

Application of Nucleus Exchange Technique to Lignin Structural Analysis*

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Abstract

The nucleus exchange method was originally developed for producing phenols from lignin. This method is a combination of alkylation and dealkylation in the presence of boron trifluoride and phenol. This method includes three reaction steps, which proceed simultaneously in the single reaction system containing phenol and boron trifluoride: first, the formation of diphenylmethane type structures by phenolation of the side chains; second, the exchange of lignin phenyl nuclei for phenol; finally, the demethylation of methoxyl groups. That is, in this process, C_α-aryl carbon-carbon linkages in phenylpropane units are selectively and quantitatively cleaved, releasing phenyl nuclei typified by guaiacol from guaiacyl (softwood) lignins. The guaiacol subsequently is partially demethylated to catechol. In the present paper, the application of nucleus exchange technique to lignin structural analysis is discussed in detail, following the theory, mechanism, and procedure of nucleus exchange method.

Key words: Lignin structural analysis, Nucleus exchange, Nitrobenzene oxidation, Lignin modification, Diphenylmethane

Introduction

A chemical method to degrade lignin in a medium consisting of boron trifluoride and excess phenol was originally developed for producing phenols from lignin and lignin-like materials (FUNAOKA et al. 1978a, b, 1980a, b, 1982a, b, c, d, e, f, g, ABE and FUNAOKA 1987). This method is a combination of alkylation and dealkylation reactions. In this reaction, C_α-aryl carbon-carbon linkages in phenylpropane units are selectively and quantitatively cleaved, releasing phenyl nuclei typified by guaiacol (1) (see Fig. 1 for structures of compounds) from guaiacyl (softwood) lignins. The guaiacol subsequently is partially demethylated to catechol (2). The key step in these reactions was termed nucleus exchange (NE) by the original authors.

Phenyl nuclei in lignins may be classified into non-condensed, diphenylmethane (DPM) type, and condensed nuclei, as shown in Fig. 2. Non-condensed nuclei include non-condensed type (Ga) and non-condensed type (Ga') in DPM-type units for guaiacyl lignins and Sa and Sa' types for syringyl lignins. DPM-type units include non-condensed (Ga') and condensed (Gb') types for guaiacyl lignins and Sa' and Sb' for syringyl lignins. Condensed nuclei include DPM-type condensed (Gb') and other type condensed (Gb) nuclei for guaiacyl lignins and Sb' and Sb for syringyl lignins. It should be kept in mind that there is essentially no DPM moiety in intact

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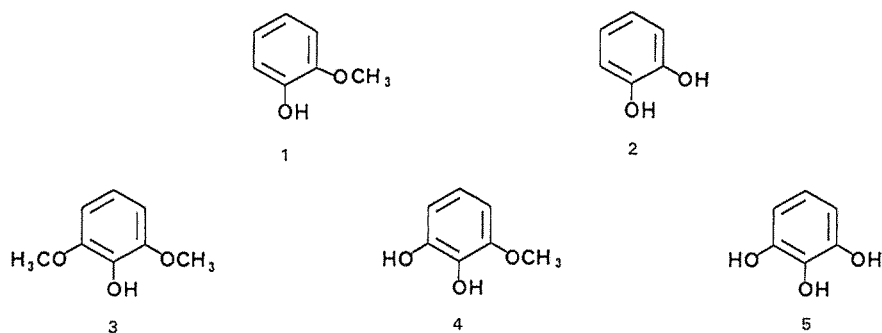


Fig. 1. Main polyhydric phenols generated from lignins in the nucleus exchange method.

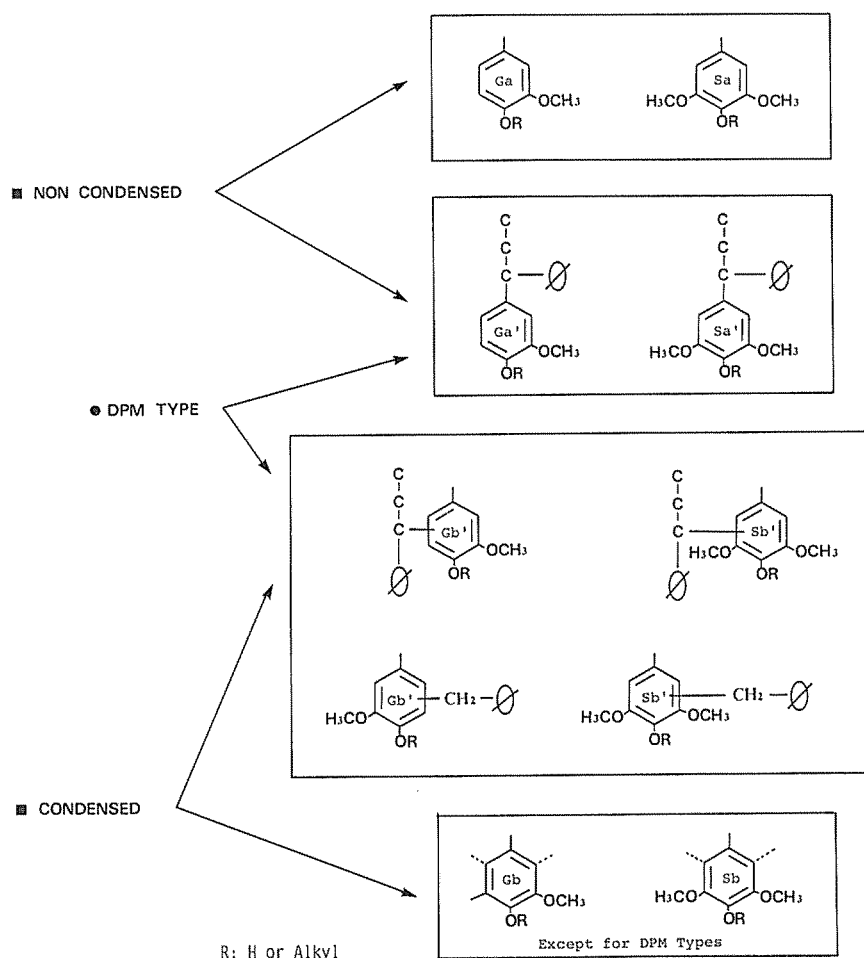


Fig. 2. Types of lignin phenyl nuclei.

protolignins. Consequently, for softwood protolignins, the phenyl nuclei are of types Ga and Gb. For hardwood protolignins, the phenyl nuclei include Ga, Gb, Sa, and Sb. However, the frequency of Sb units in hardwood protolignins is negligible.

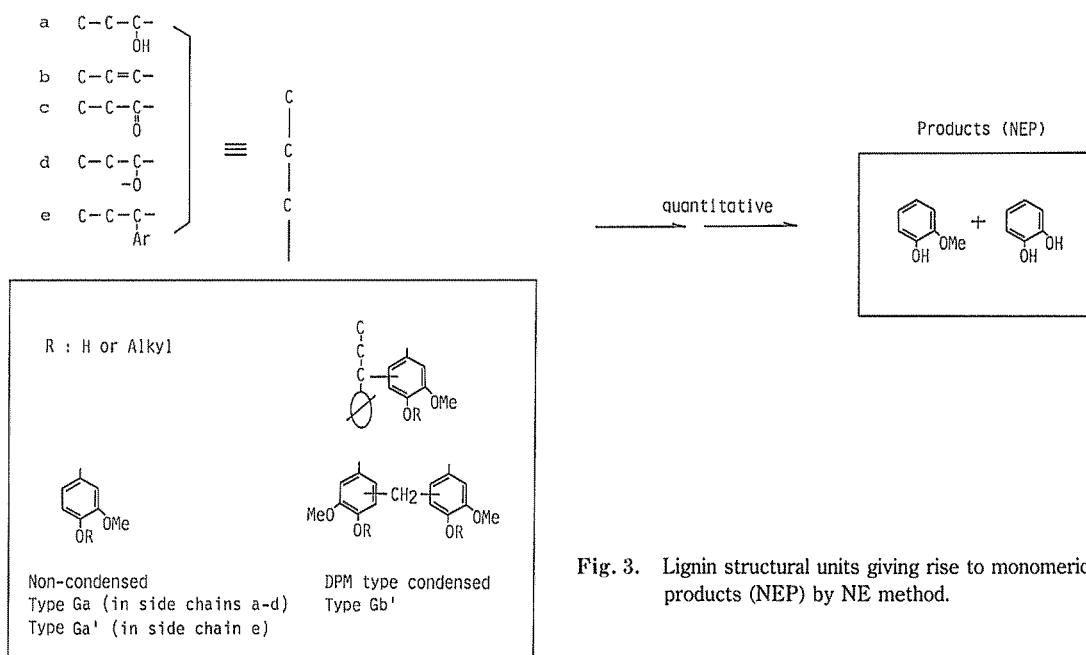


Fig. 3. Lignin structural units giving rise to monomeric products (NEP) by NE method.

The NE method quantitatively converts the non-condensed (Ga) and DPM-type (Ga', Gb') guaiacyl nuclei having side chain structures of a, b, c, d, and e (Fig. 3) into guaiacol and catechol. Quantitative yields of pyrogallol-1,3-dimethyl ether, pyrogallol-1-methyl ether, and pyrogallol are obtained from non-condensed (Sa) and DPM-type (Sa', Sb') syringyl nuclei.

Since the quantity of DPM-type units in protolignins is negligible, the sum of guaiacol and catechol (mol%) thus represents the quantity of non-condensed phenyl nuclei in softwood protolignins. In addition to guaiacol and catechol, pyrogallol-1,3-dimethyl ether (3), pyrogallol-1-methyl ether (4), and pyrogallol (5) (Fig. 1) can be formed from the release of syringyl nuclei from hardwood lignins and subsequent demethylation of these nuclei. Thus, the sum of (1) and (2) and the sum of (3), (4), and (5) represent the quantities of non-condensed phenyl nuclei in guaiacyl and syringyl units of hardwood protolignins, respectively. Consequently, unlike the other lignin degradation reactions, the NE reaction provides a quick and convenient means to determine quantitatively the extent of condensation of phenyl nuclei in intact protolignins. Furthermore, by applying the NE reaction to intact hardwood lignins, true syringyl/guaiacyl (S/G) ratios can be obtained for these lignins. So far, this is the only technique by which true S/G ratios of hardwood protolignins can be obtained with a single chemical reaction.

A combination of the NE method and nitrobenzene oxidation can be used to analyze the structural changes in lignin phenyl nuclei during various modification processes (FUNAOKA et al. 1983a, b, c, 1984, 1987b, 1988, 1989a, b, c, 1990a, b, c, CHIANG and FUNAOKA 1988, 1990a, b, CHIANG et al. 1988, 1989a, b). This paper will describe the use of the NE method to analyze the proportion of condensed and non-condensed phenyl nuclei in protolignins first and then its application to structural analysis of modified lignins.

Reaction Mechanism

In the NE reaction, lignin is degraded by boron trifluoride in the presence of excess phenol. The key step

in the reaction is the dealkylation of DPM-type structural units formed during the reaction as a result of phenolation at the side chain C_{α} -positions of phenylpropane units. To bring this reaction mechanism into clear focus, it is appropriate to discuss the alkylation and dealkylation of simple aromatic compounds.

Under acidic conditions, the alkylation and dealkylation of aromatic compounds are reversible reactions involving several steps forming π - and σ -complexes. However, dealkylation proceeds only under more drastic conditions as compared to alkylation. Nevertheless, this is not always the case. For example, if the aromatic compound is of the DPM-type, the dealkylation may proceed under mild conditions since the cations formed are resonance stabilized (Fig. 4). This statement is supported by the fact that DPM derivatives may be degraded even at room temperature by aluminum chloride to yield benzene, alkylbenzene and alkyl-diphenylmethane together with some resinous substances (TSUGE and TASHIRO 1962, 1965).

Based on the mechanism shown in Fig. 4, it is also clear that the cations formed after dealkylation may recombined with released phenyl nuclei as well as with the starting DPM compounds to form resin-like polymers (RADZIEWANOWSKI 1894, ODA and OGATA 1941, TSUGE and TASHIRO 1962, 1965). However, if to the reaction system shown in Fig. 4, another aromatic compound is added in excess, the newly formed cations will preferably react with this aromatic compound (Fig. 5). Consequently, the dealkylated phenyl nuclei (e.g. compound I in Fig. 5) remain as monomer units in the reaction system. The reaction scheme in this figure illustrates the basic

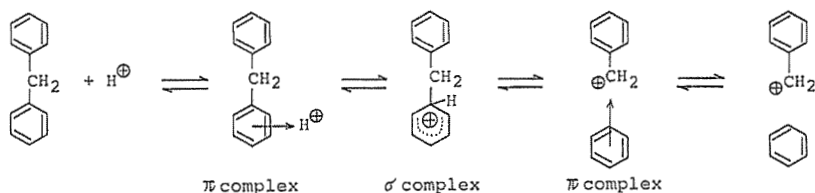


Fig. 4. Dealkylation of the phenyl nuclei in diphenylmethane type structures.

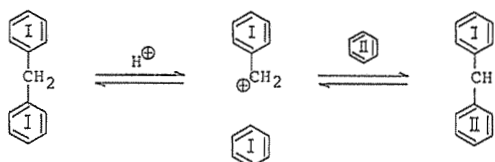


Fig. 5. Dealkylation of the phenyl nuclei in the presence of excess aromatic compound (II).

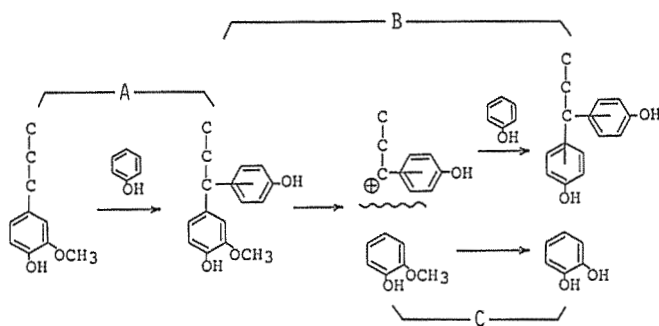


Fig. 6. Formation of catechol from softwood lignin by NE method.

principle of the NE reaction.

In the NE reaction system, lignin phenyl nuclei are converted into polyhydric phenols, such as catechol from guaiacyl nuclei and pyrogallol from syringyl nuclei according to the three reaction steps shown in Fig. 6. The step A is the phenolation of phenylpropane side chains to form DPM-type structural units, the step B is the exchange of the phenyl nuclei in lignin for phenol, and the step C is the demethylation of methoxyl groups. The quantitative character of these reactions is described below.

Phenolation

The reaction of lignin with phenol under acidic conditions has been demonstrated to occur predominantly at the C_α -position of the phenylpropane side chain (ISHIKAWA 1958, KRATZL et al. 1962, TAI et al. 1968, FUNAOKA et al. 1977). However, the phenolation reaction proceeds rapidly under even milder conditions if boron trifluoride is used as a catalyst. Using a series of monomeric guaiacyl and syringyl lignin model compounds having hydroxyl, carboxyl, carbonyl, ethylenic, and ether functionalities at the C_α -position of the side chain, it was demonstrated that almost all of the model compounds were quantitatively phenolated at the C_α -position using boron trifluoride as the acid after a 10-minute reaction at 40°C (FUNAOKA et al. 1980a). Moreover, at higher temperatures, all the model compounds listed above were quantitatively phenolated at the C_α -position to offer DPM-type structures. Furthermore, quantitative phenolation occurred with both phenolic and non-phenolic model compounds (FUNAOKA et al. 1980a, FUNAOKA 1984). Confirmation of this quantitative phenolation reaction was demonstrated by titrating the water formed during the reaction with phenol using a Karl-Fischer water titrator (FUNAOKA et al. 1980a). For instance, for every mole of phenol reacted with the benzylic hydroxyl group in 4-hydroxy-3-methoxy benzyl alcohol, one mole of water was released.

Nucleus exchange

The formation of DPM-type moieties in lignin through phenolation at the C_α -positions of the phenylpropane side chains is a key step for facilitating the release of phenyl nuclei such as guaiacol, in the NE reaction. As was stated previously, the dealkylation of DPM derivatives is facilitated because of the formation of resonance stabilized intermediate carbonium ions. Similarly, using the lignin model compounds mentioned previously, it has shown that after the formation of DPM type moieties, guaiacol was liberated quantitatively from non-condensed guaiacyl models, and pyrogallol-1,3-dimethyl ether from non-condensed syringyl models under mild conditions (40 to 60°C) (FUNAOKA et al. 1980a, b, 1982e, 1987a, FUNAOKA 1984). The sites from which guaiacol or pyrogallol-1,3-dimethyl ether was released were attacked immediately by the phenol, thus insuring that the liberated guaiacol or pyrogallol-1,3-dimethyl ether was preserved in monomeric form.

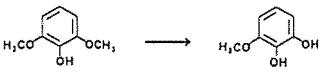

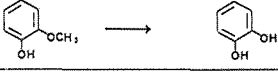
The liberation of phenyl nuclei is a second-order reaction (FUNAOKA et al. 1980a, b, FUNAOKA 1984). The reaction rate increases in the presence of a group capable of stabilizing the intermediate carbonium ions after the liberation of the phenyl nuclei. The rate is decreased by etherification of the phenolic hydroxyl group. This indicates that the phenolic hydroxyl group contributes to an increase in the electron density of the aromatic ring, thus facilitating the coordination of boron trifluoride to the aromatic ring. Because the coordination of boron trifluoride to the phenolic hydroxyl group by methoxyl groups in syringyl nuclei is hindered, the rate at which pyrogallol-1,3-dimethyl ether is liberated is slower than that for the release of guaiacol from guaiacyl units. Therefore, to overcome these obstacles for obtaining quantitative yields of guaiacol or pyrogallol-1,3-dimethyl ether, the NE reaction should be carried out at elevated temperatures such as 100°C and above. At these

temperatures, partial demethylation of guaiacol to catechol and of pyrogallol-1,3-dimethyl ether to pyrogallol-1-methyl ether occurs.

Demethylation

Unlike the two previous reaction steps (A & B in Fig. 6), demethylation proceeds slowly in the NE reaction sequence. This demethylation is a second-order reaction and becomes significant only at temperatures above 100°C (FUNAOKA et al. 1978b). When using guaiacol as a model compound to study demethylation in the NE method, it was found that extensive demethylation occurred in the temperature range of 160 to 180°C. However, at any time and temperature investigated, the sum of guaiacol and catechol concentrations was always equivalent to the starting concentration of guaiacol. This indicated that guaiacol and catechol were completely stable in the NE reaction system.

Table 1. Rate constants and activation energies for the demethylation of the methoxyl group in the nucleus exchange reaction

	Rate constant at 130°C (mole·mole ⁻¹ ·min ⁻¹)	Activation energy (Kcal)
	8.58×10^{-2}	24.09
	2.33×10^{-2}	28.45
	8.52×10^{-3}	30.55

Pyrogallol-1,3-dimethyl ether (3 in Fig. 1) is demethylated to pyrogallol-1-methyl ether (4) which in turn is demethylated to pyrogallol (5) by the NE treatment. The rates for demethylating 3 to 4 and 4 to 5 were found to be greater than the rate for demethylating guaiacol (1) to catechol (2), as shown in Table 1 (FUNAOKA et al. 1982e). This observation was explained mechanistically in detail in previous publications (FUNAOKA et al. 1982e, FUNAOKA 1984). As was demonstrated, the demethylation of syringyl nuclei occurred in the temperature range of 90 to 140°C. However, only at temperatures lower than 110°C was the sum of 3, 4, and 5 equivalent to the starting concentration of 3. At temperatures higher than 110°C, secondary modification of pyrogallol (5) occurred, lowering the combine yield of 3, 4, and 5 (FUNAOKA et al. 1982e, FUNAOKA 1984). Consequently, when dealing with syringyl lignins, a low temperature should be used to obtain reliable quantitative information.

Response of various structural units in lignins to the NE treatment

The above discussion demonstrates that lignin structural units, such as non-condensed guaiacyl and non-condensed syringyl nuclei having hydroxyl, carboxyl, carbonyl, double bond, and ether functionalities at the C_α-position of the side chain, give quantitative yields of catechol derivatives from guaiacyl nuclei and pyrogallol derivatives from syringyl nuclei in the NE reaction. The ether linkages in the predominant structural β-aryl ether units of lignin are easily cleaved by the NE reagents under mild conditions. This was demonstrated by the rapid liberation of guaiacol at 40°C from β-aryl ether model compounds, such as ω-(2-methoxyphenoxy)-

acetoveratrone and ω -(2-methoxyphenoxy)apocynol methyl ether (FUNAOKA et al. 1980a, FUNAOKA 1984). At this temperature (40°C), demethylation does not occur. Consequently, the formation of guaiacol is due entirely to the cleavage of β -aryl ether linkage releasing the guaiacol moiety from these model compounds. Cleavage of the β -aryl ether linkage was found to be a second-order reaction in which the rate determining step was phenolation at the C_α -position. In other words, the rate at which β -aryl ether linkages are cleaved is influenced by the reactivity of C_α -functional groups on the side chain towards phenolation. After phenolation, cleavage of the β -aryl ether linkage and the NE reaction occur simultaneously (FUNAOKA et al. 1980a, FUNAOKA 1984).

α -Aryl ether linkages in lignin structural units are also efficiently cleaved in the NE reaction system. Thus, guaiacol is released quantitatively from dehydrodiisoeugenol (FUNAOKA et al. 1980a).

The 5-5 linkage in biphenyl units is stable in the NE reaction system, while the hydroxyl groups in 2,2'-dihydroxydiphenyl are dehydrated forming a furan ring (FUNAOKA et al. 1982b).

Procedure of NE Method

Sample preparation

Air-dried wood sample is ground successively in a Wiley mill and a vibrational mill to pass an 80 mesh screen. The wood meal is extracted continuously with benzene-ethanol (2:1 by volume) in a Soxhlet extractor for 48 hours to remove the extractives. The extracted sample is first air-dried and then dried over P_2O_5 under vacuum for at least one week to completely remove moisture. A moisture-free sample is required for the NE reaction, since boron trifluoride will be deactivated to some extent by moisture of the sample.

In order to obtain quantitative yields of the NE products, the particle size of wood meal must be small enough to pass a 80 mesh screen (FUNAOKA et al. 1985).

Preparation of reagent

The reagent used in the method is a mixture of phenol, boron trifluoride-phenol complex, and xylene. Xylene is used as an inert reaction medium. The standard composition of the mixture (by volume) is listed below.

Phenol (reagent grade)	19 parts
Xylene (reagent grade)	10 parts
Boron trifluoride-phenol complex	4 parts

This composition is used for the analysis of wood protolignins or pulp lignins. For isolated lignins, such as milled wood lignin and kraft black liquor lignin, three parts of boron trifluoride-phenol complex are used with the amounts of phenol and xylene shown above.

Prior to mixing these three reagents, the xylene is dried over anhydrous sodium sulfate. The phenol is liquified at 40 to 50°C just before mixing. All glasswares should be moisture-free, and the preparation of the mixture should be done as quickly as possible. The steps involved are described below.

The required volume of dry xylene is measured into a graduated cylinder and a portion is transferred to a reagent bottle. Also measured out in a cylinder and quickly transferred to the same reagent bottle are the appropriate amounts of liquified phenol and the boron trifluoride-phenol complex. After each of the additions, the cylinder is washed well with the remaining portion of the xylene which is also transferred to the reagent

bottle. The bottle is sealed and stored in a warm, dark, moisture-free chamber.

The reagent should be warmed and mixed well prior to use. The phenol should be completely dissolved. If the reagent is used daily, or, in other words if the reagent is contacted with air moisture frequently, it is advisable to prepare a fresh quantity after a three-month period. However, the reagent should be checked periodically to confirm its reactivity.

Description of procedures

The NE treatment is usually carried out in stainless steel autoclave (5 ml volume) with 85 mg of wood meal and 3 ml of the reagent. Depending on the sample size (~ 28 mg) may also conveniently be used a stainless steel tube (0.6 cm diameters, 7.5 cm length) fitted with screw caps at both ends. The autoclave is heated in an oil bath or other available device in which the temperature may be controlled to $\pm 1-2^\circ\text{C}$ and which is provided with a means of shaking the autoclave continuously during the reaction. The detailed procedure is described below.

After the addition of wood meal and the reagent in an autoclave with two small stainless steel balls, the autoclave is sealed, shaken manually for about 5 minutes, then heated at $180 \pm 1-2^\circ\text{C}$ for 4 hours. The autoclave is shaken throughout the heating operation.

At the end of the reaction, the autoclave is removed from the heat source and cooled in a cold water bath. The contents are carefully transferred with ethyl ether into a 100 ml beaker. A small amount of ethyl ether is added to the autoclave and the residual material on the wall is scraped with a spatula. This solution is also added to the 100 ml beaker. This procedure is repeated until the washings become clear. The cap is also washed with ethyl ether which is combined with other ether washings.

A known amount of internal standard (bibenzyl in benzene) is added to the combined reaction solution and washing in the 100 ml beaker and the ether insoluble materials are filtered through a glass fiber filter paper (Whatman GF/A) which subsequently is washed several times with ethyl ether. The combine filtrate and washings from this operation are transferred to a separatory funnel and a NaCl-saturated solution is added to deactivate the boron trifluoride. The funnel is shaken vigorously and the ether layer is removed and concentrated to about 30 ml by film evaporation. The concentrate is transferred to a glass vial fitted with a Teflon-lined cap and dried overnight over anhydrous sodium sulfate.

A 0.05 ml aliquot of the ether solution is pipetted into a small glass vial which contains a few drops of pyridine and 0.1 ml of N,O-bis(trimethylsilyl)acetamide. The vial is stopped with a Teflon-lined cap and the TMS derivatization is carried out at room temperature for 1 hour. The TMS derivatives are determined by gas chromatography.

Quantitative analysis of products by gas chromatography

A Hewlett Packard 5890 A Gas Chromatograph equipped with a computerized integrator (HP 9122+HP 3393 A or equivalent) and a FID detector are used. The separation is carried out on a 50 m crosslinked methyl silicone capillary column (Hewlett Packard HP-1, 50 m \times 0.2 mm \times 0.33 μm). The column temperature is held at 170°C for 14 minutes and then is raised to 180°C at a rate of $5^\circ\text{C}/\text{min}$. After 8 minutes at 180°C , the column is heated again to a final temperature of 270°C at a rate of $30^\circ\text{C}/\text{min}$. The final temperature is maintained for 15 min. The injector and detector temperatures are 250 and 270°C , respectively. Helium is used as the carrier gas. A typical chromatogram is shown in Fig. 7.

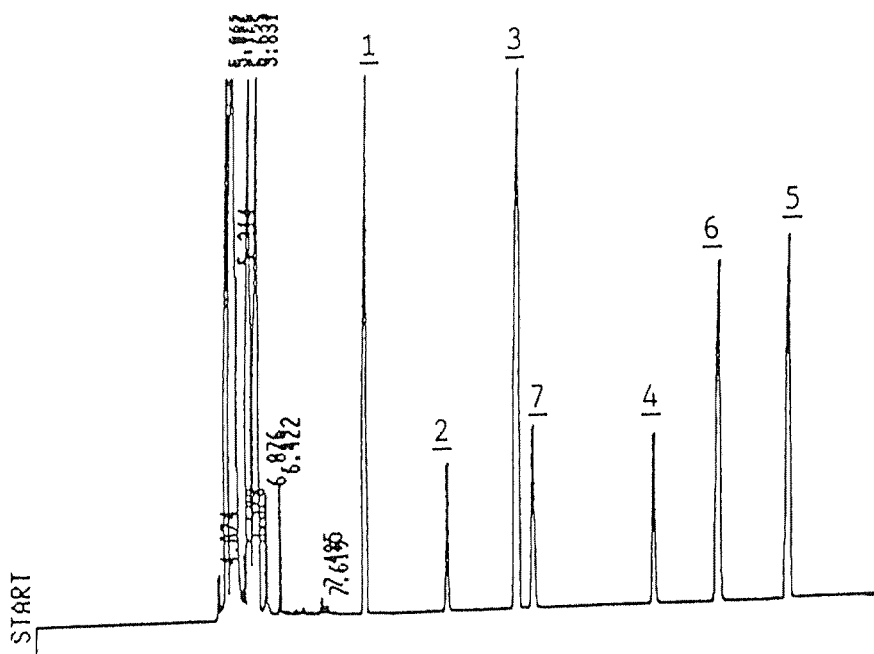


Fig. 7. Gas chromatogram of nucleus exchange products (NEP).

1: Guaiacol, 2: Catechol, 3: Pyrogallol-1, 3-dimethyl ether, 4: Pyrogallol-1-methyl ether, 5: Pyrogallol, 6: Internal standard (bibenzyl), 7: Diphenylether (by-product from phenol).

Applying the same GC separation conditions described above, calibration curves are made for guaiacol, catechol, pyrogallol-1,3-dimethyl ether, pyrogallol-1-methyl ether, and pyrogallol using the authentic compounds. For each compound, a linear relationship is established between (W_p/W_i) and (A_p/A_i) , as shown in Eq. (1).

$$(W_p/W_i) = a + b (A_p/A_i) \quad (1)$$

where W_p and W_i are the weights of the product and the internal standard, respectively, A_p and A_i are the peak areas of the product and the internal standard, respectively. From the calibration curves, the yields of NE products can be calculated and expressed either as weight% (Y_w) based on lignin or mole% (Y_m) based on lignin according to Eqs. (2) and (3), respectively.

$$Y_w (\text{weight}\%, \text{ based on lignin}) = W_p/W_i \quad (2)$$

$$Y_m (\text{mole}\%, \text{ based on lignin}) = (W_p/MW_p) \times (MW_{C-9}/W_i) \quad (3)$$

where W_i is the weight of lignin, MW_p is the molecular weight of the product, and MW_{C-9} is the molecular weight of a C-9 lignin unit. W_i can be determined from the Klason+acid soluble lignin contents. To obtain the molecular weight of the C-9 unit, milled wood lignin is isolated from the corresponding wood samples (CHIANG and FUNAOKA 1988). From the elemental and methoxyl content analysis, a C-9 empirical formula and thus, the molecular weight can be obtained. When a sample such as a kraft lignin is used, a C-9 molecular weight for this lignin should be determined and used in Eq. (3).

Structural Analysis of Protolignin

Quantitative determination of non-condensed and condensed guaiacyl units in softwood protolignins

For softwood protolignins, the yields of the NE products (NEP(o)_g, guaiacol+catechol) represents the amount of non-condensed guaiacyl units.

$$\text{Non-condensed guaiacyl units (Ga), mole\%,} = \text{NEP(o)}_g = Y_{mg}\% + Y_{mc}\% \quad (4)$$

where Y_{mg} and $Y_{mc}\%$ are mole% (based on lignin) yields of guaiacol and catechol, respectively.

Since the numbers of syringyl and p-coumaryl units are usually insignificant for most of the common normal softwood species, then:

$$\text{Condensed guaiacyl units (Gb), mole\%,} = 100 - \text{NEP(o)}_g \quad (5)$$

Quantitative determination of non-condensed and condensed units in hardwood protolignin

There are two principle types of phenyl nuclei, guaiacyl and syringyl, in most of the common hardwood protolignins. There being no substituent on ring positions 2 and 6, syringyl units in hardwood protolignins are of the non-condensed type. Consequently, there are non-condensed and condensed guaiacyl and non-condensed syringyl units in hardwood protolignins. The quantity of non-condensed guaiacyl units can be obtained in the same way as for softwood protolignins described above. Thus, after the standard NE treatment (at 180°C for 4 hours), the mole% of NEP(o)_g represents the non-condensed guaiacyl units and can be calculated according to Eq. (4). However, in order to obtain the quantity of condensed guaiacyl units, the quantity of non-condensed syringyl units must be determined.

To obtain quantitative information for syringyl units, the NE treatment is carried out first at 110°C for 4 hours to obtain the NE products (NEP(o)_s¹¹⁰, pyrogallol-1,3-dimethyl ether(3)+pyrogallol-1-methyl ether(4)+pyrogallol(5) from syringyl units. At temperatures higher than 130°C, pyrogallol tends to undergo secondary modification reactions, which lower the total yield of the products (see Section "Demethylation"). This problem is prevented when the reaction temperature is 110°C. However, the quantity of 3+4+5 at 110°C (NEP(o)_s¹¹⁰) is only a fraction of the maximum yield of NEP(o)_s from syringyl units. Consequently, the NEP(o)_s¹¹⁰ has to be multiplied by a factor of "f" to obtain the quantitative yield of NEP(o)_s. Because of the similarity between guaiacyl and syringyl units in their responses to the NE reaction (FUNAOKA et al. 1982e), the factor "f" can be conveniently obtained from the ratio of quantitative NEP(o)_g yield obtained at 180°C to NEP(o)_g yield at 110°C (NEP(o)_g¹¹⁰):

$$\text{NEP(o)}_g / \text{NEP(o)}_g^{110} = f \quad (6)$$

and

$$\begin{aligned} &\text{NEP(o)}_s(\text{quantitative mole\% yield of 3+4+5 from syringyl units}) \\ &= \text{NEP(o)}_s^{110} \times f = \text{NEP(o)}_s^{110} \times [\text{NEP(o)}_g / \text{NEP(o)}_g^{110}] \end{aligned} \quad (7)$$

From the known quantity of non-condensed syringyl units, Sa, (NEP(o)_s), the quantity of total guaiacyl units is calculated according to Eq. (8). From Eq. (9), the quantity of condensed guaiacyl units is then obtained.

$$\text{Total guaiacyl units (Ga and Gb), mole\%,} = 100 - \text{NEP(o)}_s \quad (8)$$

$$\begin{aligned}
 &\text{condensed guaiacyl units (Gb), mole\%,} \\
 &= 100 - \text{syringyl units (Sa)} - \text{non-condensed guaiacyl units (Ga)} \\
 &= 100 - \text{NEP(o)}_s - \text{NEP(o)}_g
 \end{aligned}
 \tag{9}$$

Discussion of method

Confirmation of the quantitative nature of NE reaction on lignin

Model compounds selected to represent various non-condensed structural elements in lignin have been reacted with the NE reagents and found to yield quantitative amount of guaiacol and catechol (FUNAOKA et al. 1980a, b, 1982a, b, c, FUNAOKA 1984). This finding was validated by subjecting spruce milled wood lignin (MWL) to the same sequence of reactions and comparing the results with pertinent data obtained using other analytical methods (Table 2) (FUNAOKA et al. 1980a).

The sum of the quantities of benzyl alcohol, conjugated carbonyl groups, non-cyclic and cyclic benzyl ethers, and ethylenic double bonds on the side chains of guaiacyl units, has been found to be 0.803 mole/OCH₃ (MARTON 1964). This figure represents the total quantity of these structures for both condensed and non-condensed guaiacyl units. The fraction of non-condensed guaiacyl units in softwood milled wood lignin was reported to be 50 to 60% (LUDWIG et al. 1964). Consequently, the content of non-condensed guaiacyl units having the above mentioned side chain structures should be 0.40 to 0.48 mole/OCH₃. This value is in excellent agreement with that for the combine yields of guaiacol and catechol from this MWL after the NE treatment (Table 2).

Table 2. Comparison of the quantities of reactive sites of spruce milled wood lignin and the nucleus exchange product (NEP)

Functional groups	Quantity (mole/OCH ₃)
Carbonyl	
Coniferyl type	0.031
α -Carbonyl	0.073
Benzyl alcohol and ether	
Non-cyclic	0.438
Cyclic	
Phenylcoumaran	0.115
Pinoresinol	0.104
Ethylenic double bond	
Coniferyl type* ¹	0.042
Total	0.803
Non-condensed type* ²	0.40–0.48
Nucleus exchange product (NEP)	0.42

*¹ Calculated by subtracting the amount of coniferyl aldehyde from the value by MARTON (1964), which contains two types of double bonds, coniferyl alcohol and coniferyl aldehyde.

*² Calculated by multiplying the ratio of non-condensed units, 0.50–0.60 (LUDWIG et al. 1964), by the total amount of reactive sites.

The use of NE method for protolignins

The NE method provides a rapid and relatively simple means of determining the degree of condensation in protolignins. As shown in Table 3, the quantities of non-condensed (Ga) and condensed (Gb) guaiacyl units have been determined for 14 softwood protolignins using the NE reaction.

In general, the fraction of condensed guaiacyl units in softwood protolignins is in the range of 43 to 55%. The extent of condensation in guaiacyl units has been estimated mainly using milled wood lignins isolated from various softwood species (ADLER et al. 1961, LUDWIG et al. 1964, LENZ 1968, MOROHOSHI et al. 1971). For protolignins, TERASHIMA et al. (1979, 1987) and TOMIMURA et al. (1979) have demonstrated, using radiotracer methods, that about 50% of the guaiacyl units are of the condensed type. Their finding is in excellent agreement with the results of the NE method applied to protolignins (Table 3). However, unlike the NE reaction, their methods are usually laborious and sometimes require sophisticated instrumental techniques.

Qualitatively, it has been demonstrated by Meshituka and NAKANO (1985) that guaiacyl lignin in the middle lamella region is more condensed than the lignin in the secondary wall region. Quantitatively, this has been

Table 3. Condensed and non-condensed guaiacyl units in softwood protolignins

Species	mole% based on lignin	
	Condensed	Non-condensed
Douglas-fir* ¹ (<i>Pseudotsuga menziesii</i>)	50	50
Western hemlock* ² (<i>Tsuga heterophylla</i>)	55	45
Spruce (<i>Picea abies</i>)	48	52
Yezo spruce (<i>Picea jezoensis</i>)	47	53
Glehn's spruce (<i>Picea glehnii</i>)	45	55
Slash pine (<i>Pinus elliottii</i>)	50	50
Japanese red pine (<i>Pinus densiflora</i>)	43	57
Japanese black pine (<i>Pinus thunbergii</i>)	48	52
Japanese larch (<i>Larix leptolepis</i>)	55	45
Japanese fir (<i>Abies firma</i>)	49	51
Japanese hemlock (<i>Tsuga sieboldii</i>)	48	52
Sugi (<i>Cryptomeria japonica</i>)	45	55
Hinoki (<i>Chamaecyparis obtusa</i>)	45	55
Japanese torreyia (<i>Torreya nucifera</i>)	53	47

*¹ From CHIANG and FUNAOKA (1988)

*² From CHIANG et al. (1989b)

The rest of the data are from FUNAOKA et al. (1986)

confirmed by applying the NE method to secondary wall- and middle lamella-enriched fractions isolated from Douglas-fir wood meal (CHIANG and FUNAOKA 1989a). As shown in Table 4, the ratio of condensed guaiacyl units/non-condensed guaiacyl units is about unity for both whole wood and the secondary wall-enriched fraction, whereas a ratio of about 2.5/1 is found for the middle lamella-enriched fraction. This suggests that the frequencies of β -5, 5-5, and 4-O-5 types of linkages are significantly higher in the middle lamella lignin than in the secondary wall lignin. This further indicates that the polymerization of lignin in the middle lamella region is mainly of the "bulk type", generating a highly condensed lignin macromolecule.

The quantities of non-condensed and condensed units in hardwood protolignins have been reported infrequently due mainly to the difficulty of analyzing these heterogeneous lignins by conventional methods. However, the NE method again provides facile means to analyze the heterogeneity of hardwood lignins. The various types of units in sweetgum protolignin, analyzed by the NE method, are shown in Table 5 (CHIANG and FUNAOKA 1990a). Sweetgum protolignin has a syringyl/guaiacyl (S/G) ratio of 1.56. This value is the true ratio of syringyl to guaiacyl contents and differs from the conventional S/V ratio [(syringaldehyde + syringic acid)/(vanillin + vanillic acid)] obtained from alkaline nitrobenzene oxidation. Nitrobenzene oxidation gives monomeric products (vanillin and vanillic acid) from non-condensed guaiacyl units with the conversion rate of 0.7–0.8, whereas the NE method quantitatively converts the non-condensed guaiacyl units into guaiacol and catechol. Consequently, it should be stressed that the S/V ratios obtained by nitrobenzene oxidation of lignin preparations may be misleading and deviate significantly from the true relationship between the quantities of syringyl and guaiacyl units. The S/G ratio of 1.56 for sweetgum is in agreement with the estimated S/G ratios obtained in a solid state ^{13}C NMR study (MANDERS 1987).

Table 4. Characteristics of Douglas-fir lignin

	Whole wood	Secondary wall (SW)	Middle lamella (ML)
Lignin content	29.8 (% on lignin)	25.9 (% on SW fraction)	46.2 (% on ML fraction)
Condensed	50/50	48/52	71/29
Non-condensed			

Table 5. Characterization of hardwood protolignin by the NE method

Sample	Guaiacyl, mole% on lignin			Syringyl, mole% on lignin		S/G* ¹	S/V* ²
	Non-condensed	Condensed	Total	Non-condensed	Total		
Sweetgum (Liquidambar styraciflua)	22	17	39	61	61	1.56	2.82

*¹ S/G = Total syringyl units/Total guaiacyl units

*² S/V = (Syringaldehyde + Syringic acid)/(Vanillin + Vanillic acid) from nitrobenzene oxidation.

Structural Analysis of Modified Lignin

In the below section, the abbreviations are used as follows:

NC(o)_g and NC(o)_s: Non-condensed guaiacyl and syringyl units in the original lignin, respectively.

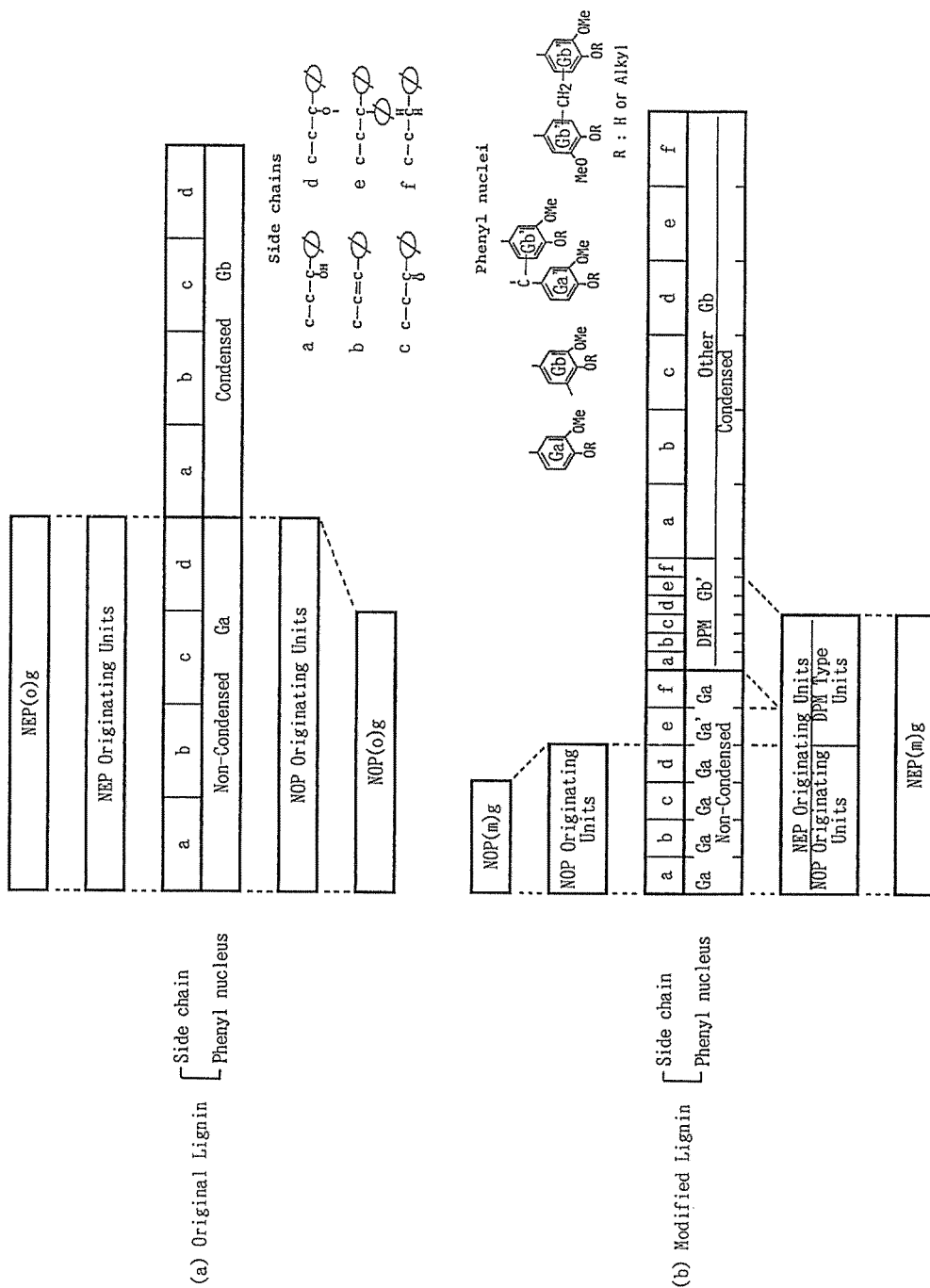


Fig. 8. Relationship between softwood lignin structures and yields of nucleus exchange (NEP) and nitrobenzene oxidation (NOP) products.

NC(m)_g and NC(m)_s: Non-condensed guaiacyl and syringyl units in the modified lignin, respectively.

NEP: NE Reaction products.

NEP(o)_g and NEP(m)_g: NE Reaction products (guaiacol and catechol) from guaiacyl units in original and modified lignins, respectively.

NEP(o)_s and NEP(m)_s: NE Reaction products (pyrogallol-1,3-dimethyl ether, pyrogallol-1-methyl ether and pyrogallol) from syringyl units in original and modified lignins, respectively.

NOP: Nitrobenzene oxidation products.

NOP(o)_g and NOP(m)_g: Nitrobenzene oxidation products (vanillin and vanillic acid) from guaiacyl units in original and modified lignins, respectively.

NOP(o)_s and NOP(m)_s: Nitrobenzene oxidation products (syringaldehyde and syringic acid) from syringyl units in original and modified lignins, respectively.

Compared with protolignin, the structures of modified lignins are much more complicated because of the various structural changes, such as secondary condensation and dehydration, during some treatments. Fig. 8 schematically illustrates the structure of modified softwood lignin. Among those structural units, only the units with α -saturated side chains [the side chain f in Fig. 8 (b)] give neither NEP nor NOP. Furthermore, there are the following two significant differences between NEP and NOP:

1. NEP originating units: non-condensed units (Ga) with side chains of a to d, DPM type non-condensed units (Ga') with side chain of e, and DPM type condensed units (Gb') with side chains of a to e.

NOP originating units: non-condensed units (Ga) with side chains of a to d.

Namely, DPM type units (Ga' and Gb') do not give NOP (Fig. 9).

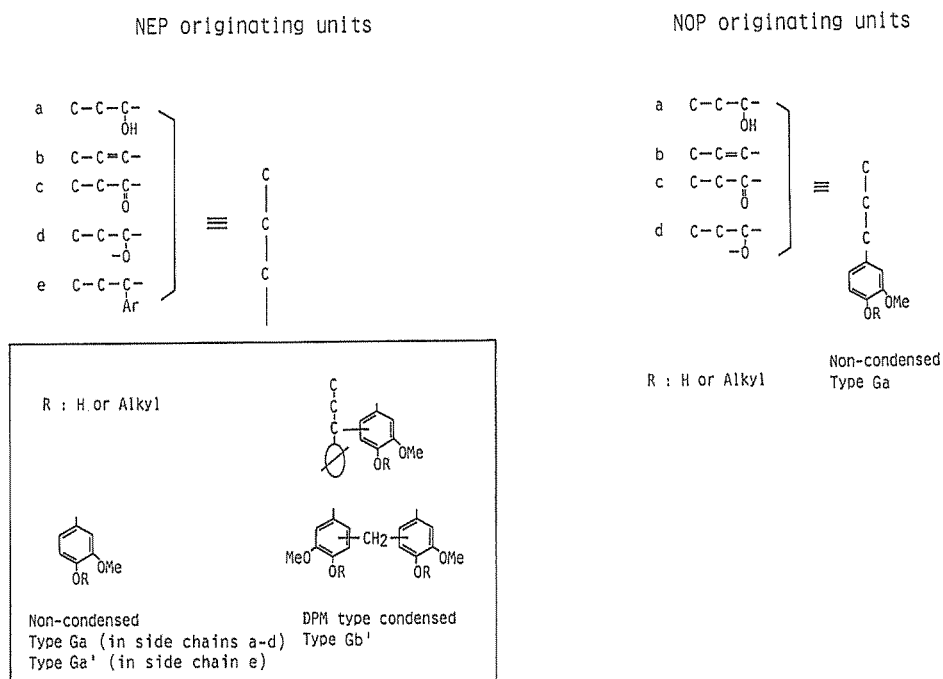


Fig. 9. Lignin structural units giving monomeric products, NEP and NOP, by NE method and nitrobenzene oxidation, respectively.

2. The NEP's are formed quantitatively from all NEP originating units, whereas the formation of NOP is not quantitative (there is a maximum conversion of non-condensed units, Ga, into NOP) [Fig. 8(b)].

If the presence of α -saturated structures may be neglected because of their trace amounts, Fig. 8 can be simplified to Fig. 10. Similarly, hardwood modified lignin can be illustrated as Fig. 11. The quantities of non-condensed (Ga, Sa), DPM type (Ga' and Gb', Sa' and Sb'), and other type condensed (Gb, Sb) units can be estimated rapidly by a combination of NEP and NOP. So far, it has been very difficult by conventional analytical methods, even by modern NMR techniques to distinguish quantitatively between non-condensed (Ga, Sa) and DPM type non-condensed (Ga', Sa'), and between condensed (Gb, Sb) and DPM type condensed (Gb', Sb').

In the calculation of various units in modified lignins by NE technique, the estimation of non-condensed units (Ga, Sa) is of the utmost importance. Non-condensed (Ga, Sa) units are those that have escaped condensation reactions during the modification process, suggesting that the modification degree of the units (Ga, Sa) is much lower than other units. Therefore, it is likely that the conversion rate of non-condensed units (Ga, Sa) into NOP in modified lignins is similar to that in protolignin. Of course, various structural changes of side chains might occur during modification processes. However, the formation of NOP-high yielding structures might be set off against that of NOP-low yielding structures, resulting in almost unchanged conversion rate to NOP. In fact, the variations of NOP yields during various modification processes usually have a fair correspondence with the condensation of lignin, and the NOP yield has been used as an index for condensation reactions during modifications (KRATZL 1960). This means that the NOP yield is influenced mainly by structural changes in the side chains and phenyl nuclei caused by condensation (the formation of types Ga', Gb' and Gb in guaiacyl, and types Sa', Sb' and Sb in syringyl). In other words, the conversion rate of non-condensed units (Ga, Sa) that are not associated with condensed structures into NOP cannot be regarded to be changed significantly during modification.

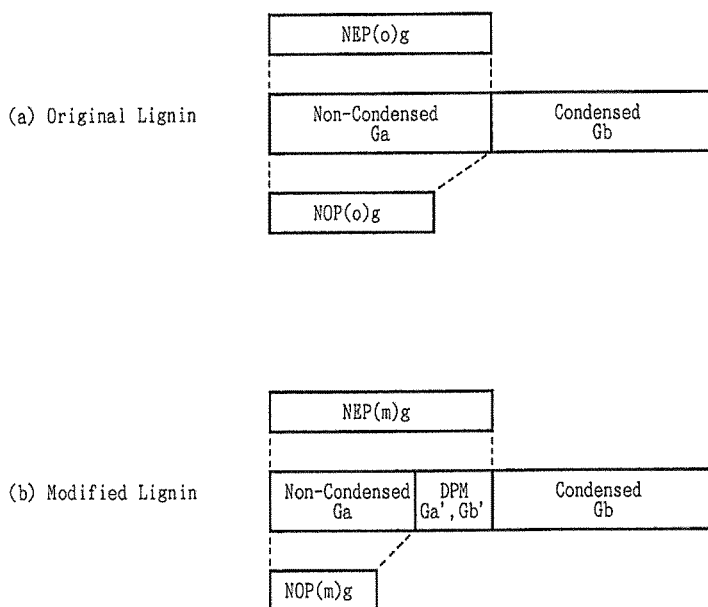


Fig. 10. Structural units of softwood lignin and the yields of NEP and NOP.

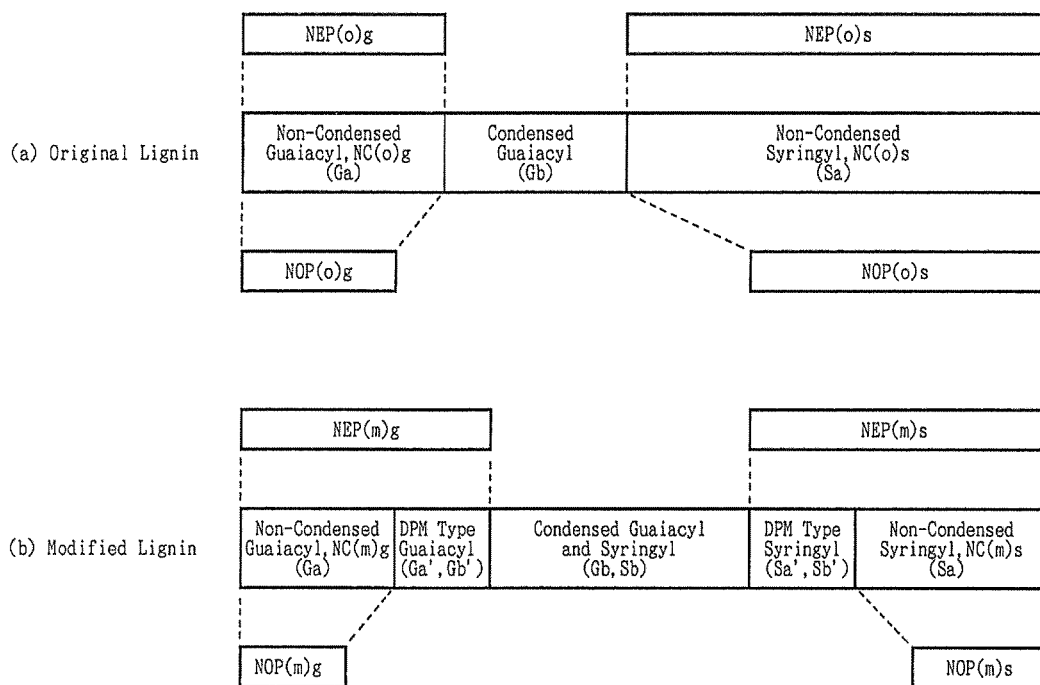


Fig. 11. Structural units of hardwood lignin and the yields of NEP and NOP.

Experimental procedure

The procedure of NE method was described in detail previously. The nitrobenzene oxidation is carried out as follows: extractive- and moisture-free wood sample (40 mg, 80 mesh pass), 2 N sodium hydroxide (3.2 ml) and re-distilled nitrobenzene (0.2 ml) are placed in a stainless steel autoclave (5 ml in volume) with two stainless steel balls and heated at 170°C for 3 hours in an oil bath. After the reaction, the mixture is transferred quantitatively into a separatory funnel with 2 N sodium hydroxide solution and is extracted 3 times with ethyl ether. The aqueous layer is separated, to which a known amount of internal standard (2,6-dimethoxyphenol in dioxane) is added, and then acidified to pH 2 with 18% hydrochloric acid. The acidic solution is saturated with sodium chloride and extracted 3 times with ethyl ether in a separatory funnel. The ether extracts are combined and then dried over anhydrous sodium sulfate overnight. After the evaporation of ethyl ether, the products are converted to their trimethylsilyl derivatives with N,O-bis(trimethylsilyl)acetamide in pyridine and analyzed by gas chromatography. The same gas chromatograph system as for the NE products is used. However, the column temperature is 160°C initially and is raised after 14 minutes to the final temperature of 270°C at a rate of 5°C/min.

Quantitative calculation of various lignin structural units

Softwood modified lignin

$$\text{Assuming that } \text{NOP(m)}_g / [\text{NC(m)}_g, \text{Ga}] = \text{NOP(o)}_g / \text{NC(o)}_g \quad (10)$$

various units in modified softwood lignin can be calculated as shown below.

$$\begin{aligned} \text{Non-condensed guaiacyl units [NC(m)}_g, \text{ Ga], mole\%,} &= \text{NOP(m)}_g / [\text{NOP(o)}_g / \text{NC(o)}_g] \\ &= \text{NOP(m)}_g / [\text{NOP(o)}_g / \text{NEP(o)}_g] \end{aligned} \quad (11)$$

$$\begin{aligned} \text{DPM type units (Ga' and Gb'), mole\%,} &= \text{NEP(m)}_g - [\text{NC(m)}_g, \text{ Ga}] \\ &= \text{NEP(m)}_g - \text{NOP(m)}_g / [\text{NOP(o)}_g / \text{NEP(o)}_g] \end{aligned} \quad (12)$$

$$\text{Condensed units (Gb), mole\%,} = 100 - \text{NEP(m)}_g \quad (13)$$

In the above calculations, it should be noted that the α -saturated units, if present, and the units with diphenyl ether linkages are counted as condensed units (Gb) because α -saturated units do not give NEP and diphenyl ether linkages are stable under NE conditions.

Hardwood modified lignin

Similarly, various units in modified hardwood lignin can be calculated as follows:

$$\text{Assuming that } \text{NOP(m)}_g / [\text{NC(m)}_g, \text{ Ga}] = \text{NOP(o)}_g / \text{NC(o)}_g \quad (14)$$

$$\text{Non-condensed guaiacyl units [NC(m)}_g, \text{ Ga], mole\%,} = \text{NOP(m)}_g / [\text{NOP(o)}_g / \text{NEP(o)}_g] \quad (15)$$

$$\begin{aligned} \text{DPM type guaiacyl units (Ga' and Gb'), mole\%,} &= \text{NEP(m)}_g - [\text{NC(m)}_g, \text{ Ga}] \\ &= \text{NEP(m)}_g - \text{NOP(m)}_g / [\text{NOP(o)}_g / \text{NEP(o)}_g] \end{aligned} \quad (16)$$

$$\text{Assuming that } \text{NOP(m)}_s / [\text{NC(m)}_s, \text{ Sa}] = \text{NOP(o)}_s / \text{NC(o)}_s \quad (17)$$

$$\text{Non-condensed syringyl units [NC(m)}_s, \text{ Sa], mole\%,} = \text{NOP(m)}_s / [\text{NOP(o)}_s / \text{NEP(o)}_s] \quad (18)$$

$$\begin{aligned} \text{DPM type syringyl units (Sa' and Sb'), mole\%,} &= \text{NEP(m)}_s - [\text{NC(m)}_s, \text{ Sa}] \\ &= \text{NEP(m)}_s - \text{NOP(m)}_s / [\text{NOP(o)}_s / \text{NEP(o)}_s] \end{aligned} \quad (19)$$

$$\text{Condensed guaiacyl and syringyl units (Gb and Sb), mole\%,} = 100 - \text{NEP(m)}_g - \text{NEP(m)}_s \quad (20)$$

If total guaiacyl and syringyl units can be known,

$$\text{Condensed guaiacyl units (Gb), mole\%,} = \text{Total guaiacyl} - \text{NEP(m)}_g \quad (21)$$

$$\text{Condensed syringyl units (Sb), mole\%,} = \text{Total syringyl} - \text{NEP(m)}_s \quad (22)$$

Analysis of softwood and hardwood modified lignins

The NE method is especially useful as an analytical tool when used to analyze the content of total DPM moieties in lignins modified by various chemical processes such as pulping. The formation of DPM-type structural units in lignin is an important reaction and occurs readily under both acidic and alkaline conditions. For instance, the α -positions on the lignin side chains may be attacked readily by adjacent phenyl nuclei under both acidic and alkaline conditions or merely by heating to form C_α -aryl linkages (DPM type of structures) (NIMZ 1969, FUNAOKA et al. 1978a, GIERER 1982, FUNAOKA et al. 1988, 1989a, b, c, 1990a, b, c, d). It is also known that condensation of phenyl nuclei with formaldehyde derived from the γ -carbons yields DPM type structures (GIERER 1982, YASUDA et al. 1982). DPM type structural units greatly influence the color of lignin and other properties such as its reactivity and solubility. So far, the quantity of these units secondarily formed within the

lignin molecule can only be determined without isolation of the lignin by a technique combining the NE method and nitrobenzene oxidation.

It has been well known for a long time that the acid treatment of wood makes the lignin molecule rigid mainly because of condensation. However, it has been so far difficult to elucidate condensed structures in acid-modified lignins because of its rigidity, less reactivity, and less solubility. Highly condensed lignins can be readily depolymerized to give ether soluble monomeric and oligomeric fractions by the NE method because DPM type condensed structures, main condensed structures, are selectively cleaved (FUNAOKA et al. 1989a, b, 1990a). In order to elucidate the relationship between the condensation at C_α -positions and the acidic treatment conditions, spruce wood meal (*Picea jezoensis*) were treated under various acidities and the resulting lignins were analyzed by the NE technique. The treatments with only water (Fig. 12) and with concentrated acid (Fig. 13) caused selective condensation at C_α -positions, whereas the selectivity of C_α -condensation got lower in the treatment with diluted acid (FUNAOKA et al. 1988, 1989a, b, c, 1990a, b, c, d). That is, diluted acid treatment of lignin leads to the diversification of reactivity of lignin side chains which is undesirable for utilization.

During pulping, depolymerization of lignin is always accompanied by condensation reactions. In general, the latter leads to increased molecular weight of lignin and the solubilization of lignin is retarded or inhibited. However, it still remains unknown when, and to what extent, condensation reactions occur in wood or in pulp residual lignins during pulping. Among various condensations, DPM type units can be considered to be the major condensation products present in residual lignins. At various steps of kraft delignification the quantities of units that were condensed into the DPM type moieties in residual lignins were determined by the NE technique. In kraft pulping of Douglas-fir, the formation of DPM type structures did not occur before the pulping temperature had reached 170°C. As delignification proceeded, the quantity of DPM type moieties in residual lignins increased steadily. After 2 hours at 170°C ($-\kappa 30$) the residual lignin consisted of 54 mol% units

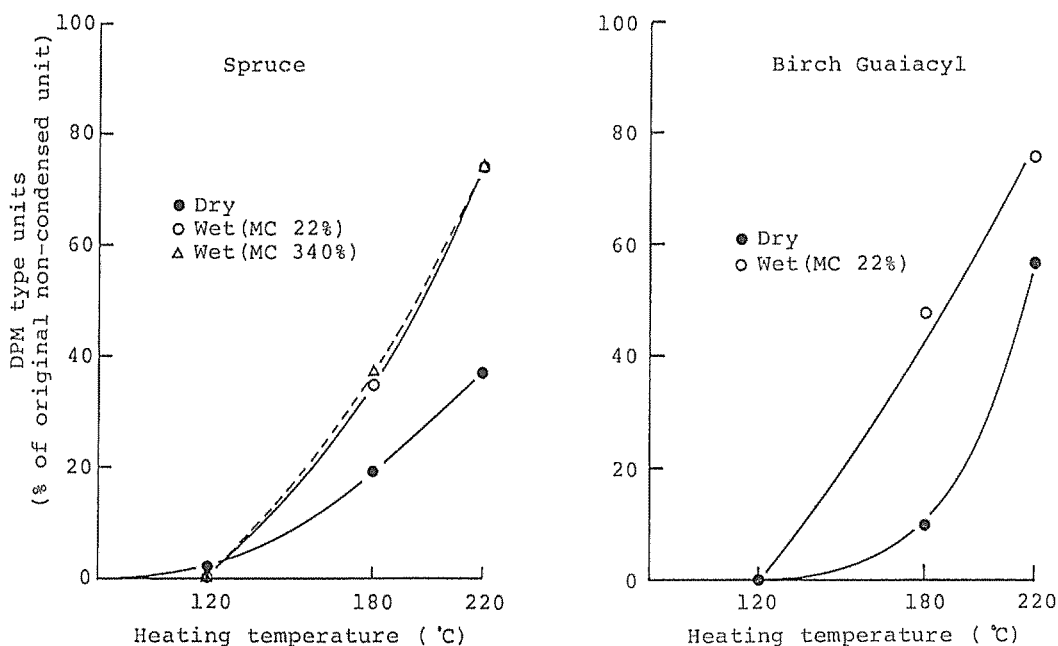


Fig. 12. Conversion of non-condensed to diphenylmethane type units in lignin during heating.

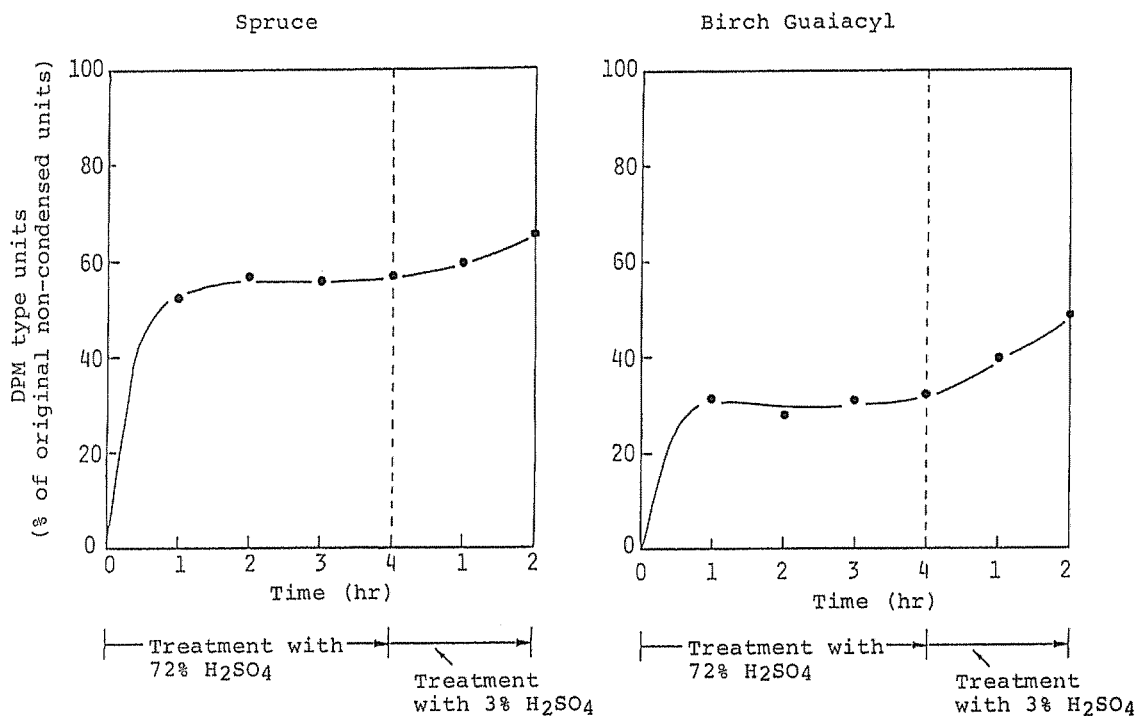


Fig. 13. Conversion of non-condensed to diphenylmethane type units in lignin during the treatment with sulfuric acid.

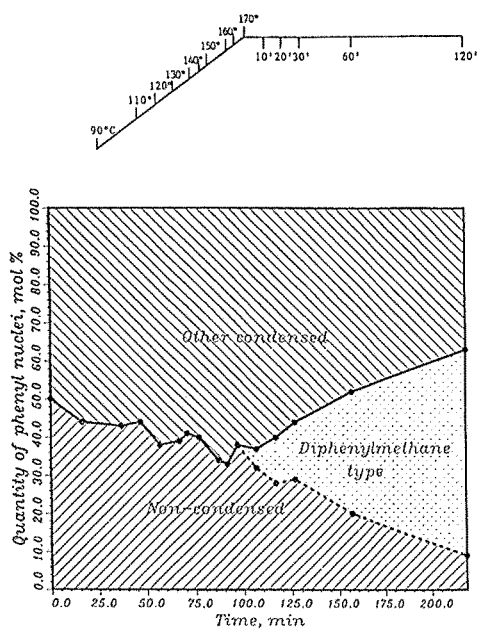


Fig. 14. Quantities and types of phenyl nuclei in residual lignin during kraft pulping of Douglas-fir.

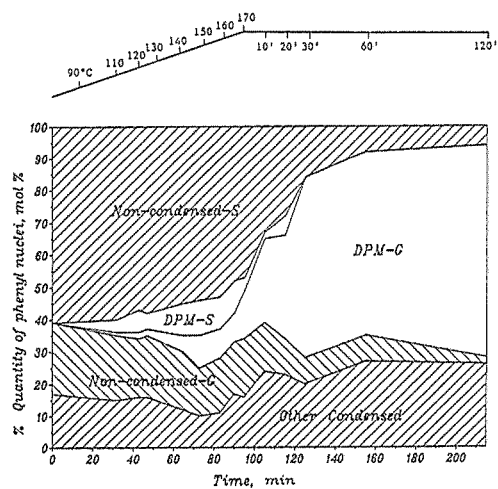


Fig. 15. Quantities and types of phenyl nuclei in residual lignins during kraft pulping of seetgum.

(Ga' and Gb') that were associated with the DPM type moieties, 37 mol% other type of condensed (Gb) and 9 mol% non-condensed units (Ga) (CHIANG and FUNAOKA 1988) (Fig. 14). On the other hand, in kraft pulping of sweetgum, the formation of guaiacyl-syringyl types of diphenylmethane moieties occurred already at 110°C and reached the maximum at around 150°C. These condensed units were dissolved at higher temperatures (> 160°C) and after 30 min at 170°C, no syringyl unit was found associated with DPM type moieties in residual sweetgum lignins. At the end of pulping ($-\kappa 20$), the residual sweetgum lignin consisted of 66 (Ga', Gb') and 26 (Gb) mol% of guaiacyl units that were associated with DPM type moieties and other types of condensed units, respectively (CHIANG and FUNAOKA 1990a) (Fig. 15).

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リグニン構造解析への核交換手法の応用

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核交換法は、リグニンの側鎖-芳香核間結合を選択的かつ定量的に解裂させる手法である。本法は、三フッ化ホウ素およびフェノールの存在下における芳香核のアルキル化と脱アルキル化のコンビネーションに基づく。核交換法とニトロベンゼン酸化分解の併用によって、複合体からリグニンを単離することなくリグニンの芳香核組成が迅速に計算される。本手法は、特にリグニンの二次縮合構造として重要なジフェニルメタン型構造単位の解析手段として有用である。