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STRUCTURAL STUDIES OF AN ARABINOXYLAN ISOLATED FROM *Litsea glutinosa* (LAURACEAE)

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ABSTRACT

A water-soluble arabinoxylan (D-xylose and L-arabinose in the molar ratio 1.0:3.4) was isolated from the mucilaginous bark of *Litsea glutinosa* (Lauraceae). The results of methylation analysis, partial hydrolysis, and ^{1}H- and ^{13}C-n.m.r. spectroscopy indicated a backbone of (1→4)-linked β-D-xylopranosyl residues substituted at both positions 2 and 3 with side chains composed of either single or (1→3)-linked arabinofuranosyl residues. Both α-L- and β-L-arabinofuranosyl residues were present. It is possible that side chains composed of two β-L-arabinofuranosyl residues are attached mainly at O-2 of some xylosyl residues.

INTRODUCTION

Litsea glutinosa (Lauraceae) is a moderate-sized tree found in the low-hill-country areas of Sri Lanka. The mucilaginous bark is used as a demulcent and mild astringent for diarrhoea and dysentery, the roots and leaves are used for poulticing sprains and bruises, and the oil extracted from the seeds is used in the treatment of rheumatism[1].

A polysaccharide composed of arabinose and xylose has been isolated[2] from *Litsea chinensis* Lamb. (*L. glutinosa* Lour.) but the structural features were not reported. We now report on a water-soluble polysaccharide isolated from the mucilaginous bark of *L. glutinosa*.

RESULTS AND DISCUSSION

The polysaccharide had a high protein content (18.3%). The carbohydrate content[3] of the crude polysaccharide (CPS), obtained after deproteinisation[4], was 81%, the protein content was reduced to 7%, and the uronic acid content[5] was low (1.5%). Arabinose and xylose were the major neutral sugars present, but small proportions of glucose and galactose were also detected (Table I). The CPS was purified by the Cetavlon method[6] and the neutral fraction was subjected to gel

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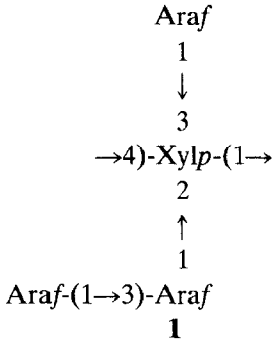
TABLE I

SUGAR COMPOSITION OF THE NATIVE AND DEGRADED POLYSACCHARIDES FROM *L. glutinosa*

Sugar	*Mole %*				
	CPS	*PPS*	*PS1*	*PS2*	*PS3*
Arabinose	71	74	55	39	21
Xylose	21	22	40	56	74
Galactose	3	2	2	2	3
Glucose	5	2	3	3	2

filtration on Sephacryl S-400 to give the pure polysaccharide (PPS), eluted in the void volume. The hexose content of the PPS was less than that of CPS (Table I), and L-arabinose and D-xylose were present in the molar ratio 3.4:1.0. The absolute configurations of the sugars were determined by the method of Gerwig *et al.*[7].

Methylation analysis (Table II) of CPS and PPS revealed terminal arabinofuranosyl, 3-linked arabinofuranosyl, and fully substituted xylosyl residues. The stoichiometry of the products indicates a structural unit of the type **1**.



1

The mass spectrum of a g.l.c. peak of low intensity (4%) revealed a small proportion of 2,4- and 3,4-linked xylosyl residues in PPS. Larger proportions of these two residues, as well as some 4-linked xylosyl residues, were detected in CPS and may have arisen from less-substituted polymeric impurities.

The polysaccharide gave highly viscous, aqueous solutions, and a product (PS1) suitable for n.m.r. spectroscopy was prepared by heating PPS in 0.1M trifluoroacetic acid for 5 min at 100°. Methylation analysis (Table II) of PS1 revealed that substantial cleavage of arabinofuranosyl residues had occurred, mainly of single-unit side chains linked to positions 2 and 3 of the xylosyl residues, since approximately equal proportions of 2,4- and 3,4-linked xylosyl residues were formed. Sugar analysis (Table I) indicated an increased proportion of arabinosyl residues in PS1, probably as a result of incomplete hydrolysis of xylosyl residues.

The ^{1}H-n.m.r. spectrum of PS1 contained signals for anomeric protons

TABLE II

METHYLATION ANALYSIS OF THE NATIVE AND DEGRADED POLYSACCHARIDES FROM *L. glutinosa*

Sugar[a]	*Mole %*				
	CPS	*PPS*	*PS1*	*PS2*	*PS3*
2,3,5-Ara	43	45	29	26	11
2,3,4-Xyl	0	0	0	0	3
2,5-Ara	21	25	20	15	9
2,3-Xyl	4	—	15	35	68
3-Xyl					9
2- and 3-Xyl	10	4	23	17	
Xyl	22	26	12	8	0

[a]2,3,5-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, *etc.*

attributable to α-L-arabinofuranosyl and β-L-arabinofuranosyl residues[8] (Table III). The doublet (*J* 7.5 Hz) of low intensity at δ 4.47 was assigned to β-D-xylopyranosyl residues[8]. The broad peak of higher intensity at δ 4.50 was probably due to highly substituted β-D-xylopyranosyl residues (Fig. 1a).

Partial depolymerisation of PS1 with 0.125M sulphuric acid for 40 and 80 min at 80° gave the degraded polysaccharides PS2 and PS3, respectively. Sugar and methylation analyses of PS2 and PS3 (Tables I and II) indicated that a progressive cleavage of arabinofuranosyl residues had occurred during acid hydrolysis. The decrease in the proportion of fully substituted xylopyranosyl residues was accompanied by an increase in the content of 4-linked xylosyl residues, which indicated a backbone of highly substituted (1→4)-linked β-D-xylopyranosyl residues.

The proportions of 2,4- and 3,4-linked xylosyl residues were greater in PS1

TABLE III

^{1}H-N.M.R. DATA FOR THE DEGRADED POLYSACCHARIDES PS1–PS3 FROM *L. glutinosa*

Sample	*δ (p.p.m.)*	$J_{1,2}$ *(Hz)*	*Integral*	*Assignment*
PS1	5.39, 5.31, 5.28, 5.24	n.r.[a]	1.9	α-L-Ara*f*
	5.11	4.6	1.0	β-L-Ara*f*
	4.55	broad		
	4.47	7.5	3.0	β-D-Xyl*p*
PS2	5.31, 5.28, 5.24	n.r.	1.9	α-L-Ara*f*
	5.12	4.5	1.0	β-L-Ara*f*
	4.58	broad		
	4.48	7.5	3.9	β-D-Xyl*p*
PS3	5.31, 5.28	n.r.	1.3	α-L-Ara*f*
	5.12	4.5	1.1	β-L-Ara*f*
	4.58	broad	1.0	
	4.48	7.5	7.5	β-D-Xyl*p*

[a]Signal not resolved.

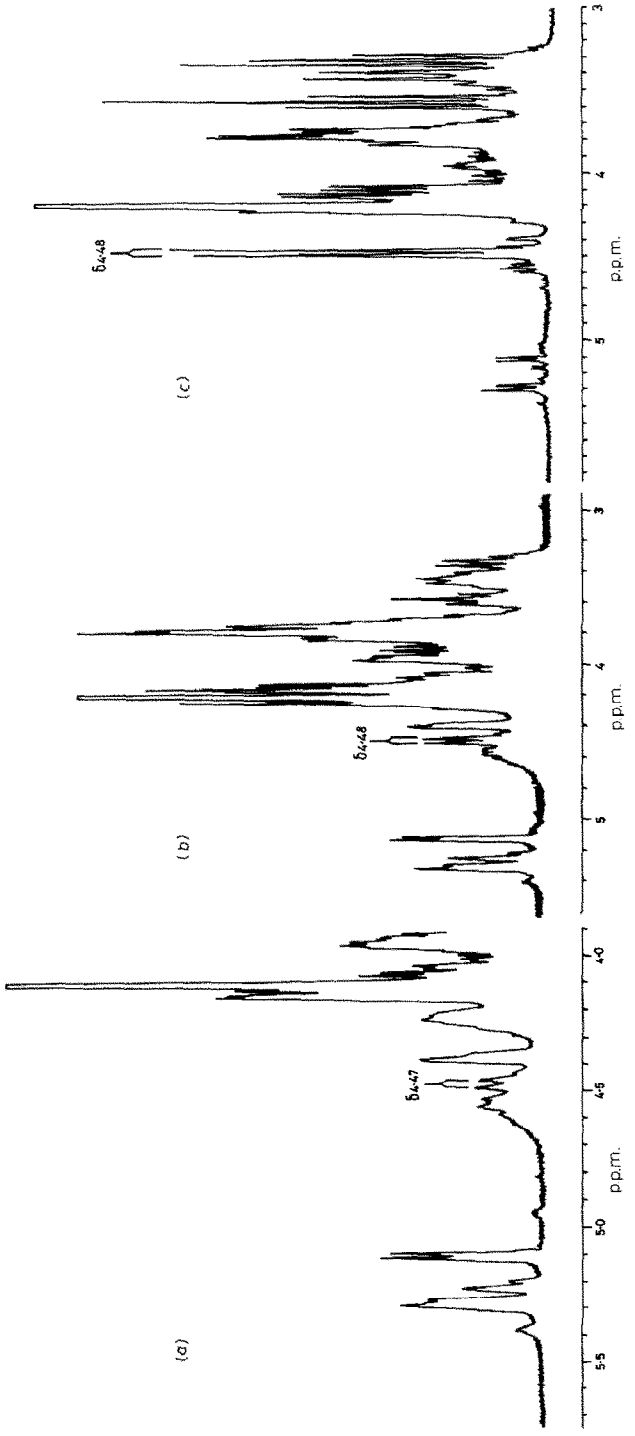


Fig. 1. ^{1}H-N.m.r. spectra of degraded arabinoxylans: (a) PS1, (b) PS2, (c) PS3.

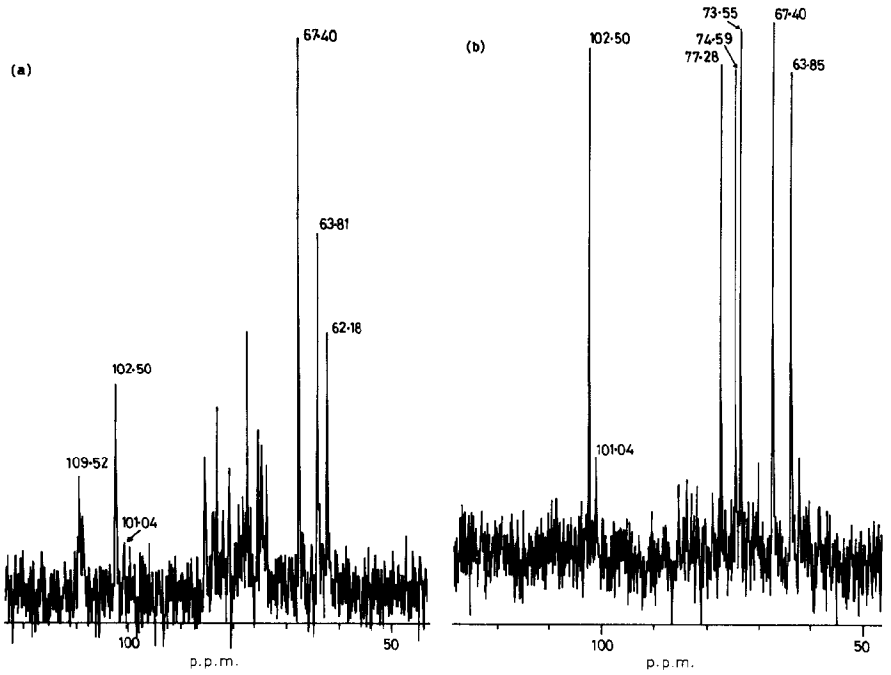


Fig. 2. ^{13}C-N.m.r. spectra of the degraded arabinoxylans: (a) PS2, (b) PS3.

and PS2 than in PPS (Table II). The mass spectrum of the peak due to these two residues suggested the presence of approximately equal proportions of 3,4- and 2,4-linked xylosyl residues in PS1 and PS2, and PS3 contained only 2,4-linked xylosyl residues. Hence, the results of methylation analysis indicated that disaccharide side-chains composed of (1→3)-linked arabinofuranosyl residues were attached to O-2 of some xylosyl residues in PS3. These observations suggested that PPS comprised a backbone of (1→4)-linked xylopyranosyl residues, a large proportion of which was fully substituted with side chains of arabinofuranosyl residues and a small proportion was unsubstituted. It is likely that there were side chains of either one or two arabinofuranosyl residues attached at both O-2 and O-3 of xylosyl residues. The side chains that are less readily hydrolysed probably carry β-arabinofuranosyl residues that are more resistant than α-arabinofuranosyl residues[9]. These side chains may be attached mainly to O-2 of some xylosyl residues.

The ^{1}H-n.m.r. spectrum of PS2 contained unresolved signals for anomeric protons at δ 5.31, 5.28, and 5.24 (α-L-arabinofuranosyl residues) and a signal at δ 5.12 (d, *J* 4.80 Hz) assigned to β-L-arabinofuranosyl residues[8] (Table III). The signal at δ 4.48 (d, *J* 7.5 Hz), assigned to less-substituted β-xylopyranosyl residues, was of increased intensity and the broad peak at δ 4.55 was also present (Fig. 1b). The ^{13}C-n.m.r. spectrum of PS2 contained signals for anomeric carbons which were assigned tentatively to α-L-arabinofuranosyl (109.52 and 108.65 p.p.m.)[10], β-D-

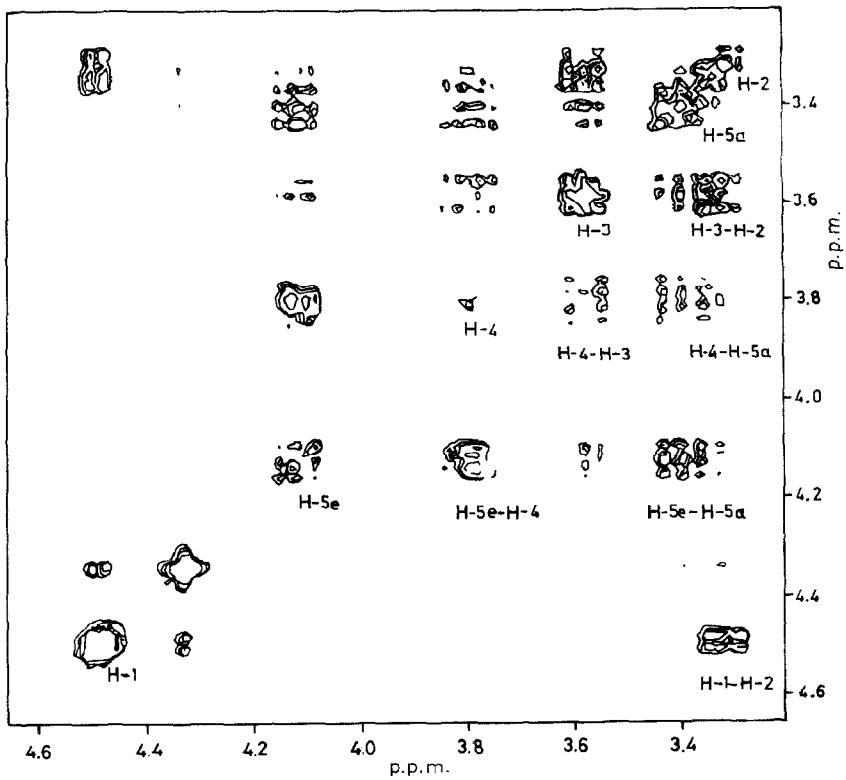


Fig. 3. Homonuclear ^1H–^1H 2D-COSY spectrum of PS3.

xylopyranosyl (102.50 p.p.m.)[11], and β-L-arabinofuranosyl (101.04)[10] residues (Fig. 2a) by comparison with literature data. The upfield shift of the signal due to the xylopyranosyl residue could be due to heavy substitution of these residues.

The ^1H-n.m.r. spectrum of PS3 contained signals for anomeric protons at δ 5.31 and 5.28 (unresolved), and 5.11 (d, *J* 4.76 Hz). The signal at δ 4.48 (d, *J* 7.5 Hz) was sharp and of high intensity (Fig. 1c) showing the presence of a large proportion of unsubstituted (1→4)-linked β-xylopyranosyl residues. The ^{13}C-n.m.r. spectrum of PS3 contained two signals (102.50 and 101.04 p.p.m.) for anomeric carbons, whereas those at 109–108 p.p.m., attributed to α-arabinofuranosyl residues, were of low intensity (Fig. 2b) which reflects the loss of α-arabinofuranosyl residues noted above. The high-intensity signals at 77.28, 74.59, 73.55, and 63.85 p.p.m. were attributed to C-4, C-3, C-2, and C-5, respectively, of the xylopyranosyl residues by comparison with data in the literature[10]. The signal at 77.26 p.p.m. was attributed to C-4, as methylation analysis and ^1H-n.m.r. evidence confirmed the presence of (1→4)-linked xylopyranosyl residues.

The homonuclear 2D-COSY n.m.r. spectrum of PS3 (Fig. 3) was used to

TABLE IV

SELECTED ^{1}H- AND ^{13}C-N.M.R. DATA FOR THE DEGRADED POLYSACCHARIDE PS3

δ (p.p.m.)	*Assignment*	*J (Hz)*
4.48	H-1	$J_{1,2}$ 7.43
4.12	H-5*e*	$J_{5e,4}$ 5.47, $J_{5e,5a}$ 11.33
3.78	H-4	$J_{4,5a}$ 10.26
3.53	H-3	$J_{3,4}$ 9.53, $J_{2,3}$ 9.58
3.37	H-5*a*	
3.28	H-2	
102.50	C-1	
77.28	C-4	
74.59	C-3	
73.55	C-2	
63.85	C-5	

assign all the ^{1}H resonances of the xylosyl residues. Similar results were obtained by the difference decoupling experiment and it was also possible to determine the coupling constants (Table IV). These results accord with data in the literature[12].

The unusual occurrence of α- and β-L-arabinofuranosyl residues in the same polysaccharide has been reported also for an arabinoglucuronomannan isolated from suspension-cultured tobacco cells[13] and a water-soluble polysaccharide from the leaves of *Neolitsea cassia* (Lauraceae)[14]. In the latter study, the presence of β-arabinofuranosyl residues was deduced from the signals for H-1 at δ 5.1 (d, *J* 4.0 Hz) and for C-1 at δ_C 110.35. In the present investigation, the signal at δ_C 101.04 has been assigned tentatively to β-L-arabinofuranosyl residues[10,15]. Heteronuclear n.m.r. studies of suitably degraded polysaccharides are necessary to confirm this assignment.

EXPERIMENTAL

General methods. — For g.l.c., a Varian 3300 instrument with a flame-ionisation detector was used with capillary columns of DB-1 for methylated alditol acetates, and DB-225 for alditol acetates with a temperature programme (170° for 2 min, 170→220° at 3°.min^{-1}). G.l.c.–m.s. was carried out at the Department of Organic Chemistry, University of Stockholm, with a Hewlett–Packard 5970 instrument, using an SE-54 capillary column. Neutral sugars were determined after hydrolysis of the polysaccharide with 2M trifluoroacetic acid for 1 h at 120°, reduction with $NaBH_4$, and acetylation. Glycosyluronic acid residues were determined by a decarboxylation method[5]. The absolute configurations of xylose and arabinose in the pure polysaccharide (1 mg) were determined by the method of Gerwig *et al.*[7]. Methylation and work-up were performed as decribed by Waeghe *et al.*[16] and the methylated sugars were reduced with $NaBD_4$. The identities of the partially methylated alditol acetates were established by g.l.c.–m.s. and comparison with reference compounds.

The ^1H- (85°) and ^{13}C-n.m.r. spectra (70°) of solutions in D_2O were recorded at the Department of Organic Chemistry, University of Stockholm, with a JEOL GX-270 instrument, using 3-trimethylsilylpropanoate-d_4, δ 0.00 (^1H), or 1,4-dioxane, δ 67.4 (^{13}C), as internal references.

Isolation and purification of the water-soluble polysaccharide. — Stem bark of *L. glutinosa* was collected from Matale in the Central Province of Sri Lanka. The fresh bark (930 g) was crushed and extracted with cold benzene (2.5 L, 24 h), then methanol (2.5 L, 24 h), and air-dried. The dry bark (490 g) was extracted twice with distilled water (2 × 2.5 L) for 24 h each at 27°, dialysed, and centrifuged. The supernatant solution was concentrated under reduced pressure at >40°, treated with an excess of ethanol, and centrifuged. A solution of the residue (4.2 g) in the minimum volume of water was deproteinised[4], dialysed, and freeze-dried to give the crude polysaccharide (CPS, 3.2 g). A solution of CPS (500 mg) was dissolved in 0.25M sodium chloride (200 mL) and purified using the Cetavlon method[6]. The neutral fraction was eluted from a column of Sephacryl S-400 with water, and the single broad peak eluted in the void volume was freeze-dried to give the pure polysaccharide (PPS, 460 mg).

Partial hydrolysis. — (*a*) PPS (50 mg) was heated with 0.1M trifluoroacetic acid (5 mL) at 100° for 5 min, then dialysed against distilled water for 24 h. The retentate was concentrated and freeze-dried, and the residue (29.3 mg) was eluted from a column of Sephadex G-25 with water. The fraction eluted in the void volume was freeze-dried to give PS1 (23 mg).

(*b*) Samples (50 mg) of PPS were hydrolysed with 0.125M sulphuric acid (5 mL) at 80° for 40 and 80 min, respectively. Each partial hydrolysate was neutralised ($BaCO_3$), passed through Amberlite IR-120 (H^+) resin, and precipitated with ethanol. Each product was eluted from a column (2.26 × 70 cm) of Bio-Gel P-2 with water. The fractions eluted in the void volume were collected and freeze-dried to give PS2 (18 mg) and PS3 (14 mg).

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