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Are hemicelluloses from *Podocarpus lambertii* typical of gymnosperms?

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Abstract

Podocarpus lambertii, a Podocarpaceae, is a member of the most diverse family of conifers and *Araucaria angustifolia*, which belongs to the Araucariaceae family, is a typical gymnosperm from Southern Brazil. A structural characterisation of their hemicelluloses was carried out. The hemicelluloses were obtained by sequential and selective extraction with aqueous alkaline solvents, which resulted in the preparation of arabinoxylans (AGX) with different Xyl:Ara ratios, indicating the existence of distinct polysaccharides. Some are more exposed in the extraction process (higher arabinose content) and others which interact strongly with cellulose, had molecular mass differences, confirmed by HPSEC analysis. Isolated arabinoxylans and galactoglucomannans (GGM) had, by chemical and spectroscopic analysis, structural features similar to those found in other coniferous species. The hemicelluloses of *P. lambertii* and *A. angustifolia* had GGM:AGX ratios of 1.0:1 and 2.3:1, respectively. This proportion found in *A. angustifolia* is common to other gymnosperms. However, the content of AGX in *P. lambertii* is higher than expected, since that coniferous plants contain mainly GGM with a lower proportion of acidic arabinoglucuronoxylans. This discrepancy, added to the peculiar morphologic characteristics from the *Podocarpus* genus should probably be significant in the evolutionary process.

Keywords: Hemicelluloses; Secondary cell wall; *Podocarpus lambertii*; *Araucaria angustifolia*; Arabinoxylan; Galactoglucomannan

1. Introduction

Podocarpaceae is, morphologically and ecologically, the most diverse family of conifers (Kelch, 1998). It has a distinctive characteristic, namely the presence of an ovule-supporting structure, denominated epimatium. This feature plays a part in a controversy since the epimatium was homologised with the periantum or the second integument of the angiosperm ovule (Tomlinson, 1992). On the other hand Florin (1954), interpreted cone development as a homologue of the ovuliferous scale of the other conifers, e.g. Pinaceae.

The polysaccharides of the cell wall have been frequently considered as taxonomic indicators. Lufrano and Caffini (1981) compared the composition of the mucilage from the leaves of four different species of *Chorisia* by phytochemical analysis, suggesting a chemotaxonomic approach at genus and species levels. Reid and Edwards (1995) suggested that low-galactose galactomannans are present in the seeds of species belonging to the more primitive Caesalpiniaceae and medium to high galactose galactomannans are from more recent Fabaceae. The fine structure of galactomannans and arabinans from the seeds of *Schizolobium parahybae* and *S. amazonicum* have been also used as a chemotyping parameter (Petkowicz, Sierakowski, Ganter, & Reicher, 1998) and the results support the suggestion of Rizzini (1986) that they are not different species. Carpita and Gibeaut (1993) proposed molecular models for primary cell walls, involving their main polysaccharides and protein constituents and considered those from dicotyledonous species as Type I cell walls and from graminaceous monocotyledonea as Type II. Carpita, McCann, and Griffing (1996), then showed the components of primary cell wall to be a taxonomic character in the monocotyledonous species, ordering different groups. The hemicelluloses from secondary cell walls also have this potential since structurally distinct polysaccharides are components of cell walls from gymnosperms and angiosperms. Woody tissues from coniferous plants contain mainly galactoglucomannans (GGM) with a lower amount of arabinoglucuronoxylan, while angiosperms contain higher amounts of glucuronoxylan (Fengel & Wegener, 1989; Kubackova, Karacsonyi, & Bilisics, 1992; Sjöström, 1993). In a classical publication,

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Timell (1964) reported that in gymnosperms, the relative proportion of arabino-(4-*O*-methylglucurono)xylan varies considerably, even for species belonging to the same genus, consisting of about one third to one quarter of total hemicelluloses in the wood, whilst the major part is constituted by *O*-acetyl-GGM.

We now carry out a structural characterisation of the hemicelluloses from the stem of a Podocarpaceae (*Podocarpus lambertii*) and an Araucariaceae (*Araucaria angustifolia*), known in Brazil as pinheiro-bravo and pinheiro do Paraná, respectively. Both are native species of Southern Brazil, where *A. angustifolia* is considered to be a typical gymnosperm.

2. Experimental

2.1. Analysis of wood components

Total sugar—Determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using xylose and glucose as standards.

Uronic acids—Determined by the *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), using glucuronic acid as standard.

Lignin—Determined gravimetrically, by the Klason method (Adams, 1965).

Phenolic compounds—Described by Bochicchio, Ganter, and Reicher (2000), using the Folin-Ciocalteau reagent as in the Hartree method (Hartree, 1972). Coniferylic acid was used as standard.

Protein—Determined by the colorimetric method of Peterson (1977), using bovine serumalbumin as standard, and the method of Kjeldahl (Kabat & Mayer, 1964).

Monosaccharide composition—Polysaccharide fractions were hydrolysed with 1 M trifluoroacetic acid (5 h, 100 °C) or 72% w/v sulfuric acid (1 h, 0 °C), followed by dilution to 8% w/v (5 h, 100 °C), neutralisation with barium carbonate and filtration. Hydrolysates were reduced with sodium borohydride and acetylated with pyridine-acetic anhydride (1:1 v/v), at room temperature. The resulting alditol acetates were analysed by gas-liquid chromatography (GLC) using model, HP 5240, at 250 °C with a DB-225 column.

2.2. Preparation of hemicelluloses

The milled woody tissues of *P. lambertii* and *A. angustifolia* (30 g; 120 mesh) were extracted with toluene:ethanol (2:1, v/v) for 20 h in a Soxhlet apparatus and the dried material was then submitted to delignification by the chlorite method (Timell, 1965). In this 40% sodium chlorite (24.5 ml), acetic acid (3.5 ml) and distilled water (490 ml) were added to the defatted samples and the temperature maintained at 70 °C for 6 h (Bochicchio et al., 2000). The residues were filtered, washed with ethanol and dried. The delignified residues were submitted as follows to three procedures for extraction of hemicelluloses:

1. Initial extraction with 4 M NaOH, followed by 4 M KOH (*P. lambertii*; Fig. 1a).
2. Initial extraction with 4 M KOH, followed by 4 M NaOH (*P. lambertii*; Fig. 1b).

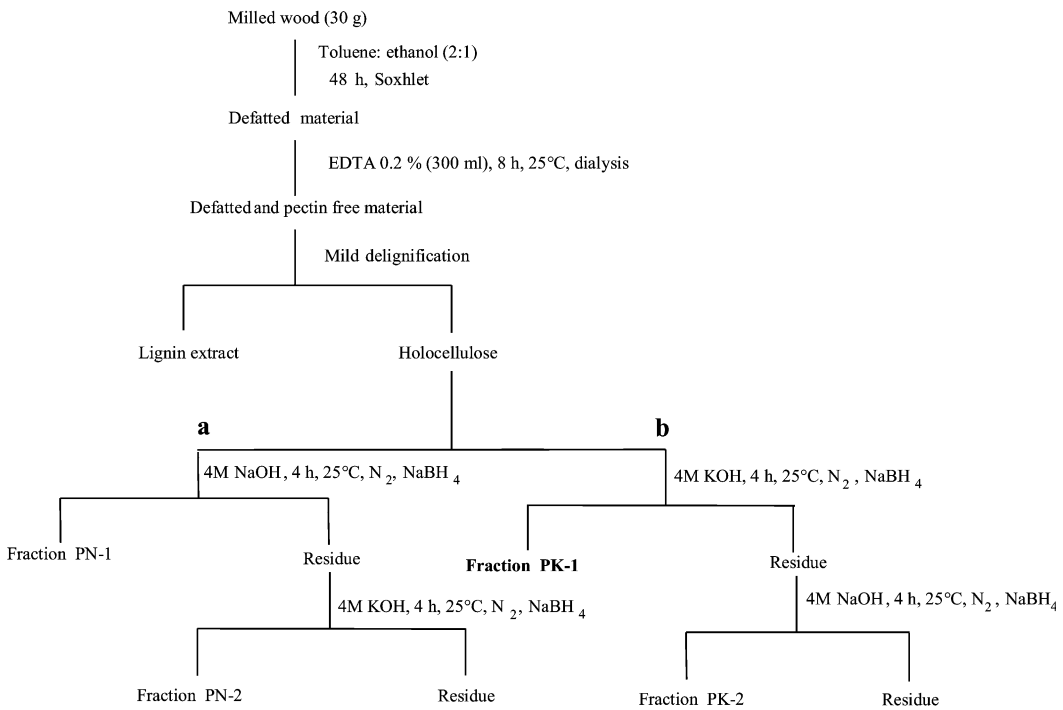

Fig. 1. Selective alkaline extraction of the stem from *P. lambertii*.

3. Sequential extraction with 1, 2, and 4 M KOH (twice), followed by 4 M NaOH (twice) (*P. lambertii*; Fig. 2). A similar procedure was used for *A. angustifolia*, beginning with 0.25, 0.5, 1, 2 and 4 M KOH, followed by 1 and 4 M NaOH (Fig. 3).

In each extraction step, a ratio of 1 g to 10 ml of solvent was used, at 25 °C for 4 h.

The alkaline extracts containing hemicellulosic fractions were acidified to pH 5 with acetic acid and dialysed for 3 days, and 4 volumes ethanol were then added to the solution. The final residue was suspended in water, neutralized, and dried.

Fractions of acidic arabinoxylan (AGX) (KOH soluble) from *P. lambertii* (PK-1; Fig. 1b; PK-A and PK-F; Fig. 2) and *A. angustifolia* (AK-A to AK-E; Fig. 3) were studied. The final extraction steps with NaOH, yielded fractions of galactoglucomannan (GGM) from *P. lambertii* (PK-G; Fig. 2) and *A. angustifolia* (AK-F; Fig. 3), which were submitted to structural analysis.

2.3. Analysis by HPSEC

The elution profiles of AGX of PK-1, PK-A and PK-F from *P. lambertii* and AK-A to AK-F from *A. angustifolia* were determined by high performance size-exclusion chromatography (HPSEC), using a WATERS 510 HPLC pump at 0.6 ml/min, with four gel permeation columns in series with exclusion sizes of one million to five thousand, using a refractive index (RI) detector. The eluent was 0.1 mol/l $NaNO_3$ with 200 ppm NaN_3. Samples, previously filtered in membrane (0.22 μm; Millipore), were injected (250 μl loop) at a 2 mg/ml concentration.

2.4. Structural characterisation

2.4.1. Methylation

The GGM fractions, PK-G and AK-F, were pre-*O*-methylated using the Haworth method (Haworth, 1915; Hirst & Percival, 1960), followed twice by that of Ciucanu and Kerek (1984). AGX fractions, PK-1, PK-A and AK-A, were methylated directly by the Ciucanu and Kerek method (twice). The methylated AGX fractions were subjected to methanolysis with methanol–HCl (2 ml), 2 h, 65 °C, followed by neutralisation with $AgCO_3$. The samples were hydrolysed with 0.5 M H_2SO_4 (1 ml), 6 h, neutralised with $BaCO_3$ and filtered. The methylated GGM fractions were directly hydrolysed by Saeman's method (Saeman, Moore, Mitchell, & Millet, 1954). The partially methylated alditol acetates were analysed by GLC VARIAN 3300 at 220 °C, coupled to a FINNIGAN MAT mass spectrometer, using DB-225 and DB-210 (30 m × 0.25 mm i.d.) capillary columns. Helium was used as the carrier gas.

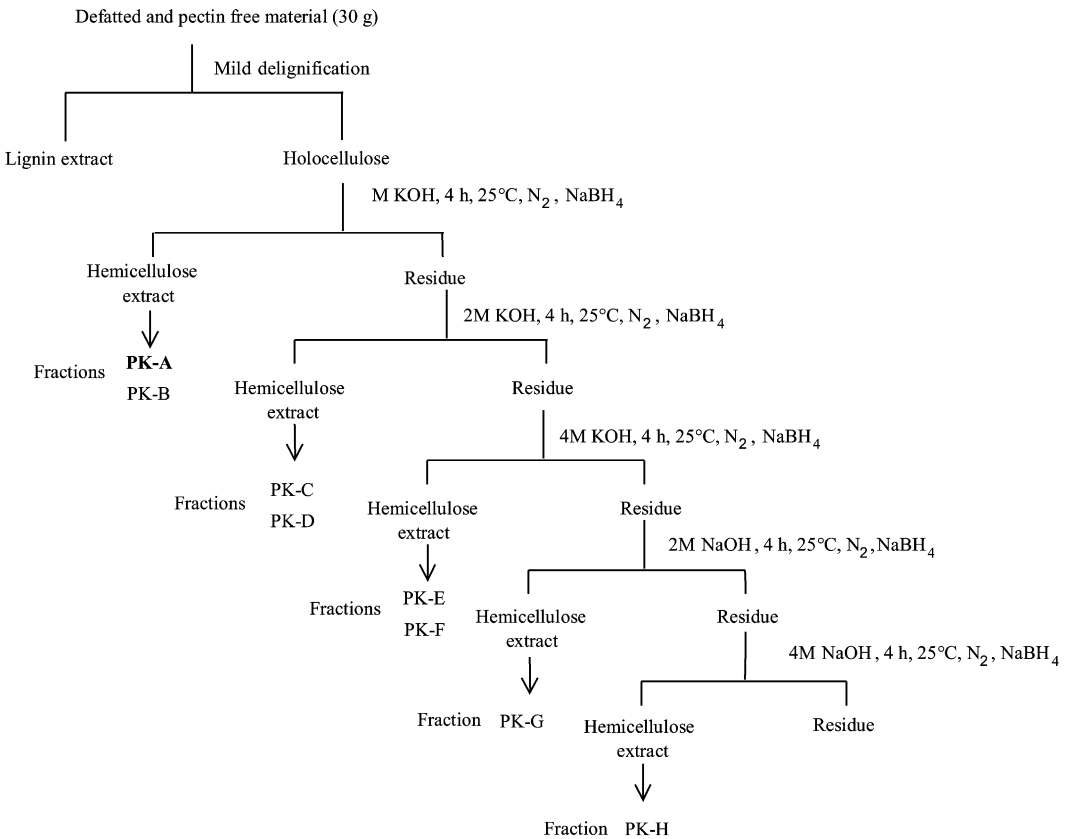

Fig. 2. Sequential alkaline extraction from *P. lambertii*.

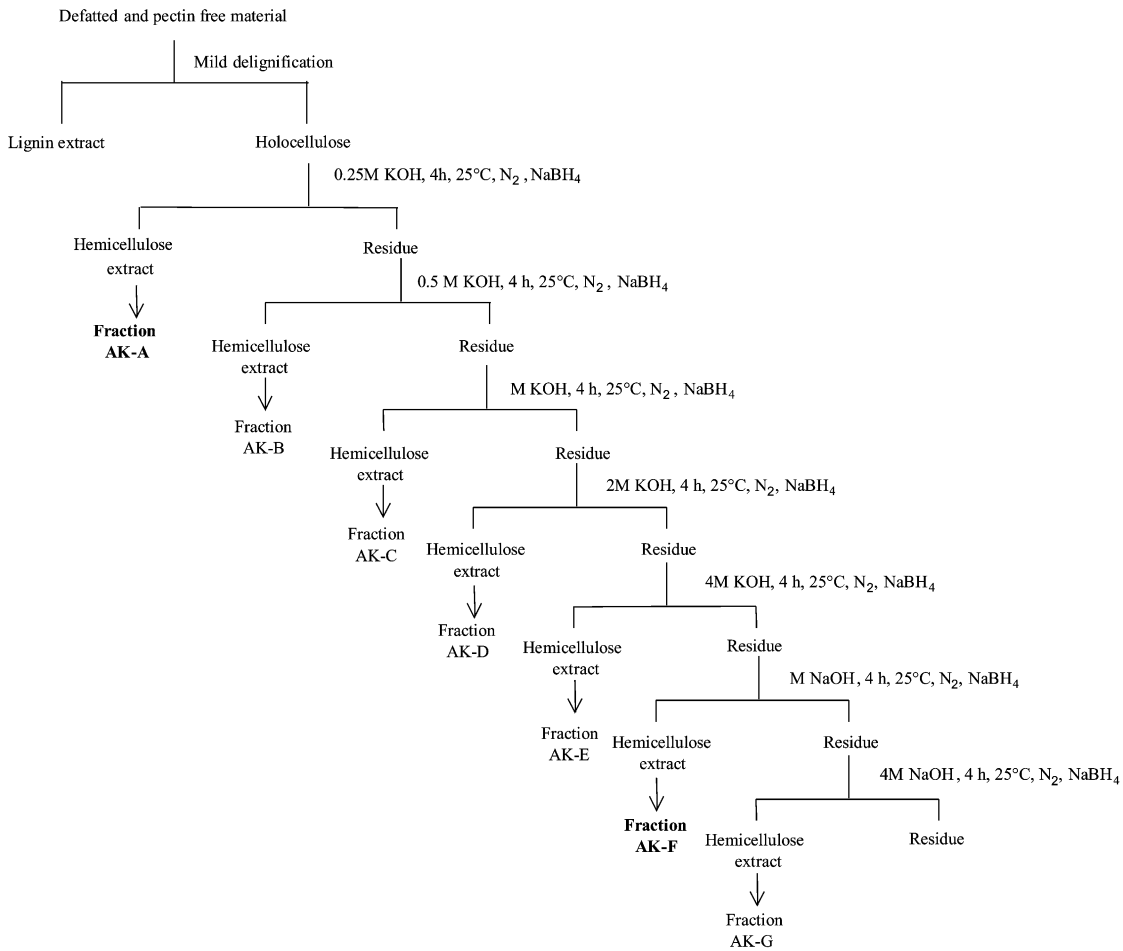

Fig. 3. Sequential alkaline extraction from *A. angustifolia*.

The partially methylated monosaccharides were reduced, acetylated and analysed by GC-MS.

2.4.2. NMR spectroscopy

Examinations were carried out by ^{13}C and ^{13}C DEPT for the AGX fraction (PK-1; AK-A), in D_2O at 30 °C and $^{1}H,^{13}C$ HMQC for the GGM fraction (PK-G; AK-F), in 1% NaOH in D_2O at 30 °C, both using a Bruker 400 MHz DRX spectrometer (shifts expressed as δ ppm, relative to Me_4Si, $\delta = 0$).

3. Results and discussion

P. lambertii, now used as a source of hemicelluloses, is native to Brazil, belonging to the Podocarpaceae family, gymnosperm division. The stem contains 32% lignin, a value common for other wood gymnosperms (Sarkanen & Hergert, 1971). The powdered wood was defatted with toluene:ethanol to remove lipids (0.8%) and then with EDTA to extract pectic material (0.1% w/w).

As the yield of hemicelluloses from *P. lambertii* was very low (0.5% w/w) when extracted with aqueous alkali, delignification by the chlorite method was carried out according to Timell (1965). In the present investigation, only half the amounts of sodium chlorite and acetic acid were employed. This modification resulted in the liberation of hemicelluloses from *P. lambertii* with a similar efficiency to alkali treatments (Bochicchio et al., 2000). Subsequent extraction with 4 M NaOH (Fig. 1a) gave rise to greater yields of hemicelluloses (PN-1 15.4% w/w; Fig. 1a; Table 1). The monosaccharide composition of PN-1 (Man:Xyl 1.2:1 ratio) indicates that it contains a mixture of acidic arabinoxylans (AGX) and GGM and that the relative proportion of xylose in PN-1 was higher than expected for gymnosperms, showing a higher content of GGM, in agreement with other investigations (Adams, 1965; Aspinall, 1959; Croon & Lindberg, 1958; Dutton & Hunt, 1960; Fengel & Wegener, 1989; Tyminski & Timell, 1960). A similar result for PN-1 was found when the delignified wood of *P. lambertii* was totally hydrolysed (cell wall fraction; Table 1; Man:Xyl 1.0:1 ratio).

Table 1
Monosaccharide composition of the cell wall and fractions obtained by sequential alkali extraction from *P. lambertii* wood

Fraction	Yield[a] (g%)	Monosaccharide composition (% mol)							
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids
Cell wall[b]	–	2.0	0.3	7.9	25.0	24.0	10.8	30.0	nd
PN-1 (4 M NaOH)	15.4	1.2	0.8	4.6	30.5	36.6	5.1	11.5	9.7
PN-2 (4 M KOH)	0.1	2.8	0.8	18.9	31.3	10.3	4.8	6.1	25.0

[a] From delignified residue.
[b] Hydrolysis with sulfuric acid (Saeman et al., 1954).

Table 2
Monosaccharide composition of the fractions obtained by selective alkali extraction from *P. lambertii* wood

Fractions	Yield[a] (g%)	Monosaccharide composition (mol%)								Phenolics (%)[b]
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids	
PK-1 (4 M KOH)	12.6	0.8	0.3	8.2	41.2	8.0	6.8	5.8	28.9	11.6
PK-2 (4 M NaOH)	6.5	0.3	0.1	1.3	5.8	62.4	2.7	19.7	7.7	5.3
Residue	–	1.4	–	4.5	10.0	28.5	6.7	48.9	nd	nd

[a] From delignified residue.
[b] Bochicchio et al. (2000) and Hartree (1972).

When the residue from the NaOH extract was then treated with 4 M KOH a fraction rich in xylose was obtained, although in low yield (PN-2; 0.1% w/w). This indicated that initial extraction with NaOH liberated 99% of the total available hemicelluloses.

An alternative extraction process was performed using aqueous KOH as the first solvent (Fig. 1b), which is selective to xylans (Capek et al., 2000; Timell, 1964, 1965). Results are shown in Table 2.

The main monosaccharide unit in PK-1 (4 M KOH; 12.6% w/w) was xylose (41.1%), indicating the presence of an arabinoxylan. In PK-2 (4 M NaOH; 6.5% w/w), mannose (62.4%) was the principal monosaccharide, indicating the presence of a galactoglucomannan. It is interesting to observe that the yields of PK-1 and PK-2 (19.1% w/w) are consistent with that of the fraction extracted initially with NaOH (PN-1; Table 1). This result confirms the efficiency and selectivity of KOH for obtaining arabinoxylan.

The yield of PK-1 is about twice greater than that of PK-2. However, this is probably due to the high content of phenolics, higher in PK-1 (Table 2). These were determined using the Folin-Ciocalteau reagent (Hartree, 1972), using coniferylic acid as standard (Bochicchio et al., 2000). Considering that the final residue of these extraction containing about 5% of hemicelluloses, this set of experiments confirms that *P. lambertii* wood contains similar amounts of AGX and GGM.

Treatment with increasing concentration of alkali can expose different AGX (Du Pont & Selvendran, 1987; Izydorczyk & Biliaderis, 1995; Sun, Lawther, & Banks, 1996). Therefore, another extraction strategy was used, beginning with a milder extraction (two times M KOH) to 4 M KOH and NaOH (Fig. 2). Results are shown in Table 3. The initial treatment with M KOH resulted in extraction of ~65% of available hemicelluloses, whose arabinose content was ~20% (PK-A). A similar arabinose content

Table 3
Monosaccharide composition of the fractions obtained by sequential alkaline extractions from *P. lambertii* wood

Fraction	Yield[a] (g%)	Monosaccharide composition (mol%)							
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids
PK-A (1 M KOH)	13.8	2.4	0.5	20.7	36.9	12.4	13.0	5.1	9.0
PK-B (1 M KOH)	1.0	1.3	0.3	17.5	26.2	22.8	13.1	6.8	12.0
PK-C (2 M KOH)	2.2	0.2	0.4	6.7	31.7	29.1	5.2	9.8	16.9
PK-D (2 M KOH)	1.0	0.8	0.5	5.0	19.8	38.4	7.2	13.6	14.7
PK-E (4 M KOH)	0.7	0.7	0.4	4.7	22.9	38.3	5.2	12.9	13.9
PK-F (4 M KOH)	0.2	1.4	0.5	6.4	21.5	33.0	17.0	11.1	9.1
PK-G (4 M NaOH)	1.6	0.3	0.1	1.3	3.0	71.3	2.3	18.6	3.1
PK-H (4 M NaOH)	0.8	0.4	0.1	1.8	5.9	67.3	2.5	19.0	3.0

[a] From delignified residue.

Table 4
Monosaccharide composition of the cell wall and fractions obtained by sequential alkaline extractions from *A. angustifolia*

Fraction	Yield[a] (g%)	Monosaccharide composition (mol%)							
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids
Cell wall[b]		1.3	–	4.4	15.9	36.0	7.1	35.3	nd
AK-A (0.25 M KOH)	4.0	1.6	–	14.2	49.4	3.6	8.0	6.1	17.1
AK-B (0.5 M KOH)	1.3	0.9	–	10.6	57.6	7.9	6.1	3.3	13.6
AK-C (1 M KOH)	1.4	0.6	–	9.0	49.1	12.2	4.8	5.3	19.0
AK-D (2 M KOH)	1.8	0.6	0.2	7.9	43.9	16.1	5.2	6.0	20.1
AK-E (4 M KOH)	0.6	0.3	0.3	8.5	42.2	25.7	3.4	6.1	13.5
AK-F (M NaOH)	0.4	0.3	–	2.9	12.0	57.6	5.2	15.6	6.4
AK-G (4 M NaOH)	2.0	0.8	–	1.7	7.3	67.9	2.1	15.7	4.5

[a] From delignified material.
[b] Hydrolysis with sulfuric acid (Saeman et al., 1954).

was found for PK-B, although this fraction was obtained in a lower yield. The Xyl:Ara ratio in subsequent fractions (PK-C to PK-F) increased with concentration of alkali, suggesting the presence of different molecular families. PK-A was further submitted to structural analysis.

It was observed that comparing PK-B until PK-F (Table 3), mannose and glucose increased and the mannose to glucose ratio was almost constant (~3.5:1), indicating that GGM with the same structure are probably present in these fractions. The existence of arabinose and xylose in these fractions indicate that AGX are also present. An increase in the concentration of KOH was employed in order to obtain GGM free of AGX, as can be observed in PK-G, obtained with NaOH (71.3% mannose; 18.6% glucose. Thus the stem from the pinheiro do Paraná (*A. angustifolia*), a typical gymnosperm from Southern Brazil, were used for comparative analysis.

Initially, delignified wood of *A. angustifolia* gave in a Man:Xyl ratio of 2.3:1 (cell wall fraction; Table 4), in agreement with to other studied gymnosperm species (Croon & Lindberg, 1958; Fengel & Wegener, 1989; Kooiman & Adams, 1961; Meier, 1958; Tyminski & Timell, 1960), and in contrast with the 1.0:1 ratio obtained for *P. lambertii,* under the same experimental conditions (Table 1).

Knowing that 1 M KOH proved to be efficient in extracting most available hemicelluloses from secondary cell wall, the extraction process was initiated with 0.25 M KOH (Table 4). This concentration extracted 4% w/w of hemicellulose (AK-A) with a relativity high arabinose substitution (Xyl:Ara (3.5:1) ratio). The subsequent fractions (AK-B to AK-G) yielded about 7.5% w/w of hemicelluloses with lower degrees of substitution. Successive treatments with KOH extracted AGX and the final treatment with NaOH solubilised the available GGM, according to the results found with *P. lambertii*.

Fractions AK-A (AGX) and AK-F (GGM) were further submitted to structural analysis.

3.1. HPSEC analysis

PK-1, PK-A and PK-F (*P. lambertii*), AK-A to AK-F (*A. angustifolia*) water-soluble fractions, rich in AGX, were submitted to HPSEC analysis. The filtration process (0.22 μm pore) eliminated GGM contamination. This polysaccharide, due to its low water solubility, was retained in the membrane.

Fraction PK-1, obtained directly with 4 M KOH (Fig. 4), showed two distinct peaks with predominance of peak II (lower molar mass). Fraction PK-A (initial extraction with 1 M KOH; Fig. 1b) resulted in a heterogeneous elution profile with two peaks, although showing greater amounts of AGX with a high molecular mass. It is interesting to observe that PK-F (final extraction with 4 M KOH; Fig. 2) gives

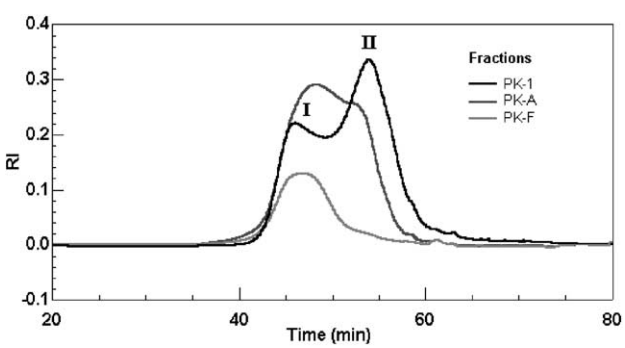

Fig. 4. HPSEC of the AGX (fractions PK-1, PK-A and PK-F) from *P. lambertii*.

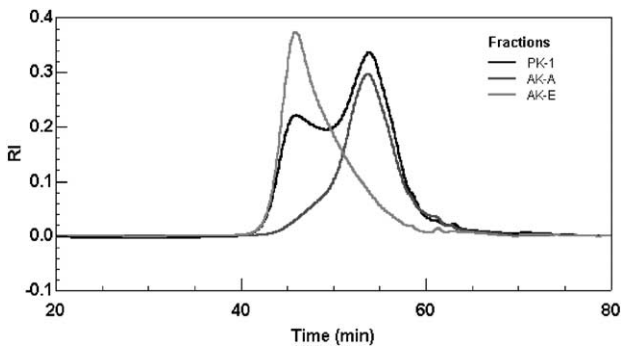

Fig. 5. HPSEC of the fractions KOH soluble PK-1 (*P. lambertii*), AK-A and AK-E (*A. angustifolia*).

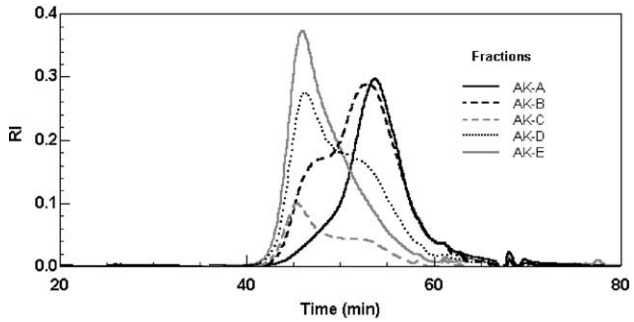

Fig. 6. HPSEC of the KOH fractions from *A. angustifolia*.

Table 5
^{13}C NMR signals of the fraction PK-1 from *P. lambertii*

	Chemical shift (ppm)				
	C-1	C-4	C-5	C-6	OMe
→ 4)-βXyl*p*-(1-	101.5	73.5	62.8	–	
→ 4)-βXyl*p*-(1-substituted	101.2			–	
→ 3)-αAra*f*-(1-	107.5		61.2	–	
→ 2)-4-*O*Me-αGlcA*p*-(1-	97.4			176.6	59.6

only one AGX (corresponding to peak I), suggesting that it is probably intimately linked with other cell wall components, while more highly substituted AGX of low molecular mass could be more exposed, because it was obtained with a low concentration of alkali.

This hypothesis can be demonstrated comparing AGX of *P. lambertii* and *A. angustifolia* (Fig. 5). Fraction AK-A, extracted by 0.25 M KOH from *A. angustifolia,* was practically homogeneous, with a peak corresponding to PK-1 (*P. lambertii*) of lower molecular mass (peak II). The increase in concentration of alkali (4 M KOH; AK-F) liberated only AGX with higher molecular masses (Fig. 4), the same for peak I from PK-1 and PKF (Fig. 4). Fig. 6 confirms these results where the molecular mass increased with the alkali concentration from AK-A to AK-E from *A. angustifolia*.

These results confirm the existence of a family of AGX, some being more exposed (higher arabinose content, Table 4) and others interacting strongly with cellulose, with molecular mass differences between these AGX. Izydorczyk and Biliaderis (1995), studying hemicelluloses from cereals, demonstrated the existence of the several families of AGX, obtained by diverse extraction methods.

3.2. Structural characterisation of AGX fractions

AGX from *P. lambertii* and *A. angustifolia* (PK-1 and AK-A, respectively) were submitted to NMR and methylation analysis.

The ^{13}C NMR examination of PK-1 (Table 5) gave a complex spectrum, whose anomeric region contained two signals, corresponding to β-Xyl*p*, substituted at O-2 (δ 101.2) and non-substituted (δ 101.5) with α-arabinofuranosyl units attached to O-3 of a monosubstituted xylose residue in the main chain (δ 107.5). The 4-*O*-substituted β-Xyl*p* units in the main chain is confirmed by a δ 73.5 signal. The C-5 signal at δ 62.8 (Ara*f*) and δ 61.2 (Xyl*p*), were indicated by inverted ^{13}C DEPT signals (Fig. 7).

The presence of 4-*O*-Me-α-uronic acid was confirmed by signals at δ 97.4 and δ 176.6 corresponding to C-1 and C-6, respectively; a signal at δ 59.6 showed the *O*Me groups. The presence of fragments of lignin was demonstrated by a signal

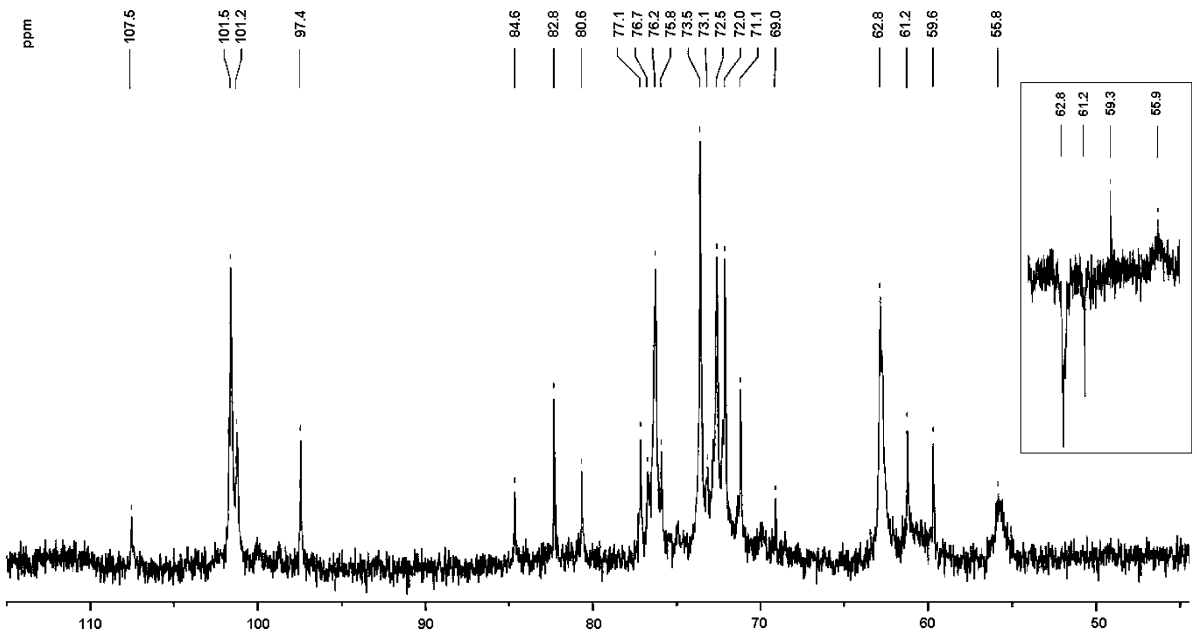

Fig. 7. ^{13}C NMR and ^{13}C DEPT spectrum of the fraction PK-1 in D_2O.

Table 6
Analysis of the partial methylated monosaccharides from the acidic arabinoxylan (fractions PK-1 and AK-A)

O-methyl alditol acetate	Fragments (*m/z*)	*P. lambertii* (PK-1) (%mol)	*A. angustifolia* (AK-A) (%mol)
2,3,5-Me_3 Ara	87, 101, 117, 129, 145, 161	12.3	15.3
2,3-Me_2 Ara	87, 101, 117, 129, 189	3.2	–
6-Deoxy-2 Me Hex	87, 99, 117, 129, 159, 173, 201	1.2	–
2,3,4-Me_3 Xyl	87, 101, 117, 129, 145, 161	3.2	2.8
2,3-Me_2 Xyl	87, 101, 117, 129, 161, 189	59.8	59.9
6-Deoxy-3 Me Hex	87, 101, 117, 129, 143, 189, 203	1.3	–
2-Me Xyl	87, 99, 129, 145, 189	9.0	9.4
3-Me Xyl	85, 87, 99, 113, 117, 129, 159	10.0	9.6

Table 7
^{13}C NMR signals of the fraction PK-G from *P. lambertii*

	Chemical shift (ppm)					
	C-1	C-2	C-3	C-4	C-5	C-6
→ 4)-Man*p*-(1-	100.2	70.3	71.7	76.4	75.3	60.7
→ 4)-Glc*p*-(1-	102.7	73.2	75.0	78.6	74.5	60.5

in δ 55.8, arising from methoxyl groups (Wallace et al., 1995), released after chlorite treatments. Similar assignments of all signals were obtained to AK-A.

These NMR data were complemented with methylation analysis, where the partially *O*-methylated alditol acetates obtained by hydrolysis from the fractions were identified by GC-MS. The results are listed in Table 6.

The (1 → 4) linkage in the main chain of the PK-1 was identified through 2,3-Me_2Xyl (59.8%). This peak could be also, under the experimental conditions used, 3,4-Me_2Xyl, however, this is not likely. The main chain is partially substituted at O-3 by arabinose (2,3,5-Me_3Ara) or uronic acids probably at O-2. The 2,3-Me_2Ara can arise from an ether linkage at O-5 of arabinose units with phenolic residues from the wood lignin, as shown by Izydorczyk and Biliaderis (1994), Sjöström (1993) and Wallace et al. (1995), who investigated hemicelluloses of the straw cell wall, also identifying the presence of this methylated derivative and attributing it to an intermediary arabinose between xylose of the main chain and terminal arabinose. Rhamnose or fucose derivatives were also identified, according to its monosaccharide composition. The presence of these monosaccharides in all hemicellulose fractions

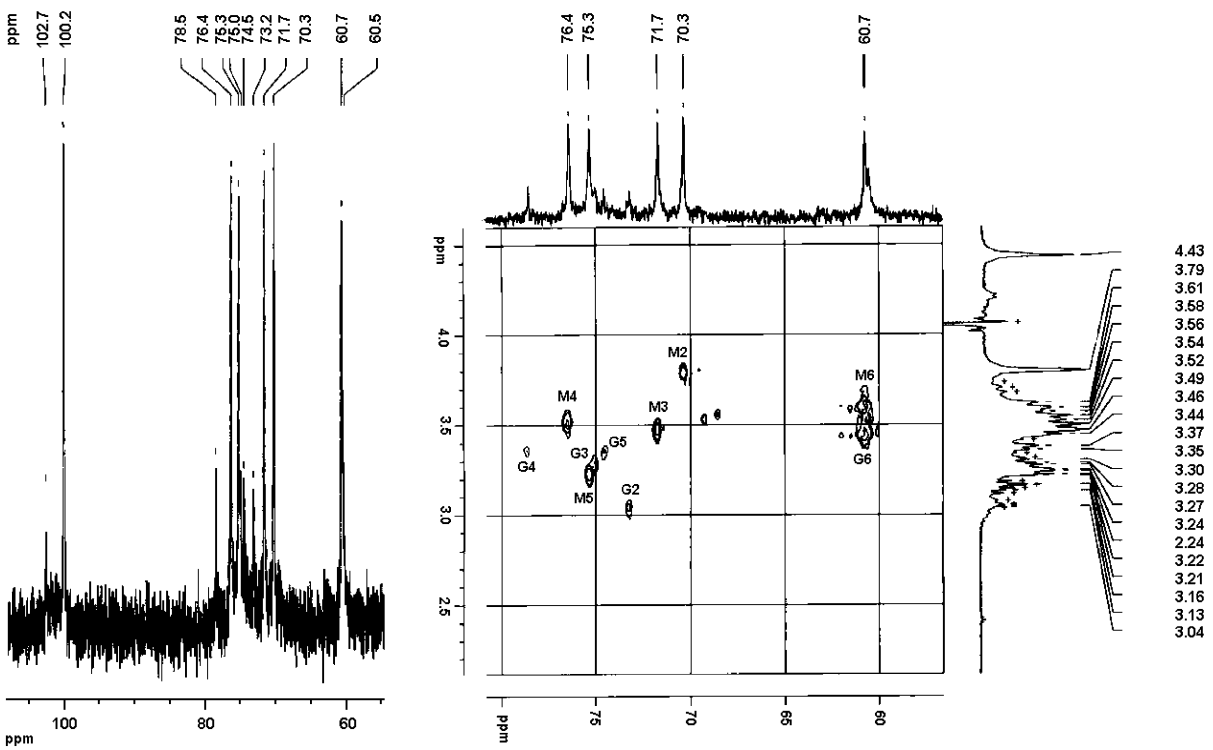

Fig. 8. ^{13}C NMR and HMQC spectrum of the fraction PK-G in 1% NaOD.

Table 8
Analysis of the partial methylated monosaccharides from the galactoglucomannan (fractions PK-G and AK-F)

O-methyl alditol acetate	Fragments (*m/z*)	*P. lambertii* (PK-G) (mol%)	*A. angustifolia* (AK-F) (mol%)
2,3,6-Me_3Man	99,101,113,117,129,131,161,173	71.0	75.2
2,3,6-Me_3Glc	99,101,113,117,129,131,161,173	23.3	19.0
2,3,4,6-Me_4Man	87,101,117,129,145,161,205	1.8	1.0
2,3,4,6-Me_4Glc	87,101,117,129,145,161,205	0.8	0.4
2,3,4,6-Me_4Gal	87,101,117,129,145,161,205	1.1	1.5
2,3-Me_2Man	87,101,117,129,143,203	2.0	1.8
2,3-Me_2Glc	87,101,117,129,143,203	–	1.1

from *P. lambertii* suggest the possibility of their participation of the AGX. The AGX of *A. angustifolia* (AK-A) showed similar structural characteristics for the AGX of *P. lambertii*, except for the absence of the 2,3-*O*-methylated derivative of arabinose (Table 6). Neither rhamnose nor fucose derivatives were found. The monosaccharide composition and structural analyses of AK-E (methylation and NMR), demonstrated structural characteristics similar to the other AGX, except for lower 3-*O*-Me Xyl contents in AK-E (data not shown).

3.3. Structural characterisation of GGM fractions

After exhaustive extraction of AXG by KOH, the GGMs were sequentially obtained by NaOH extraction (Fig. 2) with low AGX contaminants. The monosaccharide ratios of *A. angustifolia* (Man:Gal:Glc 4.0:1:0.1) and *P. lambertii* (Man:Gal:Glc 3.5:1:0.1) show them to be GGM ratios typical of other gymnosperms. The selected fractions (PK-G; *P. lambertii*; Fig. 2 and AK-F; *A. angustifolia*; Fig. 3) were submitted to structural elucidation.

^{13}C NMR examination of PK-G indicated twelve signals characteristics of a linear glucomannan (Table 7). The low amount of galactose did not manifest itself in the spectrum.

The signals of the anomeric carbons indicate Man*p* and Glc*p* units with a β configurations. The possible partial overlapping of C-6 signals of Man*p* and Glc*p* was resolved through HMQC spectrum (Fig. 8), which amplified the separation through carbon and proton correlation.

The methylated derivatives were obtained in order to confirm the NMR data. Table 8 indicates the partially methylated alditol acetates from PK-G and AK-F, which was approximately consistent with the Man:Glc:Gal ratio.

The 2,3,6-Me_3 Man and 2,3,6-Me_3Glc components represent (1 → 4) linked mannosyl and glucosyl residues, respectively. Both are from the main chain, being eventually substituted by galactosyl residues (2,3,4,6-Me_4Gal). In PK-G (*P. lambertii*) only mannose is substituted at O-6 (2,3-Me_2Man; 2.0%), whilst in AK-F (*A. angustifolia*) mannosyl and glucosyl residues are attached to galactose, both through O-6.

4. Conclusions

Acidic AGX and GGM were found in *P. lambertii* and *A. angustifolia*, aqueous NaOH and KOH being used in order to obtain the hemicelluloses. With NaOH, mixtures of AGX and GGM were obtained whereas KOH was selective for AGX. HPSEC analysis showed the existence of a family of AGX, in both species, some being more exposed and others interacting strongly with cellulose. Increases in concentration of alkali liberated only AGX with a higher molecular mass. It should be to emphasize the use of HPSEC has not been common in the comparative analysis of AGX in different species. Isolated AGX and GGM showed structural features similar to those found in other coniferous species. However, the Man:Xyl ratio of *P. lambertii* is 1.0:1 (cell wall fraction; Table 1), contrasting with Man:Xyl ratio from *A. angustifolia* that is 2.3:1 (cell wall fraction; Table 4). This is a proportion obtained in other investigations and which confirms a greater presence of GGM in gymnosperms (Croon & Lindberg, 1958; Fengel & Wegener, 1989; Kooiman & Adams, 1961; Meier, 1958; Sjöström, 1993; Tyminski & Timell, 1960). The discrepancy in *P. lambertii*, added to peculiar morphologic characteristics should probably be significant in the evolutionary process of the *Podocarpus* genus.

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