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0144-8617(94)00073-5

Carbohydrate Polymers **26** (1995) 19–23

0144-8617/95/$09.50 + .00

A water-soluble polysaccharide from the leaves of *Litsea gardneri* (Lauraceae)

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(Received 19 May 1994; revised version received 12 September 1994; accepted 13 September 1994)

A neutral polysaccharide isolated from the water extract of the leaves of *Litsea gardneri* was composed mainly of xylose (70%) and small, approximately equimolar amounts of arabinose, mannose, galactose and glucose. Methylation analysis combined with GLC-MS, ^{1}[H] and ^{13}[C]NMR spectroscopy of the native and degraded polysaccharides indicated the presence of a $1 \rightarrow 4$ linked β-D-xylopyranosyl backbone. It is possible that some arabinofuranosyl residues were incorporated in the backbone of the polymer. Side chains were attached to *O*-2 of some of the xylopyranosyl residues in the polymer chain. The available evidence suggests that the side chains may be either single units of terminal arabinofuranosyl/xylopyranosyl residues or multiple unit side chains containing both arabinosyl and xylosyl residues.

INTRODUCTION

Litsea gardneri (Sinh. Talan) (Lauraceae) is a moderately sized tree which is endemic to Sri Lanka. It is found in the rainforests of the Knuckles range and the main mountain massif of Sri Lanka up to an altitude of 6000 feet. Previous chemical investigation of *L. gardneri* has led to the isolation of alkaloids (Bandara *et al.*, 1989).

Two species of *Litsea*, *L. chinensis* (Haq *et al.*, 1974) and *L. glutinosa* (Herath *et al.*, 1990) have been studied previously. A polysaccharide has been isolated from the alkali-soluble substance of the bark of *L. chinensis*. Starch was separated from the extract by precipitation with iodine. The residual polysaccharide upon hydrolysis showed the presence of D-xylose and L-arabinose (Haq *et al.*, 1974). The polysaccharide from *L. glutinosa* was found to have a backbone of $(1 \rightarrow 4)$ linked β-D-xylopyranosyl residues substituted at both *O*-2 and *O*-3 with short side chains composed of arabinofuranosyl residues (Herath *et al.*, 1990).

Aqueous decoctions of many plant species belonging to the family Lauraceae are used in the preparation of native medicines in Sri Lanka (Jayaweera, 1981) and led to our interest in the water-soluble polysaccharides from this family. This paper describes the isolation, fractionation and structural studies of a water-soluble polysaccharide isolated from the leaves of *L. gardneri*.

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MATERIALS AND METHODS

General methods

Evaporations were carried out *in vacuo* at a temperature less than 40°C. Blowing and co-distillations were performed by flushing air through stainless steel needles while the samples were maintained at a temperature <40°C. GLC analysis were carried out using either a Varian 3300 instrument or Hewlett Packard 5890 instrument equipped with a flame ionization detector. Alditol acetates and partially *O*-methylated alditol acetates were analysed using DB-1, DB-225 or HP-5 capillary columns. GLC-MS studies were carried out using a Hewlett Packard 5970 MSD instrument. ^{1}[H] and ^{13}[C]NMR spectra of poly- and oligosaccharides were recorded in D_2O solutions using a JEOL GX 400 or GSX 270 MHz instrument. The carbohydrate contents were determined by a GLC method (Albersheim *et al.*, 1967) using D-allose as the internal standard. Hydrolyses were performed with 2 N trifluoroacetic acid for 2 h at 120°C. Methylation analyses were carried out according to a modified Hakamori procedure (Harris *et al.*, 1984).

Gel permeation chromatography

A column of Bio-Gel P-2 ($2{\cdot}5 \times 90$ cm) irrigated with 1% *n*-butanol in water was standardized using a mixture of dextran-10, stachyose, raffinose, sucrose and

glucose. The gel chromatogram was recorded using a Waters RI 403 refractometer coupled with an SE-120 recorder. Fractions of 6 ml were collected at a flow rate of 20 ml/h.

NMR spectroscopy

NMR spectra of poly- and oligosaccharide solutions in deuterium oxide were recorded at 70°C using sodium 3-trimethylsilylpropionate-d_4, 0·00 ^{1}[H], 1,4-dioxane, 67·4 ^{13}[C] or acetone, 31·0 ^{13}[C] ppm as internal references.

Isolation of the neutral polysaccharide (NPS)

Dry leaves (250 g) of *L. gardneri* collected in the Sinharaja forest reserve of Sri Lanka were extracted with methanol at 20°C for 6 and 24 h, respectively. The extract was filtered and the residue was air-dried. The dry residue (174 g) was extracted with distilled water, filtered, dialysed and freeze dried (3·4 g, 1·8%). The water-soluble material was treated with Cetavlon (Scott, 1965) and the aqueous phase was dialysed and freeze-dried to obtain the neutral polysaccharide, NPS, (284 mg, 9·0%). The NPS was chromatographed on a column of Bio-Gel P-2 (2·5 × 90 cm) irrigated with 1% *n*-butanol in water and the purified polysaccharide fraction PPS was separated and freeze-dried (190 mg, 67%).

Partial acid hydrolysis of PPS

Two samples of PPS (25 mg each) were hydrolysed with 0·1 M TFA (100 ml) at 100°C for 30 and 60 min, respectively. The hydrolysates were concentrated on a rotavapor and chromatographed on the standardized column of Bio-Gel P-2. The fractions were monitored using an RI 403 refractometer. Hydrolysis of PPS for 30 min gave a polymeric fraction PS1 (16 mg, 64%) which eluted with the void volume. Hydrolysis of PPS for 60 min gave a fraction PS4 (3·2 mg, 13%) which corresponded to a tetrasaccharide in the standard gel chromatogram.

RESULTS AND DISCUSSION

Dry leaves of *L. gardneri* (250 g) were extracted with cold methanol. The residue obtained was air-dried and extracted with distilled water at room temperature. The crude polysaccharide (3·4 g, 1·8%) obtained from the dialysed water extract was purified by the Cetavlon method (Scott, 1965) and a neutral polysaccharide fraction (NPS) was isolated (284 mg, 9·0%). The NPS was passed through Bio-Gel P-2 and one fraction (PPS) was eluted at the void volume (190 mg, 67% of NPS).

The carbohydrate contents of NPS and PPS determined by GLC method (Albersheim *et al.*, 1967) using D-allose as the internal standard, were found to be 87 and 91%, respectively. The glycosyl composition determined by the same method showed that both NPS and PPS were composed mainly of xylose (70%). Small amounts of arabinose, mannose, galactose and glucose were also detected (Table 1). L-arabinose (67–74%) and D-xylose (22–25%) were the major neutral sugars found in the water-soluble arabinoxylans isolated from the mucilaginous stem bark of *L. glutinosa* (Herath *et al.*, 1990) and the leaves of *Neolitsea cassia* (de Silva *et al.*, 1986) belonging to the same family.

The ^{1}[H] NMR spectrum of NPS (Fig. 1a) showed signals at δ 5·24 (1 H, br s, $W_{1/2}$ = 8 Hz), 4·62 (1·4 H, d, J = 7·0 Hz) and 4·46 (11 H, d, j ï= 7·3 Hz). The doublet at δ 4·46, which was the most intense peak in the anomeric region of the spectrum, was assigned to β-D-xylopyranosyl residues (Bock & Thogersen, 1982). the ^{13}[C] NMR spectrum of NPS showed four anomeric signals at δ 102·68, 102·54, 102·00 and 98·71. The signal at δ 102·54 was of high intensity and confirmed the presence of β-linked D-xylopyranosyl residues (Herath *et al.*, 1990). The signals of low intensity at δ 102·68 and 102·00 were tentatively assigned to β-D-glucopyranosyl (Bock & Thogersen, 1982) and β-L-arabinofuranosyl residues by comparison with reported data (Gorin & Mazurek, 1975; Ritchie *et al.*, 1975). The appearance of the ^{1}[H] NMR spectrum of PPS was similar to that of NPS. The relative intensities of the anomeric signals indicated that the water-soluble polysaccharide from *L. gardneri* was a β-D-xylan.

The glycosyl linkage composition of PPS was determined by GLC and GLC-MS analysis of the per-*O*-methylated alditol acetates. The polysaccharide was methylated according to a modified Hakamori procedure (Harris *et al.*, 1984) and the per-*O*-methylated alditol acetates were analysed on a DB-225 capillary column (Table 2).

The 2,3,4-, 2,3- and 3- pentitols were identified as 2,3,4-xylitol, 2,3-xylitol and 3-xylitol, respectively by the relative retention time and indicated the presence of a 1 → 4 linked xylopyranosyl backbone containing some branch points in PPS. The relative proportion of 2,3,4-xylitol, 3-xylitol and 2,3,5-arabinitol suggested the presence of single unit side chains of either terminal xylopyranosyl or terminal arabinofuranosyl residues

Table 1. Glycosyl composition of the native and degraded polysaccharides

Sugar residue	NPS	PPS	PPS_1[a]	PS_4[b]
Arabinose	9	8	9	22
Xylose	70	70	70	78
Mannose	10	6	6	—
Galactose	6	8	7	—
Glucose	6	7	7	—

[a] PS_1 was eluted at the void volume of Bio-Gel P-2 column.
[b] PS_4 was eluted at the tetrasaccharide region of the Bio-Gel P-2 column.

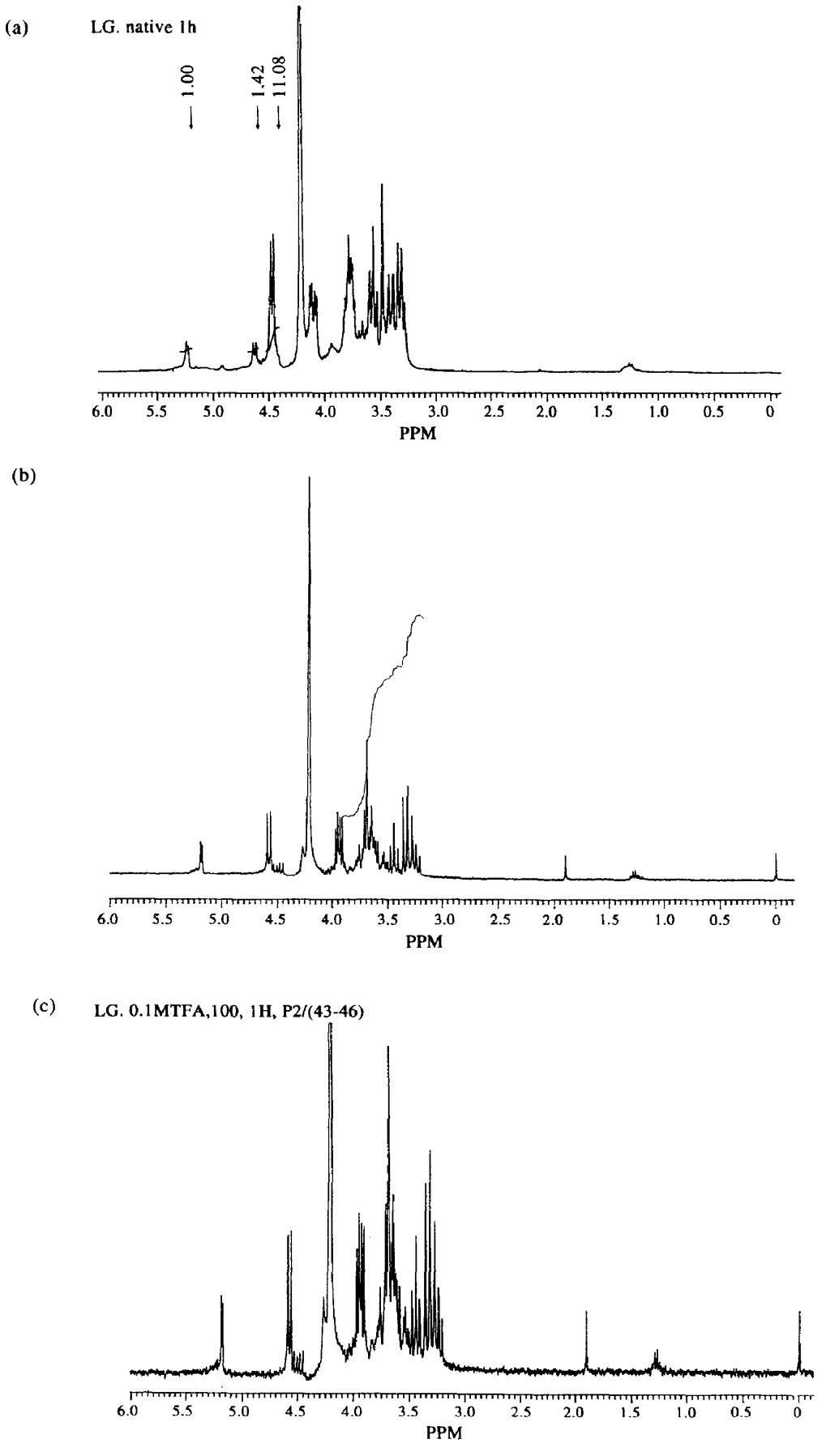

Fig.1 ^{1}H NMR spectra of (a) NPS; (b) PS1; and (c) PS4 from *Litsea gardneri*.

Table 2. Methylation analysis of PPS and degraded polysaccharides

Sugar residue	r.r.t.	% Value in PPS	PS_1	PS_4	Structural unit assigned
2,3,5 pent		4	—	—	Ara*f*-(1 →
2,3,4 pent	0·63	13	4	30	Xyl*p*-(1 →
2,3 pent	0·85	0·8	6	—	→ 5)Ara*f*(1 →
2,3 pent	0·87	63	74	70	→ 4)-Xyl*p*-(1 →
2,3,4,6 hex	1·00	0·4	2	—	Glc*p*-(1 →
3 pent	1·18	17	6	—	→ 4)-Xyl*p*-(1 → 2 ↑
2,3,6 hex		0·4	5	—	
2,3,6 hex		0·6	—	—	
2,3,4 hex		—	3	—	
2,4 hex		0·4	—	—	

r.r.t, retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol on a HP-5 capillary column. Temp. programme (185°(8) → 250° (5°C/min)).

attached to *O*-2 of some of the xylopyranosyl residues in the backbone of PPS (see Fig. 2a). The arabinoxylan from *L. glutinosa* was found to have the same type of backbone composed of 1 → 4 linked β-D-xylopyranosyl residues but was more highly branched (Herath *et al.*, 1990).

A small amount of 2,3-Ara*f* (0·8%) was detected in PPS. It is possible that these residues as well as some of the 2,3-Xyl*p* residues may be present in either the side chains or in the backbone of the polymer. Hence the presence of multiple unit side chains in the polymer cannot be ruled out (Fig. 2b).

Partial acid hydrolysis of PPS

A polymeric fraction PS_1 and an oligomeric fraction PS_4 were separated by gel permeation chromatography (Bio-Gel P-2) of the partial acid hydrolysates. The elution volume of PS_4 corresponded to that of a tetrasaccharide.

Sugar analysis of PS_1 showed that the composition of the polysaccharide was unchanged after hydrolysis (Table 1, column 2). Methylation analysis of PS_1 gave a higher proportion of 2,3-Ara*f* and hexosyl residues and suggested the presence of some arabinosyl and hexosyl residues in the backbone of the xylan. The higher proportion of Ara*f* residues (6%) detected in PS1 suggests that these residues are more likely to form part of the backbone of the polysaccharide because hydrolysis of the side chains, which is more likely to take place at the arabinosyl linkages, would result in loss of these arabinosyl residues.

The sugar composition of PS_4 showed the presence of arabinose and xylose (Table 1, column 3). The ^{1}[H] NMR spectrum of PS_4 (Fig. 1c) was similar to that of PS_1 and suggested a regular basic structure in the polysaccharide. The coupling constant of the signal at δ 5·18 (1 H, d, J = 3·3 Hz) indicated the presence of β-L-arabinosyl residues (Capon & Thacker, 1964) while the signal at δ 4·57 (3 H, d, J = 7·7 Hz) was assigned to β-D-xylopyranosyl residues. The sugar composition and

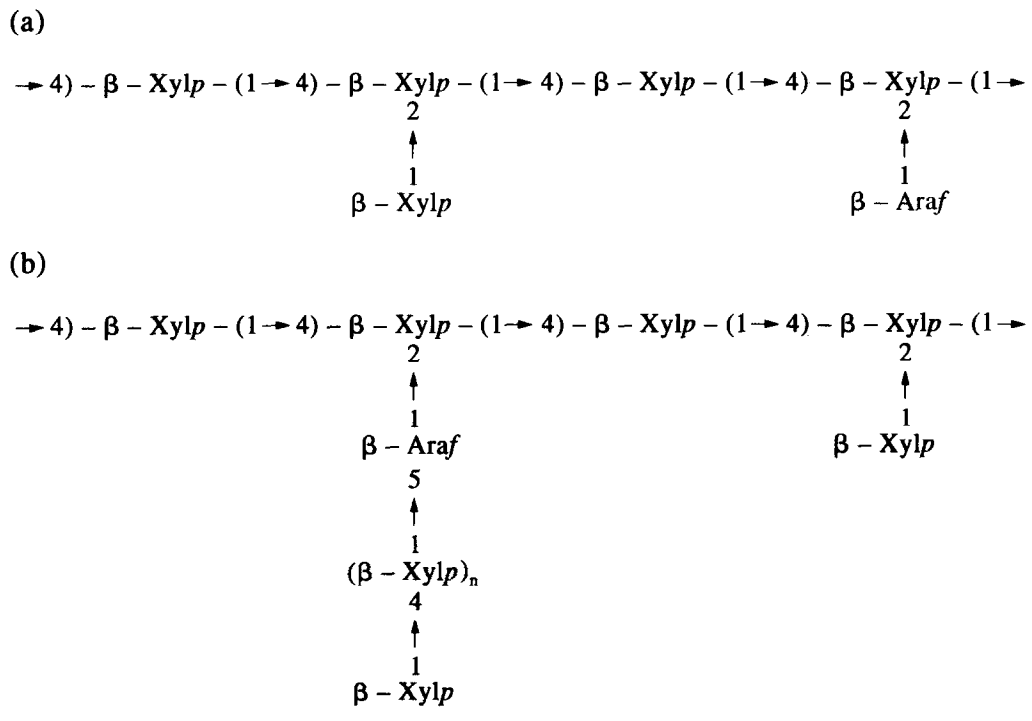

Fig.2.

the ^{1}[H] NMR integral of PS_4 supported the presence of three xylosyl and one arabinosyl residue in the tetrasaccharide. Signals which could be attributed to α-arabinofuranosyl residues were not observed in the ^{1}[H]- and ^{13}[C]- NMR spectra of these samples (Fig. 1).

Methylation analysis of PS_4 showed the presence of terminal xylopyranosyl residues and (1 → 4) linked xylosyl residues (Table 2). The absence of the arabinosyl residues in the methylation analysis of PS_4 may be attributed to the degradation by base during methylation, of the arabinosyl sugar residue at the reducing end of PS_4. The ^{1}[H]-NMR spectrum of PS_4, however, provides evidence for the presence of one Ara*f* residue in the tetrasaccharide. Hence PS_4 is probably a tetrasaccharide having the structure shown below and may have been derived from either the backbone of the polymer or from a side chain.

β-Xyl*p*-(1 → 4)-β-Xyl*p*-(1 → 4)-β-Xyl*p*-(1 → 5)-
β-Ara*f*-(1 →.

These results indicated that the PPS isolated from the leaves of *L. gardneri* contained a (1 → 4) linked β-D-xylopyranosyl backbone of which some of the xylopyranosyl residues were substituted either with a terminal xylopyranosyl/arabinofuranosyl residue or multiple unit side chains composed of both xylosyl and arabinosyl residues. The structure of PS_4 and stoichiometry of the methylation analyses suggests that some arabinosyl residues were incorporated in the backbone of the polysaccharide. This polysaccharide was less branched than the water-soluble polysaccharides previously isolated from *L. glutinosa* (Herath *et al.*, 1990) and *N. cassia* (de Silva *et al.*, 1986) belonging to the same family.

ACKNOWLEDGEMENTS

The authors thank the International Program in Chemical Sciences, Uppsala University, Sweden, for financial support. This work was sponsored by the Natural Resources, Energy and Science Authority of Sri Lanka.

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