



NOTE

Characterization of Lignin Isolated from Iranian *Carpinus betulus* Wood

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Lignin isolated from *Carpinus betulus* by acidolytic dioxane method were characterized using alkaline nitrobenzene oxidation, elemental analysis, molecular weight analysis, GC-MS or gas chromatography-mass spectrometry, FT-IR and ¹H NMR spectroscopy. The results showed that M_w (weight average molecular weight) and M_n (number average molecular weight) of the lignin is 8377.3 g/mol and 5152.8 g/mol, respectively. The ratio of S unit to G unit is 3.88 and C₉ formula of *Carpinus betulus* lignin is C₉H^{al}_{2.296}H^{ph}_{1.751}O_{2.104}(OH)^{ph}_{0.477}(OH)^{al}_{1.409}(OCH₃)_{1.76}.

Key Words: Lignin, Iranian *Carpinus betulus*, Wood.

Lignin is the second most abundant aromatic (phenolic) polymer whose main function is to cement the cellulose fibers in plants. It is generally obtained from black liquor, a waste discharged from paper mills in large quantities and which can cause a major problem of disposal^{1,2}. On the other hand, as the production of lignin amounts to more than 50 million tons per year, there has been increasing interest in the development of economically valuable new applications and examples can be found on the web page of the International Lignin Institute. Currently, much of the lignin produced by the paper industry is consumed as a fuel. Lignin is a large, cross-linked macromolecule with molecular masses in excess of 10,000 u. It is relatively hydrophobic and aromatic in nature. Different types of lignin have been described depending on the means of isolation¹⁻³. There are three monolignol monomers, methoxylated to various degrees: *p*-coumaryl alcohol (P), coniferyl alcohol (G) and sinapyl alcohol (S).

Sample preparation: A 55 years old *Carpinus betulus* wood cut chipped and milled to 40 meshes and extracted with ethanol: toluene, ethanol and hot water solvent for preparation of extractive free wood powder. The extraction of dioxane lignin was performed with dioxane: HCl 2 mol/L (9:1) at reflux temperature for 3 h. After evaporation of the solvent, raw extracted lignin purified by Bjorkman method. Purified lignin acetylated and characterized with elemental analysis, molecular weight analysis, gas chromatography, FTIR and ¹H NMR spectroscopy.

Nitrobenzene oxidation: Alkaline nitrobenzene oxidation of *Carpinus betulus* was carried out according to Mun's modiWed method³. 1 g of a dried extractive free wood meal (40-60 mesh), 35 mL of 2 M NaOH and 2 mL of freshly distilled nitrobenzene are placed in a 50 mL stainless steel bomb. The bomb is sealed tightly with a screw cap fitted with a Teflon gasket and heated at 170 °C for 2.5 h in an electrically preheated. The bomb was shaken occasionally and after the heating period, the oxidation mixture was transferred to a liquid-liquid extractor and extracted continuously with chloroform for 4 h to remove nitrobenzene reduction products and excess nitrobenzene. The oxidation mixture was acidified to pH = 3-4 with conc. HCl and further extracted continuously with chloroform for 48 h. The solvent from the second chloroform solution was removed at 40 °C under reduced pressure to obtain the nitrobenzene oxidation mixture²⁻⁶.

Acetylation of lignin: 100 mg purified lignin was added in 2 mL of dried pyridine-acetic anhydride (1:1) for 72 h. The solution then added to a 10 fold volume of ice-cold water and acetylated sample was recovered as a precipitate and washed successively with water and dried.

Molecular weight: The weight average molecular weight (M_w) and number average (M_n) molecular weight *Carpinus betulus* acetylated lignin were determined. The samples were dissolved in tetrahydrofuran (THF) and 20 μL was injected to the column. The column was operated at 30 °C and eluted

with THF at a flow rate of 1 mL/min. The column was calibrated using polystyrene standards.

Infrared spectra were recorded by using a Shimadzu FTIR spectrometer model 8201 PC (El Mansouri & Salvadó). The dried samples were embedded in KBr pellets in the concentration of about 1 mg/100 mg KBr. The spectra were recorded in the transmittance mode in the range 4000-400 cm^{-1} .

The NMR spectra of 20 mg acetylated lignin solution in 0.5 mL CDCl_3 were. TMS Solvent was used as internal standard (7.25 ppm). The C, H and N analysis of purified lignin samples were carried out using C, H, N analyzer and O was determined by difference. The methoxyl content in lignin samples was determined in accordance of JIS P8013 1972 standard.

Molecular weight: The weight average (M_w) and number average (M_n) molecular weight and polydispersity of *Carpinus betulus* lignin is 8377.3 g/mol and 5152.8 g/mol respectively (Fig. 1).

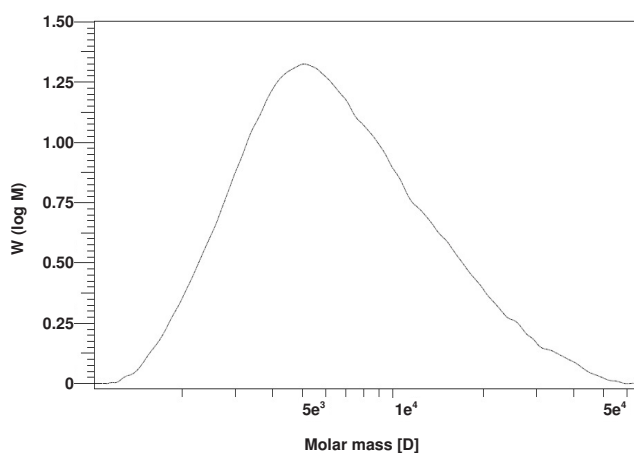


Fig.1. GPC chromatogram of *Carpinus betulus*

GC-MS analysis: GC analysis was conducted using an Agilent gas chromatograph equipped with capillary column (30m \times 0.5mm), column temperature was programmed to increase from 50 to 260 $^{\circ}\text{C}$ at the rate of 6 $^{\circ}\text{C}/\text{min}$. Column flow rate was 10 mL/min and split ratio was 10. Fig. 2. shows the GC chromatogram of *Carpinus betulus* lignin.

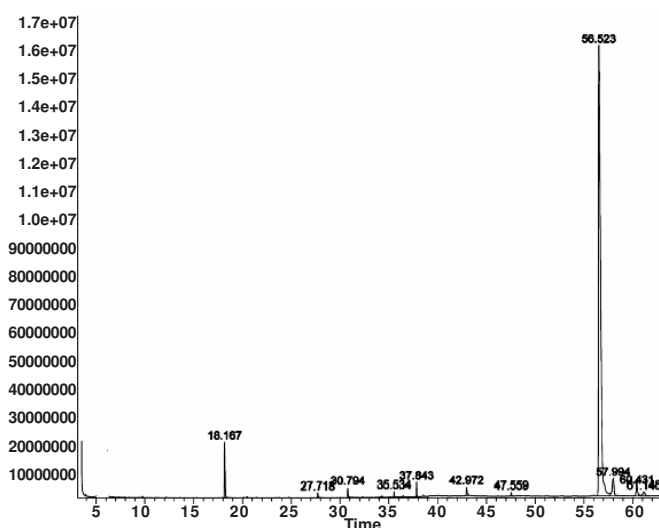


Fig. 2. GC-MS Chromatogram of *Carpinus betulus*

^1H NMR spectroscopy: Table-1 listed the position of signal assigned and their proton area calculated from the integration of ^1H -NMR spectra⁴. The area ratio of free aliphatic and phenolic hydroxyl groups were determined from the corresponding acetate signals. The ratio of $(\text{OH})_{\text{al}} / (\text{OH})_{\text{ph}}$ is 2.95. The lignin showed two peaks in the aromatic proton region, which corresponded to guaiacyl units (δ 6.9) and syringyl units (δ 6.6). Lignin showed stronger peak in syringyl units region (δ 6.6) than guaiacyl units region (δ 6.9) and the ratio is 3.88⁵.

TABLE-1
 ^1H NMR DATA OF THE ACETYLATED *Carpinus betulus* LIGNIN

Region δ (ppm)	Attribution	Area (arbitrary units)	% area / total area
> 8	Other	0.223	0.6
6.95	Aromatic region in G.	1.603	4.29
6.63	Aromatic region in S.	2.544	6.8
5.75-6.25	benzylic region	1	2.67
3.95-5.20	Aliphatic region	4.57	12.22
3.55-3.95	Methoxyl	12.81	26.34
2.20-2.50	Aromatic acetoxy region	3.47	10.8
1.60-2.20	Aliphatic acetoxy region	10.25	31.96
<1.60	Nonoxygenated aliphatic region	0.912	2.44

FTIR spectroscopy: FTIR spectra were recorded and the assignment made by some studies³⁻⁶. The band at 1600 cm^{-1} was assigned to the aromatic skeletal vibrations, 1507 cm^{-1} assigned to the aromatic skeletal vibrations coupled with C-H in plane deformations, 1460 cm^{-1} assigned to C-H deformations asymmetric in methyl, methylene and methoxyl group.

Elemental analysis and C_9 formula: The average C_9 formula was calculated from the elemental analysis and methoxyl content⁶. The result of elemental analysis for *Carpinus betulus* purified dioxin lignin was 5.9 % H, 55 % C, 38.1 % O. The number of methoxyl groups per C_9 unit was 1.66. The C_9 formula for *Carpinus betulus* wood was: $\text{C}_9\text{H}^{\text{al}}_{2.296}\text{H}^{\text{ph}}_{1.751}\text{O}_{2.104}(\text{OH})^{\text{ph}}_{0.477}(\text{OH})^{\text{al}}_{1.409}(\text{OCH}_3)_{1.76}$

Conclusion

It was concluded that dioxane lignin extracted from *Carpinus betulus* was syringyl-guaiacyl type. The methoxyl content of this lignin was similar to hardwood lignin. The weight average molecular weight (M_w) was comparable to temperate hardwood lignin.

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