

## KRAFT PULPING CHARACTERISTICS OF THREE MOROCCAN EUCALYPTI. PART 2. COMPARISON OF THE GUAIACYL FRACTION OF THE NATIVE LIGNINS BY A NOVEL METHOD

Ericka F. Alves,<sup>a</sup> Raymond C. Francis,<sup>a,\*</sup> Badr. Barcha,<sup>b</sup> and Mohammed El Moussaouiti<sup>b,\*</sup>

In Part 1 of this series it was observed that one of the eucalypti (EGC 39) was more reactive than the other two in kraft and soda-AQ (SAQ) cooking. However, the lignin in EGC 39 contained equal or less syringyl (S) units than the other two eucalypti. In the present research an attempt was made to compare the guaiacyl (G) fraction of the three lignins. The approach was to use SAQ treatment to cleave  $\beta$ -O-4 bonds in dimeric units containing uncondensed guaiacyl A-rings (those rearranging to quinone methides). The coniferyl alcohol, vinylguaiacol and isoeugenol generated from  $\beta$ -O-4 cleavage are then trapped as dimers by ethylguaiacol that is included in the SAQ liquor. Research with sugar maple (*Acer saccharum*) showed that the estimate of these structures (uncondensed G- $\beta$ -O-4) by this approach was in close agreement with traditional but more tedious methods such as permanganate oxidation and <sup>31</sup>P NMR. It was also shown that the lignin in the EGC 39 hybrid contained a higher concentration of uncondensed G- $\beta$ -O-4 structures than the other two eucalypti lignins.

*Keywords:* Eucalyptus; Sugar maple; Alkaline pulping; Quinone methide; Uncondensed structures

*Contact information:* a : Department of Paper & Bioprocess Engineering (PBE) and the Empire State Paper Research Institute (ESPRI), SUNY College of Environmental Science & Forestry, 1 Forestry Drive, Syracuse, NY 13210 USA; b: Laboratoire de Chimie Physique générale 1, Faculté des Sciences, Université Mohammed V, Rabat, Morocco; \* Corresponding author: francis@syr.edu or elmoussa@fsr.ac.ma

### INTRODUCTION

High yields of *p*-coumaryl, coniferyl, and sinapyl alcohols are obtained when uncondensed  $\beta$ -O-4 structures in biomass lignins are treated with Soda-AQ (SAQ) liquor at elevated temperatures. The mechanism in Fig. 1 is frequently cited as an explanation (Gierer et al. 1979; Landucci 1980; Venica et al. 2008), but a credible free-radical mechanism is also supported by substantial data (Dimmel 1985; Smith and Dimmel 1994a,b). A longstanding concern in SAQ pulping is the possibility that these three lignin monomers rearrange to quinone methides (QMs) and become involved in condensation reactions with lignin and carbohydrate moieties. It was recently observed that ethylguaiacol (EG) is quite effective at condensing with monomeric QMs, and the dimers generated react only slowly in further condensation (Kanungo et al. 2009, 2011). The prior data also suggested that uncondensed  $\beta$ -O-4 structures in sugar maple (*Acer saccharum*) were cleaved at a high rate during SAQ cooking at 165°C. Also, EG appeared to trap coniferyl alcohol (CA) and its transformation products, vinylguaiacol (VG) and isoeugenol (IE), to form dimers **1** – **3** (Fig. 2) at high yields. The reactions schemes for the transformation of CA to VG and IE are in the literature (Mortimer 1982). The objective of this research was to further investigate SAQ + EG cooking too see if EG trapping of CA + VG + IE could be used to estimate the concentration of  $\beta$ -O-4 in native lignin, where the A-ring (one

forming QM in Fig. 1) is an uncondensed G unit, i.e. the concentration of uncondensed G- $\beta$ -O-4. Research would be performed with sugar maple along with the three eucalypti.

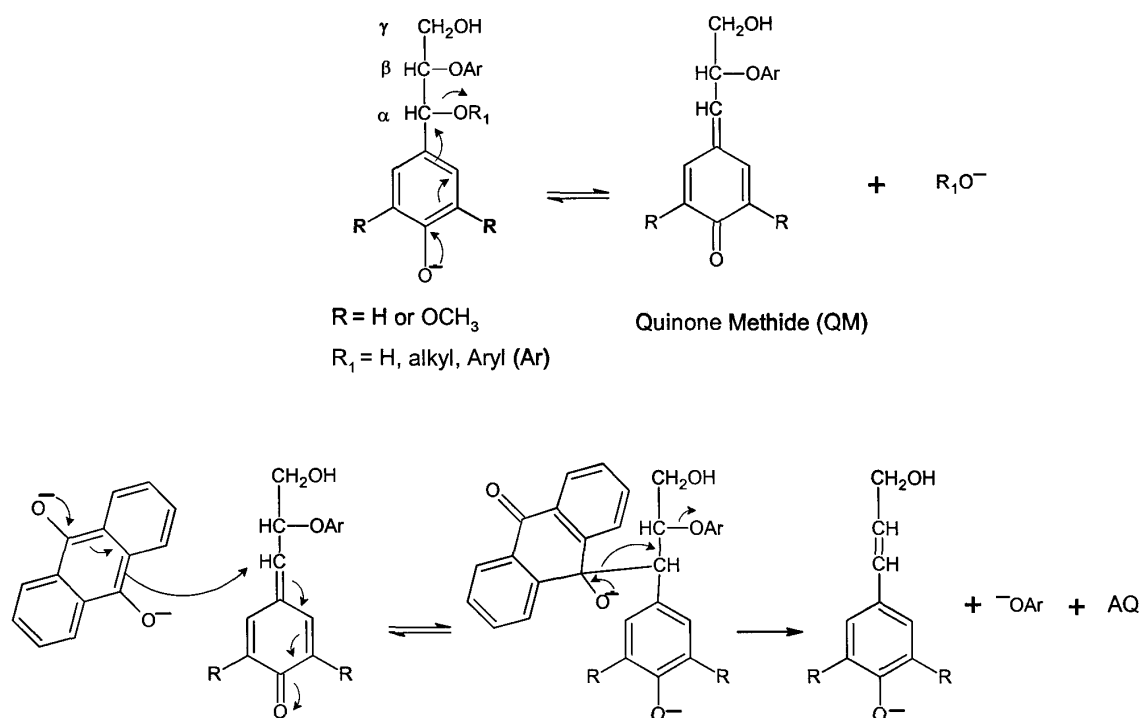


Fig. 1. SAQ depolymerization of lignin to generate *p*-coumaryl, coniferyl, and sinapyl alcohols

## MATERIALS AND METHODS

### SAQ Delignification in the Presence of Ethylguaicol (EG)

The following substrates were added to 60 mL of 0.4 M NaOH in stainless steel autoclaves; 5.0 g (OD basis) of wood meal ground to 15 mesh and extracted with ethanol-toluene; 250 mg of EG (1.64 mmoles); and 23 mg of AQ. The slurries were shaken vigorously to ensure good mixing and the autoclaves heated for 60 minutes 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 minutes to 163°C and 46 minutes at that temperature (H-factor 441). The increase in product yields was statistically insignificant when treatment time was increased to 75 min. After 60 minutes, the autoclaves were cooled, and the alkaline product mixtures were filtered through two sheets of Whatman #1 filter paper then through a 1.2  $\mu\text{m}$  Magna nylon supported plain filter (GE Osmonics). Both filtrations were aided by vacuum, and the filter cakes were washed with ca. 15 mL of 0.1 M NaOH. Each liquid was then acidified and extracted (3x75 mL) by dichloromethane (DCM) with the first extraction mixture being left overnight to ensure a good phase separation. The final DCM extract was dried over  $\text{Na}_2\text{SO}_4$  then reduced to a low volume by evaporation under vacuum. The internal standard, benzhydrol, was then added. A fraction of the DCM solution (100  $\mu\text{L}$ ) was added to another vial along with 100  $\mu\text{L}$  of BSTFA [N,O-bis(trimethylfluoromethylsilyl)acetamide] and a drop of pyridine. The mixture was allowed to sit at room temperature overnight or for ca. 30 minutes at 40°C before being analyzed by gas chromatography - mass spectrometry (GC/MS).

## GC/MS and Other Analyses

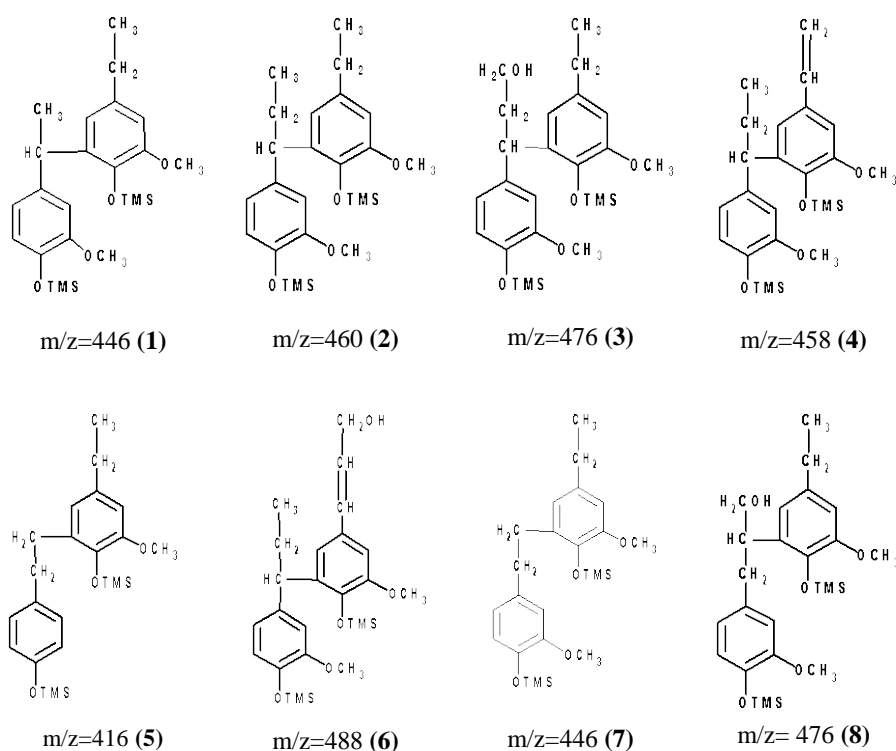
GC-MS analyses were performed using a Thermo Scientific Finnigan Trace GC Ultra GC coupled to Thermo MAT95XP double focus magnetic sector mass spectrometer. The column used was a 30 m x 0.25 mm ID., Rtx®-5MS (5% diphenyl/95% dimethylpolysiloxane) capillary column (film thickness 0.25  $\mu\text{m}$ ). Helium at a flow rate of 1 mL/min was used as the carrier gas. About 1  $\mu\text{L}$  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 initial temperature 110°C (hold for 4 min); a ramp from 110°C 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 minutes. Ionization was carried out at a 70 eV impact voltage in an ion chamber heated at 280°C. The MS range scanned was 45-800 m/z at a rate of 0.6 scan/second. Peak identification was carried out on the basis of mass fragmentation patterns, and by comparing the MS data with those in the Pfleger-Mauer-Weber, Wiley, and NIST libraries.

Lignin content analyses and S:G ratio by nitrobenzene oxidation (NBO) and methoxyl analyses are reported in Part 1 of this series (El Moussaouiti et al. 2012). All analyses were performed in duplicate or triplicate.

## RESULTS AND DISCUSSION

### Results for Sugar Maple Wood Meal

The maple wood meal was SAQ delignified in the presence of EG, and it appeared as if a high fraction of the estimated CA that would be generated was trapped as dimers **1** – **4** and **6** – **8** in Fig. 2.



**Fig. 2.** Dimers produced from SAQ + AQ treatment of hardwood meal (after silylation)

The wood meal contained 24.8% lignin, and the S:G ratio of that lignin was 1.25. A reasonable estimation for the molecular weight of a C<sub>9</sub> unit in the lignin of a hardwood like sugar maple is 210 Daltons (Masingale et al. 2009) The number of mmoles of C<sub>9</sub> unit used was approximately 5.90 (0.248 x 5000 mg ÷ 210). The yields of the dimers obtained from sugar maple are documented in Table 1.

A segment of the chromatogram from the GC-MS analysis is shown in Fig. 3, and the minor peaks beyond the one at 36.91 min (for dimer **3**) were larger than those observed in the initial research of Kanungo et al. (2011). Based on the limited data collected by Kanungo et al. (2011) it was decided that the reproducibility on the yield of the minor peaks beyond dimer **3** was less than satisfactory, and they were not included in the initial tabulation of those authors. Instead, the chromatogram selected by them was one with large peaks for **1** to **3** and very small peaks for the minor products. That chromatogram was selected to demonstrate that the three dimers from sinapyl alcohol that would correspond to **1** to **3** were not detected at significant yields. However, in subsequent research these minor products, **4** - **8** in Fig. 3 (sugar maple) and Fig. 4 (EGC 241; *E. grandis* x *E. camaldulensis*) were detected more reproducibly, and it was decided that they should be included in the tabulation of condensation products.

**Table 1.** Yields of Residual EG and Dimers Generated in SAQ+EG Cooking of Maple Wood

Compound	m/z	Yield, mmoles <sup>1</sup>	Derived from CA <sup>3</sup>
EG		1.15 <sup>2</sup>	
VG-EG ( <b>1</b> )	446	0.10	0.10
IE-EG ( <b>2</b> )	460	0.06	0.06
CA-EG ( <b>3</b> )	476	0.24	0.24
IE-VG ( <b>4</b> )	458	0.02	0.04
VP-EG β-linked ( <b>5</b> ) <sup>4</sup>	416	0.02	0
IE-CA ( <b>6</b> )	488	0.01	0.02
VG-EG β-linked ( <b>7</b> )	446	0	0
CA-EG β-linked ( <b>8</b> )	446	0.01	0.01

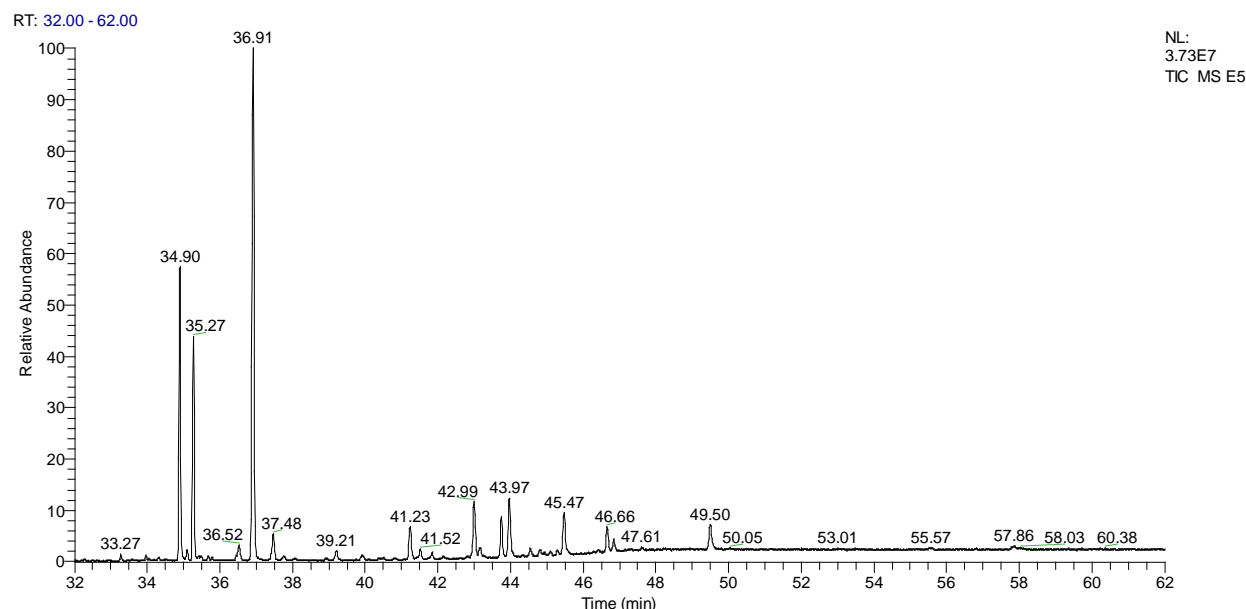
<sup>1</sup> From 5.90 mmoles of C<sub>9</sub> units, see text  
<sup>2</sup> Initial dose of EG = 1.64 mmoles (250 mg)  
<sup>3</sup> mmoles of monomers (0.47 in total)  
<sup>4</sup> Vinylphenol (VP) derived from *p*-coumaryl alcohol (H unit)

Dimers **1** – **8** are assigned to the peaks in Fig. 4, 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 rare 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 CA monomers. Therefore, although only 0.02 mmole of **4** was detected (Table 1), the dimer was produced from 0.04 mmole of CA, and as such the yield of this product is almost as significant as that of **2**.

### Assignment of Dimers from MS Data

The spectra used for the assignments of **1** – **3** have already been presented and analyzed and in the case of **1** and **3**, spectra for the GC/MS peaks from both the un-derivatized and silylated product mixtures were presented and analyzed (Kanungo et al. 2011). The spectrum assigned to **3** is presented in Fig. 5, and the assignment is conclusive (Kanungo et al. 2011). There are well established mechanisms to explain the  $\alpha$ -5 condensation responsible for the generation of **3**. These mechanisms are both ionic (Gierer 1970, 1980, 1985) and free-radical in nature (Dimmel et al. 1994; Smith and Dimmel 1994a,b; Dimmel 1996). However,  $\beta$ -5 condensation is much less common in alkaline pulping chemistry. Justification for the assignment of  $\beta$ -5 structures will be attempted before a possible mechanism for their formation is discussed. The spectrum assigned to **8**, the  $\beta$ -5 counterpart of **3**, is presented in Fig. 6. Cleavage of the  $\alpha$ -5 bond in **3** or the  $\beta$ -5 bond in **8** would result in fragments with  $m/z = 253$  and  $223$ , respectively. Both peaks, albeit very small, are observed in Fig. 5, while a more sizeable  $m/z = 253$  peak is observed in Fig. 6. Cleavage of the  $C_1$ - $C_\alpha$  would give fragments with  $m/z = 281$  and  $195$ . The  $m/z = 281$  fragment is observed in Fig. 6 but not Fig. 5. Cleavage of the  $C_\alpha$ - $C_\beta$  bond in **8** would give fragments with  $m/z = 267$  and  $209$ , and both fragments are observed in Fig. 6. Interestingly, the  $M^+ - 15$  peak is large in Fig. 5 but small in Fig. 6. This phenomenon appears to be common to all the  $\beta$ -5 dimers. Another example is shown in Fig. 7, a spectrum assigned to **5**. Cleavage of the  $C_\alpha$ - $C_\beta$  bond in **5** would give fragments with  $m/z = 237$  and  $179$ , and both fragments are observed in Fig. 7. Once again, the  $M^+ - 15$  peak is very small relative to the  $M^+$  peak (Fig. 7).

The MS for **4**, **6**, and **7** are presented in Figs. 8 through 10, and the assignments for **6** and **7** are fairly conclusive. The assignment for **4** is based on the similarity of its MS to that of **6** (Figs. 8 and 9).

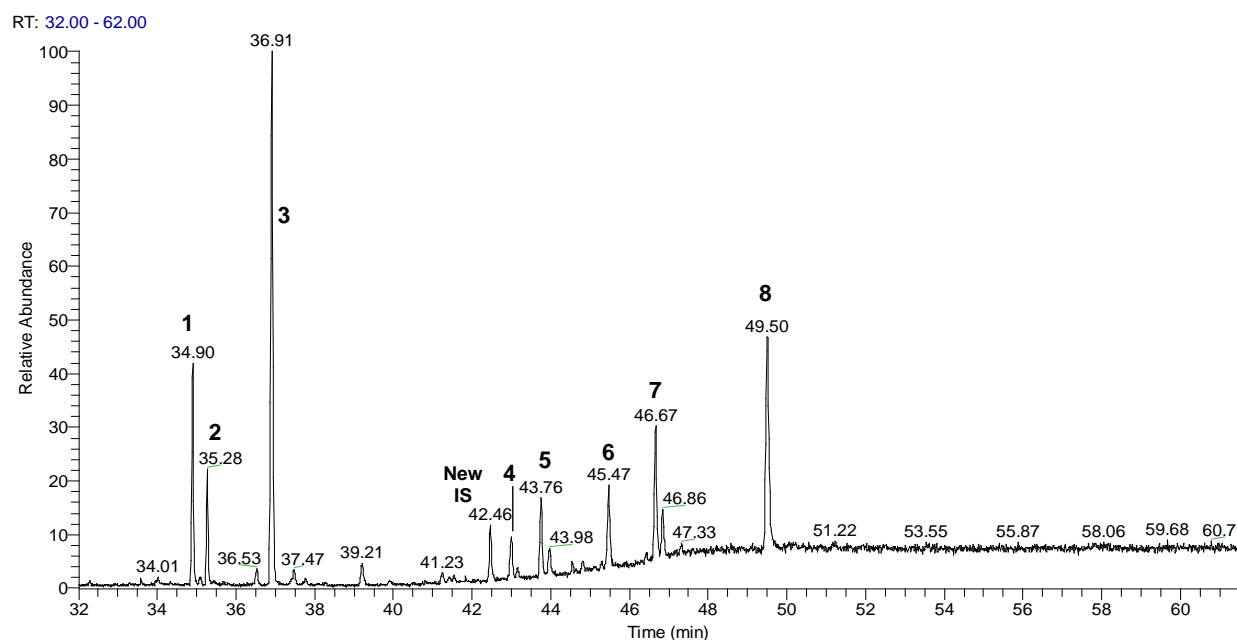


**Fig. 3.** A section of GC/MS chromatogram showing dimeric products from SAQ+EG treatment of sugar maple wood meal at a 12:1 liquor to wood ratio

### A Possible Mechanism to Explain $\beta$ -5 Condensation

The  $\beta$ -5 condensation pattern is not normally seen in alkaline pulping chemistry. However, it was clearly seen in one case where 2,6-xyleneol was added to soda cooking (1.0 M NaOH at  $150^\circ$ ) of a  $\beta$ -O-4 dimer. The mole ratio of 2,6-xyleneol to the  $\beta$ -O-4 dimer was  $>1.0$  (Gierer and Pettersson 1977). Similar to EG in the present case, the

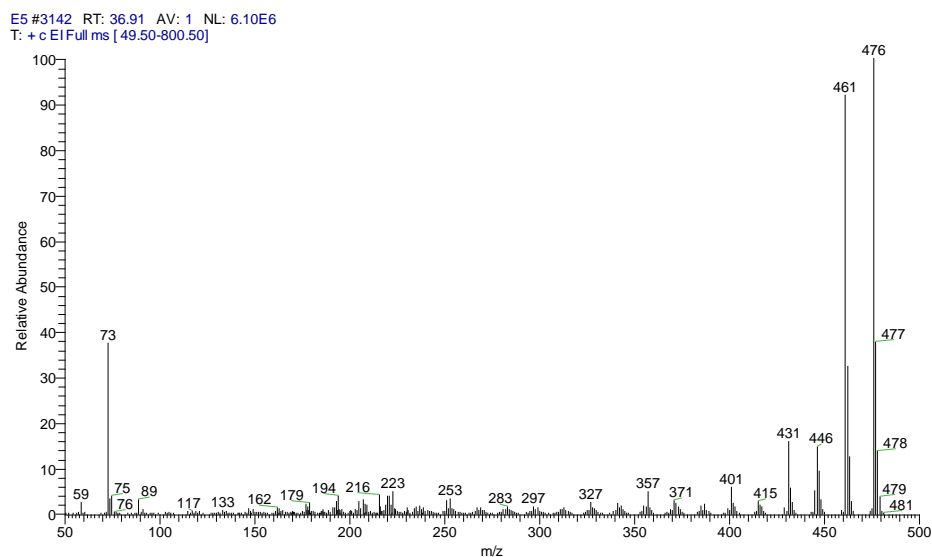
xylenol generated an alkali-stable phenolate and contained two electron-donating groups. The mechanism proposed by those authors involved the xylenol adding to the QM as a para-cyclohexadienone carbanion, resulting in ring condensation with the  $\alpha$ -carbon of the  $\beta$ -O-4 dimer. The xylenol, now with a C-C bond para to the phenolate group, once again rearranged to a para-cyclohexadienone carbanion, cleaving the neighboring  $\beta$ -O-4 bond with simultaneous migration of the xylenol from the  $\alpha$ -carbon to the  $\beta$ -carbon (Gierer and Pettersson 1977). A fairly similar mechanism with EG rearranging to an ortho-cyclohexadienone carbanion is shown in Fig. 11. The product is a  $\beta$ -5 dimer containing a vinylic group. Unlike the previous research by Gierer and Pettersson (1977), reducing agents were involved in the present research. The vinylic group in the end product of Fig. 11 could have been reduced by the anthrahydroquinone dianion or anthrahydroquinone radical anion to give **5**, **7** and **8** in Fig. 2. In enzymatic processes, vinylphenols are known to be reduced to ethylphenols (Chatonnet et al. 1992), while CA is reduced to dihydroconiferyl alcohol (Savage and Forster 2001).



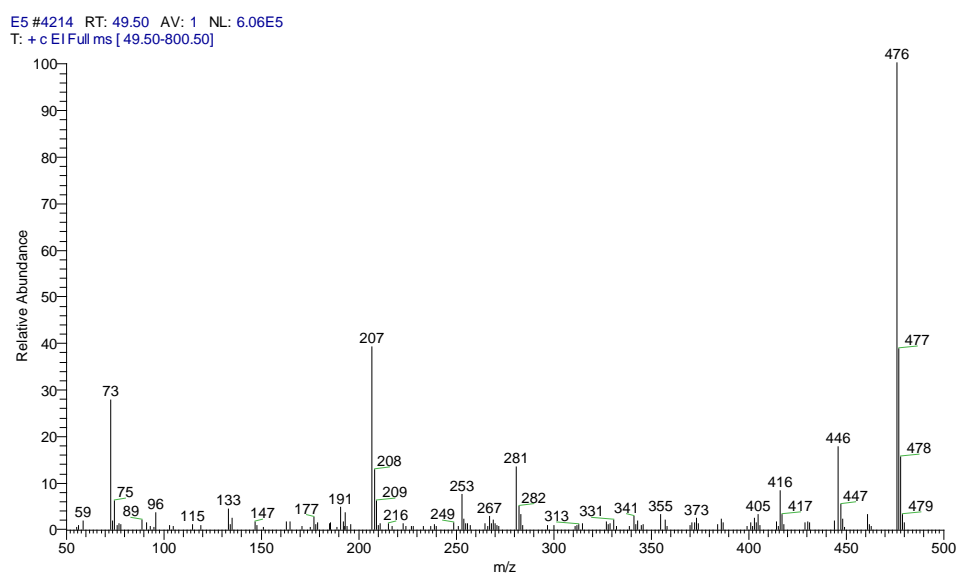
**Fig. 4.** A section of GC/MS chromatogram showing dimeric products from SAQ+EG treatment of EGC 241 wood meal at a 12:1 liquor to wood ratio

### Yield of CA Derived Dimers as Related to Lignin Structure

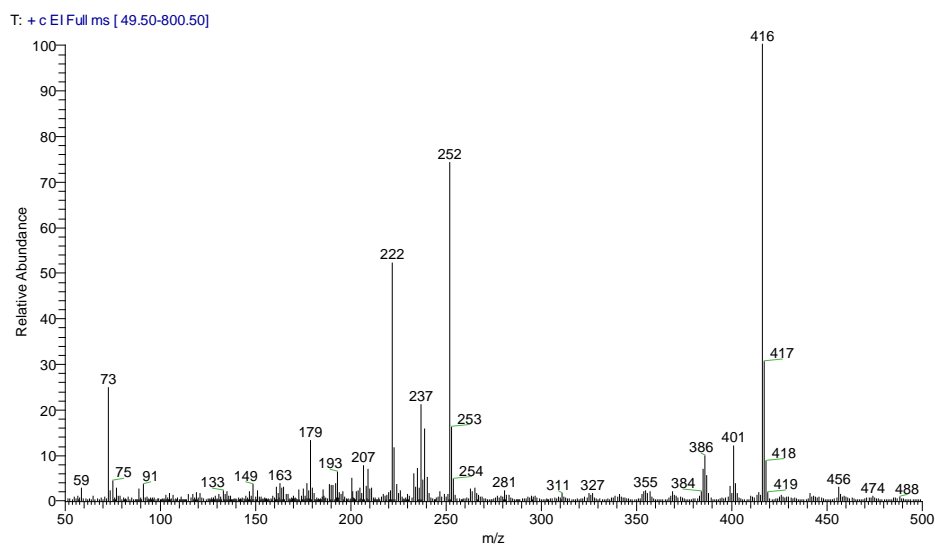
A discussion on the expected yield of CA that should be generated from the maple wood meal will now be attempted. As previously discussed, the wood meal contained 24.8% lignin, the S:G ratio of that lignin was 1.25 and the number of mmoles of  $C_9$  unit used was approximately 5.90. A credible estimate of the percentage of the  $C_9$  units containing either an  $\alpha$ -OH or an  $\alpha$ -ether and involved in  $\beta$ -O-4 linkages is ca. 30% (30 dimers/100  $C_9$ ) (Adler 1977), and one mole of CA would be generated from one mole of  $\beta$ -O-4 dimer (Fig. 1). The CA would come from the A-ring, the one forming the QM (Fig. 1). If it is assumed that 43.5% of the  $C_9$  units were guaiacyl and 54.5% were syringyl (S:G = 1.25), then A rings would constitute ca. 30% of the  $C_9$  units in the lignin, and 43.5% of them would be guaiacyl. The total guaiacyl A-rings would be ca. 0.77 mmole (5.90 x 0.30 x 0.435).



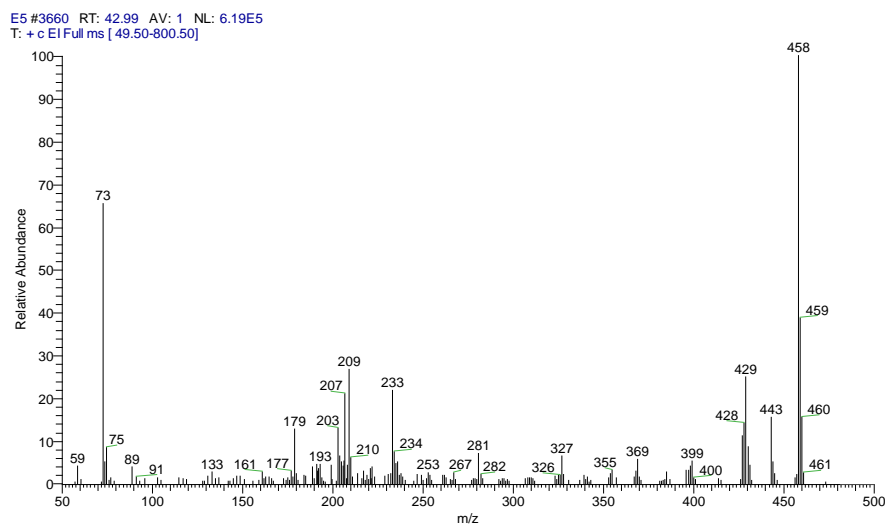
**Fig. 5.** Mass spectrum for the compound **3**, an  $\alpha$ -5 linked CA-EG dimer with MW of 476



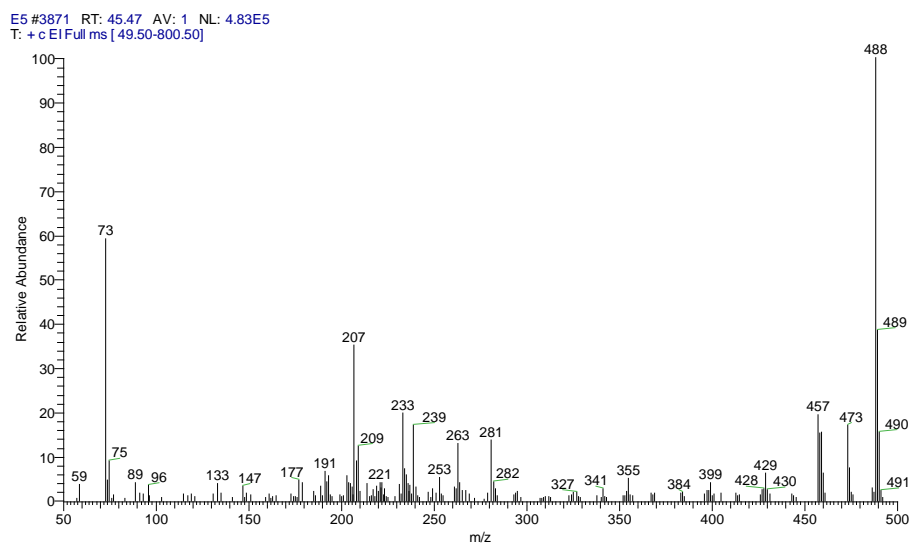
**Fig. 6.** Mass spectrum for the compound **8**, a  $\beta$ -5 linked CA-EG dimer with MW of 476



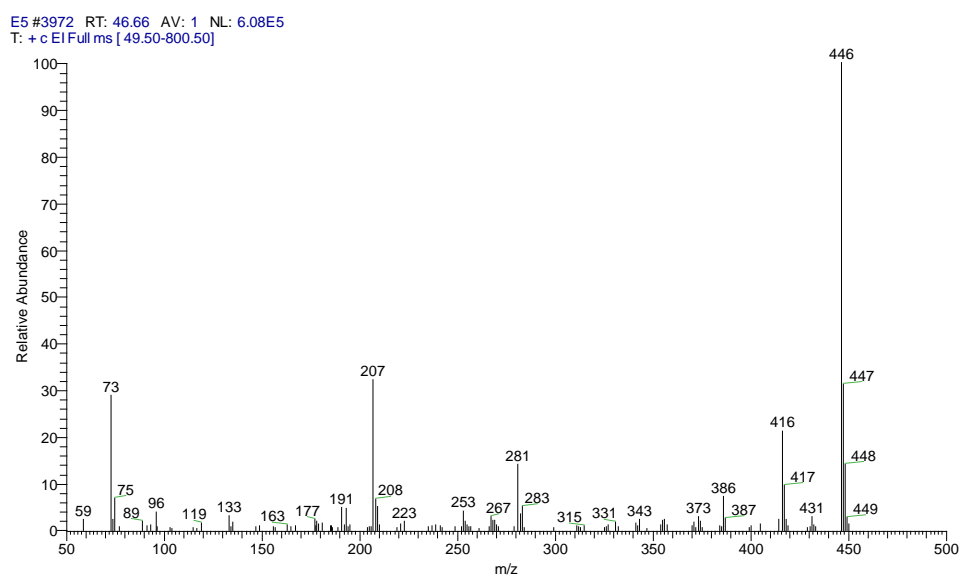
**Fig. 7.** Mass spectrum for the compound **5**, a  $\beta$ -5 linked VP-EG dimer with MW of 416



**Fig. 8.** Mass spectrum for the compound **4**, an  $\alpha$ -5 linked IE-VG dimer with MW of 458



**Fig. 9.** Mass spectrum for the compound **6**, an  $\alpha$ -5 linked IE-CA dimer with MW of 488



**Fig. 10.** Mass spectrum for the compound **7**, a  $\beta$ -5 linked VG-EG dimer with MW of 446



In all probability, CA would only be produced from uncondensed guaiacyl A-rings. An uncondensed  $C_9$  unit is defined as one not containing a C-C bond at any ring position except for C-1 (sidechain) nor connected to another  $C_9$  unit by a diaryl ether linkage. The total yield of CA derived dimers was 0.47 mmole (Table 1). If it is assumed that all the  $\beta$ -O-4 dimers with uncondensed A-rings were converted to CA then the uncondensed fraction of the total guaiacyl A-rings would be 61% (0.47/0.77).

Evtuguin et al. (2001) analyzed *Eucalyptus globulus* by permanganate oxidation and found that 61% of the G units were uncondensed. Using the same permanganate oxidation technique, Bose et al. (2009) later obtained values of 65.4%, 65.4%, and 69.0%, respectively for the fraction of uncondensed G units from two poplar wood meals along with that from a 1:1:1 mixture of sugar maple (*Acer saccharum*), paper birch (*Betula papyrifera*), and cottonwood (*Populus deltoides* Bartr.). The belief that the G fraction of hardwood lignin is approximately 60% uncondensed is supported by data from  $^{31}\text{P}$  NMR. Wu and Argyropoulos (2003) prepared milled wood lignin (MWL) and enzymatic mild acidolysis lignin (EMAL) from aspen (*Populus tremuloides*). The fraction of the total  $C_9$  units that was phenolic was ca. 20% for both samples. When analyzed by  $^{31}\text{P}$  NMR, the average value for uncondensed phenolic G units in EMAL and MWL was 0.35 mmole/g, while the concentration of total condensed phenolic units was 0.25 mmole/g. If it is assumed that 80% of the condensed phenolics are in the G fraction, then one arrives at ca. 0.35 mmole/g of uncondensed and 0.20 mmole/g of condensed phenolic units (ca. 64% uncondensed).

Finally, it should be noted that the consumption of EG in Table 1 was 0.49 mmoles (1.64-1.15). Therefore, EG appears to react almost exclusively with CA and its transformed products (VG and IE). Of the total EG consumption of 0.49 mmoles, VG, IE and CA consumed 0.41 mmoles.

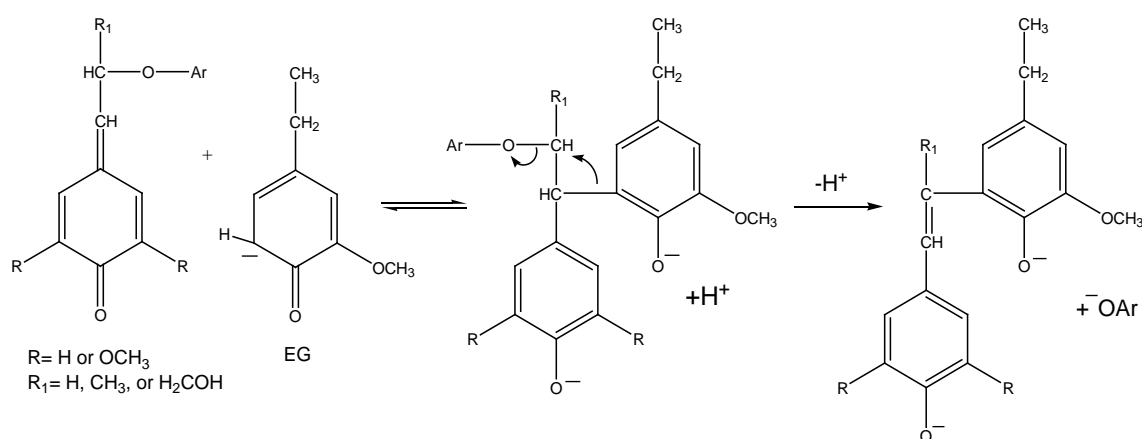


Fig. 11. Proposed mechanism for the formation of  $\beta$ -5 dimers during SAQ + EG treatment

### SAQ + EG Analysis of the Three Eucalypti

The lignin content, % S units, % G units, total number of  $C_9$  units, and mmoles of G units are summarized in Table 2 for the wood meals from sugar maple and the three eucalypti. It can be seen that even though the eucalypti all contained more lignin than the sugar maple, each of them afforded less G units for the SAQ + EG treatment. The percentages of S units and G units were calculated from the average S:G based on nitrobenzene oxidation (NBO) and methoxyl analyses as discussed in Part 1 (El Moussaoui et al. 2012).

**Table 2.** Lignin Content and Distribution of S Units and G Units for the Four Hardwood Meals

Sample	Lignin Content, %	%S <sup>1</sup> Units	%G <sup>1</sup> Units	Total mmoles <sup>2</sup> of C <sub>9</sub> units	mmoles <sup>2</sup> of G units
Sugar Maple	24.8	54.5 <sup>3</sup>	43.5	5.90	2.57
<i>E. camaldulensis</i>	30.6	65.5	32.5	7.22	2.35
EGC 39	27.8	62.0	36.0	6.56	2.36
EGC 241	28.9	63.0	35.0	6.82	2.39

<sup>1</sup>Average value from NBO and methoxyl analyses (El Moussaoui et al. 2012).  
<sup>2</sup>In 5g of wood meal and assuming C<sub>9</sub> molecular wt. of 210 for maple and 212 for eucalypti  
<sup>3</sup>S and G units by NBO only

The yields of dimers **1 - 8** from SAQ + EG treatment are summarized for the three eucalypti in Table 3. The total yield of CA derived dimers from EGC 39 was ca. 10% higher than for the other two eucalypti. Even though this is a relatively small difference, there were only minor variations in yields between duplicate analyses for all three samples. As shown in Fig. 4, the GC/MS peaks are well resolved, and area ratio of peaks relative to the internal standard was very repeatable and reproducible. The dimer yield from EGC 39 was equal or higher than for the other two eucalypti for 7 out of the 8 dimers. The SAQ + EG treatment method allows one to monitor small differences in the G fraction of hardwood lignins, and this is usually not possible with most of the other methods of lignin analyses.

**Table 3.** Yields of Dimers **1 – 8** Generated from the Three Eucalypti<sup>1</sup>

Compound	<i>E. camaldulensis</i>	EGC 39	EGC 241
VG-EG ( <b>1</b> )	0.06	0.07	0.06
IE-EG ( <b>2</b> )	0.02	0.02	0.02
CA-EG ( <b>3</b> )	0.16	0.16	0.15
IE-VG ( <b>4</b> ) <sup>2</sup>	0.01	0.02	0.01
VP-EG $\beta$ -linked ( <b>5</b> ) <sup>3</sup>	0.02	0.03	0.02
IE-CA ( <b>6</b> ) <sup>2</sup>	0.01	0.02	0.02
VG-EG $\beta$ -linked ( <b>7</b> )	0.02	0.03	0.03
CA-EG $\beta$ -linked ( <b>8</b> )	0.07	0.06	0.06
Total CA Derived	0.37	0.42	0.38

<sup>1</sup> From 5 g of wood meal                      <sup>2</sup> Derived from two CA molecules  
<sup>3</sup> Not CA derived and excluded in the summation

The importance of being able to monitor small differences in lignin structure is demonstrated by the results in Table 4, where % delignification by SAQ + EG and % uncondensed G- $\beta$ -0-4 in the G fraction are calculated. The kappa number for *E. camaldulensis*, EGC 39, and EGC 241 after SAQ + EG was 37.9, 31.7, and 38.6, respectively, and this would indicate that the EGC 39 would be best suited for the production of pulp with a low kappa number. However, when kappa number and recovered fiber yield were used to calculate % delignification in SAQ + EG treatment, the values for the three eucalypti fell in a narrow range of 88% to 90%. The EGC 39 pulp had a significantly lower kappa number but the lignin content of the EGC 39 chips was also lower than for the other two samples. The number of uncondensed G- $\beta$ -0-4 dimeric units per 100 guaiacyl C<sub>9</sub> was calculated as 15.7, 17.8, and 15.9 for *E. camaldulensis*, EGC 39 and EGC 241, respectively. Since the EGC 39 contained equal or less S units than the other two eucalypti the two extra uncondensed G- $\beta$ -0-4 dimeric units in the G

fraction of its lignin is a likely contributor to its slightly higher reaction rate in kraft pulping (Part 1) and SAQ + EG treatment in the present investigation. It should be noted that all three eucalypti were delignified to a higher degree than was sugar maple (Table 4). The likely explanation is the higher number of G units that was involved in the treatment of the maple wood meal (Table 2).

**Table 4.** Extent of Delignification and Ratio of Uncondensed G- $\beta$ -O-4 to Total G Units Derived from SAQ + EG Treatment

Sample	Fiber <sup>1</sup> Recovery, %	Kappa <sup>1</sup> Number	% Delign. <sup>2</sup>	Mmoles of G- $\beta$ -O-4	G- $\beta$ -O-4, % of total G <sup>3</sup>
Sugar Maple	60	41.9	85	0.47	18.3
E. camaldulensis	59	37.9	89	0.37	15.7
EGC 39	61	31.7	90	0.42	17.8
EGC 241	61	38.6	88	0.38	15.9

<sup>1</sup>Recovered fibers after SAQ + EG  
<sup>2</sup>Residual lignin in fibers calculated as 0.15 x kappa number  
<sup>3</sup>Values given in Table 2

## CONCLUSIONS

It appears that when 15 mesh meal samples from hardwoods were SAQ delignified to an H-factor of 441, almost all of the  $\beta$ -O-4 linkages with uncondensed guaiacyl A-rings were cleaved. The primary product from  $\beta$ -O-4 cleavage was CA, which was ultimately trapped in high yield by EG. When sugar maple was analyzed by SAQ + EG, the estimation of uncondensed G- $\beta$ -O-4 concentration was in close agreement with results previously reported for hardwoods by traditional but more tedious methods such as permanganate oxidation and <sup>31</sup>P NMR. The SAQ + EG treatment method involves 100% of the native lignin and the entire procedure takes less time than would be required for lignin isolation for spectroscopic analyses. Also, the lignin yield after most isolation procedures is generally <70%, i.e. if significant modifications are to be avoided. The time requirement for SAQ + EG would be approximately equal to that for the pre-methylation or first step in permanganate oxidation analysis (Gellerstedt 1992; Bose et al. 1998). Of all the G units that were A-rings in  $\beta$ -O-4 linkages in sugar maple lignin, it was found that ca. 60% of them were uncondensed (18 out of 30). The method was then used to compare the three eucalypti, and it was observed that the lignin in EGC 39 that was more reactive contained 17.8 uncondensed G- $\beta$ -O-4 dimeric units per 100 G units. The other two eucalypti that were less reactive contained only 15.7 and 15.9 uncondensed G- $\beta$ -O-4 dimeric units per 100 G units.

## ACKNOWLEDGEMENTS

We deeply appreciate the financial and technical support obtained from Cellulose du Maroc. We thank all of its members and in particular the Director of Production, Dr. Abdelaziz Rmili. For research performed in Syracuse, New York, the financial support from NSF Grant CHE 0848391, Andritz Inc and the Empire State Paper Research Associates (ESPRA) is deeply appreciated. Finally, we are most grateful to Dr. Lucian Lucia of North Carolina State University for his assistance in formulating the mechanism to explain  $\beta$ -5 condensation that is shown in Fig. 11.

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Article submitted: August 18, 2011; Peer review completed: January 9, 2012; Revised version received: January 31, 2012; Second revised version: February 8, 2012; Accepted: February 8, 2012; Published: February 11, 2012.