Characterization and Comparison of Acetosolv and Milox Lignin Isolated from Crofton Weed Stem

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ABSTRACT: Crofton weed (*Eupatorium adenophorum* Spreng) is an invasive species in China, which has damaged native ecosystems and caused great economic losses. To use the weed instead of simple control and management of it, the weed stem of was delignified by Acetosolv and Milox processes to obtain lignin. The lignins (Acetosolv lignin, AL; and Milox lignin, ML) were characterized and compared through several analysis methods. It was found that both of AL and ML were syringyl-guaiacyl type (GS) lignin, but that the chemical composition and structure of the two lignins were somewhat different. The obtained C_9 expanded formulas for AL and ML were $C_9H_{9.16}O_{3.50}N_{0.13}(OCH_3)_{0.97}(Ph{-}OH)_{0.34}Ac_{0.22}$ and $C_9H_{7.96}O_{3.13}N_{0.13}(OCH_3)_{1.23}(Ph{-}OH)_{0.51}Ac_{0.14}$, respectively. According to an elementary analysis, the two lignins had similar higher heat value of 20.0–21.0 MJ kg^{-1}, which were similar to that of raw coal. Gel permeation chromatography analysis indicated the two lignins had similar molecular weights. Notwithstanding that the Ultraviolet, Fourier transform infrared, and ^{1}H-NMR spectra illustrated that the two lignins had similar chemical functional groups, the AL showed more acetyl groups signals. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 1295–1302, 2009

Key words: FTIR; gel permeation chromatography (GPC); NMR; Crofton weed; lignin characterization

INTRODUCTION

Crofton weed (*Eupatorium adenophorum* Spreng) is a tufty, semishrubby, perennial herbaceous plant with the ability to adapt to different environmental conditions. It is native to Central America, mainly Mexico; but is now, however, widely distributed in the United States of America, Australia, New Zealand, and many countries in Southeast Asia.[1] It is reported that this weed has caused thereat to these countries.[2] Many farmlands, pasture fields, and forests have been destroyed by the weed. Since the 1950s, this exotic plant has spread rapidly across the Southwest of China, damaging native ecosystems and causing great economic losses.[3] Much attention has been paid to the management of this weed, and several methods have been developed to control it, including manual, chemical and biological control. Nevertheless, no obvious progress has been achieved. The weed is still spreading from Southwest to Northeast at a rate of 10 to 60 km per year in China.[4]

A promising way to control this weed is to exploit it. In our previous work, a potential pathway was proposed by using it as a feedstock for the production of fermentable sugars and further production of fuel ethanol.[5] However, this proposal was only based on the utilization of the stem's carbohydrates. Being similar to most of cellulosic biomass, the stem of the weed mainly consists of cellulose, hemicellulose, and lignin, all of which are natural polymers. As a byproduct of pulping process, lignin has many potential applications, such as being used for the production of absorbents, fuels, dispersants, polymer substitutes, among others.[6] As a polymer substitution, lignin has been used as macromonomer for synthesis of polyurethane,[7,8] lignophenolic resins,[9,10] resins for wood adhesives,[11] and others.[6] In conventional pulping process (Kraft pulping), lignin is always used as fuels for compensating of energy consumption. During last decades, to overcome the existing problems of Kraft pulping, some pulping processes using organic solvents for delignification known as "organosolv pulping" have been developed.[12] One of the most important advantages of organosolv pulping is its remarkable byproduct recovery potential. The chemical recovery process in organosolv pulping allows isloating lignin as a solid materials and carbohydrates as syrup, both of which display promising chemical feedstocks properties.[13] It seems that organosolv pulping is also a biomass-refining process, which separates lignocellulosic biomass into cellulose fibers, lignin, and syrup. Therefore, in this work, two well-known organosolv pulping processes, namely Acetosolv and Milox processes, which were developed by Nimz et al.[14] and Poppius-Levlin et al.,[15] respectively, were used for isolating lignin from crofton weed

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stem. In particular, the biomass was delignified with acetic acid and formic and performic acid, respectively, under atmospheric pressure at the solvent's boiling point temperature.

The characteristics of isolated lignins depend both on the kind of vegetal material (species, geographic location, etc.) and on the nature of the delignification process. Herbaceous straw lignins are always very different from woody species' lignins. Straw lignins are composed of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) monomeric units, whereas wood lignins are composed mainly of either G or G and S units.[14] However, so far as we know, no article referring to lignin isolated from crofton weed stem has been published. In this work, the Acetosolv lignin (AL) and Milox lignin (ML) were precipitated from the pulping liquors, followed by being purified and analyzed with several chemical and instrumental analysis techniques. This work, thus, can provide some basic data for further studying the lignin of the weed stem, and might serve as a step for utilization and control of this weed.

EXPERIMENTAL

Materials

Crofton weed was obtained from Lincang city, Yunnan province in Southwestern China. Its stem was air dried, crushed, and screened. The fraction collected between 20 and 40 mesh was used for all the experiments. According to our previous work,[5] the chemical compositions of the weed stem were determined to be 4.52% ash, 18.16% cold-water extractives, 18.12% hot-water extractives, 47.54% 1%-NaOH extractives, 8.86% benzene-ethanol extractives, 37.14% cellulose, 66.22% holocellulose, and 16.42% Klason lignin. Each datum was the average of at least duplicate tests.

Preparation of AL and ML

AL

The weed stem was delignified with 93% (v/v) acetic acid with 0.1% (w/w of liquid phase) HCl as catalyst at boiling point temperature (107°C) for 3 h. The liquid-to-solid ratio was selected as 10 : 1 (v/w). After cooking, the spent liquor was filtered out and concentrated under reduced pressure at 60°C. Then, seven volumes of water were poured into the concentrated spent liquor to precipitate the lignin. The crude Acetosolv lignin was separated by centrifugation and washed with deioned water and lyophilized.

ML

The two-stage Milox delignification process of crofton weed stem was carried out at boiling point temperature (105°C) with 88% (w/w) formic acid at a liquid-to-solid ratio of 10 : 1 (v/w) for 1.5 h in the first stage; and a 2% (based on raw materials) H_2O_2 solution was added for further delignification at 80°C for 3 h in the second stage of reaction. The subsequent procedure for separation of lignin from spent liquor was the same as that of AL isolation.

Lignin purification

The lignin purification procedure was carried out according to Pan's methodology.[16] The crude lignin was dissolved in a 90% (v/v) acetic acid solution and then 10 volumes of water were added. Precipitated lignin was centrifuged off, washed, and lyophilized. The obtained lignin was dissolved in 1,4-dioxane-ethanol (8 : 2, v/v) and the precipitate was removed by centrifugation. The supernatant was added drop by drop to five volumes of diethyl ether-petroleum ether (1 : 1, v/v) while stirring. The precipitate was centrifuged off, washed two times with diethyl ether-petroleum ether (1 : 1, v/v), and finally lyophilized.

Lignin acetylation

Lignins (0.3 g) were dissolved in 1.5 mL of pyridine, before adding 1.5 mL of acetic anhydride. The mixture was left for 72 h at room temperature and then poured into 30 mL of ice water with stirring. The precipitate was filtered out, washed with water, and finally lyophilized.

Elementary analysis and function groups

C, H, and N analyses were carried out in the Department of Thermal Engineering, Tsinghua University, using the CE440 Elementary Analyzer (EAI, Chelmsford, MA).The accuracy of analysis was ±0.15% absolute plus ±0.15% relative. The oxygen content was estimated based on the assumption that the samples only contain the C, H, N, and O elements. The sample's higher heating value (HHV) was calculated according to the Dulong formula[17]:

$$\mathrm{HHV(MJ/kg)} = 0.3383 \times \mathrm{C} + 1.442 \times (\mathrm{H} - \mathrm{O}/8) \quad (1)$$

where H and O represent the weight percentages of carbon, hydrogen, and oxygen, respectively.

The methoxyl contents in AL and ML were determined with the modified Viebock-Schwappach method.[18] The content of phenolic hydroxyl group was determined by employing ultraviolet (UV) spectroscopy technique (Δε method). This method is based on the difference of absorption of phenolic units at 300 and 360 nm in neutral and alkaline solutions.[19] The content of acetyl group (Ac) was determined according to NREL LAP-017 methodology.[20]

TABLE I
Lignin Yield and Contents of Some Components in the Treated Solid Obtained by Acetosolv and Milox Pulping Processes

Pulping process	SR (%)	Contents of some compositions in the treated solid (%)					DD (%)	LY (g/g)
		Glucan	Xylan	Klason lignin	Ac	For		
Acetosolv	61.15	52.09	19.83	16.76	4.91	0.33	37.23	0.040
Milox	41.0	70.88	4.25	18.69	0.64	3.25	60.82	0.101

SR, solid recovered; Ac, acetyl group; For, formyl group; DD, degree of delignification; LY, lignin yield per gram of raw materials.

Analysis of lignin and carbohydrates

The carbohydrates content of the lignin were determined as monosaccharides after hydrolysis according to the NREL LAP-002 methodology (Determination of Structural Carbohydrates and Lignin in Biomass).[20] Acid-soluble lignin was determined according to the procedure described in NREL LAP-004 protocol.[20] The content of protein was calculated according by multiplying the nitrogen content by 6. Klason lignin was calculated by deduction of contents of polysaccharides, acid-soluble lignin and protein.

Molecular weight

Average molecular weights of the acetylated lignin samples were estimated by gel permeation chromatography (GPC) on an HPLC system (Shimadzu, Japan). The columns were calibrated with authentic polystyrenes. Tetrahydrofuran was used as eluent at a flow rate of 0.7mL/min.

Spectroscopy

UV

The spectra of the lignins in dioxane and alkaline solution were recorded. A measure of 8 to 9 mg of lignin were dissolved in 5 mL of dioxane-water solution (9 : 1, v/v) followed by a 50-fold dilution. Similarly, 6–7 mg lignin were dissolved in 5 mL pH = 12 NaOH solution followed by a 50-fold dilution. Subsequenlty, the spectra were recorded using a UV8500 spectrophotometer (Techcomp Limited, Shanghai, China).

Fourier transform infrared (FTIR)

IR spectra were recorded by using a NICOLET 560 FTIR spectrometer (Nicolet Company, Madison, WI). The dried samples were embedded in KBr pellets with an approximate concentration of 1 mg/100 mg KBr. The spectra were recorded in the absorption band mode in the range of 4000–400 cm^{-1}.

^{1}H-NMR

Spectra were recorded for solutions about 10 mg of acetylated lignin contained in 1 mL $CDCl_3$. Tetramethylsilane was used as an internal standard using a JOEL JNM–ECA600 spectrometer (JOEL, Japan).

RESULTS AND DISCUSSION

Lignin yields and components of treated solid

Table I gives the lignin yields and the contents of some components in the treated solids obtained by Acetosolv and Milox pulping processes. It can be seen that more solid was dissolved in Milox process. Glucan was the most component for both of the Acetosolv-treated and Milox-treated solids, whereas Acetosolv-treated solid contained more xylan and acetyl group. Milox process caused much more dissolve of xylan and lignin. It can also be seen that the Milox-treated solid had much higher formyl group content, indicating the happening of formylation during Milox pulping. It can also be known that Milox process had much higher degree of delignification and lignin yield, which illustrated that Milox process was more suitable for delignification of the weed stem.

Elementary analysis and functional groups of Acetosolv lignin and Milox lignin

The results of the elementary analysis of functional groups of AL and ML are shown in Table II. It can be seen that the two lignins had similar C, H, O,

TABLE II
Elementary Analysis and Functional Groups of AL and ML Isolated from Crofton Weed Stem

Lignin	Elemental analysis and functional groups (% of lignin)							HHV(MJ/kg)
	C	H	O	N	OCH_3	Ph—OH	Ac	
AL	58.23	5.22	35.62	0.86	14.03	2.73	4.36	20.8
ML	59.12	4.77	35.26	0.85	17.90	4.07	2.76	20.5

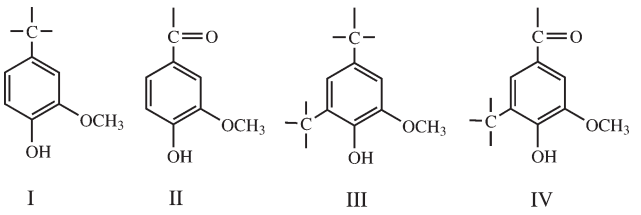

Figure 1 Models of Ph—OH in lignin.

and N contents, but ML contained more methoxyl (OCH_3) and phenolic hydroxyl (Ph—OH) groups. The nitrogen content was mainly derived from the protein-lignin complexes formed during delignification.[20] The two lignins had similar HHV to that of raw coal ($\sim$ 20.9 MJ kg^{-1}) and about 1/2 that of crude oil ($\sim$ 41.9 MJ kg^{-1}). However, compared with acetic acid lignin isolated from birch (BL) and fir (FL), the C, H, OCH_3, and Ph—OH contents in these two lignins were relatively lower, whereas the O and N contents were higher.[21] Formyl group was also found in the two lignins, with their contents in AL and ML being 0.43% and 0.64%, respectively, which were much lower than those of acetyl group. However, ML contained a litter higher formyl group than AL, probably because of the introduction of this group during formic acid delignification. Nevertheless, the experimental results indicated that the formylation action of lignin was not significant during Milox process.

It is interesting to find that ML had higher methoxyl group content. Actually Milox is an oxidative process and causes demethylation. The lower methoxyl group content in AL might be due to the introduction of acetyl group, which increased the molecular weight of per C_9 unit. On the other hand, the result indicted that no significant demethylation of lignin happened during Milox process in this study, because the H_2O_2 loading is relatively low, but more details should be investigated, especially for studying the properties of MWL of the weed.

The Ph—OH in the lignin can be of several types as shown in Figure 1. In Types I and III, no conjugation liaison is formed with the benzene ring. According to the experimental data obtained with the $\Delta\varepsilon$ method, the total Ph—OH contents of Types I and III ($Ph—OH_{(I+III)}$) for AL and ML were 2.38% and 3.64 %, respectively, but the total Ph—OH contents of Types II and IV ($Ph—OH_{(II+IV)}$) were only 0.36% and 0.43%, respectively, for the two lignins. This showed that most of the phenolic hydroxyl group exited as Types I and III. However, the OCH_3 and Ph—OH contents in AL were lower than those in ML, but the acetyl group (Ac) content of AL was higher. It showed that the hydroxyl groups were partially acetylated during the Acetosolv delignification. On the other hand, the cleavage of β-aryl ether linkage during solvolytic breaking of lignin skeleton also caused the formation of Ph—OH.[22] Milox process had higher degree of delignification, which indicated that more β-aryl ether linkage was cleaved to form Ph—OH.

Phenolic hydroxyl group is an important functional group reflecting the solubility and chemical reaction ability of lignin. It indicated that ML would be more active for modification than AL in the preparation of corresponding derivatives.

According to the elementary analysis and the analyzed functional groups, the C_9 formulas of AL and ML are summarized and compared with some other acetic acid lignins in Table III. It can be seen that there are many differences between AL and ML, especially concerning Ph—OH and Ac. Compared with Rice straw lignin (RL), AL and ML had less oxygen, hydrogen, nitrogen contents, and Ac in C_9 unit, but higher methoxy group contents. However, compared with wood lignin (ML and FL), the weed stem lignin had less Ph—OH and Ac, but much higher oxygen and nitrogen contents. This indicates that the chemical compositions of lignin also greatly depend on the species biomass.

Chemical compositions of AL and ML

The chemical compositions of AL and ML showed in Table IV indicated that the two lignins had comparative glucose, arabinose, and protein contents, but that ML contained more xylose and acid-soluble lignin. Xylose was the major sugar in both of the

TABLE III
Comparison of C9 Formulas of AL and ML Isolated from Crofton Weed Stem with Some Other Acetic Acid Lignins

Lignin	C_9 expanded formula	C_9 weight
AL	$C_9H_{9.16}O_{3.50}N_{0.13}(OCH_3)_{0.97}(Ph—OH)_{0.34}Ac_{0.22}$	216.59
ML	$C_9H_{7.96}O_{3.13}N_{0.13}(OCH_3)_{1.23}(Ph—OH)_{0.51}Ac_{0.14}$	215.90
RL2[a]	$C_9H_{11.79}O_{2.36}N_{0.35}(OCH_3)_{0.62}(Ph—OH)_{0.33}Ac_{0.28}$	199.24
BL[a]	$C_9H_{9.84}O_{1.61}N_{0.03}(OCH_3)_{1.40}(Ph—OH)_{0.60}Ac_{0.52}$	220.06
FL[a]	$C_9H_{6.51}O_{1.12}N_{0.05}(OCH_3)_{1.26}(Ph—OH)_{0.83}Ac_{0.88}$	224.08

[a] The C_9 expanded formula was determined according to the elementary analysis and functional groups performed by Pan and Sano.[21]

TABLE IV
Chemical Compositions of AL and ML

Lignin	Glucose (%)	Xylose (%)	Arabinose (%)	Protein (%)	Acid-soluble lignin (%)	Klason lignin (%)
AL	0.25	1.43	0.06	5.16	1.06	92.04
ML	0.79	3.23	0.09	5.10	1.67	89.12

lignins, which was similar to those of acetic acid lignin isolated from rice straw and birch.[21] The higher content of acid-soluble lignin in ML can be explained by the higher Ph—OH content which increased its solubility in acid solution. The difference between the two lignins illustrated that the delignification process had significant influence on the chemical compositions of lignin.

UV spectra

Figure 2 shows the UV spectra of AL and ML in dioxane-water solution (9 : 1, v/v) and pH = 12 NaOH solution. The two lignins showed three absorption maxima at 210–220, 240–250, and ~ 280 nm in alkaline solution, but only showed two absorption maxima at ~ 250 and ~ 280 nm in dioxane-water solution. For typical of HGS lignin, there are three absorption maxima at 230–250, ~ 280, and ~ 315 nm. The absorption at ~ 280 nm originated from nonconjugated phenolic units in lignin, whereas the absorption at ~ 315 nm corresponded to conjugated phenolic units in ferulic and *p*-coumaric acids.[21,23] However, as shown in Table V, in dioxane-water solution, the maximum absorption around 280 nm for the AL and ML shifted to 277 and 278 nm, and shifted to 282 and 283 nm in alkaline solution, respectively. It has been known that the substitutions of an extra methoxyl group in the 5-position shift the maxima to lower wave length,[24] whereas the dissociation of phenolic group in alkaline solution increases the conjugation of oxygen atom with the benzene ring and shifts the maxima to higher wavelength. There was no absorption observed at ~ 315 nm for AL and ML. It indicated that AL and ML were not the typical HGS lignins. Compared with the reported UV spectra of Acetosolv lignins isolated from rice straw,[21] birch,[21] fir,[21] and bagasse,[23] it can be known that AL and ML were more similar than woody lignin rather than herbaceous lignin.

From Table V it can be known that the absorptivity (ε) of ML was larger than that of AL, which was probably because that ML had more Ph—OH, which increased the electron density in benzene ring and increased the absorptivity at ~ 280 nm.

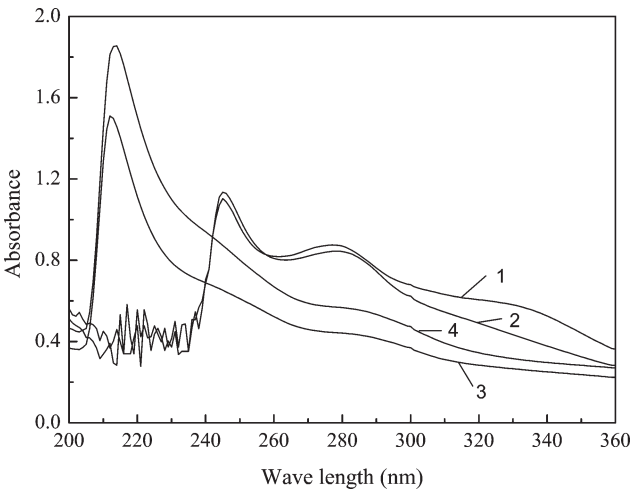

Figure 2 UV spectra of Acetosolv lignin and Milox lignin isolated from crofton weed stem. 1-Acetosolv lignin in dioxane-water (9 : 1) solution; 2-Milox lignin in dioxane-water (9 : 1) solution; 3-Acetosolv lignin in pH = 12 solution; 4-Milox lignin in pH = 12 solution.

FTIR spectra

Infrared spectroscopy is frequently used for investigating the structure of lignin. The FTIR spectra of AL and ML are shown in Figure 3. The corresponding bands and the assignments are listed in Table VI. The calibrations of the bands were performed based to the figures reported in the literatures.[14,16,19] The band at ~ 1510 cm^{-1} (aromatic skeletal vibrations) was used as a reference to estimate the relative intensities of the other bands.

From Figure 3, it can be known that the two lignins had very similar signals. The absorption peak at ~ 3400 cm^{-1} belonged to the stretching of O—H groups, inclusive of R—OH and Ar—OH. The relative intensities ($A_{\sim 3400}/A_{\sim 1510}$) of bands for AL and ML were of 0.88 and 0.90, respectively. This indicated that ML contained more —OH than AL. Both of the lignins had strong signals at 1720 cm^{-1}, which was attributed to unconjugated C=O stretching. The

TABLE V
λ Max and Absorptivity (ε) at ~ 280 Nm for AL and ML

Lignin	λ_{max} in dioxane-water solution (nm)	ε, L g^{-1} cm^{-1}	λ_{max} in pH = 12 solution (nm)	ε, L g^{-1} cm^{-1}
AL	277	18.42	282	17.61
ML	278	19.87	283	22.18

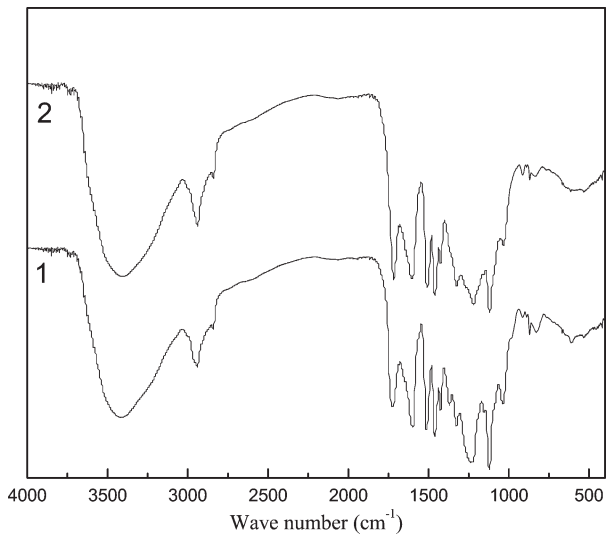

Figure 3 FTIR spectra of Acetosolv lignin (1) and Milox lignin (2).

band at 1675–1655 cm^{-1} is assigned to the conjugated C=O stretching; however, very weak signals were found for the two lignins, indicating that the conjugated C=O contents in AL and ML were relatively low. Both of the two lignins had apparent characteristic bands for the aromatic skeletal vibrations at ~ 1600, ~ 1510, and ~ 1420 cm^{-1}. AL had an obvious signal at 1369 cm^{-1}, which was due to aliphatic C—H stretching in CH_3 (not in OMe). It further reflected the occurrence of acetylation for AL. Further evidences for more carbonyl moieties in AL had the stronger intensities at ~ 1230 and ~ 1020 cm^{-1}. According to the literature,[16,23] the band at 1166 cm^{-1} is assigned to *p*-hydroxy phenyl propane (H) unit, which is also typical for annual (HGS) lignins. However, this band was not found for AL and ML. On the other hand, the strong signals corresponding to G (1230–1210 cm^{-1}) and S units (1130–1120 cm^{-1}) were clearly found for AL and ML. It further indicated that the two lignins were more similar to wood lignin than annual plant lignin.

^{1}H-NMR spectra

To further investigate the chemical structure of AL and ML, ^{1}H-NMR was used for analyzing the type of proton in the lignin. The spectra are shown in Figure 4. The chemical shifts (Δ) listed in the literatures and their corresponding signal assignments for ^{1}H-NMR spectra of the two lignins are listed in Table VII.[18,24,25,26] Both of the two lignins had signals in the range of 8.00–9.00 ppm, which were assigned to proton in carbonyl and aldehyde groups. The signal intensity for ML was stronger. This could be attributed to the introduction of aldehyde group during the formic delignification process. The integrals of signals between 6.28 and 7.28 ppm are attributed to aromatic protons in syringylpropane and guaiacylpropane structures. The marked signals in this range indicate the existence of syringyl and guaiacyl units in the two lignins, which is also reflected in the FTIR spectra. It has been found that the small signals in 7.3–7.8 ppm are associated with (1) the aromatic protons in Positions 2 and 6 in the structures containing a C=O group, (2) the aromatic protons in Position 2, (3) the 6 of *p*-hydroxyphenyl units

TABLE VI
FTIR Absorption Bands of AL and ML

		AL	ML
Band (cm^{-1})	Assignment	Band location (cm^{-1})	
3400–3300	O—H stretching	3400	3390
~ 2940	C—H asymmetric stretching in methyl and methylene group.	2940	2940
2850–2830	C—H symmetric stretching in methyl and methylene group.	2830	2840
1720–1680	C=O stretching in unconjugated ketone, carbonyl, and ester groups	1720	1720
1670–1640	C=O stretching in conjugated p-substituted aryl ketones	1640	1640
1610–1590	Aromatic skeletal vibrations plus C=O stretching	1590	1600
1515–1505	Aromatic skeletal vibrations	1513	1506
1470–1460	C—H deformations (asymmetric in —CH_3 and —CH_2—)	1460	1460
~ 1420	Aromatic skeletal vibrations combined with C—H in-plane deformations	1420	1420
1365–1370	Aliphatic C—H stretching in CH_3 (not in OMe) and phenolic OH	1369	—
1230–1210	C—C plus C—O plus C=O stretching ($G_{condensed} > G_{etherified}$, typical of G units)	1230	1210
1166	Typical for HGS lignins	—	—
1140	Aromatic C—H in-plane deformation	1140	1140
1130–1120	Typical of S units; also C=O stretching and symmetric stretching of C—O—C	1130	1120
1035–1030	Aromatic C—H in-plane deformation (G >S) plus C—O deformation in primary alcohols plus C=O stretching (unconjugated)	1030	1030
915–910	C—H out of plane (aromatic rings)	910	910
870– 860	C—H out of plane in Positions 2, 5, and 6 (G units)	868	868
830–810	C—H out of plane in Positions 2 and 6 of S units	820	820

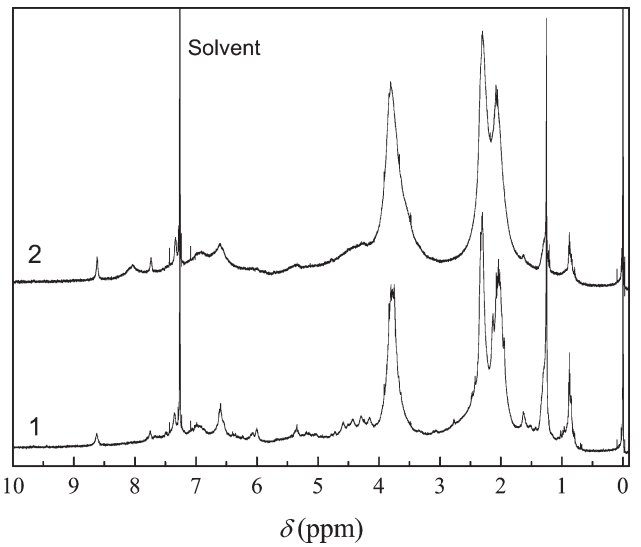

Figure 4 ^{1}H-NMR Spectra of acetylated Aetosolv lignin (1) and Milox lignin (2) isolated from crofton weed stem.

conjugated with a double bond, (4) the proton in C_α= C_β structures, and (5) the aromatic protons in *p*-coumaric and ferulic acids.[24,19] However, in the FTIR spectra of the two lignins, the typical band for *p*-hydroxy phenyl propane unit at 1166 cm^{-1} was not found. Hence, it is more reasonable to assign the 7.3–7.8 ppm signals to aromatic protons in Positions 2 and 6 in structures containing a C=O group. ML had weaker signals in 4.0–4.9 ppm, which was mainly assigned to proton in β–O–4 structures. It further confirmed the occurrence of the cleavage of the β–O–4 structure during Milox delignification, as shown in reaction (2). The stronger signal in 2.22–1.60 ppm for AL also confirmed the existence of aliphatic acetates.

TABLE VII
Chemical Shifts (δ) and Signal Assignments for ^{1}H-NMR Spectra of Acetylated AL and ML

δ (ppm)	Assignment
11.5–8.00	Proton in carboxyl and aldehyde group
7.80–7.30	Proton in *p*-hydroxyphenyl; aromatic protons in Positions 2 and 6 in structures containing a α-C=O group
7.30–7.28	$CDCl_3$ (solvent)
7.28–6.80	Aromatic proton in guaiacyl units
6.80–6.28	Aromatic proton in syringyl units
6.28–5.75	Hα of β–O–4 and β–1 structures
5.75–5.18	Hα of β–5 structure
5.18–4.90	H of xylan residue
4.90–4.30	Hα and Hβ of β–O–4 structures
4.30–4.15	Hγ of β–1, β–5, β–O–4, and β–β
4.15–3.00	H of methoxyl groups
2.50–2.22	H of aromatic acetates
2.22–1.60	H of aliphatic acetates
1.60–0.38	H in aliphatic groups

TABLE VIII
Average Molecular Weights of AL and ML

Lignin	M_w	M_n	M_w/M_n
AL	1900	610	3.10
ML	1890	660	2.88

Molecular weight

The weight-average (M_w) and number-average (M_n) molecular weights and the polydispersity (M_w/M_n) of the two lignins are summarized in Table VIII. It can be seen that the two lignins had similar M_w, M_n, and polydispersity. The M_w of these two lignins were in the M_w range of bagasse Acetosolv lignin, but M_n were somewhat lower.[21] The molecular weight of lignin had a significant effect on the characteristics of lignin, such as fusiability, solubility, thermal degradation, and reaction activity in polymerization with other monomers. This will be investigated in our continued work.

CONCLUSION

Both of AL and ML isolated from crofton weed stem were GS lignins. Nevertheless, the chemical composition and structure of the two lignins were somewhat different. AL contained more acetyl group and less phenolic hydroxyl group than ML because of acetylation in the Acetosolv delignification. The C_9 expanded formulas for AL and ML were $C_9H_{9.16}O_{3.50}N_{0.13}(OCH_3)_{0.97}(Ph{-}OH)_{0.34}Ac_{0.22}$ and $C_9H_{7.96}O_{3.13}N_{0.13}(OCH_3)_{1.23}(Ph{-}OH)_{0.51}Ac_{0.14}$, respectively. According to the elementary analysis, the two lignins had similar high heat value to that of raw coal. The Analysis of the chemical compositions showed that AL had a higher Klason lignin content but a lower carbonhydrate content. The UV spectra displayed absorption maxima at 277 and 278 nm for AL and ML in dioxane-water solution (9 : 1, v/v), but at 282 and 283 nm, respectively in pH = 12 solution. FTIR and ^{1}H-NMR spectra illustrated that the two lignins had similar chemical functional groups, with AL showing more signals of acetyl groups. GPC analysis showed that these two lignin had similar molecular weights. Nevertheless, more information will be given in a continued work for a deeper understanding of the chemical and physical properties of the two lignins, including solubility in different solvents, alkaline nitrobenzene, and permanganate oxidation, pyrolysis as well as the analysis of the degradation products. However, this study can provide some data for further utilization of weed lignin and control of this invasive weed, and also serve as a step for using the lignin as macromonomers for some polymer synthesis.

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