Studies on Wood Phenolics (Part V)*,**
Chemical Properties and NMR Analysis
of Milled Wood Lignins

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Abstract——TMSi (trimethylsilyl)-ethers of MWL's prepared by heating MWL's with reagent of pyridine-HMDS-TMSiCl were dissolved in carbon tetrachloride, and analysed by NMR spectrometry. Large peaks of TMSi protons derived from hydroxyl groups appeared in high magnetic shielded ranges, and enabled to determine hydroxyl protons which were generally difficult to analyse by NMR. Some chemical properties such as distribution of molecular size and chemical analysis of three kinds of MWL's were also examined. The molecular wight ($\overline{M}n$) of the MWL's of Metasequoia, beech and bamboo were 2610, 2700 and 1740, respectively.

Introduction

Since NMR analysis of lignin was first subjected to methanol lignin by Bland[1], several studies[2~7] have been reported on the application of this technique in lignin chemistry. In the most cases, however, methanol lignin and lignin acetate have been used in deuterochloroform solution, except only the case in which lignin itself was dissolved in deutero dimethylsulfoxide. Morohoshi et al.[7], for example, estimated hydroxyl content of Yezo spruce-MWL (*Picea jezoensis*) as acetate.

TMSi-derivatives have been often used for gas-chromatography as thermal stable compounds having lower boiling points. In the previous papers, TMSi-polyphenols were analysed by gas-chromatography[8] and NMR[9], and carbon tetrachloride was used for the NMR analysis of a series of TMSi-polyphenols. The present authors have found that trimethylsilylation of MWL is suitable for determination of hydroxyl groups by NMR spectrometry in terms of the following points; 1) the ethers are simply prepared in a short time, 2) the cost of the solvent carbon tetrachloride is much cheaper than deuterated solvents which are usually used for NMR analysis, and 3) the peaks of hydroxyl groups are amplified as much as nine times by substitution of TMSi group.

Thus, NMR analysis of hydroxyl groups of the TMSi-MWL's is reported in the present investigation.

Experimental

MWL's of Metasequoia (*Metasequoia glyptostroboides*), beech (*Fagus crenata*) and bamboo (*Phyllostachys pubescence*) were selected as samples. The extractives of Metasequoia were studied by Sato et al.[8], but the lignin has scarcely been studied.

Sample Preparation——Heartwood of Metasequoia, beech wood, and bamboo were milled and MWL's prepared by Björkman's method[11], respectively. The sample named BO was milled with a small scale machine (Sanei Co., BO type) for which 60 g of wood meal was charged with toluene, and sample B-1 was milled with a big machine (300 g wood meal was charged with toluene) equipped with water-jackets. Milled wood was extracted with *p*-dioxane-water (9:1 v/v) and purified by an ordinary method. Yield of MWL from Metasequoia in the case of BO type was very low (1 % of Klason lignin) and those from beech and from bamboo were 6 to 10 % (by B-1) and 30 % (by B-1), respectively, and a little changes in yields due to milling time were observed.

One gram of each sample was hydrolyzed with 30 ml of 2 % sulfuric acid at reflux temperature for 2 hours. The yields based on MWL were 88.1 %, 84.1 % and 92.8 % for Metasequoia, beech and bamboo, respectively.

Methyl-, Acethyl- and TMSi Derivatives of MWL——Methylation of hydroxyl group was carried out as follows; one gram of wood meal was suspended in 15 ml of *p*-dioxane to which added the same volume of ether saturated with diazomethane, and the suspension was kept for over a night. The addition of diazomethane was repeated four times for several days and the methylation of phenolic hydroxyl groups was completed. The solution was condensed and dried up under a reduced pressure (ME-1). Alternatively, total hydroxyl groups in lignin were methylated with dimethyl sulfate. Acetylation was done with acetic anhydride and sodium acetate by refluxing for 2 hours. TMSi ethers of MWL's were prepared in the combination of pyridine (3 ml), HMDS (0.5 ml) and TMSiCl (0.5 ml) for 50 mg of samples with reflux of one hour. The solution was condensed and the residue was dissolved in carbon tetrachloride. Then, the solution was filtered and used for NMR analysis.

Analysis of Samples——A vapor pressure osmometer (Hewlett-Packered Co., Type 302) was used for determination of molecular weight. Samples in the range of 10 to 25 g/l were dissolved in methyl cellosolve and measured at 65°C. Benzil was chosen as a reference compound and **K** value was 255. For determination of the molecular distribution of MWL's, each 50 mg was dissolved in 3 ml of dimethyl sulfoxide and applied on a Sephadex-column (1.5 × 50 cm) in which 5 g of Sephadex

G-100 was packed. Every 4 ml of eluate was collected and absorption at 280 nm using a portion dilluted 10 times by a UV spectrometer (Hitachi 124 Type). Phenolic hydroxyl groups were calculated from the values of $\Delta\varepsilon$ curves according to Goldschmidt's method[12]. Namely, equal volume of buffer solution of pH 6 or 12 and sample solution of methyl cellosolve were mixed, and the intensity of absorbtion was read in the range of UV wavelength. Mean values were obtained from α-conidendrin (ε, 3100) and vanillyl alcohol (ε, 2400). Determination of methoxyl group was achieved by a volumetric method (Pyridine Method) using a Shibata's Apparatus for methoxyl. Following three kinds of NMR instruments were operated at room temperature; A, Minimer (JEOL, MH-60 Type, 60 MHz); B, R-22 Type (Hitachi Co., 90 MHz); C, A-60 Type (Varian Co., 60 MHz). Deutero chloroform and carbon tetrachloride were used for the acetate-MWL and the TMSi-MWL as solvents, respectively. Tetramethylchlorosilane (TMSi) or t-buthanol was added in a tube as an internal standard.

Results and Discussion

Molecular weight, elementary analysis and methoxyl content of each MWL were summarized in Table 1. Analytical data of isolated MWL's were presented on the upper column, and of hydrolyzed MWL (MWL-H) were on the lower. Since Marton has found that the ratio of $\overline{M}w$ to $\overline{M}n$ of lignin is between 3 to 4, actually the weight average molecular weight is supposed to be three or four times larger than the values in Table 1. The lower values in bamboo MWL are supposed to be related with fast growing and incomplete lignification of the bamboo.

Methoxyl contents of Metasequoia MWL were greatly changed by hydrolysis. It would be, perhaps, due to some contaminants such as cyclitols, because the data of MWL-H was more close to these of Real's data[10] (15.8 %). Beech-MWL was little changed in methoxyl content by hydrolysis. However, methoxyl content of bamboo-MWL was increased by hydrolysis (ca. 4 %), and this fact was considered

Table 1. Elementary, group and molecular weight analysis of MWL's.

Sample	M.W. (Mn)	Elem. contents			Methox. (%)	Emp. formula
		C %	H %	O %		
Metasequoia MWL (BO)	2610	60.79	5.78	33.43	10.10	$C_9H_{9.03}O_{3.35}(OCH_3)_{0.62}$
Metasequoia MWL-H	—	58.71	5.85	35.43	17.20	$C_9H_{8.62}O_{3.45}(OCH_3)_{1.15}$
Beech MWL (B1)	2700	58.40	5.80	35.80	18.29	$C_9H_{8.40}O_{3.47}(OCH_3)_{1.24}$
Beech MWL-H	—	54.43	5.98	39.59	17.15	$C_9H_{9.66}O_{4.34}(OCH_3)_{1.25}$
Bamboo MWL (B1)	1740	59.42	5.68	34.90	16.67	$C_9H_{8.22}O_{3.36}(OCH_3)_{1.09}$
Bamboo MWL-H	—	60.10	5.87	34.03	20.71	$C_9H_{7.94}O_{3.03}(OCH_3)_{1.38}$

to be due to the partial elimination of p-coumaric acid moiety esterified with lignin.

Empirical formulae of the MWL's according to Freudenberg's method[13] were given in Table 1. The water content as adduct was generally larger than the data expected by Freudenberg's hypothesis in which the content of such water was approximately 0.4 mol and did not exceed two oxygen atoms per an aryl propane unit. It is conceivable that the difference is due to the drying method and no correction for ash content.

In Figure 1, the molecular weight distribution curves of MWL's were shown.
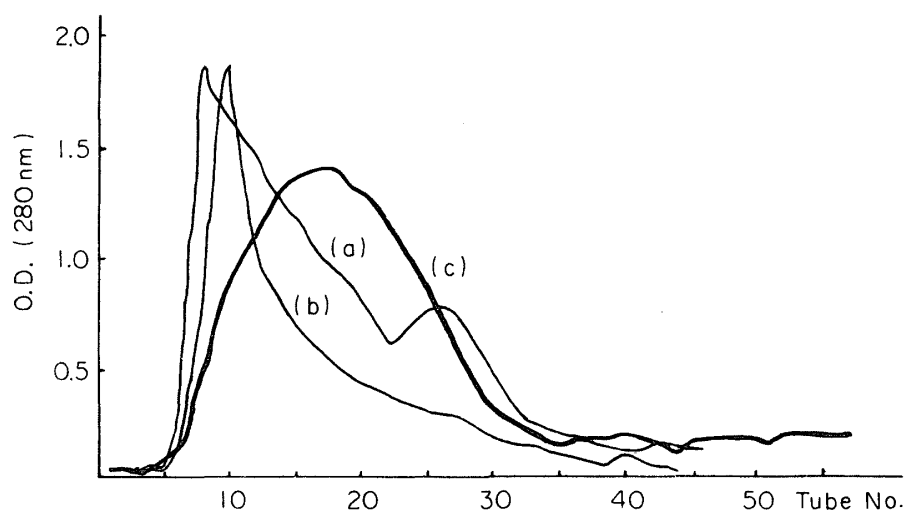
a) Metasequoia MWL, b) Beech MWL, c) Bamboo MWL.

Fig. 1. Elution curves of MWL (Sephadex G-100).
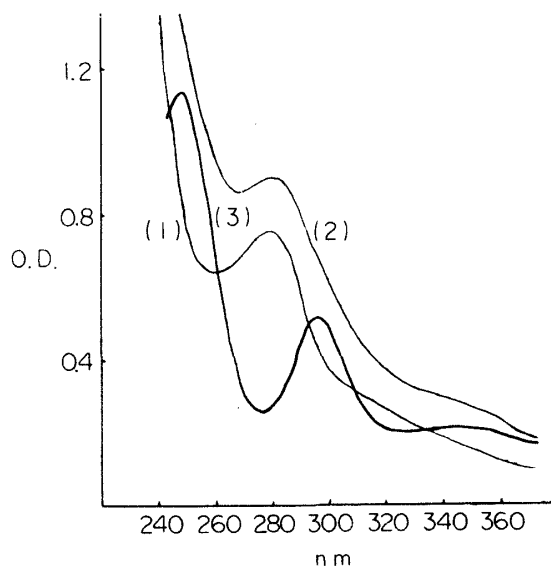
(1) pH 6, (2) pH 12 (3)$\Delta\varepsilon$ [(2)−(1)].

Fig. 2. UV absorption curves of metasequoia MWL.

Different types of curves were found by each kind of MWL. Double peaks are observed in Metasequoia-MWL and the low molecular weight of bamboo-MWL is expected from the curve C. In Figure 2, $\Delta\varepsilon$ curve of Metasequoia-MWL was shown with UV absorption curves which showed the maximum peaks at 279 nm in pH 6 and 282 nm in pH 12. These values were in agreement with those of softwood lignin. Maximum peaks from $\Delta\varepsilon$ curve were at 250 nm and 296 nm, and the phenolic content calculated from the value was 3.64 % (0.43/C₉) which was slightly higher than the value, 0.32, found by Freudenberg with spruce lignin.

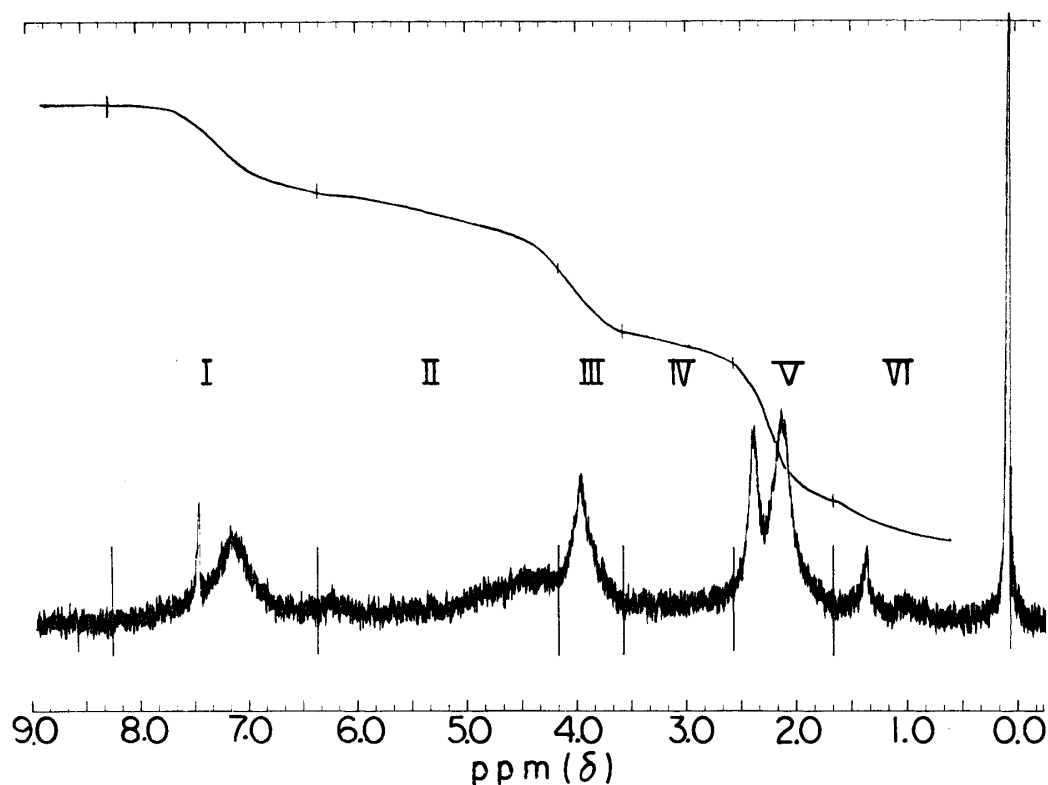A NMR chart of Metasequoia MWL-acetate divided to 6 regions according

Fig. 3. NMR chart of metasequoia MWL (acetate). (MH-60)
Regions were divided into 6 parts due to Bland's method.

Table 2. Distribution of prosons in MWL-acetate.

Range ppm (δ)	1	2	3	4	5	6	7	8	9	10	Total (%)
		~8.00~	6.28~	5.74~	5.18~	3.95 ~	3.24~	2.40~	2.19~	1.58~	
Sample		arom.				methox.		arom. acet.	aliph. acet.		
Metasequoia (BO)	—	19.9	3.2	2.7	12.8	14.1	7.7	11.5	20.5	7.7	100
Beech (B1)	—	13.5	2.9	0.9	15.1	27.7	5.6	6.2	25.0	3.1	100
Bamboo (B1)	1.0	18.8	2.9	1.2	16.2	25.4	6.1	6.8	19.3	2.3	100

to Bland was shown in Figure 3. Alternately, the same chart was divided to 10 regions after Lenz[4] and summarized in Table 2, where the 2, 6, 8 and 9th are shown as percentages of the aromatic, methoxyl, aromatic acetoxy and aliphatic acetoxy protons, respectively. From these results, the methoxyl content of Metasequoia-MWL is conceivable to be lower. Since different instruments often gave different relative intensity to the same samples through NMR measurement, the combination of modulation and intensity of high frequency on the instrument should be cared. On the above data, mean values were chosen from multiple measurements.

Comparison was made on the elementary composition and the methoxyl content of Metasequoia MWL methylated with diazomethane (ME-1) and with dimethyl sulfate (ME-2), respectively, in Table 3. The increment of methoxyl content of the MWL after diazomethane-methylation was 6.3 % ($0.39/C_9$) in comparing with

Table 3. Elementary and methoxyl analysis of Metasequoia MWL.

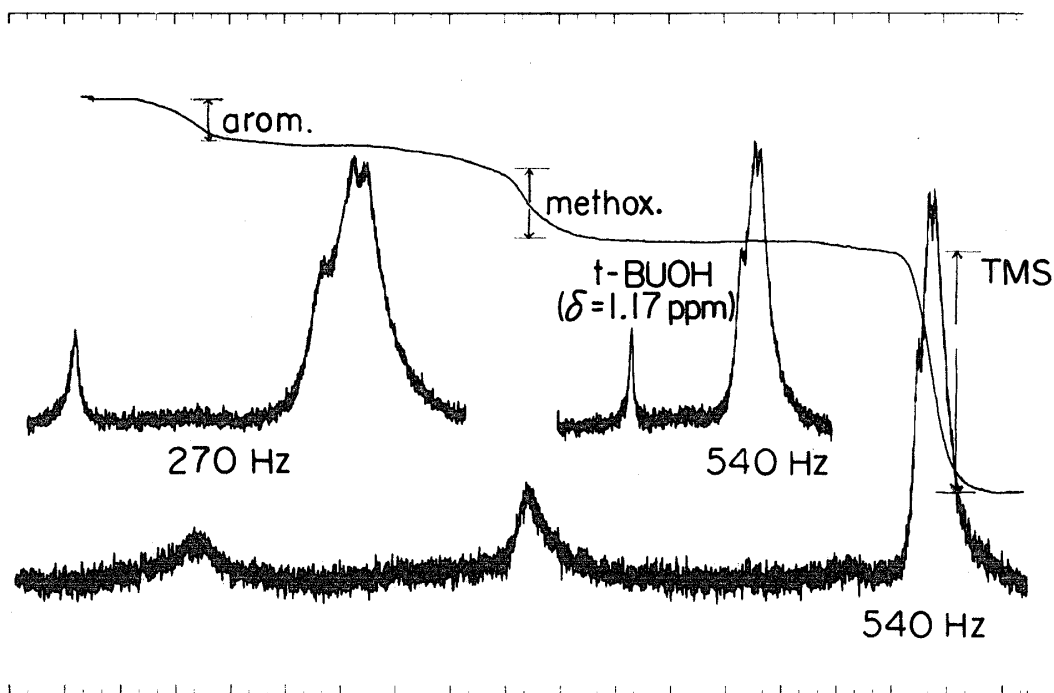| Sample	Elem. analysis			Methox. (%)	Emp. formula
	C %	H %	O %		
MWL (BO)	60.79	5.78	33.43	10.10	$C_9H_{9.03}O_{3.35}(OCH_3)_{0.62}$
MWL-ME 1 (BO)	61.91	6.59	31.50	16.36	$C_9H_{9.65}O_{2.81}(OCH_3)_{1.02}$
MWL-ME 2 (BO)	63.24	6.38	30.38	25.43	$C_9H_{7.83}O_{2.18}(OCH_3)_{1.66}$
MWL-acetate (BO)	59.94	5.26	34.80	9.77	——

Fig. 4. NMR chart of TMS-metasequoia MWL. (MH-60)

untreated one. In the sample, ME-2, an additional increment of the methoxyl content was $0.61/C_9$, and final increment was found to be $1.66/C_9$. From the data of ME-1 and $\varDelta \varepsilon$ method, the hydroxyl content of Metasequoia showed similar values. The increment by ME-2 was less than the value from spruce MWL (ca. 1.0), but the reason was not clear. TMSi-MWL showed brown color, and was viscous liquid which is soluble in carbon tetrachloride. An example of NMR chart from TMSi-MWL was shown in Figure 4.

TMSi groups introduced were shown as large peaks on the chart at the range of 0.4 to 0.35 ppm (δ). In the lower magnetic shielded field, aromatic protons (7.5 to 6.3 ppm) and methoxyl protons (4.1 to 3.3 ppm) appeared as smaller peaks. Assignment of the peaks at the range of TMSi-protons were followed in the previous paper using t-buthanol as an internal standard, and aromatic and aliphatic TMSi protons were successfully distinguished. Different patterns from different wood species were also observed in the range of TMSi-protons as shown in Figure 5. Aromatic TMSi protons were marked with an arrow. New peaks of hexamethyl-disiloxane[9] which are not found in freshly prepared TMSi-MWL appeared on the chart of old TMSi-MWL stored for several days.
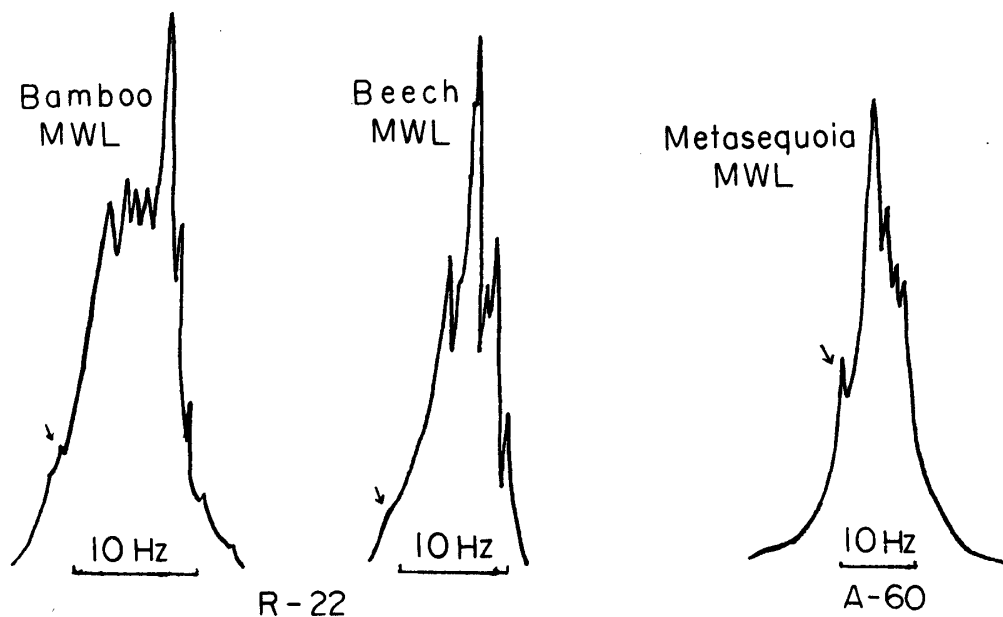

Fig. 5. Details of TMS groups in NMR charts of TMSi-MWL.

Quantitative determination of the functional groups of TMSi-MWL was carried out as follows by using NMR. Integral intensity (A) shown as percentages was first divided by the atomic weights of protons which was contained in each functional group to obtain the divisor named (B). After that, each divisor (B) was further divided by the methoxyl values from rational formulae (expressed by C_9) and

Table 4. Distribution of protons in TMSi-MWL.

Protons Sample	Aromatic			Methoxyl			Trimethylsilyl		
	a %	b	c	a %	b	c	a %	b	c
Metasequoia MWL (BO)	6.94	6.88	0.94	14.58	4.82	0.66	78.48	8.65	1.19
Metasequoia MWL-ME 1 (BO)	11.19	11.10	1.39	25.17	8.32	1.05	63.64	7.01	0.88
Metasequoia MWL-ME 2 (BO)	20.00	19.84	1.89	52.50	17.36	1.66	27.50	3.03	0.29
Beech MWL (B1)	8.50	8.43	1.30	24.18	8.00	1.24	67.32	7.42	1.15
Bamboo MWL (B1)	7.00	6.94	0.92	25.00	8.27	1.19	68.00	7.50	1.00

obtained the ratio (C). This ratio (C) which means the number of functional groups per C_9, were shown in Table 4. In the case of Metasequoia, the result from TMSi-MWL were shown as $0.31/C_9$, $0.59/C_9$ and $1.19/C_9$ for aromatic, aliphatic and total hydroxyl content, respectively. These values are in good agreement with the data from elementary and functional analysis. The numbers of protons due to aromatic nuclei were little changed, but the contents of total hydroxyl groups were not fixed. Mean values from several samples were $1.40/C_9$ to $1.27/C_9$. The values on beech-MWL were supposed to be resonable, but the values from bamboo-MWL gave a small content of total hydroxyl groups and the reason was still unknown.

At present, all data from TMSi-metnod does not always coincided with the data from other techniques, but further study including correction about ash and adduct-water would be expected.

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