Lignin from Tree Barks: Chemical Structure and Valorization

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Lignins from different tree barks, including Norway spruce (*Picea abies*), eucalyptus (*Eucalyptus globulus*), mimosa (*Acacia dealbata*) and blackwood acacia (*A. melanoxylon*), are thoroughly characterized. The lignin from *E. globulus* bark is found to be enriched in syringyl (S) units, with lower amounts of guaiacyl (G) and *p*-hydroxyphenyl (H) units (H/G/S ratio of 1:26:73), which produces a lignin that is highly enriched in β-ether linkages (83%), whereas those from the two *Acacia* barks have similar compositions (H/G/S ratio of ≈5:50:45), with a predominance of β-ethers (73–75%) and lower amounts of condensed carbon–carbon linkages; the lignin from *A. dealbata* bark also includes some resorcinol-related compounds, that appear to be incorporated or intimately associated to the polymer. The lignin from *P. abies* bark is enriched in G units, with lower amounts of H units (H/G ratio of 14:86); this lignin is thus depleted in β-O-4′ alkyl–aryl ether linkages (44%) and enriched in condensed linkages. Interestingly, this lignin contains large amounts of hydroxystilbene glucosides that seem to be integrally incorporated into the lignin structure. This study indicates that lignins from tree barks can be seen as an interesting source of valuable phenolic compounds. Moreover, this study is useful for tailoring conversion technologies for bark deconstruction and valorization.

Introduction

The search for an alternative to replace fossil fuels for the production of chemicals, products, and energy has found in lignocellulosic biomass the most widespread and available source of renewable raw materials. With agricultural crops being mostly intended for food production, forest biomass and residues from both agricultural and forestry industries are seen as the most reliable sources of biomass for the production of biofuels, bioproducts, and value-added chemicals, especially if inserted in a biorefinery context with full resource valorization and a zero-waste philosophy.[1,2]

In this regard, tree barks, which are generated in significant amounts as waste from the wood industries (e.g., timber or pulp and paper industries) or as residues resulting from forest management, are considered potential upgradable side streams for value-added applications.[3,4] These widely available and low-cost residues are mostly used as solid fuel for the production of energy and heat or for horticultural use, despite the high chemical potential that this lignocellulosic biomass might offer. The rationale for valorization of bark is its high availability, chemical richness, and structural diversity, allowing for the targeting of multiple products. However, its higher complexity requires better knowledge and understanding of its composition and structure, and perhaps more demanding and adequate processing routes. Moreover, as tree barks contain significant amounts of lignin (sometimes with higher lignin content than their respective woods), bark deconstruction routes should target this abundant aromatic polymer for the production of fuels, chemicals and materials that are nowadays produced from fossil resources.[5,6] Many studies have focused on the deconstruction and uses of the different bark fractions,[3,4,7,8] but only a few have addressed in detail the characterization of the lignin fraction,[9–11] even though lignin is a major component in these forest wastes that can be valorized as a source of platform chemicals, biofuels, and biobased materials.

Lignin is a complex phenylpropanoid polymer that has a structural role in plant cell walls while also providing hydrophobicity and protection against pathogens. Lignin is synthesized by the oxidative radical polymerization of three main hydroxycinnamyl alcohols—*p*-coumaryl, coniferyl, and sinapyl al-

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cohols (so-called monolignols)—to produce a branched backbone of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units.[12] Besides the three canonical monolignols, a growing number of other phenolic compounds have also been documented as behaving as true lignin monomers. These include phenolic compounds, such as monolignol ester conjugates, catechols, and methoxycatechols, arising from the truncated biosynthesis of monolignols or ferulate esters, among others. In addition, phenolic compounds derived from other biosynthetic pathways have also been found incorporated into the lignin of several plants. Such compounds include the flavone tricin, which is present in the lignins of grasses and other monocots,[13–15] the hydroxystilbenes piceatannol, isorhapontigenin, and resveratrol, which are incorporated into the lignin of palm fruit endocarps,[16,17] and their corresponding *O*-glucosylated counterparts (astringin, isorhapontin and piceid), which have recently been reported to be incorporated into the lignin of Norway spruce bark,[18] and the hydroxycinnamic amides tyramine ferulate, which is found in the lignins of some Solanaceae,[19] and diferuloylputrescine, which is found in the lignin of maize kernels.[20] These new additions to the family of lignin precursors show that this biopolymer is far more complex than previously thought, providing further evidence that any phenolic compound present in the lignifying zone of the cell wall can be incorporated into the lignin polymer through similar oxidative reactions.[12,21] More importantly, these discoveries greatly expand the range of valuable phenolic compounds that can be obtained from lignins, thus enhancing the value of what is considered a waste product of forestry and agricultural activities.

The overall lignin content and the composition and relative abundance of the different monomeric units, especially the S/G ratio and the distribution of the different linkage types and functional groups, are important parameters to understand the lignin structure, as well as its chemical properties and reactivities. Detailed knowledge of the lignin structure is a prerequisite to optimize and tailor the conditions for processing, aiming at lignocellulosic deconstruction for subsequent valorization of their components. In this sense, the present study focuses on the comprehensive structural characterization of lignins from the barks of a series of trees, including the softwood Norway spruce (*Picea abies*) and the hardwoods eucalyptus (*Eucalyptus globulus*), mimosa (*Acacia dealbata*), and blackwood acacia (*A. melanoxylon*). Eucalyptus and Norway spruce are representative of the major hardwood and softwood species used by the timber and pulp and paper industries in Europe, with bark accounting for roughly 10–15% of the bole mass,[7,8,22] which is generated as waste in large quantities at industrial sites. For the *Acacia* species, the continuous fight against these invasive species also generates large amounts of bark residues, since a common method to mitigate their proliferation is removing the bark without felling the tree, thus preventing sprouting from the stump. The detailed characterization of the lignin structure of these barks will be highly relevant for the further valorization of these abundant lignocellulosic wastes.

Results and Discussion

Composition of the main constituents of the barks

The abundances of the main constituents (namely, the contents of dichloromethane, ethanol and water extractives, Klason lignin, acid-soluble lignin, polysaccharides, and ash) of the different barks selected for this study are shown in Table 1. A wide diversity in the content of the different constituents was observed among all barks. In general terms, all barks had a high content of extractives, which were particularly prominent for *A. dealbata* bark, accounting for about 46% of the total bark, most of which were due to polar compounds. A high content of polar extractives has also been reported for other *Acacia* species, such as *A. mangium*, accounting for 38% of the total bark.[3] Great differences were also observed in the content of structural polysaccharides, with the barks of the two *Acacia* species showing the lowest amounts of structural polysaccharides (≈21–29%) when compared to *E. globulus* (≈61%) and *P. abies* (≈48%). One interesting feature regarding the composition of polysaccharides of *P. abies* bark was the higher content of xylose (≈5%) with respect to mannose (≈3%), which is in contrast to that found in its respective wood (≈7% xylose vs. ≈14% mannose) and what is commonly found in the woods of other conifers.[8] Regarding the lignin content, the bark from *A. melanoxylon* presented a very high value (≈55%), which might be the result of polyphenolics condensation during the Klason lignin determination procedure, implying that these compounds are intimately associated to the lignocellulosic matrix, even after successive extractions with dichloromethane, ethanol, and water. *A. dealbata* bark presented the lowest lignin content, which accounted for

Table 1. Abundance of the main constituents (wt% dry basis) of the different barks (average of three replicates). PA: *P. abies*; EG: *E. globulus*; AD: *A. dealbata*; AM: *A. melanoxylon*.

Components	PA[a]	EG[b]	AD	AM
Extractives				
dichloromethane	5.4±0.1	0.9±0.1	1.0±0.0	2.2±0.1
ethanol	4.5±0.3	2.3±0.1	37.5±0.3	5.9±0.2
water	10.3±1.3	6.6±0.2	7.8±0.8	6.1±0.7
Lignin				
Klason lignin	25.9±0.8	18.9±1.1	16.7±0.3	54.3±0.4
acid-soluble	0.9±0.1	3.0±0.1	1.9±0.1	1.0±0.1
Polysaccharides				
rhamnose	0.5±0.1	0.5±0.1	0.2±0.1	0.3±0.1
arabinose	5.2±0.4	1.6±0.1	1.9±0.1	2.2±0.1
galactose	2.0±0.1	1.6±0.1	1.2±0.1	1.5±0.1
glucose	26.8±0.1	37.5±1.6	19.0±0.6	12.4±0.1
xylose	4.6±0.2	15.2±0.2	3.7±0.7	2.2±0.1
mannose	2.6±0.1	0.4±0.1	0.6±0.2	0.5±0.1
galacturonic acid	5.6±0.1	1.7±0.1	0.5±0.1	0.8±0.2
glucuronic acid	0.3±0.1	0.1±0.1	0.6±0.1	0.8±0.1
acetic acid	0.4±0.1	2.7±0.1	0.9±0.1	0.6±0.1
Ash	3.9±0.1	5.4±0.2	3.3±0.1	5.6±0.1

[a] From reference [9]. [b] From reference [7].

roughly 19%. The lignin contents in *P. abies* and *E. globulus* barks accounted for 26.8% and 21.9%, respectively. In general terms, the lignin content in barks was found to be higher than in their respective woods, as was also observed for other tree species, such as willow and cork oak.[11,23]

In this work, the structural characteristics of the lignins from the different barks were thoroughly addressed. For this, the "milled-bark" lignin (MBL) preparations were isolated according to the classical protocol,[24] and were subsequently analyzed by various techniques, including analytical pyrolysis, in the absence and in the presence of tetramethylammonium hydroxide (TMAH), derivatization followed by reductive cleavage (DFRC), and 2D NMR spectroscopy.

Lignin composition as determined by Py-GC/MS

The composition of the lignins isolated from the different barks was first addressed by pyrolysis–gas chromatography–mass spectrometry (Py-GC/MS; Figure 1). The identities and relative molar abundances of the released lignin-derived phenolic compounds are listed in Table 2. Significant differences were observed among the different lignins. The lignin from *P. abies* bark exhibited a composition typical of softwoods (Figure 1A), with the release of phenolic compounds derived mostly from G lignin units (≈78% of all phenolic compounds), including guaiacol (peak 2), 4-methylguaiacol (peak 4), 4-ethylguaiacol (peak 6), and 4-vinylguaiacol (peak 7), together with lower amounts of compounds derived from H lignin units (≈22% of all phenolic compounds), including phenol (peak 1), 4-methylphenol (peak 3), and 4-ethylphenol (peak 5). The lignin from *E. globulus* bark released phenolic compounds derived mostly from S lignin units (≈70%; Figure 1B), including syringol (peak 10), 4-methylsyringol (peak 13), 4-ethylsyringol (peak 16), 4-vinylsyringol (peak 18), syringaldehyde (peak 25), and acetosyringone (peak 26), together with lower amounts of compounds derived from G lignin units (≈28% of all phenolics) and from H lignin units (≈2% of all phenolics), with a S/G ratio of approximately 2.5. In the case of the barks from the two *Acacia* species, the pyrograms showed a similar distribution of lignin-derived phenolic compounds (Figure 1C,D), with a predominance of those derived from G lignin units (≈53–57%), alongside lower amounts of compounds derived from S lignin units (≈33–36%) and H lignin units (≈10–12%), and with similar S/G ratios of around 0.6–0.7. However, and surprisingly, the lignin from *A. dealbata* bark also released high amounts of resorcinol (Figure 1C, peak 15, accounting for ≈35% of all phenolic compounds), which is absent in the pyrograms of the other bark lignins. Resorcinol is a phenolic compound that is not derived from lignin units and its peculiar structure, with two hydroxy groups in *meta* position, suggests that it may derive from moieties with flavonoid/hydroxystilbene skeletons that might be incorporated or intimately associated to the lignin polymer. It is well known that some flavonoids, such as the flavone tricin, are incorporated into the lignins in grasses and other monocotyledons.[13–15] Likewise, hydroxystilbenes, particularly piceatannol, have been found incorporated into the lignins of palm fruit endocarps.[16,17] Resorcinol was released from the lignin of *A. dealbata* bark even after exhaustive extraction with different solvent systems, reinforcing the idea that it belongs to phenolic moieties that are strongly associated to the lignin polymer.

It has been reported that the lignins from other barks, such as that from cork oak (*Quercus suber*) bark, also include ferulates in their structure.[23,25] However, ferulates (and *p*-hydroxycinnamates in general) cannot be analyzed by Py-GC/MS, owing to decarboxylation during pyrolysis.[26,27] The occurrence of ferulates, and other *p*-hydroxycinnamates, in these lignins can, however, be evaluated by performing pyrolysis in the presence of tetramethylammonium (TMAH), a methylating reagent that prevents decarboxylation during pyrolysis and releases intact *p*-hydroxycinnamates (as their permethylated derivatives).[26,27] Figure 2 shows the chromatograms of the compounds released during Py-TMAH of the different lignins. The identities and relative molar abundances of the released compounds are listed in Table 3. The distribution of the lignin-derived compounds follows the same trend as those released by conventional pyrolysis. The lignin from *P. abies* bark released predominantly G lignin units with minor amounts of H lignin units, whereas the lignin from *E. globulus* bark released mostly S lignin units with lower amounts of G and H lignin units and the lignins from the two *Acacia* species released similar amounts of G and S lignin units, with minor amounts of H lignin units. More importantly, the Py-TMAH chromatograms (Figure 2) also showed the release of *p*-hydroxycinnamates (as their methyl derivatives), which are incorporated into these lignins, including the methyl derivatives of *p*-coumarates (peak 29, *p*CA), ferulates

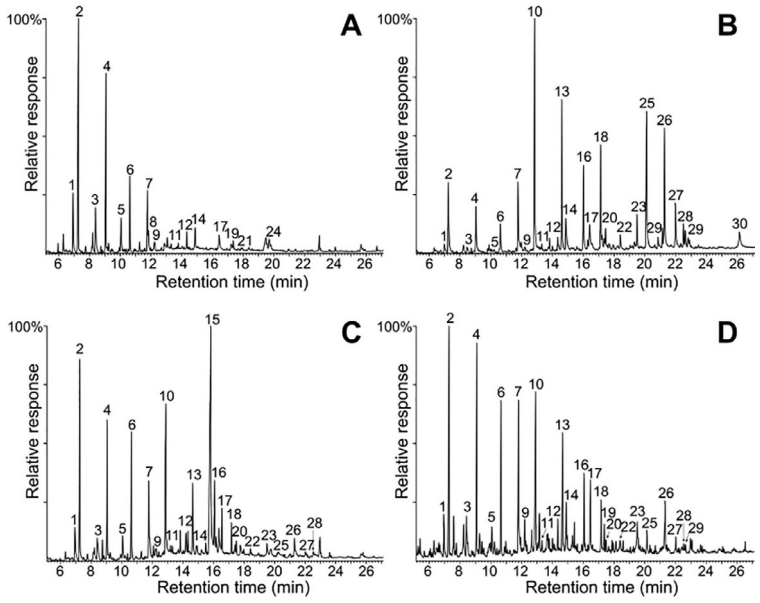

Figure 1. Py-GC/MS chromatograms of the MBLs isolated from the barks of *P. abies* (A), *E. globulus* (B), *A. dealbata* (C), and *A. melanoxylon* (D). The identities and relative abundances of the lignin-derived phenolic compounds released are listed in Table 2.

Table 2. Identities and relative molar abundances of the lignin-derived phenolic compounds released after Py-GC/MS of the MBLs isolated from the different barks. PA: *P. abies*; EG: *E. globulus*; AD: *A. dealbata*; AM: *A. melanoxylon*.

Entry	Compound	PA	EG	AD	AM
1	phenol	10.2	1.0	3.7	5.0
2	guaiacol	32.8	6.9	11.2	15.2
3	4-methylphenol	6.3	0.9	2.2	3.1
4	4-methylguaiacol	19.9	4.4	6.6	12.5
5	4-ethylphenol	3.5	0.1	1.6	2.0
6	4-ethylguaiacol	6.6	2.5	6.1	8.4
7	4-vinylguaiacol	6.1	5.4	5.1	9.2
8	4-vinylphenol	2.0	0.0	0.0	0.0
9	eugenol	0.6	0.2	0.5	1.3
10	syringol	0.0	17.6	10.1	10.8
11	*cis*-isoeugenol	0.5	0.5	0.3	0.6
12	*trans*-isoeugenol	1.5	1.1	1.0	1.7
13	4-methylsyringol	0.0	9.5	4.1	7.0
14	vanillin	3.0	3.5	0.7	3.1
15	resorcinol	0.0	0.0	35.3	0.0
16	4-ethylsyringol	0.0	5.2	3.7	3.8
17	acetoguaiacone	3.5	2.2	1.7	3.8
18	4-vinylsyringol	0.0	6.6	1.3	2.8
19	guaiacylacetone	0.9	0.8	0.5	1.2
20	4-allylsyringol	0.0	1.0	0.5	0.5
21	propiovanillone	0.3	0.6	0.3	0.5
22	*cis*-4-propenylsyringol	0.0	1.0	0.4	0.7
23	*trans*-4-propenylsyringol	0.0	2.1	1.2	1.5
24	dihydroconiferyl alcohol	2.3	0.0	0.0	0.0
25	syringaldehyde	0.0	10.9	0.3	1.3
26	acetosyringone	0.0	8.1	1.1	2.7
27	syringylacetone	0.0	3.4	0.3	0.7
28	propiosyringone	0.0	1.2	0.3	0.3
29	syringyl vinyl ketone	0.0	0.7	0.1	0.3
30	*trans*-sinapaldehyde	0.0	2.6	0.0	0.0
	H [%]	22.0	2.0	11.7[a]	10.1
	G [%]	78.0	28.1	52.5[a]	57.4
	S [%]	0.0	69.9	35.9[a]	32.5
	S/G ratio	0.0	2.5	0.7	0.6

[a] Relative abundances calculated without resorcinol (Figure 1, peak 15).

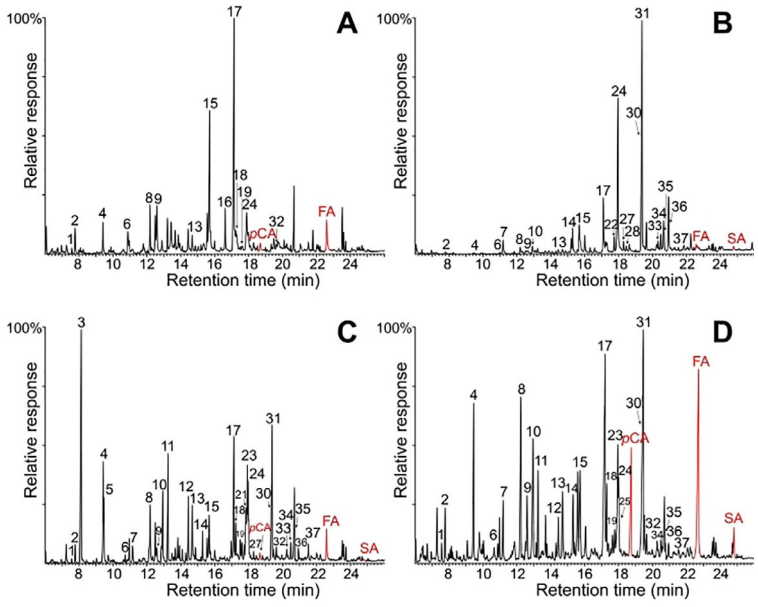

Figure 2. Py-TMAH-GC/MS chromatograms of the MBLs isolated from the barks of *P. abies* (A), *E. globulus* (B), *A. dealbata* (C), and *A. melanoxylon* (D). The identities and relative abundances of the lignin-derived phenolic compounds released are listed in Table 3.

(peak 38, FA), and sinapates (peak 39, SA), that were particularly abundant in the lignin from *A. melanoxylon* bark (accounting for ≈5% *p*CA, ≈10% FA, and ≈1% SA among all released compounds). The lignin from *E. globulus* bark released only trace amounts of ferulates and sinapates. It is important to remark the occurrence of *p*-hydroxycinnamates in the lignins of these tree barks, as they have not been reported in the lignins of their respective woods.

Py-TMAH of the lignin from *A. dealbata* bark (Figure 2C) also released significant amounts of other phenolic compounds (as their methyl derivatives) that derived neither from lignin nor from *p*-hydroxycinnamate moieties, such as 1,3-dimethoxybenzene (peak 3), 2,4-dimethoxytoluene (peak 5), and methyl 2,4-dimethoxybenzoate (peak 21), and which are absent from the rest of the lignins studied here. These compounds are structurally related to the resorcinol released by Py-GC/MS from the lignin of *A. dealbata* bark (Figure 1C and Table 2) and may arise from moieties with flavonoid/hydroxystilbenoid skeletons incorporated or intimately associated to the lignin polymer. The lignin from *P. abies* bark also released significant amounts of a compound that was not derived from lignin or *p*-hydroxycinnamates, namely methyl 3,5-dimethoxybenzoate (peak 16), which was absent from the rest of the lignins and that arose from hydroxystilbene moieties that are integrally incorporated into the lignin polymer (see below).

Analysis by derivatization followed by reductive cleavage

Additional information regarding the lignin monomeric units, as well as other phenolic units potentially incorporated into the lignin polymer, was obtained through chemical degradation by a method called derivatization followed by reductive cleavage (DFRC), a chemical degradative method that cleaves β-ether bonds in lignin and releases the corresponding lignin monomers involved in these linkages.[28] The chromatograms of the compounds released from these lignins are shown in Figure 3. The lignin from *P. abies* bark released the *cis* and *trans* isomers of the guaiacyl (*c*G and *t*G) lignin monomers (as their acetate derivatives), as corresponds to a conifer lignin, whereas the lignins from the barks of *E. globulus*, *A. dealbata*, and *A. melanoxylon* also released the *cis* and *trans* isomers of the syringyl (*c*S and *t*S) lignin monomers (as their acetate derivatives). Interestingly, the chromatogram of the DFRC degradation products released from *P. abies* bark also showed a series of peaks that were identified by comparison with authentic standards as the hydroxystilbenes resveratrol, isorhapontigenin, and piceatannol (peaks 1–3 in Figure 3A). The release of these compounds during DFRC indicates that at least a part of the hydroxystilbenes are incorporated into the lignin polymer of *P. abies* bark as β-ether-linked structures (those cleaved by the DFRC degradation method). Hydroxystilbenes have also been found incorporated into the lignins of other plant tissues, such as palm fruit endocarps, where they have been shown to behave as authentic lignin mono-

Table 3. Identities and relative molar abundances of the compounds released after pyrolysis-TMAH of the MBLs isolated from the different barks. PA: *P. abies*; EG: *E. globulus*; AD: *A. dealbata*; AM: *A. melanoxylon*.

Entry	Compound	Origin	PA	EG	AD	AM
1	4-methoxystyrene	H	1.0	0.0	0.3	0.8
2	1,2-dimethoxybenzene	G	4.0	0.5	1.3	2.3
3	1,3-dimethoxybenzene	FL/ST	0.0	0.0	20.1	0.0
4	3,4-dimethoxytoluene	G	3.7	0.3	6.0	6.7
5	2,4-dimethoxytoluene	FL/ST	0.0	0.0	3.4	0.0
6	4-methoxybenzaldehyde	H	3.7	0.7	0.2	1.0
7	1,2,3-trimethoxybenzene	S	0.0	2.5	1.0	2.2
8	3,4-dimethoxystyrene	G	5.3	1.2	4.2	7.4
9	methyl 4-methoxybenzoate	H	6.2	0.9	1.1	2.8
10	3,4,5-trimethoxytoluene	S	0.0	1.4	4.0	5.8
11	1,3,5-trimethoxybenzene	FL/ST	0.0	0.0	6.4	3.1
12	2,4,6-trimethoxytoluene	FL/ST	0.0	0.0	3.4	1.7
13	3,4-dimethoxypropenylbenzene	G	1.9	0.8	3.3	3.3
14	3,4,5-trimethoxystyrene	S	0.0	3.3	1.3	2.6
15	3,4-dimethoxybenzaldehyde	G	22.1	8.8	3.2	5.0
16	methyl 3,5-dimethoxybenzoate	FL/ST	3.7	0.0	0.0	0.0
17	methyl 3,4-dimethoxybenzoate	G	28.5	8.6	7.3	11.6
18	3,4-dimethoxyacetophenone	G	2.2	1.7	2.0	2.4
19	1-(3,4-dimethoxyphenyl)-2-propanone	G	0.8	0.1	1.1	0.7
20	1-(3,4,5-trimethoxyphenyl)-1-propene	S	0.0	1.5	0.6	0.9
21	methyl 2,4-dimethoxybenzoate	FL/ST	0.0	0.0	3.9	0.0
22	*cis*-1-(3,4-dimethoxyphenyl)-2-methoxyethylene	G	2.5	1.2	0.9	1.0
23	methyl 3,4-dimethoxy-benzeneacetate	G	3.8	0.8	4.3	2.0
24	3,4,5-trimethoxy-benzaldehyde	S	0.0	23.9	3.4	3.8
25	*trans*-1-(3,4-dimethoxy-phenyl)-2-methoxyethylene	G	2.3	1.3	0.9	0.8
26	*cis*-1-(3,4-dimethoxyphenyl)-3-methoxyprop-1-ene	G	0.8	0.0	0.4	0.3
27	*cis*-1-(3,4-dimethoxy-phenyl)-1-methoxyprop-1-ene	G	0.9	1.5	0.5	0.2
28	*trans*-1-(3,4-dimethoxy-phenyl)-1-methoxyprop-1-ene	G	0.3	0.9	0.3	0.2
29	methyl *trans*-4-*O*-methyl-*p*-coumarate	*p*CA	1.0	0.0	0.7	5.0
30	3,4,5-trimethoxy-acetophenone	S	0.0	10.1	2.8	2.5
31	methyl 3,4,5-trimethoxy-benzoate	S	0.5	19.1	4.9	8.9
32	*trans*-1-(3,4-dimethoxy-phenyl)-3-methoxyprop-1-ene	G	0.7	0.0	0.7	0.7
33	1-(3,4,5-trimethoxyphenyl)-2-propanone	S	0.0	1.2	0.7	0.7
34	1-(3,4,5-trimethoxyphenyl)-2-methoxypropane	S	0.0	1.7	0.7	0.6
35	*cis*-1-(3,4,5-trimethoxy-phenyl)-2-methoxyethylene	S	0.0	2.1	1.1	0.7
36	*trans*-1-(3,4,5-trimethoxy-phenyl)-2-methoxyethylene	S	0.0	2.1	0.8	0.7
37	methyl 3,4,5-trimethoxy-benzeneacetate	S	0.0	0.8	1.0	0.5
38	methyl *trans*-4-*O*-methyl-ferulate	FA	4.0	0.5	1.7	10.3
39	methyl *trans*-4-*O*-methyl-sinapate	SA	0.0	0.6	0.1	1.0

H: *p*-hydroxyphenyl units; G: guaiacyl units; S: syringyl units; *p*CA: *p*-coumarates; FA: ferulates; SA: sinapates; FL/ST: flavonoids/stilbenoids.

mers.[16,17] Moreover, the chromatogram also showed the release of significant amounts of glucose (as its peracetate), which indicates that the hydroxystilbenes are incorporated into this lignin as their corresponding *O*-glucosides (Figure 3E), namely resveratrol-*O*-glucoside (piceid), isorhapontigenin-*O*-glucoside (isorhapontin), and piceatannol-*O*-glucoside (astringin).

The lignin from *A. dealbata* bark also released some phenolic compounds as their acetate derivatives (peaks 4–7 in Figure 3C), but their structures could not be fully established. The mass spectra of these compounds showed a molecular ion peak at *m/z* 440 and four consecutive losses of 42 mass units (fragments at *m/z* 398, 356, 314, and 272) that indicate the presence of four hydroxy groups (as acetates) in the structure (Figure 3F). The spectra are similar to those of acetylated tetrahydroxychalcones, but comparison with authentic standards of the common chalcones 2,3′,4,4′-tetrahydroxychalcone, 2′,3,4,4′-tetrahydroxychalcone (butein), and 2′,4,4′,6′-tetrahydroxychalcone (naringenin chalcone) ruled out this type of structure. Additional work is still in progress to fully identify the structure of these compounds, which might correspond to polyphenolic moieties incorporated or closely associated to the lignin from *A. dealbata* bark and that could be at the origin of the resorcinol released during Py-GC/MS and the related compounds released during Py-TMAH.

An interesting feature of the DFRC degradation method is that it cleaves β-ether linkages but leaves γ-esters intact, and therefore is also a powerful tool to identify monolignol ester conjugates with different acyl groups attached to the γ-OH of the lignin side chain, such as acetates and *p*-coumarates, which are common components in the lignins of many plants.[29–34] However, no traces of *p*-coumaroyl monolignol ester conjugates could be detected among the DFRC degradation products, most probably because they are below the detection limit, even in the case of the lignin from *A. melanoxylon* bark, which has the highest *p*-coumarate content, as indicated

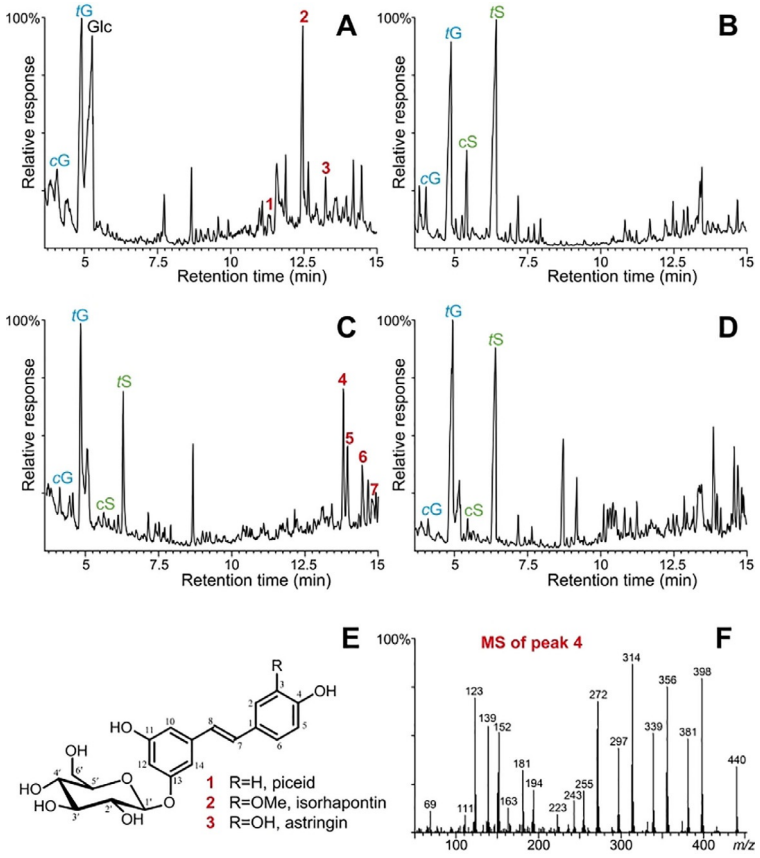

Figure 3. A–D) Chromatograms of the DFRC degradation products released from the lignins isolated from the barks of *P. abies* (A), *E. globulus* (B), *A. dealbata* (C), and *A. melanoxylon* (D). *c*G, *t*G, *c*S and *t*S are the normal *cis*- and *trans*-coniferyl (G) and sinapyl (S) alcohol monomers (as their acetate derivatives). Peaks in red color correspond to the different hydroxystilbene compounds: **1**: resveratrol; **2**: isorhapontigenin; **3**: piceatannol, as their acetyl derivatives. Glc: glucose (as the acetate derivative). Peaks 4–7 correspond to unidentified isomeric compounds with a molecular ion at *m/z* 440. E) Structures of the hydroxystilbene glucosides. F) mass spectrum of peak **4** released from the lignin of *A. dealbata* bark.

by Py-TMAH. Moreover, the lignin from some barks, such as that from cork oak bark, also showed significant levels of native acetates acylating the γ-OH.[23] To analyze the occurrence of native acetate groups attached to the γ-OH of the lignin side chain, the original DFRC protocol was slightly modified (so-called DFRC′) by replacing acetylating reagents for propionylating ones.[29,31] The chromatograms of the DFRC′ degradation products released from the lignins isolated from each of the barks are shown in Figure 4. The chromatograms show the release of originally γ-acetylated guaiacyl (cG_{ac} and tG_{ac}) and syringyl (cS_{ac} and tS_{ac}) lignin units, confirming that naturally occurring acetates acylate the γ-OH groups of these lignins, particularly in the lignin from *P. abies* bark, with up to 7% of acetylated G units. This finding was somewhat unexpected, as the lignins from conifer woods are not acetylated at the γ-OH,[31] and this is the first report of a lignin from a conifer tissue that shows significant levels of acetylation at the γ-OH. The lignin from *E. globulus* bark also showed some levels of acetylation of the lignin side chain, which occurred predominantly over the S lignin units (8% of the total S units are acetylated) whereas G units were barely acetylated (2% of the G units). This is the opposite to what occurred in the corresponding *E. globulus* wood, where G lignin units were preferentially acetylated.[31] In contrast, the lignins from the two *Acacia* barks were scarcely acetylated at the γ-OH.

Lignin structural units and interunit linkages as elucidated by 2D NMR

The lignins isolated from the different barks were also analyzed by 2D HSQC-NMR spectroscopy, which provided useful information regarding the lignin composition and the proportion of the different interunit linkages. The side-chain ($\delta_C = 50–98$ ppm; $\delta_H = 2.5–6.8$ ppm) and the aromatic/unsaturated ($\delta_C = 98–155$ ppm; $\delta_H = 5.8–7.8$ ppm) regions of the spectra are shown in Figure 5. The main lignin substructures found are displayed at the bottom.

The aromatic/unsaturated regions of the spectra showed signals from the aromatic rings and unsaturated side chains of the different H, G, and S lignin units, as well as from *p*-hydroxycinnamates (ferulates, FA, and *p*-coumarates, *p*CA) and cinnamaldehyde end groups (J). The lignin from *P. abies* bark showed signals from G and H lignin units, whereas the spectrum of the lignin from *E. globulus* bark showed signals from S and G lignin units, whereas signals from H units were barely detected. The lignins from the barks of the two *Acacia* species presented signals from all three S, G, and H lignin units. Signals from *p*-coumarates (*p*CA) and ferulates (FA) were only detected in the spectrum of the lignin from *A. melanoxylon* bark, corroborating the Py-TMAH data that indicated the occurrence of significant amounts of *p*-hydroxycinnamates in this lignin. Strong signals from cinnamaldehyde end groups (J) were also observed in the spectra of *E. globulus* and *A. melanoxylon* barks, and with lower intensity, in the lignin from *A. dealbata* bark.

However, the most remarkable feature in this region of the spectra was the presence of strong signals at around $\delta_C = 100–110$ ppm/$\delta_H = 6.0–6.5$ ppm in the spectra of the lignins from *P. abies* and *A. dealbata* barks, which are related to the atypical phenolic compounds released from these lignins by Py-GC/MS, Py-TMAH, and DFRC. In the case of *A. dealbata* bark, these signals (Figure 5, gray) are related to the resorcinol released during Py-GC/MS, the similar compounds released during Py-TMAH, and the still unknown phenolic compounds released during DFRC. However, extensive NMR analysis using different techniques (HSQC, HMBC, HSQC-TOCSY) failed to fully establish their structure. These signals seem to derive from polyphenolic moieties with flavonoid/hydroxystilbenoid skeletons, including condensed tannins, that are apparently incorporated or closely associated to the lignin polymer but whose structure remains elusive to us. However, in the case of the lignin from *P. abies* bark, the new signals (Figure 5, pink) could be unambiguously assigned (with the aid of authentic standards) to the hydroxystilbenes (principally isorhapontigenin, but also piceatannol and

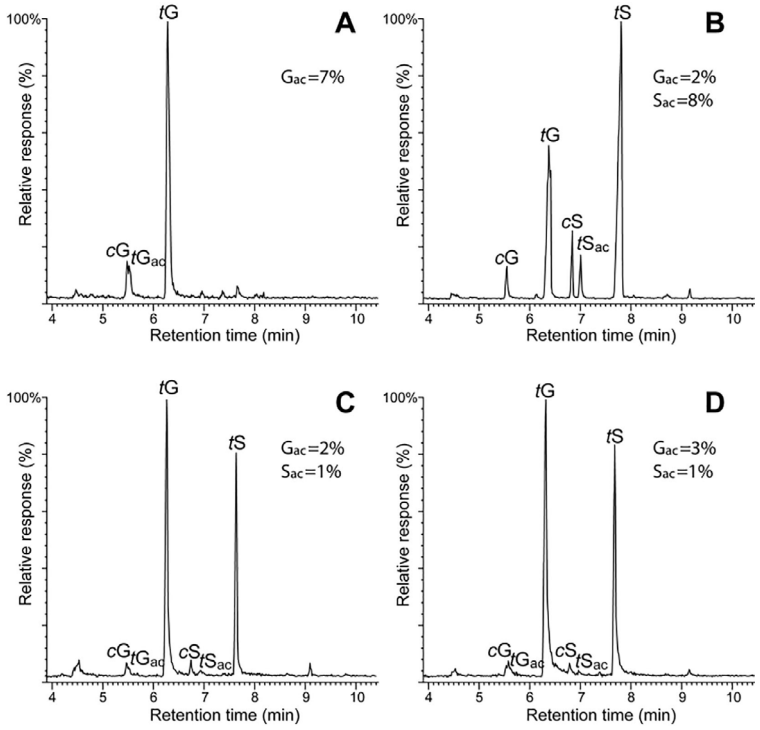

Figure 4. Reconstructed ion chromatograms (sum of the ions at *m/z* 222, 236, 252, and 266) of the DFRC′ degradation products released from the lignins isolated from the barks of *P. abies* (A), *E. globulus* (B), *A. dealbata* (C), and *A. melanoxylon* (D). *c*G and *t*G are the normal *cis*- and *trans*-coniferyl (G) alcohol monomers (as their propionylated derivatives; *m/z* 236); *c*S and *t*S are the normal *cis*- and *trans*-sinapyl (S) alcohol monomers (as their propionylated derivatives; *m/z* 266); tG_{ac} is the γ-acetylated *trans*-coniferyl (G) alcohol monomer (as the propionylated derivative; *m/z* 222); tS_{ac} is the γ-acetylated *trans*-sinapyl (S) alcohol monomer (as the propionylated derivative; *m/z* 252).

resveratrol) that were released during DFRC. These signals are similar to those previously observed in the spectra of the lignins from palm fruit endocarps, which were assigned to the hydroxystilbene piceatannol incorporated into the lignin structure.[16,17] In addition, the occurrence in the aliphatic–oxygenated region of the spectrum of strong signals from glucose at $\delta_C = 72.9$ ppm/$\delta_H = 3.17$ ppm (Glc_2), $\delta_C = 76.5$ ppm/$\delta_H = 3.24$ ppm (Glc_3 and Glc_5), $\delta_C = 69.3$ ppm/$\delta_H = 3.17$ ppm (Glc_4), and $\delta_C = 60.2$ ppm/$\delta_H = 3.79$–3.40 ppm (Glc_6), together with the occurrence of a signal for the linkage between the hydroxystilbene and the glucose moieties in the HMBC spectrum at $\delta_C \approx 158$ ppm/$\delta_H \approx 4.7$ ppm,[18] conclusively confirmed that these hydroxystilbenes are glucosylated and that the phenolic compounds that are incorporated into the lignin polymer are the corresponding *O*-glucosides, namely isorhapontin (isorhapontigenin-*O*-glucoside), astringin (piceatannol-*O*-glucoside), and piceid (resveratrol-*O*-glucoside). Piceid, astringin, and isorhapontin are known compounds occurring among the extractives from *P. abies* bark.[35–37] However, these compounds are highly soluble in water and other solvents and, as the bark was subjected to exhaustive extraction with different solvents (dichloromethane, ethanol and water) aimed at removing all the extractives prior to lignin isolation (and the MBL preparation was additionally exhaustively washed with different organic solvents), it is possible to assume that the hydroxystilbene glucosides observed are linked to the lignin by covalent bonds and do not correspond to residual free molecules. This assumption is also supported by the absence from the HSQC spectrum of signals from the unsaturated bonds that evidenced their participation in radical coupling reactions. In addition, exhaustive analysis by diffusion-ordered spectroscopy (DOSY) also confirmed that hydroxystilbene glucosides were integrally incorporated into the lignin polymer of *P. abies* bark.[19] Interestingly, the occurrence of significant amounts of hydroxystilbene glucosides in a 'milled bark tannin-lignin" fraction isolated from Norway spruce bark was previously reported, although the authors suggested without any experimental evidence that they were linked to the condensed tannin moiety instead of the lignin polymer.[9]

The aliphatic–oxygenated regions of the spectra gave information on the different interunit linkages present in lignin. The most prominent signals in this region of the spectra corresponded to typical lignin substructures (Figure 5), including signals from β-O-4′ alkyl–aryl ethers (**A**), β–5′ phenylcoumarans (**B**), β–β′ resinols (**C**), 5–5′ dibenzodioxocins (**D**), spirodienones (**F**), and cinnamyl alcohol end groups (**I**). In addition, the HSQC spectrum of the lignin from *P. abies* bark also showed other signals that were assigned to substructures involving hydroxystilbenes glucosides, including signals for a benzodioxane ($\mathbf{P_b}$) structure arising from 8-O-4′ coupling of two hydroxystilbene glucosides, signals for a phenylcoumaran structure ($\mathbf{P_c}$) involving 8–10′ coupling of two hydroxystilbene glucosides, as well as signals for a benzodioxane structure (**V**) formed by β-O-4′ cross-coupling of coniferyl alcohol and astringin, and that presented similar correlations to those signals observed for the incorporation of the hydroxystilbene piceatannol in the lignins of palm fruit endocarps.[16,17] The definitive assignments of the structures involving the incorporation of hydroxystilbene glucosides into the lignin polymer were attained by detailed HSQC-TOCSY and HMBC experiments, as already described.[18] The occurrence of these structures ($\mathbf{P_b}$, $\mathbf{P_c}$, **V**) conclusively demonstrates that hydroxystilbene glucosides behave as true lignin monomers in *P. abies* bark, participating in radical coupling reactions during lignification and being integrally incorporated into the lignin structure.

The relative abundances of the main lignin interunit linkages and end groups, as well as the abundances of the different lignin units (H, G, and S), *p*-hydroxycinnamates (*p*CA and FA), and hydroxystilbene glucosides (P) of the lignins from the different barks, estimated from volume integration in the HSQC spectra, are shown in Table 4. Important differences were found among the lignins from the different barks. The lignin from *P. abies* bark presented mostly G lignin units, with lower amounts of H lignin units (H/G ratio of 14:86), in agreement with the data obtained from Py-GC/MS. In addition, this lignin contained large amounts of hydroxystilbene glucosides (36 units per 100 aromatic lignin units), mostly isorhapontin, incorporated into its structure. This composition makes this

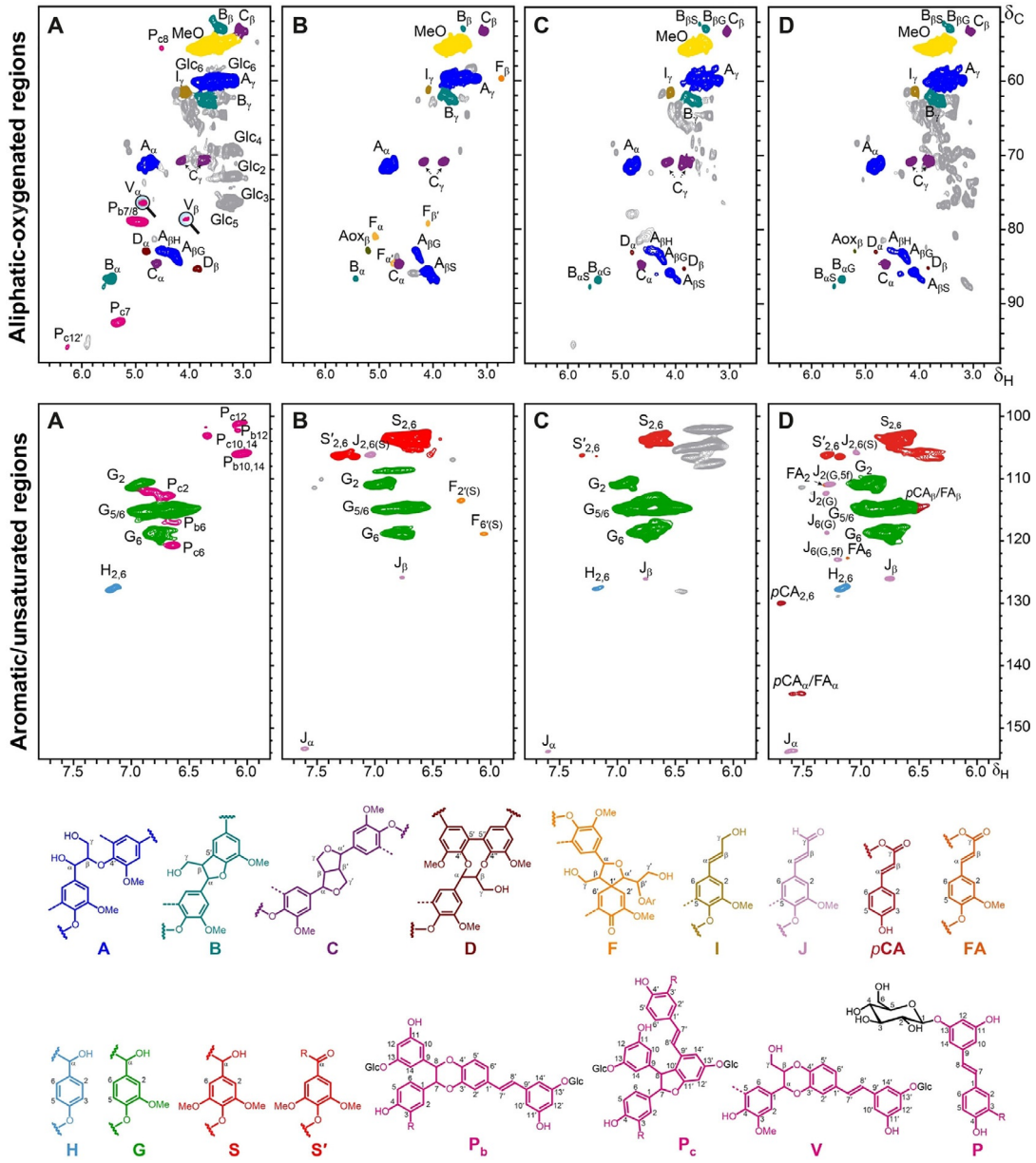

Figure 5. Side-chain (δ_C = 50–98 ppm; δ_H = 2.5–6.8 ppm) and aromatic (δ_C = 98–155 ppm; δ_H = 5.8–7.8 ppm) regions of the 2D HSQC-NMR spectra of the MBLs isolated from the barks of *P. abies* (A), *E. globulus* (B), *A. dealbata* (C), and *A. melanoxylon* (D). The main lignin structures identified are depicted at the bottom. **A**: β-O-4′ alkyl-aryl ethers; **B**: β-5′ phenylcoumarans; **C**: β-β′ resinols; **D**: 5-5′ dibenzodioxocins; **F**: β-1′ spirodienones; **I**: cinnamyl alcohol end groups; **J**: cinnamaldehyde end groups; ***p*CA**: *p*-coumarates; **FA**: ferulates; **H**: *p*-hydroxyphenyl units; **G**: guaiacyl units; **S**: syringyl units; **S′**: Cα-oxidized syringyl units; **P**: hydroxystilbene glucosides (isorhapontin, R = OCH_3; astringin, R = OH; piceid, R = H); $\mathbf{P_b}$: 8-O-4′/3′-O-7 benzodioxane structures involving isorhapontin (R = OCH_3), astringin (R = OH) or piceid (R = H) units; $\mathbf{P_c}$: 8–10′/11′-7 phenylcoumaran structures involving isorhapontin (R = OCH_3), astringin (R = OH) or piceid (R = H) units; **V**: β-O-4′/3′-O-α benzodioxane structure formed by cross-coupling of astringin (R = OH) and coniferyl alcohol.

lignin highly condensed, with a low abundance of β-O-4′ ether linkages (44% of all interunit linkages) and a high abundance of condensed linkages, mostly phenylcoumarans (20%), dibenzodioxocins (5%), resinols (4%), and other condensed linkages involving coupling of hydroxystilbene glucosides (benzodioxanes $\mathbf{P_b}$, 13%; phenylcoumarans $\mathbf{P_c}$, 12%; benzodioxanes **V**, 2%). By contrast, the lignin from *E. globulus* bark contained mostly S lignin units and lower amounts of G and H lignin units (H/G/S ratio of 1:26:73; S/G ratio of 2.8), in agreement with the Py-GC/MS data. This composition makes this lignin highly enriched in β-O-4′ ether linkages (83% of all interunit linkages) and depleted in condensed linkages, consisting mostly of resinols (8%), spirodienones (5%), and phenylcoumarans (4%). The lignins from *A. dealbata* and *A. melanoxylon* barks presented a rather similar composition, with a slight predominance of G over S lignin units (S/G ratio of 0.9) and with lower amounts of H units (≈4–5%). This composition produced a lignin with a high content of β-O-4′ linkages (≈73–75%), and with a lower abundance of condensed linkages, such as phenylcoumarans (12%), resinols (≈7–8%), dibenzo-

Table 4. Structural characteristics (lignin interunit linkage types, end groups, aromatic units, and S/G ratio, *p*-hydroxycinnamate and hydroxystilbene contents) from volume integration of $^{1}H/^{13}C$ correlation signals in the HSQC spectra of the MBLs isolated from the different barks. PA: *P. abies*; EG: *E. globulus*; AD: *A. dealbata*; AM: *A. melanoxylon.*

Components	PA	EG	AD	AM
Lignin interunit linkages [%]				
β-O-4′ aryl ethers (**A**)	44	83	73	75
phenylcoumarans (**B**)	20	4	12	12
resinols (**C**)	4	8	8	7
dibenzodioxocins (**D**)	5	0	4	4
spirodienones (**F**)	0	5	3	2
benzodioxanes ($\mathbf{P_b}$)	13	0	0	0
phenylcoumarans ($\mathbf{P_c}$)	12	0	0	0
benzodioxanes (**V**)	2	0	0	0
Lignin end-groups[a][%]				
cinnamyl alcohol end-groups (**I**)	2	2	3	3
cinnamaldehyde end-groups (**J**)	0	4	2	6
Lignin aromatic units				
H [%]	14	1	5	4
G [%]	86	26	50	51
S [%]	0	73	45	45
S/G ratio	0	2.8	0.9	0.9
***p*-Hydroxycinnamates[b]**				
p-coumarates *p*CA [%]	0	0	0	4
ferulates FA [%]	0	0	0	4
Hydroxystilbene units P [%][b]	36	0	0	0

[a] Expressed as a fraction of the total lignin interunit linkage types **A**–**V**. [b] *p*-Coumarate, ferulate and hydroxystilbene contents are expressed as percentages of total lignin content (H + G + S = 100).

dioxocins (4%), and spirodienones (≈2–3%). The main differences among the lignins from the two *Acacia* barks were the occurrence of small amounts of *p*-coumarates and ferulates in the lignin from *A. melanoxylon* bark, that could not be detected in the spectrum of the lignin from *A. dealbata* bark (although *p*-coumarates and ferulates occurred at lower levels in this lignin, as indicated by Py-TMAH), as well as the occurrence of large amounts of still unknown polyphenolic compounds that are presumably incorporated or closely associated to the lignin of *A. dealbata* bark and are absent in the lignin from *A. melanoxylon* bark.

Bark lignin valorization potential

Lignin, the most abundant natural polymer with an aromatic skeleton, has long been considered a waste product of the pulping industry, although its combustion is important for the internal energy supply to the process. Nowadays, lignin is increasingly seen as an attractive renewable feedstock for producing chemicals, materials, and fuels that are currently obtained from fossil resources,[5,6] with a potential market value estimated at about 12 billion € by 2020–2025 for new lignin-based products.[38] Barks have the advantage of often presenting higher lignin contents than their respective woods, making them interesting raw materials for obtaining lignin for different uses. The best example is the bark of *A. melanoxylon*, with 55% lignin content, which represents 72% of the structural cell-wall components.

Although the variability and complexity of the lignin structure in barks can be seen as a limitation for their industrial extraction and subsequent transformation and utilization, it can also provide a wide range of possibilities and specific end uses for each of the lignin polymers for the production of different phenolic compounds for diverse chemical and pharmaceutical industries. The high S/G ratio of the lignin from *E. globulus* bark, together with its enrichment in β-ether units indicates a more reactive and easier to depolymerize lignin that will result in a higher yield of monomers; on the other hand, the enrichment of G lignin units in the lignin from *P. abies* bark, despite being a more condensed lignin and, therefore, more difficult to depolymerize, could be advantageous for example for the production of high added-value compounds such as vanillin for use in the flavor and fragrance industry or for the synthesis of epoxy resins.[39,40]

The significant content of *p*-hydroxycinnamates in the lignin from *A. melanoxylon* bark, which have not been described in lignins from woods, could make this bark an unconventional source of *p*-hydroxycinnamates, which are mostly produced from grass lignins. More important is the occurrence of significant amounts of hydroxystilbene glucosides that are integrally incorporated into the lignin of *P. abies* bark, which makes this an interesting feedstock for obtaining highly valuable hydroxystilbenes. Bark lignins enriched in resorcinol moieties, such as that from *A. dealbata* could serve as raw materials for the production of resorcinol–formaldehyde resin, which is mainly used as thermosetting binders for wood. Resorcinol is considerably more reactive than phenol, but it is less used in resins preparation, owing to its higher cost. In this sense, *A. dealbata* bark lignin, after undergoing a thermochemical depolymerization process, could be considered a valuable raw material for the industrial preparation of resorcinol-containing resins for wood adhesives or other commercial uses, such as semiconductor photocatalysts[41] and organic aerogels.[42] Finally, as occurs with lignins from other sources, the aromatic nature of bark lignins makes them suitable materials to mimic and replace phenol in polyurethane (PU) formulation and in phenol–formaldehyde (PF) resins for adhesives formulation in medium density fiberboards (MDF) manufacture.[43,44]

Conclusions

This study provided a comprehensive characterization of the lignins from the barks of several species, including the softwood *P. abies*, and the hardwoods *E. globulus*, *A. dealbata*, and *A. melanoxylon.* A wide diversity in the content, composition, and structure of the lignin polymers was observed among the different barks. This knowledge will be of great help for the development of efficient conversion technologies of these lignocellulosic materials, that have been considered as waste and will aid in their full valorization. The occurrence in some of these lignins of phenolic compounds that are different from the traditional monolignols, such as the hydroxystilbene glucosides present in the lignin of *P. abies* or the still unknown poly-

phenolic compounds apparently incorporated into the lignin of *A. dealbata*, expands the range of products that can be obtained from these lignins, thus enhancing the value of these waste materials that are produced in high abundance at low cost by forestry operations and by the timber and pulp and paper industries.

Experimental Section

Samples

Barks from *P. abies* and *E. globulus* were collected after debarking in industrial sites at a sawmill near Jyvaskyla, Finland, and at The Navigator Company pulp mill located in Setúbal, Portugal, respectively. Both samples were manually sorted to remove wood contamination from the debarking process. The barks from the two Acacia trees were collected directly from trees at Sintra (*A. melanoxylon*, ≈40 year-old specimens) and Buçaco (*A. dealbata*, ≈6 year-old specimens), Portugal. The barks were air-dried, knife milled and successively Soxhlet extracted with dichloromethane (2 L, 24 h), ethanol (2 mL, 24 h) and water (2×2 mL, 24 h). The Klason lignin content was determined in the extractive-free material following the TAPPI standards T222 om-88 (and corrected for ash and protein contents), whereas the acid-soluble lignin was determined spectrophotometrically following the TAPPI method UM250 om-83. The composition of polysaccharides was determined in the Klason lignin hydrolysates as neutral monosaccharides, glucuronic acid, galacturonic acid, and acetates through separation by a Dionex ICS-3000 High Pressure Ion Chromatographer, using an Aminotrap plus Carbopac SA10 column. All chemical analyses were made in triplicate.

Lignin isolation

The "milled-bark" lignin (MBL) preparations were obtained from extractive-free samples using ball-milling conditions, as previously described.[33] The ball-milled materials (≈80 g) were extracted with 90:10 *v/v* dioxane/water mixture (2 L) under continuous stirring in the dark for 12 h. The solution was centrifuged and the supernatant, which contained the lignin, was then collected by decantation. This extraction process was repeated three times, using fresh dioxane/water mixture each time, and the supernatants combined. Crude lignins were obtained after removal of the solvent on a rotary evaporator at 40 °C and the isolated lignins were subsequently purified as described previously.[33] The yields of the crude MBLs (calculated as the percentage of the Klason lignin content) were 37% (*P. abies*), 21% (*E. globulus*), 49% (*A. dealbata*), and 11% (*A. melanoxylon*).

Analytical pyrolysis

Pyrolysis of the lignins was performed at 500 °C (1 min) in a 3030 micro-furnace pyrolyzer (Frontier Laboratories Ltd., Fukushima, Japan) connected to a GC 7820A (Agilent Technologies, Inc., Santa Clara, CA) and an Agilent 5975 mass-selective detector. The column used was a 30 m×0.25 mm i.d., 0.25 μm film thickness, DB-1701 (J&W Scientific, Folsom, CA). The GC oven was heated from 50 °C to 100 °C at 20 °C min^{-1} and then ramped to 280 °C at 6 °C min^{-1} and held for 5 min. Helium (1 mL min^{-1}) was used as the carrier gas. For the pyrolysis in the presence of tetramethylammonium hydroxide (Py-TMAH), the lignins were mixed with a droplet of TMAH (25 wt% in methanol) prior the pyrolysis. The released compounds were identified by comparison of their mass spectra with those present in the NIST and Wiley mass spectral libraries and by comparison with reported data.[45]

Derivatization followed by reductive cleavage (DFRC)

DFRC degradation was performed according to the classical procedure,[28] and the details have been described previously.[33] Briefly, the lignin (≈10 mg) was first treated with 8:92 *v/v* acetyl bromide/acetic acid mixture (2.5 mL) under stirring (2 h, 50 °C), after which it was dried. Powdered Zn (50 mg) and a 5:4:1 *v/v/v* dioxane/acetic acid/water mixture (2.5 mL) were added and allowed to react for 40 min at room temperature. The liquid phase was removed, treated with saturated ammonium chloride solution (3 mL) and then extracted with dichloromethane (10 mL, then 2×5 mL). After evaporating the organic phase to dryness, the lignin degradation products were acetylated with acetic anhydride/pyridine prior to analysis by GC-MS. To evaluate the occurrence of native acetate groups attached to the lignin, the original DFRC method was slightly modified by using propionylating reagents (denoted as DFRC′) instead of acetylating ones, as previously described.[29,31] The DFRC and DFRC′ lignin degradation products were analyzed by on a Saturn 4000 GC-MS apparatus (Varian, Walnut Creek, CA). The column used was a 12 m×0.25 mm i.d., 0.1 μm film thickness, DB5-HT (J&W Scientific, Folsom, CA). Helium (2 mL min^{-1}) was used as the carrier gas. The samples were injected directly onto the column by using a septum-equipped programmable injector (Varian 8200 autosampler, Varian, Folsom, CA) that was heated from 120 °C (0.1 min) to 330 °C at a rate of 200 °C min^{-1} and held until the end of the analysis. The GC oven was heated from 120 °C (1 min) to 380 °C (10 min) at a rate of 10 °C min^{-1}. The GC-MS transfer line was set to 300 °C.

NMR spectroscopy

2D NMR spectra were recorded on an AVANCE III 500 MHz instrument (Bruker, Karlsruhe, Germany) fitted with a cryogenically cooled 5 mm TCI gradient probe with inverse geometry, at the NMR facilities of the General Research Services of the University of Seville (SGI-CITIUS). The MBL sample (≈40 mg) was dissolved in [D_6]DMSO (0.75 mL). The residual DMSO signal (δ_C = 39.5 ppm; δ_H = 2.49 ppm) was used as the internal reference. The HSQC experiments used the Bruker standard pulse programs "hsqcetgpsisp2.2". The detailed NMR experimental conditions were described previously,[46] and the signals were assigned according to reported values.[13,18,33,46,47] Quantifications of lignin units and interunit linkages were performed as described previously.[10,33] Briefly, the signals used to quantify the relative abundances of the aromatic units were $H_{2,6}$, G_2, $S_{2,6}$, $pCA_{2,6}$, FA_2, and P_{c12}/P_{b12}—as signals $H_{2,6}$, $S_{2,6}$, and $pCA_{2,6}$ involve two proton–carbon pairs, their volume integrals were halved. The various interunit linkages were quantified via the volume integrals of the $\mathbf{A}_\alpha$, $\mathbf{B}_\alpha$, $\mathbf{C}_\alpha$, $\mathbf{D}_\alpha$, $\mathbf{F}_\alpha$, $\mathbf{P}_{b7}$, $\mathbf{P}_{c7}$, and $\mathbf{V}_\alpha$ correlation signals. The relative abundances of cinnamyl alcohol end groups (**I**) were estimated by integration of the signal $\mathbf{I}_\gamma$, which was also halved as it involves two proton–carbon pairs, whereas the abundances of cinnamaldehyde end groups (**J**) was determined by integration of the signal $\mathbf{J}_\alpha$ and comparing that with $\mathbf{I}_\alpha$.

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Conflict of interest

The authors declare no conflict of interest.

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