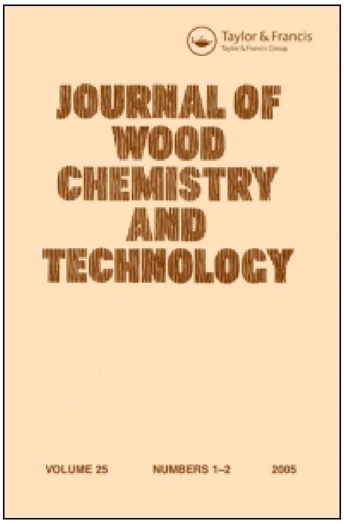

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On the Determination of β-Aryl Ether Units in Wood Lignin by an Ozonation Method

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ABSTRACT

The determination of lignin structures by ozonation is rather unique and unlike other analytical techniques is based largely on the formation of side-chain oxidation products. Also, it can readily reveal the proportion of the two stereoisomers of β-aryl ether structures from the yields of erythronic (E) and threonic (T) acids. We have observed that the relative yields of these two acids vary significantly depending on how they are determined. For example, the E/T ratio of these two

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tetronic acids from the ozonation of spruce wood lignin in situ varies from 1.10 to 1.57 based on their analysis as the free acid and lactone derivatives, respectively. Thus, the work-up procedure of the reaction products is very important to the ozonation technique.

Key Words: Ozonation; β-Aryl ether units; Erythro/threo ratio; Lignin analysis.

INTRODUCTION

Structural studies of lignin in wood or pulp samples[1] can be pursued either in situ or on the isolated lignin preparations. The major advantage of the latter approach is that the lignin sample can be analyzed by physical techniques, notably the proton, ^{13}C, and ^{31}P NMR spectrometry.[2–4] However, the lignin preparations[5] are often difficult to obtain quantitatively from the wood or pulp substrates. On the other hand, the in situ approach can reveal the intrinsic nature of the whole lignin component based on the characteristics of the lignin degradation products. The common lignin degradation techniques,[6–10] include acidolysis, thioacidolysis, nitrobenzene oxidation, permanganate oxidation, and ozonation. Each of these methods has its pros and cons, as it can only reveal certain types of the noncondensed or condensed units.

Among the lignin degradation methods, the ozonation process[10] is rather unique, as it unlike others is based on products derived largely from the side-chain unit. This technique was developed earlier by the Nakano group[11–13] to determine the major types of lignin structures (Fig. 1). These include the formation of erythronic (1_a) and threonic (1_b) acids from the β-*O*-4 units I, and of dicarboxylic acids 2 from the β-5 and β-1 structures (II). Also, Matsumoto et al.[11] suggested that ozonation of the α-aryl condensed units III would yield the dicarboxylic acid 3. Subsequently, Sarkanen et al.[10,14] reported a similarity between the ozonation and acidic permanganate oxidation reactions in lignin degradation while ozonation is a better technique. They also identified the presence of 3,4-dihydroxybutanoic acid 4 among the ozonolysis products of Klason lignin samples, and this acid was assumed being derived from decarboxylation of the dicarboxylic acid 3. We have recently confirmed this proposed pathway based on the ozonation products of an α-condensed dimer of III.[15,16] Also, we have been using this technique to complement our studies[15,16] on the characterization of the pulp residual lignin.

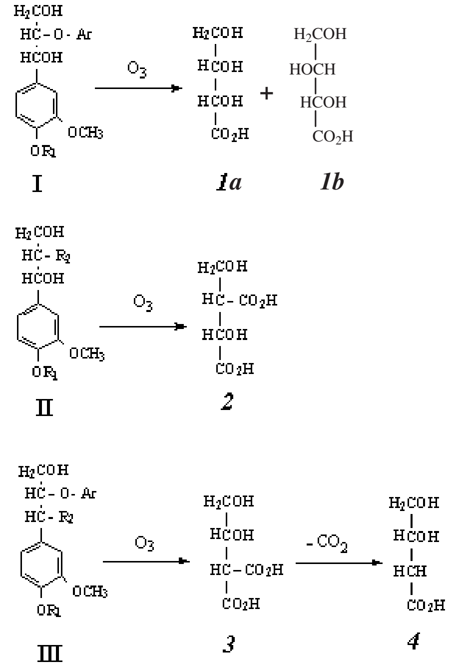

Figure 1. Ozonation products characteristic of the lignin units.

β-Aryl Ether Units in Wood Lignin

Recently, the use of ozonation as an analytical technique has continued to attract much attention.[17–23] Perhaps one of the salient applications of ozonation in lignin chemistry is in determination of the β-*O*-4 aryl ether structure, especially to reveal its relative proportion of the erythro- (E) and the threo- (T) subunits, since there is a stereospecific conversion of these units to the respective tetraonic acids (1_a and 1_b). This structural information is a subject of both fundamental and practical interest, as it relates to the nature of lignin biosynthesis as well as to the lignin reactivity.[24,25]

Table 1 summarizes the reported E/T ratios for the spruce wood lignin and milled wood lignin (MWL) determined by the NMR[26–28] and ozonation[10–13] methods. It is evident that all the values are very similar ($\sim$1), except a considerably higher ratio of 1.5 for the spruce wood lignin reported by Taneda et al.[13] Interestingly, Sarkanen et al.[10]

Table 1. The erythro/threo (E/T) ratios of β-*O*-4 aryl ether structures in the spruce wood lignin.

Material	E/T	Method	Ref.
MWL	~1	^{1}H NMR	Hauteville et al.[26]
MWL	~1	^{1}H NMR	Lundquist[27]
MWL	1.0	^{31}P NMR	Jiang and Argyropoulos[28]
MWL	1.1	Ozonation	Taneda et al.[13]
Wood	1.5	Ozonation	Taneda et al.[13]
Wood	1.05	Ozonation	Sarkanen et al.[10]

obtained a low ratio of 1.05 for the same wood species by ozonation. We have been interested in understanding the contributing factors to this apparent discrepancy in two ozonation studies.

The overall ozonation procedure is consisted of three basic steps: ozonation, saponification, and derivatization. A careful examination of the procedures used by the Taneda et al.[13] and by the Sarkanen et al.[10] reveals a major difference between the saponification and the derivatization stages. In the Taneda's case, the ozonation mixture after saponification was passed through a cation exchange column in the H^+ form to remove the sodium ions. This work-up procedure leads to the lactonization of erythronic (1_a) and threoinc (1_b) acids, and the resulting lactone products were actually determined. In the Sarkanen's method, the saponification solution was treated with a cation exchange in the NH_4^+ form to avoid the lactone formation. The resulting ammonium tetronates were then silylated and analyzed. The extent to which these two procedures may affect the resulting E/T ratio of lignin is the subject of this study.

In this article, we have determined the formation of erythronic (1_a) and threonic (1_b) acids from ozonation of the wood meal samples for a softwood (Norway spruce) and a hardwood (aspen) species, and their yields were compared with three different work-up procedures for the ozonation products.

EXPERIMENTAL

Materials

Samples of the extractive-free wood meal (40–60 mesh) were prepared by extraction with acetone overnight and air-dried. The species studied were Norway spruce (*Picea abies*) and aspen (*Populus tremuloides*).

The total (Klason plus acid-soluble) lignin contents of spruce and aspen wood were 28.8 and 22.2%, respectively.

Ozonation

The ozonation of wood meal sample (100 mg) followed the general procedure of Tsutsumi et al.[14] using an Union Carbide ozone generator (model SG4050). It was conducted in a mixed solvent (100 mL) consisting of acetic acid, water, and methanol (16:3:1) placed in a plastic bottle. A flow of oxygen containing ~2.5% of ozone was bubbled through the reaction mixture at the rate of about 0.6 L/min at 0°C for 2 h. The residual ozone was removed by aeration for 30 min. The reaction mixture was filtered and evaporated to dryness in vacuo at 40°C. The residue was dissolved in distilled water (20 mL) and saponified (by adjusting the mixture to pH 11 with 0.5 M NaOH)) under nitrogen and room temperature overnight. The saponification solution was then worked up for the GC analysis.

Work-Up Procedures

Three different work-up methods were examined. The first one (Method A) followed the procedure of Tsutsumi et al.[14] The saponification mixture was passed through a column filled with 15 mL of Amberlite IR-120 (NH_4^+) cation exchange resin. A 1 mL of an internal standard solution containing 1 mg of pentaerythritol was added to the total effluent collected (250 mL). The product mixture was then evaporated to dryness in a vacuum evaporator, and the dry residue was then trimethylsilylated in pyridine (1 mL) with *N*,*O*-*bis*(trimethylsily)trifluoracetamide (BSTFA) (1 mL) containing 1% chlorotrimethylsilane.

The second procedure (Method B), was similar to Method A except that the saponification mixture was passed through a column filled with 15 mL of Amberlite IR-120 (H^+) cation exchange resin. Also, both pentaerythritol and vanillic acid were added to serve as internal standards.

The third one (Method C) was identical to Method B except that the final dry product mixtures were heated in an oven at 70°C for 10 min prior to trimethylsilylation.

Analytical Methods

The lignin (Klason plus acid-soluble) content of wood samples was determined according to the Tappi Standard Methods. A Hewlett

Packard 5890A gas chromatograph equipped with flame ionization detectors, a computerized integrator, and a Quadrex fused silica capillary column (50 m × 0.25 mm × 0.25 μm) packed with a 007 series methyl silicone was used to analyze the ozonation products. The temperature profile was: held at 150°C for 0.5 min, 150–270°C (4°C/min), and final temperature (20 min). Authentic samples were used in the product identification and quantification.

RESULTS AND DISCUSSION

In this study, the yields of erythronic (E) and threonic (T) acids from ozonation of the Norway spruce and aspen wood meal were determined, especially with respect to how they were impacted by the work-up procedures for GC analysis. The ozonation mixture was processed in three variations. In Method A, the saponfication mixture was treated with a cation exchange resin in the NH_4^+ form. This leads to the analysis of two tetronic acids (E and T) as the free-acid derivatives. In Method B, the saponification mixture was treated with a cation exchange resin in the H^+ form. This results in lactonization of the tetronic acid, and the resulting erythronic γ-lactone and threonic γ-lactone were then trimethylsilylated for analysis. In Method C, the procedure was identical to Method B, with the exception that the final dry products were heated at 70°C for 10 min prior to the trimethylsilylation in an attempt to promote the lactonization process.

Norway Spruce Wood Lignin

Figure 2 illustrates the gas–liquid chromatograms of ozonation products from the spruce wood meal prepared by the three different procedures noted earlier. Since the same amounts of wood sample and internal standard were used in all the cases, the relative intensity of peaks may provide a qualitative indication of the product yield. Several interesting features are evident. In Method A, the peak intensity of No. 2 (erythronic acid), No. 3 (threonic acid), and No. 1 (pentaerythritol standard) was approximately equal. In Method B, the peak 4 (threonic acid γ-lactone) was significantly smaller than that of No. 5 (erythronic acid γ-lactone). Also, these two peaks along with the peak 1 (pentaerythritol standard) were very small as compared to that of Method A. Thus, it appears that the lactonization of threonic and

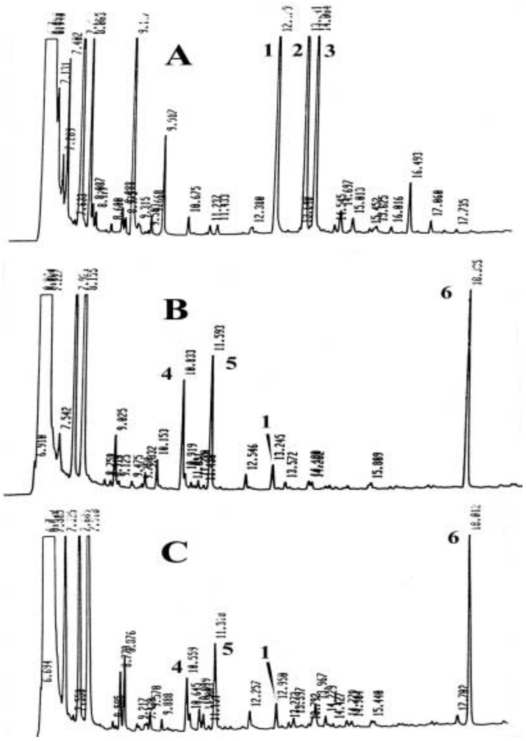

Figure 2. Gas–liquid chromatograms of the ozonation products from Norway spruce wood meal prepared by three different work-up procedures: A (Method A), B (Method B), and C (Method C). The numbered peaks are: 1. Internal standard (pentaerythritol). 2. Erythronic acid. 3. Threonic acid. 4. Threonic γ-lactone. 5. Erythronic γ-lactone. 6. Internal standard (vanillic acid).

erythronic acids were not completed or the resulting lactones were not stable under the conditions used. Also, pentaerythritol was unstable, and is not appropriate as an internal standard for Method B. In Method C, a heating of the dry products prior to trimethylsilylation drastically reduced the peak intensity of threonic acid γ-lactone (No. 4) and erythronic acid γ-lactone (No. 5).

Table 2 illustrates that the work-up procedure of ozonation products for the GC analysis has significant impacts on the total yields of erythronic and threnoic acids as well as the apparent E/T ratio of the β-aryl ether structures in the spruce wood lignin. As indicated, the total yield of two tetronic acids determined as the free-acid derivative (15%) (Method A)

Table 2. Influence of work-up procedures on the total yield of erythronic (E) and threonic (T) acids and the E/T ratio from ozonation of Norway spruce wood meal.

Method	Form of cation exch. resin	Tetronic acid	
		Yield (%)	E/T
A	NH_4^+	14.96	1.10
B	H^+	7.54	1.57
C[a]	H^+	3.90	2.00

[a] Heated at 70°C for 10 min prior to trimethylsilylation.

was nearly twice that of Method B based on the formation of the lactone products (8%). In contrast, the E/T ratio from Method A was considerably lower than that of Method B (1.10 vs. 1.57). Interestingly, the value of 1.10 is practically identical to that reported by Sarkanen et al. (1.05), whereas the ratio of 1.57 matches well with that of Taneda et al. (1.5) (Table 1). This confirms that the inconsistent data on the spruce wood lignin (Table 1) can be largely attributed to the difference in the work-up procedures. Additionally, a heating of the dry product mixture prior to trimethylsilylation resulted in a further yield reduction of the tetronic acids (from 8 to 4%), and an increase in the E/T ratio (from 1.57 to 2.00).

Aspen Wood Lignin

Table 3 further illustrates that the work-up procedure has a considerable impact on the yield of erythronic and threonic acids as well as their relative proportion from ozonation of the aspen wood meal. As indicated, the total yield of these two tetronic acids was higher determined by Method A as the free-acid form (27%) than by Method B as the lactone products (23%), whereas the E/T ratio was lower for the Method A (1.87 vs. 2.61). Also, heating of the dry products prior to trimethylsilation further reduced the total yield of two tetronic acids (23 vs. 13%), and increased the E/T ratio (2.35 vs. 2.61).

CONCLUSIONS

This study clearly demonstrates that the work-up procedure has a considerable impact on the total yield of erythronic and threonic acids

Table 3. Influence of work-up procedures on the total yield of erythronic (E) and threonic (T) acids and the E/T ratio from ozonation of aspen wood meal.

		Tetronic acid	
Method	Form of cation exch. resin	Yield (%)	E/T
A	NH_4^+	26.61	1.87
B	H^+	22.68	2.35
C[a]	H^+	13.12	2.61

[a] Heated at 70°C for 10 min prior to trimethylsilylation.

from ozonation of the wood lignin. Our data confirm that the determination of these acids by first conversion into the ammonium salt forms followed by silyation and GC analysis is a preferred method.[10,14,17] One should avoid to analyze these acids as the lactone products, as this approach may yield several artifacts. This procedure, as indicated in Tables 1 and 2 (Method B), gave a low yield of tetronic acids (as the lactone products) and a high E/T ratio. These erroneous data are likely resulted from a combination of incomplete lactonization and the loss of products during the work-up process. Also, we observed that pentaerythritol cannot be used an internal standard for this procedure, as it is unstable under the prevailing acidic conditions.

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