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Trapping of *p*-Coumaryl and Coniferyl Alcohol during Soda–Anthraquinone Treatment: A Means of Estimating Uncondensed *β*-*O*-4 Structures in Native Lignin

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Supporting Information

ABSTRACT: In most native lignins, at least 50% of the phenylpropane (C_9) units are involved in *β*-*O*-4 linkages. It was recently observed that ethylguaiacol (EG) was efficient at trapping coniferyl alcohol generated from the cleavage of uncondensed *β*-*O*-4 dimeric structures during soda–anthraquinone (AQ) or SAQ delignification of sugar maple wood meal. Some of the coniferyl alcohol was transformed to vinylguaiacol and isoeugenol, and the *α*-carbon atom in all three monomers formed C–C bonds with the C-5 position of EG. In the present research, eucalyptus and sugar cane bagasse meals were also investigated, and the yields of uncondensed *β*-*O*-4 structures in the nonsyringyl fraction were quantitated. The estimates of the uncondensed fraction of the lignin in the three samples (assuming S units are 90–95% uncondensed) were in close agreement with results from traditional but more tedious methods such as permanganate oxidation or spectroscopic methods requiring a sample representative of native lignin.

KEYWORDS: *hardwoods, bagasse, soda–AQ, coniferyl alcohol, p-coumaryl alcohol*

INTRODUCTION

In alkaline pulping of biomass to generate papermaking fibers, the *β*-*O*-4 bond connecting uncondensed aromatic rings is known to be cleaved at a relatively high rate.[1,2] The bond is also labile during thermochemical treatments of lignin for chemical and fuel syntheses.[3,4] An uncondensed aromatic ring is defined as one not containing a C–C bond at any ring position except for C-1 (side chain) nor connected to another C_9 unit by a diaryl ether linkage. On the other hand, condensed aromatic rings demonstrate low reaction rates in both alkaline pulping[5] and catalytic hydrogenation treatments.[6,7] It is therefore of great interest to find and develop relatively fast and nontedious methods for the estimation of the fraction of uncondensed rings in the lignin in biomass samples.

The soda–anthraquinone (SAQ) pulping process uses NaOH and anthraquinone to catalyze the depolymerization of lignin and generate lower molecular weight polymers with a high concentration of phenolic hydroxyl groups. One of the principal mechanisms believed to be occurring during SAQ pulping is described in Figure 1. High yields of *p*-coumaryl, coniferyl, and sinapyl alcohols are obtained when uncondensed *β*-*O*-4 structures in biomass lignins are treated with SAQ liquor at elevated temperatures. *p*-Coumaryl alcohol is obtained from *p*-hydroxyphenylpropane (H) units, coniferyl alcohol from guaiacyl (G) units, and sinapyl alcohol from syringyl (S) units in the lignin. The mechanism in Figure 1 is frequently cited as being responsible for the formation of three substituted cinnamyl alcohols,[8] but a credible free-radical mechanism is also supported by substantial data.[9] The three substituted cinnamyl alcohols above are generated from the A ring identified in Figure 1. An uncondensed *β*-*O*-4 structure is defined as one containing an uncondensed A ring. It was recently observed that ethylguaiacol (EG) was quite effective at condensing with monomeric quinone methides and the dimers generated react only slowly in further condensation.[2,10] The prior data also suggested that uncondensed *β*-*O*-4 structures in sugar maple (*Acer saccharum*) and three eucalypti were cleaved at a high rate during SAQ cooking at 165 °C.[2,11] Also, EG appeared to trap coniferyl alcohol and its transformation products, vinylguaiacol and isoeugenol, to form dimers **1**–**3** (Figure 2) at high yields.[2] The reactions schemes for the transformation of coniferyl alcohol (CA) to vinylguaiacol (VG) and isoeugenol (IE) are in the literature.[1] Products expected from the condensation between EG and sinapyl alcohol were not observed.[2]

There was evidence from the prior data to suggest a positive correlation between the concentration of uncondensed guaiacyl A rings (as defined in Figure 1) and the extent of SAQ delignification.[11] It is known that most of the syringyl rings (~90%) in hardwood lignin are uncondensed.[5,12] Syringyl rings also solubilized at a higher rate than guaiacyl rings during alkaline pulping.[5] Therefore, a high syringyl content and/or a high concentration of uncondensed guaiacyl A rings would be a preferred trait for the lignin in hardwoods being used to produce alkaline chemical pulps. Nimz[13] proposed a structural model for the lignin in beech (*Fagus silvatica*) hardwood. The model consisted of 25 C_9 units and contained 8 *β*-*O*-4 dimeric

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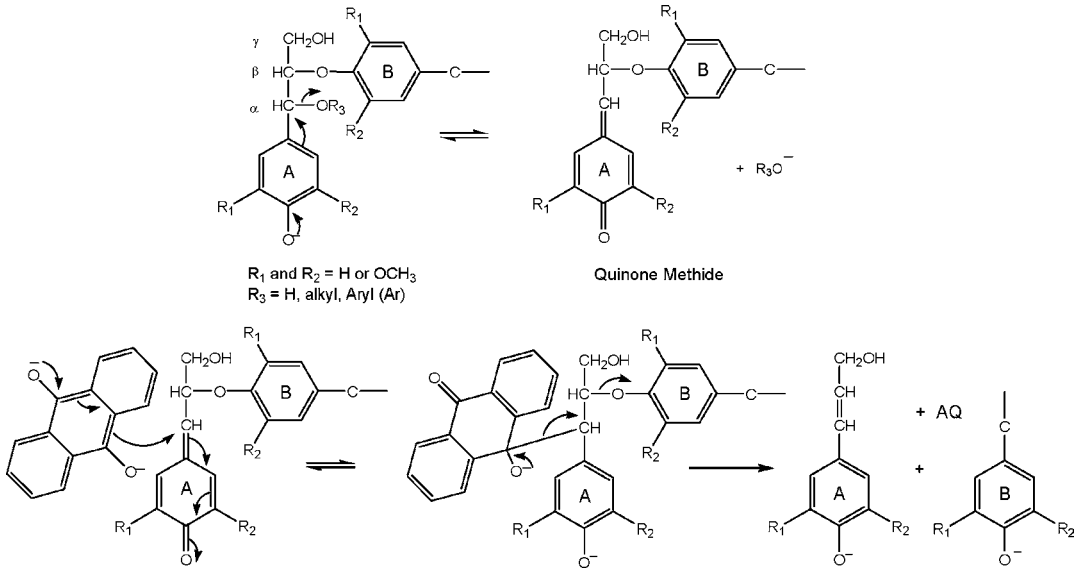

Figure 1. SAQ depolymerization of lignin to generate *p*-coumaryl alcohol (R_1 and R_2 = H), coniferyl alcohol (R_1 = H; R_2 = OCH_3), and sinapyl alcohol (R_1 and R_2 = OCH_3) from A rings.

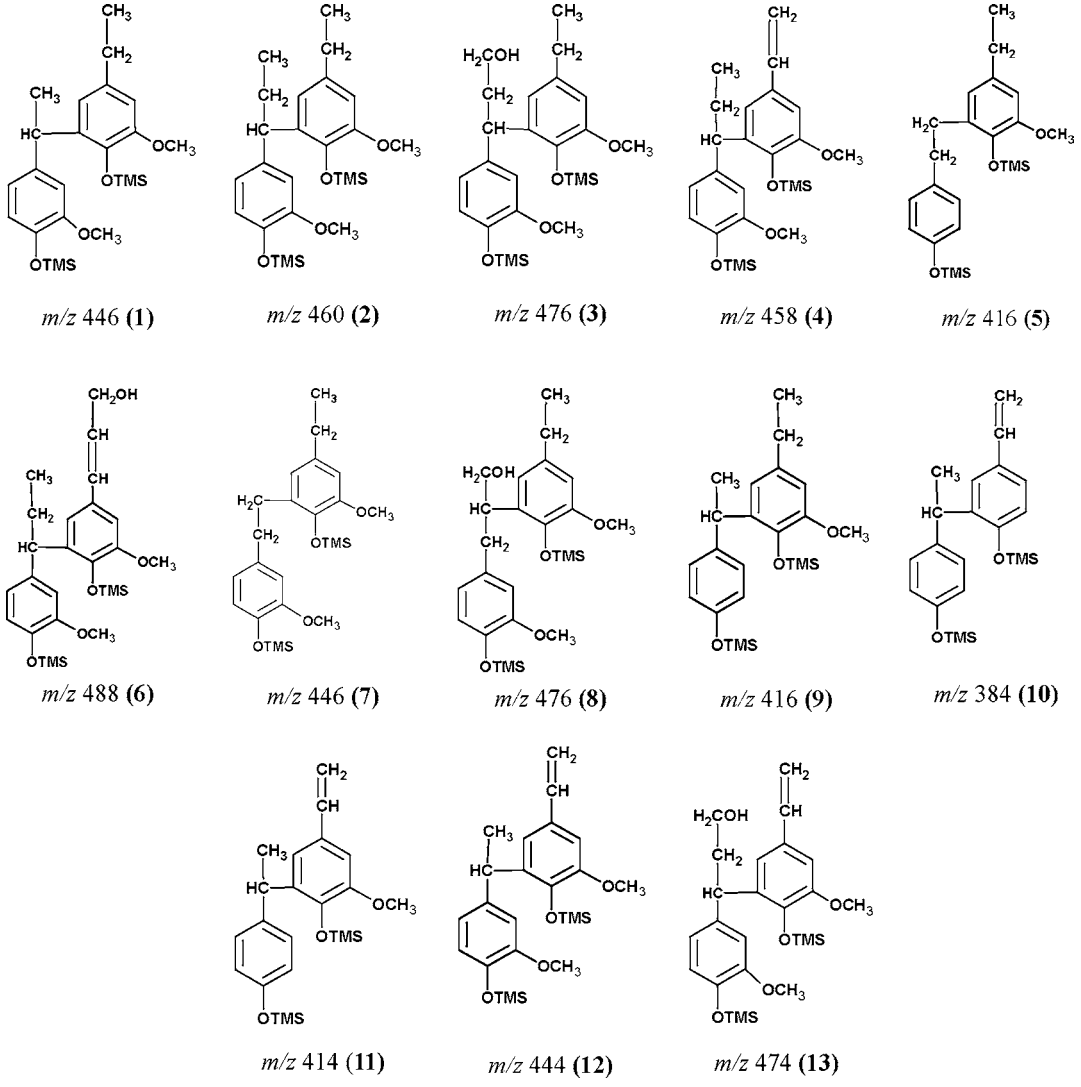

Figure 2. Dimers produced from SAQ + AQ treatment of hardwood and bagasse meal (after silylation).

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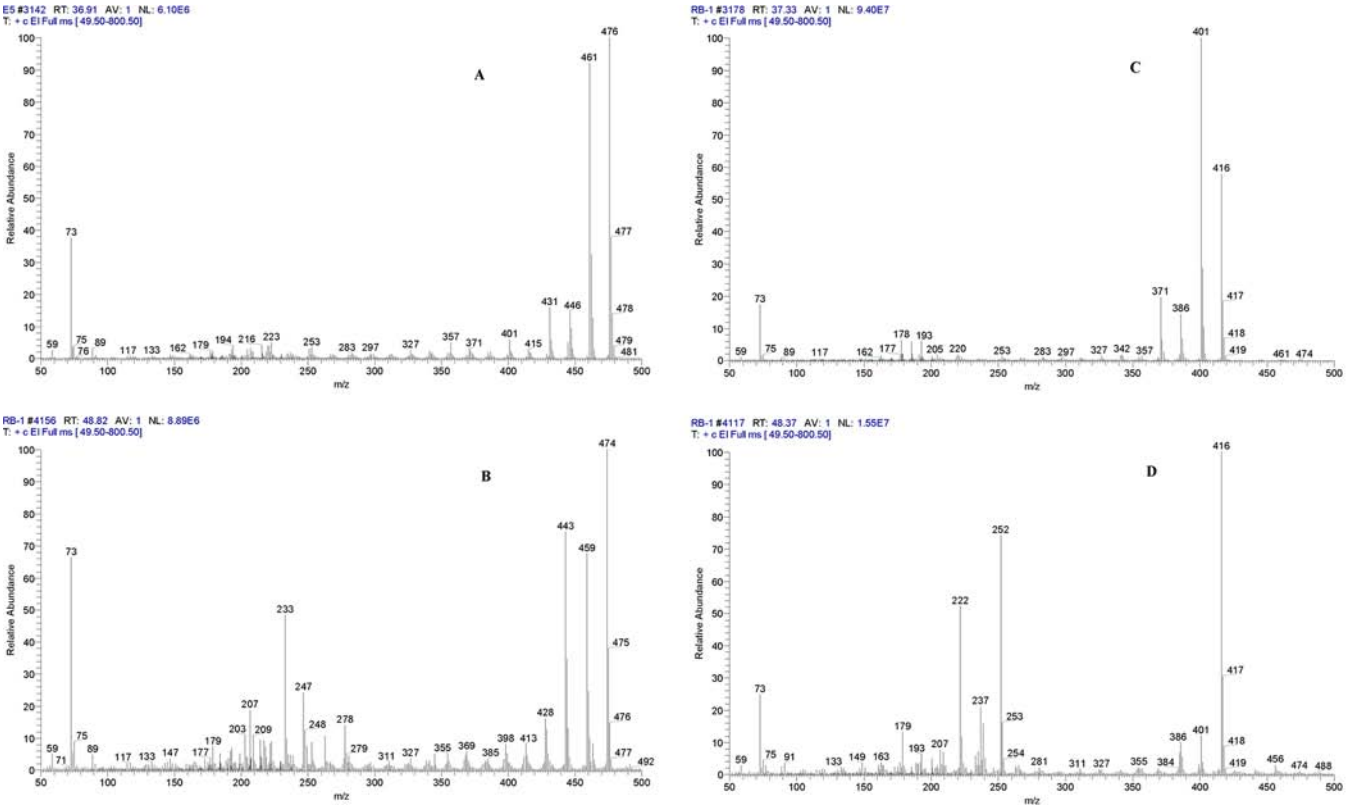

Figure 3. Mass spectrum for **3**, an α-5 linked CA−EG dimer with M_r of 476 (A); mass spectrum for **13**, an α-5 linked CA−VG dimer with M_r of 474 (B); mass spectrum for **9**, an α-5 linked VP−EG dimer with M_r of 416 (C); and mass spectrum for **5**, a β-5 linked VP−EG dimer with M_r of 416 (D).

units (16 C_9 units). Three of the eight A rings (37.5%) were condensed.[13] This distribution of ∼60% uncondensed and ∼40% condensed β-*O*-4 units is believed to be typical for hardwood lignins (to be discussed later).

The objective of this research was to further investigate SAQ + EG cooking to see if EG trapping of coniferyl alcohol and *p*-coumaryl alcohol along with their transformation products could be used to estimate the concentration of uncondensed β-*O*-4 in a wider range of native lignins. In addition to the temperate hardwood (sugar maple) and *Eucalyptus camaldulensis* from Morocco, sugar cane bagasse (*Saccharum officinarum*), which is known to contain significant amount of H units, was investigated.

MATERIALS AND METHODS

Biomass Samples. Mature sugar maple trees were harvested from a forest in Central New York, while the *E. camaldulensis* (6−8 years old) were harvested in Gharb in the North−West of Morocco. The depithed bagasse sample was kindly donated by the Bangladesh Forest Research Institute (BFRI) in Chittagong, Bangladesh. The two hardwoods were debarked by hand and converted to chips and then to 15 mesh (1.30 mm) wood meal using a Wiley mill. The bagasse was similarly converted to a 15 mesh meal, and all three samples were extracted with ethanol−toluene in accordance with Tappi Method T 204 om-88.

SAQ Delignification in the Presence of EG. The following substrates were added to 60 mL of 0.4 M NaOH in stainless steel autoclaves: 5.0 g (oven-dried or OD basis) of extracted biomass meal from above, 250 mg of EG (1.64 mmol), and 23 mg of anthraquinone (AQ). The slurries were shaken vigorously to ensure good mixing, and the autoclaves were heated for 60 min at 165 °C. Based on accurate vapor pressure measurements with deionized water only, the internal temperature profile in the autoclaves that were used was estimated to be 14 min to 163 °C and 46 min at that temperature (H-factor 441). The increase in product yields was statistically insignificant when treatment time was increased to 75 min. After 60 min, the autoclaves were cooled, and the alkaline product mixtures were filtered through two sheets of Whatman no. 1 filter paper and then through a 1.2 μm Magna nylon supported plain filter (GE Osmonics, Minnetonka, MN). Both filtrations were aided by vacuum, and the filter cakes were washed with approximately 15 mL of 0.1 M NaOH. Each liquid was then acidified and extracted (3 × 75 mL) by dichloromethane with the first extraction mixture being left overnight to ensure a good phase separation. The internal standard, benzhydrol, was added to the final dichloromethane extract, which was dried over Na_2SO_4 and then reduced to a low volume by evaporation under vacuum. A fraction of the dichloromethane solution (100 μL) was added to another vial along with 100 μL of *N*,*O*-bis(trimethylflouromethyl-silylacetamide) (BSTFA) and a drop of pyridine. The mixture was allowed to sit at room temperature overnight or for approximately 30 min at 40 °C before being analyzed by gas chromatography/mass spectrometry (GC/MS).

GC/MS and Other Analyses. GC/MS analyses were performed using a Thermo Scientific Finnigan Trace GC ultra GC coupled to a Thermo MAT95XP double focus magnetic sector mass spectrometer. The column used was a 30 m × 0.25 mm i.d., 0.25 μm, Rtx-5MS (5% diphenyl/95% dimethylpolysiloxane) capillary column (Restek Corporation, Bellefonte, PA). Helium at a flow rate of 1 mL/min was used as the carrier gas. About 1 μL of sample was injected and analyzed using a split ratio of 20:1. The injector temperature was 240 °C, and the column temperature profile was as follows: initial temperature 110 °C (hold for 4 min); a ramp from 110 to 260 °C at 5 °C/min followed by a 5 min hold; then, a second ramp at 5 °C/min to 300 °C and a hold at this maximum temperature for 20 min. Ionization was carried out at a 70 eV impact voltage in an ion chamber heated at 280 °C. The MS range scanned was m/z 45−800 at a rate of 0.6 scans/s. Peak identification was carried out on the basis of mass fragmentation

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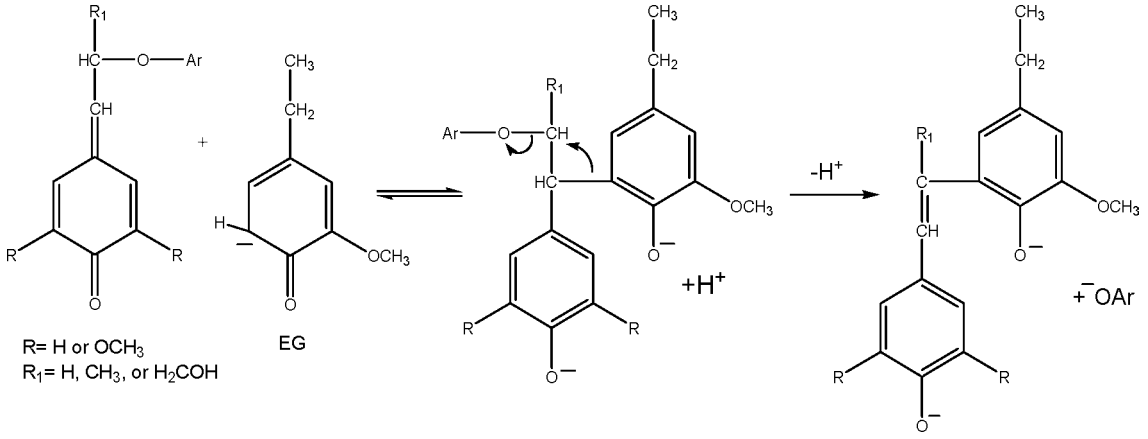

Figure 4. Proposed mechanism for the formation of β-5 dimers during SAQ + EG treatment.

patterns and by comparing the MS data with those in the Pfleger–Mauer–-Weber, Wiley, and NIST libraries. A different Rtx-5MS column with the same specifications was used for the bagasse and the retention times for identical compounds were longer that those observed with the hardwoods.

Lignin content analyses and S/G ratio by nitrobenzene oxidation were performed by previously reported methods.[14] An organosolv lignin was prepared from sugar maple using the methods of Pan et al.,[15] and its methoxyl content was determined. Methoxyl content analyses on organosolv lignin and wood meals were performed by Galbraith Laboratories, Knoxville, TN using the hydriodic acid or Zeisel method. The derivation of the S and G content of the lignin in the *E. camaldulensis* from the methoxyl content of the wood meal is discussed elsewhere.[11]

RESULTS AND DISCUSSION

Assignment of Dimers from MS. The dimers obtained from SAQ + EG treatments of hardwoods and bagasse (Figure 2) fell into three main classes: (1) α-5 linkage between EG and coniferyl alcohol (CA), *p*-coumaryl alcohol (*p*-CMA), or their transformation products; (2) α-5 linkage between vinylguaiacol (VG) or vinylphenol (VP) and CA, *p*-CMA, or their transformation products; and (3) β-5 linkage between EG and CA, *p*-CMA, or their transformation products.

The spectra used for the assignments of **1**–**3** have already been presented and analyzed, and in the case of **1** and **3**, mass spectra for both the underivatized and silylated dimers were presented and analyzed.[2] The spectrum assigned to **3** (coniferyl alcohol–EG, α-linked) is presented in Figure 3A, and the assignment is conclusive.[2] The spectrum for the corresponding dimer (**13**) with vinylguaiacol instead of EG is shown in Figure 3B. In a previous investigation with monomers, a 2 Da difference was also seen between ethylguaiacol- and vinylguaiacol-linked dimers for the M^+ and $[M-15]^+$ fragments.[10] The difference between α-5 and β-5 condensed products is best demonstrated by the spectra in Figure 3C and D, where the molecular ion had *m*/*z* 416 in both cases (vinylphenol–EG). The α-5 dimer (**9**) in Figure 3C is typical of all the α-5 dimers with M^+, $[M-15]^+$, $[M-30]^+$, and $[M-45]^+$ being major peaks. The $[M-15]^+$ peak is much smaller relative to the M^+ peak for the β-5 dimer (**5**) in Figure 3D. This was a common attribute for all of the β-5 dimers (**5**, **7**, **8**). Cleavage of the Cα–Cβ bond in **5** would give fragments with *m*/*z* 237 and 179, and both fragments are observed in Figure 3D. There are well-established mechanisms to explain the α-5 condensation responsible for the generation of **3** or **13**. These mechanisms are both ionic[8] and free-radical in nature.[9] However, β-5 condensation is much less common in alkaline pulping chemistry.

Possible Mechanism to Explain β-5 Condensation. The β-5 condensation pattern is not normally seen in alkaline pulping chemistry. However, it was clearly seen in one case where 2,6-xylenol was added to soda cooking (1.0 M NaOH at 150 °C) of a β-*O*-4 dimer. The mole ratio of 2,6-xylenol to the β-*O*-4 dimer was >1.0.[16] Similar to EG in the present case, the xylenol generated an alkali-stable phenolate and contained two electron-donating groups. The mechanism proposed by those authors involved the xylenol adding to the QM as a *para*-cyclohexadienone carbanion, resulting in ring condensation with the α-carbon of the β-*O*-4 dimer. The xylenol, now with a C–C bond *para* to the phenolate group, once again rearranged to a *para*-cyclohexadienone carbanion, cleaving the neighboring β-*O*-4 bond with simultaneous migration of the xylenol from the α-carbon to the β-carbon.[16] A fairly similar mechanism with EG rearranging to an *ortho*-cyclohexadienone carbanion is shown in Figure 4. The product is a β-5 dimer containing a vinylic group. Unlike the previous research,[16] reducing agents were involved in the present research. The vinylic group in the end product of Figure 4 could have been reduced by the anthrahydroquinone dianion or anthrahydroquinone radical anion to give **5**, **7**, and **8** in Figure 2. In enzymatic processes, vinylphenols are known to be reduced to ethylphenols,[17] while CA is reduced to dihydroconiferyl alcohol.[18]

Hardwood Results. All of the key properties of the lignin in sugar maple and *E. camaldulensis* wood meals were determined while those for bagasse were estimated, based on literature values. Properties of bagasse lignin will be discussed under the Bagasse Results section. The sugar maple had a lignin content of 24.8% while *E. camaldulensis* had a value of 30.6%. For sugar maple, 5.0 g of wood meal contained 5.90 mmol of C_9 units while the value was 7.22 mmol for *E. camaldulensis*, that is, assuming C_9 molecular weights of 210 and 212, respectively. The estimated S/G/H ratio for *E. camaldulensis* was 0.655:0.325:0.02 by both nitrobenzene oxidation and methoxyl analyses.[11] The corresponding ratio for sugar maple by nitrobenzene oxidation was 0.545:0.435:0.02. An organosolv lignin was also extracted from sugar maple, and it contained 1.53 OCH_3/C_9. This methoxyl content corresponds almost exactly to the S/G/H ratio obtained by nitrobenzene oxidation. The yields of dimers from SAQ + EG treatment of both hardwoods are documented in Table 1. A response factor, relative to silylated benzhydrol, of 0.85 was used for **5**, **9**, and

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Table 1. Yields of Residual EG and Dimers Generated in SAQ + EG Cooking of Maple, *E. camaldulensis*, and Bagasse Meals

compd	*m/z*	sugar maple dimers, mmoles[a]	*E. camaldulensis* dimers, mmoles[a]	bagasse dimers, mmoles[a]
EG		1.15[b]		
VG−EG (**1**)	446	0.10 (0.10)[c]	0.06 (0.06)[c]	0.17 (0.02)[d]
IE−EG (**2**)	460	0.06 (0.06)	0.02 (0.02)	0.02
CA−EG (**3**)	476	0.24 (0.24)	0.16 (0.16)	0.09 (0.01)
IE−VG (**4**)	458	0.02 (0.04)	0.01 (0.02)	0.03
VP−EG *β*-linked (**5**)	416	0.02 (0)	0.02 (0)	0.02
IE−CA (**6**)	488	0.01 (0.02)	0.01 (0.02)	0
VG−EG *β*-linked (**7**)	446	0 (0)	0.02 (0.02)	0
CA−EG *β*-linked (**8**)	476	0.01 (0.01)	0.07 (0.07)	0
VG−EG (**9**)	416	0	0	0.32 (0.03)
VP−VP (**10**)	384	0	0	0.11 (0.01)
VP−VG (**11**)	414	0	0	0.09 (0.01)
VG−VG (**12**)	444	0	0	0.04
CA−VG (**13**)	474	0	0	0.02

[a]From 5.90 mmol of C_9 units for maple, 7.22 mmol for *E. camaldulensis*, and 6.27 mmol for bagasse (see text). [b]Initial dose of EG = 1.64 mmol (250 mg). [c]Monomers derived from coniferyl alcohol (0.47 mmol in total for maple and 0.37 mmol for the eucalypt). [d]Standard deviation based on three complete analyses with one analysis performed on a different occasion from the other two.

10 while a value of 0.95 was used for **6** and 0.90 for the remaining nine dimers. The approaches used to arrive at these estimated values were previously presented.[2]

When the maple and *E. camaldulensis* wood meals were SAQ delignified in the presence of EG, it appeared as if a high fraction of the estimated coniferyl alcohol that would be generated was trapped as dimers **1**−**4** and **6**−**8** (Figure 2). There was no detection of syringyl containing dimers at the 0.01 mmol level from either wood meal. A segment of the chromatogram from the GC/MS analysis of the products from sugar maple is shown in Figure 5A while a similar segment for *E. camaldulensis* is shown in Figure 5B.

Dimers **1**−**8** are assigned to the peaks in Figure 5B, which also contained the peak for a second internal standard, 3,3′,4′-trimethoxy-4-hydroxystilbene. The idea was to use a larger concentration of the regular internal standard, benzhydrol, for more accurate quantification of dimers **1**−**3** and a lower concentration of the stilbene for more accurate quantification of dimers **4**−**8.** No significant difference was obtained when using two internal standards instead of one, and the practice was abandoned. Benzhydrol elutes at a retention time that is in between those of the monomers and dimers. Very rarely are there any small peaks eluting in the region of the chromatogram where benzhydrol elutes, and as such, its peak area is always precise. One reason for the inclusion of **4**−**8** in the product yield summation is that some of these dimers were ultimate products of two coniferyl alcohol monomers. Therefore, although only 0.02 mmol of **4** was detected when sugar maple was treated (Table 1), the dimer was produced from 0.04 mmol of coniferyl alcohol, and as such, the yield of this product is almost as significant as that of **2**.

A credible estimate of the percentage of the C_9 units containing either an *α*-OH or an *α*-ether and involved in *β*-*O*-4 linkages is approximately 30% (30 dimers or 60 monomers/100 C_9),[12] and one mole of coniferyl alcohol would be generated from one mole of *β*-*O*-4 dimer (Figure 1). The maple wood meal contained 24.8% lignin, and the lignin was estimated to contain 43.5% G units and 54.5% S units for a S/G ratio of 1.25. By assuming that A rings constitute approximately 30% of the C_9 units in the lignin and 43.5% of them are guaiacyl units, the total guaiacyl A rings would be approximately 0.77 mmol (5.90 mmol × 0.30 × 0.435). In all probability, coniferyl alcohol would only be produced from uncondensed guaiacyl A rings. The total yield of coniferyl alcohol derived dimers was 0.47 mmol (Table 1). If it is assumed that all the *β*-*O*-4 dimers with uncondensed A rings were converted to coniferyl alcohol, then the uncondensed fraction of the total guaiacyl A rings would be 61% (0.47/0.77). By using the corresponding data for *E. camaldulensis* lignin (presented above) and the dimer yield data from Table 1, a value of 53% would be obtained for the uncondensed fraction of the total guaiacyl A rings (0.37/0.70). The 0.70 mmol value is obtained as follows: 7.22 mmol × 0.30 × 0.325.

Gellerstedt et al.[5] analyzed the lignin in a minimally delignified *Betula verrucosa* kraft pulp (24% lignin removal) by permanganate oxidation and found that 62% of the G rings were uncondensed. Using the same permanganate oxidation technique, Evtuguin et al. analyzed *Eucalyptus globulus* and found that 61% of the G rings were uncondensed.[19] The belief that the G fraction of hardwood lignin is approximately 60% uncondensed is also supported by data from ^{31}P NMR. Wu and Argyropoulos prepared milled wood lignin (MWL) and enzymatic mild acidolysis lignin (EMAL) from aspen (*Populus tremuloides*).[20] The fraction of the total C_9 units that was phenolic was approximately 20% for both samples. When analyzed by ^{31}P NMR, the average value for uncondensed phenolic G units in EMAL and MWL was 0.35 mmol/g, while the concentration of total condensed phenolic units was 0.25 mmol/g. If it is assumed that 80% of the condensed phenolics are in the G fraction, then one arrives at approximately 0.35 mmol/g of uncondensed and 0.20 mmol/g of condensed phenolic G units (ca. 64% uncondensed). The assumption above that the S fraction contains a minor fraction of the condensed rings is supported by both permanganate oxidation[5] and ^{1}H and ^{13}C NMR studies.[12] Both approaches lead to the conclusion that approximately 90% of the S rings are uncondensed. The S fraction of the 24% delignified *Betula verrucosa* kraft pulp above was 93% uncondensed.[5]

Finally, it should be noted that the consumption of EG in the treatment of sugar maple (Table 1) was 0.49 mmol (1.64 − 1.15). Therefore, EG appears to react almost exclusively with coniferyl alcohol and its transformed products (vinylguaiacol and isoeugenol). Of the total EG consumption of 0.49 mmol, coniferyl alcohol, vinylguaiacol, and isoeugenol consumed 0.41 mmol. The yield of dimers containing two coniferyl alcohol derived monomers was 0.06 mmol (Table 1).

Bagasse Results. When the solvent extracted bagasse (23.7% lignin content) was treated with SAQ and EG, it was obvious that the yields of dimers were much higher than for maple or *E. camaldulensis*. Actually, the total product yield from bagasse was approximately twice that of the maple (Table 1) even though the lignin content of the samples was

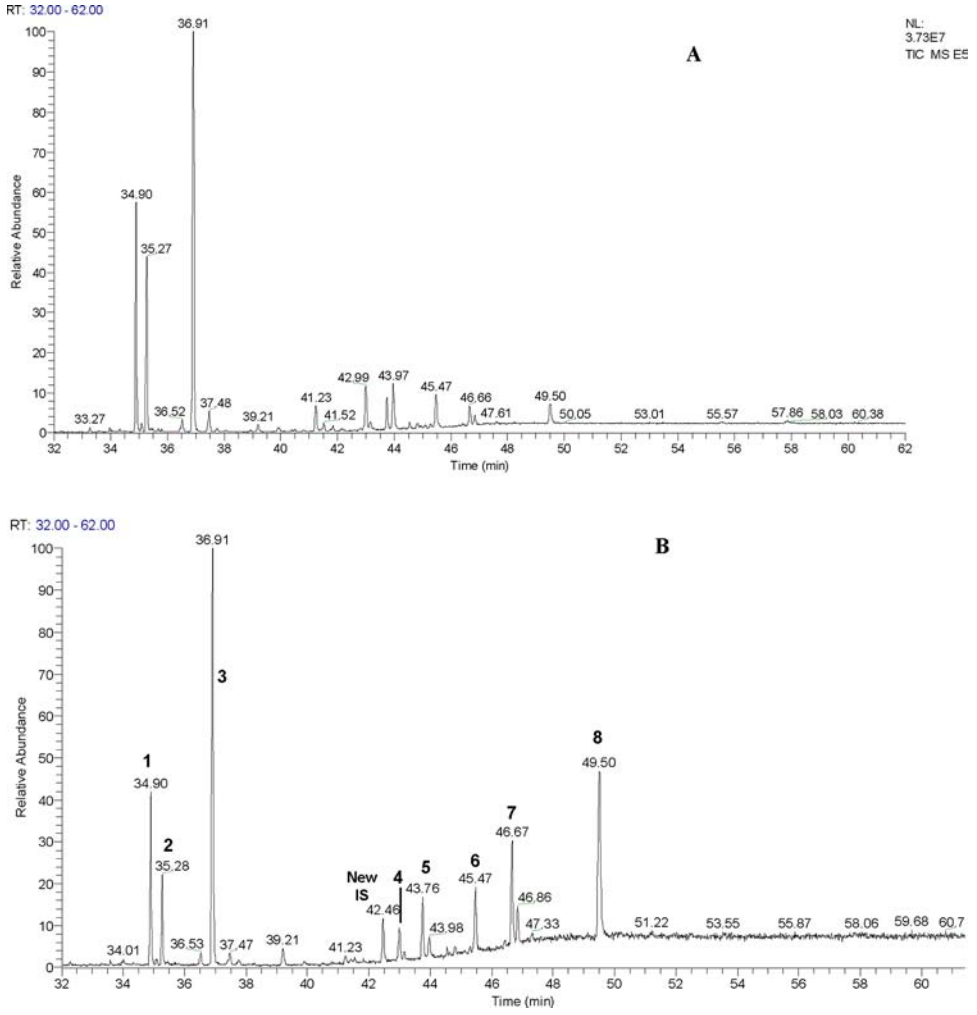

Figure 5. Section of GC/MS chromatogram showing dimeric products from SAQ + EG treatment of sugar maple (A) and *E. camaldulensis* (B) wood meal at a 12:1 liquor to wood ratio.

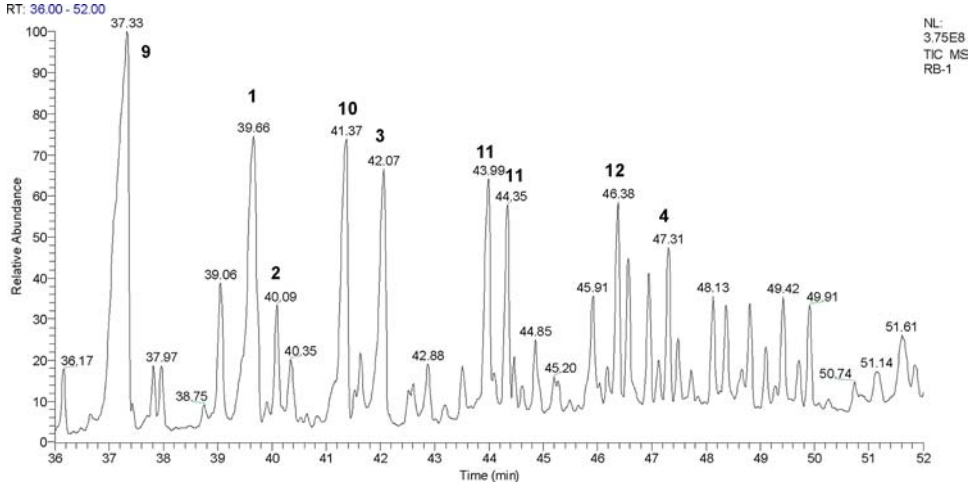

Figure 6. Dimeric products from SAQ + EG treatment of bagasse.

approximately equal (23.7% vs 24.8% for maple). A typical GC/MS chromatogram for the bagasse reaction products is shown in Figure 6 ,and peaks for the major dimers are identified on it. Nada et al.[21,22] analyzed bagasse lignin recovered from the black liquors of organosolv, soda, SAQ, and kraft pulping and found that their methoxyl contents consistently fell in the range of 0.98−1.05 OCH_3/C_9. Chen et al.[23] performed alkaline nitrobenzene oxidation on bagasse meal and obtained a high total yield of substituted benzaldehyde + benzoic acids. The distribution was 21.3%, 17.6%, and 19.2% from H, G, and S units, respectively, for a total of 58.1 mmol/100 mmol of C_9 units. Those results

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afforded a distribution of 36.7% H units, 30.3% G units, and 33.0% S units for bagasse lignin and a methoxyl content of 0.96 OCH_3/C_9. However, severe alkaline treatment was recently used to hydrolyze *p*-coumaric acid and ferulic acid from the bagasse from seven sugar cane hybrids with lignin content in the range of 20−25%.[24] After correction for degradation of the acids during the severe alkaline treatment, the authors concluded that the average value for *p*-coumaric acid ester and ether linked to bagasse samples was 6.1 wt % while the corresponding value for ferulic acid was 1.2 wt %.

The yields of nitrobenzene oxidation products from above[23] have to be corrected for *p*-coumaric and ferulic acids that would hydrolyze very early in both the nitrobenzene and SAQ + EG treatments that are performed in NaOH solutions at temperatures >160 °C. In the SAQ + EG treatment, the acids could decarboxylate to form vinylphenol and vinylguaiacol, respectively,[25] and be miscounted as coming from *β-O*-4 structures when they condense with EG. When these two acids were subjected to the standard nitrobenzene oxidation treatment, *p*-coumaric acid afforded a 52% molar yield of *p*-hydroxybenzaldehyde while ferulic acid afforded vanillin at 63% yield.[26] Therefore, the nitrobenzene oxidation yields of substituted benzaldehydes and benzoic acids from H and G units[23] have to be corrected for contributions from hydrolyzed *p*-coumaric and ferulic acids. Starting with 5.0 g of bagasse meal and assuming the hydrolysis of 6.1 wt % *p*-coumaric and 1.2 wt % ferulic acids, one would obtain 1.86 mmol of *p*-coumaric acid (5000 × 0.061/164) and 0.31 mmol ferulic acid (5000 × 0.012/194). By assuming that these acids would end up as either acid insoluble or acid soluble lignin, they would contribute 7.3% of the 23.7% total lignin with the remaining 16.4% coming from traditional lignin polymers. The number of millimoles of polymeric C_9 units (after hydrolyses of *p*-coumarate and ferulate) would be 4.10 if a C_9 molecular weight of 200 is assumed, and the total number of aromatic rings would be 6.27 mmol (4.10 + 1.86 + 0.31).

The molar yield of *p*-hydroxybenzaldehyde from *p*-coumaric acid would be ∼0.97 mmol (1.86 × 0.52) while the corresponding yield of vanillin from ferulic acid would be ∼0.20 mmol (0.31 × 0.63). Based on the yield data of Chen et al.,[23] the total yields of substituted benzaldehyde + benzoic acid from H, G, and S units that would be obtained for this bagasse sample would be 1.34 mmol of H products (0.213 × 6.27), 1.10 mmol of G products (0.176 × 6.27), and 1.20 mmol of S products (0.192 × 6.27). When the 0.97 mmol of H products from *p*-coumaric acid and 0.20 mmol of G products from ferulic acid are subtracted, yields of 0.37 mmol of H products, 0.90 mmol of G products, and 1.20 mmol of S products (2.47 mmol in total) are obtained for the polymeric lignin fraction. The H, G, and S percent distributions of the polymeric lignin would be 15.0% (0.37/2.47), 36.4%, and 48.6%, respectively. However, another correction is needed to the nitrobenzene oxidation method to account for the fact that the reaction products come almost exclusively or totally from uncondensed C_9 units. When nitrobenzene oxidation data was used to estimate the S and G fractions of the two hardwoods, the yield of guaiacyl products was divided by 0.666 (fraction of uncondensed G units) while the syringyl product yield was divided by 0.96 (fraction of uncondensed S units).[14] Based on the high yield of products from uncondensed guaiacyl A rings that was observed for bagasse (Table 1), the uncondensed G fraction for bagasse is likely to be >66.6%. If an uncondensed fraction of 80% is assumed for the G fraction of bagasse lignin and 95% for the H and S fractions, then the corrected nitrobenzene yields for the polymeric lignin fraction would be 0.39 mmol of H products (0.37/0.95), 1.13 mmol of G products, and 1.26 mmol of S products (2.78 mmol in total). The H, G, and S percent distributions of the polymeric lignin would become 14.0% (0.39/2.78), 40.6%, and 45.3%, respectively, and the molar distribution of the 4.10 mmol of C_9 units would be ∼0.58 mmol, ∼1.66 mmol, and ∼1.86 mmol, respectively. The S/G ratio of the polymeric bagasse lignin would be 1.12.

The yields of the key dimers from bagasse are shown in Table 1. Once again, there was no detection of syringyl containing dimers at the 0.01 mmol level. A significant finding was that vinylphenol-containing dimers dominated over *p*-coumaryl alcohol-containing dimers and vinylguaiacol-containing dimers dominated over coniferyl alcohol-containing ones. Actually, no *p*-coumaryl alcohol-containing dimers were detected. Also, the ratio of coniferyl alcohol-containing dimers to vinylguaiacol-containing dimers was >2.0 for the two hardwoods but <0.5 for bagasse. Elimination of the *γ*-carbon as formaldehyde from *p*-coumaryl and coniferyl alcohols is believed responsible for the formation of vinylphenol and vinylguaiacol. This formaldehyde elimination reaction might have been aided by a catalyst(s), and the catalytic effect might have been greater in the case of bagasse. As discussed previously, there would have been ∼0.58 mmol of H units in the polymeric lignin, but the yield of vinylphenol-containing dimers was 0.65 mmol (Table 1). Clearly, some of the vinylphenol must have come from hydrolyzed *p*-coumaric acid.

It is known that the thermal decarboxylation of *p*-coumaric acid to vinylphenol and CO_2 is catalyzed by acid, base, and microwave irradiation.[25] Therefore, *p*-coumaric acid and EG (1.64 mmol of each) were treated identically to the bagasse meal, and low yields of **9** and **10** were observed. The duplicate yield of **9** was 0.17 mmol while that of **10** was 0.06 mmol. Since two moles of *p*-coumaric acid would be required to generate one mole of **10** (a vinylphenol−vinylphenol dimer), the total conversion of *p*-coumaric acid to **9** and **10** was 0.29 mmol or 18%. The initial amount of hydrolyzable *p*-coumaric acids in the bagasse was estimated at 1.86 mmol, and if it is assumed that they were converted to vinylphenol-containing products at 18% yield, then 0.33 mmol of vinylphenol-containing products (Table 1) did not come from polymeric lignin but from *p*-coumaric acids. Similarly, there was 0.31 mmol of hydrolyzable ferulic acids in the bagasse, and if it is assumed that they also converted to vinylguaiacol-containing products at 18% yield, then 0.06 mmol of vinylguaiacol-containing products have to be subtracted from the total in Table 1. The bagasse data in Table 1 are presented in Table 2 with those corrections and with the total number of *p*-coumaryl alcohol and coniferyl alcohol derived monomers calculated. The corrected monomer yield was 0.32 mmol for *p*-coumaryl alcohol derived products and 0.49 mmol for coniferyl alcohol derived products.

The breakdown of the 4.10 mmol of C_9 units in the polymeric lignin was previously estimated as ∼0.58 mmol of H units, ∼1.66 mmol of G units, and ∼1.86 mmol of S units. The lignin in wheat straw is reported to contain a very high percentage of the C_9 units (∼77%) connected by *β-O*-4 linkages.[27] If it is assumed that 40% of the C_9 units in bagasse lignin contain either an *α*-OH or an *α*-ether and involved in *β-O*-4 linkages (40 dimers or 80 monomers/100 C_9), then the total number of A rings that would be H would be approximately 0.23 mmol (0.58 × 0.40) while the corresponding value for G rings would be approximately 0.66 mmol (1.66

Table 2. Yields of Monomers Derived from H and G Units in Polymeric Lignin of Bagasse and Application of Correction for Conversion of *p*-Coumaric Acid and Ferulic Acid to Dimers

compd	*m/z*	dimeric yield, mmoles	derived from *p*-coumaryl alcohol	derived from coniferyl alcohol
VG–EG (**1**)	446	0.17		0.17
IE–EG (**2**)	460	0.02		0.02
CA–EG (**3**)	476	0.09		0.09
IE–VG (**4**)	458	0.03		0.06
VP–EG β-linked (**5**)	416	0.02	0.02	
VG–EG (**9**)	416	0.32	0.32	
VP–VP (**10**)	384	0.11	0.22	
VP–VG (**11**)	414	0.09	0.09	0.09
VG–VG (**12**)	444	0.04		0.08
CA–VG (**13**)	474	0.02		0.04
total *p*-CMA derived			0.65 (0.32)[a]	
total CA derived				0.55 (0.49)[b]

[a]Corrected for vinylphenol-containing products derived from *p*-coumaric acid, see text. [b]Corrected for vinylguaiacol-containing products derived from ferulic acid, see text.

× 0.4). Of the total guaiacyl A rings, 0.49 mmol or 74% would be uncondensed. If it is assumed that 74% of the G fraction and 95% of the H and S fractions of bagasse lignin are uncondensed, then one arrives at 5.72 mmol out of total 6.27 mmol or ∼91% of the C_9 units being uncondensed. The summation is 0.74(1.66) + 0.95(0.58 + 1.86) + 1.00(1.86 + 0.31) = 5.72. The uncondensed A rings in the H fraction by SAQ + EG was higher than the total estimated A rings (0.32 mmol vs 0.23 mmol). This is likely an indication that the amount of ester- and ether-linked *p*-coumaric acid in the bagasse was actually higher than 6.1 wt %. That amount gave 0.33 mmol of vinylphenol-containing products during SAQ + EG analysis. If 8.0 wt % *p*-coumaric acid was to be assumed, then that should give 0.43 mmol of vinylphenol-containing products during SAQ + EG analysis and the amount of vinylphenol-containing products coming from polymeric lignin would decrease to 0.22 mmol (0.65 − 0.43). However, if the ester and ether-linked *p*-coumaric acid content were to be increased to 8.0 wt %, then the amount of polymeric lignin would decrease to 14.5 wt %. Both the G and S fractions of the overall lignin would decrease, and the uncondensed fraction of the G rings would increase beyond 74%. The total analysis will not be repeated because it would end in a conclusion that the uncondensed fraction of bagasse lignin is even higher than 91%, which is a very high value.

The conclusion of an uncondensed fraction of ∼91% is in the line with that of da Silva Perez et al. [28] who recovered bagasse lignin for an organosolv pulping effluent and analyzed it by ^{31}P NMR. Out of a total of 63 phenolic hydroxyl groups (PhOH)/100 C_9 units, only 8 of those structures were condensed. In a later investigation where ^{31}P NMR was used to analyze another bagasse organosolv lignin sample, it was found that 85% of the PhOH groups were connected to uncondensed C_9 units.[29] The slightly lower percentage of uncondensed C_9 units for organosolv lignin as compared to the present case (85% vs 91%) could be due to acid-catalyzed condensation during the pulping processes.[8] Formic acid was used in one investigation[28] while H_2SO_4 was used in the other.[29]

The very high percentage of uncondensed C_9 units would indicate that not many C_9 units in bagasse lignin are joined together by 5-5 biphenyl, β-5, and 4-*O*-5 diaryl linkages and this lignin should depolymerize at a much higher rate than hardwood or softwood lignin. When the delignified meals were recovered from SAQ + EG treatment, the pulp or solid yield was 60 ± 1.0% in the cases of the two hardwoods and bagasse. The lignin content of the pulps was 6.3 wt % for maple, 5.7 wt % for *E. camaldulensis*, but only 1.4% for bagasse.

The SAQ + EG treatment method involves 100% of the native lignin, and the entire procedure takes much less time than would be required for lignin isolation for spectroscopic analyses. Also, the lignin yield from woods and pulps is generally <70% after most isolation procedures, that is, if significant modifications are to be avoided. The time requirement for SAQ + EG would be approximately equal to that for the premethylation or first step in permanganate oxidation analysis.[30]

■ ASSOCIATED CONTENT

Supporting Information

Mass spectra used for assignment of **1**–**13** in Figure 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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