

Structural Characterization of Lignin from Leaf Sheaths of
“Dwarf Cavendish” Banana PlantLÚCIA OLIVEIRA,^{†,‡} DMITRY V. EVTUGUIN,^{*,§} NEREIDA CORDEIRO,^{†,‡}
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Dioxane lignin (DL) isolated from leaf sheaths of banana plant (*Musa acuminata* Colla var. *cavendish*) and in situ lignin were submitted to a comprehensive structural characterization employing spectroscopic (UV, FTIR, solid state ¹³C CP-MAS NMR, liquid state ¹³C and ¹H NMR) and chemical degradation techniques (permanganate and nitrobenzene oxidation). Results obtained showed that banana plant leaf sheath lignin is of HGS type with a molar proportion of *p*-hydroxyphenyl (H)/guaiacyl (G)/syringyl (S) units of 12:25:63. Most of the H units in DL are terminal phenolic coumarates linked to other lignin substructures by benzyl and C_γ-ester bonds in contrast to ferulates that are mainly ether linked to bulk lignin. It is proposed that banana plant leaf sheath lignin is chemically bonded to suberin-like components of cell tissues by ester linkages via essentially hydroxycinnamic acid residues. β-O-4 structures (0.31/C₆), the most abundant in DL, comprise mainly S units, whereas a significant proportion of G units is bonded by β-5, 5–5', and 4-O-5' linkages contributing to ca. 80% of condensed structures in DL.

KEYWORDS: Lignin; leaf sheaths; *Musa acuminata* Colla var. *cavendish*; banana plant; ¹³C NMR; ¹H NMR; FTIR; lignin degradation methods

INTRODUCTION

Banana production is an important sector of agricultural activity in Macaronesia (a group of islands in the East Atlantic) reaching 200–250 thousand tons annually. In Madeira Island (Portugal) banana plantations contribute significantly to the regional economy. Among the cultivated species, “Dwarf Cavendish” (*Musa acuminata* Colla var. *cavendish*) is nowadays the most important one in terms of occupied area and productivity comprising ca. 60% of the total banana production in Madeira. After harvesting of a single bunch of bananas, a great amount of agricultural residues are produced. In Madeira these agricultural residues on plantations reach around 15 000 tons/year. Pseudo-stems and foliage are usually left in the plantation soil to be used as organic fertilizer. However, these banana tree counterparts and some other byproducts of banana processing such as rachis could represent an economically interesting renewable source of fiber material. Recent preliminary studies on the utilization of pseudo-stems and rachis from Dwarf Cavendish as raw materials for papermaking (1) and composite materials (2, 3) respectively, have shown rather promising results.

Further investigations on the potential industrial utilization of Dwarf Cavendish banana residues require detailed knowledge of their chemical composition. Literature on the chemical and structural analyses of Dwarf Cavendish components is quite scarce and dispersed, and it often refers to different *Musa* species. Previous chemical studies on *Musa* species included general chemical composition (4, 5) and extractives analysis (6–8) in different morphological parts of *sapientum* and *cavendish* species and general polysaccharides and lignin characterization in leaf sheath fibers of *Musa textilis* (9, 10). A comprehensive study on the chemical composition and structure of major components from Dwarf Cavendish has not yet been done.

As part of a research project aiming to find new applications for the banana plant vegetal residues produced after the harvesting of fruits, we have been studying the chemical composition of the plant tissues from different morphological regions of Dwarf Cavendish, harvested in Madeira Island (5, 7, 8). Among the structural components of Dwarf Cavendish, lignin is one of the most abundant (10–24%) and is found in all morphological regions of this *Musa* species. Lignin is an important structural polymer that determines significantly the mechanical steadiness of plants (11) and their behavior in chemical, mechanical, and thermal processing. In the present study, the lignin structure from leaf sheaths of Dwarf Cavendish was investigated. Lignin was characterized by wet chemistry

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and spectroscopic (UV, FTIR, and NMR) methods and size exclusion chromatography (SEC).

MATERIALS AND METHODS

Preparation of Plant Material. Randomly selected mature "Dwarf Cavendish" banana plants were harvested on a banana plantation in Funchal (Madeira Island, Portugal). Banana plants were separated into five different morphological parts: petioles/midrib, leaf blades, floral stalk, leaf sheaths, and rachis. The pseudo-stems were separated from foliage, manually separated into leaf sheaths (major part of the pseudo-stem) and floral stalk, and air-dried. The fraction corresponding to leaf sheaths was used in this study. The plant material was milled in a Retsch AS200 and sieved to 40–60 mesh fraction. This fraction was submitted to successive extractions with ethanol/toluene (2:1 v/v) mixture and water. The extractives contents were 4.2% and 6.2% (oven dried (o.d.) material), respectively.

Alkaline Pre-extraction of Plant Sawdust. The alkaline extraction of extractive-free plant sawdust was performed in nitrogen atmosphere with 0.3% (0.075 mol/L) NaOH solution for 1 h (liquid-to-plant sawdust ratio 50:1) under reflux. Extracted sawdust was thoroughly washed with hot distilled water until the filtrate was neutral, dried at room temperature, and used for further analysis. The lignin content was determined in the extractive-free samples and in the alkali pre-extracted samples by the Klason method according to Tappi Standard T204 om-88. The lignin contents of extractive-free and in alkali pre-extracted samples were 13.3% and 7.8% (o.d. material), respectively.

Isolation of Lignin. Lignin was isolated by acidolysis from alkali pre-extracted sawdust in a nitrogen atmosphere by the dioxane method adapted from a previously published procedure (12). The pre-extracted sawdust was submitted to three sequential extractions (30 min each) with 200 mL of dioxane–water (9:1, v/v) solution containing 0.2 M HCl at reflux under a nitrogen atmosphere. The powder was washed then with dioxane–water (9:1, v/v) (without HCl). Each portion of extract was concentrated separately to around 40 mL; resulting concentrates were then combined and lignin was precipitated by addition of the dioxane solution in cold water. The lignin was centrifuged, washed with water until neutral pH, and freeze-dried.

Lignin Purification. The isolated lignin was submitted to an extraction step with dioxane:methanol (9:1, v/v) solution under stirring at room temperature for 2 h. Then, the dissolved lignin was once more precipitated in cold water, centrifuged, and freeze-dried. This procedure was repeated to ensure that all the lignin was extracted. All the purified lignin fractions were combined. The amount of purified lignin represented about 30% of lignin in the alkali pre-extracted plant, based on Klason lignin.

To identify the aliphatic compounds in the purified lignin, it was washed three times with chloroform at room temperature for 24 h with constant stirring and dried under vacuum (13). This lignin, representing about 17% of the lignin in the alkali pre-extracted plant, based on Klason lignin, was then submitted to structural characterization analysis.

Chloroform Extract Analysis. Twenty milligram samples of each extract were dissolved in 10 mL of 1 M KOH in 10% aqueous methanol. The mixtures were heated at 100 °C, under nitrogen, for 1 h. The reaction mixtures were cooled, acidified with 1 M HCl to pH ~2, and then extracted three times with dichloromethane (3 × 30 mL). The solvent was evaporated to dryness. Before gas chromatographic–mass spectrometric (GC–MS) analysis, nearly 20 mg of each dried sample (hydrolyzed extracts) was dissolved in 250 μ L of pyridine and the compounds were converted into trimethylsilyl (TMS) derivatives by the addition of 250 μ L of bis(trimethylsilyl)trifluoroacetamide and 50 μ L of trimethylchlorosilane. After the mixture had been kept at 70 °C for 30 min, the derivatized extracts were analyzed by GC–MS (14). GC–MS analyses were performed using a Trace Gas Chromatograph 2000 Series equipped with a Finnigan Trace MS mass spectrometer, using helium as carrier gas (35 cm/s), equipped with a 30 m × 0.32 mm i.d., 0.25 μ m DB-1 capillary column from J&W Scientific (Folsom, CA). The chromatographic conditions were as follows: initial temperature, 80 °C for 5 min; temperature rate, 4 °C/min; final temperature, 285 °C for 10 min; injector temperature, 290 °C; transfer-line

temperature, 290 °C; split ratio, 1/100. Butanedioic acid was used as internal standard.

Chemical Analysis. The permanganate oxidations of in situ and isolated lignin samples as well as the alkaline nitrobenzene oxidation of in situ lignin were performed as previously described (15, 16). The analysis of neutral sugars in the lignin sample was performed as previously reported (17). Methoxyl group analysis was performed by the Zeisel procedure (18), and the elemental composition was determined using a LECO CHNS-932 instrument.

Analysis by Size Exclusion Chromatography (SEC). Weight-average molecular weights (M_w 's) of lignins dissolved in dimethylformamide (0.5% w/v) with 0.1 N LiCl were determined by SEC using a PL-GPC 110 chromatograph (Polymer Laboratories, UK) equipped with a Plgel 10 μ m precolumn and two 300 × 7.5 mm Plgel (5 μ m) Mixed D columns (Polymer Laboratories, UK). The precolumn, column, injection system, and a refractive index detector were maintained at 70 °C. The eluent (0.5% w/v LiCl in DMF) was pumped at a flow rate of 0.9 mL/min. The SEC columns were calibrated using lignin preparations previously characterized by electrospray ionization mass spectrometry (ESI/MS).

Analysis by UV Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and 13 C Cross-Polarization Magic Angle Spinning (CP-MAS) NMR. Ultraviolet (UV) spectra were recorded in 2-methoxyethanol on a JASCO V-560 UV/vis spectrophotometer using 1 cm thick cells. FTIR spectra were recorded on a Mattson 7000 FTIR spectrometer using KBr pellets (1/250 mg). The spectra resolution was 4 cm^{-1} , and 64 scans were averaged. ^{13}C solid-state NMR spectra were recorded at 100.6 MHz (9.4 T) on a Bruker Avance 400 spectrometer. A 7-mm double bearing Bruker rotor was spun in air at 5.0 kHz. In all experiments the ^1H and ^{13}C 90° pulses were ca. 4 μ s. The CP-MAS spectra were recorded with a 5 s recycle delay and a 2 ms contact time.

Analysis by ^1H NMR. The ^1H NMR spectrum of the acetylated lignin in chloroform (CDCl_3) solution (2% concentration) was obtained using a Bruker Avance 300 spectrometer, operating at 300 MHz, at room temperature. The acquisition parameters used were as follows: 12.2 μ s pulse width (90°); 3 s relaxation delay; 300 scans.

Analysis by ^{13}C NMR. ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer operating at 75.5 MHz. Lignin sample was dissolved in $\text{DMSO}-d_6$ (ca. 23% concentration); the mixture was placed into a 5-mm-diameter tube and the spectrum was recorded at 318 K with TMS as internal reference. The inverse gated decoupling sequence, which allows quantitative analysis and comparison of signal intensities, was used with the following parameters: 4.1 ms pulse width (90° pulse angle); 12 s relaxation delay; 16 K data points; 18 000 scans.

RESULTS AND DISCUSSION

Isolation of Dioxane Lignin. Leaf sheaths represent a major biomass source of the banana tree, containing long fibers suitable for potential applications in papermaking and in biocomposites. The isolation of dioxane lignin for structural studies was carried out from extractive-free leaf sheaths, which were additionally alkali pre-extracted to eliminate the tannins. The yield of lignin isolated from leaf sheaths (ca. 30% based on Klason lignin in the alkali pre-extracted plant) was about half that reported for other annual plants (19, 20). This can be explained by a strong structural association of in situ Dwarf Cavendish lignin with polysaccharides and lipophilic compounds in leaf sheath tissues as discussed below. For the same reason our efforts on the isolation and subsequent purification of lignin using known procedures for milled wood (21) failed. In the latter case a lignin yield below 5% was obtained and the former was highly contaminated with aliphatic substances and polysaccharides.

It should be noted that the alkaline extraction of leaf sheaths before the dioxane lignin isolation led to the elimination not only of condensed tannins but also of a lignin fraction rich in *p*-hydroxyphenyl (H) type structures. As revealed by analysis of nitrobenzene oxidation (NO) products of leaf sheath material

Table 1. Elemental Analysis and Methoxyl Group Contents of Banana Plant Leaf Sheaths Dioxane Lignins before (DLne) and after (DLe) Chloroform Extraction

sample	C, %	O, %	H, %	OCH ₃ , %	empirical formula on C9	Mppu, g/mol
DLne	65.1	28.3	6.6	18.3		
DLe	61.9	33.6	6.7	17.4	C ₉ H _{9.78} O _{2.71} (OCH ₃) _{1.09}	194.9

before and after alkaline extraction, the proportion of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) structural units (H/G/S) in lignin changed from 12:25:63 to 6:23:71.

Purification of Dioxane Lignin. The isolated banana plant leaf sheath dioxane lignin (DLne) contained a small amount of residual polysaccharides (~1%) and showed a relatively high percentage of carbon in the elementary analysis (Table 1). This last observation and the abundant band at 2938 and 2850 cm⁻¹ in the FTIR spectrum (Figure 1), corresponding to C–H stretching in aliphatic moieties (22), indicated a significant contamination of DLne with lipophilic substances. This proposition was confirmed by a solid-state ¹³C NMR spectrum (Figure 2), which revealed intensive resonances at 10–35 ppm assigned to –CH– and –CH₂– moieties in aliphatic chains (23, 24). Since these aliphatic compounds were not removed after exhaustive organic solvent and alkaline extractions, it is reasonable to propose their strong bonding or even chemical linking to lignin. If the lignin–polysaccharide linkages in annual plants are not surprising, a rather strong structural association of lignin with aliphatic compounds deserves particular interest.

To eliminate adsorbed aliphatic compounds, DLne was extracted with chloroform, which resulted in a purified dioxane lignin (DLe). The removal of aliphatic compounds from parent DLne was confirmed by FTIR (Figure 1) and solid-state ¹³C NMR spectra (Figure 2). However, the extraction with chloroform caused also a partial DLne fractionation. Thus, the FTIR spectrum of DLe (Figure 1) showed an intensity decrease at

1323 cm⁻¹, assigned to syringyl ring breathing with C_{Ar}–OCH₃ stretching (25). Simultaneously, the ratio A₁₄₆₂/A₁₅₉₅ related to the amounts of methoxyl groups in lignins (26) decreased in the FTIR spectrum of DLe. This indicates that during DLne extraction with chloroform a certain proportion of lignin rich in S units was removed jointly with aliphatic compounds. Such a proposition was additionally confirmed by a ¹³C CP-MAS NMR spectrum (Figure 2), showing a significant decrease of resonance at 152 ppm in DLe assigned to C-3/C-5 resonances in S units (27). Simultaneously, the proportion of guaiacyl (G) units in DLe increased slightly as follows from an enhanced group of nonresolved signals at 110–120 ppm assigned to C-2/C-5/C-6 resonances in G units (27). Additionally, a remarkable signal around 159 ppm in DLne assigned to C-4 in H type structures (27) decreased dramatically after extraction, indicating the partial removal of these structures. The decrease of S units in DLe explains the lower amounts of methoxyl groups in this lignin when compared to DLne (Table 1).

Thus, it can be concluded that DLne isolated from the leaf sheaths is of the HGS type lignin in which a certain lignin fraction rich in H and S units is strongly structurally associated with aliphatic compounds. The nature of those aliphatic structures was evaluated through the analysis of low molecular weight compounds in saponified chloroform extract by GC–MS. The total ion chromatogram and the identified peaks are presented in the Supporting Information. Among more than 70 compounds detected in the extract, 58 were identified. Long chain saturated/unsaturated fatty acids, hydroxy acids, dicarboxylic acids, and minor amounts of sterols, followed by *p*-coumaric and ferulic acids, represented the major part of identified compounds. Notable amounts of long chain hydroxy acids (2-hydroxytetracosanoic, 24-hydroxytetracosanoic, 26-hydroxyhexacosanoic, and 28-hydroxyoctacosanoic acids among others) and *p*-coumaric and ferulic acids may originate from

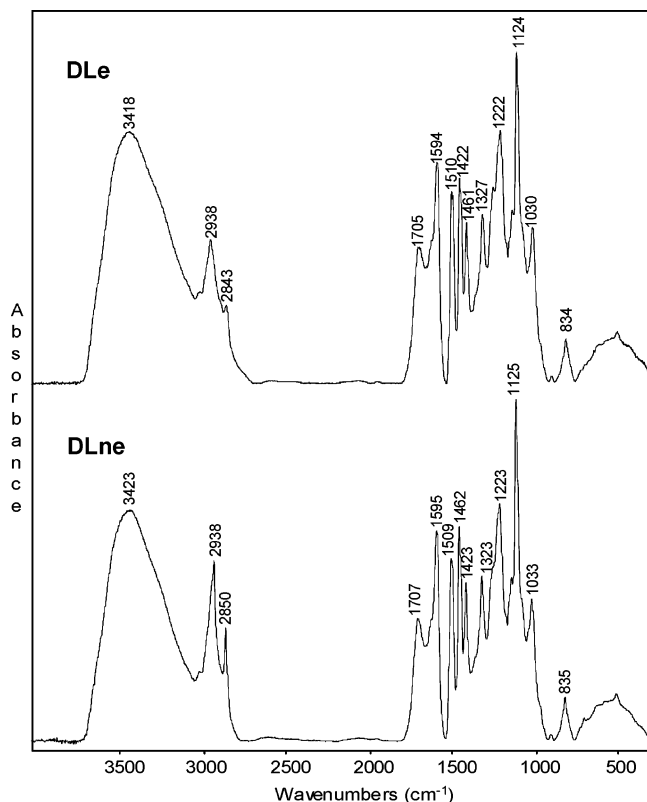
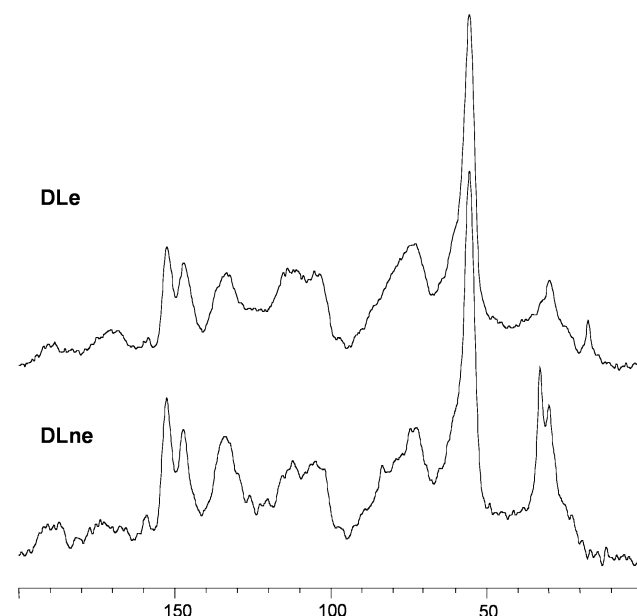
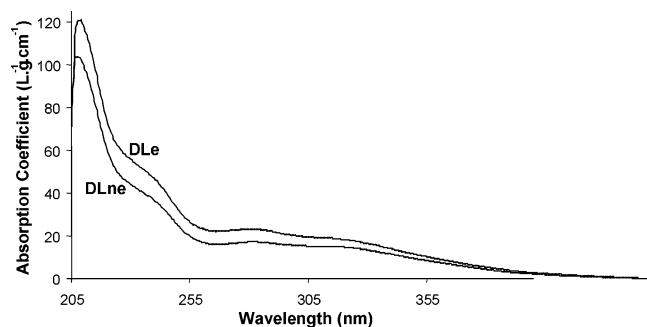
**Figure 1.** FTIR spectra of banana plant leaf sheath lignin before (DLne) and after (DLe) chloroform extraction.**Figure 2.** Solid-state ¹³C CP-MAS NMR spectra of banana plant leaf sheath lignin before (DLne) and after (DLe) chloroform extraction.

Table 2. Molar Proportions of Permanganate Oxidation Products (1–10)^{a,d}

sample	1	2	3	4	5	6	7	8	9	10
leaf sheaths ^b	46	31	6	6	tr.	2	7	1	1	nd
leaf sheaths ^c	35	36	9	5	2	4	5	2	2	nd
DLne	22	32	27	5	3	2	5	1	3	tr
DLe	21	33	24	6	3	2	6	1	4	tr

^a See **Figure 5** for structural assignments. ^b Ethanol–toluene and water-extracted leaf sheaths. ^c Alkali-extracted leaf sheaths. ^d nd, nondetected; tr, traces. The yields of permanganate oxidation products varied from around 17 to 21% (w/w).

**Figure 3.** UV spectra of dioxane lignin of banana plant leaf sheaths before (DLne) and after (DLe) chloroform extraction.

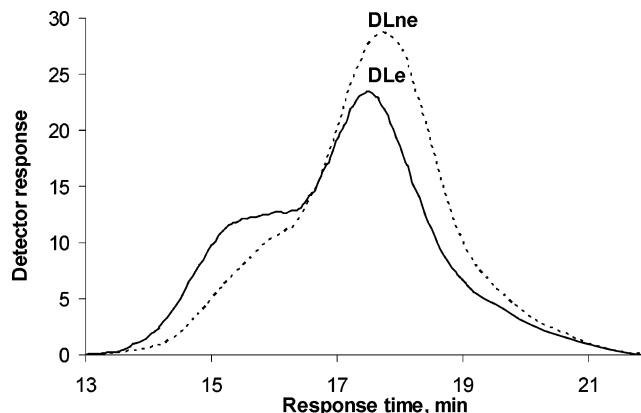
suberin-like structures, saponified during the alkaline extraction of leaf sheath sawdust before the dioxane lignin isolation. In fact, the ester linked *p*-coumaric and ferulic acids residues were even considered as a part of suberin material in suberized tissues (28, 29). Moreover, the suberin lamellae components are chemically linked to the lignin of primary cell wall in certain tissues of annual plants (29). A significant proportion of chloroform extract was represented by relatively high molecular weight suberin-like and lignin compounds that could not be analyzed by GC–MS.

Analysis of Lignin Structure by UV and FTIR Spectroscopies. The preliminary structural information on nonpurified (DLne) and purified (DLe) dioxane lignins was gained from their UV and FTIR spectra. The UV spectra of leaf sheath lignins (**Figure 3**) showed the main absorption bands typical for dioxane lignins from annual plants (19).

As can be seen from UV spectra, DLe revealed an extinction coefficient of the characteristic absorption maximum at 280 nm corresponding to the $\pi \rightarrow \pi^*$ transition in the aromatic ring (30) higher than that of DLne. This can be explained by the presence of non-lignin counterparts in DLne discussed above. The absorption around 310 nm indicates the presence of structures containing unsaturated moieties conjugated with aromatic moieties. This band at 310 nm was previously reported as indicative of the presence of hydroxycinnamic acid type structures (31).

The presence of hydroxycinnamic acid type structures in dioxane lignins was supported by the FTIR spectra (**Figure 1**). This is shown by characteristic bands at 1705 and 1630 cm^{-1} , assigned to C=O stretching of carboxylic acids, and by the stretching of C=C moieties conjugated with aromatic rings, respectively (25). Since the analysis of the chloroform extract of DLne showed the presence of *p*-coumaric and ferulic acids, it could be proposed that these structural units are also present in the leaf sheath lignin.

Molecular Weight Distribution. The analysis of DLne and DLe by SEC showed bimodal molecular weight distribution curves for both samples (**Figure 4**). The appearance of the shoulder at elution time of 15–16 min could be explained by the presence of a lignin fraction chemically linked to suberin-

**Figure 4.** GPC molecular weight distribution of dioxane lignins obtained from banana plant leaf sheaths before (DLne) and after (DLe) chloroform extraction.

like compounds that affected the hydrodynamic volume of lignin macromolecules. The proportion of this fraction after DLne extraction with chloroform increases (DLe sample), because the low molecular weight lignin fraction and free aliphatic fatty acids were removed. This explains the higher molecular weight found for DLe than for DLne, namely 3325 and 2350 Da, respectively.

Permanganate Oxidation Analysis. The permanganate oxidation (PO) analysis of preliminary ethylated dioxane and in situ lignins, followed by GC and GC–MS analysis of the oxidation products, allowed the identification and quantification of 10 carboxylic acid methyl esters (**Table 2**; **Figure 5**). These products reflect the molar proportions of different substructures.

The relative abundance of products 1–3 after PO analysis of banana plant leaf sheaths (**Table 2**) was very close to that reported previously for nodes of reed (*Arundo donax*) (20), meaning that the proportion of noncondensed H (product 1), G (product 2), and S (product 3) units in those species should be similar. It could also be proposed that most of the H units (analogously to reed) are represented by *p*-coumaric acid type structures. The significantly higher proportion of H units detected in banana plant leaf sheaths after PO analysis (35–46 mol %, **Table 2**), when compared with the results obtained by NO analysis (6–12 mol %), indicate that most of them are terminal phenolic units, since only these are accessible to the PO analysis. At the same time a small proportion of H units is found in the composition of 5–5' biphenyl type structures as revealed from product 10 detected in the PO analysis of DLne and DLe lignins (**Table 2**).

Although the proportions of condensed structures (products 4–10, **Table 2**) in the in situ and dioxane lignins (DLne and DLe) are similar, the proportion of noncondensed structures (products 1–3) in those samples is rather different. During the acidolytic isolation procedure, part of the β -O-4 linkages in lignin are broken, yielding phenolic end units accessible for PO analysis and giving higher abundances of product 3 than those

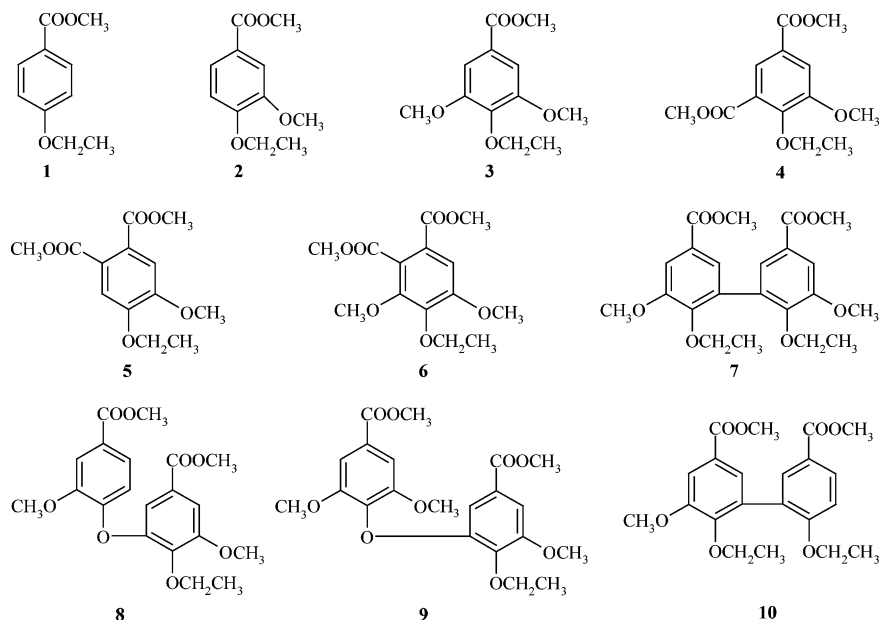


Figure 5. Carboxylic acid methyl ester structures obtained from permanganate oxidation analysis.

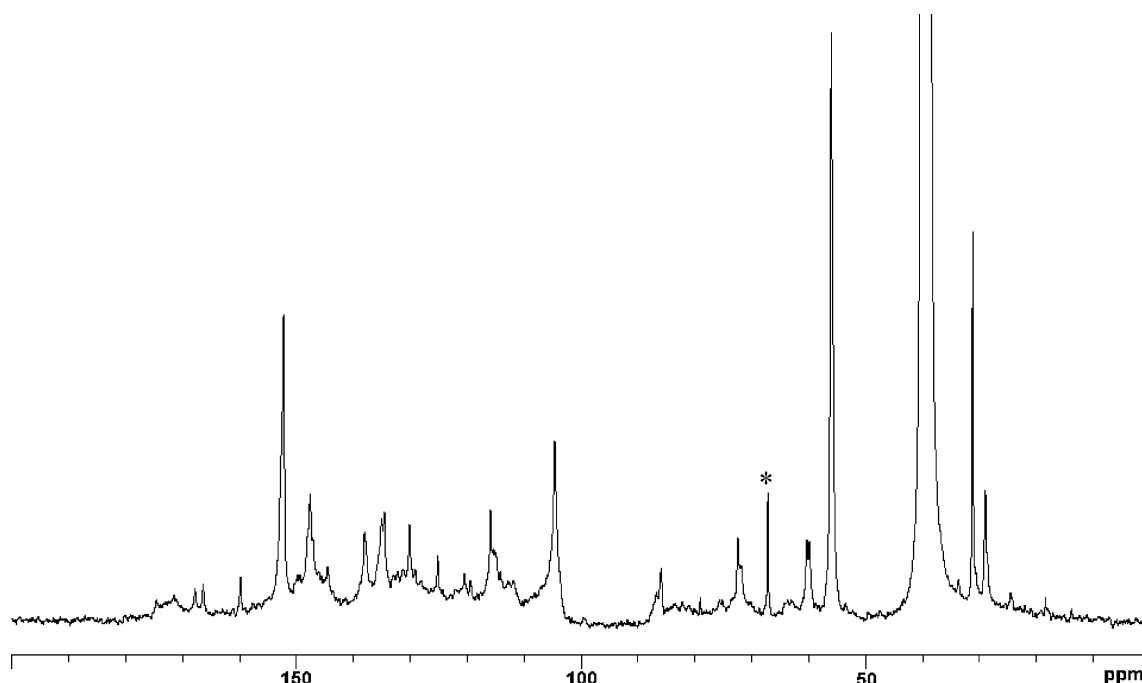


Figure 6. ^{13}C NMR spectrum of dioxane lignin (DLe) from banana plant leaf sheaths (*, solvent contamination).

found for in situ lignin. Therefore, the increased proportion of product **3** and the diminished proportion of product **1** after PO of DLne and DLe, when compared to the in situ lignin (Table 2), are explained by the partial cleavage of ether/ester linkages between S and H units during the acidolytic isolation procedure.

It may be concluded based on results of PO analysis that most of the condensed structures of Dwarf Cavendish lignin are represented by phenylcoumarin (β -5') and biphenyl (5-5') type structures (products **4**, **5**, and **7**, respectively) followed by diaryl ether type structures (products **8** and **9**). The relatively small amounts of product **6** (2–4 mol %) indicate the presence of α -6' (isotaxiresinol type) or β -6' (phenylisochroman type) structures suggested previously in wood lignins (32, 33).

Relative to the chloroform extraction step, the proportions of PO products **1**–**10** obtained for DLne and DLe were very similar (Table 2). Hence, no significant structural differences were found in these lignins though DLe represents a much more

pure sample. For those reasons further structural analyses employing NMR techniques were carried out only with DLe.

^{13}C NMR Analysis. The quantitative ^{13}C NMR spectrum of purified banana plant leaf sheath dioxane lignin (DLe) is presented in Figure 6. The near absence of typical polysaccharide signals between 90 and 103 ppm in the ^{13}C NMR spectrum confirms the low content of associated polysaccharides in DLe determined by analysis of neutral sugars (~1%). According to previously published methodology (12), the regions at 103–156 and at 159–162 ppm in ^{13}C NMR spectrum were assigned to aromatic carbon resonances and, after correction for the presence of olefinic structures, the calculation of all structural elements in DLe was carried out per one aromatic ring (C_6) [molar contribution of structural unit = integral of characteristic carbon resonance in the structure/(corrected integral at 103–156 + 159–162 ppm/6)].

The resonances at 167.8 and 166.4 ppm were assigned to carbons in carboxyl and ester groups, respectively, in *p*-coumaric and ferulic acid structures (34, 35). A series of signals at 159.0–162.0 ppm indicate the presence of *p*-coumaric structures in DLe and are assigned to C-4 in the aromatic ring. A small resonance at 61.8 ppm was assigned to C_γ in cinnamyl alcohol type structures (36). Using these assignments, the integral of signals at 103–156 ppm ($I_{103-156}$) was corrected for the presence of olefinic structures ($I_{103-156(\text{corrected})} = I_{103-156} - 2I_{166.0-168.0} - 2I_{61.5-62.0}$).

Characteristic tertiary carbon resonances from S units at 103.0–110.0 ppm (C-2,6), G units at 110.0–125.0 ppm (C-2,5,6), and quaternary carbon resonances from H units at 159.0–162.0 ppm (C-4) were used to assess the H:G:S ratio in DLe (12). This ratio was 7:43:50. However, the proportion of S units may be seriously overestimated since these structures are less condensed than G units (Table 2) and contribute more strongly to the number of tertiary carbon signals. The amounts of H type structures, other than *p*-coumarates, were insignificant (<0.02/C₆) as revealed from integrals of the signals at 156–158 ppm (C-4 in corresponding structures) and were not considered in calculations.

The amount of methoxyl groups per aromatic ring (1.10/C₆), calculated based on the correspondent carbon signal centered at 56.1 ppm (Figure 6), was very similar to the number of methoxyl groups per statistical phenylpropane unit (1.09/C₉) calculated based on the elemental analysis and the analysis of methoxyl groups (Table 1).

The amount of *p*-coumaric acid structures assessed based on resonances at 159.0–162.0 ppm was 0.07/C₆, and the amount of ferulic acid structures (0.05/C₆) was calculated from the difference of integrals at 166.0–168.0 ppm (sum of *p*-coumarates and ferulates) and 159.0–162.0 ppm (only *p*-coumarates). *p*-Coumaric acid structures in DLe are mainly terminal phenolic units, because the signal at 159.8 ppm (C-4 in phenolic *p*-coumarates) is prevalent over the signal at 160.8 ppm (C-4 in non-phenolic *p*-coumarates). Therefore, a significant part of *p*-coumaric acid structures should be ester-linked to other lignin substructures. In the ¹³C NMR spectrum of DLe the characteristic resonances at 75.0–76.0 ppm (C_α in benzyl ester of *β*-O-4 structures) and 64.0–65.0 ppm (C_γ in *γ*-esterified *β*-O-4 structures) were observed. The total amount of benzyl and *γ*-ester structures (0.09/C₆), calculated from the corresponding integrals, was almost 50% more intense than the integral of signals at 166.0–167.0 ppm (C_γ in esterified *p*-coumarates and ferulates, 0.06/C₆), indicating that hydroxycinnamic acid structures are not the only ones ester-linked to bulk lignin. These could be the suberin-like compounds, although the latter are normally linked to lignin by ester linkages via ferulates (29). The intense resonances at 29.0 and 31.2 ppm (Figure 6) confirm that, despite the removal of the major proportion of aliphatic compounds during DLne extraction with chloroform, part of them still remained in the purified lignin sample. A group of nonresolved signals at 171–173 ppm in the ¹³C NMR spectrum (Figure 6) showed the presence of ester-linked fatty/hydroxy acids. Nonesterified *p*-coumarates and ferulates of total amount 0.06/C₆ (integral at 167.0–168.0 ppm) should be linked to other lignin substructures by ether or carbon–carbon bonds. Since most *p*-coumaric acid structures (0.07/C₆) are terminal phenolic units and the total amount of *p*-coumarates and ferulates is 0.12/C₆, almost all ferulic acid units (0.05/C₆) should be ether-linked, because the total number of hydroxycinnamic structures with free carboxyl groups (nonphenolic ones) was 0.07/C₆ (resonance at 168.8 ppm).

Table 3. Data on Different Structural Elements in Banana Plant Leaf Sheath Dioxane Lignin (DLe) As Revealed by ¹³C NMR

structural element	number/C ₆
OCH ₃	1.10
<i>β</i> - <i>β</i> + <i>β</i> -5 structures	0.08
<i>β</i> -O-4 structures without C _α =O	0.27
<i>β</i> -O-4 structures with C _α =O	0.04
<i>β</i> -O-4/ <i>α</i> -O-4 structures	0.03
benzyl and C _γ -ester structures	0.09
coniferyl alcohol structures	0.02
<i>p</i> -coumaric acid structures	0.07
ferulic acid structures	0.05
H:G:S ratio	7:43:50

The lignin structures involving *β*-O-4 linkages without C_α=O group were calculated based on the integral of signals at 59.0–61.0 ppm assigned to the C_γ resonance (36). These are the most frequent structures in DLe, representing about 0.27/C₆. The frequency of *β*-O-4 structures with C_α=O group (0.04/C₆) was assessed based on the small signal at 63.0 ppm, assigned to C_γ in corresponding structures (37). These amounts were confirmed by the integral at 196.0–198.0 ppm assigned to carbon resonance in C_α=O groups of *β*-O-4 structures (38). The amount of *β*-*β* and *β*-5 structures in DLe lignin was rather small (0.08/C₆) as calculated from integral of signals at 51.5–54.0 ppm assigned to C_β in corresponding structures (36, 37). The characteristic resonance at 79.0 ppm assigned to C_α in *β*-O-4 structures with *α*-O-4 linkages (39) allowed the confirmation of the existence of noncyclic *α*-O-4 structures in DLe and their quantification (0.03/C₆). The data on frequency of occurrence of different DLe structures are summarized in Table 3.

Comparing the intensities of the signals at 152.1 ppm (C-3,5 in etherified *β*-O-4 linked S units) and 149.0–149.5 ppm (C-3 in etherified *β*-O-4 linked G units), the ratio of which is about 8:1, it is possible to conclude that S units are mainly ether-linked contributing to linear molecular fragments, whereas G units are mainly nonetherified and frequently linked by carbon–carbon bonds representing terminal phenolic lignin units. This explains the much higher abundance of G than S units in PO analysis (Table 2). It should be noted that part of the linear low molecular weight fragments comprised mainly of *β*-O-4-linked S units was extracted with chloroform during DLne purification as discussed above. This explains the underestimation of the proportion of S structural units in DLe (H:G:S = 7:43:50, Table 3) when compared to the in situ lignin of leaf sheaths before the dioxane lignin isolation, the H:G:S ratio of which was 6:23:71 as determined by NO analysis.

¹H NMR Analysis. Proton NMR spectroscopy was used to confirm the quantitative estimations of some structural elements done by ¹³C NMR and to obtain additional structural information on lignin. All calculations were made per C₉ unit using the resonance of methoxyl protons at 3.60–4.00 ppm as an internal standard. The ¹H NMR spectrum of acetylated leaf sheath lignin DLe is presented in Figure 7.

The abundance of *β*-O-4 structures was estimated based on the resonance of H_α at 5.8–6.2 ppm (40). Thus, the value obtained for the content of *β*-O-4 structures without C_α=O (0.26/C₉) is practically equal to that determined by ¹³C NMR spectroscopy per C₆ (0.27/C₆). The amounts of cyclic/noncyclic *α*-O-4 structures (0.09/C₉) were calculated based on the resonance at 5.4–5.6 ppm (C_α resonances). The estimated frequency of occurrence of *β*-*β* structures was suggested to be about 0.04/C₉ as determined from the integral at 3.0–3.15

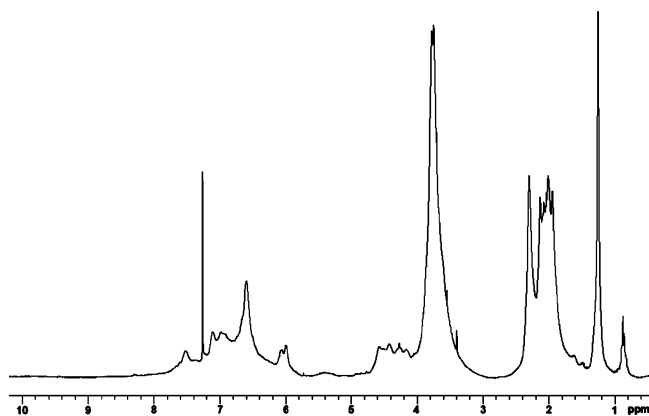


Figure 7. ^1H NMR spectrum of dioxane lignin (DLe) from banana plant leaf sheaths.

ppm (C_β in corresponding structures). If the amount of β -5 and β - β structures is $0.08/\text{C}_6$, as revealed from the ^{13}C NMR spectrum (**Table 3**), and the amount of β - β structures is $0.04/\text{C}_9$ as revealed from the ^1H NMR spectrum, the estimated content of β -5 structures calculated by difference should be $0.04/\text{C}_9$. In this case the number of cyclic α -O-4 bonds ($0.04/\text{C}_9$) in β -5' structures can be subtracted from the total amount of α -O-4 structures calculated from the ^1H NMR spectrum ($0.08/\text{C}_9$), giving the amount of noncyclic α -O-4 moieties ($0.04/\text{C}_9$), which is very similar to that obtained from the ^{13}C NMR spectrum ($0.03/\text{C}_6$).

The signals between 0.8 and 1.6 ppm, assigned to CH_3 and CH_2 in saturated aliphatic chains, respectively, suggest once more the presence of aliphatic compounds linked to lignin (total amount $1.36/\text{C}_9$). The existence of suberin-like aliphatic chains covalently bound to the lignin polymer with resonances similar to above was already reported for monocotyledonous plants (41). The presence of proton signals from suberin-like aliphatic structures (including hydroxy acids) at 1.5–2.2 ppm did not allow the correct quantification of aromatic and aliphatic hydroxyl groups, the acetate derivatives of which showed resonances in the same region (42). The broad signal around 7.4–7.6 ppm was assigned to H-2/H-6 aromatic protons and H_α in p -coumaric acid structures (38), confirming their significant amounts in DLe.

The results described here represent the first comprehensive study on the leaf sheaths of Dwarf Cavendish. It was suggested that lignin in leaf sheath tissues is structurally associated with suberin-like components, which complicate its isolation and characterization. It is suggested that banana plant leaf sheath lignin is chemically bonded in the cell tissues to suberin-like components, probably by ester linkages via essentially hydroxycinnamic acid residues. However, the direct esterification of lignin with carboxylic moieties of fatty acids cannot be completely excluded, as follows from the balance of ester bonds in DLe.

Leaf sheath lignin is composed of H, G, and S units. Their molar ratio (H:G:S), before alkaline extraction of starting material, was 12:25:63 as assessed by NO. Practically all H units are represented by coumarates linked to other lignin substructures by benzyl and C_γ -ester bonds. A notable proportion of coumarates should be linked to linear fragments constituted by β -O-4-linked S units. Ferulates are less abundant in banana plant leaf sheath lignin than coumarates. The β -O-4 structures are the most abundant in lignin ($0.31/\text{C}_6$). The frequency of occurrence of β -5 and β - β structures in dioxane lignin from banana plant leaf sheaths is remarkably lower, being almost half that in dioxane lignins of other annual plants such as kenaf (19)

and reed (20). In contrast, the amount of biphenyl and diaryl ether type structures is rather significant, approaching even the values reported for G type lignins from softwood (15).

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Supporting Information Available: Table containing data of the components identified in the chloroform extract from banana plant leaf sheath dioxane lignin (DLne). Figure containing the total ion chromatogram of chloroform extract obtained from dioxane lignin isolated from leaf sheaths of Dwarf Cavendish banana plant. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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