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# STRUCTURE OF AN ARABINOXYLAN FROM THE BARK OF *Persea macrantha* (LAURACEAE)

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## ABSTRACT

An arabinoxylan isolated from the delignified bark of *Persea macrantha* is composed of L-arabinose (73%) and D-xylose (27%). Partial hydrolysis gave 3-*O*-D-xylopyranosyl-L-arabinose, 4-*O*-D-xylopyranosyl-D-xylose, *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose, and *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose. Methylation analysis and periodate oxidation have been performed on the native and the degraded polysaccharides. It is suggested that the polysaccharide contains a backbone consisting of (1→4)-linked β-D-xylopyranosyl residues, each of which is substituted both at O-2 and O-3 with L-arabinofuranosyl and 3-*O*-D-xylopyranosyl-L-arabinofuranosyl groups. Some of the D-xylosyl residues of the latter side-chains are further substituted either at O-2 and O-4, or at O-3 and O-4, with L-arabinofuranosyl groups.

## INTRODUCTION

The bark of *Persea macrantha* (Nees) Kostermans[1] (family, Lauraceae), which has a characteristic, pleasant odour, is used extensively in making incense sticks as a cheap substitute for *Cinnamomum iners*[2]. The bark is a rich source of a mucilage that is somewhat inferior in its binding properties compared to the bark mucilage of *C. iners*. We have described an arabinoxylan[3] that is a major constituent of the bark of *C. iners*, and now report on a water-soluble, major polysaccharide present in the bark of *P. macrantha*.

## RESULTS AND DISCUSSION

Delignification[4] of the bark powder gave a white, insoluble residue and an aqueous extract. The polysaccharide in the aqueous extract was precipitated with ethanol and obtained (28%) as a pale-yellow powder. This material was reprecipitated with ethanol (3 vol.) from its solution in aqueous acetic acid after neutralisation with aqueous sodium hydroxide, and then contained L-arabinose (73%) and D-xylose (27%) as revealed by g.l.c. analysis of the derived alditol acetates[5]; nitrogen, *O*-acetyl

groups, and uronic acids were absent. The sugar composition remained unaltered even after barium hydroxide fractionation[6]. However, attempts to fractionate with Fehling's solution[7] were unsuccessful, as the reagent failed to precipitate the polysaccharide. The polysaccharide was excluded from Sephadex G-200. Free-boundary electrophoresis[8] of the polysaccharide in 0.1M sodium tetraborate gave a single, symmetrical peak. Thus, the polysaccharide appeared to be a single species and was designated as an arabinoxylan.

Partial hydrolysis of the arabinoxylan gave, in addition to L-arabinose and D-xylose, four oligosaccharide fractions. The oligosaccharides were isolated and purified by preparative paper chromatography, and characterised as 3-*O*-D-xylopyranosyl-L-arabinose, 4-*O*-D-xylopyranosyl-D-xylose, *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose, and *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose. On graded hydrolysis of the arabinoxylan, L-arabinose and 3-*O*-D-xylopyranosyl-L-arabinose were released under very mild conditions, suggesting that L-arabinose is probably present in the furanosidic form.

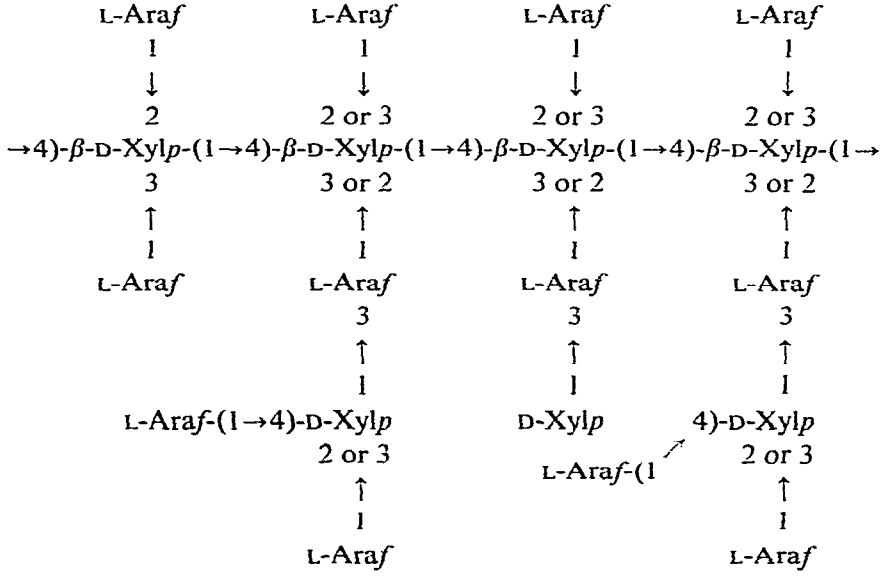
Methylation analysis[9] of the arabinoxylan gave 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-*O*-methyl-L-arabinose, 2-*O*- and 3-*O*-methyl-D-xylose, and D-xylose in the molar ratios 9.5 : 1.0 : 2.4 : 2.0 : 2.4. The relative proportions of 2-*O*- and 3-*O*-methylxylose could not be determined, as they were not separated by g.l.c.; however, their presence was inferred from their characteristic mass-spectral fragments. The formation of 2,3,5-tri-*O*-methyl-L-arabinose in very high proportion indicated that the majority of the L-arabinose residues are present as terminal, non-reducing, furanosidic residues. The presence of D-xylose in the hydrolysate of the methylated polysaccharide, and the formation of 4-*O*-D-xylopyranosyl-D-xylose and the corresponding xylotriose and xylotetraose on partial hydrolysis, suggested that the polysaccharide contains a xylan backbone consisting of (1→4)-linked D-xylopyranosyl residues. Formation of 2,3,4-tri-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose, and 2,5-di-*O*-methyl-L-arabinose, and the isolation of 3-*O*-D-xylopyranosyl-L-arabinose by partial hydrolysis, indicated that 3-*O*-D-xylopyranosyl-L-arabinosyl groups are present as side-chains attached to the xylan backbone. The formation of a relatively small proportion of 2,3,4-tri-*O*-methyl-D-xylose and a large proportion of 2,3,5-tri-*O*-methyl-L-arabinose indicates that some of the D-xylosyl residues of these side-chains are further substituted either at O-2 and O-4, or at O-3 and O-4, with L-arabinofuranosyl end-groups.

The polysaccharide reduced[10] 0.43 mol of periodate per pentosyl residue, and the formic acid liberated was negligible. Borohydride reduction of the periodate-oxidised arabinoxylan followed by acid hydrolysis gave (p.c.) glycerol, L-arabinose, and D-xylose.

Acid hydrolysis of the arabinoxylan under very mild conditions gave a degraded polysaccharide, $[\alpha]_D$ −125°, in addition to L-arabinose, D-xylose, and 3-*O*-D-xylopyranosyl-L-arabinose. The degraded polysaccharide was composed of L-arabinose (22%) and D-xylose (78%). Methylation analysis[9] of the degraded polysacchar-

ide gave 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*- and 3-*O*-methyl-D-xylose, and D-xylose in the molar ratios 13:8:10:52:17:1. The formation of a high proportion of 2,3-di-*O*-methyl-D-xylose confirms the presence of a (1→4)-D-xylan backbone. Identification of 2,3,4-tri-*O*-methyl-D-xylose and 2,5-di-*O*-methyl-L-arabinose in almost equal proportions indicated that an appreciable amount of the 3-*O*-D-xylopyranosyl-L-arabinosyl side-chains had survived the mild hydrolysis with acid. Hence, the degraded polysaccharide is essentially a linear, (1→4)-linked D-xylan carrying some of the L-arabinofuranosyl and 3-*O*-D-xylopyranosyl-L-arabinofuranosyl side-chains originally present in the arabinoxylan. The highly negative specific rotation of the degraded polysaccharide indicated the preponderance of β-glycosidic linkages. The degraded polysaccharide reduced 0.97 mol of periodate per pentosyl residue, which accords with the results of methylation analysis. Acid hydrolysis of the periodate-oxidised, degraded polysaccharide gave (p.c.) mainly glycerol and small proportions of D-xylose and L-arabinose.

Based on the foregoing results, the average structural features of the arabinoxylan can be tentatively represented as shown in **1**. It is structurally similar to the arabinoxylan previously reported from *C. iners*, in relation to the xylan backbone and side-chains, but the 3-*O*-D-xylopyranosyl-L-arabinosyl side-chains in the *P. macrantha* arabinoxylan are further substituted with L-arabinofuranosyl groups. This difference in branching pattern of the side-chain could be partly responsible for the poorer binding properties of the bark powder of *P. macrantha*.




**1**

## EXPERIMENTAL

*General.* — The bark powder of *P. macrantha* was obtained from the local market. Descending paper chromatography (p.c.) was performed on Whatman No. 1 and 3MM papers with *A*, 1-butanol–benzene–pyridine–water (5:1:3:3, upper layer); *B*, 1-butanol–acetic acid–water (4:1:5, upper layer); *C*, ethyl acetate–pyridine–water (8:2:1); *D*, 1-butanol–pyridine–water (6:4:3); and detection with *p*-anisidine hydrochloride[11] and alkaline silver nitrate[12]. G.l.c. of the sugar derivatives was performed on a Willy Giede GCHF 18.3 gas chromatograph fitted with a flame-ionisation detector and a stainless-steel column (2 m × 4 mm) containing 3% of OV-225 on Gas Chrom Q (100–120 mesh), with nitrogen as the carrier gas. Retention times for the partially methylated sugars were determined relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

Unless otherwise stated, the native, the degraded, and the periodate-oxidised polysaccharides and the oligosaccharides were completely hydrolysed with 0.25M sulphuric acid at 100° for 6–16 h. The hydrolysates were neutralised ($BaCO_3$), filtered, deionised with Amberlite IR-120 ($H^+$) and IRA-400 ($CO_3^{2-}$) resins, and concentrated under diminished pressure below 45°. The residues were examined by p.c., and the sugars were also analysed by g.l.c. as their alditol acetates[5]. The Amberlite IRA-400 ($CO_3^{2-}$) resin was eluted with 2M formic acid, the eluate was concentrated to dryness, and the residue was examined by p.c. for acidic sugars.

Oligosaccharides were converted into their methyl glycosides by treatment with dry, 2% methanolic hydrogen chloride overnight at room temperature. The oligosaccharides were either reduced with sodium borohydride or converted into their methyl glycosides before methylation by the Hakomori procedure[13]. The permethylated products were hydrolysed with 0.25M sulphuric acid, reduced with sodium borohydride, and acetylated. The resulting, partially methylated alditol acetates were analysed by g.l.c. and g.l.c.–m.s.[9].

*Isolation of the polysaccharide.* — Finely powdered, dark-brown bark (10 g) was stirred with water (500 mL) and delignified[4] with sodium chlorite in the presence of acetic acid. The insoluble residue was collected and dried (6.9 g). After neutralising the filtrate with 5% aqueous sodium hydroxide, ethanol (3 vol.) was added with stirring. The precipitate was collected, suspended in water, and dialysed successively against running tap-water and distilled water. The polysaccharide was recovered by precipitation with ethanol (2 vol.) and centrifugation, and dried to give a pale-yellow solid (2.8 g).

*Purification of the polysaccharide.* — The polysaccharide (1 g) was dissolved in 5% acetic acid (1 L) and centrifuged, the clear solution was neutralised with 10% aqueous sodium hydroxide, and ethanol (3 vol.) was added with continuous stirring. The precipitate was collected and a suspension in water (50 mL) was dialysed against running tap-water for 24 h and distilled water for 24 h. The polysaccharide (900 mg), recovered either by lyophilisation or by the addition of excess of ethanol, was completely and sharply precipitated from its aqueous solution with barium hydroxide.

Fehling's solution did not precipitate the polysaccharide. Free-boundary electrophoresis of the polysaccharide (1% solution) in 0.1M sodium tetraborate gave a single, symmetrical peak.

*Arabinoxylan. — Sugar composition.* The polysaccharide was completely hydrolysed, and the hydrolysate was fractionated into neutral and acidic portions using Amberlite IRA-400 ($CO_3^{2-}$) resin. P.c. of the neutral sugars revealed xylose and arabinose, with traces of glucose and galactose. The acidic portion did not contain any uronic acid. The sugars in the hydrolysate were isolated by preparative p.c. and their absolute configurations were determined by measuring their specific rotations. G.l.c. analysis of the derived alditol acetates gave L-arabinose (73%) and D-xylose (27%).

*Methylation analysis.* The polysaccharide (10 mg) was methylated twice by the Hakomori method[13]. The product, which showed negligible absorption for hydroxyl, was hydrolysed with 90% formic acid (2 mL) at 100° for 2 h and then, after evaporation of the formic acid, with 0.25M sulphuric acid for 8 h. The resulting, partially methylated sugars were converted into their alditol acetates. G.l.c. and g.l.c.–m.s.[9] indicated the presence of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-*O*-methyl-L-arabinose, 2-*O*- and 3-*O*-methyl-D-xylose, and D-xylose in the molar ratios 9.5:1.0:2.4:2.0:4.0.

*Partial hydrolysis.* The polysaccharide (600 mg) was hydrolysed with 0.125M sulphuric acid (60 mL) at 100° for 45 min, to give a maximum yield of small oligosaccharides. The hydrolysate was neutralised, deionised, and concentrated. P.c. (solvent *A*) of the residue revealed oligosaccharide fractions **1**–**4** ($R_{Glc}$ 0.86, 0.76, 0.33, and 0.12, respectively) in addition to L-arabinose and D-xylose. These oligosaccharides were isolated by preparative p.c. (yields: 10, 12, 8, and 5 mg, respectively), and their homogeneity was ascertained by p.c.

*Fraction* **1**. $R_{Glc}$ 0.86 (solvent *A*). Acid hydrolysis gave (p.c.) D-xylose and L-arabinose in equal proportions. Reduction with sodium borohydride followed by acid hydrolysis gave D-xylose and L-arabinitol. Periodate oxidation of the methyl glycoside followed by acid hydrolysis and p.c. gave only L-arabinose as the intact sugar. Borohydride reduction followed by methylation analysis gave 1,2,4,5-tetra-*O*-methyl-L-arabinitol and 2,3,4-tri-*O*-methyl-D-xylose in equal proportions. Hence, fraction **1** was 3-*O*-D-xylopyranosyl-L-arabinose.

*Fraction* **2**. $R_{Glc}$ 0.76 (solvent *A*). Acid hydrolysis gave (p.c.) only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no D-xylose. Borohydride reduction followed by methylation analysis gave 1,2,3,5-tetra-*O*-methyl-D-xylitol and 2,3,4-tri-*O*-methyl-D-xylose in equal proportions. Hence, fraction **2** was 4-*O*-D-xylopyranosyl-D-xylose.

*Fraction* **3**. $R_{Glc}$ 0.33 (solvent *A*). Acid hydrolysis gave only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no D-xylose. Methylation analysis of its methyl glycoside gave 2,3,4-tri-*O*-methyl-D-xylose (1 mol) and 2,3-di-*O*-methyl-D-xylose (2 mol). Hence, fraction **3** was *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose.

*Fraction* **4**. $R_{Glc}$ 0.12 (solvent *A*). Acid hydrolysis gave only D-xylose. Periodate oxidation of the methyl glycoside followed by borohydride reduction and acid hydrolysis gave (p.c.) glycerol, but no D-xylose. Methylation analysis of its methyl glycoside gave 2,3,4-tri-*O*-methyl-D-xylose (1 mol) and 2,3-di-*O*-methyl-D-xylose (3 mol). Hence, fraction **4** was *O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-*O*-D-xylopyranosyl-(1→4)-D-xylose.

*Periodate oxidation*[10]. — The polysaccharide (100 mg) was treated with 45mM sodium metaperiodate (100 mL) at room temperature in the dark. Periodate consumption and the liberation of formic acid were monitored by titration against standard sodium thiosulphate and sodium hydroxide, respectively. The periodate consumption, 0.43 mol per pentosyl residue, became constant after 18 h, and the formic acid liberated was negligible. The reaction mixture was treated with ethylene glycol, dialysed, reduced with sodium borohydride, and hydrolysed with 0.25M sulphuric acid at 100° for 6 h. The resulting sugars were examined by p.c., which revealed glycerol, L-arabinose, and D-xylose.

*Partially degraded arabinoxylan.* — The arabinoxylan (300 mg) was hydrolysed with 0.125M sulphuric acid (100 mL) at 80° for 1 h. The hydrolysate was neutralised ($BaCO_3$), deionised, and concentrated under reduced pressure. Addition of excess of ethanol precipitated a degraded polysaccharide (25 mg), $[\alpha]_D$ −125° (*c* 0.2, water).

Acid hydrolysis of the degraded polysaccharide and g.l.c. of the resulting sugars as their alditol acetates indicated the presence of D-xylose (72%) and L-arabinose (28%).

The degraded polysaccharide (10 mg) was methylated by the Hakomori procedure[13]. The product was hydrolysed with 90% formic acid (2 mL) for 2 h at 100° and, after evaporation of the formic acid, with 0.25M sulphuric acid for 6 h at 100°. The resulting, methylated sugars were converted into their alditol acetates. G.l.c. and g.l.c.–m.s. indicated the presence of 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,5-di-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2-*O*- and 3-*O*-methyl-D-xylose, and D-xylose in the molar ratios 13:8:10:52:17:1.

The degraded polysaccharide (10 mg) was treated with 45mM sodium metaperiodate (10 mL) at room temperature in the dark. After 24 h, the periodate consumption was 0.97 mol per pentosyl residue. The reaction mixture was treated with ethylene glycol, dialysed, reduced with sodium borohydride, and hydrolysed. P.c. of the hydrolysate revealed mainly glycerol with small proportions of D-xylose and L-arabinose.

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