An Improved Method for Isolating Lignin in High Yield and Purity

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When pre-extracted wood is ground to a fine powder using a porcelain ball mill, the resulting material may easily be degraded by cellulolytic enzymes, eliminating ~90% of the carbohydrates. The residue still contains large amounts of carbohydrates which may readily be removed by an optimized mild (0.01 mol/L) acidolysis stage. The resulting enzymatic/acidolysis lignin (EAL) is of significantly higher yield (~ 2.5 times greater) than the corresponding milled-wood lignin preparation. Detailed structural analyses of the EAL produced from poplar and spruce wood samples when compared to their milled-wood lignin counterparts showed no marked differences. The proposed methodology offers lignin preparations in higher yields than any other known protocol, with minimal work up and solvent requirements.

Lorsque du bois pré-extrait est moulu en fine poudre à l'aide d'un moulin à bille de porcelaine, la matière produite est facilement dégradable par les enzymes cellulolytiques, ce qui permet d'éliminer environ 90% des hydrates de carbone. Le résidu contient encore de grandes quantités d'hydrates de carbone, qu'on peut facilement éliminer lors d'une étape d'acidolyse douce (0,01 mol/L) optimisée. La lignine enzymatique/acidolyse (EAL) obtenue offre un rendement beaucoup plus élevé (~2.5 fois plus grand) que la préparation correspondante de lignine de bois moulue. Des analyses structurelles détaillées de l'EAL produite à partir d'échantillons de peuplier et d'épinette n'ont montré aucune différence marquée lorsqu'on les compare à leurs contreparties de lignine de bois moulu. La méthodologie proposée offre des préparations de lignine avec un rendement plus élevé que tout autre protocole connu, avec des besoins moindres en matière de solvant et de traitement final.

INTRODUCTION

Although lignin has been studied for more than 150 years, its structural details continue to emerge. One of the problems that one encounters when studying lignin is whether the sample examined is representative of the overall material within the wood or the plant species from which it was isolated. This is because to date there is no method that may be defined as ideal for the isolation of a highly representative and totally unaltered native lignin. For example, an early lignin preparation technique used strong mineral acids, such as 40% hydrochloric acid, or 3:1 (v/v) mixture of hydrochloric and 80% phosphoric acids [1]. While such methods could produce large amounts of material that could be described as lignin, such drastic conditions cause irreversible reactions that severely alter the structure of the isolated material. To date, the Björkman method of lignin isolation allows the isolation of milled-wood lignin (MWL) by extensive ball milling of the wood sample followed by extraction with neutral solvents at room temperature [2,3]. This methodology is generally accepted as the state of the art as far as lignin isolation methods are concerned. It offers lignin at moderate yields (up to 32% for various wood species) with minimal structural alterations.

Detailed studies on the process of MWL have shown that milling time has a significant effect on the yield, the functional group content and the actual topochemistry (middle lamella lignin versus secondary wall lignin) of the isolated material [4,5]. Despite its documented advantages, the MWL isolation procedure is confronted with the question of the representative nature of the isolated samples. At such moderate yields of isolation, does the lignin obtained represent the overall material present in the starting wood or plant species? In addition, due to the extensive milling, some depolymerization reactions may occur, altering the structure of the native material [4,6,7].

Additional efforts to improve the process of isolating lignin, in a relatively unaltered state, included those of Pew and Weyna that treated ball-milled wood with commercial cellulase enzymes, aimed at selectively degrading the wood polysaccharides. Their effort allowed nearly all the lignin in aspen and spruce wood to be present in the residue. However, these lignins contained about 10–12% carbohydrates and as such were not characterized [8]. Chang et al. [9] developed the cellulolytic enzyme lignin (CEL) procedure. This method is equally applicable to hardwoods and softwoods and is based on extracting enzymatically treated wood meal successively with 96 and 50% aqueous dioxane. The procedure affords two lignin fractions. For spruce wood, the yields of these two fractions were 27.8 and 29.2%, and their carbohydrate contents were 4.3 and 8.9%, respectively. The CEL procedure offers a significantly improved yield (over the traditional MWL procedure) with small differences in the properties. The disadvantages of the CEL procedure are that high dosages of cellulolytic enzymes are required (causing eventual protein contamination) as well as large amounts of solvents involving a rather laborious process.

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The existence of lignin–carbohydrate complexes (LCC) was first proposed by Björkman in 1957 [10]. Ensuing research showed that some parts of the lignin are covalently linked with carbohydrates [11]. The fact that some of the LCCs actually dissolve in acidified aqueous media or in dioxane–water mixtures [12,13] represents an obstacle to isolating lignin in high yield and purity. The selective scission of such linkages could offer tangible benefits in isolating pure lignin in high yields.

In a recent study, we proposed a novel residual kraft lignin isolation procedure composed of an initial mild enzymatic hydrolysis stage, followed by a mild acid hydrolysis stage [14]. This combination produced residual lignin samples of high yield and purity with no obvious structural alterations. Our study, on the residual lignin front, paved the way to developing an improved methodology for isolating lignin from wood by including a step that selectively and mildly cleaves most of the LCC bonds.

In the present paper, we report that cellulolytic enzymes are highly effective in degrading ball-milled wood powder. The residue obtained when this material is treated with a mild acid offers lignin (enzymatic/acidolysis lignins (EAL)) in high yields and purity, with reduced carbohydrate content. Detailed structural analyses of the new lignins, isolated from spruce and aspen wood, are compared with MWL isolated by conventional means.

EXPERIMENTAL

Preparation of EALs and MWLs from Poplar and Black Spruce

Populus tremuloides and *Picea marianna* wood chips were ground in a Wiley mill. They were then acetone extracted over a period of 48 h, air dried and finally dried in a vacuum oven set at room temperature. An amount of 230 g of dry extracted powder was used for poplar EAL, poplar MWL and spruce EAL. For spruce MWL, 80 g of dry extracted powder were used. This was ground in a 13.5 L ball mill jar in the presence of 365 balls which occupied 18% of the active jar volume. The grinding period was 14 days with a speed of rotation of 36 rpm.

The milled wood was thoroughly mixed and used to prepare both MWLs and EALs. Poplar MWL and black spruce MWL were prepared according to the standard Björkman procedure and applying the Brownell modifications [3]. The procedure for preparing poplar and black spruce EAL is described below. The yield data discussed throughout this paper were actually gravimetric yield data and were based on the amount of Klason and UV lignin of the original wood species. Furthermore, the purity data were based on the sum of Klason and UV lignin contents of a specified sample.

Cellulolytic Enzymatic Treatment

The ground wood meal (40 g o.d.) was treated with cellulase (Iogen Industrial Cellulase, Iogen Corp., Ottawa, ON, Canada) having an activity of ~1300 carboxyl methyl cellulose (CMCase) units mL^{-1}. This enzyme preparation is also known to contain a significant amount of hemicellulase activity. Repeated optimization experiments indicated that a low ratio of enzyme to wood meal should be maintained. This was found to be 400 units g^{-1} of o.d. wood meal. Such enzyme charges minimized the protein contamination of the final products as we reported earlier [14]. The enzymatic hydrolysis was carried out at 40°C over a period of 48 h at pH 4.5 (phosphate buffer) and a consistency of 5%. The water bath used was equipped with an orbital shaker set at 240 rpm. After the enzymatic hydrolysis, the resulting crude lignin, which contained carbohydrate impurities, was collected by centrifugation and washed (X2) with acidified (pH = 2) deionized water followed by freeze drying.

Mild Acidic Hydrolysis and Recovery of EAL

The crude lignin obtained from the mild enzymatic treatment was suspended in acidic dioxane–water (85:15 v/v) mixture. An amount of 5 g of crude lignin was suspended in 100 mL of acidified dioxane–water solution (i.e. for the 0.01 mol/L acid concentration: 98.6 mg of 37% hydrochloric acid were mixed with 100 mL of the dioxane–water mixture). This mixture was then refluxed (boiling point 86°C) under nitrogen for 2 h. The resulting solution was filtered and the solid residue was washed with fresh dioxane–water (85:15 v/v) and finally with fresh dioxane until the filtrate was clear. The combined filtrates were then neutralized with sodium bicarbonate. The neutralized solution was rotary evaporated (at 30°C) until a thick solution was obtained. This solution was added dropwise to 1 L of acidified deionized water (pH = 2.0). The precipitated lignin was isolated by centrifugation, washed and freeze dried. The isolated lignin was washed with high-pressure liquid chromatograph (HPLC) grade hexane to remove any additional extractives and vacuum dried at room temperature.

Chemical Analyses

The Klason, UV lignin and carbohydrate (gas chromatography method) contents of the isolated lignins were determined by using standard PAPTAC methods. The elemental and methoxyl analyses were carried out by Schwarzkopf Microanalytical Laboratories, Woodside, New York, USA.

Quantitative ^{31}P-Nuclear Magnetic Resonance (NMR) Spectroscopy

Quantitative ^{31}P-NMR spectra were obtained on a Varian 300 MHz spectrometer, using pyridine/$CDCl_3$ (1.6/1 v/v) as the solvent, cholesterol as the internal standard, chromium acetylacetonate as the relaxation reagent and 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxapholane as the phosphitylation reagent [15,16].

Derivatization Followed by Reductive Cleavage (DFRC)

The DFRC procedure was nearly identical to that developed by Lu and Ralph [17,18] with minor but significant modifications related to the actual amounts of the reagents and lignin. An amount of 12.5 mL of a mixture of acetyl bromide and acetic acid (1/9 v/v) was added to a lignin sample (50 mg) in a 50 mL round-bottom flask. The reaction was allowed to take place at 50°C for 3 h under stirring. The solvent was then rapidly evaporated to dryness under reduced pressure, using a rotary evaporator connected to a vacuum pump and a cold trap. The residue was then dissolved in 12.5 mL of acidic solution (dioxane/acetic acid/water 5/4/1) and 250 mg of zinc dust were added. The mixture was then stirred at room temperature for 30 min. The reaction mixture was quantitatively transferred to a 50 mL saturated ammonium chloride solution in a separating funnel using in total 50 mL of HPLC grade dichloromethane. An amount of 100 µL of internal standard solution (80 mg of cholesterol in 2 mL of dichloromethane) was added for subsequent quantitative ^{31}P-NMR analyses. The aqueous layer was extracted with HPLC-grade dichloromethane (3 x 20 mL). The combined extracts were dried with anhydrous sodium sulphate, filtered and evaporated to dryness. To completely eliminate acetic acid, ~20 mL of dioxane/water (85/15) solution was added and the sample was freeze dried. The dry powder was used for subsequent quantitative ^{31}P-NMR analyses.

Quantitative Determination of Methane Carbons (CH) using Distortionless Enhancement by Polarization Transfer (DEPT) Experiment

Quantitative ^{13}C-NMR spectra were collected using the DEPT sequence [19,20] on a Varian Mercury 300 (75.5 MHz for ^{13}C observation). Both dried lignin samples (~200 mg) and internal standard compounds pentafluorobenzene (~90 mg) were accurately weighed, and dissolved in 700 µL of dimethyl sulphoxide-d_6. Chromium acetylacetonate should never be used during the DEPT sequence since it adversely affects the relaxation of the magnetization and consequently the quantitative reliability of the experiment. The acquisition time varied from 30~60 h. For more detailed acquisition and processing parameters, one may consult our earlier publication [21].

RESULTS AND DISCUSSION

Optimization of the HCl Concentration During Acid Hydrolysis

The presence of actual linkages between lignin and carbohydrates in wood has been extensively studied. However, LCCs are very complex and the precise nature and amount of these linkages is far from being understood [11]. The most frequently quoted types of LCC bonds are benzyl ethers, benzyl esters, and phenyl glycoside linkages [22,23]. Both benzyl ethers and phenyl glycoside linkages can be easily cleaved under mildly acidic conditions [1]. Consequently, we examined if, after the milling and the enzymatic treatment, a mild acidic treatment could cleave the lignin carbohydrate linkages and recover the lignin in high

yield and purity. At this point, however, the HCl concentration should be chosen carefully to avoid cleaving the ether linkages within lignin units and to avoid promoting acid-induced condensation reactions.

The HCl concentration had an obvious effect on the yield, purity and functional group content of the resulting EAL (Figs. 1,2). As shown in Fig. 1, the purity of poplar EAL increased very slightly with the HCl concentration. For the same wood species, surprisingly, the opposite trend was observed, as far as the lignin yield was concerned. More specifically, the yield was decreased from 69.9 to 56.0% when the HCl concentration was increased from 0.01 to 0.05 mol/L. This could be rationalized on the basis that, at higher HCl concentrations, acidic degradation of lignin might occur, resulting in actual delignification and the generation of low molecular weight lignin derivatives [24] which are not recovered during the ensuing lignin isolation procedure followed in this work. Demethoxylation reactions were also evident when the HCl concentration was increased.

DFRC followed by quantitative ^{31}P-NMR [25,26] was used to determine the functional group content and β-O-4 linkages of EALs obtained under different HCl concentrations. As shown in Fig. 2, the condensed hydroxyl group content increased rapidly from 0.29 to 0.40 mmol/g when the HCl concentration was increased from 0.01 to 0.025 mol/L, while it didn't continue to increase at the higher HCl concentration of 0.05 mol/L. The term condensed phenolic units refers only to those aromatic structures with C5 substituents other than methoxyl. We thus explore structures such as biphenolic units, diaryl methanes and diaryl ethers bearing one or two free phenolic hydroxyl groups. Notably, the frequency of β-O-4 bonds decreased by ~16% when the HCl concentration was changed from 0.01 to 0.05 mol/L.

Yield, Purity, Elemental Composition and Functional Group Content of EALs

Table I shows that the concerted effect of ball milling, cellulolytic action and mild acid hydrolysis offered significant yield improvements over the traditional MWL procedure. For poplar wood, the yield of EAL was 69.9%, while for spruce, the yield of EAL was 75.3%. Compared to the corresponding MWLs, these are 2.4 times greater for poplar and 2.6 times greater for spruce.

The purity of EAL from both poplar and spruce was also higher than that of the corresponding MWLs. When one compares the yield and purity of EAL to that of CELs [9], it becomes apparent that the EALs are of greater purity and yield than either CEL fraction. More specifically, for spruce CEL, the combined yields of the fractions obtained after extractions with 50 and 96% dioxane were no more than 57% [9], compared to 75.3% for EAL. CEL from the hardwood sweetgum was obtained at a combined yield of 68% containing 5.3% carbohydrates [9], while for the hardwood poplar, EAL was obtained at 69.9% yield containing less than 4.3% carbohydrates (Table I).

The functional group content of EAL as determined by quantitative ^{31}P-NMR is shown in Table II (for actual spectra, see Fig. 3). It is

TABLE I
YIELD, PURITY AND ELEMENTAL COMPOSITION OF EAL AND MWL

	Poplar		Black Spruce	
	EAL[a]	MWL	EAL[a]	MWL[b]
Yield[c] (%)	69.9	28.7	75.3	28.5
Purity[d] (%)	87.4	84.7	91.3	88.3
Carbohydrates (%)	<4.3	<8.0	<7.7	<9.6
C (%)	58.9	59.2	61.0	61.0
H (%)	5.8	5.9	5.6	5.7
O (%)	35.0	34.8	33.4	33.3
N (%)	0.2	<0.1	<0.1	<0.1
OCH_3[e] (%)	18.3	16.6	12.5	13.5
C_9 formula	$C_9H_{8.52}O_{3.56}(OCH_3)_{1.26}$	$C_9H_{8.96}O_{3.57}(OCH_3)_{1.12}$	$C_9H_{8.43}O_{3.23}(OCH_3)_{0.78}$	$C_9H_{8.52}O_{3.19}(OCH_3)_{0.84}$
Molecular Weight of C_9 Unit	212.5	208.6	192.2	193.6

(a) HCl concentration for mild acidolysis was 0.01 mol/L.
(b) Spruce MWL isolated using Björkman procedures; for isolation of EALs and poplar MWL (see Experimental).
(c) Based on Klason lignin content of extracted ground wood meal.
(d) Purity based on sum of Klason and acid-soluble lignins.
(e) Methoxyl group content was not corrected for lignin purity.

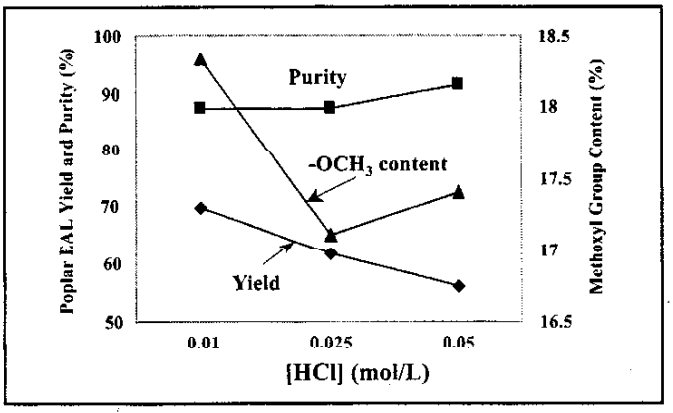

Fig. 1. The effect of HCl concentration on the yield, purity and methoxyl content of poplar EAL.

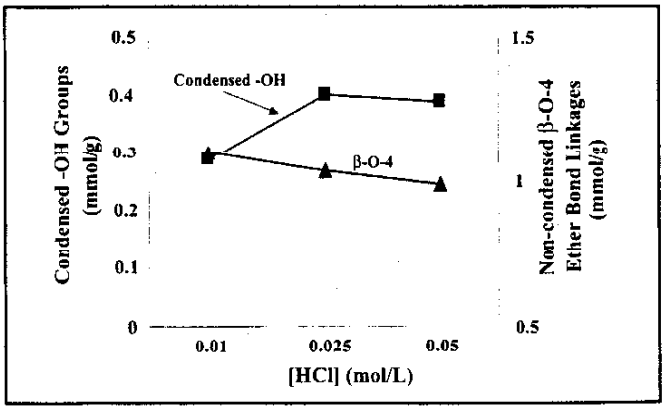

Fig. 2. The effect of HCl concentration on the abundance of β-O-4 linkages and condensed phenolic hydroxyl groups, for poplar EAL.

known that milling liberates phenolic hydroxyl groups [10]. Since, however, in our work the poplar EAL and MWL were prepared under identical milling conditions, it is not surprising to find that the total phenolic hydroxyl groups of these two samples were nearly identical (Table II). Furthermore, these nearly identical values indicate no evidence of liberation of phenolic hydroxyl groups resulting from acid hydrolysis of the ether bonds in lignin. For spruce wood, the total phenolic hydroxyl group content of EAL and MWL were 0.23/C_9 and 0.21/C_9, respectively. For the same wood species, the literature value (determined by the periodate method) is ~0.20/C_9 [9], which compares favorably to that of EAL.

It is significant to note that the methoxyl group content of poplar EAL was ~2% greater that that of the corresponding MWL (Table I). The fact that the secondary wall of hardwood lignin is enriched in syringyl units [27] (resulting in a higher methoxyl group content) leads to the conclusion that EAL contains more secondary wall lignin than MWL, and consequently is more representative of the overall lignin. This is also in agreement with the findings of Lee et al. [4] who have examined the structural details of MWL as a function of milling time. Their work showed that, as the milling time was extended, the syringyl content of the isolated MWL increased. The EAL samples from poplar were subjected to 14 days of milling prior to a mild acidolysis.

The aliphatic hydroxyl group data for poplar EAL and MWL were 0.83/C_9 and 0.94/C_9, respectively, which compares reasonably to the literature range of 1.1~1.15/C_9 [22] considering the uncertainties associated with carbohydrate contaminants and their effect on such data. Similarly, the aliphatic hydroxyl content of spruce EAL and MWL was 0.94/C_9 and 0.8/C_9, respectively, with corresponding literature data being 1.1~1.2/C_9 [22].

The condensed phenolic hydroxyl group content of poplar EAL was ~25% greater than that of poplar MWL. However, a comparison of the condensed phenolic hydroxyl group content of spruce EAL with the corresponding MWL indicated that the latter contained 32% more condensed phenolic groups. Despite the fact that softwood lignin is composed of guaiacyl C_9 units, which contain a free C_5 aromatic position, the isolated EAL contained fewer condensed structures than MWL. The greater condensed phenolic hydroxyl content in the hardwood EAL may be due to the liberation of 5-5' biphenyls present in specific dibenzodioxocin moieties, as will be discussed in more detail in future publications.

DEPT Edited Quantitative ^{13}C-NMR Spectra

Recent research in our laboratory has allowed the development of novel protocols for acquiring quantitative ^{13}C-NMR spectra of lignins using internal reference standards. Among others, pentafluorobenzene has been shown to be an excellent internal standard for the acquisition of DEPT spectra. Although the

TABLE II
FUNCTIONAL GROUP CONTENT OF EAL AND MWL AS DETERMINED BY QUANTITATIVE ^{31}P-NMR[a]

Functional Group	Integrated Chemical Shift Range (ppm)	Poplar		Black Spruce	
		EAL	MWL	EAL	MWL
Aliphatic –OH	149.2–146.0	3.91	4.53	4.92	4.13
Guaiacyl –OH	140.0–138.8	0.33	0.37	0.72	0.67
p-Hydroxyl –OH	138.2–137.4	0.14	0.17	0.06	0.09
Syringyl –OH	143.1–142.38	0.23	0.24		
Ratio of G:H:S[b]		1.44:0.61:1	1.54:0.71:1		
Ratio of H:G[b]		0.43	0.48	0.09	0.13
Total Uncondensed Phenolic –OH		0.70	0.72	0.78	0.76
Condensed –OH	144.5–143.1 142.38–141.5	0.29	0.22	0.30	0.44
Total phenolic –OH		1.00 (0.21/C_9)	1.00 (0.21/C_9)	1.09 (0.21/C_9)	1.20 (0.23/C_9)
Total –OH		4.90	5.56	6.00	5.33
Carboxylic acids	135.5–134.5	0.11	0.14	0.09	0.11

(a) In mmol/g; error ±0.02.
(b) Refers to units bearing free phenolic OH; standardized by expressing the syringyl phenolics as unity.

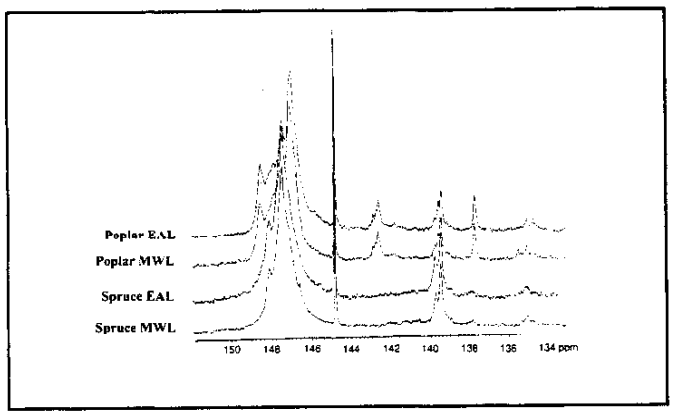

Fig. 3. Quantitative ^{31}P-NMR spectra of EALs and MWLs from poplar and black spruce.

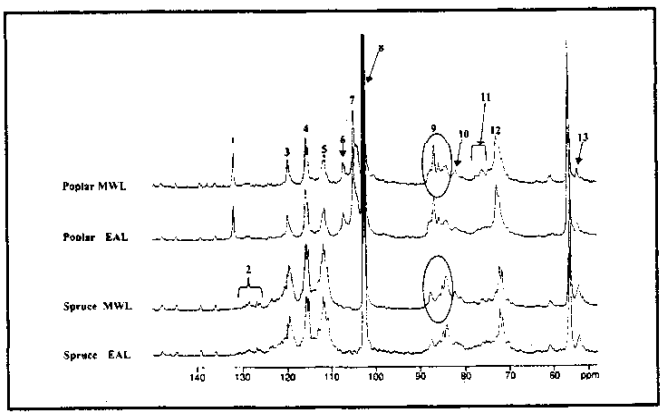

Fig. 4. DEPT edited quantitative ^{13}C-NMR spectra of EALs and MWLs.

inverse gated proton decoupled ^{13}C-NMR spectral data are known to be very reliable for the quantification of various lignin carbon environments, the method suffers from two major disadvantages. Primarily, the sensitivity of this experiment is significantly lower than the routine 1D ^{13}C-NMR experiment, due to the loss of the nOe effect. Secondly, extensive signal overlapping occurs and hence signal assignments are difficult; as such, quantitative structural information should be treated with caution. These difficulties can be somewhat circumvented by the DEPT experiment. For these reasons, we used the DEPT experiment to acquire quantitative information for all lignins isolated in this work with the aim to compare their methine carbons on a quantitative basis.

In general, the quantitative data for the various methine carbon environments indicated that there were only minor differences between the various EAL lignins and the corresponding milled wood lignins (Table III).

It is interesting to observe that only the softwood lignins contained the unsaturated C_α and C_β environments (set of signals 2, Fig. 4), indicative of the unsaturated aldehydic sidechains (Ar – CH = CH – CHO). As anticipated, signals 3 and 5, indicative of the C_6 and C_2 carbons in G-units, are significantly reduced (by ~70%) in the poplar samples as opposed to the spruce samples.

For the poplar lignins, the set of signals indicated as 9 in the spectral data of Fig. 4 is thought to originate from the various aliphatic C_β carbons with minor C_α contributions of ring IV in dibenzodioxocin (DBDO) moieties (Fig. 5). ^{31}P-NMR spectroscopy has shown the potential of resolving the various DBDO structures represented in Fig. 5 and this will be the subject of another communication. Due to the significant overlap with the signals at 87.7, 85.9 and 85.4, however, the quantitative data that emerge from the integration of the overall area are prone to significant errors. The set of signals labeled as 11 could be attributed to C_2, C_3 and C_4 carbons in xylan units which seem to be more effectively removed by the acidolysis of the poplar sample than its spruce counterpart (i.e. signal 11 EAL data). Finally, it is notable that non-significant differences were observed between the EAL and the MWL samples for signal 13 assigned to C_β/C_β' in G- or S units with β-β′ linkages or C_β in G- or S-units with β-5′ linkages.

CONCLUSIONS

When ball-milled wood is subjected to a mild cellulase treatment followed by mild (0.01 mol/L HCl) acidolysis, the resulting material (EAL) is obtained in significantly greater yield than the corresponding MWL. Structural analyses between MWLs and the lignins produced by the above protocol offer no evidence of marked differences.

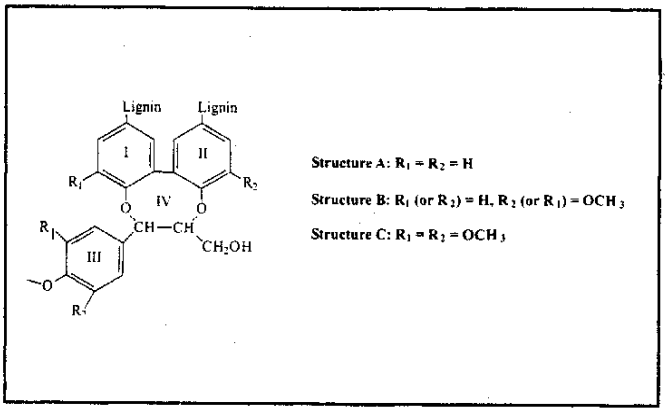

Fig. 5. The DBDO structures present in lignins. For poplar lignins, it is anticipated that all structures (A, B, C) are present while, for spruce, structure C predominates.

TABLE III
SIGNAL ASSIGNMENTS AND METHINE (CH) CARBON CONTENTS OF EALs AND MWLs OBTAINED BY QUANTITATIVE DEPT EDITED ^{13}C-NMR SPECTROSCOPY[a]

Signal No.	Chemical Shift (ppm)	Signal Assignment[b,c]	Methine Carbon Content (mmol/g)			
			Poplar		Spruce	
			EAL	MWL	EAL	MWL
1	132.0	C_2/C_6 in p-hydroxybenzoates	0.23	0.21		
2	129.3, 127.0	C_β in Ar–CH = CH–CHO C_α in Ar–CH = CH–CHO			0.33	0.30
3	119.9	C_6 in G-units	0.41	0.46	1.48	1.33
4	115.8, 115.3	C_3/C_5 in p-hydroxybenzoates (for hardwood) or C_5 in etherified and non-etherified G-units	0.73	0.78	1.35	1.14
5	111.5	C_2 in G-units	0.62	0.68	1.80	1.60
6	107.2	C_2/C_6 in etherified or not S-units with α–C = O, or C_2/C_6 in S-units with α-O-4′ linkage	0.52	0.51		
7	104.8, 104.1	C_2/C_6 in etherified or not S-units C_2/C_6 in S-units with β-O-4 and C_αHOH	1.22	1.21		
8	102.9, 102.6, 102.2	C_1 in pentafluorobenzene (internal standard)				
9	87.7	C_β in S-units with β-O-4 (both *threo* and *erythro*)(for poplar); For spruce, the signal at 87.7 ppm has not been assigned.				
	86.9	C_β in dibenzodioxocins (DBDO) structures[d]	1.29	1.32	1.25	1.06
	85.9,85.4	C_β in G-units or S-units with β-O-4′ (both *threo* and *erythro*)				
	84.5	C_α in DBDO structures[d]				
10	82.1	Unassigned	0.39	0.49	0.39	0.37
11	77.2, 76.2	$C_2/C_3/C_4$ in xylan units	0.48	0.61	0.69	0.41
12	72.9,72.3,71.7	Unassigned, and C_α in G-units	1.33	1.61	1.30	0.90
13	54.5,54.1, 53.4	C_β/C_β' in G-units or S-units with β-β′ linkages C_β in G-or S-units with β-5′ linkages	0.32	0.35	0.37	0.40

(a) Errors = ±0.04.
(b) Spectral assignments are based on [21,28,29].
(c) H = *p*-hydroxyphenylpropane; G = guaiacylpropane; S = syringylpropane.
(d) Assigned on the basis of model compound studies.

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REFERENCES

1. ADLER, E., "Lignin Chemistry-Past, Present and Future", *Wood Sci. Technol.* 11:169–218 (1977.)
2. BJÖRKMAN, A., "Studies on Finely Divided Wood 1. Extraction of Lignin With Neutral Solvents", *Svensk Papperstidn.* 59:477–485 (1956).
3. BROWNELL, H.H.; "Isolation of Milled Wood Lignin and Lignin Carbohydrate Complex", *Tappi* 48:513–518 (1965).
4. LEE, Z.Z., MESHITSUKA, G., CHO, N.-S. and NAKANO, J., "Characteristics of Milled Wood Lignins Isolated with Different Milling Times", *Mokuzai Gakkaishi* 27(9):671–677 (1981).
5. WHITING, P. and GORING, D.A.I., "The Morphological Origin of Milled Wood Lignin", *Svensk Papperstidn.* R120–122(1981).
6. LUNDQUIST, K., "Wood" in Methods in Lignin Chemistry, S.Y. Lin and C.W. Dence, Eds., Springer, Berlin, Germany Ch. 3.1 (1992).
7. BRUNOW, G., LUNDQUIST, K. and GELLERSTEDT, G., "Lignin" in Analytical Methods in Wood Chemistry, Pulping and Papermaking, Springer, Berlin, Germany, Ch. 4 (1998).
8. PEW, J.C. and WEYNA, P., "Fine Grinding, Enzyme Digestion and the Lignin–Cellulose Bonds in Wood", *Tappi* 45(3):274–256 (1962).
9. CHANG, H.M., COWLING, E.B., ALDLER, E. and MIKSCHE, G., "Comparative Studies on Cellulolytic Enzyme Lignin and Milled Wood Lignin of Sweetgum and Spruce", *Holzforschung* 29(5):153–160 (1975).
10. BJÖRKMAN, A., "Studies on Finely Divided Wood. V. The Effect of Milling", *Svensk Papperstidn.* 60.243–251 (1956).
11. YAKU, F., TANAKA, R. and KOSHIJIMA, T., "Lignin Carbohydrate Complex. Part 4. Lignin as Side-Chain of the Carbohydrate in Björkman LCC", *Holzforschung* 35(4):177–181 (1981).
12. IVERSEN, T., WESTERMARK, U. and SAMUELSSON, B., "Some Comments on the Isolation of Galactose-Containing Lignin Carbohydrate Complexes", *Holzforschung* 41(2):119–121 (1987).
13. KOSHIJIMA, T., YAKU, F. and TANAKA, R., "Fractionation of Björkman LCC from Pinus Densiflora", *Proc. Appl. Polym. Symp.* 28:1025–1039 (1976).
14. ARGYROPOULOS, D.S., SUN, Y.J. and PALUS, E., "Isolation of Residual Kraft Lignin in High Yield and Purity", *J. Pulp Paper Sci.* 28(2):50–53 (2002).
15. ARGYROPOULOS, D.S., "Quantitative Phosphorus-31 NMR Analysis of Lignin: a New Tool for the Lignin Chemist", *J. Wood Chem. Technol.* 14:81 (1994).
16. GRANATA, A. and ARGYROPOULOS, D.S., "2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, a Reagent for the Accurate Determination of the Uncondensed and Condensed Phenolic Moieties in Lignins", *J. Agric. Food Chem.* 43(6):1538–1544 (1995).
17. LU, F. and RALPH, J., "Derivatization Followed by Reductive Cleavage (DFRC Method), A New Method for Lignin Analysis: Protocol for Analysis of DFRC Monomers", *J. Agric. Food Chem.* 45:2590–2592 (1997).
18. LU, F. and RALPH, J., "The DFRC Method for Lignin Analysis.1. A New Method for β-Aryl Ether Cleavage: Lignin Model Studies", *J. Agric. Food Chem.* 45:4655–4660 (1997).
19. BENDALL, M.R. and PEGG, D.T., "Complete, Accurate Editing of Decoupled Carbon-13 Spectra Using DEPT and a Quaternary-Only Sequence", *J. Magn. Reson.* 53:272–296 (1983).
20. DODDRELL, D.M., PEGG, D.T. and BENDALL, M.R., "Distortionless Enhancement of NMR Signals by Polarization Transfer", *J. Magn. Reson.* 48:323–327 (1982).
21. XIA, Z.C., AKIM, L.G. and ARGYROPOULOS, D.S., "Quantitative ^{13}C NMR Analysis of Lignins With Internal Standards", *J. Agric. Food Chem.* 49(8):3573–3578 (2001).
22. ALEN, R., "Structure and Chemical Composition of Wood" in Forestry Resources and Sustainability, J. Gullichsen and H. Paulapuro, Eds., Fapet Oy, Helsinki, Finland (2000).
23. CHEN, C.L., "Determination of Total and Aliphatic Hydroxyl Groups" in Methods in Lignin Chemistry, Ch. 7.1, 409–422, Y. Lin and C.W. Dence, Eds., Springer-Verlag, Berlin, Germany (1992).
24. LUNDQUIST, K., "Low-Molecular Weight Lignin Hydrolysis Products", Proc. Appl. Polym. Symp. John Wiley & Sons, Inc., New York, NY, USA 1393–1407 (1976).
25. ARGYROPOULOS, D.S., JURASEK, L., KRISTOFOVA, L., XIA, Z., SUN, Y. and PALUS, E., "Abundance and Reactivity of Dibenzodioxocins in Softwood Lignin", *J. Agric. Food Chem.* 50(4):658–666 (2002).
26. TOHMURA, S. and ARGYROPOULOS, D.S., "Determination of Arylglycerol-β-aryl Ethers and other Linkages in Lignins Using DFRC/^{31}P NMR", *J. Agric. Food Chem.* 49(2):536–542 (2001).
27. FERGUS, B.J. and GORING, D.A.I., "The Location of Guaiacyl and Syringyl Lignins in Birch Xylem Tissue", *Holzforschung* 24(H4):113–117 (1970).
28. NIMZ, H.H., ROBERT, D., FAIX, O. and NEMR, M., "Carbon-13 NMR Spectra of Lignins. 8. Structural Differences Between Lignins of Hardwoods, Softwoods, Grasses and Compression Wood", *Holzforschung* 35(1):16–26 (1981).
29. BARDET, M., FORAY, M.F. and ROBERT, D., "Use of the DEPT Pulse Sequence to Facilitate the ^{13}C NMR Structural Analysis of Lignin", *Makromol. Chem.* 186:1495–1504 (1985).

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