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STRUCTURE OF AN L-ARABINO-D-XYLAN FROM THE BARK OF *Cinnamomum zeylanicum*

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ABSTRACT

An arabinoxylan isolated from the bark of *Cinnamomum zeylanicum* was composed of L-arabinose and D-xylose in the molar ratio 1.6:1.0. Partial hydrolysis furnished oligosaccharides which were characterised as α-D-Xyl*p*-(1→3)-L-Ara, β-D-Xyl*p*-(1→4)-D-Xyl, β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl, β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl, xylopentaose, and xylohexaose. Mild acid hydrolysis of the arabinoxylan gave a degraded polysaccharide consisting of L-arabinose (8%) and D-xylose (92%). Methylation analysis indicated the degraded polysaccharide to be a linear (1→4)-linked D-xylan in which some xylopyranosyl residues were substituted at O-2 or O-3 with L-arabinofuranosyl groups. These data together with the results of methylation analysis and periodate oxidation of the arabinoxylan suggested that it contained a (1→4)-linked β-D-xylan backbone in which each xylopyranosyl residue was substituted both at O-2 and O-3 with L-arabinofuranosyl, 3-*O*-α-D-xylopyranosyl-L-arabinofuranosyl, and 3-*O*-L-arabinofuranosyl-L-arabinofuranosyl groups.

INTRODUCTION

The bark of *Cinnamomum zeylanicum* has a characteristic pleasant odour and is used as a flavouring agent and spice in food preparations[1]. The bark contains a significant amount of a hitherto unstudied mucilaginous substance which consists mainly of a water-extractable L-arabino-D-xylan and an alkali-extractable D-glucan. We have described[2] the chemical nature of the mucilaginous material and the structure of a novel arabinoxylan present in the bark of a closely related species, *viz. Cinnamomum iners*, and now report on the structure of an arabinoxylan from the bark of *C. zeylanicum*.

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RESULTS AND DISCUSSION

The extractive-free bark powder, on treatment with water, gave a viscous solution and an insoluble residue. Dialysis and lyophilisation of the aqueous extract yielded a polysaccharide (6% of the dry bark) which was composed of mainly L-arabinose and D-xylose with a trace of glucose. Extraction of the residue with alkali and precipitation with ethanol gave a crude glucan (10%) consisting of D-glucose (90.1%), L-arabinose (4.7%), and D-xylose (5.2%).

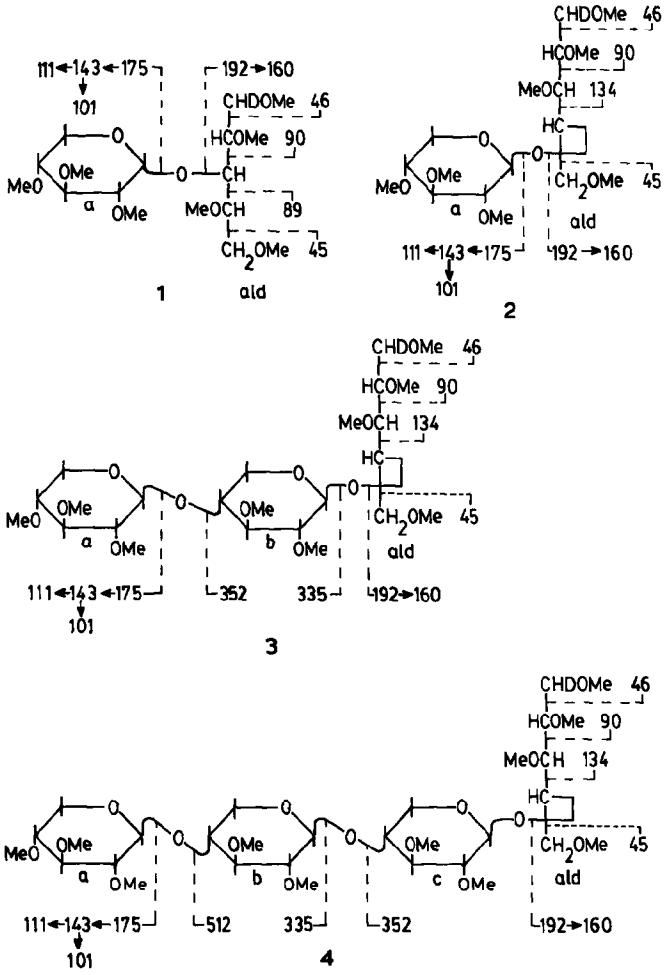
The water-extractable arabinoxylan, after purification by precipitation from its aqueous solution with barium hydroxide[3], gave a single peak on sedimentation analysis[4] and was composed of L-arabinose and D-xylose in the molar ratio 1.6:1.0. It was readily and sharply precipitated from an aqueous solution with barium hydroxide[3] and Fehling's solution[5], and the sugar composition remained unaltered on repeated reprecipitation. Thus, the arabinoxylan appeared to be a single species.

Partial hydrolysis of the arabinoxylan gave, in addition to L-arabinose and D-xylose, a series of oligomers, out of which six (*A–F*) were isolated homogeneous by preparative p.c. and found to have $[\alpha]_D$ values (water) of +179°, −28°, −46°, −61°, −68°, and −75°, respectively[6]. *A–F* were characterised on the basis of (*a*) sugar analysis before and after borohydride reduction, (*b*) methylation analysis using g.l.c. and g.l.c.–m.s.[7], and (*c*) borodeuteride reduction, methylation, and e.i.-m.s. of the methylated oligosaccharide-alditols. The mass fragments are designated according to the scheme of Kochetkov and Chizhov[8].

Oligosaccharide *A* (R_{Glc} 0.86, solvent *A*), on acid hydrolysis, gave D-xylose and L-arabinose in equal proportions. Borohydride reduction and then acid hydrolysis gave (p.c.) xylose and arabinitol. Methylation analysis of the alditol of *A* yielded 1,2,4,5-tetra-*O*-methylarabinitol and 2,3,4-tri-*O*-methylxylose in equal proportions. E.i.-m.s. of the methylated alditol of *A* (see **1**) gave ions at *m/z* 111 (aA_3, 9.5%), 143 (aA_2, 54), 175 (aA_1, 42), 192 ($aldJ_2$, 9), and 252 ($aldJ_1$, 23); ions at *m/z* 133 and 134 were absent. These data suggested *A* to be (1→3)-linked, and the $[\alpha]_D$ value (+179°) indicated the linkage to be α. Therefore, *A* is 3-*O*-α-D-xylopyranosyl-L-arabinose.

The results of acid hydrolysis, before and after borohydride reduction, and methylation analysis showed oligosaccharide *B* (R_{Glc} 0.73) to be a (1→4)-linked xylobiose. E.i.-m.s. of the methylated alditol (**2**) of *B* gave ions at *m/z* 133 and 134 in addition to those observed for **1**. The $[\alpha]_D$ value of −28° indicated the linkage to be β. Therefore, *B* is 4-*O*-β-D-xylopyranosyl-D-xylose.

The compositional and methylation analysis data suggested oligosaccharides *C* (R_{Glc} 0.32) and *D* (R_{Glc} 0.13) to be (1→4)-linked xylotriose and xylotetraose, respectively. E.i.-m.s. of the methylated alditol (**3**) of *C* gave ions at *m/z* 111 (aA_3, 21%), 143 (aA_2, 83), 175 (aA_1, 69), 192 ($aldJ_2$, 7), 252 ($aldJ_1$, 3), 303 (abA_2, 2.8), 320 ($baldJ_3$, 0.2), 335 (abA_1, 0.1), 352 ($baldJ_2$, 13), 412 ($baldJ_1$, 0.2), 133, and 134, whereas that (**4**) of *D* gave ions at *m/z* 111 (aA_3, 28%), 143 (aA_2, 100), 175 (aA_1,



86), 192 (aldJ$_2$, 50), 151 (aldJ$_1$, 2.4), 171 (abA$_3$, 0.12), 303 (abA$_2$, 3), 320 (caldJ$_3$, 2.8), 335 (abA$_1$, 0.15), 352 (caldJ$_2$, 10), 412 (caldJ$_1$, 0.22), 512 (bcaldJ$_2$, 0.05), 133, and 134. These data, together with the $[\alpha]_D$ values, suggested *C* and *D* to be β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl and β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xyl, respectively.

Oligosaccharides *E* and *F* were not characterised but, from their behaviour in p.c. and sugar compositions before and after borohydride reduction, it was inferred that they were xylopentaose and xylohexaose, respectively.

Methylation analysis of the arabinoxylan gave the results in Table I. The presence of a large proportion of D-xylose in the hydrolysate of the methylated polysaccharide and the formation of β-D-Xyl*p*-(1→4)-D-Xyl and higher oligosaccharides containing exclusively (1→4)-linked β-D-xylopyranosyl residues on partial hydrolysis of the native polysaccharide indicated the presence of a (1→4)-linked

β-D-xylan backbone in which each residue was substituted at both O-2 and O-3. The formation of 2,3,5-tri-*O*-methyl and 2,5-di-*O*-methyl derivatives of arabinose suggested the exclusive presence of arabinose in the furanose form. The formation of 2,3,4-tri-*O*-methylxylose in the methylation analysis and the formation of α-D-Xyl*p*-(1→3)-L-Ara on partial hydrolysis of the arabinoxylan demonstrated that some of the terminal xylopyranosyl groups were attached to O-3 of the L-arabinofuranosyl residues which, in turn, were attached most probably to the xylan backbone. The formation of a relatively high proportion of 2,5-di-*O*-methylarabinose indicated that a significant proportion of the non-terminal L-arabinofuranosyl residues were substituted at O-3, presumably by L-arabinofuranosyl groups.

The arabinoxylan, on mild acid hydrolysis, gave a degraded polysaccharide, which had $[\alpha]_D$ −108° and was composed of D-xylose (92%) and L-arabinose (8%). The results of methylation analysis of the degraded polysaccharide are given in Table 1. The formation of a high proportion of 2,3-di-*O*-methylxylose and low proportions of 2-*O*- and 3-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, and 2,3,4-tri-*O*-methylxylose indicated the degraded polysaccharide to be essentially a (1→4)-linked D-xylan with a few L-arabinofuranosyl units attached at O-2 or O-3. The high negative $[\alpha]_D$ value suggested the preponderance of β linkages. These results supported the presence of a (1→4)-linked β-D-xylan backbone in the arabinoxylan.

On periodate oxidation[9], the arabinoxylan consumed 0.48 mol of oxidant per mol of pentosyl residue. The oxidised polysaccharide contained L-arabinose and

TABLE 1

METHYLATION ANALYSIS DATA FOR THE ARABINOXYLAN (*A*) AND THE DEGRADED POLYSACCHARIDE (*B*)

Alditol acetate of[a]	T[b]	*Molar proportions*		*Characteristic mass fragments* (*m/z*)	*Linkage*
		A	*B*		
2,3,5-Me_3-Ara	0.44	4.6	1.0	45, 87, 101, 117, 129, 145, 161, 205	Ara*f*-(1→
2,3,4-Me_3-Xyl	0.58	1.0	2.1	87, 101, 117, 129, 145, 161	Xyl*p*-(1→
2,5-Me_2-Ara	0.89	4.2	—	45, 87, 99, 113, 117, 159, 173, 189, 201, 233	→3)-Ara*f*-(1→
2,3-Me_2-Xyl	1.18	—	14.6	87, 101, 117, 129, 161, 189	→4)-Xyl*p*-(1→
2- and 3-Me-Xyl	2.12	—	1.3	87, 117, 129, 145, 189, 201, 261	→3,4)-Xyl*p*-(1→ →2,4)-Xyl*p*-(1→
Xyl	3.5	4.5	—	85, 103, 115, 127, 145, 187, 217, 289	→2,3,4)-Xyl*p*-(1→

[a]2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methylarabinose, *etc.* [b]Retention times relative to that of 2,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on 3% OV-225.

D-xylose in the molar ratio ~1.0:1.2. These results accord with the methylation analysis data.

Thus, the native arabinoxylan isolated from the bark of *C. zeylanicum* contained a (1→4)-linked β-D-xylan backbone in which each D-xylopyranosyl residue was substituted at both O-2 and O-3 with L-arabinofuranosyl, 3-*O*-α-D-xylopyranosyl-L-arabinofuranosyl, and 3-*O*-L-arabinofuranosyl-L-arabinofuranosyl groups.

Arabinoxylans containing a highly branched (1→4)-linked β-D-xylan backbone having substitutents at both O-2 and O-3 have been isolated from corm sacs of *Watsonia pyramidata*[10], barks of *Cinnamomum iners*[2] and *Persea macrantha*[11], and leaves of *Litsea polyantha*[12,13] and *Neolitsea cassia*[14]. The arabinoxylan obtained from the bark of *C. iners*[2] bears a close resemblance in its structure to that reported here for *C. zeylanicum*.

EXPERIMENTAL

General. — A finely powdered commercial sample of the bark of *Cinnamomum zeylanicum* and authentic bark collected from the local forest were used. Unless stated otherwise, all hydrolyses were performed with 0.25M sulphuric acid at 100° for 8–10 h. The hydrolysates were neutralised ($BaCO_3$), filtered, deionised with Dowex 50W-X8 (H^+) and Dowex 2-X8 ($HCOO^-$) resins, concentrated under reduced pressure below 40°, and analysed by p.c. for neutral sugars. The neutral sugars were converted into their alditol acetates and analysed by g.l.c. The anion-exchange resin was eluted with M formic acid, and the eluate was concentrated and analysed by p.c. for acidic sugars. The enantiomeric configurations of the individual sugars were determined by their $[\alpha]_D$ values after isolation by preparative p.c. Whatman Nos. 1 and 3MM papers were used for p.c. with *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*, 1-butanol–pyridine–water (6:4:3). Sugars were detected with *p*-anisidine hydrochloride[15] and alkaline silver nitrate[16].

Other analytical procedures used have been reported[17].

Isolation of the polysaccharide. — The bark powder (15 g) was extracted continuously first with methanol–benzene (2:1) and then with acetone. A portion (10 g) of the residue (12.0 g) was stirred with water (500 mL, and 250 mL × 2), and the combined extracts were centrifuged, concentrated to 200 mL, dialysed against distilled water, and lyophilised to yield the crude arabinoxylan (850 mg). The water-insoluble residue was stirred with aqueous 5% sodium hydroxide (250 mL, and 150 mL × 2). The combined extracts were centrifuged, ethanol (2 vol.) was added, the precipitate was collected by centrifugation, and a solution in water (250 mL) was dialysed against distilled water and then lyophilised to give a crude glucan (1.3 g). The polysaccharides were separately hydrolysed, the resulting sugars were converted into their alditol acetates, and the products were analysed by g.l.c. The arabinoxylan contained xylose and arabinose together with a trace of glucose, and

the glucan contained glucose (90.1%), arabinose (4.7%), and xylose (5.2%); uronic acids were absent from each polysaccharide fraction. Individual sugars were isolated by preparative p.c., and their $[\alpha]_D$ values indicated that the xylose and glucose were D and the arabinose was L.

Purification of the arabinoxylan. — A solution of the polysaccharide (500 mg) in cold aqueous 5% acetic acid (250 mL) was centrifuged and then dialysed. Aqueous, saturated barium hydroxide was added until precipitation was complete, the precipitate was collected by centrifugation, and a solution in cold aqueous 10% acetic acid was dialysed and then lyophilised to give the arabinoxylan (460 mg). The arabinoxylan (10 mg) was hydrolysed with acid, the released sugars (uronic acids absent) were converted into their alditol acetates, and the products were analysed by g.l.c. which revealed derivatives of arabinose and xylose in the molar ratio 1.6:1.0.

Sedimentation analysis of an aqueous 0.5% solution of the arabinoxylan at pH 6.0 was carried out in a Beckman Model E analytical ultracentrifuge.

Partial hydrolysis of the arabinoxylan. — The polysaccharide (500 mg) was hydrolysed with 0.125M sulphuric acid at 100° for 1 h. P.c. (solvent *A*) of the hydrolysate revealed, in addition to arabinose and xylose, a series of oligosaccharides *A*–*F* (R_{Glc} 0.86, 0.73, 0.32, 0.13, 0.06, and 0.02) which were isolated by preparative p.c. (solvent *A*) in yields of 12, 9, 3.6, 4, 1.8, and 2 mg, respectively. Each oligosaccharide was (*a*) hydrolysed with acid before and after borohydride reduction and the products were examined by p.c.; (*b*) reduced with sodium borohydride and then methylated by the Hakomori procedure[18], the product was isolated by reversed-phase chromatography using Sep-Pak C_{18} cartridges[19] and hydrolysed with acid, and the resulting sugars were analysed as their alditol acetates by g.l.c. and g.l.c.–m.s.[6]; and (*c*) reduced with sodium borodeuteride and methylated[18], and the product was isolated by reversed-phase chromatography[19] and analysed by e.i.-m.s.[20].

The degraded polysaccharide. — The arabinoxylan (100 mg) was hydrolysed with 0.125M sulphuric acid (10 mL) at 80° for 70 min. The hydrolysate was neutralised, deionised, and concentrated to 2 mL. Ethanol (10 mL) was added and the precipitate (degraded polysaccharide; 28 mg), collected by centrifugation, had $[\alpha]_D$ −108° (*c* 0.5, water). Acid hydrolysis and g.l.c. of the released sugars as their alditol acetates gave derivatives of arabinose (8%) and xylose (92%).

Methylation analysis. — The arabinoxylan and the degraded polysaccharide (5 mg of each) were separately methylated twice by the Hakomori procedure[18]. The products were hydrolysed with aqueous 90% formic acid at 100° for 2 h and, after evaporation of the formic acid, with 0.25M sulphuric acid at 100° for 8 h. The sugars were converted into their alditol acetates and analysed by g.l.c. and g.l.c.–m.s.[7]. The results are given in Table I.

Periodate oxidation. — The arabinoxylan (100 mg) was treated with M sodium hydroxide (25 mL) at room temperature for 3 h, and the solution was neutralised with cold aqueous 50% acetic acid, dialysed, and oxidised with 45mM sodium

periodate in a total volume of 100 mL at room temperature in the dark. The periodate consumption was monitored by titration of aliquots with 0.1M sodium thiosulphate. After 48 h, the excess of periodate was reduced with ethylene glycol (0.5 mL), the solution was dialysed and then treated with sodium borohydride, and the product was hydrolysed and analysed by p.c. The sugars were also analysed as their alditol acetates by g.l.c., which gave arabinose and xylose in the molar ratio ~1.0:1.2.

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