Isolation and structural characterization of the milled-wood lignin from *Paulownia fortunei* wood

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ABSTRACT

The chemical structure of the milled-wood lignin isolated from *Paulownia fortunei* wood was investigated. The lignins were characterized by analytical pyrolysis and two-dimensional NMR that indicated a predominance of guaiacyl (G) over syringyl (S) units, and only showed small amounts of *p*-hydroxyphenyl (H) units, with a H:G:S molar ratio of 1:59:40. The heteronuclear single quantum correlation (HSQC) NMR spectrum gave additional information about the relative abundances of the different inter-unit linkages present in the lignin structure. Paulownia lignin showed a predominance of β-O-4′ aryl ether linkages (62% of total side-chains), followed by β-β′ resinol-type (12%) and β-5′ phenylcoumaran-type structures (11%) and with lower amounts of other condensed structures such as spirodienone (3%) and dibenzodioxocin (2%) structures. The high lignin content (*ca.* 23% Klason lignin), together with the low S/G ratio and the abundance of condensed (carbon–carbon linked) structures (together with the low abundance of the most labile ether-linked structures) could contribute to the low reactivity of paulownia lignin during alkaline pulping.

1. Introduction

Paulownia is a fast-growing shade tree native to China and South-East Asia that is grown commercially for the production of hardwood timber (Bergmann, 1998). Paulownia wood has also been investigated as a promising raw material for the production of chemical pulp (Olson and Carpenter, 1985; Rai et al., 2000; Jiménez et al., 2005; Caparrós et al., 2007, 2008). The most suitable species of paulownia for this purpose is *Paulownia fortunei*, with an average fiber length of 1.42 mm (Rai et al., 2000). This species is characterized by its fast development and a uniform and regular growth (Ede et al., 1997). Under the appropriate conditions, a 5–7-year-old tree can reach about 15–20 m high and the annual production is as high as 150 tons/ha (Jiménez et al., 2005).

The growing interest in paulownia wood as a promising chemical feedstock has stimulated recent studies on the structural characteristic of its main constituents. Among these, the composition and structural features of xylans and extractives isolated from paulownia wood have recently been addressed (Gonçalves et al., 2008; Silvestre et al., 2005). However, there is a lack of studies regarding the structural characteristics of the lignin polymer, despite its great influence during the industrial processing of wood, such as pulping and papermaking. Pulping and bleaching performances are highly dependent on the relative content, structure and reactivity of the three wood structural biopolymers, cellulose, hemicelluloses and lignin. Particularly, the lignin content and its composition in terms of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) moieties as well as the nature of the different inter-unit linkages present in its structure are important factors in pulp production affecting the delignification rates, chemical consumption and pulp yields. The lower the lignin content and the proportion of G-lignin and condensed units, the less energy and chemicals are needed for alkaline pulping and subsequent bleaching. It has already been shown that higher S/G ratios in hardwoods implied higher delignification rates, less alkali consumption and therefore higher pulp yields (González-Vila et al., 1999; del Río et al., 2005).

It is therefore clear that, in order to optimize the use of paulownia wood as raw material for paper pulp manufacture, more information needs to be acquired on the composition and structure of its lignin polymer. In this paper, we report the composition and structural characteristics of the lignin from the wood of *P. fortunei*. For this purpose, the milled-wood lignin (MWL) of paulownia wood was isolated and characterized by pyrolysis–gas chromatography/mass spectrometry (Py–GC/MS) and two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy.

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2. Materials and methods

2.1. Samples

The wood was collected from 3-year-old trees of *Paulownia fortunei*, provided by the University of Huelva, Spain. The air-dried wood was milled using a knife mill (Janke and Kunkel, Analysenmühle), and successive extracted with acetone in a Soxhlet apparatus for 8 h and hot water (3 h at 100 °C). Klason lignin content was estimated as the residue after sulfuric acid hydrolysis of the pre-extracted material according to Tappi rule T222 om-88 (Tappi, 2004). The MWL samples (Björkman, 1956) were obtained from grounded wood prepared in a Retsch cutting mill to pass through a 100-mesh screen. This sawdust was successively extracted with acetone for 8 h in a Soxhlet extractor and with hot water (100 °C) for 3 h. The extractive free sawdust was finely ball-milled in a Retsch S100 centrifugal ball mill with toluene (*ca.* 50 h) using agate jar and balls. After this treatment the milled wood was submitted to an extraction (3× 12 h) with dioxane–water (9:1, v/v) (25 ml of solvent/g of milled wood). The solution was centrifuged and the supernatant subsequently evaporated at 40 °C at reduced pressure until dryness. The residue obtained (raw MWL) was redissolved in a solution of acetic acid/water 9:1 (v/v) (20 ml of solvent/g of raw MWL). The solution was then precipitated in water and the precipitated residue was separated by centrifugation, milled in an agate mortar and subsequently dissolved in a solution of 1,2-dicloromethane:ethanol (1:2, v/v). The mixture was then centrifuged to eliminate the insoluble material, the supernatant was precipitated in diethyl ether and the obtained residue was separated by centrifugation. This residue was then resuspended in petroleum ether and centrifuged again to obtain the final purified MWL fraction, which was dried under a N_2 current. The final yield was *ca.* 20% of the original Klason lignin content. Underivatized and acetylated MWL, after 48 h treatment in acetic anhydride-pyridine (1:2) were analyzed as described below.

2.2. Py–GC/MS

Pyrolysis of MWL (approximately 100 μg) was performed with a 2020 micro-furnace pyrolyzer (Frontier Laboratories Ltd.) connected to an Agilent 6890 GC/MS system equipped with a DB-1701 (Agilent J&W) fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5973 mass selective detector (EI at 70 eV). The pyrolysis was performed at 500 °C. The oven temperature was programmed from 40 °C (1 min) to 300 °C at 6 °C min^{-1} (10 min). He was the carrier gas (1 ml min^{-1}). The compounds were identified by comparing their mass spectra with those of the Wiley and NIST libraries and reported in the literature (Faix et al., 1990; Ralph and Hatfield, 1991). Peak molar areas were calculated for the lignin-derived products, the summed areas were normalized to 100, and the data for two repetitive analyses were averaged and expressed as percentages.

2.3. NMR spectroscopy

2D-NMR spectra of underivatized and "in vitro" acetylated MWL were recorded at 25 °C in a Bruker AVANCE 500 MHz, equipped with a z-gradient triple resonance probe. Around 40 mg of MWL were dissolved in 0.75 mL of dimethylsulfoxide (DMSO)-d_6, and HSQC (heteronuclear single quantum correlation) spectrum was recorded. The spectral widths were 5000 and 13,200 Hz for the ^{1}H and ^{13}C dimensions, respectively. The number of collected complex points was 2048 for ^{1}H dimension, with a recycle delay of 5 s. The number of transients was 64, and 256 time increments were always recorded in ^{13}C dimension. The $^1J_{CH}$ used was 140 Hz. The J-coupling evolution delay was set to 3.2 ms. Squared cosine-bell apodization function was applied in both dimensions. Prior to Fourier transformation, the data matrixes were zero filled up to 1024 points in the ^{13}C dimension. The central solvent (DMSO) peak was used as an internal reference (δ_C 40.1; δ_H 2.50 ppm). HSQC cross-signals were assigned by comparing with the literature (Ralph et al., 1999, 2004b; Liitiä et al., 2003; Capanema et al., 2004, 2005; Ibarra et al., 2007a,b; Rencoret et al., 2008; Martínez et al., 2008; del Río et al., 2008).

A semiquantitative analysis of the intensities of the HSQC cross-signal intensities was performed (Liitiä et al., 2003; Heikkinen et al., 2003; Zhang and Gellerstedt, 2007). Since the cross-signal intensity depends on the particular $^1J_{CH}$ value, as well on the T_2 relaxation time, a direct analysis of the intensities is elusive. Thus, integration was performed separately for the different regions of the spectra, which contain signals corresponding to chemically analogous C–H pairs, with similar $^1J_{CH}$ coupling values. In the aliphatic oxygenated region, inter-unit linkages were estimated from C_α–H_α correlations, except for structures **I** described below where the C_γ–H_γ correlations were used, and the relative abundance of side-chains involved in the different substructures and terminal structures were calculated. In the aromatic region, C–H correlations from S and G units were used to estimate the S/G ratio, and the comparison of the intensities of the C_β–H_β correlations of **I** and **J** structures were used to estimate the abundances of the latest. The amount of phenolic and alcoholic hydroxyls in MWL was estimated by integrating the methyl signals of the corresponding acetates, after "in vitro" acetylation (compared with underivatized MWL). The percentage of phenolic aromatic units was calculated by referring the phenolic acetate signal to the total number of aromatic rings (H + G + S), which was estimated from the intensity of the methoxyl signal (δ_C/δ_H 56/3.7 ppm) and taking into account the H:G:S ratio.

3. Results and discussion

The lignin content of *P. fortunei*, estimated as Klason lignin, is relatively high (22.7%), in agreement with previous works (Jiménez et al., 2005; Gonçalves et al., 2008). This content is comparable to other hardwoods commonly used for papermaking, such as eucalypt, with lignin content around 18–23% (Rencoret et al., 2007). However, the delignification reactions and therefore the pulping efficiency is not only affected by the lignin content but is also greatly influenced by the lignin composition and structure. Therefore, we have thoroughly studied the lignin composition and structure of paulownia wood. For this, the MWL was isolated by aqueous dioxane extraction from finely ball-milled wood according to the classical lignin isolation procedure (Björkman, 1956). MWL is considered to be representative of the whole native lignin in the plant, in spite of its low yield and the possibility of some modifications during milling (Holtman et al., 2006). The lignin composition was analyzed by means of Py–GC/MS, which allows for the rapid characterization of the lignin H:G:S composition, and then a more detailed characterization of the lignin substructures present was performed by 2D-NMR of the isolated MWL, that gives information on the different inter-unit linkages present.

3.1. Py–GC/MS

Py–GC/MS is a rapid and sensitive technique for analyzing the composition of lignin (Faix et al., 1990; Ralph and Hatfield, 1991). It has been widely applied for the calculation of S/G ratios in different hardwoods (Rodrigues et al., 1999; Yokoi et al., 1999, 2001; del Río et al., 2005; Rencoret et al., 2007, 2008; Marques et al., 2008). The pyrogram of paulownia MWL is shown in Fig. 1 and the identities and relative molar abundances of the released lignin compounds are listed in Table 1.
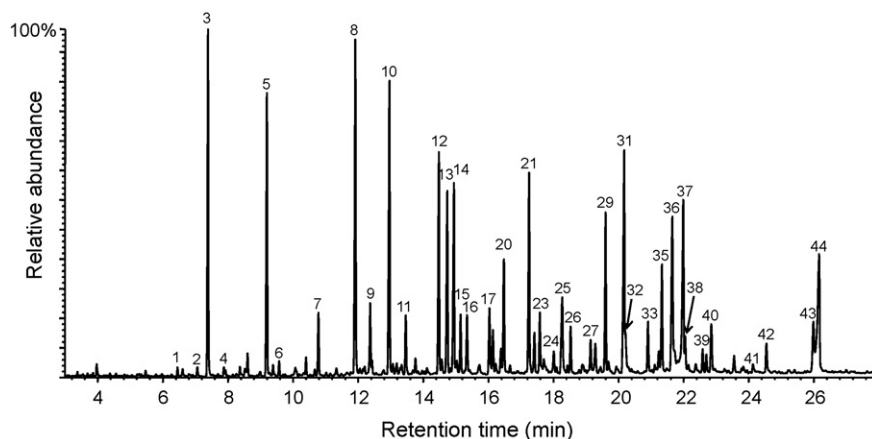
Fig. 1. Py–GC/MS chromatogram of the MWL isolated from *Paulownia fortunei* wood. The numbers refer to the compounds listed in Table 1.

Table 1

Identification and relative molar abundance (%) of the compounds identified in the Py–GC/MS of MWL from *Paulownia fortunei* wood.

Label	Compound	MW (amu)	Relative abundance (%)	Origin
1	4-Hydroxybenzaldehyde	122	0.2	H
2	Phenol	94	0.3	H
3	Guaiacol	124	9.0	G
4	Methylphenol	108	0.2	H
5	4-Methylguaiacol	138	6.8	G
6	Dimethylphenol	122	0.3	H
7	4-Ethylguaiacol	152	1.5	G
8	4-Vinylguaiacol	150	8.3	G
9	Eugenol	164	1.5	G
10	Syringol	154	7.5	S
11	*cis*-Isoeugenol	164	1.3	G
12	*trans*-Isoeugenol	164	4.8	G
13	4-Methylsyringol	168	4.1	S
14	Vanillin	152	4.8	G
15	Propine-guaiacol	162	1.3	G
16	Propine-guaiacol	162	1.4	G
17	Homovanillin	166	1.7	G
18	4-Ethylsyringol	182	0.9	S
19	Vanillic acid methyl ester	182	0.5	G
20	Acetoguaiacone	166	2.6	G
21	4-Vinylsyringol	180	4.2	S
22	Guaiacyl-acetone	180	0.9	G
23	4-Allylsyringol	194	1.1	S
24	Propiovanillone	180	0.5	G
25	Guaiacyl vinyl ketone	178	2.2	G
26	*cis*-Propenylsyringol	194	0.8	S
27	Propine-syringol	192	0.6	S
28	Propine-syringol	192	0.7	S
29	*trans*-Propenylsyringol	194	3.0	S
30	Dihydroconiferyl alcohol	182	0.2	G
31	Syringaldehyde	182	5.3	S
32	*cis*-Coniferyl alcohol	180	0.8	G
33	Homosyringaldehyde	196	1.0	S
34	Syringic acid methyl ester	212	0.3	S
35	Acetosiryngone	196	2.0	S
36	*trans*-Coniferyl alcohol	180	4.3	G
37	*trans*-Coniferaldehyde	178	4.7	G
38	Syringylacetone	210	0.5	S
39	Propiosyringone	210	0.4	S
40	Syringyl vinyl ketone	208	1.0	S
41	Dihydrosinapyl alcohol	212	0.2	S
42	*cis*-Sinapyl alcohol	210	0.6	S
43	*trans*-Sinapyl alcohol	210	1.5	S
44	*trans*-Sinapaldehyde	208	4.0	S
	% H		1.1	
	% G		59.1	
	% S		39.8	
	S/G		0.67	

Among them, guaiacol and syringol-type phenols, derived from the guaiacyl (G) and syringyl (S) lignin units respectively, typical of hardwoods, were identified. Only small amounts (*ca.* 1%) of phenol-type compounds derived from *p*-hydroxycinnamyl (H) lignin units could be detected among the released compounds. The most important compounds identified were guaiacol (**3**), 4-methylguaiacol (**5**), 4-vinylguaiacol (**8**), syringol (**10**), *trans*-isoeugenol (**12**), 4-methylsyringol (**13**), vanillin (**14**), 4-vinylsyringol (**21**), syringaldehyde (**31**), *trans*-4-propenylsyringol (**29**), *trans*-coniferyl alcohol (**36**), *trans*-coniferaldehyde (**37**) and *trans*-sinapaldehyde (**44**). Surprisingly, the G-lignin-derived phenols were released in higher abundances than the respective S-lignin-derived phenols, which is unusual for a hardwood, with a H:G:S composition of 1:59:40, and a molar S/G ratio of 0.66. The predominance of G-lignin units in paulownia wood, in contrast to other hardwood species, such as eucalypt, with a high predominance of S-lignin units (Rencoret et al., 2007), is unfavorable for alkaline delignification due to the lower reactivity of the G-lignin compared to S-lignin in alkaline systems (Chang and Sarkanen, 1973; Tsutsumi et al., 1995). Therefore, the low lignin S/G ratio of paulownia will adversely affect the pulping efficiency. It has already been shown for other hardwoods, such as eucalypt woods, that higher S/G ratios imply higher delignification rates, less alkali consumption and therefore higher pulp yield (González-Vila et al., 1999; del Río et al., 2005). Therefore, for paulownia wood, the low S/G ratio, together with its high lignin content (*ca.* 23%), would make this wood more resistant to alkaline cooking, requiring a higher alkali charge and therefore implying a lower pulp yield.

3.2. 2D-NMR

2D-NMR is a powerful tool for lignin structural characterization providing information of the structure of the inter-unit linkages present (Balakshin et al., 2003, 2005; Capanema et al., 2001, 2004, 2005; Ibarra et al., 2007a,b; Ralph et al., 1999, 2004b; Rencoret et al., 2008; del Río et al., 2008; Martínez et al., 2008) and enabled the description of new lignin substructures such as the dibenzodioxocins (Karhunen et al., 1995) and the spirodienones (Zhang and Gellerstedt, 2001; Zhang et al., 2006) during recent years.

The HSQC NMR spectrum of paulownia MWL showed three regions corresponding to aliphatic, side-chain and aromatic ^{13}C–^1H correlations. The aliphatic (non-oxygenated) region showed signals with no structural information, excepting those of the acetate methyls that were used to estimate the phenolic and aliphatic hydroxyls in MWL after "in vitro" acetylation (signals with δ_C/δ_H around 21/2.2–2.3 and 21/1.8–2.1 ppm, respectively). The side-chain region (δ_C/δ_H 50–95/2.5–6.0 ppm) and the aromatic region

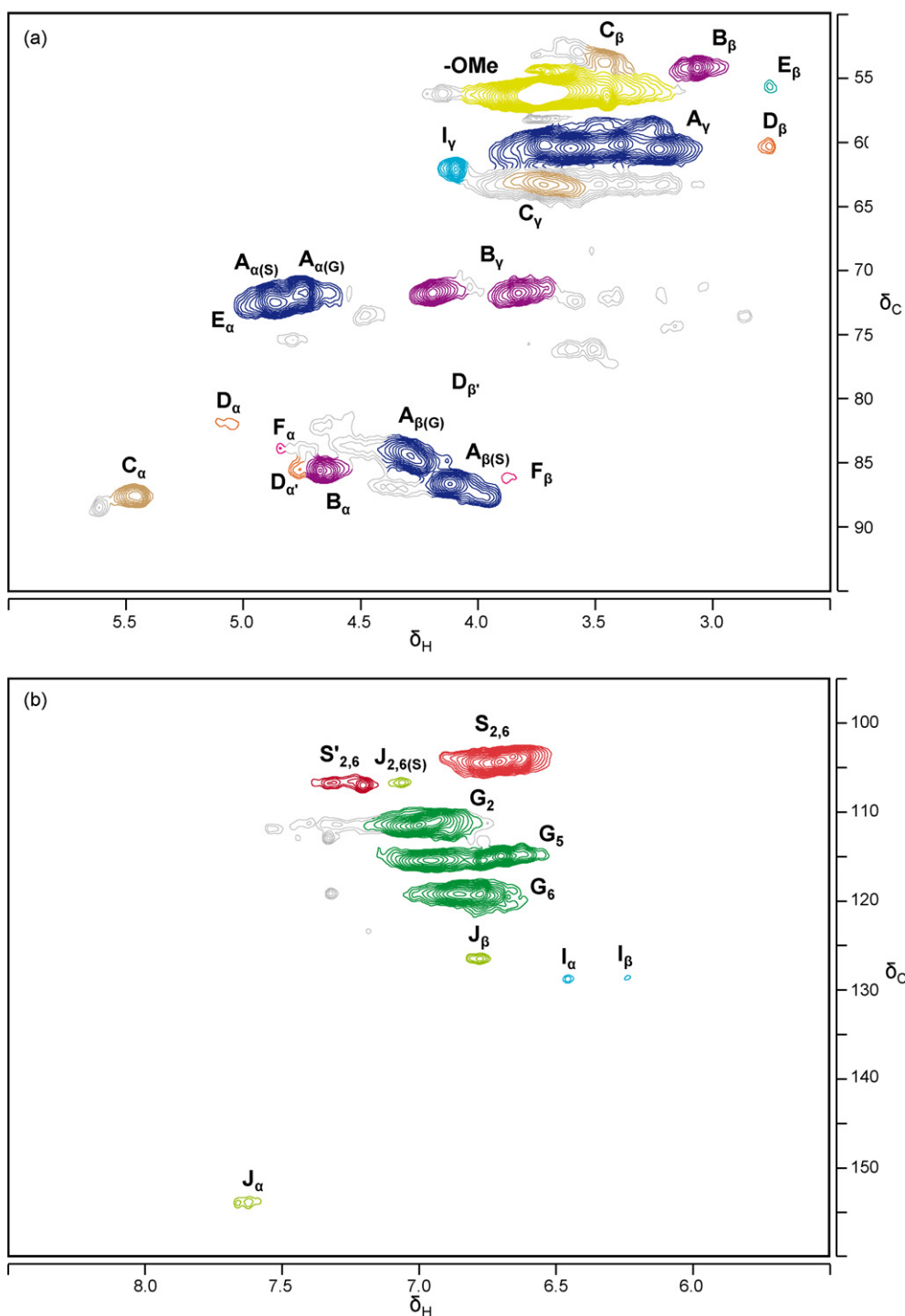**Fig. 2.** (a) Expanded side-chain region (δ_C/δ_H 50–95/2.5–6.0 ppm), and (b) expanded aromatic region (δ_C/δ_H 95–160/5.5–8.5 ppm), of the HSQC spectrum of the MWL from *Paulownia fortunei* wood. See Table 2 for signal assignment and Fig. 3 for the main lignin structures identified. Different colors were used to identify the main cross-signals and their corresponding structures.

(δ_C/δ_H 95–160/5.5–8.5 ppm) of the HSQC spectrum of paulownia MWL are shown in Fig. 2. The main lignin cross-signals assigned in the HSQC spectrum are listed in Table 2 and the main substructures present are depicted in Fig. 3.

The side-chain region of the spectrum (Fig. 2a) gives useful information about the different inter-unit linkages present in paulownia lignin. The spectrum shows prominent signals corresponding to β-O-4′ aryl ether linkages (substructure **A**). The C_α–H_α correlations in β-O-4′ substructures were observed at δ_C/δ_H 71.6/4.74

and 72.4/4.86 ppm for structures linked to G or S-lignin units, respectively. Likewise, the C_β–H_β correlations were observed at δ_C/δ_H 84.1/4.28 ppm for β-O-4′ structures linked to G lignin units and at δ_C/δ_H 86.4/4.11 ppm for β-O-4′ structures linked to S-lignin units. The C_γ–H_γ correlations in β-O-4′ substructures were observed at δ_C/δ_H 60.1/3.40 and 3.72 ppm, partially overlapped with other signals. In addition to β-O-4′ aryl ether substructures, other linkages were also observed in important amounts. Strong signals for resinol (β-β′/α-O-γ′/γ-O-α′ linkages) substructures (**B**)

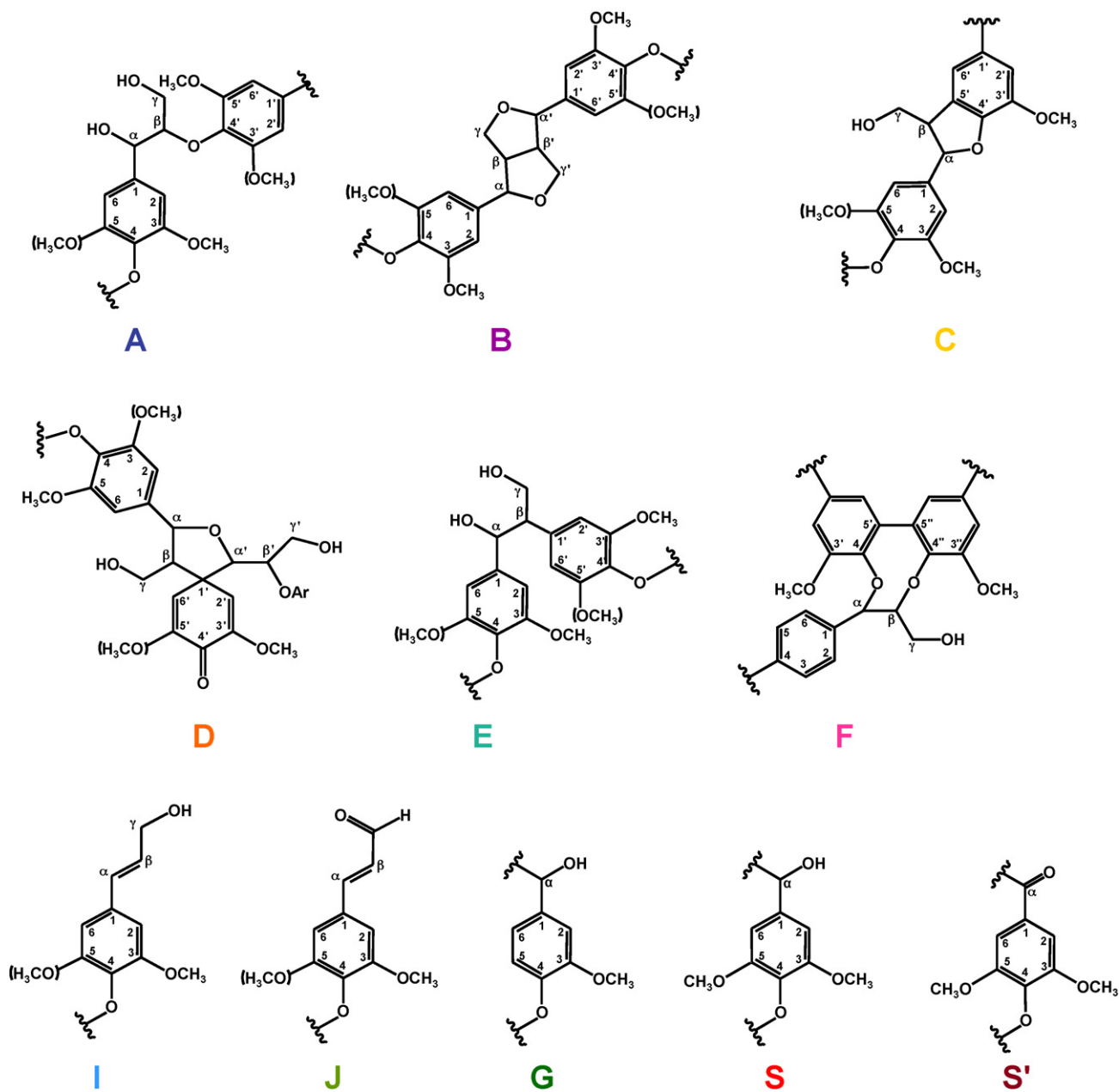**Fig. 3.** Main structures present in *Paulownia fortunei* MWL: (A) β-O-4′ aryl ether linkages; (B) resinol structures formed by β-β/α-O-γ′/γ-O-α′ linkages; (C) phenylcoumaran structures formed by β-5′/α-O-4′ linkages; (D) spirodienone structures formed by β-1′/α-O-4′ linkages; (E) β-1′ linkages; (F) dibenzodioxocin structures formed by 5′-5″/α-O-4′/β-O-4″ linkages; (I) cinnamyl alcohol end-groups; (J) cinnamaldehyde end-groups; (G) guaiacyl units; (S) syringyl units; (S′) C_α-oxidized syringyl units.

were observed in the spectrum, with their C_α–H_α, C_β–H_β and the double C_γ–H_γ correlations at δ_C/δ_H 85.5/4.67, 54.1/3.06 and 71.7/3.83 and 4.19 ppm, respectively. Phenylcoumaran (β-5′/α-O-4′ linkages) substructures (**C**) were also found in important amounts in paulownia MWL, the signals for their C_α–H_α and C_β–H_β correlations being observed at δ_C/δ_H 87.4/5.47 and 53.7/3.46 ppm, respectively, and that of C_γ–H_γ correlation overlapping with other signals around δ_C/δ_H 63.2/3.72 ppm. Small signals corresponding to spirodienone (β-1′/α-O-α′ linkages) substructures (**D**) could also be observed in the spectra, their C_α–H_α, $C_{\alpha'}$–$H_{\alpha'}$, C_β–H_β and $C_{\beta'}$–$H_{\beta'}$ correlations being at δ_C/δ_H 81.7/5.09, 86.6/4.39, 60.3/2.75 and 79.8/4.11 ppm, respectively. Other small signals in the side-chain region of the HSQC spectra corresponded to C_β–H_β correlations (at δ_C/δ_H 55.6/2.76 ppm) of conventional β-1′ substructures (**E**) (Lundquist, 1987). Interestingly, dibenzodioxocin (5′-5″/α-O-4′/β-O-4″ linkages) structures (**F**) could also be observed in the

HSQC spectrum of paulownia MWL, although in low amounts, with their C_α–H_α and C_β–H_β correlations at δ_C/δ_H 83.6/4.83 and 85.9/3.88 ppm, respectively. Dibenzodioxocins are important lignin structures in softwoods (Karhunen et al., 1995; Ralph et al., 2004a) where they act as branching points, but have rarely been found in hardwoods (Ämmälahti et al., 1998; Kukkola et al., 2004). Finally, other small signals observed in the HSQC spectrum are the C_γ–H_γ correlations (at δ_C/δ_H 61.9/4.09 ppm) assigned to cinnamyl alcohol end-groups (**I**). The olefinic correlations of the cinnamyl structures were observed in the aromatic region of the spectrum.

The main cross-signals in the aromatic region of the HSQC spectrum (Fig. 2b) corresponded mainly to the benzenic rings of the different lignin units. Cross-signals from syringyl (**S**) and guaiacyl (**G**) lignin units could be observed in the spectrum. The S-lignin units showed a prominent signal for the $C_{2,6}$–$H_{2,6}$ correlation at δ_C/δ_H 104.7/6.69 ppm, while the G units showed different corre-

Table 2
Assignments of ^{13}C–^1H correlation signals in the HSQC spectrum of the MWL from *Paulownia fortunei* wood.

Labels	δ_C/δ_H (ppm)	Assignment
C_β	53.7/3.46	C_β–H_β in phenylcoumaran substructures (**C**)
B_β	54.1/3.06	C_β–H_β in resinol substructures (**B**)
E_β	55.6/2.76	C_β–H_β in β-1′ substructures (**E**)
-OMe	56.2/3.73	C–H in methoxyls
A_γ	60.1/3.40 and 3.72	$C_\gamma H_\gamma$ in β-O-4′ substructures (**A**)
D_β	60.3/2.75	C_β–H_β in spirodienone substructures (**D**)
I_γ	61.9/4.09	C_γ–H_γ in cinnamyl (sinapyl/coniferyl) alcohol end-groups (**I**)
C_γ	63.2/3.72	C_γ–H_γ in phenylcoumaran substructures (**C**)
$A_{\alpha(G)}$	71.6/4.74	C_α–H_α in β-O-4′ linked to a G unit (**A**)
B_γ	71.7/3.83 and 4.19	C_γ–H_γ in resinol substructures (**B**)
$A_{\alpha(S)}$	72.4/4.86	C_α–H_α in β-O-4′ linked to a S unit (**A**)
$D_{\beta'}$	79.8/4.11	$C_{\beta'}$–$H_{\beta'}$ in spirodienone substructures (**D**)
D_α	81.7/5.09	$C_\alpha H_\alpha$ in spirodienone substructures (**D**)
F_α	83.6/4.83	C_α–H_α in 5-5′ (dibenzodioxocin) substructures (**F**)
$A_{\beta(G)}$	84.1/4.28	C_β–H_β in β-O-4′ linked to a G unit (**A**)
B_α	85.5/4.67	C_α–H_α in resinol substructures (**B**)
F_β	85.9/3.88	C_β–H_β in 5-5′ (dibenzodioxocin) substructures (**F**)
$D_{\alpha'}$	86.6/4.39	$C_{\alpha'} H_{\alpha'}$ in spirodienone substructures (**D**)
$A_{\beta(S)}$	86.4/4.11	C_β–H_β in β-O-4′ linked to a S unit (**A**)
C_α	87.4/5.47	C_α–H_α in phenylcoumaran substructures (**C**)
$S_{2,6}$	104.7/6.69	$C_{2,6}$–$H_{2,6}$ in etherified syringyl units (**S**)
$J_{2,6(S)}$	106.8/7.06	$C_{2,6}$–$H_{2,6}$ in sinapaldehyde end-groups (**J**)
$S'_{2,6}$	107.0/7.32 and 7.19	$C_{2,6}$–$H_{2,6}$ in oxidized (C_α=O) phenolic syringyl units (**S′**)
G_2	111.6/6.99	C_2–H_2 in guaiacyl units (**G**)
G_5	115.4/6.72 and 6.94	C_5–H_5 in guaiacyl units (**G**)
G_6	119.5/6.77	C_6–H_6 in guaiacyl units (**G**)
J_β	126.7/6.77	C_β–H_β in cinnamyl aldehyde end-groups (**J**)
I_β	129.0/6.23	C_β–H_β in cinnamyl alcohol end-groups (**I**)
I_α	129.0/6.45	C_α–H_α in cinnamyl alcohol end-groups (**I**)
J_α	153.8/7.63	C_α–H_α in cinnamyl aldehyde end-groups (**J**)

Table 3
Structural characteristics (percentage of lignin side-chains forming different inter-unit linkages **A–F** and cinnamyl end-groups **I, J**, referred to as the total side-chains; H, G and S contents, S/G ratio, phenolic content, as % of H + G + S after MWL acetylation, and phenolic-to-aliphatic hydroxyl ratio) from integration of ^{13}C–^1H correlation signals in the HSQC spectrum of MWL from *Paulownia fortunei* wood.

Linkage relative abundance (% side-chains involved)	
β-O-4′ structures (**A**)	62
Resinols (**B**)	12
Phenylcoumarans (**C**)	11
Spirodienones (**D**)	3
β-1′ structures (**E**)	1
Dibenzodioxocins (**F**)	2
Cinnamyl alcohol end-groups (**I**)	4
Cinnamyl aldehyde end-groups (**J**)	5
Percentage of lignin units	
H (%)	0
G (%)	60
S (%)	40
S/G ratio	0.66
Phenolic content (%)	16
Phenolic/aliphatic hydroxyls ratio	0.14

The relative abundances of the main inter-unit linkages (referred to as the total side-chains) present in the paulownia MWL, as well as the relative abundance of the H, G and S units and the molar S/G ratio, calculated from the HSQC spectrum, are shown in Table 3. In addition, the phenolic content and the phenolic-to-aliphatic hydroxyl ratio were estimated from the HSQC after MWL acetylation, and are also included in the table. The main substructure present in paulownia MWL was the β-O-4′ aryl ether ones (**A**), that accounts for 62% of all side-chains, followed by the β-β′ resinol substructures (**B**) that involved 12% of all side-chains and the β-5′ phenylcoumaran structures (**C**) with 11%. The rest of substructures, such as spirodienones (**D**), β-1′ structures (**E**), dibenzodioxocins (**F**), and cinnamyl end-groups (**I** and **J**) were present in lower proportions. Comparing to other hardwoods commonly used for papermaking, such as eucalypt wood (Rencoret et al., 2008), the lignin from paulownia presents a lower proportion of β-O-4′ ether linkages, and a higher proportion of carbon-carbon linked (condensed) structures (such as β-β′, β-5′, 5-5′ and β-1′) and a lower S/G ratio. Since the β-O-4′ ether linkages are cleaved to a high extent during alkaline cooking, while condensed linkages resist alkaline cooking conditions (Gierer, 1985; Gierer and Norén, 1980; Ibarra et al., 2007a), the relatively high content of condensed structures in the lignin from paulownia wood will make this wood more resistant to alkaline delignification.

4. Conclusions

The MWL isolated from *P. fortunei* has been characterized by Py–GC/MS and 2D-NMR techniques. A predominance of guaiacyl (G) over syringyl (S) lignin moieties was found in this wood lignin, with a H:G:S molar composition of 1:59:40. The main inter-unit linkages present in paulownia MWL were the β-O-4′ ether bonds (62% of all side-chains) followed by β-β′ resinol-type (12%) and β-5′ phenylcoumaran substructures (11%), and with lower amounts of β-1′, spirodienone and 5-5′ dibenzodioxocin substructures. The high lignin content (*ca.* 23% Klason lignin) together with the high amounts of G-lignin units (S/G ratio of 0.66) and condensed structures would contribute to the lower reactivity of paulownia lignin during pulping.

lations for C_2–H_2 (δ_C/δ_H 111.6/6.99 ppm), C_5–H_5 (δ_C/δ_H 115.4/6.72 and 6.94) and C_6–H_6 (δ_C/δ_H 119.5/6.77 ppm). The double C_5–H_5 signal revealed some heterogeneity among the G units especially affecting the C_5–H_5 correlation, probably because it is due to different substituents at C_4 (e.g. phenolic or etherified in different substructures). Signals corresponding to $C_{2,6}$–$H_{2,6}$ correlations in C_α-oxidized S-lignin units (**S′**) were observed at δ_C/δ_H 106.8/7.32 and 107.0/7.19. Signals of H-lignin units were not detected in the HSQC spectrum, in agreement with the low proportions of H units (*ca.* 1%) observed by Py–GC/MS. The NMR estimation of the S/G ratio of paulownia MWL is included in Table 2, and accounts for 0.66, in agreement with the results obtained upon Py–GC/MS. Other signals in this HSQC region of the spectrum are from cinnamyl alcohol end-groups (**I**), with their C_α–H_α and C_β–H_β correlations observed at δ_C/δ_H 129.0/6.23 and 129.0/6.45 ppm, respectively; and cinnamaldehyde end-groups (**J**), with the C_α–H_α and C_β–H_β correlations observed at δ_C/δ_H 153.8/7.63 and 126.7/6.77 ppm, respectively. The total relative content of the cinnamaldehyde end-groups was estimated by comparison of the intensities of the C_β–H_β correlations in cinnamyl alcohols (**I**) and aldehydes (**J**). The aromatic cross-signals of the cinnamyl alcohol end-groups overlapped with the same signals in lignin S and G units. However, this was not the case for some of the cinnamaldehyde aromatic cross-signals revealing the presence of (i) sinapaldehyde end-groups (**J**$_{(S)}$), characterized by an unique $C_{2,6}$–$H_{2,6}$ correlation signal at δ_C/δ_H 106.8/7.06 ppm, and (ii) coniferaldehyde end-groups (**J**$_{(G)}$), characterized by C_2–H_2 and C_6–H_6 correlation signals at around δ_C/δ_H 111/7.4 and 123./7.2 ppm, respectively.

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