

QUANTITATIVE LIGNIN ANALYSIS BASED ON PERMANGANATE OXIDATION

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Qualitative lignin analysis relies rather much on studies of lignin degradation products. As concerns precise quantification of lignin's composition such studies in general have obvious limitations. Aromatic acids obtained on permanganate oxidation of pretreated lignins (cleavage of ethers and alkylation of phenolic groups) offer a possibility to estimate the amounts of differently substituted aromatic units in lignins. An equation is derived for the calculation of the gross composition of lignins based on the yields of methoxy-substituted aromatic acids obtained on permanganate oxidation of lignins with methylated phenolic groups. The equation could also be used for the calculation of the phenolic content in a lignin sample based on permanganate oxidation data, provided that such data are available for a similar lignin sample with known phenolic content. Literature data for milled wood lignin from spruce are used to exemplify the calculations.

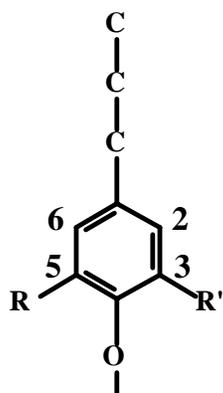
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INTRODUCTION

Degradation methods such as acidolysis (Lundquist 1992) and thioacidolysis (Rolando et al. 1992; Öennerud et al. 2003) provide lignin degradation products that in general can be traced to particular lignin structures with confidence. However, the analyses comprise primarily monomeric degradation products, and the examinations of dimeric degradation products are incomplete. Quantitative studies using these techniques are therefore essentially restricted to "uncondensed" units ("uncondensed" and "condensed" units are defined in Fig. 1). One group of lignin degradation methods [thioacetolysis (Nimz 1974), thioacidolysis/Raney Ni treatment (Lapierre et al. 1991), DFRC (Lu and Ralph 1998; Peng et al. 1998)] involves a degradation step and a subsequent reduction step. In these cases both monomeric and dimeric products are thoroughly investigated. Even so, the results represent "uncondensed" units to a greater extent than "condensed" units. The limitations of the degradation/reduction methods also pertain generally for hydrogenolysis (Sakakibara 1992). Degradation methods that permit the analysis of both "condensed" and "uncondensed" lignin units are permanganate oxidation (Erickson et al. 1973b; Gellerstedt 1992), nitrobenzene oxidation (Chen 1991), and the nucleus exchange method (Funaoka et al. 1992). Ozonation (Akiyama et al. 2005

and preceding work) offers a possibility to quantitatively analyze different types of side chains in lignins.



H R=R'= H
G R= H, R'= OCH₃
S R=R'= OCH₃

Fig.1. H, G and S represent different types of "uncondensed" lignin units. H, G and S units bound to another phenylpropane unit in additional ring positions (2, 3, 5 or 6) are designated "condensed" units.

Much attention has been paid to calculations of the composition of lignins based on results from permanganate oxidation studies (Erickson et al. 1973b; Morohoshi and Glasser 1979; Chen 1991; Gellerstedt 1992; Bose et al. 1998). A schematic description of the reaction sequence involved in these studies is shown in Fig. 2; the aromatic acids are usually analyzed as methyl esters.

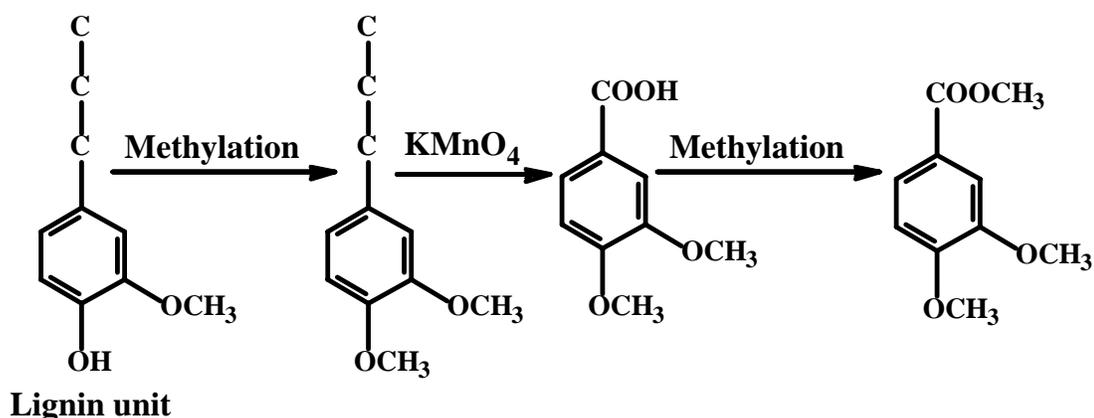


Fig.2. Schematic description of the reactions involved in methylation/permanganate oxidation/esterification of lignin leading to esters of methoxylated aromatic acids.

Analysis of lignins by permanganate oxidation is advantageous, since the degradation reaction has been thoroughly studied (Erickson 1973a), and convenient methods for the analysis of the degradation products have been worked out (Erickson 1973a). Furthermore, this degradation method specifically concerns phenolic lignin units (Fig. 2). In this paper we introduce an alternative method for the calculation of the compositions of lignins based on degradation products from permanganate oxidation studies. In conformity with all the earlier published calculation methods we have assumed that all the individual types of carbon atoms, attached to aromatic nuclei and susceptible to permanganate oxidation, are converted to carboxyl groups to the same extent. For the sake of simplicity we have restricted the presentation here to spruce lignin. We think that the principles for the calculations presented may be applicable to other degradation methods than permanganate oxidation.

MATERIALS AND METHODS

Conditions Used in the Cited Experimental Work

Results from permanganate oxidation studies of spruce lignin (milled wood lignin, MWL) published by Miksche and co-workers (Erickson et al. 1973b) are used as an example in the calculations presented in this paper. Erickson et al. (1973a,b) methylated the phenolic groups in MWL (dimethyl sulfate/KOH). The methylated lignin was degraded to aromatic acids by oxidation with $\text{KMnO}_4/\text{NaIO}_4$ complemented with a H_2O_2 treatment in a subsequent reaction step. The obtained aromatic acids were esterified with diazomethane and analysed by gas chromatography.

Deduction of Equations

The calculations are based on the yield (mg/100 mg lignin) of permanganate oxidation/esterification products **I-VII** (Fig. 3) and the amount of "trace constituents".

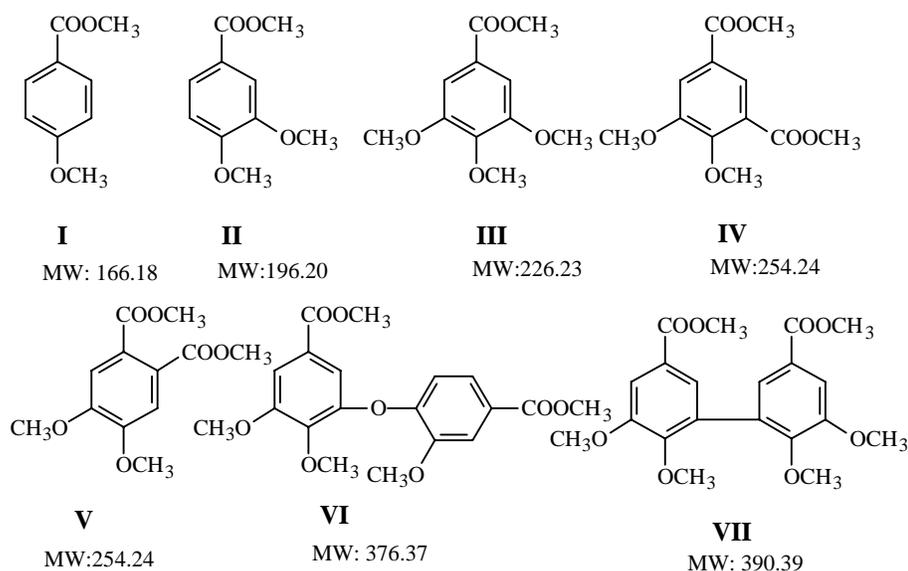


Fig.3. Esters obtained on methylation/permanganate oxidation/esterification of spruce lignin.

Esters of "trace constituents" constituted 3-5 % of the total of the esters (Erickson et al. 1973b). As an approximation we have assumed that 3-5 % of the spruce lignin sample consists of "trace constituents." Judged by the composition of the "trace constituents" (Larsson and Miksche 1969) we assume in the calculations that they consist of equal amounts of H and G units. The calculations are based on assumed or determined contents of phenolic groups (phenolic groups/phenylpropane unit). It is only aromatic acids from phenolic lignin units with protected phenolic group (alkylation, often methylation) that are analyzed in permanganate oxidation studies (Fig. 2). An equation (**Equation A**) has been derived for the calculations. It is assumed that all the individual types of carbon atoms, attached to aromatic nuclei and susceptible to permanganate oxidation, are converted to carboxyl groups to the same extent. The fraction of these carbon atoms that is converted to carboxylic groups is designated F. The conversion factor F can be calculated using **Equation A**. This equation is based on the condition that the amount (weight) of phenolic units in the lignin sample investigated must be equal to the amount of units (weight) derived from esters **I-VII** using the conversion factor F together with the phenolic fraction of the "trace constituents" (assumed to be phenolic to the same extent as the rest of the lignin). Note that the extent of conversion to dicarboxylic acids is determined by F². The determined factor F is used to calculate the distribution of different types of units in the lignin sample investigated. Furthermore it is possible to calculate the phenolic content in a lignin sample from **Equation A** provided the factor F is known. **Equation A**:

$$\frac{I(\text{mg}) \times 157}{166.2 \times F} + \frac{II(\text{mg}) \times 187}{196.2 \times F} + \frac{III(\text{mg}) \times 217}{226.2 \times F} + \frac{IV(\text{mg}) \times 187}{254.2 \times F^2} + \frac{V(\text{mg}) \times 187}{254.2 \times F^2} + \frac{2 \times VI(\text{mg}) \times 187}{376.4 \times 2 \times F^2} +$$

$$\frac{2 \times VII(\text{mg}) \times 187}{390.4 \times F^2} + Ph \times \text{Trace (total amount in the sample, mg)} = Ph \times [100 (\text{lignin sample, mg}) - \text{Carbohydrates (mg)}]$$

[The degradation of a 100 mg sample of the lignin is considered, Ph is the fraction of the lignin units that is phenolic, designations and molecular weights of the degradation products (esters **I-VII**) appear in Fig. 3, the average lignin unit weights 157, 187 and 217 (corresponding to H, G and S units, Fig. 1) are taken from Larsson and Miksche (1971)].

When the factor F is known, it is possible to calculate the distribution of lignin units corresponding to esters **I-VII** and "trace constituents". For example, the fraction (%) of "uncondensed" G units (represented by ester **II**) is obtained by dividing the calculated amount (mmol) of such units:

$$\frac{II(\text{mg}) \times 100}{196.2 \times F}$$

with the total amount (mmol) of calculated lignin units [trace constituents assumed to consist of equal amounts of H and G units (Larsson and Miksche 1969)]:

$$\frac{I(\text{mg})}{166.2 \times F} + \frac{II(\text{mg})}{196.2 \times F} + \frac{III(\text{mg})}{226.2 \times F} + \frac{IV(\text{mg})}{254.2 \times F^2} + \frac{V(\text{mg})}{254.2 \times F^2} + \frac{2 \times VI(\text{mg})}{376.4 \times F^2} + \frac{2 \times VII(\text{mg})}{390.4 \times F^2} +$$

$$\frac{Ph \times \text{Trace (total amount in the sample, mg)}}{(157 + 187)/2}$$

A second equation (**Equation B**) for the determination of the conversion factor *F* and the calculation of the distribution of units has been presented (Parkås et al. 2004). This equation is based on the same premises as **Equation A**, excepting that it is assumed that a pretreatment has resulted in a complete cleavage of phenolic ethers connecting lignin units (excepting the very stable diaryl ethers). **Equation B** (the designations are the same as those in **Equation A**):

$$\frac{I(\text{mg}) \times 157}{166.2 \times F} + \frac{II(\text{mg}) \times 187}{196.2 \times F} + \frac{III(\text{mg}) \times 217}{226.2 \times F} + \frac{IV(\text{mg}) \times 187}{254.2 \times F^2} + \frac{V(\text{mg}) \times 187}{254.2 \times F^2} + \frac{2 \times VI(\text{mg}) \times 187}{376.4 \times F^2} + \frac{2 \times VII(\text{mg}) \times 187}{390.4 \times F^2} + \text{Trace (total amount in the sample, mg)} = 100 (\text{lignin sample, mg}) - \text{Carbohydrates (mg)}$$

Excel Files for the Calculations

Excel files for the calculations are available on the website:

<http://www.sikt.chalmers.se/lignin/>

Further details regarding the calculations are also given at this web-site. The Excel files can alternatively be obtained from the authors on request. The files are designed to solve a second order equation (**Equation A** or **Equation B**), which gives the conversion factor *F*. Based on this, the distribution of lignin units is calculated simultaneously. Calculations based on **Equation A** (Method Phenolic) and **Equation B** (Method Quadratic) are exemplified in the Excel-files. Calculations based on **Equation B** are also discussed by Parkås et al. (2004). The Excel-files can readily be adapted to calculations involving derivatives of the degradation acids other than **I-VII** (e.g. ethyl esters instead of methyl esters). Similarly unit weights can be adjusted to the type of lignin analyzed.

RESULTS AND DISCUSSION

General

Aromatic carboxylic acids obtained on permanganate oxidation of methylated lignins were subjected to detailed studies by Freudenberg and co-workers (Freudenberg 1968), and this approach to lignin analysis has later been extensively employed by several groups (Erickson et al. 1973b; Morohoshi and Glasser 1979; Chen 1991; Gellerstedt 1992; Bose et al. 1998; Tamminen and Hortling 1999). Figure 2 shows the reaction sequence involved in these studies; the aromatic acids are usually analyzed as methyl esters, but in some studies the initial methylation of phenolic groups has been replaced by an ethylation. A method for the analysis of the non-esterified acids has also been described (Javor et al. 2003). Important improvements of the lignin analysis based on permanganate oxidation were introduced by Miksche and co-workers (Erickson et al. 1973a). Experiments with lignin model compounds have shown that diarylmethane structures give only low yields of aromatic acids on permanganate oxidation (Erickson et al. 1973a; Meguro et al. 1998). However, such structures are not present in significant amounts in MWL of spruce (Erickson et al. 1973b). Another complication is that pretreatment with CuO/NaOH (to cleave ethers) results in formation of small amounts of

biphenyls (Bose et al. 1998). It is important to be aware of that it is only the phenolic portion of the lignin samples that is analyzed.

Determination of the Gross Composition of Lignins

Calculation of the structure of MWLs based on results from permanganate oxidation was introduced by Miksche and co-workers (Larsson and Miksche 1971; Erickson et al. 1973b). The calculations are based on the assumption that all the individual types of carbon atoms susceptible to permanganate oxidation and attached to aromatic nuclei are converted to carboxyl groups to the same extent. A further assumption is that a pretreatment with CuO/NaOH results in a complete cleavage of ethers connecting the lignin units (excepting the very stable diaryl ethers). An equation for this type of calculations was derived by Parkås et al. (2004) (**Equation B**, see Materials and Methods). A factor (F) for the conversion of carbon substituents to carboxyl groups can be calculated using this equation. Other groups (Gellerstedt 1992; Chen 1991) employ a value of conversion factor F based on permanganate oxidation experiments with lignin model compounds. A drawback, in this case, is that there is no connection between the magnitude of the factor and the actual yields of aromatic acids obtained from the particular lignin sample investigated. It has been recognized by two groups (Morohoshi and Glasser 1979; Bose et al. 1998) that a CuO/NaOH pretreatment to cleave ethers does not result in a complete cleavage of ether bonds and that some decomposition of lignin units may occur during this treatment. They have modified the conversion factor F in different ways to compensate for this. It is a fact (cf. Bose et al. 1998) that it is solely the phenolic portion of a lignin sample that give rise to esters **I-VII** (Figs. 2 and 3). We have derived an equation (**Equation A**, see Materials and Methods) in which this fact is taken into account, i.e. it is only the phenolic fraction of the samples that is considered in the calculations.

Erickson et al. (1973b) have published yields of **I-VII** (Fig. 3) obtained on permanganate oxidation of CuO/NaOH pretreated spruce MWL. Using their data we have applied **Equation A** to calculations of the distribution of units in spruce MWL. Table 1 shows the calculated distribution of units in spruce lignin assuming different phenolic contents. Undoubtedly the phenolic content of CuO/NaOH treated spruce MWL is high, but to our knowledge, it has not been determined experimentally. However, Bose et al. (1998) have determined the phenolic fraction of the lignin in CuO/NaOH treated spruce wood as 69%. One of the columns in the table shows a distribution of units practically identical with that obtained when the equation presented by Parkås et al. (2004) (**Equation B**) is applied. It is obvious from calculations based on **Equation A** (Table 1) that, provided the yields of esters are unchanged, a lower phenolic content (determined or assumed) requires a larger conversion factor (F), which in turn implies larger amounts of "uncondensed" units. The composition of the non-phenolic fraction is not known. It follows that the calculated distribution of lignin units is reliable only if the phenolic content is high and specified in the calculations. In that case the influence of the composition of the non-phenolic fraction on the gross composition of the lignin can be neglected.

The percentages of different types of units corresponding to esters **I-VII** given by Erickson et al. (1973b) are based on considerations similar to those expressed in

mathematical form in **Equation B** (cf. Parkås et al. 2004). However, their choice of conversion factor *F* is not based on precise calculations (cf. Larsson and Miksche 1971). Their results fairly closely correspond to those calculated based on **Equation A**, assuming a phenolic content of approx. 85 %. Since this is not very far from a probable phenolic content, the structural conclusions drawn by Erickson et al. (1973b) agrees fairly well with those derived from calculations based on **Equation A**. However, **Equation A** suggests a slightly lower proportion of "condensed" units.

Table 1. Distribution (%) of the lignin units in a spruce MWL sample (assumed carbohydrate content 1%) that gives rise to esters **I-VII** (Fig. 3). The distribution is calculated (**Equation A**) based on the yields of these esters (Erickson et al. 1973b) obtained on esterification of aromatic acids formed on permanganate oxidation of the CuO/NaOH treated and methylated sample assuming 70%, 80%, 90% or 96.5% phenolic units after the CuO/NaOH treatment. Regarding the interpretation of permanganate oxidation results in terms of structural elements in lignin, see Erickson et al. (1973 b).

	Amount (mg/100 mg lignin)	Distribution assuming 70% phenolic units (gives <i>F</i> =0.69)	Distribution assuming 80% phenolic units (gives <i>F</i> =0.63)	Distribution assuming 90% phenolic units (gives <i>F</i> =0.57)	Distribution assuming 96.5% phenolic units*** (gives <i>F</i> =0.55)
I	0.7	1.59	1.53	1.48	1.45
II	29.8	57.31	55.18	53.30	52.19
III	0.5	0.83	0.80	0.78	0.76
IV*	5	10.75	11.42	12.00	12.35
V	1.1	2.37	2.51	2.64	2.72
VI	2.1	6.10	6.48	6.81	7.01
VII	6	16.81	17.84	18.76	19.31
Trace**	4	4.24	4.23	4.22	4.22

* Assumed to originate from β -5 linked units (a contribution from 5-5 linked units is unlikely here since CuO/NaOH treatment results in extensive ether cleavage).

** Trace constituents, assumed to consist of equal amounts of **H** and **G** units (Larsson and Miksche 1969).

*** Comparable with calculations based on **Equation B**

Determination of Phenolic Groups in a Lignin Sample based on the Yields of I-VII on Permanganate Oxidation

It is possible to calculate the phenolic content in a lignin sample from the yields of **I-VII** using **Equation A**, provided that the conversion factor *F* is known. A probable factor *F* can be calculated using **Equation A** if data (phenolic content, yields of **I-VII**)

for a related lignin sample are known. Insertion of the yields of **I-VII** from spruce MWL reported by Erickson et al. (1973b) and the phenolic content (0.26) in spruce MWL adopted by these authors in **Equation A** gives the conversion factor (F) 0.70. Insertion of this factor and the yields of **I-VII** from CuO/NaOH treated MWL [reported by Erickson et al. (1973b) and shown in Table 1] in **Equation A** gives the phenolic content 0.69. Interestingly, this phenolic content in CuO/NaOH treated spruce MWL agrees with the value (also 0.69) reported by Bose et al. (1998) for the lignin in CuO/NaOH treated spruce wood. However, it should be kept in mind in this context that untreated spruce MWL is not an ideal reference lignin for CuO/NaOH treated lignin. Gellerstedt (1992) has estimated the phenolic content in lignins based on permanganate oxidation. In this case a conversion factor for formation of aromatic acids was selected based on model compound studies.

CONCLUSIONS

1. The calculation method developed (exemplified using the cited experimental work) improves the comparability of results obtained with different samples, since it is based on the fact that the phenolic content of the lignin sample examined defines the theoretical yield of the methoxylated acids obtained on methylation/permanganate oxidation.
2. The equations derived for the calculations make it possible to treat input data in a consistent way. "Trace constituents" and carbohydrate content are considered in the equations.
3. The solution of the equations using Excel-files is convenient. The Excel-files are readily adapted to different types of derivatives of the degradation acids. Similarly the parameters used in this paper can readily be replaced by others.

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