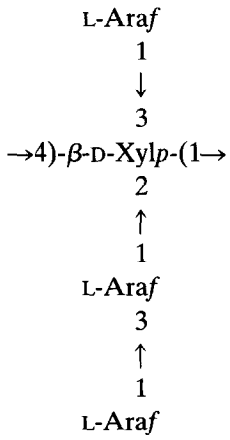
On permethylation, acid hydrolysis, and g.l.c. and g.l.c.–m.s. analysis of the released sugars, as their alditol acetates, the Smith-degraded product gave the results presented in Table II. L-Arabinose was released exclusively as the 2,3,5-tri-*O*-methylated sugar, suggesting that all of the O-3-substituted inner L-arabinofuranosyl residues present in the native polysaccharide had become nonreducing end-groups after Smith degradation, and that these residues were attached directly to the D-xylan backbone, *i.e.*, the side chains containing O-3 substituted L-arabinose residues were present as L-arabinofuranosyl-(1→3)-L-arabinofuranosyl units. The occurrence of 3-*O*-methylxylose indicated that the side-chain substituents attached to O-3 and O-2 of the D-xylan backbone were (nonreducing) L-arabinofuranosyl and L-arabinofuranosyl-(1→3)-L-arabinofuranosyl groups, respectively. 2,3-Di-*O*-methylxylose had arisen because a significant proportion of O-3-substituted L-arabinofuranosyl residues (which resisted periodate oxidation and were exposed as end-groups during Smith degradation) were hydrolyzed under the acid hydrolysis conditions employed, thus resulting in the exposure of both 2- and 3-hydroxyl groups in some of the D-xylosyl residues of the D-xylan backbone.

Partial hydrolysis of the Smith-degraded polysaccharide gave, in addition to L-arabinose and D-xylose, D-xylobiose, D-xylotriose, D-xylotetraose, and higher oligosaccharides, suggesting that the D-xylan backbone was intact even after the Smith degradation.

Periodate oxidation of the Smith-degraded product followed by borohydride reduction, acid hydrolysis, and p.c. examination indicated the presence of glycerol and D-xylose in approximately equal proportion. Partial hydrolysis of the Smith-degraded and periodate-oxidized product released xylose, xylobiose, xylotriose, and traces of xylotetraose, demonstrating that a substantial proportion of the D-xylan backbone in the water-extractable arabinoxylan had resisted the second

periodate oxidation. These results accord with the methylation analysis data for the Smith-degraded polysaccharide.

Based on the results of these studies, it was proposed that the water-extractable arabinoxylan is built up from a repeating unit having the following structure.



Structure of the alkali-extractable arabinoxylan. —Methylation analysis of the alkali-extractable arabinoxylan (see Table II) gave mainly 2,3-di-*O*-methylxylose together with smaller proportions of 2,3,5-tri-*O*-methylarabinose and 2-*O*- and 3-*O*-methylxylose suggesting that it contained a linear, (1→4)-linked D-xylan having a few L-arabinofuranosyl groups attached at O-2 or O-3.

Partial hydrolysis[2] of the alkali-extractable arabinoxylan also furnished (1→4)-linked xylobiose, xylotriose, xylotetraose, and xylopentaose which had chromatographic mobilities identical to those of the corresponding oligosaccharides formed in the case of the degraded polysaccharide from the water-extractable arabinoxylan. This result, together with the methylation analysis data, suggested that the alkali-extractable polysaccharide was essentially a (1→4)-linked β-D-xylan having a limited number of branches consisting of L-arabinofuranosyl groups attached at O-2 or O-3.

On periodate oxidation[12], the alkali-extractable arabinoxylan consumed 1.01 moles of the oxidant per mole of pentosyl residue. The oxidized product, after borohydride reduction and acid hydrolysis, gave glycerol (major) and D-xylose. These results were in agreement with the methylation analysis data for the polysaccharide.

The results of the foregoing analyses suggested that the alkali-extractable arabinoxylan is essentially a linear, (1→4)-linked β-D-xylan having a limited number of branches consisting of L-arabinofuranosyl end-groups at O-2 or O-3.

Conclusion. — Highly branched arabinoxylans containing a (1→4)-linked β-D-xylan backbone on which L-arabinofuranosyl groups are substituted at both O-2

and O-3 have been reported recently from the bark of *Cinnamomum iners*[2], *C. zeylanicum*[3], *Persea macrantha*[4], and the leaves of *Litsea polyantha*[14,15] and *Neolitsea cassia*[16]. In the bark arabinoxylans[2–4], the side-chain substituents are both (nonreducing) L-arabinofuranosyl and α-D-xylopyranosyl groups, whereas in the arabinoxylans from the leaves[14–16], the latter are absent. Furthermore, it has been reported that an arabinoxylan isolated from *Litsea polyantha* contains side chains consisting of 3-*O*-substituted L-arabinofuranosyl residues and nonreducing L-arabinofuranosyl end-groups attached to the D-xylan backbone at O-3 and O-2, respectively, whereas in the arabinoxylan from *Neolitsea cassia*[16], similar side-chains are attached to the D-xylan backbone at O-2 and O-3, respectively. The structural features reported here for the water-extractable arabinoxylan isolated from the bark of *Persea americana* bears a close resemblance to that reported for the arabinoxylan from the leaves of *Neolitsea cassia*[16]. Furthermore, it is interesting that the alkali-extractable polysaccharide fractions from the barks of *C. iners*[2], *C. zeylanicum*[3], and *P. macrantha*[4] contain mainly α-D-glucans, whereas the corresponding fraction from *P. americana*, reported here, contains mainly a β-D-xylan having a few arabinose residues.

EXPERIMENTAL

General. — The bark was collected from young stems of the tree *Persea americana* grown in the Botanical Gardens of the University of Mysore, Mysore, cut into pieces, dried in an oven by circulation of warm air at 50°, and ground to a fine powder in a plate mill.

Whatman Nos. 1 and 3MM papers were used for analytical and preparative chromatography, respectively, with the following solvents (v/v): *A*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer), *B*, 1-butanol–acetic acid–water (4:1:5, upper layer), and *C*, 6:4:3 1-butanol–pyridine–water.

The carbohydrate[17], lignin[18], protein[19], *O*-acetyl group[20], phosphate[21], sulfate[22], and ash[23] contents of the polysaccharides were determined by the standard methods. The general methods and analytical procedures employed have been reported[2–4].

Isolation of polysaccharides. — The bark powder (20 g) was exhaustively extracted in a Soxhlet apparatus, first with 1:2 benzene–methanol, and then with acetone, giving extractive-free bark-powder, 17.5 g, and organic, extractable matter, 2.2 g.

The bark powder (10 g) was allowed to swell overnight with water (100 mL), stirred for 4 h, and the suspension centrifuged. The residue was further extracted with water (3 × 100 mL). The combined aqueous solution was extracted with 4:1 chloroform–1-butanol, to remove proteins, and ethanol (4 vol.) was added. The pale-brown precipitate (300 mg) thus formed was collected. For the removal of lignin, the crude polysaccharide was dissolved in water (50 mL) containing glacial acetic acid (0.15 mL). The solution was heated to 50°, while being stirred, and

sodium chlorite (1.5 g) was added in three portions at 5-min intervals. After stirring for a further 10 min, the solution was cooled, dialyzed, and lyophilized. The polysaccharide was obtained as a white powder (260 mg).

The bark residue was further treated with aqueous, 5% sodium hydroxide (3 × 100 mL); the solution was partitioned with 4:1 chloroform–methanol, and ethanol (4 vol.) was added. The precipitate (1.15 g) was collected, dissolved in water (50 mL), dialyzed, delignified as before and lyophilized. The polysaccharide was obtained as a white powder (800 mg).

Gel-permeation chromatography. — The polysaccharide fractions (25 to 30 mg each), before and after delignification, were dissolved in 50mM sodium acetate–acetic acid buffer, pH 4.5, applied to a column (2 × 85 cm) of Bio-Gel A-15m, and eluted with the same buffer. Fractions (5 mL) were collected, and aliquots were analysed for carbohydrate by the phenol–sulfuric acid method[17]. The column was calibrated with dextrans T-2000, T-500, T-40, and T-10 (Pharmacia, Uppsala, Sweden).

Sugar composition. — The polysaccharide fractions (300 mg of each) were separately hydrolyzed with 0.25M sulfuric acid for 8 h at 100°, the acid neutralized with barium carbonate, the suspension filtered, and the filtrate de-ionized by using Dowex 50W-X8 (H^+) and Dowex 2-X8 ($HCOO^-$) resins. P.c. examination of the hydrolyzate showed that the water-extractable polysaccharide contained mainly arabinose and xylose, with traces of glucose and galactose, whereas the alkali-extractable polysaccharide was composed of xylose (major) and arabinose. In each case, the anion-exchange resin was eluted with M formic acid, and the eluate evaporated, and examined by p.c., which indicated the absence of acidic sugars from both polysaccharides. Arabinose and xylose were isolated in pure form by preparative p.c. using solvent *A*, and the specific rotations indicated that the sugars respectively had the L and D configuration.

Purification of the polysaccharides. — The water-extracted polysaccharide (200 mg) was dissolved in aqueous, 5% sodium hydroxide (80 mL), Fehling solution (20 mL) was added, and the mixture was kept for 30 min. The precipitate (~15 mg) was removed, and the supernatant liquor was cooled in ice, acidified with cold M hydrochloric acid, and the polysaccharide (170 mg) precipitated with ethanol (4 vol.); this was designated water-extractable arabinoxylan. The arabinoxylan (5 mg) was hydrolyzed, and g.l.c. analysis of the released sugars as their alditol acetates showed arabinose and xylose in the molar ratio of 3:1. The purified polysaccharide (10 mg) was chromatographed on Bio-Gel A-15m as already described.

The alkali-extracted polysaccharide (200 mg) was also fractionated as just described, to give Fehling solution-precipitable polysaccharide (160 mg; designated alkali-extractable arabinoxylan), and a nonprecipitable polysaccharide (20 mg). The former was composed of xylose (95%) and arabinose (5%), and the latter of xylose (40%) and arabinose (60%). The alkali-extractable arabinoxylan (10 mg) was chromatographed on Bio-Gel A-15m as already described.

Graded precipitation. — The water-extractable and the alkali-extractable

arabinoxylans (50 mg each) were separately dissolved in water (10 mL). The polysaccharides were precipitated by the stepwise addition of 1, 2, 3, and 4 vol. of ethanol. The precipitates obtained in each step were recovered, and their sugar composition determined.

Methylation analysis. — The purified arabinoxylans (10 mg of each) were separately methylated, using dimethylsulfinyl carbanion in dry dimethyl sulfoxide and methyl iodide, according to the Hakomori procedure[9]. The permethylated products were hydrolyzed[2], and the sugars released were converted into their alditol acetates, and these analyzed by g.l.c. and g.l.c.-m.s.[10]. The results are given in Table II.

Isolation of the degraded polysaccharide. — The water-extractable arabinoxylan (500 mg) was hydrolyzed with 125mM sulfuric acid (100 mL) for 80 min at 80°. The acid was neutralized with barium carbonate, the suspension filtered, and the filtrate de-ionized and concentrated. The degraded polysaccharide (150 mg) was precipitated by the addition of an excess of ethanol; $[\alpha]_D$ −101° (*c* 0.5, water). Acid hydrolysis of the degraded polysaccharide, and g.l.c. analysis of the sugars as their alditol acetates gave arabinose and xylose in the molar ratio of 1:4.

Isolation and characterization of oligosaccharides. — The degraded polysaccharide (200 mg) was hydrolyzed with 125mM sulfuric acid (50 mL) for 45 min at 100°. The acid was neutralized with barium carbonate, the suspension filtered, and the filtrate de-ionized and concentrated. P.c. analysis showed, besides arabinose and xylose, the presence of oligosaccharides having R_{Glc} 0.72, 0.31, 0.11, and 0.06. The oligosaccharides were isolated by preparative p.c., and their specific rotations determined, and each was characterized.

The alkali-extractable polysaccharide (500 mg) was hydrolyzed as before, and the resultant oligosaccharides were isolated, and characterized.

Periodate oxidation[12]. — The arabinoxylans (100 mg each) were separately oxidized with 50mM sodium periodate (100 mL) at room temperature in the dark. The periodate consumptions, and the sugar compositions of the oxidized products were determined.

Smith degradation[13]. — The water-extractable polysaccharide (160 mg) was oxidized with 50mM sodium periodate (150 mL) for 60 h at room temperature in the dark. The oxidation product was reduced with sodium borohydride, and the reduction product hydrolyzed with 2M trifluoroacetic acid for 48 h at room temperature. The Smith-degraded polysaccharide (62 mg) was precipitated with an excess of ethanol. P.c. analysis of the supernatant liquor revealed the presence of glycerol (major) and arabinose.

Acid hydrolysis of the Smith-degraded polysaccharide and g.l.c. analysis of the sugars as their alditol acetates gave arabinose and xylose in the molar ratio of 1.0:1.8.

The Smith-degraded polysaccharide was subjected to methylation analysis, partial acid hydrolysis to release the oligosaccharides, and periodate oxidation, as described for the native arabinoxylans.

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