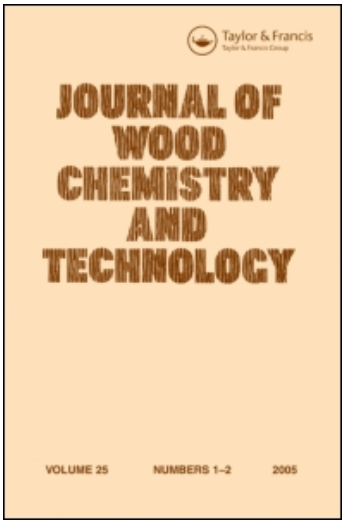

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Isolation of a Syringyl-β-O-4 Rich End-Wise Type Lignin Fraction from Birch Periodate Lignin

Nobutaka Fukagawa; Gyosuke Meshitsuka; Atsushi Ishizu

To cite this Article Fukagawa, Nobutaka , Meshitsuka, Gyosuke and Ishizu, Atsushi(1992) 'Isolation of a Syringyl-β-O-4 Rich End-Wise Type Lignin Fraction from Birch Periodate Lignin', Journal of Wood Chemistry and Technology, 12: 1, 91 — 109
To link to this Article: DOI: 10.1080/02773819208545052
URL: http://dx.doi.org/10.1080/02773819208545052

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 12(1), 91-109 (1992)

ISOLATION OF A SYRINGYL-β-O-4 RICH END-WISE TYPE LIGNIN FRACTION FROM BIRCH PERIODATE LIGNIN

Nobutaka Fukagawa, Gyosuke Meshitsuka
and Atsushi Ishizu
Department of Forest Products, Faculty of Agriculture
The university of Tokyo, Yayoi 1-1-1, Bunkyo-ku,
Tokyo, Japan

ABSTRACT

A fraction of lignin which was almost monodisperse was isolated from an acetylated birch periodate lignin, and its chemical structure was investigated by various NMR experiments. It was proved that the fraction was composed mainly of non-phenolic syringyl-β-O-4 structures.

INTRODUCTION

The morphological heterogeneity in lignin structure has been one of the most important problems with respect not only to the chemical structural study of lignin but also to the pulping chemistry.[1] As to the relative abundance of each aromatic structure in hardwood lignin, it has been found that the ratio of syringyl nuclei/guaiacyl nuclei is higher in the fiber secondary wall than in the middle lamella.[2-5]

With respect to structural dependency on dehydrogenative conditions, two types of polymer lignins, 'bulk lignin' and 'end-wise lignin' were proposed.[6] The former is thought to be formed when monomer radicals

formed via dehydrogenation of coniferyl alcohol type structures are supplied in high concentration, and the formation of dimers under these dehydrogenation conditions is followed by further polymerization to tetrameric and the higher molecular weight lignols. On the other hand, end-wise lignin is thought to be formed under the condition when the monomer radicals are supplied slowly to the lignificating area and are allowed to combined one-by-one with the radicals present on the surface of polymer molecules. Bulk lignin has higher abundance in β-β and β-5 types of linkages, phenolic hydroxyl groups and unattached side-chains than end-wise lignin. β-1 type structures are specific for end-wise lignin. In recent years, it has been reported that compound middle lamella lignins are highly condensed and have more 'bulk lignin' character than the secondary wall lignin.[7-10]

For the investigation on the structural heterogeneity of lignin with respect to these two types of polymer lignins, a nondestructive analysis is essential. A periodate oxidation combined with subsequent extraction causes little modification of side-chain structures in lignin, although phenolic hydroxyl groups may be oxidized. And NMR spectroscopy is one of the most powerful and nondestructive tools for the investigation of chemical structures. In this paper, we isolated a lignin fraction of almost mono-dispersity from an acetylated periodate lignin, and investigated its chemical structure in detail by various NMR experiments.

EXPERIMENTAL

Preparation of Periodate Lignin

To 27.6 g (oven dried weight) of birch wood meal (40-60 mesh, pre-extracted with ethanol-benzene), 2100

mL of water, 15 mL of acetic acid and 75 g of sodium metaperiodate were added and stirred for 10 min. Then the reaction mixture was allowed to stand in the dark at room temperature. After one week, the reaction mixture was filtered with a glass fiber sheet and the residue was washed with 3 L of water and 3 L of hot water. To this residue, 1200 mL of aqueous 0.1N NaOH and 30 g of $NaBH_4$ were added and kept at room temperature. After 24 hr, the reaction mixture was filtered and the residue was washed successively with water, 10% acetic acid, and hot water. Then, to this residue 600 mL of aqueous 0.5N HCl was added and stirred at room temperature. After 24 hr the reaction mixture was filtered and washed with water and hot water. Finally, the residue was dried at 45 °C under vacuum to give 8.4 g of periodate lignin.

Acetylation and Fractionation of Periodate Lignin

Five grams of periodate lignin was extracted ultrasonically with 500 mL of dioxane/H_2O (9/1, v/v) for 2.5 hr. During this period, the temperature of the mixture rose from 25 °C to 50 °C. The mixture was filtered by a glass fiber sheet, and the filtrate was evaporated to dryness at 45 °C. The residue was dried at 40 °C under vacuum, and acetylated with 20 mL of pyridine and 20 mL of acetic anhydride at room temperature for 24 hr. The reaction mixture was poured into ice-cooled water, and extracted with a mixture of dichloromethane/acetone (2/1, v/v). The organic layer was washed with 2N HCl and brine, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was extracted ultrasonically with 50 mL of MeOH for 15 min. The MeOH solution was evaporated, and the resultant product was dried under vacuum at 45 °C to give 13 mg of fraction PIL-M. The residue was further extracted ultrasonically with 50mL of acetone

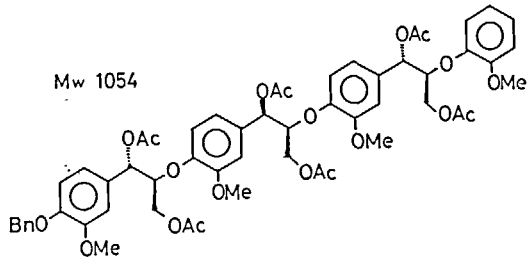

Figure 1. The structure of acetylated tetralignol

for 15 min. The acetone solution was evaporated, and the resultant product was dried under vacuum at 45 °C to give 116 mg of fraction PIL-A.

Measurement of Molecular Weight Distribution

The molecular weight distribution of an acetylated periodate lignin fraction was measured by HPLC under the following conditions.

HPLC system: Shimadzu LC-4A, column: Shimadzu GPC-801, 802 and 803, eluent: THF, flow rate: 1mL/min, detector: UV(280nm).

Acetylated tetralignol[11] (Figure 1) and acetylated birch MWL[12,13] were used as references.

NMR Experiments

All NMR spectra were recorded on Bruker AM600 and AC300 spectrometers, and their chemical shifts were referenced to tetramethylsilane.

The 1D ^{1}H spectrum was recorded at 27 °C on 36 mg of PIL-A in 1ml of degassed $CDCl_3$ at 600 MHz. The data matrix was 32k and 32 scans were accumulated.

The 1D ^{13}C spectrum was recorded at 27 °C on 100 mg of PIL-A in 0.5 ml of $CDCl_3$ at 75 MHz. The data matrix was 32k and 42000 scans were accumulated. A 2 Hz exponential line-broadening window was applied.

All of 2D experiments were acquired at 27 °C on 36 mg of PIL-A in 1 mL of degassed $CDCl_3$ at 600 MHz.

Phase-sensitive mode HMQC data were acquired using the Bruker pulse program:INVD1DP9.AU.[14] The dimension of matrix was 344 x 1K and the dimension of transformation was 1k x 2k. The minimum t_1 was 3 μs and each increment of t_1 was 10.7 μs. For each t_1 increment, 92 scans were accumulated. A $\pi/2$ phase shifted sinebell-squared window was applied to the t_1 dimension and a $\pi/4$ phase shifted sinebell-squared window was applied to the t_2 dimension.

Phase-sensitive mode HOHAHA data were acquired using the Bruker pulse program:MLEV17DW.AU.[15] The mixing time was 60 ms. The dimension of matrix was 512 x 1K and the dimension of transformation was 1k x 2k. The minimum t_1 was 1 μs and each increment of t_1 was 83 μs. For each t_1 increment, 64 scans were accumulated. A $\pi/4$ phase shifted sinebell-squared window was applied in each dimension. The data matrices were not symmetrized.

Phase-sensitive mode HMQC-HOHAHA data were acquired using the pulse program reported by Lerner *et al.*[16] The mixing time was 60 ms. The dimension of matrix was 360 x 1K and the dimension of transformation was 1k x 2k. The minimum t_1 was 1 μs and each increment of t_1 was 10.7 μs. For each t_1 increment, 288 scans were accumulated. A $\pi/2$ phase shifted sinebell-squared window was applied to the t_1 dimension, and a $\pi/4$ phase shifted sinebell window was applied to the t_2 dimension.

Phase-sensitive mode NOESY data were acquired using Bruker pulse program:NOESYPH.AU.[17] The mixing time was 150 ms. The dimension of matrix was 484 x 1K and the dimension of transformation was 1k x 2k. The minimum t_1 was 1 μs and each increment of t_1 was 83 μs.

For each t_1 increment, 128 scans were accumulated. A $\pi/4$ phase shifted sinbell-squared window was applied in each dimension. The data matrices were not symmetrized.

RESULTS AND DISCUSSION

Molecular Weight Distribution

Gel permeation chromatograms of PIL-A, PIL-M, and acetylated MWL was shown in Figure 2. PIL-A showed almost mono-dispersity and had a higher number average molecular weight (Mn) than MWL. PIL-M showed a broad polymodal pattern, and its Mn was lower than that of PIL-A.

Investigation of Chemical Structures of PIL-A by NMR

^1H (Figure 3) and ^{13}C (Figure 4) spectra of PIL-A were further correlated by two-dimensional experiments: HMQC (^1H-Detected Multiple Quantum Coherence), HOHAHA (Homonuclear Hartmann-Hahn), HMQC-HOHAHA and NOESY (Nuclear Overhauser and Exchange Spectroscopy) experiments.

Side-chain Structures

Three peaks ascribable to β-O-4 structures showed high intensities in the HMQC spectrum (Figure 5 and Table 1). The peaks ascribable to α-positions were bimodal, and the peaks of γ-positions were broad and obscure. Therefore, by the use of HOHAHA, HMQC-HOHAHA and NOESY experiments, assignments of these peaks were further investigated. In the HOHAHA spectrum (Figure 6) two series of cross peaks in vicinity (6.1ppm/4.5ppm, 4.3ppm, 3.8ppm and 6.0ppm/4.6ppm, 4.4ppm, 4.15ppm) were observed. In the HMQC-HOHAHA spectrum (Figure 7) these two networks showed slightly different chemical shifts for α- and γ-^{13}C, and the same one for β-^{13}C. Each peak of α-protons

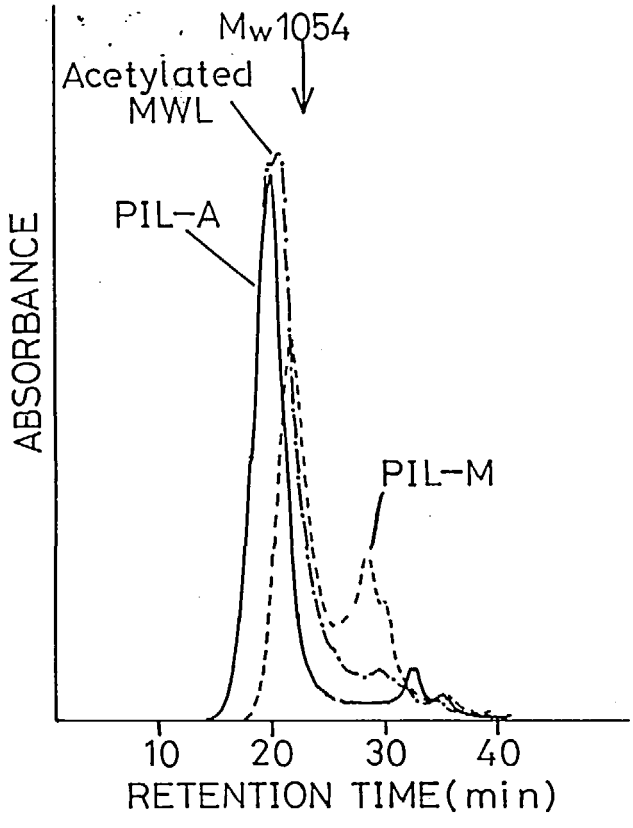

Figure 2. Gel-permeation chromatograms of PIL-A, PIL-M, and acetylated MWL.

———— PIL-A*1
- - - - - - PIL-M*2
—·—·— acetylated MWL

Thee arrow on the top of Figure indicates the position of the peak in chromatogram of acetylated tetralignol(M_W 1054) under the same conditions.

*1 Fraction extracted with acetone from MeOH insoluble fraction of acetylated Periodate lignin

*2 Fraction extracted with MeOH from acetylated periodate lignin

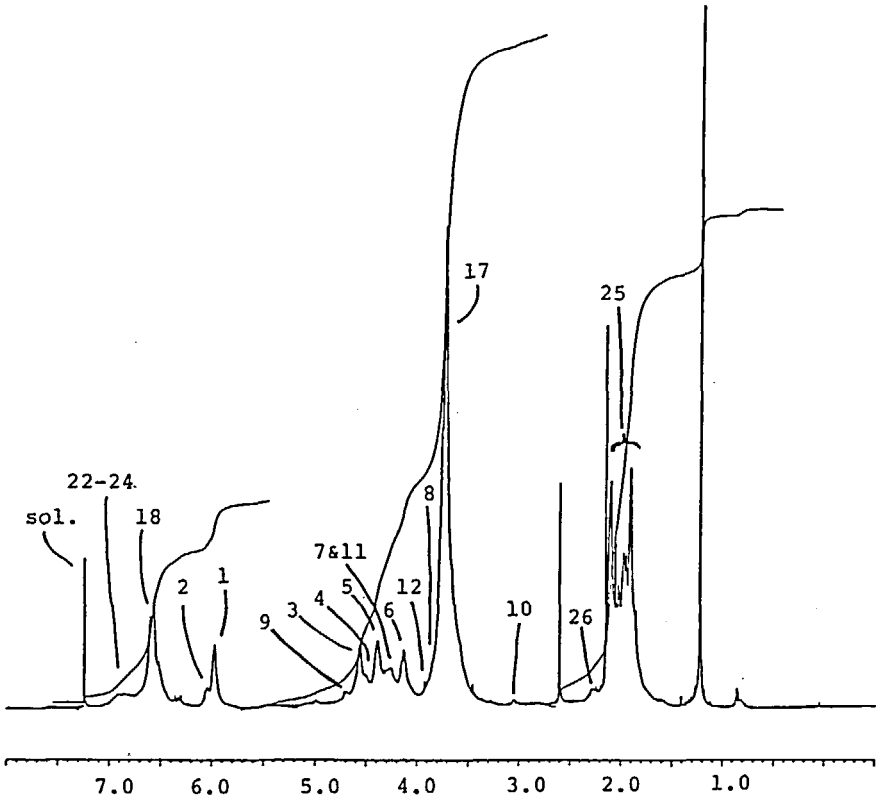

Figure 3. 1D ^{1}H spectrum of PIL-A. The peak number correspond to those in Table 1.

(6.1ppm and 6.0ppm) in two networks showed cross peaks at 6.5-6.7ppm in the NOESY spectrum (Figure 8). From the scope of the above results, these two networks are thought to be assigned to two diastereomers, threo and erythro (Table 1). On the basis of the peak areas in the 1D-^{1}H spectrum (Figure 3), the abundance ratio of these two diastereomers is about 1/3 (threo/erythro).

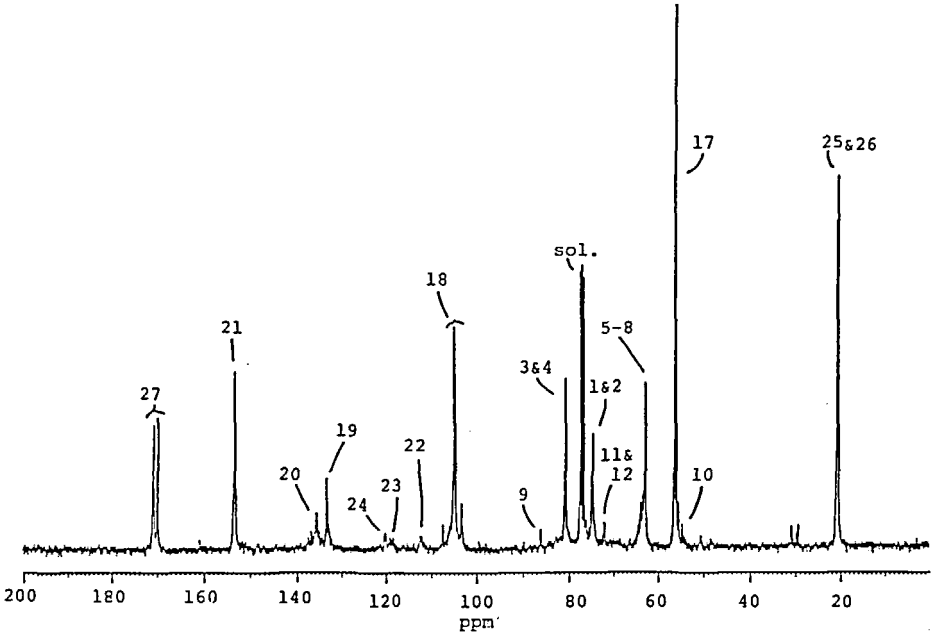

Figure 4. 1D ^{13}C spectrum of PIL-A. The peak number correspond to those in Table 1.

Four peaks for pinoresinol structures were observed in the HMQC spectrum (Figure 5 and Table 1) and the coupling network was clearly identified in the HOHAHA spectrum (Figure 6), although their intensities were much weaker than those for β-O-4 structures.

The cross peaks (3.3ppm/4.1ppm, 4.3ppm, 6.0ppm) probably ascribable to β-1 structures which are thought to be specific for 'end-wise lignin' were observed in the HOHAHA spectrum (Figure 6). Since their intensities were much weaker than those from pinoresinol structures, this type of structure was ignored for the quantitative discussion in the next section.

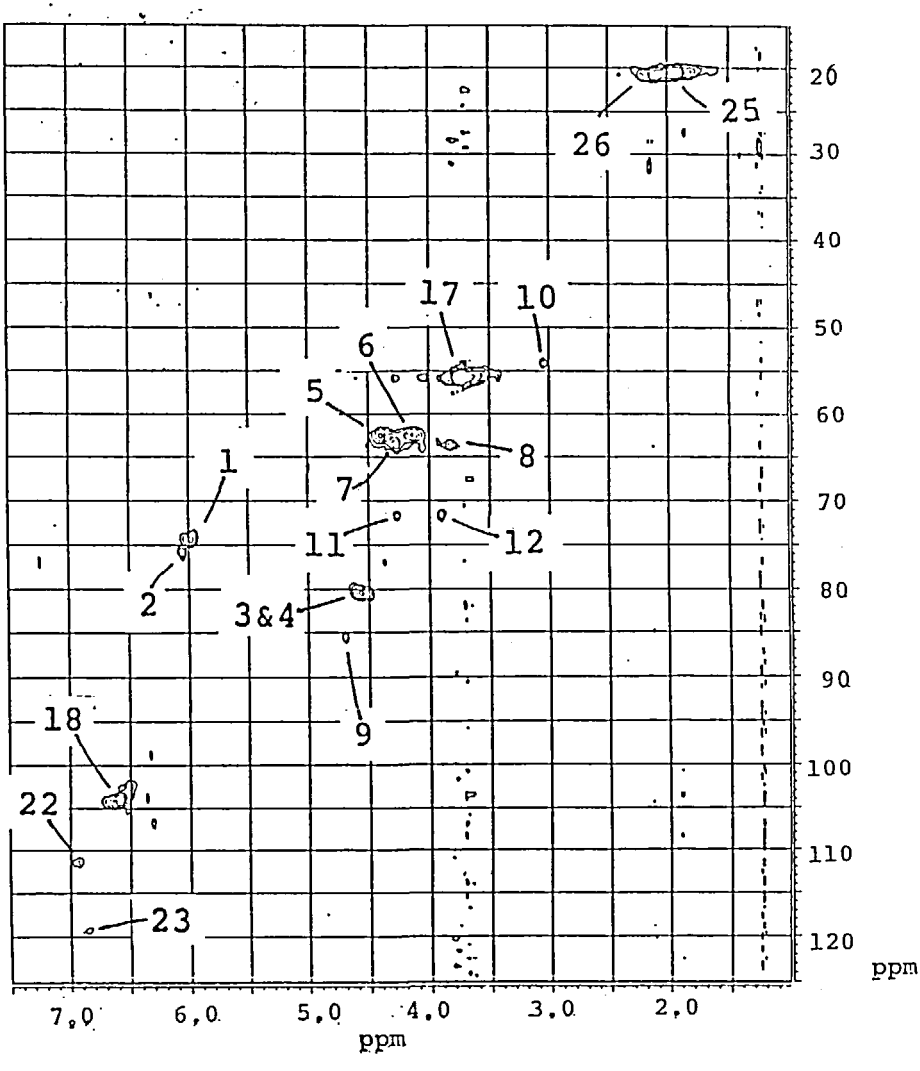

Figure 5. Phase sensitive HMQC spectrum of PIL-A. The peak number correspond to those in Table 1.

Table. 1

Assignments of Each Peak in ^{1}H- and ^{13}C-NMR Spectra of PIL-A

No.	Chemical Shift(ppm)		Assignments
	^{1}H	^{13}C	
1.	5.9-6.0	74	β-O-4-α erythro
2.	6.1	75.5	β-O-4-α threo
3.	4.5-4.6	80	β-O-4-β erythro
4.	4.5	80	β-O-4-β threo
5	4.4	62-63	β-O-4-γ erythro
6.	4.15	62-63	β-O-4-γ erythro
7.	4.3	63-64	β-O-4-γ threo
8.	3.8	63-64	β-O-4-γ threo
9.	4.7	86	Pinoresinol-α
10.	3.1	54	Pinoresinol-β
11.	4.25	72	Pinoresinol-γ (equatorial)
12.	3.9	72	Pinoresinol-γ (axial)
13.	6.0	--	β-1-α
14.	3.3	--	β-1-β
15.	4.1	--	β-1-γ
16.	4.3	--	β-1-γ
17.	3.5-3.9	55-56	Methoxyl
18.	6.5-6.75	102-105	Syringyl-2, 6
19.	--	134	Syringyl-4
20.	--	136	Syringyl-1
21.	--	153	Syringyl-3, 5
22.	6.95	112	Guaiacyl-2
23.	6.85	119	Guaiacyl-5
24.	6.7-7.0	120	Guaiacyl-6
25.	1.8-2.2	20-21	Methyl of acetoxyl on aliphatic carbon
26.	2.2-2.5	20-21	Methyl of acetoxyl on aromatic carbon
27.	--	169-171	Carbonyl of acetoxyl

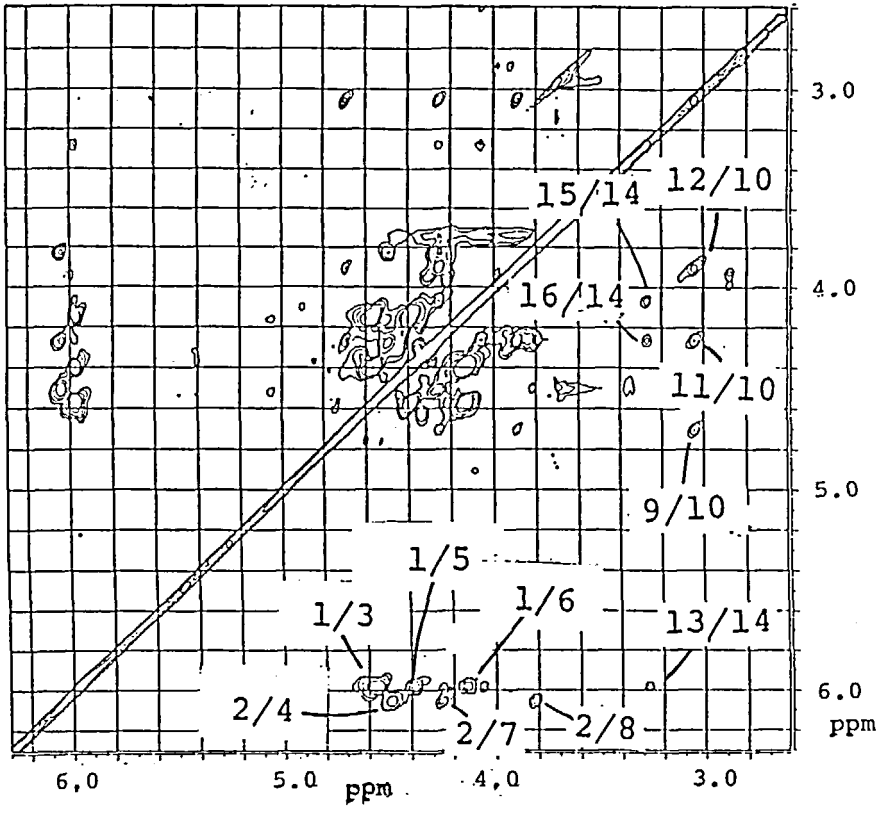

Figure 6. Phase sensitive HOHAHA spectrum of PIL-A. The peak number correspond to those in Table 1.

No peaks derived from other side-chain structures such as phenylcoumaran, coniferyl alcohol and so on, were observed either in the HMQC spectrum or the HOHAHA spectrum.

Aromatic Structures

In the HMQC spectrum (Figure 5), the peak ascribable to syringyl-2, 6 (peak 18) shows much higher intensity than those of guaiacyl-2 (peak 22) -5 (peak

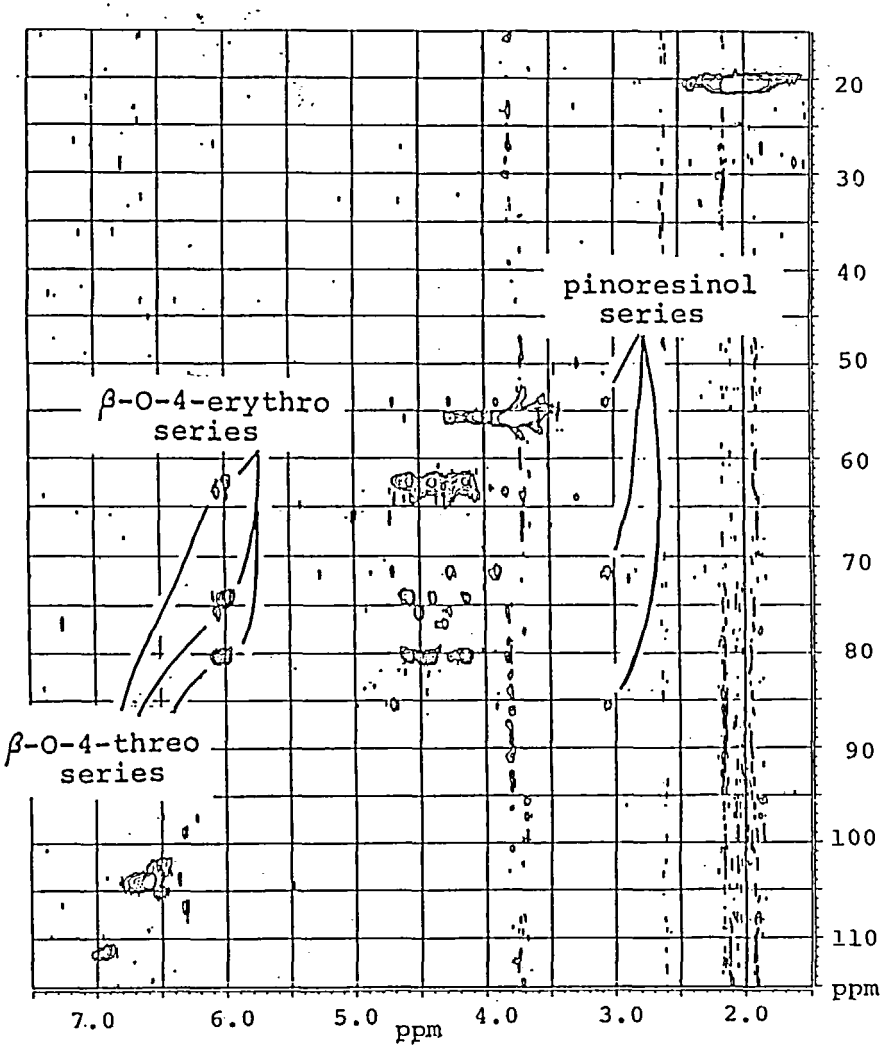

Figure 7. Phase sensitive HMQC-HOHAHA spectrum of PIL-A.

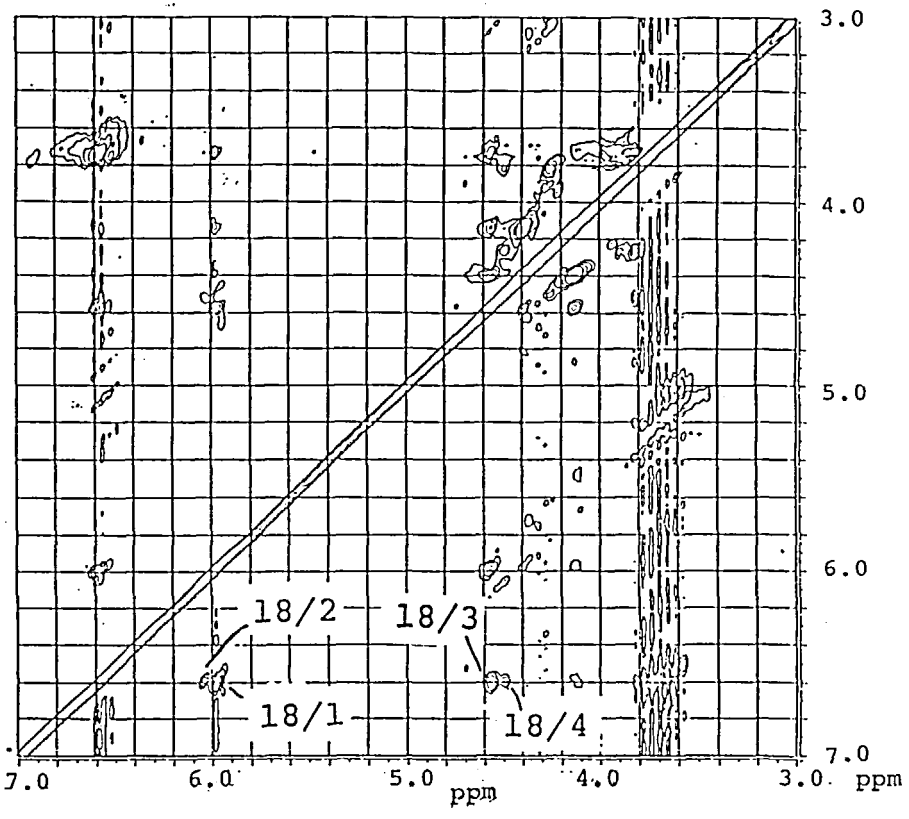

Figure 8. Phase sensitive NOESY spectrum of PIL-A. The peak number correspond to those in Table 1.

23). The peak for guaiacyl-6 which should appear at 6.7-7.0ppm, 120ppm was not seen at this contour-plot level.

Adler *et al* reported that free phenolic syringyl and guaiacyl components were readily oxidized by sodium periodate to o-quinone or muconic acid structures.[18] Since methyl protons in acetyl groups reacted with phenolic hydroxyl groups appeared as a very weak shoul-

der in the range of 2.2-2.5ppm[19, 20] of 1D ^{1}H spectrum (Figure 3), very few phenolic hydroxyl groups were contained in this fraction. Furthermore, no distinct peaks ascribable to o-quinone and muconic acid structures were observed in the HMQC spectrum (Figure 5). Although three weak peaks (6.3ppm/98ppm, 6.3ppm/107ppm and 6.35ppm/104ppm) were observed in the HMQC spectrum (Figure 5), which may be ascribable to oxidation products of phenolic hydroxyl groups, the intensities of these peaks in the 1D ^{1}H spectrum (Figure 3) were extremely low. Therefore, this lignin fraction is thought to be originally almost free of phenolic hydroxyl groups.

Carbohydrates

There are no peaks in the region (4.0-6.0ppm, 90-105ppm) of the HMQC spectrum (Figure 5) where anomeric positions of carbohydrates resonate. Moreover, no cross peaks ascribable to carbohydrate skeletons were observed in the HOHAHA spectrum (Figure 6). Therefore, PIL-A seems to be almost free of carbohydrates.

Quantitative Discussion

On the basis of the results in the previous section, each peak in the regions (6.75-7.1ppm: guaiacyl-2, 5 and 6, 6.45-6.75ppm: syringyl-2 and 6, 5.8-6.15ppm: β-O-4-α, 3.0-3.1ppm: pinoresinol-β) of the 1D ^{1}H spectrum was ascribable to a single structural group. A strong peak in the region of 3.5-3.9ppm was ascribable to two structural groups, methoxyl group and one of the non-equivalent methylene protons of threo β-O-4-γ. Therefore, the peak area of methoxyl groups can be calculated by subtracting that of threo β-O-4-γ-protons, which can be estimated on the basis of that of α-protons in 6.02-6.15ppm, from that in the region of 3.5-3.9ppm. Therefore, quantitative discus-

Table. 2

Peak Area of Each Structural Group in 1D ^{1}H Spectrum

Chemical shift	Assignments	Relative area[a)]	Abundance
3.0-3.1ppm	Pinoresinol-β	0.05	0.025x2H
3.5-3.9ppm	Methoxyl	8.9	3.0x3H
5.8-6.15ppm	β-O-4-α	1.0	1.0x1H
6.45-6.75ppm	Syringyl -2 and 6	2.3	1.2x2H
6.75-7.1ppm	Guaiacyl -2, 5 and 6	0.56	0.2x3H

a)These values were calculated relative to β-O-4-α proton.

sion of the abundance of these structural groups was possible on the basis of the 1D ^{1}H spectrum.

According to the results shown in table 2, the abundance ratio (guaiacyl + syringyl x 2)/ OMe was 0.87. It is not clear whether the derivation from 1.0 is due to the existence of OMe groups in other components than syringyl and guaiacyl units, or to experimental error. The ratio of syringyl/guaiacyl components (S/G) was calculated to be about 6. Saka et.al.[3] reported the S/G ratio of fiber secondary-wall lignin to be 88/12 by bromination and EDXA technique. It was also reported that under the conditions of sulfite cooking in which hemicellulose started to be removed, secondary wall lignin dissolved more readily than middle lamella lignin.[21, 22] Therefore, PIL-A might be derived from fiber secondary wall.

If none of guaiacyl component is bound to β-O-4 side chains, about 80% of syringyl components are bound to β-O-4 side chains. Even if all of guaiacyl

components are bound to β-O-4 side chains, about 66% of syringyl components should be bound to β-O-4 side chains. Protons of guaiacyl-2 and 6 showed no cross peak with α-protons of β-O-4 structures in the NOESY spectrum (Figure 8). Since a cross peak of high intensity was observed between α-protons of β-O-4 side chains and protons of syringyl 2 and 6, it is hard to expect that a considerable part of guaiacyl components is bound to β-O-4 side chains. Although the proportion of β-O-4 structures in other isolated lignins which are thought to be the mixture of end-wise and bulk lignins,[6] varied depending on wood species and on experimental methods, values in the range of 20-60% were reported.[23,24] Comparatively, the proportion of β-O-4 side chains in this lignin fraction was unusually high. Furthermore, PIL-A has a very low abundance of phenolic hydroxyl groups, and shows no unattached side-chains such as coniferyl alcohol and aldehyde structures. Therefore PIL-A has very characteristic structures of the 'end-wise lignin'.

CONCLUSION

A lignin fraction of a higher molecular weight than MWL and of almost mono-dispersity was obtained from an acetylated birch periodate lignin. This fraction had a very homogeneous structure mainly composed of non-phenolic syringyl-β-O-4 units which are characteristic of 'end-wise lignin'. The syringyl/guaiacyl ratio of this fraction was estimated to be about 6/1 from the ^{1}H-NMR spectroscopy.

REFERENCES

1. D. A. I. Goring, In Lignin, Properties and Materials, p.3-10, American Chemical Society, Washington, DC, 1989

2. Y. Musha and D. A. I. Goring, Wood Sci. Technol., 9, 45(1975)

3. S. Saka and D. A. I. Goring, In Biochemistry and Biodegradation of Wood Components, p.51-62, T. Higuchi(ed.), Academic,New York, 1985

4. H.-L. Hardell, G. Leary, M. Stoll and U. Westermark, Svensk Paperstidn., 83, 71(1980)

5. N. S. Cho, J. Lee, G. Meshitsuka and J. Nakano, Mokuzai Gakkaishi, 26, 527(1980)

6. K. V. Sarkanen, In Lignins, p. 150-155, K. V. Sarkanen and C. H. Ludwig (ed.), Wiley-Interscience, New-York, 1971

7. G. Meshitsuka and J. Nakano, J. Wood Chem. Technol., 5, 391(1985)

8. T. J. Eom, G. Meshitsuka and J. Nakano, Mokuzai Gakkaishi, 33,576(1987)

9. T. J. Eom, G. Meshitsuka, A. Ishizu and J. Nakano, Cellulose Chem. Technol., 22, 211(1988)

10. T. J. Eom, G. Meshitsuka and A. Ishizu, Mokuzai Gakkaishi, 35, 820(1989)

11. J. A. Hyatt, Holzforshung, 41, 363(1987)

12. Z. Z. Lee, G. Meshitsuka, N. S. Cho and J. Nakano, Mokuzai Gakkaishi, 27, 671(1981)

13. N. Fukagawa, G. Meshitsuka and A. Ishizu, J. Wood Chem. Technol., in print

14. A. Bax, R. H. Griffey and B. L. Hawkins, J. Magn. Reson., 55, 301(1983)

15. A. Bax and D. G. Davis, ibid., 65, 355(1985)

16. L. Lerner and A. Bax, ibid., 69, 375(1986) The BIRD sequence was omitted in our experiment.

17. G. Bodenhausen, H. Kogler and R. R. Ernst, ibid., 58, 370(1984)

18. E. Adler, S. Hernestam and I. Wallden, Acta Chem. Scand., 9, 319(1955)

19. C. H. Ludwig, B. J. Nist and J. L. McCarthy, J. Am. Chem. Soc., 86, 1186(1964)

20. C. H. Ludwig, B. J. Nist and J. L. MaCarthy, ibid., 86, 1196(1964)

21. D. A. I. Goring, In Liginis, p. 753-761, K. V. Sarkanen and C. H. Ludwig(ed.), Wiley-Interscience, New York, 1971

22. P. Whiting and D. A. I. Goring, Holzforshung, 36, 303(1982)

23. A. Sakakibara, In Rigunin No Kagaku, p.138-140, J. Nakano(ed.), Uni Public., Tokyo, 1990

24. Y. Matsumoto, K. Iiyama, A. Ishizu and J. Nakano, Mokuzai Gakkaishi, 28, 249(1982)