

STRUCTURAL CHARACTERIZATION OF LIGNIN IN DIFFERENT EUCALYPT SPECIES

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ABSTRACT

The chemical structure of milled-wood lignins from *Eucalyptus globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii* was investigated. The lignins were characterized by analytical pyrolysis, thioacidolysis and 2D-NMR that confirmed the predominance of syringyl over guaiacyl units, and only showed traces of *p*-hydroxyphenyl units. The HSQC-NMR spectra showed a predominance of β -O-4' ether linkages (66-72% of total side-chains), followed by β - β' resinol-type linkages (16-19% of side-chains) and lower amounts of β -5' phenylcoumaran-type (3-7% of side-chains) and β -1' spirodienone-type linkages (1-4% of side-chains). The analysis of desulphurated thioacidolysis dimers provided additional information on the carbon-carbon and diaryl ether bonds forming the most condensed moiety of the eucalypt lignins, and the type of units involved in each of the above linkage types. Interestingly, 93-94% of the total β - β' dimers included two syringyl units indicating that most of the β - β' substructures identified in the HSQC spectra were of the syringaresinol type. Moreover, three isomers of a major trimeric compound tentatively identified as arising from a β - β' syringaresinol substructure attached to a guaiacyl unit through a 4-O-5' linkage were found.

I. INTRODUCTION

Eucalypt species are grown as raw material for paper pulp manufacturing in Spain, Portugal, Brazil and other countries. Among the different eucalypt species used by the paper pulp industry, *Eucalyptus globulus* provides the best raw material for kraft pulp manufacturing in terms of the high pulp yield (Rencoret et al. 2007). Pulping and bleaching performances are highly dependent on the relative content, structure and reactivity of the wood polymers, cellulose, hemicellulose and lignin. In particular, the lignin content and its composition in terms of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units and the different inter-unit linkages are important factors in pulp production affecting the delignification rate and pulp yield. A higher S/G ratio in woods from the same eucalypt species implies higher delignification rates, less alkali consumption and therefore higher pulp yield (del Río et al. 2005). In this paper, we report the structural characterization of lignin from woods of *E. globulus*, *Eucalyptus nitens*, *Eucalyptus maidenii*, *Eucalyptus grandis* and *Eucalyptus dunnii*, which are the species most frequently grown in eucalypt plantations for papermaking. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), thioacidolysis and 2D-NMR, were used for the characterization of the milled-wood lignins (MWL) isolated from these eucalypt species.

II. EXPERIMENTAL

Samples. The woods from *E. globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii* were provided by ENCE (Pontevedra, Spain) and corresponded to 10-12 year-old trees. MWL samples were obtained by the classical procedure (Björkman 1956).

Py-GC/MS. Pyrolysis was performed with a 2020 micro-furnace pyrolyzer (Frontier Lab) connected to an Agilent 6890 GC/MS system equipped with a HP 5MS fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μ m film thickness). The pyrolysis was performed at 500°C. The oven temperature was programmed from 50°C (1 min) to 100°C at 30°C min⁻¹ and then to 300°C (10 min) at 10°C min⁻¹.

Thioacidolysis. Thioacidolysis was performed as described (Rolando et al. 1992) using 0.2 M BF₃ etherate in dioxane/ethanethiol 8.75:1. GC analysis of trimethylsilylated reaction products was performed with a Hewlett-Packard 6890 instrument using an Rtx 5 column from Restec Corporation (45 m x 0.32 μ m i.d., 0.25 μ m film thickness) and a FID detector. The temperature was programmed from 180°C to 270°C (15 min) at 40°C min⁻¹ and then to 300°C (5 min) at 4°C min⁻¹. Injector and detector were set at 250°C and 280°C respectively.

Desulphurization of thioacidolysis degradation products. Two-hundred μ L of the CH₂Cl₂ solution containing the thioacidolysis products was desulphurized as described (Lapierre et al. 1991). GC/MS analysis of the dimeric compounds was performed in a Varian Saturn 2000 ion-trap detector, using a DB-5HT fused-silica capillary column from J&W Scientific (30 m x 0.25 mm i.d., 0.1 μ m film thickness). The temperature was programmed from 50°C to 110°C at 30°C min⁻¹ and then to 320°C (13 min) at 6°C min⁻¹. The injector and transfer line were at

300°C. Trimeric compounds were analyzed using a short (12 m) capillary column of the same characteristics and a temperature program from 60°C (1 min) to 380°C (5 min) at 10°C min⁻¹. The transfer line was at 300°C, and the injector was programmed from 120°C (0.1 min) to 380°C at 200°C min⁻¹.

NMR. 2D-NMR spectra were recorded at 25°C in a Bruker AVANCE 500 MHz, equipped with a z-gradient triple resonance probe. Forty mg of MWL were dissolved in 0.75 mL of dimethylsulfoxide (DMSO)-*d*₆, and HSQC spectra were recorded. The spectral widths were 5000 and 13200 Hz for the ¹H and ¹³C dimensions, respectively. The number of collected complex points was 2048 for ¹H dimension, with a recycle delay of 5 s. The number of transients was 64, and 256 time increments were always recorded in ¹³C 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 ¹³C dimension. Signal volume integration was used to calculate the S/G ratio (from S_{2,6} and G₂+G₆ cross-signals) and percentage of side-chains involved in different inter-unit linkages (from C_α-H_α correlations, except for structures E and F).

III. RESULTS AND DISCUSSION

Py-GC/MS. All the eucalypt MWL yielded guaiacol and syringol-type phenols, derived from the G and S lignin units respectively, with molar S/G ratios in the range from 1.9 to 2.6 (**Table 1**). The lignin from *E. globulus* had the highest S/G ratio. Only traces (less than 0.1%) of *p*-hydroxycinnamyl (H) units could be detected.

2D-NMR. The side-chain and aromatic regions of the HSQC of a representative eucalypt MWL are shown in **Figure 1**. All the spectra showed prominent signals corresponding to β-O-4' ether linkages (**A**). Strong signals for resinol substructures (**B**) were observed in all spectra, and phenylcoumaran (β-5'/α-O-4) substructures (**C**) were also found, although in lower amounts. Finally, small signals corresponding to spirodienone (β-1'/α-O-α') substructures (**D**) could also be observed. Other small signals corresponded to β-O-4' substructures bearing a C_α carbonyl group (**E**), and to *p*-hydroxycinnamyl (**F**) terminal structures. No dibenzodioxocin structures could be observed in the HSQC spectra of the eucalypt MWL, in agreement with the results already reported for the lignins of *E. grandis* (Capanema et al. 2005) and *E. globulus* (Ibarra et al. 2007). The percentage of lignin side-chains involved in the main substructures and terminal structures in the eucalypt lignins are indicated in **Table 1**, which also shows (in parenthesis) the relative abundance of each of the inter-unit linkages. In all cases, the main substructure was the β-O-4' aryl ether (**A**) that corresponded to around 70% of all side-chains. The second most abundant linkage corresponded to the resinol substructure (**B**) that involved around 17% of all side-chains, the highest proportion being found in *E. dunnii*. The other linkages, such as phenylcoumaran (**C**) or spirodienone (**D**), were present in lower proportions.

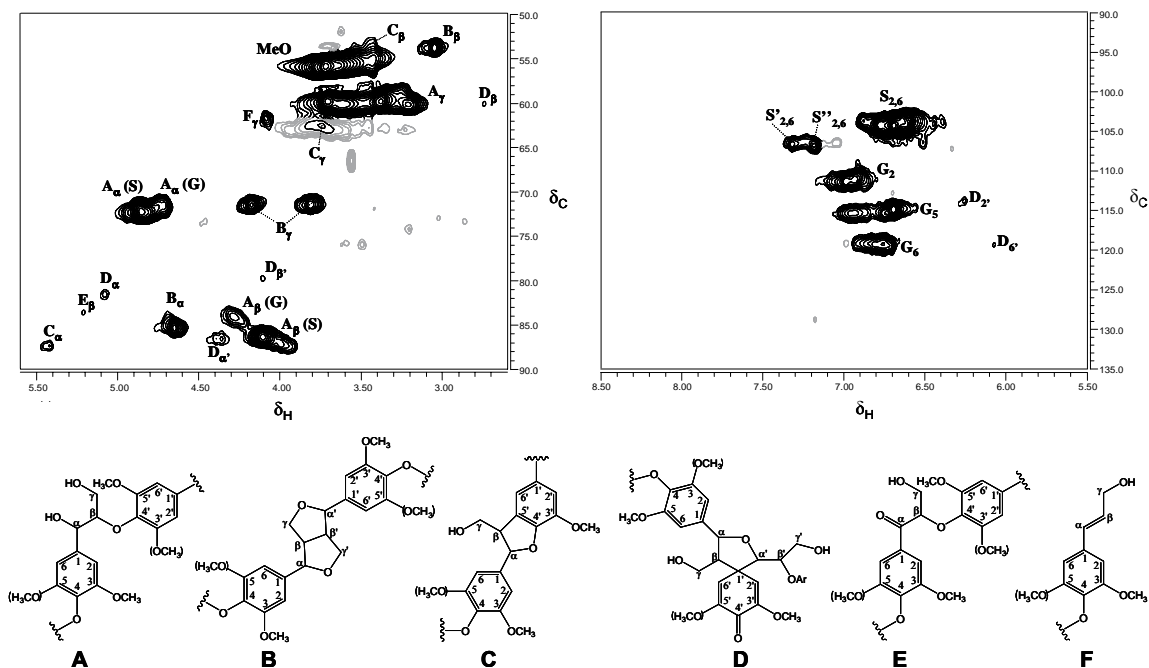


Figure 1.- HSQC spectrum (side-chain and aromatic regions) of a representative MWL (from *E. dunnii* wood). The main structures observed were: (**A**) β-O-4' linkages; (**B**) resinol formed by β-β', α-O-γ' and γ-O-α' linkages; (**C**) phenylcoumaran structures formed by β-5' and α-O-4' linkages; (**D**) spirodienone structures formed by β-1', α-O-4' linkages; (**E**) C_α-oxidized β-O-4' linkages; and (**F**) *p*-hydroxycinnamyl alcohol terminal unit.

Table 1. Percentage of lignin side-chains forming different inter-unit linkages (**A-E**) and terminal structures (**F**) from integration of ^{13}C - ^1H correlation signals in the HSQC spectra of MWL from wood of the different eucalypt species analyzed (referred to total side-chains). The relative abundance of each inter-unit linkage type (taking into account that two side-chains are required to form one resinol structure) are indicated in parentheses.

| | <i>E. globulus</i> | <i>E. nitens</i> | <i>E. maidenii</i> | <i>E. grandis</i> | <i>E. dunnii</i> |
|---|--------------------|------------------|--------------------|-------------------|------------------|
| β -O-4' (A) | 69.3 (80.4) | 71.7 (83.1) | 69.7 (80.3) | 66.9 (77.3) | 65.9 (77.0) |
| Resinol (B) | 18.2 (10.6) | 16.1 (9.3) | 16.4 (9.5) | 16.5 (9.5) | 19.0 (11.1) |
| Phenylcoumaran (C) | 2.9 (3.4) | 4.0 (4.7) | 3.6 (4.2) | 6.8 (7.9) | 4.0 (4.7) |
| Spirodienone (D) | 2.8 (3.3) | 1.3 (1.5) | 3.6 (4.2) | 2.9 (3.3) | 4.2 (4.9) |
| β -O-4'-C α =O (E) | 2.0 (2.3) | 1.3 (1.5) | 1.7 (1.9) | 1.7 (1.9) | 1.9 (2.2) |
| <i>p</i> -Hydroxycinnamyl alcohols (F) | 4.7 | 5.7 | 4.9 | 5.3 | 4.9 |
| S/G (Py-GC/MS) | 2.6 | 2.1 | 2.0 | 1.9 | 2.4 |
| S/G (NMR) | 2.9 | 2.7 | 2.4 | 1.7 | 2.7 |
| S/G (thioacidolysis) | 2.5 | 2.1 | 1.8 | 1.6 | 2.3 |

The main cross-signals in the aromatic region of the HSQC spectra (**Figure 1**) corresponded to the benzenic rings of S and G units. Signals of H units were not detected. Signals corresponding to C α -oxidized S-lignin units (S' and S'') were also observed. A NMR estimation of the molar S/G ratios in the different lignins is included in **Table 1**, ranging from 1.7 to 2.9. The highest S/G value corresponded to the *E. globulus* lignin while the lowest value corresponded to that from *E. grandis*, as observed by Py-GC/MS.

Thioacidolysis. The composition of the monomers released after thioacidolysis showed a predominance of S over G units in the etherified eucalypt lignin, and the nearly-complete absence of H units (less than 0.5% of the total), as observed by Py-GC/MS. The molar S/G ratios are shown in **Table 1** and are similar to those estimated by the two other methods, with the highest value for the *E. globulus* and the lowest for the *E. grandis* and *E. maidenii*. The dimeric compounds obtained by thioacidolysis were analyzed after Raney-Ni desulphuration. The main dimers obtained were of 5-5', 4-O-5', β -1', β -5', β - β' tetralin and phenylisochroman (including β -1'/ α -O- α' bonds) types, and their relative proportions are shown in **Table 2**. Tetralin and β -1' dimeric compounds were the most prominent thioacidolysis dimers, and appeared in similar proportion (around 30% of each). Tetralins would be formed from the resinol substructures as described by Lapierre et al. (1995). The high proportion of β - β' dimers (tetralin-type) was in agreement with the high amount (9-11%) of β - β' substructures (resinol-type) in all the eucalypt lignins (**Table 1**) that would represent around 50% of the NMR structures with C-C linked side-chains (**B-D**). More interestingly, nearly-all the β - β' dimers were of syringaresinol type (pinoresinol being absent and the G-S structure appearing as a minor substructure) (**Table 2**). β -1' dimers were anomalously prominent, as reported for degradation products from other lignins (Lapierre et al. 1995, Ralph et al. 2004). The existence of β -1' dimeric substructures in lignin has been a matter of controversy for years. Two β -1' linked substructures, spirodienones and phenylisochromans, were identified using 2D-NMR in combination with DFRC (Ralph et al. 1998, Zhang and Gellerstedt 2001).

Table 2. Relative molar percentages of the different dimer types released after thioacidolysis and Raney-nickel desulphuration of MWL from different eucalypt species analyzed, and total S/G ratio of condensed lignin.

| | 5-5' | | 4-O-5' | | β -1' | | | β -5' | | β - β' | | β -1'/ α -O- α' | | |
|--------------------|------|----|--------|----|-------------|----|----|-------------|----|--------------------|----|-------------------------------------|----|--|
| | GG | GG | SG | GG | SG | SS | GG | SG | SG | SS | GG | SG | SS | |
| <i>E. globulus</i> | 6 | 3 | 6 | 4 | 8 | 19 | 10 | 5 | 2 | 30 | 1 | 1 | 5 | |
| <i>E. nitens</i> | 10 | 3 | 7 | 3 | 5 | 24 | 7 | 4 | 2 | 30 | 1 | 1 | 3 | |
| <i>E. maidenii</i> | 6 | 2 | 5 | 3 | 8 | 18 | 12 | 5 | 2 | 32 | 1 | 1 | 4 | |
| <i>E. grandis</i> | 10 | 4 | 5 | 7 | 8 | 17 | 12 | 5 | 2 | 26 | 1 | 1 | 3 | |
| <i>E. dunnii</i> | 11 | 4 | 8 | 3 | 4 | 24 | 8 | 5 | 2 | 25 | 1 | 1 | 4 | |

Phenylisochromans are resistant toward thioacidolysis, therefore, only spirodienones could be at the origin of the β -1' dimers observed here. However, a comparison of the abundances of spirodienones (HSQC) and β -1' dimers (thioacidolysis) suggested that other β -1' structures, maybe including simple dimeric structures, are also present in eucalypt lignin. β -5' dimers were also released in important amounts (11-17%) as corresponds to a phenylcoumaran abundance near 25% of the condensed substructures in HSQC spectra (**B-D**). The three thioacidolysis dimers with the lowest abundance, i.e. 5-5', 4-O-5' and isochroman, were not detected by NMR because the abundance of the original substructures was below the NMR detection level. Dibenzodioxocins are supposed to be the main biphenyl structures in lignin (Karhunen et al. 1995), therefore, the 5-5' dimers were considered mostly as being dibenzodioxocin degradation products. On the other hand, several thioacidolysis trimers were obtained. These trimers presented a molecular ion (and base peak) at m/z 696, and a fragment at m/z 306 (characteristic of S-S tetralin dimers) and were tentatively identified as S- β - β' -S'-4'-O-5"-G" trimers. The same trimeric compound has been identified in other hardwoods (Önnerud and Gellerstedt 2003). Other thioacidolysis trimers were detected in very minor amounts. All the trimers detected were formed by addition to one of the main dimers previously described of one G unit linked by a 4-O-5' ether linkage.

IV. CONCLUSIONS

The MWL isolated from different eucalypt species (*E. globulus*, *E. nitens*, *E. maidenii*, *E. grandis* and *E. dunnii*) has been characterized by Py-GC/MS, 2D-NMR and thioacidolysis (followed by Raney-Ni desulphuration). A predominance of S over G lignin moieties was found in all eucalypt MWL. The main inter-unit linkage present was the β -O-4' followed by β - β' resinol-type substructures, with lower amounts of β -5' phenylcoumaran and β -1' spirodienone substructures. Analysis of desulphurated thioacidolysis products indicated that the β - β' substructures are almost exclusively of syringaresinol type. Trimeric compounds made of a syringaresinol moiety attached to a guaiacyl unit through a 4-O-5' linkage were also reported.

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