

The Structure of Lignins in Pulps

Part 4: Comparative Evaluation of Five Lignin Depolymerization Techniques*

N. Morohoshi and W. G. Glasser

Department of Forestry and Forest Products and
Department of Chemical Engineering, Virginia Polytechnic Institute and State University
Blacksburg, Virginia

Summary. Five potential lignin depolymerization techniques were evaluated with regard to their analytical value in combination with oxidation by permanganate and hydrogen peroxide. The methods included involved alkaline solutions of cupric oxide and nitrobenzene, acetolysis with thioacetic acid, derivatization with thioglycolic acid followed by alkaline hydrolysis, and reductive aminolysis with sodium in liquid ammonia. Gas and gel permeation chromatography served for the separation of product mixtures, and milled wood lignins of Loblolly pine and red alder were the lignin substrates. Whereas thioacetolysis proved most efficient in terms of lignin depolymerization, the side chains of its product mixtures were insufficiently oxygenated to warrant effective conversion into polycarboxy aromatic acids by permanganate. Cupric oxide and alkali were deemed most effective because of greatest overall yields of five prominent permanganate oxidation products combined with mildness (measured in terms of ratio of condensed to uncondensed degradation products). Suitable depolymerization conditions were found to be five hours reaction time at 174 °C. The results were in general agreement with data reported by Pepper et al. and Miksche et al.

Introduction

The structure of an isolated lignin is commonly evaluated by a combination of several analytical techniques which may include chemical analyses with regard to the elemental and functional group composition, various forms of spectroscopy, and depolymerization and analytical degradation of the polymeric structure of the lignin. Lignin analysis by means of the latter kind has proven particularly fruitful, and has

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yielded much valuable information on the chemical composition of lignin (for review, see Sarkanen, Ludwig 1971).

The lignin literature describes several different types of depolymerization techniques, all leading to mixtures of isolatable and identifiable low molecular weight degradation products. To judge critically their relative value in terms of generating structural information on lignin polymers, several performance criteria must be considered, including how efficiently, in terms of yield, a given method can depolymerize lignin into a mixture of monomers, dimers, trimers, and higher oligomers; how well the method can achieve its depolymerization and at the same time avoid extensive, unpredictable, and uncontrollable side reactions; and especially how well, efficiently, and conveniently low molecular weight reaction products can be separated, isolated, and quantitatively identified; because, it is only then that a depolymerization procedure may qualify as an assay procedure for monitoring structural changes in lignin.

With the aim of identifying a sensitive depolymerization technique applicable to the routine analysis of isolated lignins, a study was undertaken in which five different depolymerization procedures were evaluated. Because of the last performance criterion, the requirement for ready separation and quantitative determination of low molecular weight fragments, the techniques were evaluated in conjunction with ultimate oxidative degradation by permanganate which leads to aromatic reaction products containing only two types of substituents. Any increase in the number of substituent-types would automatically inflate the number of degradation products, thus complicating the analysis and its interpretation. The permanganate oxidation procedure was first applied to lignin by Freudenberg et al. (for review, see Chang, Allan 1971; Freudenberg, Neish 1968), and it has recently been refined by Miksche and his co-workers (Larsson, Miksche 1967; Erickson et al. 1973 a).

Experimental

Depolymerization Techniques

Sodium in Liquid Ammonia (Na/NH₃)

The procedure was patterned after work by Yamaguchi et al. (Yamaguchi et al. 1973; Yamaguchi 1973), and was as follows: Dry liquid ammonia (20 ml) and 1 g metallic sodium were placed in a 25 ml round-bottomed flask containing 200 mg of lignin (MWL). The flask was protected from atmospheric moisture with a CaCl₂-drying tube and kept in a dry ice-methanol bath at -70 to -40 °C. The mixture was allowed to stand for 48 hrs with occasional agitation. After the blue color had disappeared, ammonium chloride (0.5 g, dried over P₂O₅ in vacuum) was added, and the mixture was stirred for some time. Petroleum ether (2 ml) was then added and the solvent was evaporated. The residue was subjected to methylation with dimethyl sulfate.

Thioglycolic Acid Degradation (TGA)

The procedure was adopted from work by Holmberg (1930), Freudenberg et al. (1959), and Mogharab and Glasser (1976). Loblolly pine MWL (100 mg) was placed in a 100 ml, 3-necked round-bottomed flask fitted with a magnetic stirrer, dropping funnel and two gas-inlets. After the air in the reaction vessel had been replaced with nitrogen gas, a solution of 4 ml thioglycolic acid and 0.1 ml boron trifluoride was added. The mixture was stirred at room temperature for four days. The solvent was distilled under reduced pressure and the resulting residue was subjected to alkaline hydrolysis and methylation with dimethyl sulfate.

Thioacetic Acid Degradation (TAA)

The procedure was that of Nimz (1969). Loblolly pine MWL (200 mg) was placed in a 100-ml reaction vessel, and the air in the vessel was replaced with nitrogen gas. A solution of 4 ml thioacetic acid and 0.1 ml boron trifluoride etherate was added slowly to the vessel under reduced pressure. Nitrogen gas was introduced into the vessel which was kept under nitrogen at atmospheric pressure. The mixture was allowed to react at room temperature for three days. The mixture was evaporated at ambient temperature under reduced pressure for removal of excess thioacetic acid. After 70 ml of 5% sodium acetate had been added, the mixture was mixed thoroughly and extracted with three 60-ml portions of ethyl acetate. The degraded lignin products were completely dissolved in the ethyl acetate extracts, which were combined, dried with anhydrous sodium sulfate and evaporated under reduced pressure.

The degraded lignin residue and 40 ml 2N-NaOH in ethanol-water (3 : 1) were placed in a 250-ml round-bottomed flask and the mixture was allowed to react at 60° for 24 hours. After flushing with nitrogen gas, the mixture was neutralized with 5N-hydrochloric acid and evaporated. The residue was methylated with dimethyl sulfate.

Nitrobenzene Oxidation (NB)

The procedure used was based on work by Freudenberg et al. (1940) and Leopold (1950). Loblolly pine MWL (1 g), 40 ml 2N sodium hydroxide and 3 ml nitrobenzene were placed in a 50-ml stainless steel autoclave. The mixture was heated at 180° for 3 hours in an oil bath. The mixture was recovered, washed with 150 ml of water, and extracted with ether in a liquid-liquid extractor for five days. The alkaline solution was carefully acidified with 50% sulfuric acid and again extracted with ether. The aqueous mixture was centrifuged at 10,000 rpm for 10 min. The supernatant was extracted twice with 50 ml chloroform-acetone (1 : 1). The mix-

ture of ether and chloroform-acetone extracts, and the precipitate were subjected to methylation with dimethyl sulfate.

Cupric Oxide Oxidation (CuO)

The procedure was adopted from work by Pearl (1942). Loblolly pine MWL (1 g), 2.1 g of sodium hydroxide in 4 ml of water, and 2.5 g CuSO_4 in 10 ml water were allowed to react in a 20 ml sealed stainless steel autoclave which was heated to 174°C by immersion in an oil bath, and occasionally removed for agitation. After 5 hrs the mixture was cooled and methylated directly with dimethyl sulfate.

Oxidation Procedure

The procedure was adopted from work by Erickson et al. (1973 a), and was as follows.

Methylation with Dimethyl Sulfate

The reaction products from the depolymerization of 100 mg of lignin were dissolved into 40 ml solvent (1,2-dimethoxy-ethane : methanol : water, 35 : 35 : 30). The reaction vessel was fitted with an electrode and an automatic titrimer, and air was replaced by nitrogen. After the mixture had been adjusted to pH 11 (15 % KOH), 2 ml dimethyl sulfate was added. The reaction was carried out at room temperature for 24 hrs at constant pH. After cooling, the mixture was adjusted to pH 4 with 2M H_3PO_4 and extracted three times with 60 ml acetone-chloroform (1 : 1). The combined organic extract was washed with 20 ml water, dried with anhydrous sodium sulfate, and evaporated to dryness under reduced pressure.

Oxidation with Potassium Permanganate

The methylated lignin-derived mixture was mixed with 48 ml tert-butanol : water (3 : 1), 48 ml 5N NaOH, 120 ml 0.6M NaIO_4 and 24 ml 0.3M KMnO_4 . The mixture was stirred vigorously and heated at 82°C on a steam bath for six hours. After cooling and adding 60 ml of ethanol, the mixture was filtered through a short Celite column. The filtrate was washed with a small amount of 1 % aqueous Na_2CO_3 , cooled and extracted twice with 300 ml ether. The combined ether solutions were washed with 180 ml 1 % NaOH, and the aqueous phase was combined with the above aqueous solution. The combined alkaline solutions were neutralized with 1M H_2SO_4 and concentrated to 150 ml under reduced pressure.

Oxidation with Hydrogen Peroxide

The permanganate oxidation product mixture, 10.8 g anhydrous Na_2CO_3 , and 60 ml H_2O were placed in a 250-ml round-bottomed flask. The solution was heated to 50°C for ten minutes with agitation. In order to decompose excessive hydrogen peroxide, 1.2 g manganese dioxide was added. After the evolution of gas had ceased, small portions of manganese dioxide were added two more times. The mixture was filtered through a short Celite column, and the residue was washed with 120 ml water. The resulting aqueous phase was combined with the above filtrate. The mixture was adjusted to pH 2 with concentrated H_2SO_4 and extracted three times with 250 ml acetone-chloroform (1 : 1). The organic phase was separated and dried with anhydrous sodium sulfate. The solvent was evaporated, and the residue was weighed.

Methylation with Diazomethane

The oxidized lignin sample was placed in a 100-ml round-bottomed flask and dissolved in 100 ml methanol. An ether solution of diazomethane was added in excess, and the mixture was left in the hood for one day. After evaporation of the solvent, the residue was again dissolved, this time in 40 ml dioxane, and once more methylated with diazomethane in ether solution for one day. Following the evaporation of the solvent, the residue was mixed with a carefully weighed amount of an internal standard (pyromellitic acid tetramethyl ester) and dissolved in 24 ml dioxane. This solution was subjected to analysis by gas and gel permeation chromatography.

Gas and Gel Permeation Chromatographic Separations

Gas chromatographic separations analogous to those reported by Erickson et al. (1973a) were performed with a Varian Model 3700 using a 1.5 m column packed with 3% SP-2100. Gel permeation chromatography was carried out on Sephadex LH-20 in DMF as solvent. A detailed description of the separation and quantitative identification technique of low molecular weight degradation products, and the interpretation of the results in terms of structural information, will be given in a subsequent paper in this series.

Results and Discussion

Depolymerization and/or degradation techniques commonly applied to lignin-containing substrates have concentrated on alkaline solvolysis with white liquor of the kraft pulping process ($\text{NaOH}/\text{Na}_2\text{S}$) (Erickson et al. 1973b). Other depolymerization techniques applied to isolated lignin preparations employ reductive degradation

(sodium in liquid ammonia (Na/NH_3)) (Shorygina et al. 1963–1974; Pernemalm, Dence 1974; Yamaguchi 1975); oxidative degradation with nitrobenzene in alkaline solution (NB) (Freudenberg et al. 1940; Leopold 1950; Brink et al. 1972); acetolytic degradation with thioacetic acid followed by alkaline hydrolysis and desulfurization with Raney-nickel (TAA) (Nimz 1969; Nimz et al. 1970; Nimz, Das 1971); derivatization with thioglycolic acid (Holmberg 1930; Freudenberg et al. 1959; Mogharab, Glasser 1976; Fisher et al. 1972), followed by alkaline hydrolysis (TGA); and oxidative/hydrolytic degradation with cupric oxide in alkali (CuO/NaOH) (Pepper, Karapally 1972; Larsson, Miksche 1971). The performance of each of these five types of lignin depolymerization techniques in combination with permanganate oxidation was critically evaluated by measuring the following performance criteria: (a) total yield of mono- to hexameric fragments by gel permeation chromatography, and total yield by types of monomers and dimers by gas chromatography; (b) ratio of monomers to dimers determined by gas chromatography; (c) ratio of monomers and dimers to higher oligomers; and (d) ratio of condensed versus uncondensed degradation products. The results of these evaluations are given in Figures 1–5 and Tables 1 and 2.

Depolymerization Efficiency

The overall yield from Loblolly pine MWL of the five most prominent monomeric and dimeric ultimate degradation products from each of the five depolymerization techniques is given in Figure 1. It is apparent that depolymerization with CuO

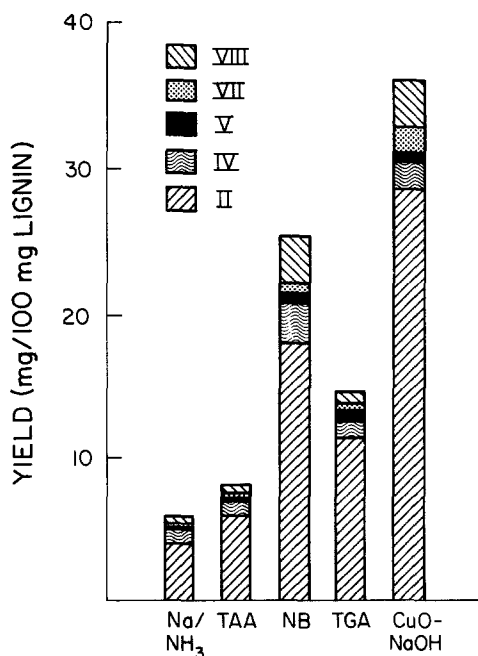


Fig. 1. Yield of degradation products from five different lignin degradation methods

generates the greatest amount of aromatic carboxylic acids on subsequent permanganate oxidation. In terms of total amounts of the five principal fragments (compounds II, IV, V, VII and VIII, Fig. 2), the CuO/NaOH technique is followed by NB, TGA, TAA, and Na/NH₃. The combined yields of these five aromatic carbo-

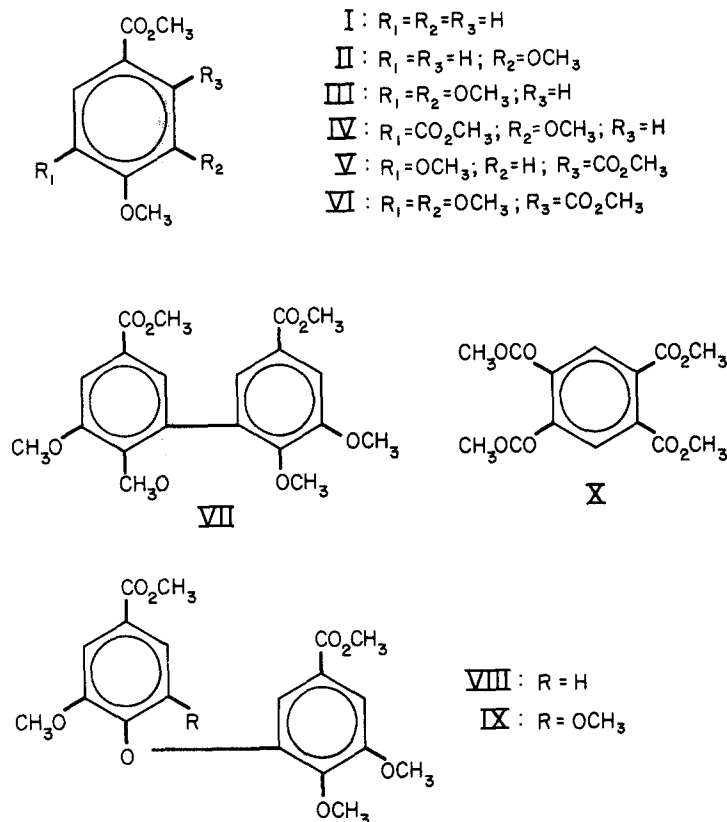


Fig. 2. Chemical structures of prominent monomeric and dimeric permanganate oxidation products

xylic acids range between 6 and 36 mg of methyl ester derivatives per 100 mg of lignin. This may be compared with 30–35% ether-solubles (based on Klason lignin) which Pepper and Karapally (1972) obtained from the CuO oxidation of spruce protolignin, and 45.2 mg total methyl esters which Larsson and Miksche (1971) obtained from the permanganate oxidation of CuO-preoxidized/prehydrolyzed spruce MWL.

Depolymerization efficiency, however, is inadequately represented by the yield of only mono- and dimeric degradation products formed on permanganate oxidation of the product mixture. Efficiency is better assessed on the basis of the molecular weight distribution of the depolymerization and oxidative degradation product mixture as determined by gel permeation chromatography. Such chromatograms of

aromatic carboxylic acids and their methyl ester counterparts are shown in Figure 3. Although the elution profile of the free acid product mixture suggests better resolution than that of the methyl esters, it was found that only the esters were eluted from Sephadex LH-20 in DMF according to their molecular weights. This observation is in accord with findings reported by Connors et al. (1978). Molecular weight distributions of degradation products can accordingly be determined only with methyl ester derivatives and not with free carboxylic acids.

On the basis of molecular weight distribution data, the five depolymerization procedures were critically evaluated in terms of the performance criteria listed in Table 1. The ratio of monomers and dimers to oligomeric degradation products is an important yardstick for measuring the depolymerization efficiency of a degradation method. This criterion is a measure of the capability of a technique to break a lignin polymer into a maximum number of small fragments. It is of interest to

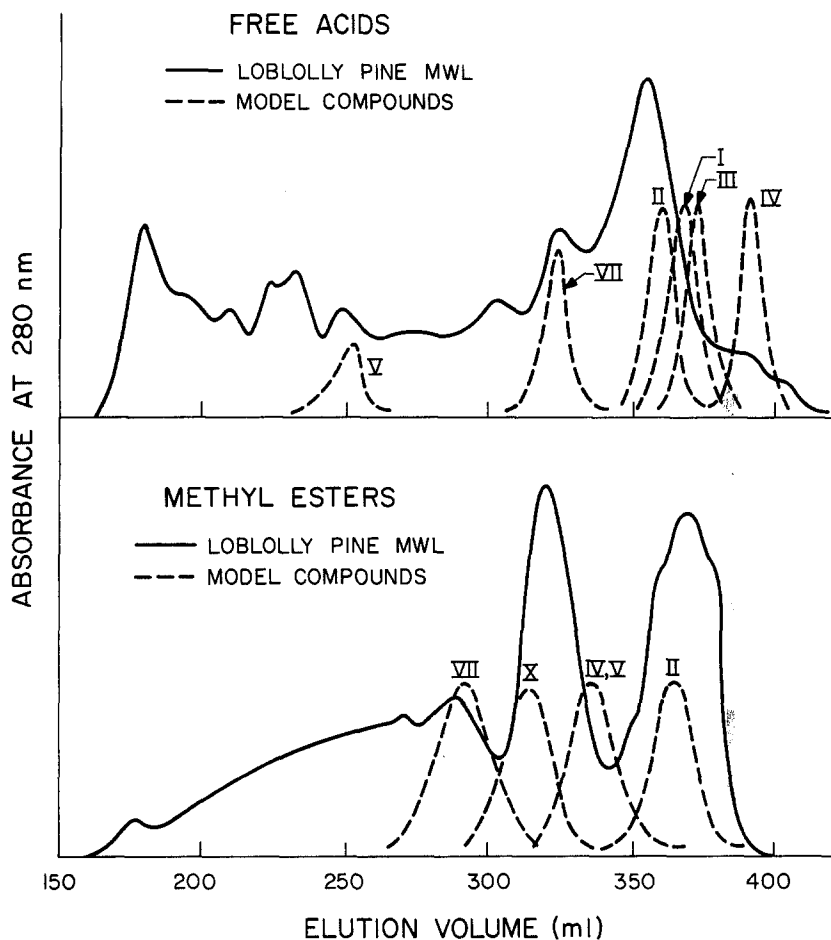


Fig. 3. Gel permeation chromatograms of permanganate oxidation product mixtures of Loblolly pine MWL as free acids and as methyl esters. Sephadex LH-20 with DMF

Table 1. Comparison of several performance criteria of five depolymerization methods

Performance criteria	Na/NH ₃	TAA	N.B.	TGA	CuO
Total yield, gravimetrically (mg ester/100 mg lignin)	9.27	11.61	48.98	68.61	78.57
Major fractions, by GPC ^a (mg ester/100 mg lignin)					
mono- and dimers	8.13	10.23	34.84	39.02	55.05
oligomers (trimers & higher)	1.41	1.38	14.14	29.59	23.52
Ratio mono- and dimers to oligomers	5.8:1	7.5:1	2.5:1	1.3:1	2.3:1
Ratio of models II: (IV + V):(VII + VIII) by gas chromatography	4.3:1.2:1	7.6:1.5:1	4.6:0.9:1	9.5:1.5:1	4.7:0.5:1
Ratio of condensed to uncon- densed type degradation products, by gas chromato- graphy	1:2.4	1:3.1	1:2.4	1:3.9	1:3.2

^a Based on quantitative determination of monomers and dimers by GC and on the assumption that the absorptivity of 280 nm is constant throughout the molecular weight range of the degradation product mixture and equal to that of monomers and dimers

note that this ratio of monomers and dimers to oligomers varies over a wide range between the five depolymerization methods investigated. Thioacetolysis (TAA) seems to be most nearly optimal in terms of generating monomers and dimers from lignin, and TGA seems to be the least efficient technique. Alkaline treatment of lignin with nitrobenzene or CuO are quite comparable to each other, but considerably less efficient with respect to generating monomers and dimers as compared to oligomers than the reductive depolymerization with Na/NH₃.

Since secondary condensation reactions may accompany depolymerization, it is important to compare the ratio of monomeric uncondensed (II, III) to condensed-type degradation products (compounds IV and V) and to dimers (VII and VIII); and the ratio of condensed to uncondensed-type degradation products ([IV + V + VI + VII + VIII]:[II + III]). Chemical structures of these monomers and dimers are shown in Figure 2. With regard to this criterion, the TGA technique and thioacetolysis (TAA) appear to be superior to the other methods, since their yield of II to the combined yield of dimeric compounds (VII and VIII) exceeds those of the three other depolymerization procedures. They also exceeded the other techniques in terms of yield of condensed-type fragments (III and IV). The highest ratio of uncondensed to condensed-type degradation products was achieved with TGA, followed by CuO and TAA. Nitrobenzene oxidation and treatment with Na/NH₃ both gave higher degrees of condensation in their monomeric and dimeric degradation products.

In summary, the evaluation by several performance criteria of five lignin depolymerization techniques suggests that thioacetolysis is optimal in producing monomers

and dimers instead of higher oligomers, and that it is also one of the mildest treatments in terms of preventing secondary condensation reactions involving the aromatic ring. However, its overall low yields disqualify it as a useful method in combination with the permanganate oxidation procedure. This finding demonstrates, in agreement with results by Erickson et al. (1973 b), the importance which side chain oxygenation has on the efficiency of mild permanganate oxidation. Only those methods which pre-oxidize lignin in the course of depolymerization seem to be capable of generating optimal and representative amounts of monomeric and dimeric degradation products. Of the two oxidative methods, alkaline treatment with nitrobenzene or CuO, the latter seems to provide higher overall yields, with less accompanying condensation side reactions. For this reason, the CuO method must be judged the best technique for depolymerizing lignin prior to permanganate oxidation. This finding is in full accord with earlier studies by Pepper et al. (1967), which suggested that CuO is a milder and more selective degradation agent for lignin than nitrobenzene. Non-oxidative depolymerization procedures seem to be less useful in combination with permanganate oxidation, although they may be more effective in cleaving prevalent ether-type interunit linkages in lignin, as is indicated by the data in Table 1.

Degradative Optimization

Attempts were made to optimize the CuO-Alkali depolymerization technique for lignin and to evaluate the effects of temperature and reaction time on yield and products. Figure 4 summarizes the results with regard to reaction temperature. In the temperature range from 100 to 174 °C, the combined yields of monomeric through hexameric degradation products (GPC yield) increase approximately sixfold, from 13 to 76 mg of methyl esters per 100 mg of lignin. The total yield of monomers and dimers (GC yield), however, increases only less than fourfold, and each of the five most prominent monomers and dimers seems to increase to its own individual extent. Since depolymerization occurs primarily by cleavage of aryl-alkyl ethers with simultaneous liberation of free phenolic OH groups, the overall slope of each curve of Figure 4 indicates total and individual free phenolic hydroxyl content per precursor type. This information is summarized in Table 2.

The precursor type with the highest percentage of free phenolic units seems to be guaiacyl units condensed with the side chain of another phenyl-propane unit. In fact, all precursors of metahemipinic acid (V) are probably phenolic units, in both Loblolly pine and red alder milled wood lignin (MWL). The syringyl analog of metahemipinic acid, trimethoxy phthalic acid, is phenolic only to the extent of approximately 70%. Results with isohemipinic acid (IV) are difficult to interpret, since 5-5' linked dimers with one phenolic and one etherified unit may also yield this fragment. One-third of all uncondensed guaiacyl units in Loblolly pine MWL appear to exist as free phenolic units, whereas this number is only one-fourth in the case of red alder MWL. Only approximately one-sixth of all uncondensed syringyl units in

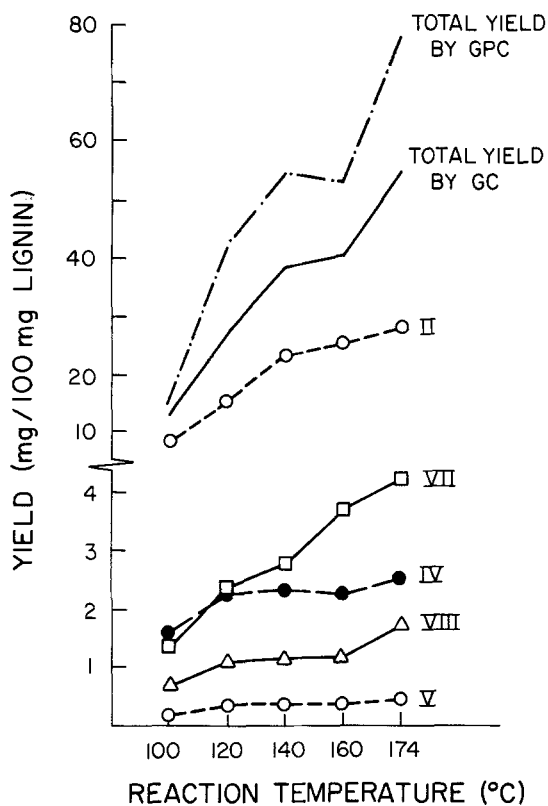


Fig. 4. Yield of degradation products vs. hydrolysis temperature. Cupric oxide/alkali

Table 2. Percent of units with free phenolic hydroxyl groups in relation to aromatic ring condensation

Type of aromatic ring	Model no.	Free phenolic units (per cent of total units in category)	
		Loblolly pine	Red alder
Guaiacyl, uncondensed	II	35	24
Syringyl, uncondensed	III	—	17
Guaiacyl, Alkyl in pos. 5	IV	59 ^a	28 ^a
Guaiacyl, Alkyl in pos. 6	V	100	100
Syringyl, Alkyl in pos. 6	VI	—	69
Guaiacyl, Aroxy in pos. 5	VIII	22	25
Guaiacyl, Aryl in pos. 5	VII	25	11

^a Incl. 5-5' linked dimers with one of the two phenolic sites free and one etherified

red alder MWL exist in the free phenolic form; the rest is involved in phenyl ether bonds. Approximately one-fourth to one-fifth of all 5-5' and 4-0-5' linked units in softwood MWL seem to be phenolic. Presumably, these differences in the reactivi-

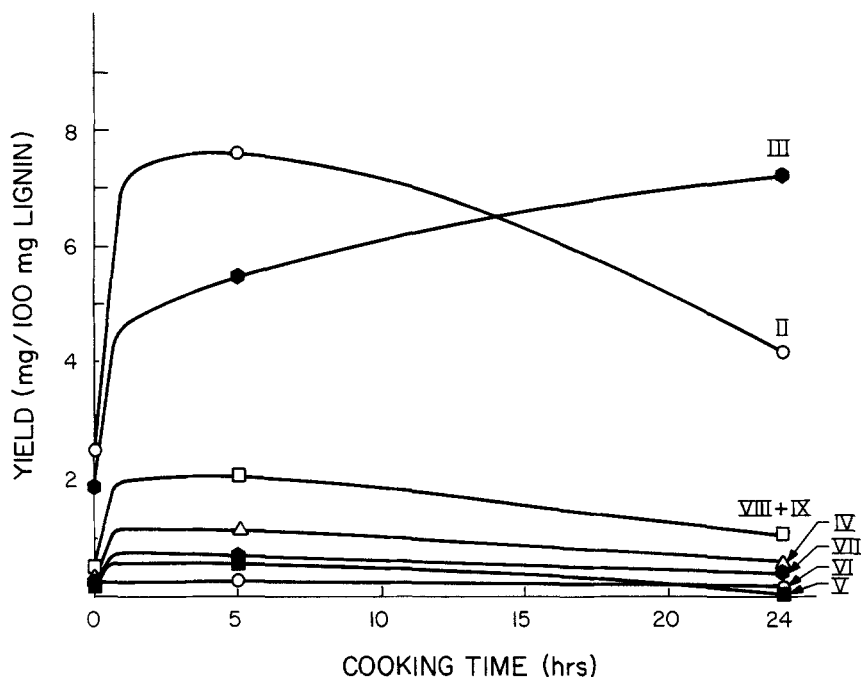


Fig. 5. Yield of major degradation products vs. hydrolysis time in Cupric oxid/alkali (red alder MWL)

ties of phenolic hydroxyl groups of phenylpropane units during lignification result from steric effects and/or electron spin density concentrations of their respective phenoxy radicals.

The effect of cooking time at 174°C on total and individual yields is shown in Figure 5. In contrast to most monomeric and dimeric aromatic carboxylic acids, which exhibit slight yield losses with increasing reaction time, trimethylgallic acid (III) shows steady yield gains. Overall, however, lignin depolymerization with CuO and alkali should be performed for not longer than 5 hours at 174°C. This agrees with previous studies (Pepper et al., 1967).

Conclusions

1. Among the five depolymerization techniques evaluated with regard to their capabilities to generate detailed analytical structural information on lignin polymers in combination with permanganate degradation, the method employing cupric oxide and alkali appeared to be most useful.

2. Acetolysis with thioacetic acid and reductive depolymerization with sodium in liquid ammonia appeared to be efficient depolymerization techniques; however, the resulting depolymerization products were difficult to oxidize with permanganate.

3. The cupric oxide/alkali method proved to be mild and gave no signs of secondary condensation reactions. This was determined by analyzing the ratio of uncondensed to condensed-type monomeric and dimeric degradation products.

4. Alkaline nitrobenzene depolymerization followed by permanganate oxidation yielded aromatic carboxylic acid mixtures with higher condensed to uncondensed mono- and dimer product mixtures, suggesting the occurrence of secondary condensation reactions. Similar results were obtained with thioglycolic acid.

5. Where alkaline depolymerization with nitrobenzene favored condensation at position 5 of the aromatic ring, the boron trifluoride catalyzed thioglycolation seemed to favor condensation at position 6.

6. Depolymerization efficiency with alkaline cupric oxide solutions was found to increase steadily in the range of 100 to 174 °C.

7. In Loblolly pine milled wood lignin, a total of ca. ten out of 28 uncondensed phenylpropane units (ca. 35 %) were found to have free phenolic hydroxyl groups, whereas other, condensed-type units were etherified to varying, higher or lesser degrees. Units condensed aliphatically in position 6 of the aromatic ring were found to be phenolic in all instances.

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N. Morohoshi
Wood Chemical Technology
Department of Forest Products
Faculty of Agriculture
Tokyo University of Agriculture and Technology
Fuchu, Tokyo 183
Japan

Prof. W. G. Glasser
Department of Forestry and Forest Products
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061
USA