Journal of Wood Chemistry and Technology, 31: 324–344, 2011

ISSN 0277-3813 print / 1532-2319 online
DOI: 10.1080/02773813.2011.562338

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FT–Raman Investigation of Milled-Wood Lignins: Softwood, Hardwood, and Chemically Modified Black Spruce Lignins

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Abstract: Raman spectroscopy is being increasingly applied to study wood and other lignin-containing biomass/biomaterials. Lignin's contribution to the Raman spectra of such materials needs to be understood in the context of various lignin structures, substructures, and functional groups so that lignin-specific features could be identified and the spectral information could be interpreted usefully. Additionally, to enhance the utility of Raman as a characterization tool, an understanding of chemical-treatment-induced changes to the lignin spectrum is important. In the present work, Raman spectra of four milled-wood lignins (MWLs)—black spruce, loblolly pine, aspen, and sweetgum—were compared, and using black spruce MWL, spectral changes brought about by alkaline hydrogen peroxide bleaching, hydrogenation, acetylation, and methylation reactions were analyzed. The band intensity changes depended upon the nature of the chemical treatments.

Keywords: Raman spectroscopy, FT–Raman, lignin, milled-wood lignin, hydrogen peroxide bleaching, diimide hydrogenation, acetylation, methylation, black spruce, loblolly pine, aspen, sweetgum

INTRODUCTION

Wood and other lignocellulosics contain lignin. The structures of lignins are highly irregular and therefore difficult to characterize. Most detailed characterization of lignin has been carried out as MWL, which is its isolated state. It has been reported that only a few differences exist between native and MWL. For example, it is known that ball milling and duration of milling lead to generation of additional carbonyl groups in lignin.[1] Lack of detailed information

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on lignin structures has hindered progress in a number of science and technology fields. Areas as diverse as lignin biosynthesis, plant cell wall lignification, forage digestibility, delignification in pulping and bleaching, biodegradation of natural materials, genetic modification of plants, and biomass utilization (e.g., biofuels) are likely to benefit from an improved understanding of lignins at the molecular level. Over the years, numerous techniques have been applied to studying lignins.[2–4] Although a large number of techniques have been applied to investigate lignins in the solution state, only a few methods are suited for *in situ* analysis. Both Raman and infrared (IR) spectroscopies fall in the latter category. Compared with IR, Raman investigations of lignins are relatively recent, and the technique needs to be advanced further for lignin applications. Raman is complementary to IR and has the added advantage that it is insensitive to water. Wet samples of lignocellulosics are easily analyzed in Raman.

Over the last 25 years, various sub-techniques in the field of Raman spectroscopy have been applied to study lignin and lignin-containing materials.[5–36] Such techniques have included visible-Raman,[5–7] micro-Raman,[8–10] FT–Raman,[11–21] Raman imaging,[22–24] UV-resonance Raman,[25–34] surface enhanced Raman,[35] and coherent anti-Stokes Raman[36] spectroscopies. An overview of most of such applications was recently published.[37] In the early days, with excitation in the visible range, it was difficult to obtain Raman spectra of acceptable quality for lignin/lignocellulosics because of lignin auto-fluorescence. Although spectra could be obtained using special sampling techniques,[38] the situation was far from satisfactory. In 1986, an FT–Raman spectrometer based on near-IR excitation (1064 nm) was developed and became commercially available thereafter. This instrument overcame the obstacle of lignin auto-fluorescence. Once FT–Raman instruments became commercially available, the use of Raman spectroscopy in the field of wood and other lignocellulosics increased significantly. The only other method that successfully avoided lignin-fluorescence was ultraviolet-visible (UV-Vis) Resonance Raman spectroscopy.[25–33] To suppress fluorescence from highly fluorescent samples, the latter method was used in conjunction with an optical Kerr gate.[28,32]

Although the application of this technique to numerous research areas has already provided useful information, fundamental Raman information on lignins is still being developed. The present study is aimed at generating such basic information on selected softwood and hardwood lignins. An additional objective of the present investigation was to determine the nature of spectral changes when a softwood (black spruce) lignin is subjected to selected chemical treatments (bleaching by alkaline hydrogen peroxide, hydrogenation by diimide-after-borohydride, acetylation by acetic anhydride, and methylation by diazomethane). The treatments were selected because they are not only important lignin reactions but because the reactions have been used in research to solve important industrial problems. For example, whereas the bleaching by alkaline hydrogen peroxide has been used to brighten both chemical and mechanical pulps,[39,40] all four treatments have been used to limit the

propensity of mechanical pulps to rapidly undergo photoyellowing upon exposure to light.[12,40] In particular, esterification, etherification, and the hydrogenation of ring-conjugated ethylenic groups were reactions that were considered useful in photostabilizing mechanical wood pulps. Similarly, acetylation and methylation of the wood has been used to give wood improved photostability, dimensional stability, and decay resistance.[41,42]

EXPERIMENTAL

Sodium silicate solution was purchased from Wilkens-Anderson, Chicago, IL. All other chemicals (most >98% pure) were purchased from Sigma-Aldrich, Milwaukee, WI.

Isolation of Milled-Wood Lignins (MWLs)

Black spruce (BS, *Picea mariana*) MWL was isolated using the modified Bjorkman procedure outlined previously.[13] The MWLs from loblolly pine (*Pinus taeda*), sweetgum (*Liquidambar styraciflua*), and aspen (Populus tremuloides) were provided by the researchers at the Forest Products Laboratory, Madison, WI. These had been isolated using methods[43] similar to the Bjorkman method described in Agarwal and Ralph.[13]

Bleaching with Alkaline H_2O_2

The treatment procedure was similar to that of Castellan et al.[44] Black spruce MWL in THF was treated at 50°C with H_2O_2 (30%) and NaOH (1 N). Alkaline hydrogen peroxide treatment of MWL resulted in elimination of the coniferaldehyde signal at 194 ppm in ^{13}C NMR spectrum of the bleached sample (in dimethyl sulfoxide-d6). This is the location where γ-CHO in coniferaldehyde contributes.[45]

Diimide-hydrogenation

First 1 g of BS MWL was treated with 50 mL 0.5 M $NaBH_4$ for 2 h at room temperature. Afterward, the excess borohydride was destroyed by slowly acidifying the reaction suspension to pH 6 with concentrated HCl. After the treatment, the contents were filtered and the MWL was washed with plenty of water until the pH was neutral. The bleached sample was dried overnight in the hood. For hydrogenation, 52 mg of $NaBH_4$ bleached BS MWL was treated with 0.0312 mL of hydrazine hydrate and 2.1 mL of 0.5 N $CuSO_4$ in 60 mL of octane. The solution was magnetically stirred and air was sparged

through fritted glass. The reaction was carried out for 2 h and the excess octane decanted off. The latter was repeated with a fresh aliquot of octane. The settled precipitate was solubilized with acetone:water (9:1) and filtered. Solvent was removed with a roto-evaporator.

Acetylation

Acetylation of BS MWL was carried out with acetic anhydride: pyridine (1:1) for 7 h at room temperature. This was followed by precipitation of the product in water, filtration on a Spectra/Por UF membrane (ME cutoff 1,000,000), thorough washing with dilute hydrochloric acid and water, and freeze-drying. The acetylated MWL in acetone-d_6 was analyzed using ^{13}C nuclear magnetic resonance (NMR) to verify that the acetylated structures were present. Upon acetylation, new bands in the region 168 to 175 ppm were detected due to the acetate carbonyl carbon signals.[46] The phenolic and aliphatic hydroxyl groups in various lignin units are known to be acetylated under the conditions used for acetylating lignin.

Methylation

Black spruce MWL (140 mg) was dissolved in 10 mL dioxane:water (9:1) and the solution stirred magnetically. The flask was cooled in an ice bath. Diazomethane (10 mL) was added to the flask containing MWL. Three more additions of diazomethane, each at 2-h intervals, were made to the reaction solution. This was followed by precipitation of methylated MWL in water, filtration on a Spectra/Por UF membrane (ME cutoff 1,000,000), thorough washing with dilute hydrochloric acid and water, and freeze-drying. The methylated MWL in acetone-d6 was analyzed using NMR to verify that the carboxyl and phenol groups were methylated. Methylation resulted in an increase of signal intensity at 56 ppm relative to 148 ppm signal, indicating that Ar-OH groups were converted to Ar-OCH_3.

Raman Spectroscopy

A Bruker RFS-100 near-IR FT–Raman spectrometer (Bruker Instruments, Inc., Billerica, MA) was used to obtain the spectra. Samples were excited using a 1064-nm Nd:YAG diode laser. For most acquisitions, the sample was pressed into the aluminum well (sampling accessory) and 1024 scans were accumulated using 600 mW of unfocused laser power. Methylated BS MWL was sampled in a shortened NMR tube (another sampling accessory). Bruker's OPUS software was used to find the peak positions, carry out baseline corrections, and manipulate spectra. For display purposes, some spectra were shifted on the intensity

scale (y-axis). The spectral data were not processed in any manner other than baseline-corrected using OPUS software (Bruker Instruments, Inc., Billerica, MA).

Because the sample of methylated MWL contained some residual deuterated acetone (from NMR analysis), its Raman spectrum was obtained in solution. Nevertheless, none of the deuterated acetone bands could be detected in Raman spectrum of methylated BS MWL and therefore, the spectrum of the sample was free of any interfering solvent bands. All other MWLs were sampled in solid state.

Qualitative comparisons among spectra (band positions, band shapes) can be carried out without any difficulty. However, for quantitative changes between treated and control BS MWL sample spectra, the spectra have to be normalized using an internal reference band. Such normalization removes/minimizes the effect of experimental variables on the band intensities. To accomplish that, spectra were first corrected for baseline variation using the rubber band correction method (in OPUS software). This was followed by spectra normalization (of treated and untreated MWLs) using a band that remained unchanged upon treatment. The latter choice depended upon what the treatment was. For instance, for the hydrogen peroxide and diimide-after-borohydride treatments, the 2940 cm^{-1} band was a good choice. In contrast, for acetylated and methylated MWLs the aromatic C-H stretch band ($\sim$3070 cm^{-1}, Table 1) was selected. The latter was necessary because both acetylation and methylation produced significant changes to the intensity at 2940 cm^{-1}, and therefore, obviously, this band could no longer fulfill the requirement for selection. Peak-height intensities were calculated using OPUS software where appropriate integration methods (to draw baselines) were used to calculate peak-height intensities in the spectra. Percentage change in band intensity was calculated as follows.

$$\%\Delta \text{ Int.} = 100 * [(I_{\text{untreated}} - I_{\text{treated}})/(I_{\text{untreated}})]$$

Moreover, for quantitative comparison between the spectra of hardwood and softwood lignins, peak height of the 3070 cm^{-1} band (aromatic C-H stretch) in the hardwood MWL spectrum was made equal to 81% of 3070 cm^{-1} band intensity of the softwood MWL. This was based, in part, on the reported values of the syringyl/guaiacyl ratios for hardwood and softwood MWLs in Table 2. Average ratios of 1.48 and 0.94 were used for hardwood and softwood lignins, respectively. The calculation for the "81% multiplier" was carried out as follows. First, although in softwood MWLs a large percentage of guaiacyl units contain 3 aromatic C-H bonds per C9-unit,[47] there are considerable unit structures where that is not the case. Therefore, the accurate number of aromatic C-Hs per C9-unit was calculated by subtracting out, from 3, the sum of the fractions of those lignin structures that do not contain a 3 C-Hs/C9-unit.[47] However, each such fraction needed to be multiplied by a factor that reflected the extent to which the C-Hs/C9-unit concentration was lower. For instance,

Table 1. Raman spectra of milled-wood lignins—band positions (cm^{-1}), relative intensities[a], and assignments

Softwoods		Hardwoods		
Black spruce	L. pine	Aspen	Sweetgum	Assignment[b]
3071 m	3070 m	3068 m	3068 m	Aromatic C−H stretch
3008 sh	3008 sh	3003 sh	3003 sh	C−H stretch in OCH_3, asymmetric
2940 m	2940 m	2939 s	2941 s	C−H stretch in OCH_3, asymmetric
2890 sh	2887 sh	2893 sh	2893 sh	C−H stretch in R_3C−H
2845 m	2846 m	2847 m	2844 m	C−H stretch in OCH_3, symmetric
1662 s	1664 s	1661 s	1664 s	C=C coniferyl alcohol + C=O coniferaldehyde
1621 sh	1620 sh	1620 sh	1620 sh	C=C stretch of coniferaldehyde/ sinapaldehyde
1597 vs	1598 vs	1595 vs	1596 vs	Aromatic ring stretch, symmetric
1508 vw	1506 vw	1501 vw	1501 vw	Aromatic ring stretch, asymmetric
1453 m	1453 m	1455 s	1456 s	CH_3 bending in OCH_3
1430 w	1434 w	1426 w	1426 w	CH_3 bend + ring stretch
1392 sh	1393 sh	1395 sh	1393 sh	Phenolic O−H bend + CH_3 bend
1363 sh	1373 sh	1367 sh	1364 sh	C−H bend in R_3C−H
1334 m	1334 m	1331 s	1331 s	Aliphatic O−H bend
1298 sh	1298 sh	—[c]	—[c]	Aryl−O of aryl−OH and aryl−O−CH_3; C−C stretch of coniferyl alcohol
1272 m	1271 m	1272 m	1273 m	Aryl−O of aryl−OH and aryl−O−CH_3; guaiacyl/syringyl ring (with C=O group) mode
1226 vw	1222 vw	1224 w	1221 w	Aryl−O of aryl−OH and aryl−O−CH_3; guaiacyl/syringyl ring (with C=O group) mode
1192 w	1192 w	1190 w	1190 w	A phenol mode
—[c]	—[c]	1156 sh	1152 sh	Unassigned
1136 m	1140 m	1130 m	1130 m	A mode of coniferaldehyde/ sinapaldehyde
1089 w	1088 w	1088 w	1088 w	Out of phase C−C−O stretch of phenol
1033 w	1031 w	1037 m	1037 m	C−O of aryl−O−CH_3 and aryl−OH
975 vw	975 vw	984 sh	982 sh	CCH and −HC=CH−deformation
928 vw	929 vw	918 sh	911 sh	CCH wag
895 vw	895 vw	899 w	899 w	Skeletal deformation
811 sh	—[c]	—[c]	—[c]	Skeletal deformation
787 w	776 w	797 w	799 w	Skeletal deformation
731 w	731 w	727 w	737 w	Skeletal deformation
637 vw	635 vw	638 w	638 w	Skeletal deformation
—[c]	—[c]	597 m	598 m	Skeletal deformation
588 vw	593 vw	588 w	588 w	Skeletal deformation

Table 1. Raman spectra of milled-wood lignins—band positions (cm^{-1}), relative intensities[a], and assignments *(Continued)*

Softwoods		Hardwoods		
Black spruce	L. pine	Aspen	Sweetgum	Assignment[b]
557 vw	557 vw	—[c]	—[c]	Skeletal deformation
534 vw	543 vw	531 m	531 m	Skeletal deformation
—[c]	—[c]	522 sh	522 sh	Skeletal deformation
—[c]	—[c]	503 vw	503 vw	Skeletal deformation
491 vw	484 vw	490 vw	490 vw	Skeletal deformation
—[c]	—[c]	472 vw	472 vw	Skeletal deformation
457 vw	465 vw	461 vw	461 vw	Skeletal deformation
—[c]	—[c]	447 vw	447 vw	Skeletal deformation
—[c]	—[c]	431 vw	431 vw	Skeletal deformation
—[c]	—[c]	417 vw	417 vw	Skeletal deformation
384 w	384 w	—[c]	—[c]	Skeletal deformation
361 w	367 w	369 m	368 m	Skeletal deformation

[a]Note: vs is very strong; s is strong; m is medium; w is weak; vw is very weak; and sh is shoulder. Band intensities are relative to other peaks in the spectrum.

[b]Some of the assignments are tentative; assignments are based on the literature.[5,6,17,51,52]

[c]No corresponding band seen.

if instead of 3 C-Hs, 2 C-Hs were present in a lignin unit, then the latter factor was 1/3. Quantities of various spruce MWL structures are known from literature[47] and were taken to represent the amounts in softwood MWLs. From this publication, the main lignin unit types with less than 3 aromatic C-Hs/C9 were 7 (dibenzodioxocin), 11 (4-O-5′), and 12 (5–5′). Using this information, the number of aromatic C-Hs/C9 was calculated to be 2.90 for softwood MWLs. The next step was to come up with the number of aromatic C-Hs/C9 for the

Table 2. Methoxyl group/C9 aryl unit concentration of different milled-wood lignins

Wood ID	$-OCH_3$/C9
Black spruce	0.95[a]
Loblolly pine	0.93[b]
Average	**0.94**
Aspen	1.44[a]; 1.47[b]
Sweetgum	1.51[a]; 1.49[b]
Average	**1.48**

[a]Musha and Goring 1975.[50]

[b]Obst and Landucci 1986.[43]

hardwood MWLs and the latter was computed simply by subtracting 0.54 (the difference between the $-OCH_3/C_9$ groups between the hardwood and softwood MWLs (Table 2)) from 2.90. This number for hardwood MWLs was 2.36. Last, to get the multiplier (81%), the number of aromatic C-Hs in hardwood MWLs was divided by the number in softwood MWLs.

RESULTS AND DISCUSSION

MWLs Spectra

Raman spectra obtained from black spruce, loblolly pine, aspen, and sweetgum MWLs are shown in Figures 1–3 and peak positions are summarized in Table 1. To better visualize the spectral features, the three figures show different regions of the spectra: Figure 1, 2700–3200 cm^{-1}; Figure 2, 1350–1850 cm^{-1}; and Figure 3, 250–1450 cm^{-1}. Raman band assignments of lignins spectra exist in the literature, but some of the assignments are tentative.[17,35] Raman features seen in the spectra are almost entirely due to lignin because although present in small amounts, amorphous hemicellulose/cellulose[48] contribution is expected to be negligible.[13] The latter expectation is supported by the Raman data of

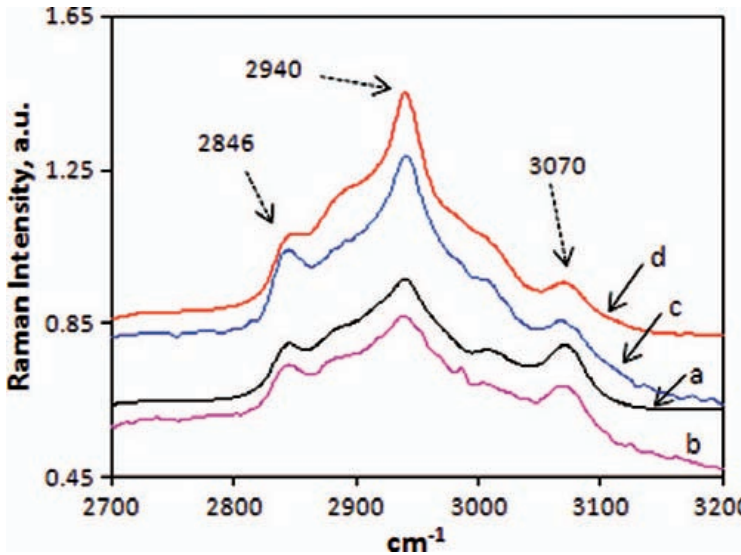

Figure 1. Raman spectra of milled-wood lignins (MWLs) in the region 2700–3200 cm^{-1}: (a) spruce; (b) loblolly pine; (c) aspen; (d) sweetgum. (color figure available online)

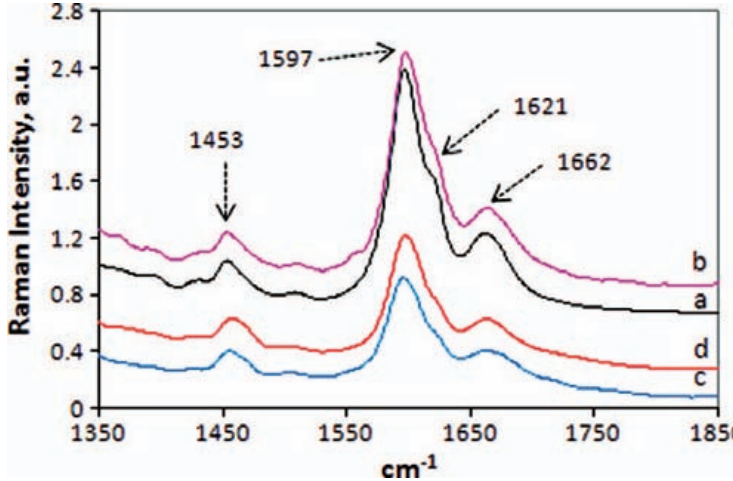

Figure 2. Raman spectra of milled-wood lignins (MWLs) in the region 1350–1850 cm^{-1}: (a) spruce; (b) loblolly pine; (c) aspen; (d) sweetgum. (color figure available online)

DHPs (dehydrogenation lignin polymers), where most of the bands detected[49] were similar to bands of MWLs (Table 1).

The band positions were very similar between the two softwood MWLs (Figures 1–3, a versus b; Table 1) and between the two hardwood MWLs

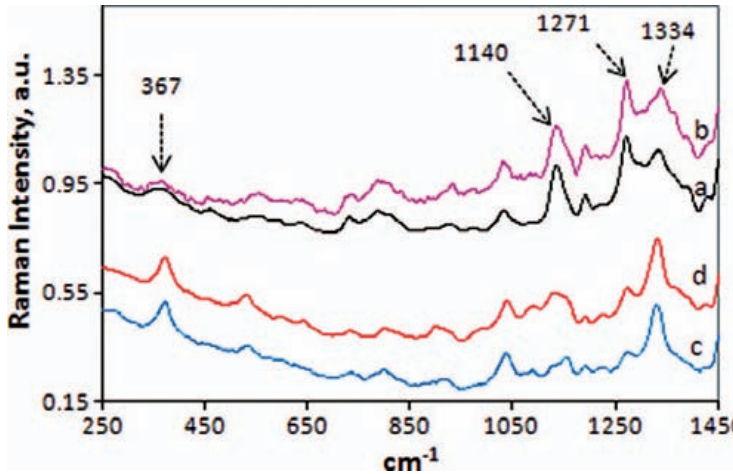

Figure 3. Raman spectra of milled-wood lignins (MWLs) in the region 250–1450 cm^{-1}: (a) spruce; (b) loblolly pine; (c) aspen; (d) sweetgum. (color figure available online)

(Figures 1–3, c versus d; Table 1). However, between the two classes of lignins (softwood versus hardwood), some differences were also present. For instance, 1298-, 557-, and 384-cm^{-1} bands present in the spectra of softwood lignins were absent in hardwood lignins' spectra. On the contrary, wavenumber positions of the bands that were only detected in the spectra of hardwood MWLs were 1156, 597, 522, 503, 472, 447, 431, and 417 cm^{-1}.

2700 to 3200 cm^{-1} Region

In lignin, both aliphatic and aromatic C-H stretches are expected to contribute in this region. In all cases, lignin bands were detected at approximately 2845, 2890, 2940, 3005, and 3070 cm^{-1}. The last band is due to the aromatic C-H stretch[17] whereas the remaining four contributions are due to aliphatic C-H stretching modes of groups, including those attached to the aromatic units. Of the aliphatic C-H stretch contributions in lignin, the 2940 cm^{-1} band is not only most prominent in the spectra of all lignins, but between the sets of softwood and hardwood MWLs, this band was more intense in the latter (Figure 1 and Table 1). This is explained by the fact that compared to softwood lignins, hardwood lignins contain higher amounts of aromatic methoxy groups (in the form of syringyl units). The $-OCH_3/C_9$-unit concentration data are listed in Table 2. Higher intensity at $\sim$2940 cm^{-1} in the spectra of hardwood MWLs is also supported by the FT–Raman data of DHPs (dehydrogenation polymer).[49] In that study, the authors found that compared to H- (p-coumaryl), HG- (p-coumaryl/guaiacyl), G-DHPs GS- (guaiacyl/syringyl), and S-(syringyl) DHPs gave more intense $\sim$2940 cm^{-1} band.

1350 to 1850 cm^{-1} Region

Table 1 lists eight Raman frequencies in this region. The region 1350–1850 cm^{-1} (Figure 2) is the most important region for lignin contributions as most group frequencies are detected here.[17,18,51] The 1500–1800 cm^{-1} interval contains bands due to aromatic rings, ring conjugated ethylenic C=C, α– and γ−C=O, and *o*- and *p*-quinones. Earlier work with black spruce wood,[13] softwood mechanical pulps,[12,20] and lignin models[18,49] was useful in assigning the 1662 cm^{-1} band to the ethylenic C=C bond in coniferyl alcohol units and γ−C=O in coniferaldehyde units in lignin. Similarly, the 1621 cm^{-1} band was found to be associated with the ring-conjugated C=C bond in coniferaldehyde units.[12,20,21] In hardwood lignins, 1661 and 1620 cm^{-1} bands would have additional contributions from sinapyl alcohol and sinapaldehyde units, respectively. Two of lignin's aromatic ring stretch modes were detected at 1597 and 1508 cm^{-1}. The intensity of the 1508 cm^{-1} band is very weak (Figure 2, Table 1), albeit somewhat higher for hardwood MWLs. In any case, this band

is not easily detected. Several bands in the rest of the region may represent mixed vibrations, as many of the modes are coupled. Also, there is likely to be overlap because of CH_3, CH_2, and CH bending modes. Furthermore, more complexity arises from specific aromatic ring vibrations that are expected to contribute here as well.

As was the case for the C-H stretch region, similarity existed between the spectra of two softwood MWLs (Figure 2). This was also the case for the hardwood lignins. However, noteworthy between softwood and hardwood sets is that for the latter, the intensity of the ~1597 and 1662 cm^{-1} bands was significantly lower compared with the spectra of softwood MWLs (Figure 2c and 2d versus Figure 2a and 2b). The spectra in Figure 2 have been normalized on the ~3070 cm^{-1} band after factoring in the $-OCH_3/C_9$-unit concentration difference (Table 2). One plausible interpretation is that the concentration of aromatic ring-conjugated structures is different between the two types of lignins. In this regard, it is important to note that the lead author of the present study previously reported finding that ring-conjugated structures significantly impact the intensity of the benzene-ring mode at 1600 cm^{-1} in lignin models.[5,17,18,51] Further support for this interpretation comes from the presence of comparatively weaker bands at 1622 and 1662 cm^{-1} in the spectra of hardwood MWLs. Contributions from coniferaldehyde/sinapaldehyde units are expected at 1622 and 1662 cm^{-1},[5,21] whereas coniferyl/sinapyl alcohol contribute at 1662 cm^{-1}.[5,21] Coniferaldehyde/sinapaldehyde and coniferyl/sinapyl alcohols have molecular structures that have aromatic-ring conjugated bonds and such conjugation is known to affect the intensity of the 1600, 1622, and 1662 cm^{-1} bands.[5,17,51]

For hardwood MWLs (Figure 2c and 2d), significant contribution from unconjugated carbonyl groups could not be detected in the frequency range 1715–1750 cm^{-1}. Usually such groups in hardwood MWLs have been detected in infrared spectroscopy (IR),[52] although the band intensities have been low. The explanation for this is that unconjugated carbonyl groups have low sensitivity in Raman and therefore their contribution remains undetected.

250 to 1350 cm^{-1} Region

Like before, there is great similarity between the Raman features of the same class of lignins (Figure 3, Table 1). Nevertheless, the two lignin sets, softwood and hardwood, show some differences. Compared with the ~367 cm^{-1} band for aspen and sweetgum (both hardwood) MWLs (Figure 3c and 3d), the softwood lignins' 367 cm^{-1} band was broader and significantly weaker (Figure 3a and 3b). This behavior of intensity enhancement was also observed for hardwood MWL bands at 531, 1037, and 1331 cm^{-1}. On the other hand, the band intensities at 1140 and 1271 cm^{-1} were higher for softwood lignins (Figure 3). A more intense 1140 cm^{-1} contribution is likely to arise from higher concentration of

cinamaldehyde structures (coniferaldehyde/sinapaldehyde units) because these structures have a contribution at about this wavenumber position.[12,21,51]

Chemical Treatment-Induced Spectral Changes

Raman spectra of control/untreated and chemically modified BS MWLs—bleached (alkaline hydrogen peroxide), hydrogenated (diimide treatment of sodium borohydride treated BS MWL), acetylated (pyridine and acetic anhydride), and methylated (diazomethane)—were compared to determine what spectral changes occurred after each treatment. The subtracted spectra (untreated and treated) are shown in Figure 4 (2500–3500 cm^{-1}) and Figure 5 (250–1850 cm^{-1}). Table 3 lists Raman frequencies of bands detected in the spectra of treated MWLs along with decline in peak intensity (% Δ Int.) for bands that were most intense in the spectrum of untreated MWL. As a result of most treatments, the intensities of the majority of bands declined, except for acetylation and methylation, where some bands showed the opposite behavior and intensity was enhanced. An additional point is that new bands seen in the spectra are listed in Table 4.

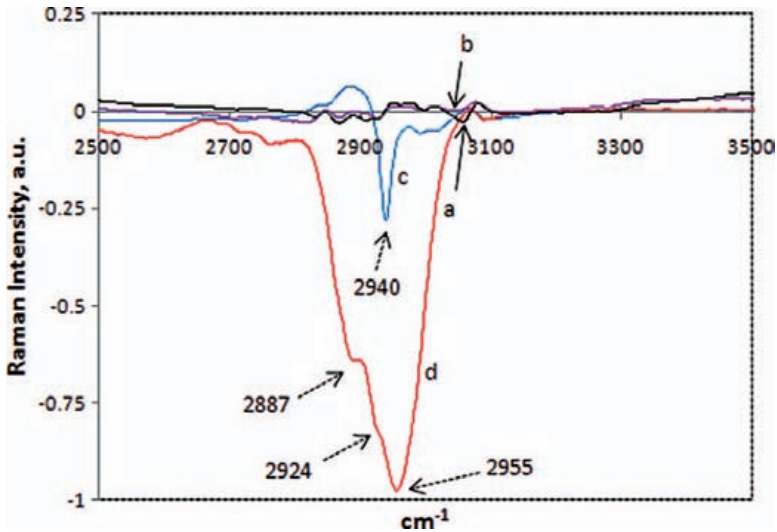

Figure 4. Subtracted Raman spectra of spruce milled-wood lignins (MWLs) in the region 2500–3500 cm^{-1}: (a) untreated minus H_2O_2 bleached; (b) untreated minus hydrogenated after borohydride; (c) untreated minus acetylated; and (d) untreated minus methylated. (color figure available online)

Table 3. Treated black spruce milled-wood lignins (MWLs)—Raman frequencies (cm^{-1}) and change in peak-intensity of most intense untreated MWL bands

Untreated	H_2O_2 bl. (% Δ Int.)	Hydro. (% Δ Int.)	Acet. (% Δ Int.)	Methy. (% Δ Int.)
3071 m[a]	3070 (sc[b])	3065 (sc)	3073 (na)	3071 (na)
3008 sh	3008 (sc[b])	3002 (sc)	3010 (nc)	3008 (nc)
2940 m	2940 (na[c])	2937 (na)	2939 (−36)	2940 (−307)
2890 sh	2883 (sc[b])	2886 (sc)	—[f] (100)	2890 (−298)
2845 m	2846 (nc[d])	2843 (nc)	2846 (sc)	2845 (nc)
1662 s	1656[e] (86)	1650[e] (100)	1674[e] (53)	1662 (94)
1621 sh	—[f] (100)	—[f] (100)	1622 (61)	1621 (100)
1597 vs	1602 (67)	1605[e] (73)	1600[d] (59)	1597 (65)
1508 vw	1508	—[f]	1508	1508
1453 m	1453 (35)	1453 (51)	1454 (49)	1453 (−111)
1430 w	1429	1435[e]	1426	1430
1392 sh	—[f]	—[f]	1391	1392
1363 sh	1360	1351[e]	1367	1363
1334 m	1334 (69)	1334 (57)	1335 (52)	1334 (50)
1298 sh	—[f]	—[f]	1300	1298
1272 m	1271 (79)	1273 (64)	1274 (58)	1272 (58)
1226 vw	1222	1224	1222	1226
1192 w	1193 (33)	1190 (50)	1195 (36)	1192 (38)
1136 m	1135 (88)	—[f] (92)	1129[d,e] (58)	1136 (78)
1089 w	1079[d,e]	—[f]	1089	1089
1033 w	1032 (35)	1032 (47)	1034 (54)	1033 (−84)
975 vw	—[f]	—[f]	972	975
928 vw	927	929	936[d,e]	928
895 vw	884[d,e]	895	907[d,e]	895
811 sh	—[f]	811	—[f]	811
787 w	781[e] (46)	789 (34)	792[e] (70)	787 (54)
731 w	731 (41)	731 (14)	730 (44)	731 (−67)
637 vw	638	641	641	637
588 vw	—[f]	—[f]	607[e]	588
557 vw	562[e]	561	548[e]	557
534 vw	535	—[f]	531	534
491 vw	484[e] vw	—[f]	490	491
457 vw	458	465[e]	461	457
361 w	368[e] (42)	370[e] (51)	366 (60)	361[e] (73)

[a]Note: vs is very strong; s is strong; m is medium; w is weak; vw is very weak; and sh is shoulder. Band intensities are relative to other peaks in spectrum.

[b]sc = Small change in intensity.

[c]na = Not applicable because this band is expected to be minimally impacted and was used in spectra normalization.

[d]nc = No change in intensity.

[e]Band shifted.

[f]Not detected.

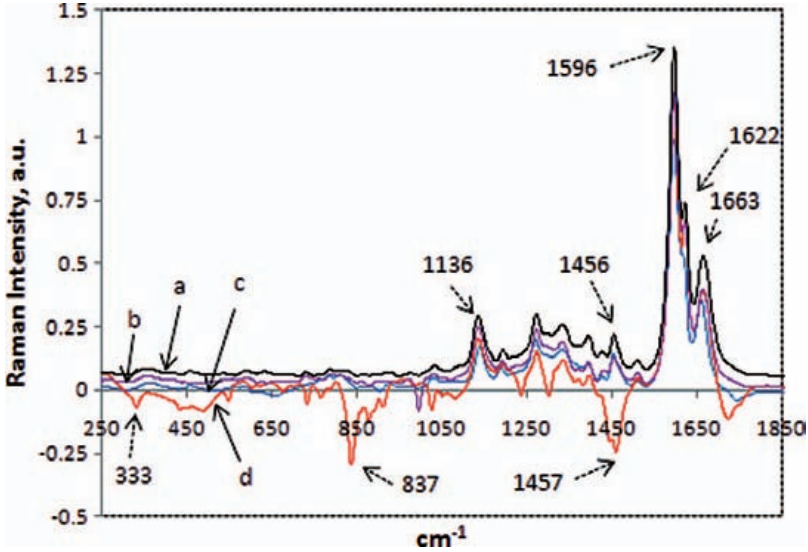

Figure 5. Subtracted Raman spectra of spruce milled-wood lignins (MWLs) in the region 250–1850 cm^{-1}: (a) untreated minus H_2O_2 bleached; (b) untreated minus hydrogenated after borohydride; (c) untreated minus acetylated; and (d) untreated minus methylated. (color figure available online)

Alkaline Hydrogen Peroxide Bleaching

Although there was not much change in the C-H stretch region (2500–3500 cm^{-1}, Figure 4a), significant changes in the intensity of most bands occurred in the region of functional groups and skeletal modes upon alkaline hydrogen peroxide bleaching (250–1850 cm^{-1}; Figure 5a, and Table 3, column 2). Based on Table 3, hydrogen peroxide bleaching caused intensity reductions in the range of 33 to 100%. Because this lignin-retaining bleaching primarily affects chromophore structures in lignin, such intensity decline implied that chromophore structures were responsible for significant intensity contributions in the Raman spectrum of unbleached MWL (spectra "a" in Figures 1–3). Most significant changes were at positions where coniferaldehyde structures contributed; for example, 1621 cm^{-1} completely disappeared, whereas 1662, 1597, and 1136 cm^{-1} peak-heights declined by 86, 67, and 88%, respectively (Table 3). This bleaching-related effect on band intensities has previously been reported.[6, 7] Additionally, contributions in the range 1660–1690 cm^{-1} due to *p*-quinones were also removed.[18,20]

Moreover, considering that chromophores-units/groups contain aromatic ring-conjugated bonds, these units contribute significantly to the intensity at wavenumbers where other lignin unit vibrations contribute. The dependence

Table 4. New Raman bands[a] (cm^{-1}) in treated black spruce milled-wood lignins (MWLs)

H_2O_2 bl.	Hydro.	Acet.	Methy.
—	—	—	2887 vs
—	—	1770 w, sh	1751 w
—	—	1742 w	1720 m
—	—	—	1303 m
—	—	—	1237 m
1156 w[b]	1154 sh[b]	1156 sh	—
—	—	—	1082 m
—	—	1015 sh	—
—	—	907 m	912 m
882 w	—	—	879 m
—	—	848 w	837 s
—	—	—	766 m
—	—	666 sh	—
620 m	—	605 sh	—
—	—	—	548 m
—	—	—	333 m

[a]Note: vs is very strong; s is strong; m is medium; w is weak; and sh is shoulder. Band intensities are relative to other peaks in spectrum.

[b]The band may have been hidden in the band profile of 1,135 cm^{-1} band in the spectrum of untreated MWL.

of intensity on ring-conjugation is a well recognized phenomenon in the field of Raman spectroscopy[53] and has been shown to exist to varying degrees for lignin models in FT–Raman spectroscopy.[51] The extent to which the conjugation effect is induced depends upon the molecular structure of a model, although in the spectrum of bleached BS lignin where intensities were significantly diminished, most Raman bands were still detected (Table 3). A few bands experienced shifts because the environments of their respective contributors were altered. The foregoing indicated that the structure of spruce lignin was not altered significantly and most of the originally present lignin sub-structures were still present. Additionally, the presence of a band at 1135 cm^{-1} in the spectrum of bleached MWL suggested the likelihood of lignin structural units other than coniferaldehyde also contributing at this wavenumber.

There were only three weak- to medium-intensity features that could be considered as new (Table 4). However, it is not clear what could have given rise to their occurrence because no new groups were added upon alkaline peroxide bleaching. On the other hand, it is possible that the bleaching resulted in unmasking of features that were previously hidden under a neighboring band. This notion gets further traction considering that, at least for the 1156 cm^{-1} band, the unmasking occurred also during hydrogenation and acetylation (Table 4).

It is unlikely that a new band at the same wavenumber position will arise for three different treatments.

Diimide Treatment (Hydrogenation)

Because total hydrogenation treatment consisted of both the borohydride and diimide treatments, the changes in the spectra are the sum total of changes brought about by each treatment individually. From the published studies of borohydride brightened mechanical pulps,[12] it is known that chromophores in pulps are modified upon reductive bleaching with $NaBH_4$. As was the case with alkaline H_2O_2 bleaching and on the basis of Table 3, which shows evidence for intensity reduction at 1662, 1621, 1597, and 1336 cm^{-1}, Raman features of chromophores are affected. The additional treatment by diimide was designed to hydrogenate the coniferyl alcohol type C=C bonds (they scatter at ~1650 cm^{-1}[5, 6,21]). That the 1662 cm–^{1} band disappeared completely (100% decline in Table 3, column 3) after combined treatment is evidence for successful hydrogenation of C=C bonds in coniferyl alcohol units. This is further supported by the observation that the 1662 cm^{-1} band declined more compared with alkaline peroxide bleaching (by 14%, Table 3 and Figure 5b). In the photoyellowing studies of mechanical pulps, such evidence was used to ensure that ring-conjugated groups were fully hydrogenated.[12] A consequence of the diimide treatment was that the aromatic ring-stretch mode of lignin had shifted to 1605 cm^{-1} (Table 3). This is likely to be due to modification/removal of units that contributed at 1600 cm^{-1} or lower wavenumbers. Comparing the effects of the two treatments (hydrogen peroxide versus hydrogenation) on other bands in the spectra, the bands that showed similar intensity changes were 3071, 3008, 2890, 2845, 1621, 1597, 1135, and 368 cm^{-1} (Table 3). In contrast, compared with hydrogen peroxide, the bands at 1453, 1190, and 1032 cm^{-1} declined more upon MWL hydrogenation, whereas peaks at 1273, 789, and 731 cm^{-1} declined less. At present, the reasons for this characteristic are not clear but may have to do with the treatment-specific chemistries involved.

As a consequence of the diimide-after-borohydride treatment, the only new feature was a shoulder at 1154 cm^{-1} (Table 4). Nevertheless, it is possible that this band existed already in the spectrum of untreated MWL and was hidden under the band profile of the stronger band at 1136 cm^{-1}.

Acetylation

The effect of acetylation on the C-H stretch region is shown in Figure 4c. It can be easily noted that acetylation resulted in the disappearing of the band at 2890 cm^{-1} (100% decline, Table 3). On the contrary, the 2940 cm^{-1} band showed a significant enhancement (36% increase, Table 3). The increased

contribution at the latter band position is due to the C-H stretches present in the acetoxy groups in acetylated lignin. It has been shown that this band could be used for quantitative monitoring of the acetylation reaction in lignin and lignocellulosics.[17]

In the 250–1850 cm^{-1} region (Figure 5c), the band intensities declined at 1662, 1621, 1597, 1453, 1334, 1272, 1192, 1136, 1033, 787, 731, and 361 cm^{-1}. The decline was significant (36 to 100%, Table 3) and the intensity decline behavior was similar to that previously observed in the spectra of bleached and hydrogenated- (hydrogen peroxide- and diimide-after-borohydride-) treated MWLs. Some bands were better resolved in the spectrum of acetylated MWL (e.g., 557 and 787 cm^{-1}), and acetylation caused some weak but distinct peaks to emerge (Figure 5c).

Two new but weak features at 1770 and 1742 cm^{-1} (Table 4) are due to the acetyl carbonyl groups. These contributions have been detected before in the IR spectrum of acetylated MWL,[52] where they were assigned to the aromatic and aliphatic acetoxy groups, respectively. Although weak, the relative band intensities at 1770 and 1742 cm^{-1} probably reflect the concentration of aromatic and aliphatic −OH groups in BS MWL. The band at 1662 cm^{-1} present in the spectrum of the untreated MWL was found to have shifted to 1674 cm^{-1}. The shift is caused by the lack of hydrogen bonding ability of the acetylated lignin units. This interpretation is supported by the solvation studies of coniferaldehyde where shifts were found to depend on the nature of the environment/solvent.[54]

Contrary to the case of bleached and hydrogenated MWLs, the acetylated lignin spectrum showed a number of new spectral features at 1156, 1015, 907, 848, 666, and 605 cm^{-1} (Table 4). These features are likely to arise either due to acetoxy derivatization or unmasking of the hidden bands (present in unacetylated MWL spectrum).

Methylation

The C-H stretch region changes are shown in Figure 4d. There are very significant increases in the intensities of bands at 2845 and 2940 cm^{-1} (symmetric and asymmetric C-H stretches, respectively). Such enhancements imply that a large number of hydroxyl groups were successfully methylated. In spruce MWL, such groups are likely to be both in lignin and residual hemicelluloses. In the 250–1850 cm^{-1} frequency region, as was the case with acetylation, methylation also resulted in significant changes in band profiles and appearance of new features (Figure 5d, Tables 3 and 4). Significant intensity reductions at 94, 100, 65, and 78% at 1662, 1621, 1597, and 1136 cm^{-1}, respectively (Table 3), implied that methylation treatment and/or reaction conditions resulted in modifying/removing most of coniferaldehyde structures. On the other hand, the intensity at 1453 and 731 cm^{-1} increased (Table 3 and Figure 5d).

The appearance of two carbonyl peaks at 1720 and 1751 cm^{-1} (Table 4) is not expected and may have something to do with the side reactions of the methylation treatment. Moreover, as mentioned in the Experimental section, these bands were not due to residual deuterated acetone. New bands also appeared at 2887, 1303, 1237, 1082, 912, 879, 837, 766, 548, and 333 cm^{-1}.

CONCLUSIONS

Most of the Raman spectral features were common between hardwood and softwood milled-wood lignins (MWLs). However, small as well as significant differences between the intensities of specific bands were present. Additionally, important changes occurred in the Raman spectrum of black spruce MWL when it was chemically modified, and these depended upon the nature of the treatment. For a number of bands, changes in band intensities were quantified. Both acetylation and methylation treatments produced large changes in the aliphatic C-H stretch region and gave rise to several new bands. That was not the case for alkaline-hydrogen peroxide and hydrogenation treatments. For the most part, spectral changes were successfully interpreted in terms of chemical-treatment induced structural changes in lignin.

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