

STRUCTURAL CHARACTERIZATION OF THE LIGNIN OF COCONUT (*COCOS NUCIFERA*) COIR

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ABSTRACT

The chemical structure of the lignin isolated from coconut coir has been comprehensively characterized in this work by analytical pyrolysis (Py-GC/MS), two-dimensional nuclear magnetic resonance (2D-NMR) and derivatization followed by reductive cleavage (DFRC). The analyses demonstrated that coconut coir lignin is a *p*-hydroxyphenyl-guaiacyl-syringyl (H-G-S) lignin, with a predominance of G-lignin units (S/G 0.24), and with a considerable amount of associated *p*-hydroxybenzoates (13%). 2D-NMR indicated that the main substructures present in this lignin include β -O-4' alkyl-aryl ethers, followed by phenylcoumarans (β -5') and resinols (β - β'). In addition, 2D-NMR indicated that coconut coir lignin is partially acylated at the γ -carbon of the side-chain. DFRC results showed that acetates are one of the acylating group and preferentially acylate the γ -OH in syringyl rather than in guaiacyl units.

I. INTRODUCTION

Plant biomass is the main source of renewable materials in Earth and represents a potential source of renewable energy and bio-based products. Biomass is available in high amounts at very low cost as forest, agricultural or industrial lignocellulosic wastes and cultures. The chemical properties of the components of lignocellulosics make them a substrate of high biotechnological value (Ragauskas et al. 2006). In this context, the enormous amounts of residual plant biomass considered as "waste", that are often disposed of by biomass burning (Levine, 1996), can be converted into various different value added products including biofuels (Sarkar et al. 2012), pulp and paper or chemicals. Among these wastes, we should refer to coconut coir, which is obtained from the fibrous husk (mesocarp) of the coconut from the coconut palm (*Cocos nucifera*), which belongs to the palm family (*Palmae*).

Cost-effective, efficient conversion of plant cell walls into their components, mainly carbohydrates and lignin, is fundamental to realising the full potential of the biomass lignocellulose feedstock. Lignin is particularly hard to degrade and represents the major barrier to efficient utilization of the plant biomass. Consequently, some mechanical, chemical, microbial or enzymatic pretreatments for lignin modification and/or removal are required, being their efficiency highly dependent of the lignin structure. For this reason, the knowledge of the exact structure and composition of the lignin polymer is important to find appropriated pretreatment methods. Up to now, the coconut coir lignin has not been characterized in detail, and this is the first step in developing the best pretreatments for fully exploiting this waste plant material. In this work, the structure of the lignin isolated from coconut coir was characterized by different analytical methods, including Py-GC/MS, 2D-NMR and DFRC.

II. EXPERIMENTAL

Coconut coir selected for this study was supplied by the Celesa pulp mill (Tortosa, Spain). The air-dried fibers were grounded in a cutting mill to pass through a 100-mesh screen and subsequently, were extracted with acetone in a Soxhlet apparatus for 8 h and with hot water (3 h at 100°C). The free sawdust was finely ball-milled in a Retsch S100 centrifugal ball mill at 400 rpm (18 h) using an agate jar and balls.

Milled-wood lignin (MWL) isolation. The MWL was isolated by aqueous dioxane extraction from finely ball-milled wood according to the classical lignin isolation procedure (Björkman, 1956).

Analytical pyrolysis. Pyrolysis of MWL (approximately 100 μg) was performed with a 2020 micro-furnace pyrolyzer (Frontier Laboratories Ltd.) connected to an Agilent 6890 GC/MS equipped with a DB-1701 fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness) and an Agilent 5973 mass selective detector (EI at 70 eV). For pyrolysis in the presence of tetramethylammonium hydroxide as a methylating agent (Py/TMAH), 100 μg of sample were mixed with approximately 0.5 μL TMAH (25%, w/w, in methanol). The compounds were identified by comparing their mass spectra with those of the Wiley and NIST libraries and those reported in the literature (Ralph and Hatfield 1991).

NMR spectroscopy. 2D-NMR spectra were recorded at 25°C in a Bruker AVANCE III 500 MHz, equipped a cryogenically-cooled 5 mm TCI gradient probe with inverse. 40 mg of MWL were dissolved in 0.75 mL of dimethylsulfoxide (DMSO)- d_6 , and HSQC (heteronuclear single quantum correlation) spectra were recorded as previously described (Rencoret et al. 2011). HSQC cross-signals were assigned by comparing with the literature (Ralph et al. 2004). A semiquantitative analysis of the volume integrals of the HSQC correlation peaks was performed as previously described (del Río et al. 2012).

DFRC (Derivatization Followed by Reductive Cleavage). The DFRC degradation was performed according to the protocol developed by Lu and Ralph (1997). The acetylated lignin degradation products were collected after rotary evaporation of the solvents and subsequently analyzed by GC/MS using mass spectra and relative retention times to authenticate the DFRC monomers. To assess the presence of naturally acetylated lignin units, a modification of the standard DFRC method using propionylating instead of acetylating reagents (DFRC') was used (Ralph and Lu, 1998). The GC/MS analyses were performed as previously described (del Río et al. 2012).

III. RESULTS AND DISCUSSION

The lignin content in the coconut coir was estimated according to the Klason method and revealed a high content in the fibers (32.1%).

Pyrolysis. The pyrolysis of coconut coir MWL released phenolic compounds that are derived from *p*-hydroxycinnamyl (H), guaiacyl (G) and syringyl (S) lignin units. The most predominant peaks were phenol, guaiacol, 4-methylguaiacol, 4-vinylguaiacol, syringol, vanillin, *trans*-isoeugenol and *trans*-coniferaldehyde. An S/G ratio value of 0.29 was obtained. The significant amount of phenol (~27% of all phenolic compounds) released upon pyrolysis is due to the presence of *p*-hydroxybenzoates in this lignin, which efficiently decarboxylate to produce phenol, which overestimates the content of H-lignin units. Therefore, this sample was also analyzed by Py/TMAH, and important amounts of 4-methoxybenzoic acid methyl ester arising from *p*-hydroxybenzoates were released. This means that the great majority of phenol released after pyrolysis in absence of TMAH comes from *p*-hydroxybenzoates.

2D-NMR. The side-chain region of the 2D-HSQC spectrum (Fig. 1a) gave useful information about the different inter-unit linkages present in coconut coir lignin. The spectrum showed prominent signals corresponding to β -O-4' alkyl-aryl ether linkages. Strong signals for phenylcoumaran (β -5'/ α -O-4' linkages) substructures (B) were also found. In addition, resinol (β - β' / α -O- γ' / γ -O- α' linkages) substructures (C) and small signals corresponding to dibenzodioxocin (5'-5''/ α -O-4'/ β -O-4'' linkages) substructures (D) were also observed. Finally, other signal corresponding to cinnamyl alcohol end-group (E) was found in the side-chain region. The cross-signals in the aromatic region of the HSQC spectrum (Fig. 1b) corresponded mainly to the benzenic rings of the different lignin units and *p*-hydroxybenzoates (PB). Cross-signals from *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) lignin units could be observed in the spectrum. Other signals in this HSQC region of the spectrum corresponded to cinnamyl alcohol end-groups (E) and cinnamaldehyde end-groups (I). The relative abundances of the main inter-unit linkages, the relative abundance of the H, G and S units and the molar S/G ratio, as well as the *p*-hydroxybenzoates calculated from the HSQC spectrum, are shown in Table 1. In addition, lignin end-groups and side-chain γ -acylation are also included in the Table 1. The S/G ratio determined upon 2D-NMR (0.24) was similar to that obtained upon Py-GC/MS (0.29).

DFRC analysis. The presence of minor acetylation of the γ -carbon of the lignin side chain in the coconut coir MWL could be observed by the DFRC' analysis, which indicated that the extent of acetylation was low and preferably on S-monomers (6%), and only 1% of G-monomers were γ -acetylated, which represents 2% of the total lignin γ -acylation. However, DFRC analysis could not firmly demonstrate that *p*-hydroxybenzoates are attached to the γ -carbon, probably because these type of DFRC products are not stable at the high temperatures required by GC/MS analysis. In any case, if they are attached to the γ -carbon, they would account for the additional 6% lignin γ -acylation shown by 2D-NMR.

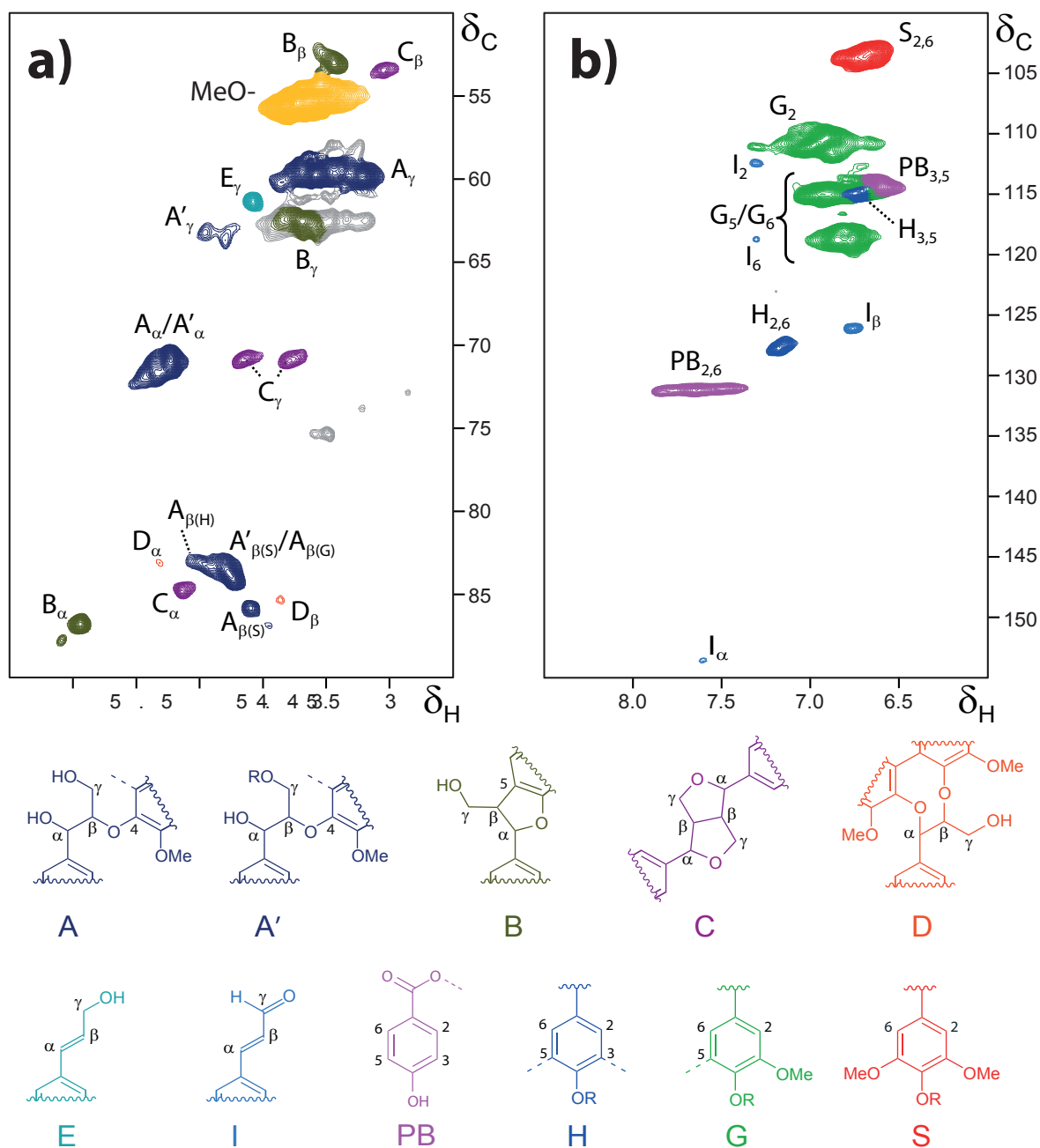


Figure 1. Side-chain region (a) and aromatic region (b) of the 2D-HSQC spectrum of MWL from coconut coir. The structures found are depicted below.

Table 1. Structural characteristics (lignin inter-unit linkages, end-groups, γ -acylation, aromatic units and S/G ratio, *p*-hydroxybenzoate content) from integration of ^{13}C - ^1H correlation signals in the HSQC spectra of the MWL isolated from coconut coir.

<u>Lignin inter-unit linkages (%)</u>		<u>Lignin side-chain γ-acylation (%)</u>	8
β -O-4' aryl ethers (A)	82	<u>Lignin aromatic units</u>	
Phenylcoumarans (B)	13	H (%)	4
Resinols (C)	4	G (%)	78
Dibenzodioxocins (D)	1	S (%)	18
<u>Lignin end-groups</u>		S/G ratio	0.24
Cinnamyl alcohol end-groups (E)	5	<u><i>p</i>-Hydroxybenzoate</u>	13
Cinnamyl aldehyde end-groups (I)	3		

IV. CONCLUSIONS

The MWL isolated from coconut coir is a *p*-hydroxyphenyl-guaiacyl-syringyl lignin type, with a predominance of guaiacyl over syringyl lignin units (S/G 0.24). A considerable amount of associated *p*-hydroxybenzoates (13%) can be found in this lignin, which is also partially acetylated at the γ -OH of the side-chain, preferentially in syringyl rather than in guaiacyl units. The main linkages presented in this lignin are β -O-4' alkyl-aryl ethers, phenylcoumarans (β -5'), resinols (β - β') and a small amount of dibenzodioxocins (5-5').

V. ACKNOWLEDGEMENT

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