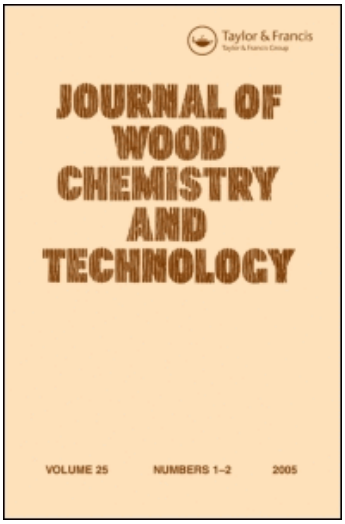

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COMPARATIVE STUDY ON MILD DEPOLYMERIZATION OF LIGNIN MODEL DEHYDROGENATION POLYMERS AND MILLED WOOD LIGNIN

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ABSTRACT

Dehydrogenation polymers (DHPs) of coniferyl alcohol prepared in three different ways (bulk, end-wise and membrane) and spruce (*Picea mariana*) milled wood lignin (MWL) were depolymerized with dry hydrogen iodide. The amounts of the only monomeric product, 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane and oligomeric products resulted from this procedure were compared. All DHPs differ noticeably from MWL in their structural organization, the bulk one being the best approximation. Yields of the diiodide were high in all cases (end-wise DHP: 45%, membrane DHP: 55%, MWL and bulk DHP: 20%). Molecular mass distributions of higher molecular mass products were similar in all the cases except end-wise DHP which demonstrated lesser degree of polymerization of high molecular mass fraction.

INTRODUCTION

The chemical structure of lignin is a matter of permanent interest of wood chemists. To date, significant progress has been

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achieved; however, even most modern structural formulae of wood lignin are of tentative character.[1,2] Numerous experimental studies on the products of lignin destruction yielded reliable information about the major types of units and interunit linkages in the polymer,[3-5] but the pattern of organization of the units in lignin macromolecules is not absolutely clear. The cause of this is in the lack of experimental approaches to provide verification for the theoretical views on the structural organization of lignin in wood. Computer simulation of lignin biosynthesis resulted in a model which describes lignin as a branched cluster polymer with crosslinkings.[6,7] No direct comparison of this model to experimental data has been possible yet because, unlike polymerization which proceeds in very mild conditions, lignin depolymerization usually requires highly reactive agents and high temperatures that lead to extensive non-specific destruction of the native polymer and numerous side reactions. Even the mildest of modern conventional methods (such as thioacydolysis)[8,9] are not free of such obstacles.

The procedure of I-cleaving (extensive depolymerization with dry hydrogen iodide)[10,11] is the mildest analytical method for use with lignins and related compounds yet known. The procedure takes about one hour at room temperature yielding a mixture of oligomers which can be analyzed by NMR and/or GPC methods.[10-12] It has been shown that I-cleaving leads to reductive release of monomeric units characterized by their NMR spectra and mixtures of oligomers which give the patterns of molecular mass distribution characteristic of certain types of lignins. Similar transformation of some lignins into the mixtures of oligomers can also be achieved by applying other iodine-containing reagents such as trimethyliodosilane[11,13-15] and acetyl iodide,[11] but hydrogen iodide is the most convenient because it adds no new carbon to the system.

In numerous studies in lignin chemistry, dehydrogenation polymer of coniferyl alcohol (DHP) was used as a convenient model of softwood lignin.[1-3,16-19] Chemical and spectral properties of DHP are considered as similar to those of coniferous lignin.[1] However, ^{13}C NMR studies[20-25] revealed that different kinds of DHPs differ between each other and from native lignin (see also[17,19,26]). Recently, it was reported that characteristic difference between DHP and MWL is the frequency of β-β bonds in DHPs;[27] to the contrary, pinoresinol structures constitute only minor structural

constituent of MWL.[28] β-β Structures of furane type or of open-ring type observed in MWL[29] were not detected in DHPs.[27] Structural similarity and difference between lignin model polymers and native lignin still remains an open question.[27,30-32]

There are three types of DHP which are of general use: bulk, end-wise, and membrane (see Experimental).[16] These three synthetic modes correspond to alternative ways of lignin biosynthesis in a cell wall. The end-wise synthesis is supposed to provide less branching than the bulk synthesis due to higher initial concentration of aroxyl radicals. Secondary wall is regarded as being formed in a similar way as the step-wise but not one-time addition[30] that yields β-O-4 pattern as the most abundant in lignin.[2] According to NMR data, the most abundant structures are β-5 in bulk DHP and β-O-4 in end-wise DHP.[27] *Erythro/threo* ratio of arylglycerol-β-aryl ether structures in MWL is closer to that in end-wise DHP than in bulk DHP.[27,30] On the other hand, hydrodynamic properties of spruce dioxane-lignin are similar to that of bulk but not end-wise DHP of ferulic acid.[32] The method of I-cleaving opens new opportunities for structural studies of lignin, and DHPs of different origins were chosen as the subject of this study to illustrate the application of the method. On the other hand, it would be important to analyze how close are DHPs to milled wood lignin (MWL) in their topological structure.

RESULTS AND DISCUSSION

The treatment of spruce MWL and the three types of DHP with dry HI resulted in extensive depolymerization of the samples in all cases. In chloroform which is a poor solvent for iodinated oligolignols, the only product found in the solution was 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane **1**, which comes from the reductive cleavage of β-O-4 bonds (Figure 1). The same product can be obtained by hydroiodination of coniferyl alcohol **2** - the precursor of lignin and the monomer of DHP.[10] In the separate experiment it was shown that HI does not reduce coniferyl aldehyde **3** to the product **1** under the chosen conditions; only the addition of HI to the carbon-carbon double bond in the side chain was observed. On the basis of the ^{1}H NMR spectrum (see Experimental), the structure **4** (Figure 1) was ascribed to the product of the reaction between coniferyl aldehyde and HI. Diiodide **1** is stable for 1-2 hours. Then it is transformed into a mixture of oligomers.

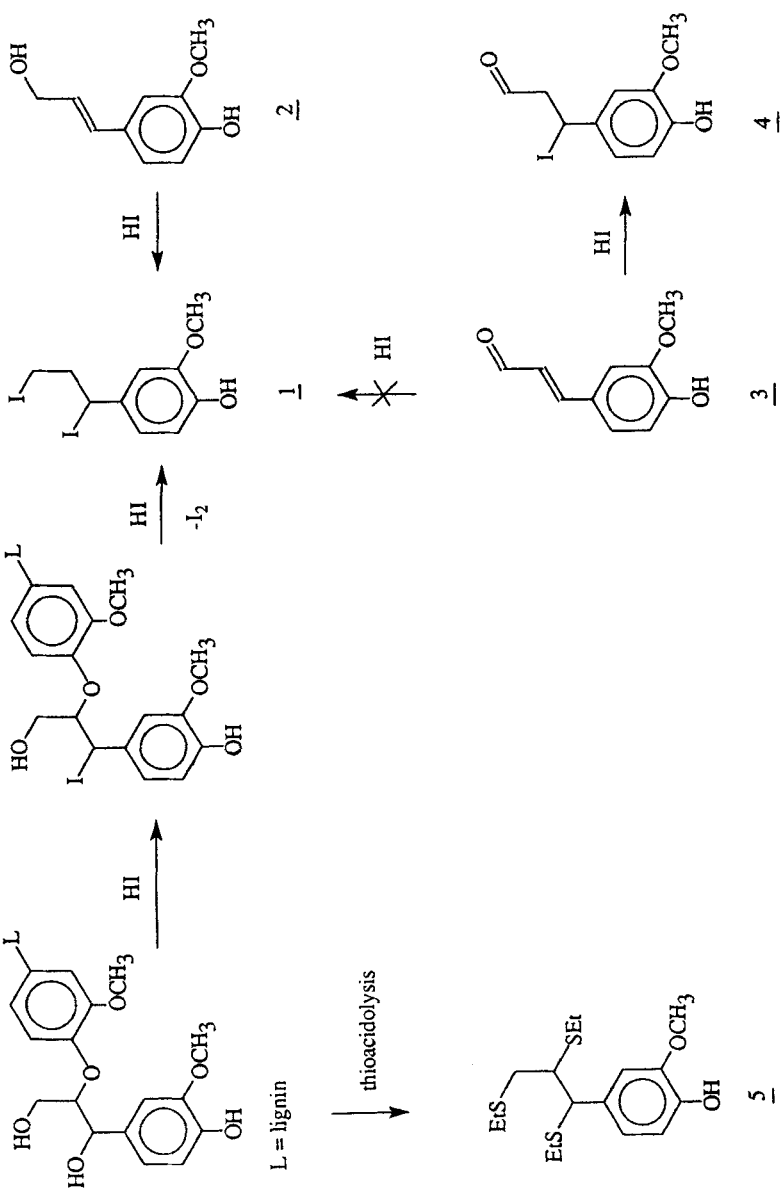

FIGURE 1. Chemical reactions of mild depolymerization of lignin.

TABLE

Yields of Diiodide 1 after I-Cleaving of DHP and MWL

Sample	Yield, %
End-wise DHP	40-45
Membrane DHP	50-55
Bulk DHP	20-22
Spruce MWL	20-22

In the experiments performed, the amount of diiodide was increasing during the treatment, and the maximum was achieved in 1 hour. After that, the concentration of diiodide was slowly decreasing. In all cases, the amount of diiodide 1 measured from ^{1}H NMR spectra (with the internal standard, see Experimental) corresponded to the maximum yield of this product of depolymerization. The values obtained (Table) were reproducible and should be treated as the upper limits of reductive splitting, which are not far from the actual amount of the iodinated monomer produced in the reaction. ^{1}H NMR monitoring of the reaction mixture is likely to be the most reliable method to control primary low molecular weight products of I-cleaving; chromatographical methods trace mostly secondary products of the reaction.[14]

Figure 2 demonstrates that diiodide 1 is practically the only product of the depolymerization of MWL, and no traces of the product of hydroiodination of the eliminated coniferyl aldehyde end-groups were observed (compare[14]). This illustrates the overwhelming predominance of splittable coniferyl alcohol polymer substructures (including end-groups) over coniferyl aldehyde end-groups. It is characteristic that, for spruce MWL, the yields of monomeric product of I-cleaving 1 obtained in this study (Table) and of monomeric product of thioacidolysis 5 reported by Brunow et al.[31] (Figure 1) are the same. Obviously, both products were formed from the same substructures of the polymer. This confirms the relevance of the information thus obtained to the primary structure of native lignin. For comparison, it is also interesting to note that, in hardwood lignin, the percentage of uncondensed

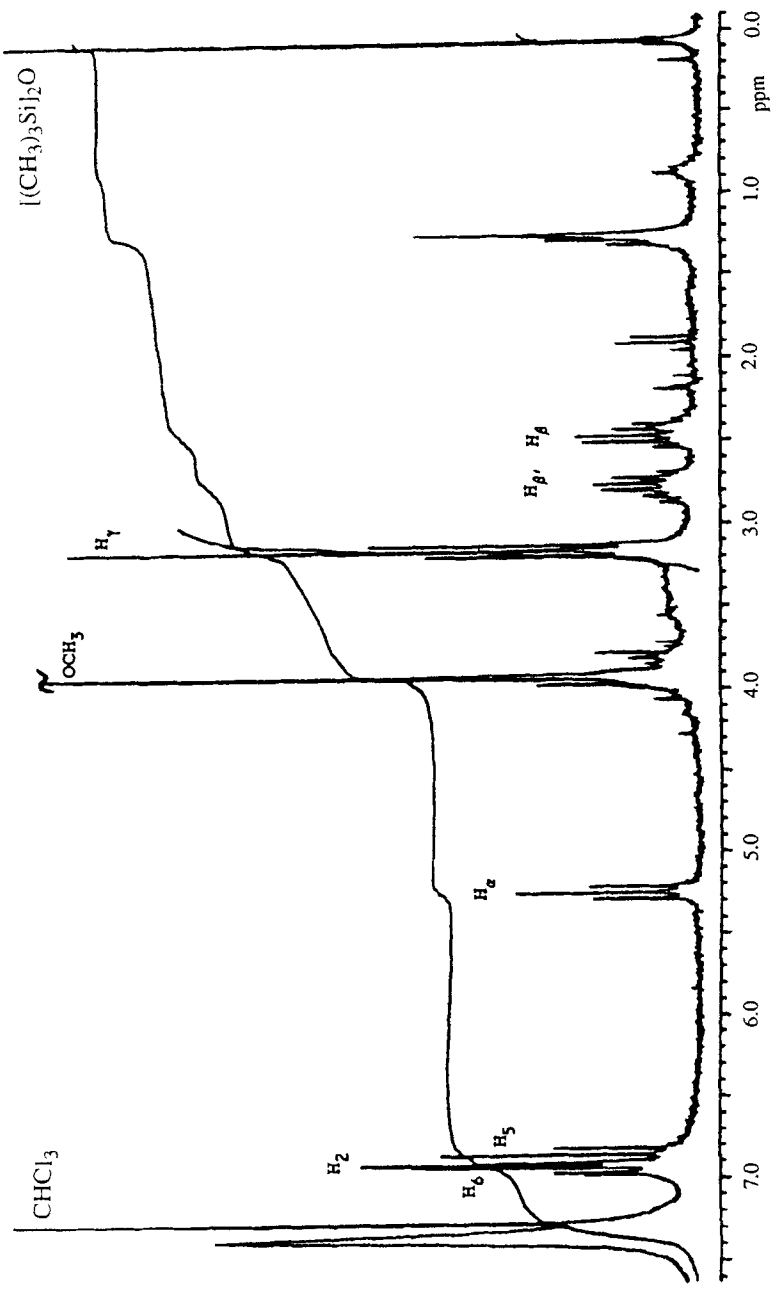

FIGURE 2. ^{1}H NMR spectrum of the $CDCl_3$ solution after depolymerization of spruce MWL with dry HI (see Experimental).

guaiacyl units having a free phenolic hydroxyl group is ca. 20%,[33] the amount close to the yield of diiodide 1 from guaiacyl (softwood) MWL.

I-cleavage is as effective for the suspended samples as for the dissolved ones (compare[10-12]). To understand this phenomenon, one has to note that the cleavage of β-O-4 bonds with HI is effective in non-aqueous media only. In these media, dissociation is suppressed, and hydrogen iodide is the weakest acid among the hydrogen halogenides.[34] Under these conditions, the strongest hydrogen bond acceptor is the α-$O_{sp}3$ atom in lignin or a lignin model compound. The molecule of HI is coordinated with the α-reaction center of lignin forming a complex or ion pair where iodine anion is placed near the reaction center because in these conditions the solvation energy which favors dissociation is much weaker than in the aqueous solution. Moreover, the direct attack of iodine anion on the α-C cationic center formed after elimination of a molecule of water is the most favorable path of the chemical reaction both in its enthalpy (no need to destroy the solvation shell) and entropy (the iodine anion is nearby). This situation is quite a bit different from that in aqueous media where strong acids such as HI are dissociated, and numerous side reactions take place.[35] A similar mechanism works in the case of a suspended polar polymer which attracts polar molecules of the reagent from the low-polar medium. The macromolecules of MWL and DHPs are rich in hydroxyl groups that grant a proton flow inside the polymer. At the same time, iodine anions are placed near the surface of the polymer held by electrostatic forces and may attack the α-cationic centers easily.

The yields of diiodide 1 from MWL and DHPs are significant (Table). It was demonstrated that, under the chosen mild conditions, HI cleaves selectively β-O-4 bonds in α-alcoholic and α-etheric structures as well as all α-etheric bonds.[10-12] Therefore, all diiodide 1 comes from coniferyl alcohol end-groups (dimension 0), the linear parts of the polymer (dimension 1) and α-branching knots (dimension 2) as it is demonstrated in Figure 3 (compare[7]). Hydrogen iodide attacks α-hydroxyl and α-etheric groups all over the lignin macromolecule. Therefore, unlike carbohydrate peeling, lignin reductive disassembling does not proceed entirely as a consequent elimination of the end-groups but rather as simultaneous splitting of β-O-4 interunit linkages in many places inside the macromolecule followed by the elimination

of non-etherified phenolic coniferyl end-groups thus formed. It is to be noted that the α-etheric bonds in lignin are unstable in hydrolysis. They undergo hydrolysis and re-etherification not only during technological processes but also *in vivo* and, therefore, can not be treated as stable elements of the primary structure of lignin.[36] The yield of diiodide 1 from MWL gives the lower limit of the amount of uncondensed units, i.e. the part of the macromolecule without carbon-carbon bonds and without any branching due to unsplittable (carbon-carbon, diaryletheric) bonds.

The topological structure of lignin was the subject of computer simulation, and the schemes of growing branched trees, cycles, and clusters of lignin were proposed by Gravitis.[6,7] However, this interesting concept has no experimental confirmation as yet. We believe that mild depolymerization with HI is essentially the best way to provide data complementary to this theoretical concept. The yield of diiodide 1 characterizes the total length of linear β-O-4 chains formed by the oxidative coupling of lignin precursors (simulated by Gravitis). In this process, α-ethers are formed as a result of the secondary reaction of nucleophilic addition.[36] Different patterns of stable interunit linkages are formed during the initial stage of radical coupling which is usually treated as a random process. A significant amount of the monomeric product 1 may result either from lengthy linear β-O-4 sections or numerous short "tails" of a star-like cluster (Figure 3). The first version looks most probable, at least in the case of end-wise and membrane DHPs. It is known that the frequency of phenolic hydroxyl group is lower in end-wise DHP than in bulk DHP that points to the linear chain organization of units in end-wise DHP.[27] Hydrodynamic measurements led to the conclusion that bulk DHP is chaotically branched and has hydrodynamic and conformational properties similar to that of dioxane-lignin; end-wise DHP demonstrated different properties, characteristic of a linear polymer.[32]

Extensive depolymerization of end-wise and membrane DHPs evidence the predominance of the selective β-O-4 pattern of radical coupling during the synthesis of both polymers, in accordance with the data on regioselectivity of chemical reactions modelling dimerization, i.e. the first stage of the formation of DHP.[37] In the bulk DHP, non-splittable (carbon-carbon, diaryletheric) linkages prevail that illustrate a decrease of

regioselectivity at higher concentrations of the monomeric precursor. In this aspect, spruce MWL is more similar to bulk than membrane and end-wise DHPs. (However, our experimental results do not confirm an assumption that β-O-4 linkages constitute only a minor part of interunit linkages in DHPs, based on NMR data[19]). Therefore, the actual mechanism of lignin biosynthesis can hardly resemble "classical" end-wise polymerization. The fact that *erythro/threo* ratio of arylglycerol-β-aryl ether structures in MWL is closer to that in end-wise DHP than in bulk DHP[27,30] cannot be regarded as a criterium of topological similarity because of differences in stereodifferentiation among chemical mechanisms involved in lignin biosynthesis and stereochemical inhomoheneity of lignin as well. The major factor contributing to both the structural inhomogeneity of plant lignin and the structural differences between DHPs and MWL may be related to a variety of enzymes responsible for lignin synthesis *in vivo*.[26,38]

Bulk DHP is most similar to MWL in another aspect. It is demonstrated by gel permeation chromatography (GPC) of higher molecular mass fraction of the products after I-cleaving (Figure 4). Lignin and lignin model DHP were extensively depolymerized after the treatment with HI. The major products were diiodide 1 and trimeric fragments. The latter can be treated as branching knots in the topological network of the polymer (Figure 3). The data obtained lead to the conclusion that the major structures in spruce MWL are linear β-O-4 chains with α-OR substituents and branching knots of degree 3. These knots belong to two major types: α-alkoxyl (aroxyl) branching and structures in which three arylpropane units are connected by carbon-carbon and/or diaryletheric bonds. The structure of these trimeric knots will be the subject of further investigation.

Molecular mass patterns of the products of depolymerization of MWL, bulk and membrane DHPs are similar (Figure 4). In end-wise DHP, the high molecular mass fraction was less polymerized than in other polymers under consideration. This means that among the three types of lignin model dehydrogenation polymers, end-wise DHP is the worst model and bulk DHP is the best model of MWL and, probably, native lignin.

CONCLUSIONS

The method of I-cleaving proved to be a powerful tool in the studies of lignin. Extensive depolymerization in mild conditions

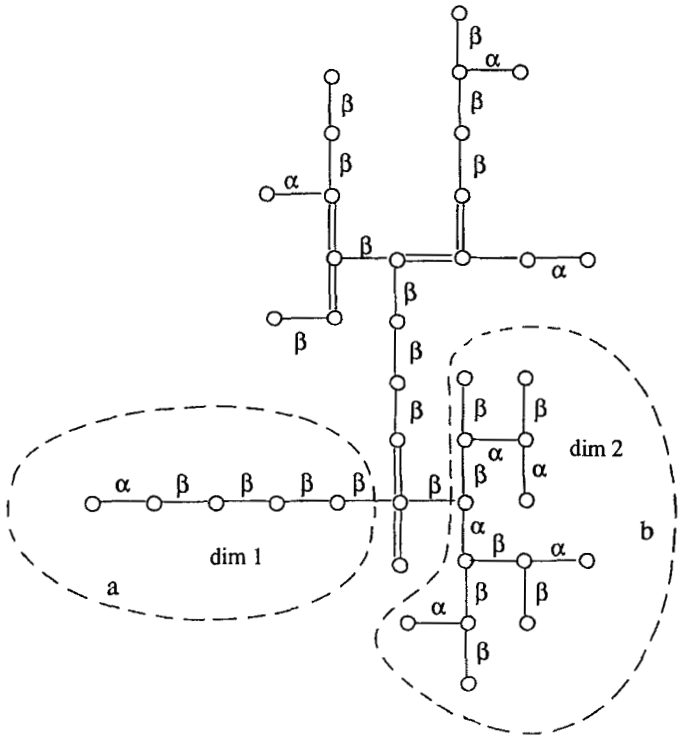

FIGURE 3. Interunit linkages in lignin: === - unsplittable bonds, β - β-O-4 bonds in alcoholic/etheric structures, α - α-etheric bonds; *a* - linear chains, *b* - star-like clusters.

demonstrated that among the major types of lignin model dehydrogenation polymers, the bulk one is most similar to the spruce milled wood lignin both in degree of conversion into monomeric units and gel permeation chromatographic patterns of oligomeric products of depolymerization. End-wise and membrane DHPs are much less cross-linked and may be depolymerized into the monomeric product with a yield of about 50%. The only monomeric product of I-cleaving in all the cases is 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane.

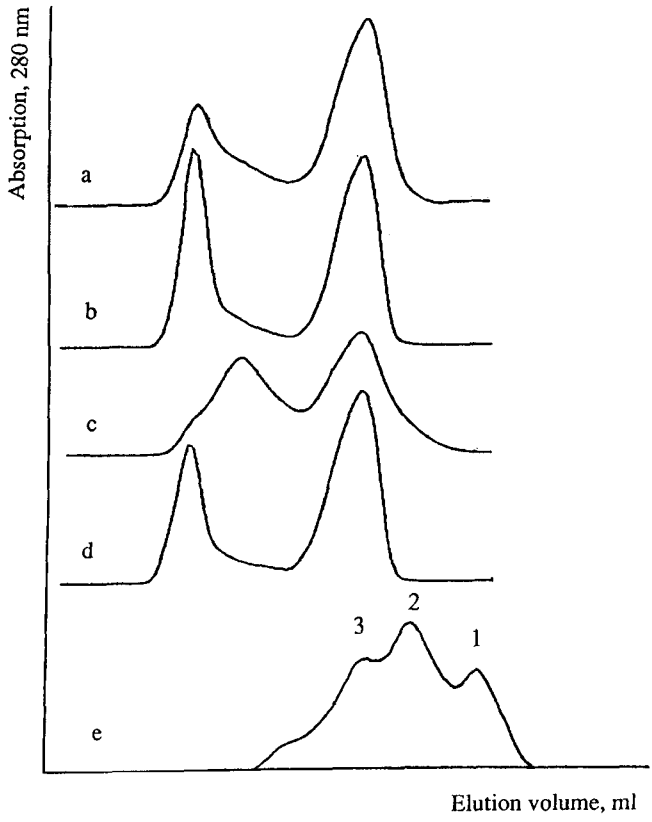

FIGURE 4. Gel permeation chromatograms of MWL and DHP treated with dry hydrogen iodide in $CHCl_3$: *a* - bulk DHP, *b* - membrane DHP, *c* - end-wise DHP, *d* - spruce MWL, *e* - the mixture of model compounds (*1* - monomeric, *2* - dimeric, *3* - trimeric; see Experimental).

EXPERIMENTAL

Materials. Spruce MWL was obtained by the standard procedure of Bjorkman.[39] Lignin model compounds were synthesized according to procedures previously reported or obtained commercially. Coniferyl alcohol **2** was synthesized by $LiAlH_4$ reduction of ferulic acid and ethyl ferulate. Horse radish peroxidase was obtained from

Tokyo Kasei (J209, 236K) and the dialysis tube from Spectrum Medical Industries, Inc. (Spectra/Por6 - wet tubing MWCO 1000).

Bulk DHP was prepared by the one-time addition of substrates according to the following procedure. The coniferyl alcohol solution (500 mg in 100 ml) and the hydrogen peroxide solution (0.5%, 25 ml) were dropped into the peroxidase solution (1 mg in 25 ml of distilled water) in a 4-neck flask (500 ml) covered with aluminum foil from respective separatory funnels for 30 minutes under nitrogen with stirring. The reaction was allowed to continue for 12 hours with stirring. The precipitated DHP was separated by centrifugation followed by membrane filtration. The collected DHP was dried *in vacuo* and dissolved in a small amount of a mixture of dichloroethane and ethanol (2:1, v/v). The solution was added drop by drop into 200 times the volume of ether with stirring. The precipitate was collected by centrifugation, washed with ether, and dried *in vacuo*.

The end-wise DHP was prepared by the gradual addition of substrates according to the following procedure. The coniferyl alcohol solution (500 mg in 250 ml) and the hydrogen peroxide solution (0.5%, 25 ml) were added into the peroxidase solution (1 mg in 25 ml of distilled water) in a 4-neck flask (2 l) covered with aluminum foil through separate silicone tubes by microtube pumps for 24 hours at the rate of 10 ml/hr under nitrogen with stirring. Stirring was continued for another 24 hours, and the enzyme solution was added every 12 hours (4 times in total). The DHP was collected by centrifugation, washed thoroughly with distilled water and dried *in vacuo*. The DHP was dissolved in a small amount of a mixture of dioxane and water (9:1, v/v) and filtered. The filtrate was freeze-dried.

The membrane DHP was prepared according to the following procedure. A dialysis tube containing the peroxidase solution (5 mg in 10 ml of distilled water) was immersed in water whose osmotic pressure was adjusted with polyethylene glycol (#4000) in a beaker (2 l) covered with aluminum foil. Through separate silicone tubes the coniferyl alcohol solution (500 mg in 100 ml of distilled water) and the hydrogen peroxide solution (0.2%, 100 ml) were added into the dialysis tube by microtube pumps for 100 hours at the rate of 1 ml/hr, and every 12 hours 1 ml of the enzyme solution was added under nitrogen with stirring (12 times in total). The tube was removed and the contents were dialyzed in distilled water for 12 hours, then for an additional 12 hours in methanol to remove low molecular weight fractions. The white

precipitate remaining in the tube was collected and dried *in vacuo*.

The solvents were purified as previously described[40]. $CDCl_3$ was commercially available.

HI treatment of samples. In ^1H NMR experiments, the samples were suspended in $CDCl_3$ (20 mg in 0.5 ml). 0.5 mm (O.D.) NMR tubes were used as reaction vessels. Dry HI was bubbled with argon flow through the suspension for 1.5 hours. The ^1H NMR spectra of the reaction mixture were recorded every 20 minutes. Before recording the last spectrum, hexamethyldisiloxane (HMDS) was added as an internal standard for the quantitative measurements. The amount of diiodide 1 was determined based on relative intensities of HMDS signal and clearly distinguished OCH_3, H_α and aromatic proton signals (Figure 2) after automatic integration. Under chosen conditions, only monomeric products remained in the solution, other products presented as suspension/colloids and gave no signal in the solution NMR spectrum. They did not disturb the determination of reproducible spectrum because very small quantities of the samples were used in the experiments (Fourier transform ^1H NMR was applied).

In the GPC analyses, dry HI was bubbled with argon flow through the sample suspended in chloroform (20 mg in 2 ml). After 1 hour, the insoluble polymer was filtered, washed with chloroform and dried *in vacuo*. The whole sample of the treated polymer (only monomeric product was removed with chloroform) was dissolved in DMSO before the GPC analysis.

NMR spectra and GPC procedures. The FT ^1H NMR spectra were recorded using a Bruker AC-200 instrument.

Gel permeation chromatograms of lignins were obtained on Sephadex LH-20 (fine) with a 500x10 mm column. DMSO + 0.03M H_3PO_4 + 0.03M LiBr was used as an eluent. Chromatograms were monitored by UV light at a wavelength of 280 nm. The samples of lignin were dissolved in DMSO (10 mg in 1 ml). Three lignin model compounds were used as standards in GPC calibration: "monomeric" 1-(4-hydroxy-3-methoxyphenyl)propan-1-ol, "dimeric" 1-(4-hydroxy-3-methoxyphenyl)-2-(2-methoxyphenoxy)ethan-1-ol, and "trimeric" 1-(4-hydroxy-3-methoxyphenyl)-1-[1-(4-hydroxyphenyl)-2-(2-methoxyphenoxy)ethoxy]propane.[11]

H^1 NMR spectrum of 1,3-diiodo-1-(4-hydroxy-3-methoxyphenyl)propane 1 (δ, ppm; J, Hz): 2.45 (H_β, m), 2.78 ($H_{\beta'}$, m), 3.16 (H_γ, t, $J_{\beta\gamma}$ 6.7), 3.92 (OCH_3, s) 5.25 (H_α, t, $J_{\alpha\beta}$ 7.5), 6.83 (H_5, d, $J_{5\text{-}6}$ 8.1), 6.90 (H_2, d, $J_{2\text{-}6}$ 2.1), 6.95 (H_6, dd); see

Figure 2. C^{13} NMR spectrum of diiodide **1** (δ, ppm, #C): 4.8 (Y), 33.8 (α), 44.3 (β), 109.9 (2), 114.5 (5), 120.2 (6), 134.4 (1), 145.7 (3), 146.5 (4). H^1 NMR spectrum of 3-(4-hydroxy-3-methoxyphenyl)-3-iodopropanal **4**, the product of the reaction between coniferyl aldehyde and HI (δ, ppm; J, Hz): 3.40 (H_β, m, $J_{\beta\beta'}$ 17.7, $J_{\alpha\beta}$ 6.6, $J_{\beta\gamma}$ 1.0), 3.56 ($H_{\beta'}$, m, $J_{\beta\beta'}$ 17.7, $J_{\alpha\beta'}$ 8.6, $J_{\beta'\gamma}$ 1.8), 3.90 (OCH_3, s), 5.57 (H_α, dd, $J_{\alpha\beta}$ 6.6, $J_{\alpha\beta'}$ 8.6), 6.82 (H_5, d, $J_{5\text{-}6}$ 8.1), 6.91 (H_2, d, $J_{2\text{-}6}$ 2.0), 6.94 (H_6, dd), 9.54 (H_γ, m).

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