

The mechanism of the Mäule colour reaction Introduction of methylated syringyl nuclei into softwood lignin

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Summary. Ezo spruce (*Picea jezoensis*) wood meal and milled wood lignin were successively reduced with sodium borohydride, methylated with methanol-HCl, oxidized with Fremy's salt, reduced with sodium dithionite, and methylated with diazomethane. Permanganate oxidation of the treated milled wood lignin showed that 0.08–0.1/C9 units of 3,4,5-trimethoxyphenyl groups were introduced into the softwood lignin. Although hardwood meal (beech, *Fagus crenata*) methylated with diazomethane gave a purple-red colour with the Mäule test, the treated softwood meal gave only a dark brown colour. The aromatic nuclei of lignin were broken down by the Mäule treatment. The consumption of permanganate by treated softwood lignin was higher than by hardwood lignin, which suggests that the guaiacyl nuclei were broken down severely. It is proposed that the purple-red colour obtained from methylated hardwood lignin with the Mäule colour test is generated by reaction of syringyl groups which were liberated by β -ether cleavage under the permanganate oxidation conditions.

Introduction

Colour tests are important for detecting and distinguishing between lignins in lignocellulosic tissues. The Mäule colour test involves sequential treatment with 1% potassium permanganate, 12% hydrochloric acid, and ammonium hydroxide, and gives an intense purple-red colour with deciduous woods and a brown shade with coniferous woods. The purple-red colour generated from the deciduous woods is due to the presence of syringyl groups in their lignin which are absent in softwood lignins.

The aromatic nuclei of softwood lignin differ from those of hardwood lignin. It is known that the difference is shown by IR spectrum and various colour reactions. The mechanism of the Mäule colour reaction has been studied by Nakano and coworkers (Migita et al. 1952, 1953; Nakano et al. 1957, 1959; Nakano, Migita 1963). Recently, Meshitsuka and Nakano (1977, 1978, 1979) proposed by model experiments that o-methoxycatechol from "end syringyl nuclei" with free phenolic hydroxyl groups was produced from hardwood lignin by demethylation with permanganate treatment. It is then chlorinated with chlorine generated from hydrochloric acid by permanganate oxidation, and this was later oxidized to chlorinated methoxy-o-quinone structures in ammonia hydroxide solution. These quinones derived from hardwood meal give an intense purple-red colour.

To test the validity of the mechanism of the Mäule colour formation further, most of the uncondensed guaiacyl nuclei in softwood lignin which have free hydroxyl groups were converted to methylated syringyl nuclei under mild conditions. The behaviour of this lignin in the Mäule colour test was compared with a methylated hardwood lignin.

Experimental

Sodium borohydride reduction

Ethanol-benzene-extracted ezo spruce (*Picea jezoensis*) and beech (*Fagus crenata*) wood meals (40–60 mesh), and milled wood lignins prepared from ezo spruce and beech wood meals, were reduced with sodium borohydride in 0.05 N sodium hydroxide aqueous solution for 48 h at room temperature. The reduced wood meal suspensions were neutralised with 0.1 M hydrochloric acid, washed successively with water and acetone, then dried in vacuo (R-NW and R-LW). Aqueous suspensions of the reduced milled wood lignins were dissolved in dioxane, and the solutions were concentrated with a rotary evaporator. The inorganic salts which precipitated from solutions by the addition of dioxane were removed by filtration. These procedures were repeated three times. The desalted reduced milled wood lignins were dissolved in dioxane, and were precipitated after dropwise addition to ether (R-NL and R-LL). These procedures were applied to the purification of the various treated wood meals and to the isolation of the various treated milled wood lignins.

Methylation of benzyl alcohol groups by methanol-hydrochloric acid

Benzyl alcohol groups of the wood meals (R-NW and R-LW) and milled wood lignin (R-NL and R-LL) were methylated with anhydrous methanol-hydrochloric acid (concentration of hydrochloric acid: 0.5 mol/L) for 12 h at room temperature (MR-NW, MR-LW, MR-NL, and MW-LL).

Fremy's salt oxidation

MR-NW, MR-LW, MR-NL, and MR-LL were oxidized in water by freshly prepared Fremy's salt (potassium nitrosodisulphonate) (Zimmer et al. 1971) for 30 min at room temperature, by the method of Adler and Lundquist (1961).

Sodium dithionite reduction

Fremy's salt-oxidized samples were added immediately to saturated sodium dithionite aqueous solution, and were kept for 10 min at room temperature.

Methylation with diazomethane

The sodium dithionite-reduced samples were suspended in ethereal diazomethane solution for 72 h at room temperature.

Permanganate oxidation

Samples prepared by the above procedures were oxidized with potassium permanganate by the method of Larsson and Miksche (1969). The mixtures methylated by diazomethane were analyzed by HPLC (Waters Model 6000A, column: μ -B-C18, 3.9 \times 300 mm, solvent: 1 : 1 water-methanol, flow rate: 1.5 ml/min, detector: Waters UV Model 440 at 254 nm).

Measurements of reflectance spectra and infrared absorption spectra

Various treated wood meals were dried in vacuo, and their reflectance spectra were measured on a Shimadzu MPS-5000 Spectrometer. Infrared absorption spectra of these samples were measured as KBr disks.

Potassium permanganate consumption during the Mäule colour reaction

Softwood and hardwood meals and chlorite holocelluloses prepared from wood meals, were treated with 1% aqueous solution of potassium permanganate for 5 min at room temperature. After filtration through glass filters, the solutions were titrated with 1 N sodium oxalate solution. The potassium permanganate consumption of lignin was calculated from both the lignin contents and permanganate consumption of wood meals and holocelluloses.

Results and discussion

Native lignin of most angiosperms (dicotyledonous and monocotyledonous) with syringyl nuclei give purple-red colour with a peak or shoulder around 520 nm by the Mäule colour test as shown in Fig. 1. Meshitsuka and Nakano (1978, 1979) showed that the purple-red colour is due to the 3-methoxy-o-quinone introduced from the oxidation and demethylation of syringyl nuclei with free phenolic hydroxyl groups by potassium permanganate. If that conclusion is correct, it is hardly possible that angiosperm meal in which all of phenolic hydroxyl groups were methylated by diazomethane, give the purple-red colour by the Mäule test. However, the methylated hardwood meal without free phenolic hydroxyl groups, which was confirmed by the $\Delta\epsilon$ i method (Goldschmidt 1954) applied to the hardwood Björkman lignin, was coloured also purple-red by the Mäule test as shown in Fig. 1.

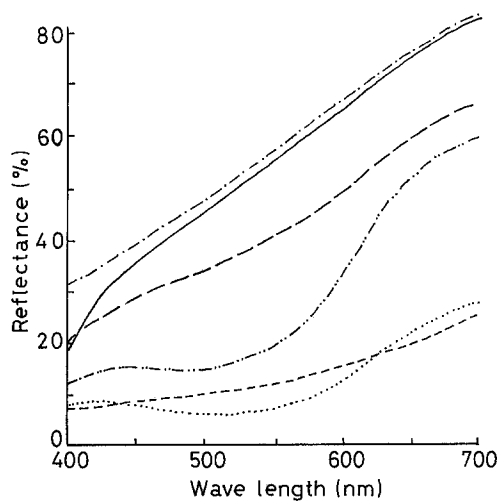


Fig. 1. Reflectance spectra of wood meals. — Untreated softwood meal, --- Untreated hardwood meal, — · — Methylated hardwood meal with CH_2N_2 , · · · · · Mäule treated softwood meal, — · — Mäule treated methylated hardwood meal

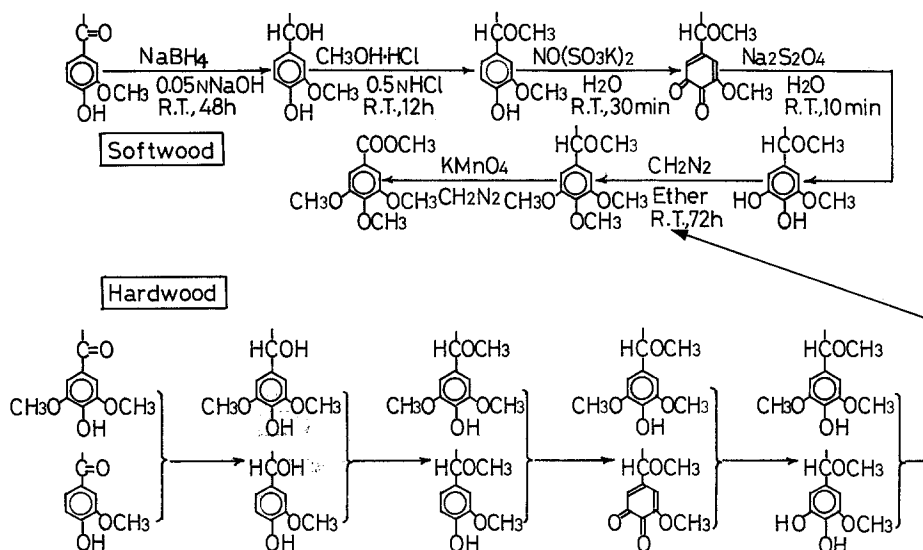


Fig. 2. Reaction scheme

This result shows that the phenolic methyl ether of syringyl nuclei is unstable toward permanganate oxidation following acid and alkaline treatment. It is demethylated easily and/or it is stable toward permanganate oxidation but new free phenolic hydroxyl groups, which are produced by the β -ether cleavage, have contributed to the purple-red colour.

Trying to find the cause of the Mäule development of the methylated hardwood meal, the introduction of the methylated syringyl nuclei into softwood lignin was done.

The reaction scheme to introduce methylated syringyl groups into softwood lignin is shown in Fig. 2. For comparison, the guaiacyl nuclei with free phenolic

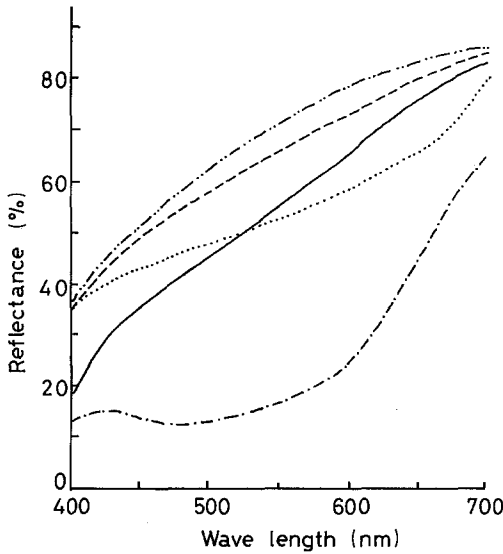


Fig. 3. Reflectance spectra of various treated softwood meals. — Untreated, --- NaBH_4 reduced, NaBH_4 reduced-MeOH·HCl methylated, —·— NaBH_4 reduced-MeOH·HCl methylated-Fremy's salt oxidized, ——— NaBH_4 reduced-MeOH·HCl methylated-Fremy's salt oxidized- $\text{Na}_2\text{S}_2\text{O}_4$ reduced

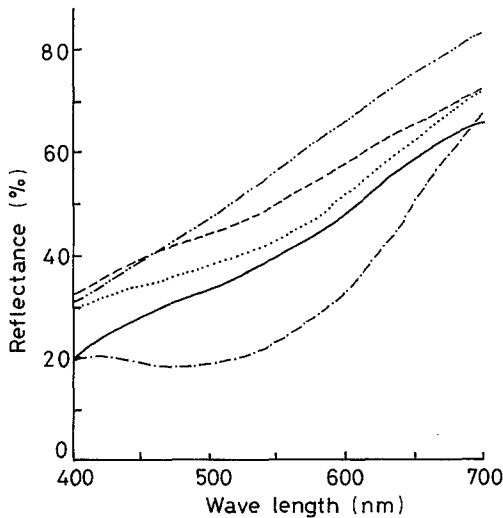


Fig. 4. Reflectance spectra of various treated hardwood meals. — Untreated, --- NaBH_4 reduced, NaBH_4 reduced-MeOH·HCl methylated, —·— NaBH_4 reduced-MeOH·HCl methylated-Fremy's salt oxidized, ——— NaBH_4 reduced-MeOH·HCl methylated-Fremy's salt oxidized- $\text{Na}_2\text{S}_2\text{O}_4$ reduced

hydroxyl groups in hardwood lignin were converted to methylated syringyl nuclei by the same procedures. The conditions of these reactions are extremely mild, and these reactions were done in heterogeneous systems. The progress of these reactions was confirmed by the measurements of the reflectance spectra of wood meals at each step. The reflectance spectra are shown in Figs. 3 and 4.

Guaiacyl nuclei with free phenolic hydroxyl groups and with a carbonyl or benzyl alcohol group at the α -position of the side chain are oxidized by Fremy's salt to methoxy-p-quinone with a strong absorption band at 420 nm in the electronic spectrum (Adler, Lundquist 1961). In the reflectance spectra of Fremy's salt-oxidized ezo-spruce and beech wood meals, the absorption band at 420 nm was not observed. The guaiacyl nuclei with free phenolic hydroxyl groups which have a

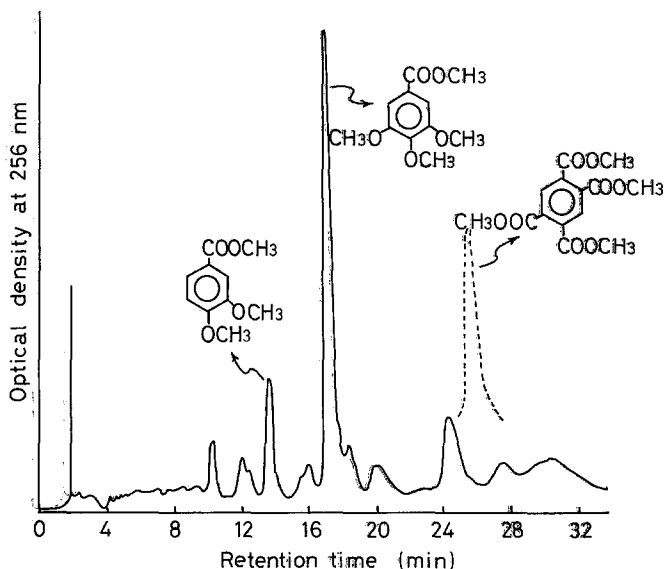


Fig. 5. HPLC of the potassium permanganate oxidation products of softwood milled wood lignin introduced syringyl nuclei

benzyl ether bond at the α -position of side chain, are oxidized to methoxy-o-quinone with a strong absorption band at about 480 nm by Fremy's salt oxidation (Teuber, Staiger 1955; Allen, Waters 1956; Zimmer et al. 1971; Iiyama, Nakano 1973). In the reflectance spectra shown in Figs. 3 and 4, the absorption bands at 480 nm were observed. These results show that the expected methoxyl-o-quinone structure was introduced. This o-quinone is reduced easily by sodium dithionite as shown in Figs. 3 and 4.

The strength of the absorption band of the Fremy's salt oxidized hardwood meal is less than that of the softwood meal, due to the small amounts of methoxy-o-quinone structures produced by the Fremy's salt oxidation. The high performance liquid chromatogram of the potassium permanganate oxidation products of the totally treated softwood milled wood lignin is shown in Fig. 5. Only small amounts of veratric acid methyl ester which originate from unreacted guaiacyl nuclei were observed. However, the 3,4,5-trimethyl gallic acid methyl ester originating from methylated syringyl nuclei was found more abundantly in the high performance liquid chromatogram. These facts show that about 85% of the uncondensed guaiacyl nuclei with free phenolic hydroxyl groups were converted to methylated syringyl nuclei.

The amounts of methylated syringyl nuclei introduced were calculated to be 0.08 to 0.1/C9 unit, because the content of the uncondensed guaiacyl nuclei with free phenolic hydroxyl groups is 0.1 to 0.12/C9 unit (Adler, Lundquist 1961).

The Mäule colour test on treated softwood lignin with 0.08–0.1/C9 units of methylated syringyl groups was examined. The purple-red colour which was noted in the hardwood meal was not observed in the treated softwood meal as shown in Fig. 6.

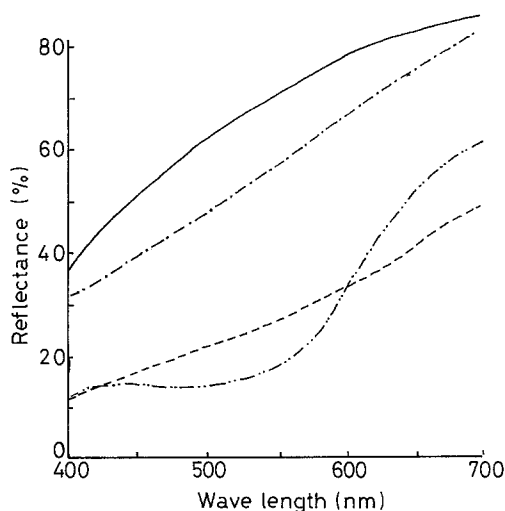


Fig. 6. Reflectance spectra of fully treated wood meals. — Fully treated softwood meal, — — Fully treated hardwood meal. - - - After Mäule treatment of fully treated softwood meal, - · - After Mäule treatment of fully treated hardwood meal

In general, aromatic nuclei with free phenolic hydroxyl groups which are ionized in alkaline media, are cleaved by oxidation with potassium permanganate, but aromatic nuclei in which free phenolic hydroxyl groups are etherified are stable towards potassium permanganate oxidation (Larsson, Miksche 1969). Thus, methylated syringyl nuclei introduced into the softwood lignin, would be expected to be stable towards potassium permanganate oxidation and not to give a positive Mäule test.

Although methylated syringyl nuclei do not produce the methoxy-*o*-quinone which gives purple-red colour, the hardwood lignin methylated by diazomethane gave the purple-red colour of the Mäule test as shown in Fig. 1. This fact shows that the syringyl nuclei with free phenolic hydroxyl groups, which are produced by the β -ether cleavage during mild permanganate oxidation, contribute to the Mäule colouring.

The wave length at maximum absorption of the chromophoric structure developed by the Mäule colour reaction of hardwood meal is 520 nm. However, that of the methoxyl-*o*-quinone structure produced by Fremy's salt oxidation is 480 nm as shown in Figs. 3 and 4. This wavelength is the same as that generated, if sulfuric acid is used instead of hydrochloric acid during the Mäule colour test. These facts show that the *o*-quinone structure in hardwood lignin is produced and is chlorinated during the Mäule colour test as shown by Meshitsuka and Nakano (1977).

The consumptions of potassium permanganate by wood components are shown in Table 1. The amounts consumed by lignins are larger than those by carbohydrates, especially that of softwood lignin which is larger than hardwood lignin. The infrared absorption spectra of wood meals before and after the Mäule colour test are shown in Fig. 7. The infrared absorption spectrum of softwood meal containing methylated syringyl nuclei maintained the characteristics of softwood meal, and was different from that of hardwood meal (Faix, Schweers 1974; Kawamura et al. 1974, 1977). The absorption band at $1,500\text{ cm}^{-1}$ assigned to aromatic nuclei

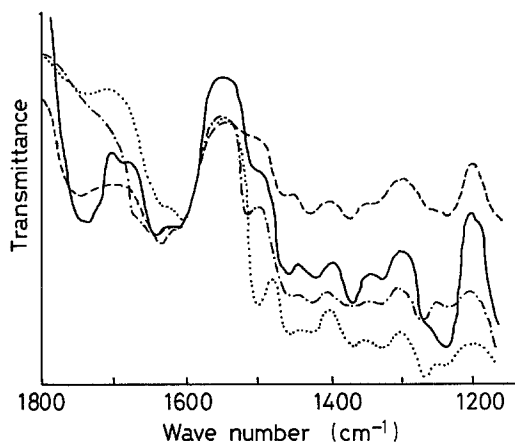


Fig. 7. Infrared absorption spectra of wood meals. — Untreated hardwood, --- Hardwood meal after Mäule treated, ····· Softwood meal introduced syringyl nuclei, —·— Softwood meal introduced syringyl nuclei after Mäule treated

Table 1. KMnO_4 consumption during colour reaction

Species	Wood meal	Carbohydrate		Lignin	
	mmol/g	mmol/g	eq/162 g	mmol/g	eq/200 g
Softwood	1.19	0.54	0.26	2.71	1.63
Hardwood	0.86	0.44	0.21	2.12	1.27

decreased, and the absorption band at around $1,650\text{ cm}^{-1}$ assigned to carbonyl groups of quinone structure increased with the Mäule colour reaction. From these facts, it seems that even if the o-quinone structure was produced in softwood lignin by potassium permanganate during the Mäule colour reaction, the o-quinone was decomposed immediately by the further oxidation with potassium permanganate. o-Quinones without o-substituent are known to be less stable than those with o-substituents (Teuber, Staiger 1955; Allen, Waters 1956; Zimmer et al. 1971).

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