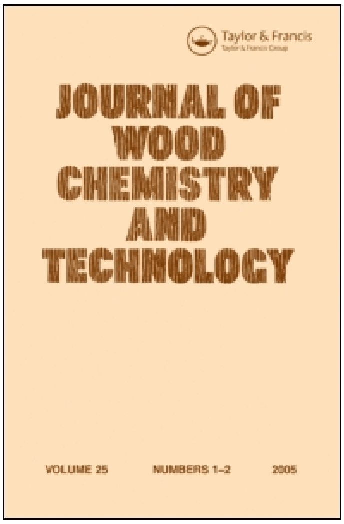

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Estimation of the Aromatic Units in Lignin by Nucleus Exchange - A Reassessment of the Method

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ESTIMATION OF THE AROMATIC UNITS IN LIGNIN BY NUCLEUS EXCHANGE - A REASSESSMENT OF THE METHOD

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ABSTRACT

A series of lignin model compounds was subjected to the nucleus exchange reaction (boron trifluoride-phenol at 180°C) to evaluate the application of the procedure to the study of wood and pulp lignin structure. The nucleus exchange reaction of the series of compounds gave rise to catechol in a range of yields, and in every case no starting material remained. In contrast to earlier reports, lignin model diphenyl ether and biphenyl compounds also gave rise to catechol. On the basis of these results and application of the nucleus exchange reaction to woodmeals and kraft pulps, we conclude that the nucleus exchange reaction does not allow accurate measurement of aromatic units in lignin.

INTRODUCTION

Chemical degradation of lignins gives rise to low molecular weight products which can provide important structural information on the lignins. A new solvolytic technique termed nucleus exchange (NE) has recently been developed by Funaoka *et al.*,[1] in which lignin is digested with phenol and boron trifluoride at 180°C for 4 h, during which time phenol displaces the lignin aromatic units. The latter are then extracted from the reaction mixture and are estimated by gas chromatography (GC). Funaoka *et al.* proposed that the reaction proceeds in three steps; condensation of phenol onto the C-α position of lignin structural units (**1**) to give the diphenylmethane (DPM) structure **2**, displacement of lignin phenolic nuclei by phenol (nucleus exchange), and demethylation of methoxyl groups of the released phenols (Figure 1).[2] The demethylation reaction probably proceeds concomitantly with the condensation and nucleus exchange. Guaiacol (**3a**) and catechol (**4a**) are the products from a softwood lignin and, in addition to these phenols, hardwood lignins give syringol (**3b**), 3-methoxycatechol (**4b**) and pyrogallol (**4c**).[3] The yields of these products have provided information on the aromatic nuclei in lignin.

From model compound studies, Funaoka and Abe found that all bonds linking lignin units are cleaved in the NE reaction with the exception of diphenyl ether and biphenyl linkages.[4,5] However, in spite of these results, they later concluded that the NE products are derived solely from non-condensed units of lignin which are capable of forming DPM structures, *e.g.* **1** (R = H or OCH_3),[5,6] because they found that the yields of NE products were in fair agreement with the expected number of non-condensed units. Furthermore, they stated that the yields of NE products from isolated lignins increased only slightly at reaction temperatures greater than 130°C,[6,7,8] which indicated that the NE products were derived from units that were very reactive under the NE conditions; presumably the non-condensed units. However, this is inconsistent with their reported NE yields from lignins, which were up to 24% higher at 180°C than at 130°C.[7] Funaoka and Abe assumed that the non-condensed units gave nucleus exchange products in quantitative yields, even though most model compounds were tested at temperatures lower than that used (180°C) for the NE reaction. They proposed NE as a method for determining the content of non-condensed units in lignin.[1]

Chiang and Funaoka also used the NE method in conjunction with nitrobenzene oxidation (NO) to estimate the content of DPM structures in modified lignins.[9] They based their method on the assumption that whereas DPM structures give NE products in quantitative yield, these structures do not give NO products. Thus, Chiang and Funaoka considered the difference in product yields from the two techniques to reflect the diphenylmethane contents of the lignins.[9] The NE-NO method has been used to determine the proportions of non-condensed, condensed and diphenylmethane structures in the kraft pulp lignins of Douglas fir[9,10,11] and of sweetgum[11,12] woods, and softwood lignins after acid[13] and heat[14] treatment of wood. One of the NE reaction products from syringyl groups of hardwood lignins, pyrogallol, is not stable at 180°C due to condensation reactions;[3] therefore, the yield of products from syringyl units at 180°C was calculated from the yield of reaction products at 110°C assuming a constant ratio of guaiacyl:syringyl products at the two temperatures.[11]

We found that the NE reaction was not a useful method for studying the lignins of eucalypt woods and kraft pulps because values obtained for the syringyl content of the lignins were erroneously high.[15] To obtain a better understanding of the NE reaction, we undertook a study of NE reactions of lignin model compounds, and we discuss the significance of these to the application of the NE technique to wood and pulp samples.

RESULTS AND DISCUSSION

NE reaction of wood, holocellulose and lignin model compounds

The NE reaction conditions employed were those suggested by Funaoka *et al.*[1] for reaction of woods, pulps or isolated lignins, *viz.* digestion with a mixture of phenol, xylene, and phenol-boron trifluoride reagent for 4 h at 180°C, except that the reaction was carried out in a steel autoclave lined with PTFE to eliminate any effect of the metal surface on the reaction. The reaction products, identified by comparison of gas chromatography-mass spectrometry (GC-MS) characteristics with those of authentic samples, are listed in Table 1. With the exception of the compounds derived from lignin nuclei (**3a**, **3b** and **4a-4c**, Figure 1), the products are the transformation products of the phenol reagent, largely *ortho* and *para* substituted alkylphenols, some of which have previously been identified by Funaoka and Abe.[1,2,7] Several of them were also present in a blank reaction mixture of the nucleus exchange reagents, which indicates that phenol is able to displace methyl groups from xylene to a minor extent. Because some of the peaks of interest overlapped with those derived from the phenol reagent (Table 1), it was necessary to examine the GC peaks by MS detection to ascertain their identity. Misidentification of the GC peaks can lead to errors in the quantitative estimation of products by GC.

TABLE 1

GC retention times of nucleus exchange reaction products[a]

Peak no.	Compound	Ret. time (min)	Present in blank
1	*o*-cresol[b]	7.91	x
2	*m*-cresol	8.09	x
3	*p*-cresol[b]	8.33	x
4	2-ethylphenol[c]	9.46	x
5	2,5-xylenol	9.78	
6	3-ethylphenol	10.05	x
7	2,4-xylenol	10.12	
8	guaiacol	10.15	
9	4-ethylphenol[c]	10.46	x
10	2-isopropylphenol	10.56	x
11	2,3-xylenol	10.66	
12	3,4-xylenol	10.92	
13	2-*n*-propylphenol	11.29	
14	4-isopropylphenol[c]	11.84	x
15	catechol	12.61	
16	4-*n*-propylphenol[c]	12.69	
17	syringol	14.73	
18	*p*-hydroquinone	14.74	x
19	diphenyl ether[d]	14.76	x
20	3-methoxycatechol	16.86	
21	dibenzofuran	17.68	x
22	bibenzyl (int. stand.)	17.76	
23	pyrogallol	18.47	
24	xanthene	20.98	

[a] Reaction mixtures were silylated prior to chromatography.

[b] cited in ref. 2 [c] cited in ref.7 [d] cited in ref. 1

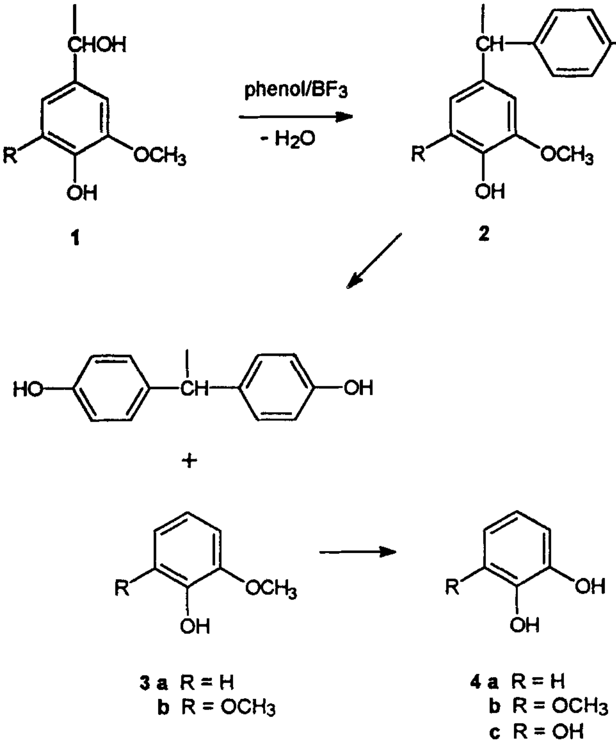


FIGURE 1. Action of boron trifluoride-phenol (nucleus exchange reaction) on lignin structures

Treatment of a eucalypt wood sample under the NE reaction conditions gave a mixture, the gas chromatogram of which is shown in Figure 2. The products deriving from lignin which were identified by MS include catechol (peak 15), 3-methoxycatechol (peak 20) and trace amounts of pyrogallol (peak 23). Guaiacol and syringol were not detected among the reaction products; the former co-elutes with 3-ethylphenol and 2,4-xylenol (peaks 6 and 7), while the latter co-elutes with *p*-hydroquinone and diphenyl ether (peaks 18 and 19).

A eucalypt holocellulose sample was subjected to the NE conditions to determine whether part of the polysaccharide fraction was converted to NE products, *e.g.* catechol. Only trace amounts of catechol were found among the

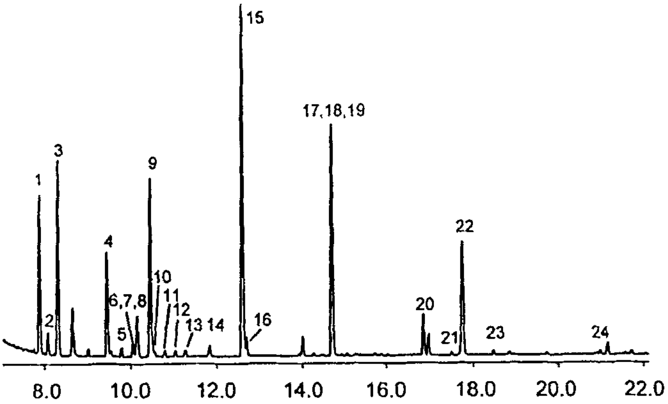
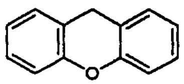


FIGURE 2. GC trace of silylated NE reaction products from eucalypt woodmeal. Peak numbers refer to compounds in Table 1.

products, and this probably arose from residual amounts of lignin in the holocellulose rather than from the polysaccharides. Ferrier *et al.*[16] found that the zinc chloride-catalysed reaction of cellulose with aqueous phenol, an analogous reaction to the NE reaction, did not give catechol as a reaction product, and xanthene (**5**) was the major neutral aromatic compound. We found xanthene among the NE products of the eucalypt holocellulose.

Previous studies on the NE reaction of some lignin model compounds have been carried out by Funaoka and Abe,[3,4,17-21] although these have generally employed lower temperatures than those used for the wood samples and thus do not give a true indication of reactions at 180°C. To test the reactivity of various lignin linkages to the NE reagent at 180°C, we subjected a range of lignin model compounds to the NE reaction at 180°C for 4 h and the products were analysed by GC-MS (Table 2). In every case, catechol (**4a**) was found as a reaction product, guaiacol was not detected, and no starting material remained. Catechol, after treatment with the NE reagent at 180°C, was recovered in 95% yield, which showed that it was stable to the NE conditions. However, guaiacol (**3a**) was completely consumed after treatment with the NE reagent for 4 h, forming catechol in 83% yield. Funaoka and Abe[17] reported that treatment of



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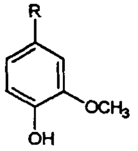
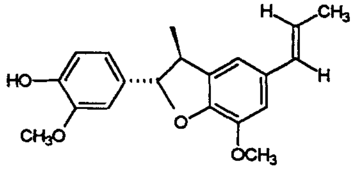


6 a R = CHO
b R = $COCH_3$
c R = $CHOHCH_3$
d R = $CH_2CH_2CH_3$



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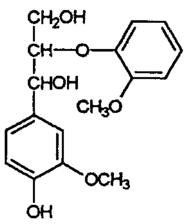

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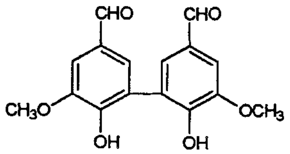

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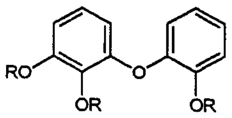
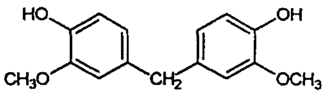


10 a R = CH_3
b R = H



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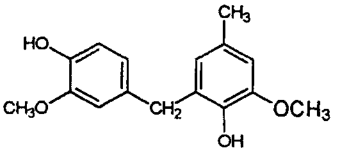

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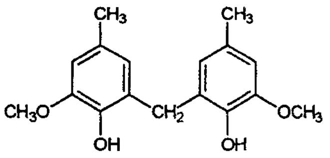

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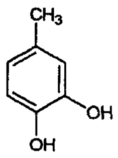

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guaiacol with the NE reagent for 2.5 h at 180°C gave a quantitative recovery of a mixture of the starting material and catechol in a 1:7 ratio.

Several 4-substituted guaiacols were treated to the NE reaction. Vanillin (**6a**) gave the highest yield of catechol (91%), acetoguaiacone (**6b**) and apocynol (**6c**) lower yields (73%) and 4-*n*-propylguaiacol (**6d**) gave the lowest yield

TABLE 2

Yields of products from nucleus exchange reaction of lignin model compounds

Compound	Catechol (% yield)	Other products (% yield)
catechol (**4a**)	95.0	
guaiacol (**3a**)	83.4	
vanillin (**6a**)	91.0	
acetoguaiacone (**6b**)	72.9	
apocynol (**6c**)	72.5	
4-*n*-propylguaiacol (**6d**)	56.8	
dehydrodiisoeugenol (**7**)	91.2[a]	
ß-ether **8**	93.0[a]	
dehydrodivanillin (**9**)	25.0[a]	
trimethoxydiphenyl ether (**10a**)	25.5[a]	pyrogallol (**3c**) (trace) trihydroxydiphenyl ether (**10b**)(34.3)
1,1'-diguaiacylmethane (**14**)	78.1[a]	
1,5'-diarylmethane (**15**)	59.3[a]	4-methylcatechol (14.9)
5,5'-diarylmethane (**16**)	44.0[a]	4-methylcatechol (9.3)

[a] 2 moles catechol formed

(57%) (Table 2). This probably reflects the reactivity of sidechains to the NE reaction,[18] since displacement of the guaiacyl group from guaiacylalkanes is known to be more difficult than displacement from C-α substituted guaiacylalkanes, as the latter can form diphenylmethane intermediates.[4]

Lignin model dimers **7-10**, representing the major linkages in lignin, were subjected to the NE reaction. As these dimers can give two moles of monomeric products from one mole of dimer, the yields of products were calculated on that basis. Both the ß-5 dimer, dehydrodiisoeugenol (**7**), and the ß-O-4 dimer **8**, gave greater than 90% yields of catechol, whereas dehydrodivanillin (**9**), a model for biphenyl linkages in lignin, gave catechol in 25% yield

(Table 2). The formation of a NE product from a biphenyl lignin model contrasts with the work of Funaoka and Abe,[4] in which dibenzofuran was the only product of NE reaction of 2,2'-dihydroxybiphenyl. In that case the product of biphenyl bond cleavage would have been phenol which could not be analysed in the presence of the excess phenol reagent.

The cleavage of the biphenyl linkage in **9** can be rationalised in terms of nucleophilic attack of phenol, catalysed by boron trifluoride, onto the C-5 aromatic carbon with displacement of the aromatic group as vanillin and formation of the new biphenyl **11** (Figure 3). Attack of phenol on compound **11** gives a further mole of vanillin, which in turn reacts with phenol to give the triphenylmethane **12**. Finally, displacement of the guaiacyl group in **12** by phenol takes place to give guaiacol, and after demethylation, catechol.

The trimethoxydiphenyl ether (**10a**), a model for the diphenyl ether units in lignin, also gave rise to catechol (25.5% yield, Table 2), together with small amounts of pyrogallol (**4c**) and the demethylated diphenyl ether (**10b**). Analogous to the reaction of the biphenyl, the cleavage of the diphenyl ether probably occurs by nucleophilic attack of phenol on the aromatic carbon bearing the oxygen atom linking the units, with displacement of the newly-formed phenol (Figure 4). When the attack is at C-1, the products are guaiacol and the biphenyl **13**, and phenol attacks the latter to give veratrole. The final product, after demethylation, is catechol (2 moles). A similar series of reactions resulting from attack of phenol at C-1' gives pyrogallol and phenol (Figure 4). The detection of only small amounts of pyrogallol in the reaction is probably because of its instability to NE reaction conditions.[3] The presence of the demethylated diphenyl ether **10b** among the reaction products indicates that the demethylation occurs before, or more likely concomitantly with, cleavage of the diphenyl ether linkage. Thus, the diphenyl ether represents a second condensed lignin structure which was found to give NE products, contrary to the assertion of Funaoka and Abe[5] that diphenyl ether linkages are stable to the NE reaction conditions.

The diphenylmethanes **14**, **15** and **16**, models of three types of structure in modified lignins, can also give rise to two moles of catechol for one mole of dimer, and they afforded catechol in 78, 59 and 44% yield, respectively. In addition to catechol, **15** and **16** yielded 4-methylcatechol (**17**) (Table 2). The lower than theoretical yields of catechol from the DPMs shows that, in contrast

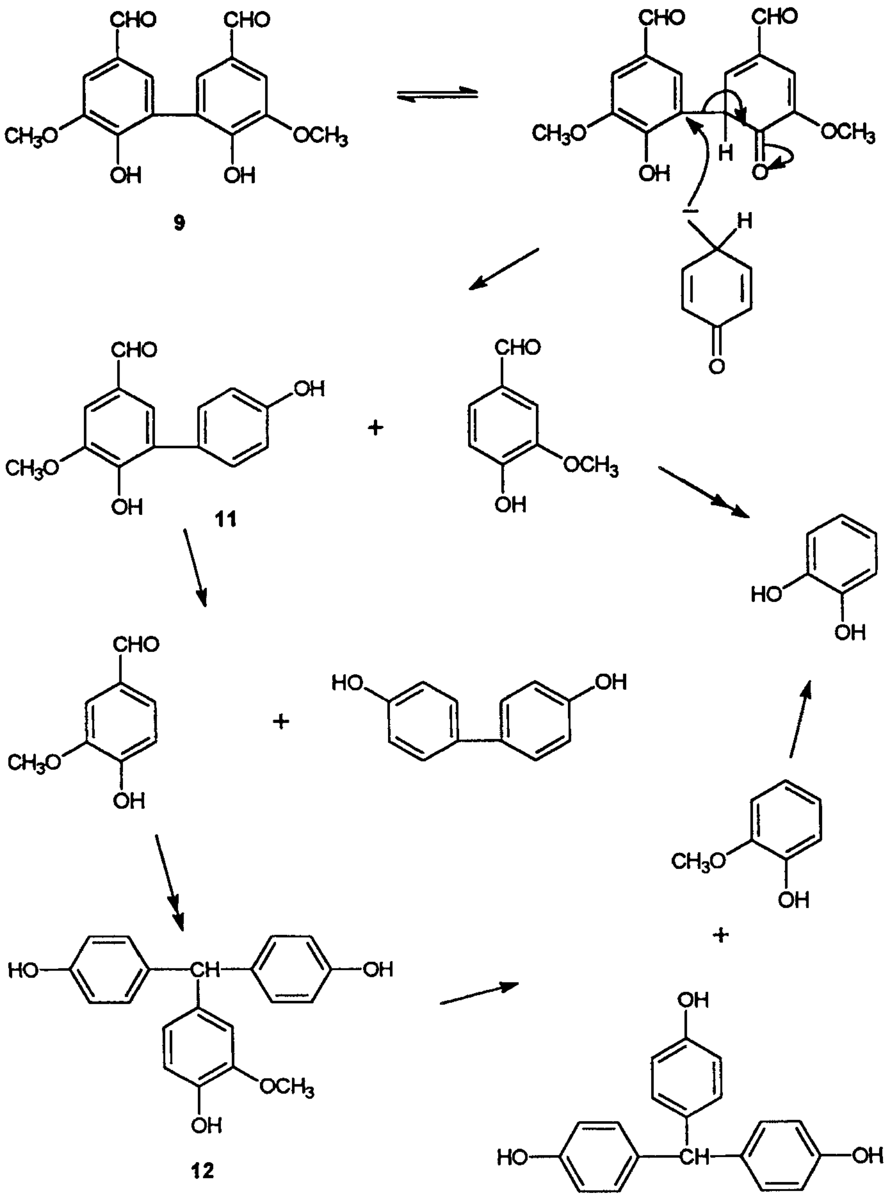

FIGURE 3. NE reaction of dehydrodivanillin (9)

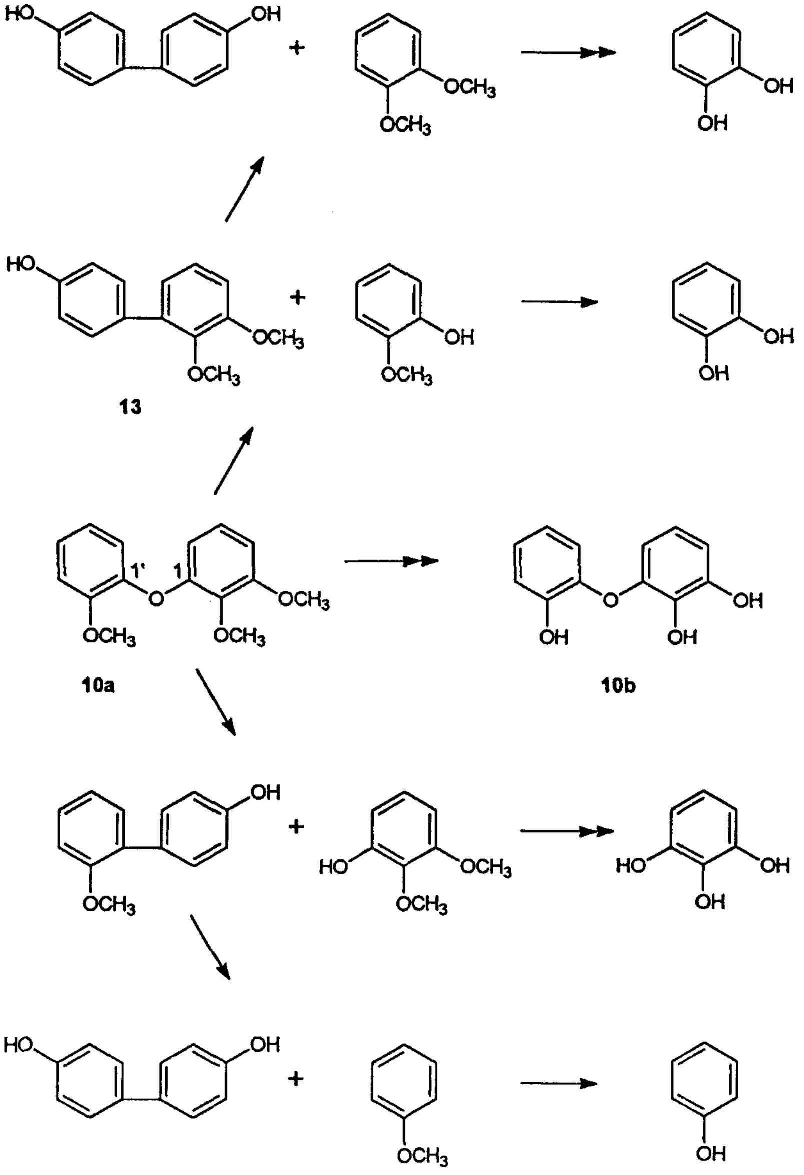

FIGURE 4. NE reaction of diphenyl ether **10a**

to the conclusion of Funaoka and Abe, there are differences in reactivity of DPM structures. The presence of 4-methylcatechol as a reaction product in addition to catechol is indicative of the relative difficulty of displacing a methyl group attached to an aromatic ring.

From the above results, it is evident that NE reaction of models of lignin structural units, including diphenylmethanes, a biphenyl compound, and a diphenyl ether, give rise to catechol in a range of yields. The polysaccharide fraction of wood gave negligible amounts of catechol.

Application to quantitative estimation of lignin structures

A sample of radiata pine woodmeal subjected to the NE reaction for 4 h at 180°C gave catechol in 53.3 mol percent yield[15] (Table 3), typical of the values obtained for softwood lignins,[5] and only trace amounts of guaiacol. The yield of catechol decreased by only 7% after extended NE reaction of the woodmeal at 180°C for 18 h. Catechol was stable to the NE conditions for the same period, also in the presence of eucalypt holocellulose, indicating that there is no evidence for reaction of catechol with the polysaccharide fraction of the woodmeal. Thus, in accordance with the work of Funaoka and Abe,[5] the conversion of guaiacyl units to NE products appears to be complete after 4 h. These authors have equated the yields of these products with the proportion of non-condensed guaiacyl units in lignin capable of forming diphenylmethane structures.[5,6,7] However, because lignin models substituted (condensed) at C-5 also give rise to NE products in a range of yields (Table 1), the yield of NE products from lignins is better interpreted as a mean of the yields from the various structural features of the lignins. Although higher yields of NE products from lignins are indicative of higher proportions of non-condensed structures, the yields cannot be used to give a quantitative estimation of these structures.

The NE reaction applied to eucalypt woodmeal and kraft pulp samples at 110°C gave guaiacol and syringol as the main products. However, at 180°C these products were not detected, and catechol and 3-methoxycatechol were the major analytes. When the method of Chiang and Funaoka[12] was used for calculation of the syringyl units in the lignin, values in excess of 100 mole percent were obtained[15] (Table 3). The calculation of yield of syringyl units at 180°C (NE conditions) is based on the ratio of guaiacyl products at 180°C and 110°C, which is assumed to be identical to the ratio of syringyl products at the

TABLE 3

NE reaction products from woods and pulps

Sample	Guaiacyl products (mol. % yield **3a,4a**)		Syringyl products (mol % yield **2b,3b,c**)	
	110°C	180°C	110°C	180°C[a]
Pinus radiata woodmeal		53.3[b]		
Pinus radiata woodmeal[c]		49.8[b]		
Eucalypt holocellulose		trace **4a**		
Eucalypt woodmeal	7.9	29.1[d]	29.9[e]	110
Eucalypt kraft pulp[f]	13.2	37.1[d]	40.9[e]	114.9
Eucalypt kraft pulp[g]	10.7	47.3[d]	36.7[e]	162.2

[a] Calculated from the ratio of guaiacyl products at 110°C/180°C[11]

[b] traces of guaiacol [c] reaction for 18 h [d] guaiacol not detected

[e] pyrogallol not detected [f] 14.3% delignified [g] 55.5% delignified

same temperatures.[12] However, this assumption is not necessarily correct because guaiacyl and syringyl units may have different relative reactivities at the two temperatures, leading to differing yields of products. Clearly, the calculation applied to the eucalypt samples (Table 3) is not valid.

The difference in yields of NE and nitrobenzene oxidation (NO) reaction products for treated woods was considered to reflect the amounts of DPM units in the lignins, based on the assumption that DPM structures do not give rise to NO products, and that the lower yields of NO products in treated woods are due entirely to the presence of DPM structures.[9-14] However, results from earlier studies[22,23] and from our own work[24] show that the yields of NO products depend on the structure of the lignin sidechain, and that various DPM structures do give rise to nitrobenzene oxidation products. Other α-methylene structures have been found in kraft lignins which could partially explain the difference in yields of NE and NO products; Gierer and Lindeberg[25] have isolated a number of dimers with α-methylene structures from the spent liquor from kraft pulping spruce woodmeal, while Gellerstedt and Robert[26] have noted (from ^{13}C NMR studies of kraft lignins) a large number of methylene groups. Thus, ascribing the difference in yield of NE and NO products to the amounts of DPM structures in the lignins[9-14] is an oversimplification, and caution must be exercised when interpreting the results of the NE reaction.

Conclusions

The nucleus exchange reaction which has been used to measure the amounts of non-condensed structures and the ratio of syringyl to guaiacyl nuclei in lignin, and, together with nitrobenzene oxidation, the amounts of diphenylmethane moieties in modified lignins, is likely to give incorrect results for the following reasons:

- NE products deriving from the phenol reagent may co-elute with the products from lignin during GC analysis.
- The yield of NE reaction products reflects an average value from all lignin structural units, as all structures give nucleus exchange products in varying yields, rather than only non-condensed units containing C-α oxygenated substituents, as assumed in the method.
- Determination of the yield of products from syringyl units based on the ratio of products derived from guaiacyl units at 110°C and 180°C is not valid.
- Estimation of the diphenylmethane structures in lignin from the difference between nucleus exchange and nitrobenzene oxidation products is flawed, because DPM units also give rise to oxidation products, contrary to the assumption in the method.

EXPERIMENTAL

NMR spectra were obtained at 100 MHz for protons and 25 mHz for carbons for deuterochloroform solutions, and chemical shifts are given in ppm from tetramethylsilane (δ 0.00). Elemental analyses were carried out by the University of Otago, Dunedin, New Zealand.

Chemicals

Unless otherwise stated, the lignin models or starting materials for synthesis were obtained from commercial sources. Apocynol (**6c**) was prepared by the reduction of acetoguaiacone (**6b**) with sodium borohydride.[27] 1-Guaiacylpropane (**6d**) was the product of Pd-C-catalysed hydrogenation of eugenol in ethanol. Dehydrodiisoeugenol (**7**) was prepared by the oxidative coupling of isoeugenol with ferric chloride.[28] The ß-ether **8** was obtained by a multistep synthesis from acetoguaiacone.[29] Dehydrodivanillin (**9**) was prepared by oxidative coupling of vanillin with sodium persulfate.[30] Diguaiacylmethane (**14**) was prepared by heating an alkaline solution of vanillyl alcohol.[31] Guaiacyl-(5-hydroxy-4-methoxy-2-methylphenyl)methane (**15**) was the product of heating an acidic solution of creosol and vanillyl alcohol.[24] Di(2-hydroxy-3-methoxy-5-methylphenyl)methane (**16**) was prepared by reaction of an alkaline solution of creosol with formaldehyde.[32]

Wood and pulp samples

Air-dried woodchips were prepared from 20-year-old *Pinus radiata* wood and a composite sample of mature mixed eucalypt species from Northern Tasmania. The woodchips were ground in a Wiley mill to pass through a screen with 420 μm apertures. The eucalypt woodmeal was kraft cooked according to Chan *et al.*[15] Holocellulose was prepared from the eucalypt woodmeal by chlorite delignification.[33]

2,2',3-Trimethoxydiphenyl ether (10a)[34]

A solution of 2-bromoanisole (5.0 g) and 2,3-dimethoxyphenol (5.2 g, 1.25 equiv.) in dry pyridine (35 mL) was vigorously stirred with anhydrous potassium carbonate (10.0 g); the mixture was refluxed under nitrogen for 22 h. After cooling, the mixture was diluted with ethyl acetate and filtered. The filtrate was washed twice with 5M hydrochloric acid, twice with 5M sodium hydroxide and was dried. After evaporation of the solvent *in vacuo*, the resulting product in hexane was adsorbed on a column of silica gel. Elution with dichloromethane gave a solid (3.04 g), which on recrystallisation from methanol gave *2,2',3-trimethoxydiphenyl ether* (**10a**), m.p. 72-3°C (Found: C, 69.04; H, 6.41. $C_{15}H_{16}O_4$ requires C, 69.22; H, 6.20%) ^{1}H NMR δ 3.84 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.89 (3H, s, OCH_3) and 6.38-7.09 (7H, m, ArH) ^{13}C NMR δ 56.1, 56.2, 61.0, 107.3, 111.4, 112.9, 119.8, 121.0, 123.3, 124.2, 140.0, 146.0, 150.9 and 154.0. MS *m/z* (%) 260 (100, $[M]^+$), 214 (69), 136 (23), 77 (20) and 95 (83).

2,2',3-Trihydroxydiphenyl ether (10b)[35]

A solution of 1M boron tribromide in dichloromethane (10 mL) was added to a solution of 2,2',3-trimethoxydiphenyl ether (500 mg) in dichloromethane (10 mL), and the mixture was kept at 20°C for 4 h. After pouring onto ice, the mixture was extracted with diethyl ether and dried. Evaporation of the solvent yielded a solid (360 mg), which after recrystallisation from dichloromethane-hexane gave *2,2',3-trihydroxydiphenyl ether* (**10b**) m.p. 135-136.5°C (Found: C, 66.18; H, 4.38. $C_{12}H_{10}O_4$ requires C, 66.05; H, 4.62%) ^{1}H NMR δ 6.31-7.01 (7H, m, ArH) and 7.99 (3H, br s, OH) ^{13}C NMR δ 110.6, 111.8, 117.5, 119.5, 119.9, 120.6, 125.0, 137.0, 145.3, 145.6, 147.4 and 148.8.

Nucleus exchange reaction

The reaction was carried out according to the method of Chiang and Funaoka.[9] The digesting reagent was a mixture of phenol, xylene, and 25% boron trifluoride-75% phenol complex in the ratio 19:10:4.5 (v:v:v). The lignin model compound (0.04 mmol) and the reagent (2.0 mL) were placed in a 10 mL PTFE-lined stainless steel sealed vessel which was heated in a rocking air bath for 4 h at 180°C. The cooled reaction mixture were transferred to a beaker with diethyl ether, and an appropriate amount of bibenzyl was added as an internal standard. The mixture was filtered and the filtrates (total volume 80 mL) were washed with saturated brine (30 mL) and dried with sodium sulfate. An aliquot of the solution (200 μL) was silylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (100 μL) for 1 h at 20°C. The excess silylating agent was removed under a stream of nitrogen, and the sample was diluted with ethyl acetate before analysis by GC. For woodmeal and pulp samples, amounts equivalent to 12 mg lignin were used, and the same procedure was adopted. The products from woodmeals and pulps were expressed as mole percent of the lignin content.

Gas chromatography (GC) and gc-mass spectrometry (GC-MS)

GC was carried out on a Hewlett-Packard 5830A chromatograph fitted with a flame ionisation detector and an HP1 megabore column (5 m by 0.53 mm ID) operated in the splitter mode. GC conditions were: injector temp. 250°C, detector temp. 280°C, oven temp. 80°C, programmed at 5°C/min to 280°C. Helium was used as carrier gas.

GC-MS analyses were carried out on a Hewlett Packard HP5890 series II chromatograph fitted with an autoinjector and an HP5971 mass selective detector. The column was a J&W bonded phase DB5 fused silica column (30 m x 0.25 mm ID) with a phase thickness 0.25 μm. Purified helium was the carrier gas with a linear velocity 30 cm/sec. The injector temperature was 250°C and the transfer line was at 280°C. The column temperature programs were those used as above. Splitless injections of 1 μL with a 0.3 min purge delay were used and full-scan (*m/z* 30-550) electron impact (70 eV) mass spectra were collected.

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REFERENCES

1. M. Funaoka, I. Abe and V. L. Chiang, In Methods in Lignin Chemistry, Chap. 6.6, S. Y. Lin and C. W. Dence (eds.), Springer-Verlag, Berlin, 1992. *pp* 369-386.
2. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **24**(4), 256 (1978).
3. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(10), 635 (1982).
4. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(8), 529 (1982).
5. M. Funaoka and I. Abe, Wood Sci. Technol., **21**, 261 (1987).
6. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(10), 627 (1982).
7. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(11), 718 (1982).
8. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **31**(8), 671 (1985).
9. V. L. Chiang and M. Funaoka, Holzforschung, **42**(6), 385 (1988).

10. V. L. Chiang, D. D. Stokke and M. Funaoka, J. Wood Chem. Technol., **9**(1), 61 (1989).

11. V. L. Chiang and M Funaoka, Holzforschung, **44**(4), 309 (1990).

12. V. L. Chiang and M. Funaoka, Holzforschung, **44**(2), 147 (1990).

13. M. Funaoka, M. Shibata and I. Abe, Holzforschung, **44**(5), 357 (1990).

14. M. Funaoka, T. Kako and I. Abe, Wood Sci. Technol., **24**(3), 277 (1990).

15 F. Chan, P. F. Nelson, K. L. Nguyen and A. F. A. Wallis, Proc. 47th Appita Annual General Conference, Rotorua, New Zealand, Vol. 2, 1993. p. 757.

16. R. J. Ferrier, W. B. Severn, R. H. Furneaux and I. J. Miller, Carbohydr. Res. **237**, 79 (1992).

17. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **24**(12), 892 (1978).

18. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **26**(5), 334 (1980).

19. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **26**(5), 342 (1980).

20. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(8), 522 (1982).

21. M. Funaoka and I. Abe, Mokuzai Gakkaishi, **28**(9), 563 (1982).

22. Leopold, B. - Acta Chem. Scand. **5**: 1393 (1951).

23. K. Kratzl, I. Wagner and O. Ettinghauser, Holzforsch. Holzverwert., **24**(2), 32 (1972).

24. F. Chan, K. L. Nguyen and A. F. A. Wallis, Proc. 48th Appita Annual General Conference, Melbourne, Australia, 1994. p. 485.

25. J. Gierer and O. Lindeberg, Acta Chem. Scand., **B34**, 161 (1980).

26. G. Gellerstedt and D. Robert, Acta Chem. Scand., **B41**, 541 (1987).

27. C. W. Bailey and C. W. Dence, Tappi, **52**(3), 491 (1969).

28. H. Erdtman, Liebig's Annalen, **503**, 283 (1933).

29. E. Adler and E. Eriksoo, Acta Chem. Scand., **9**, 341 (1955).

30. M. G. Drumond, D. P. Veloso, S. D. Santos Cota, S. A. Lemos de Morais, E. A. do Nascimento and C.-L. Chen, Holzforschung, **46**(2), 127 (1992).

31. V. S. Sultanov and A. F. A. Wallis, J. Wood Chem. Technol., **11**(3), 291 (1991).

32. S. Yasuda, K. Fujii, B. Yoon and N. Terashima, Mokuzai Gakkaishi, **26**(6), 421 (1980).

33. J. E. Stone and D. W. Clayton, Pulp Pap. Mag. Can., **61**(6), T307 (1960).

34. K.-W. Glombitza and U. Wölwer-Rieck, Liebigs Ann. Chem., (5), 261 (1988).

35. J. F. W. McOmie, M. L. Watts and D. E. West, Tetrahedron, **24**, 2289 (1968).