

# Comparison Of Milled Wood Lignin & Organosolve Lignin From Pine

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## SUMMARY

Method of preparation of milled wood lignin (MWL) and organosolve lignin such as ethanol and dioxane lignin from pine is detailed. A comparison has been made of these lignin preparations. The elementary analysis, functional group analyses, ultra-violet and infrared spectra and molecular weight of lignins prepared were recorded. Quantitative estimation of nitrobenzene oxidation products has been done by gas chromatographic technique. From all such analyses  $C_9$  formulae and the number of phenylpropane unit in a lignin molecule have been calculated. It was concluded that milled wood lignin is one in the purest form. Organosolve lignins get relatively degraded during their isolation. This difference is because of the acid used as an catalyst. The  $C_9$  formulae of the lignin preparations are:  $C_9H_{8.89}O_{2.01}(OCH_3)_{0.99}(OH)_{1.13}(\text{Phenolic-OH})_{0.18}$  for MWL;  $C_9H_{8.86}O_{2.05}(OCH_3)_{0.97}(\text{Aliphatic-OH})_{0.60}(\text{Phenolic-OH})_{0.28}$  for ethanol lignin and  $C_9H_{8.89}O_{1.95}(OCH_3)_{0.97}(\text{Aliphatic OH})_{0.82}(\text{Phenolic OH})_{0.26}$  for dioxane lignin.

## INTRODUCTION

Most of the work reported in literature on chemistry of lignin has been done on spruce occurring in Europe and North America. In India spruce is scarce, as it occurs in inaccessible regions. Chir (*Pinus roxburghii*) is an Indian conifer which is readily available and is an important raw material for pulp and paper industry. It is found in the slopes and foot-hills of Himalays from about 450-2,300 metres and is distributed in Jammu & Kashmir, Punjab, Himachal Pradesh and Uttar Pradesh. The total area under pine forest in the country is estimated to be about 8,90,000 hectare.

For understanding the mechanism of pulping it is essential to isolate lignins from the wood in its proform and ascribe structure to this lignin.

Bjorkman<sup>1</sup> opened up the possibility to isolate lignin by purely physical method. By fine

milling of wood in vibratory ball mill in presence of toluene as a non-swelling solvent followed by extraction with dioxane, he was able to isolate about 50% of lignin of the wood. Bland and Menshun<sup>2</sup> found that larger yield of lignin is obtained by mild alkali-pretreatment of wood dust before milling. Brownell<sup>3</sup> has shown that the main effect of the alkali pretreatment was to facilitate grinding by embrittlement of the wood rather than by bond cleavage.

Organosolve lignin is obtained by extracting plant material with organic solvent like alcohols, dioxane, acetone, ethylene, glycol, phenol etc. Brauns<sup>4</sup> used pure ethanol and dioxane while Holmberg and Runius<sup>5</sup> used ethanol in the presence of acid as an catalyst and Pepper and Siddiquellah<sup>6</sup> used acidified dioxane and he also tried to select the conditions that avoided excessive condensation.

## EXPERIMENTAL

**PREPARATION OF EXTRACTIVE FREE WOOD MEAL :** Pine logs from Demonstration Area of Forest Research Institute, Dehra Dun were chipped and disintegrated to produce 40-60

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mesh dust, followed by extraction with ethanol-benzene ( $1:2 \frac{V}{V}$ ) and finally washed with hot water.

#### PREPARATION OF MILLED WOOD

**LIGNIN:** Alkali-pretreatment of wood was done by stirring extractive free wood dust in 0.1N sodium hydroxide (15 ml/gm of wood dust) for 20 hrs. at about 20°C, followed by washing with distilled water till free of alkali. This dry dust was milled in a rotatory ball mill (60 r.p.m.) and stirred with 80% aqueous acetone. The acetone extract concentrated under reduced pressure until brown flakes appear. The aqueous solution was made alkaline by 0.1N sodium hydroxide solution and precipitated lignin by adding 6 M hydrochloric acid to pH 4. Lignin was washed with water until chloride ion free and finally freeze dried.

#### PREPARATION OF ORGANOSOLVE LIGNIN

**LIGNIN:** In organosolve lignin both ethanol and dioxane lignins were prepared. For ethanol lignin, acidified hydrogen chloride gas to give the required concentration of 0.2N and for dioxane, lignin dioxane and 0.2N hydrochloric acid (9:1) solvent was used. The extractive free dust was refluxed with respective solvent at 85°C for two hours in an inert atmosphere of nitrogen. The solvent was concentrated under vacuo and poured into vigorously stirred cold distilled water. Finally, purified by dissolving it in dioxane and precipitated into ether.

#### QUANTITATIVE ANALYSIS OF LIGNIN

Klason lignin or acid-insoluble lignin and soluble lignin were estimated by Tappi method T-222-Os-74 and useful method UM-250 respectively.

**ELEMENTAL ANALYSIS:** Percentage of carbon, hydrogen were determined by combustion method and the balance was oxygen.

#### QUANTITATIVE ANALYSES OF FUNCTIONAL GROUP

The analysis of total hydroxyl and phenolic hydroxyl groups were carried out by following the method of Whistler and Jeans<sup>7</sup> and Delta-Epsilon method of Goldschmid<sup>8</sup> respectively. Methoxyl group was analysed by Tappi method T-209-Su-72. Carbonyl group was determined by hydroxyl amine hydrochloride of Gierer and Soderberg<sup>9</sup>.

**MEASUREMENT OF ULTRAVIOLET AND INFRARED SPECTRUM:** Ultra-violet spectrum was measured by dissolving 20 mg lignin in 25 ml ethanol using Perkin-Elmer Model 402 UV and VIS Spectrophotometer. IR spectra was determined

by KBr disc method using Perkin-Elmer Spectrophotometer Model 735.

**DETERMINATION OF MOLECULAR WEIGHT:** Molecular weight was determined by the method of Marton and Marton<sup>10</sup> and Brady<sup>11</sup> using vapour pressure Osmometer (302 B).

**NITROBENZENE OXIDATION:** Oxidative degradation products were isolated using nitrobenzene oxidation of Stone and Blundell<sup>12</sup> with few modifications. 50 mg of lignin, 0.3 ml nitrobenzene and 4 ml of 2N sodium hydroxide were heated in a bomb at 170°C for 3 hours and extracting the oxidation products with dichloromethane.

**QUANTITATIVE ANALYSIS OF NITROBENZENE DEGRADATION PRODUCT BY GAS-CHROMATOGRAPHY:** Oxidation products were silylated using hexa-methyl disilane and trimethyl chlorosilazane and trimethyl acetamide in pyridine. The oxidation products were silylated and identified using Perkin-Elmer Model 3920 B Chromatograph equipped with differential flame ionization detector under the following conditions:

Column—Silicone SE 30, 5% on chromatoporb W.

DMCS (80–100 mesh)

Carrier gas	— Nitrogen
Gas flow rate	— 50 ml/min.
Column oven temperature	— 160°C (Isothermal)
Injection	— 0.5 $\mu$ l sample solution

**Calibration:** A reference solution containing p-hydroxy benzaldehyde, vanillin and syringaldehyde was prepared and the relative time of these authentic compounds were noted, which were 4.0, 6.7 and 10.7 min. respectively. Identification of peaks of nitrobenzene oxidation products were done by comparing their relative time. The peak area was measured using triangulation method.

#### RESULTS AND DISCUSSION

Table I shows the yield and lignin content of the lignins. A study of Table I reveals that yield of milled wood lignin and dioxane lignin is the same, but more than ethanol lignin. This suggests that dioxane is a better solvent for lignin than ethanol as the yield is higher than ethanol under the same condition of time, temperature and acid concentration. From the same table total lignin content shows that millwood lignin is purest and is less associated with non-lignin substances. Then comes ethanol finally dioxane lignin.

The acid-soluble lignin in all the preparation is very little, as is true for soft woods.

Elemental analysis of lignin is given in Table II, which indicates that lignin consists of carbon, hydrogen and oxygen. The high amount of carbon suggests its aromatic nature. Its aromatic nature is further supported by its ultraviolet spectra (Fig. 1), as discussed later.

The functional group of lignins are presented in Table III.

The composition of lignin recovered have been calculated on the average atomic composition of  $C_9$  unit, since the phenylpropane unit is the lignin basic unit. The  $C_9$  formulae of lignin are also presented in Table III.

Methoxyl content is characteristic of lignin and is a criteria of purity. The highest methoxyl content shows milled wood lignin to be purest. Milled wood lignin contains 0.99 methoxyl per  $C_9$  and is equal to a theoretical propyl guaiacyl polymer. In organosolve lignins both have the similar methoxyl content, showing similarity in their purity.

Another important functional group is hydroxyl (both phenolic and aliphatic) has been found to be 9.68 in milled wood lignin is similar to spruce milled wood lignin (10%). This value corresponds to 1.13 OH/ $C_9$  out of which 0.95/ $C_9$  is aliphatic and 0.18/ $C_9$  is phenolic in nature. Ethanol and dioxane contains respectively 0.23 and 0.26 phenolic hydroxyl/ $C_9$  which is in agreement with Arseneau<sup>13</sup> result (0.25 Ph OH/ $C_9$  for spruce dioxane lignin). But the hydroxyl content of milled wood lignin and dioxane lignin is the same, thus creating a decrease in aliphatic hydroxyls of ethanol lignin. The low value of aliphatic hydroxyl are consistent with alkylation of benzylic hydroxyl groups or hydroxyl groups alpha to an alpha carbonyl group. These findings are also supported by Farmer<sup>14</sup> from spectral studies.

The data recorded in Table III indicates the carbonyl groups per  $C_9$  unit to be higher in case of ethanol lignin than milled wood lignin. It would seem that during isolating ethanol lignin some carbonyl containing products are produced. These observations are in agreement with Hibbert's observations<sup>15</sup>. The highest carbonyl content of dioxane lignin is due to acid-catalysed solvolysis reaction which liberate carbonyl groups.

TABLE—I YIELD AND LIGNIN CONTENT OF ISOLATED LIGNINS

Sl. No.	Characteristic of lignin	Milled wood lignin %	Ethanol lignin %	Dioxane lignin %
1.	Yield (on basis of p.d. dust	3.5	2.3	3.5
2.	Acid insoluble lignin	91.59	91.02	87.47
3.	Acid soluble lignin	0.21	0.41	0.55
4.	Total lignin content	91.80	91.43	88.02

TABLE—II ANALYSES OF ISOLATED LIGNINS

Sl. No.	Characteristic of lignin	Milled wood lignin	Ethanol lignin	Dioxane lignin
1.	Carbon %	60.30	61.56	60.77
2.	Hydrogen %	6.30	6.50	6.32
3.	Oxygen %	33.40	31.94	32.91
4.	Methoxyl %	15.60	15.45	15.54
5.	Total hydroxyl %	9.68	7.38	9.48
	i) Phenolic hydroxyl %	1.55	2.07	2.25
	ii) Aliphatic hydroxyl %	8.13	5.31	7.23
6.	Carbonyl	0.17	0.20	0.21

TABLE—III COMPOSITION OF PINE LIGNIN

Sl. No.	Lignin preparations	C	H	O	OMe	Aliphatic-OH	Phenolic-OH
1.	Milled wood lignin	9.0	8.39	2.01	0.99	0.95	0.18
2.	Ethanol lignin	9.0	8.86	2.05	0.97	0.60	0.23
3.	Dioxane lignin	9.0	8.39	1.95	0.97	0.82	0.26

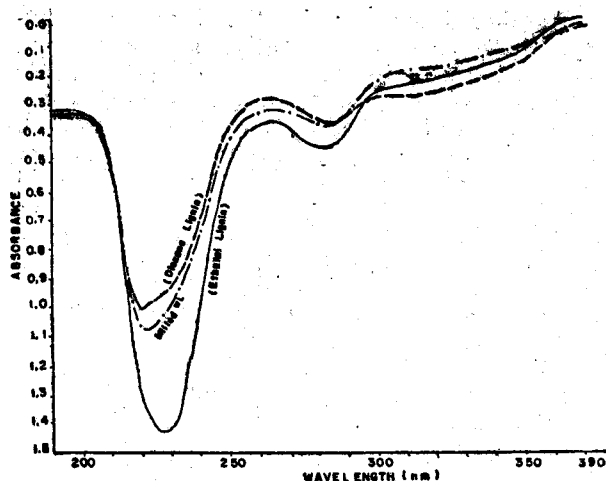


Fig. 1- ULTRAVIOLET SPECTRUM OF ISOLATED LIGNINS

The molecular weights of pine lignin are in the range of other lignin preparation as reported in literature. A perusal of Table IV shows that the molecular weight of milled wood and ethanol is nearly the same, but that of dioxane lignin is lower. Similarly the number of phenylpropane unit composing the molecule is also low. This decrease can be explained as acidolysis of lignin by refluxing in dioxane and dilute hydrochloric acid, resulting in cleavage of some phenol ether bond as well as certain minor intermanomer linkages.

A perusal of Table IV also reveals that 17 phenylpropane units in case of ethanol and 9 in case of dioxane lignin are present. The building units of organosolve lignin is less, than that of corresponding milled wood lignin (19 units). The difference may be due to hydrolysis and degradation of organosolve lignin due to the acidic condition used for the isolation of organosolve lignin.

The ultraviolet spectra of all the lignins are shown in Fig. 1. It shows a very intense peak below 230 nm and a second peak at 280-282 nm. The intensity of the lower wave length band is about 5-6 times more than that at 282 nm. These

are attributed due to oxy-substituted benzene molecule. The shoulder after 280 nm is much pronounced in organosolve lignin than in milled wood lignin which is due to more conjugated chromophores.

Identification of characteristic absorption bands caused by different functional groups is a sound basis for meaningful interpretation of IR spectra. IR spectrum is shown in Fig. 2, indicating the presence of the groups listed in front of each band<sup>16</sup>.

Position of band cm <sup>-1</sup>	Assigned group
3700-3050	OH group, hydrogen bonded
2950-2800	OH group stretching in methyl & methylene groups
1725-1710	Unconjugated carbonyl and carboxyl groups
1655-1630	Conjugated carbonyls
1595 & 1500	Aromatic skeletal vibrations
1270	Alkyl-aryl ether linkage
1220	Phenolic groups
1140-1080	dialkyl ether linkage
860 & 820	1, 2, 4 trisubstituted benzene nucleus.

The IR Spectrum of all the lignin are similar to each other. In case of IR Spectra of dioxane lignin, the intensity of the band at 1700 cm<sup>-1</sup> is higher and 1600 cm<sup>-1</sup> band intensity is lower than milled wood lignin. These changes suggest that cleavage and subsequent rearrangement of guaiacyl-glycerol side-chain. A characteristic of pine is a peak at 1725 cm<sup>-1</sup> absent in spruce, assigned to vanillate and acetate groups.

TABLE-IV MOLECULAR AND FORMULAE WEIGHT OF LIGNINS

Sl. No.	Analysis of lignin	Milled wood lignin	Ethanol lignin	Dioxane lignin
1.	Molecular weight	3260±326	3106±310	1582±158
2.	Formulae weight	169.3	176	177.6
3.	Number of phenyl propane unit per molecule	19	17	9

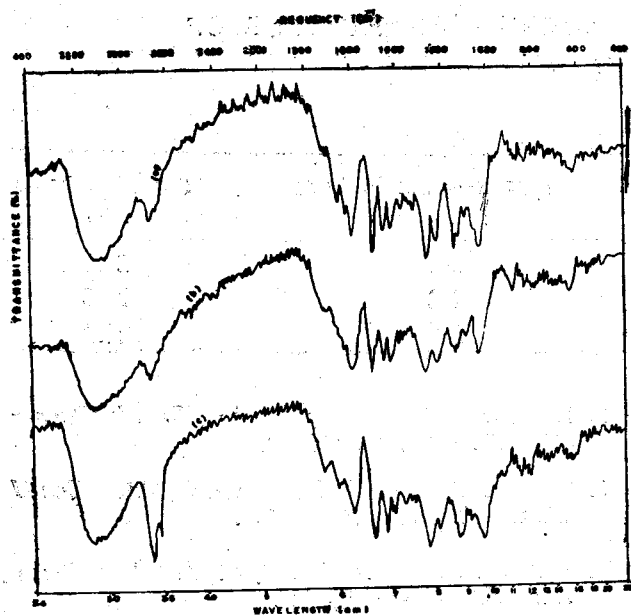


Fig. 2- INFRARED SPECTRA OF ISOLATED LIGNINS

- (a) MILLED WOOD LIGNIN
- (b) ETHANOL LIGNIN
- (c) BROMINE LIGNIN

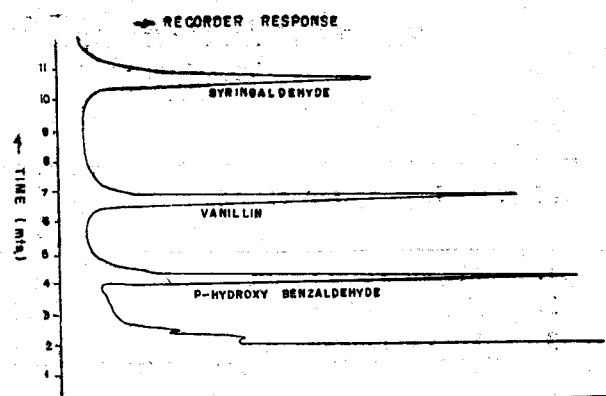


Fig. 3 GAS CHROMATOGRAM OF REFERENCE COMPOUNDS

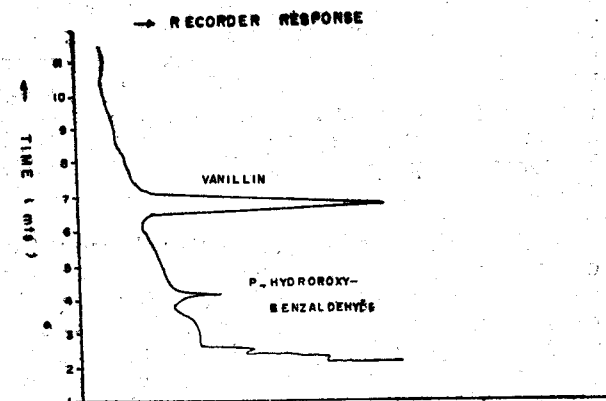


Fig. 4 GAS-CHROMATOGRAM OF MWL OXIDATION PRODUCTS

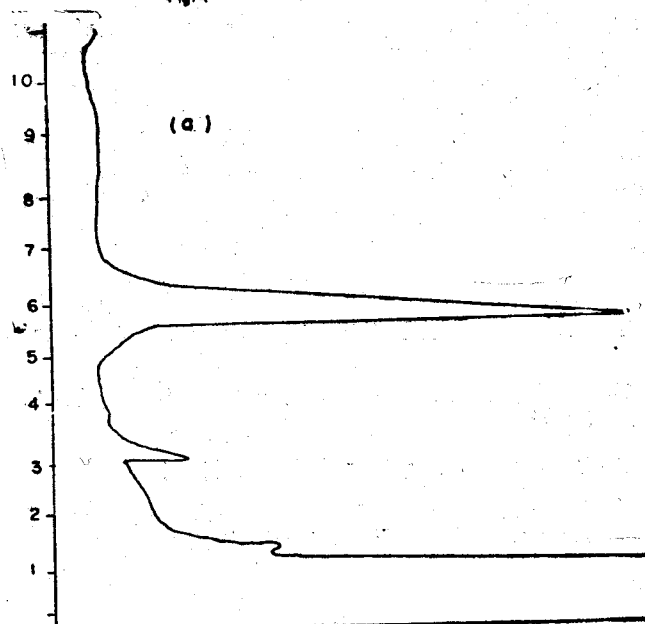
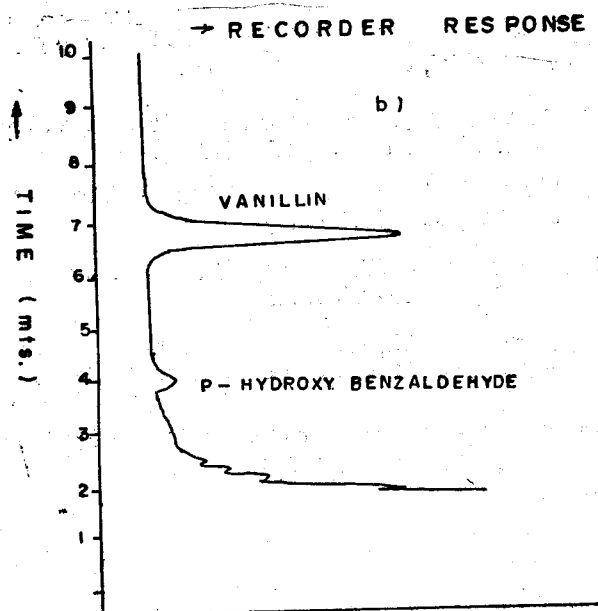


Fig. 5- GAS CHROMATOGRAM OF ORGANOSOLV LIGNIN OXIDATION PRODUCTS  
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TABLE-V ALKALINE NITROBENZENE OXIDATION PRODUCTS OF THE LIGNINS (% of lignin)

Sl. No.	Lignin preparation	Vanillin %	p-hydroxy-benzaldehyde %	Total aldehyde %
1.	Milled wood lignin	23.8	2.2	26.0
2.	Ethanol lignin	19.4	1.2	20.6
3.	Dioxane lignin	20.6	1.6	22.2

The oxidation gave an idea about the number of uncondensed unit present in lignin molecule. The peaks are given in Fig. 3. It is seen from Table V that vanillin as the main oxidation product with small quantity of p-hydroxybenzaldehyde. Whereas Pepper<sup>17</sup> and Creighton<sup>18</sup> obtained vanillin from spruce. This confirms that in contrast to the spruce, pipe lignin is dehydrogenative polymer of coniferyl and p-coumaryl alcohol.

Since the number of methoxyl per C<sub>9</sub> is constant with the value required by theoretical guaiacyl propane monomers. The inefficient conversion of guaiacyl propane units to vanilline is due to the availability of 5-position in phenylpropane unit for condensation. A comparatively lower yield of aldehydes in case of organosolve lignin than milled may be assigned to the belief that the lignin formed at the moment of liberation itself undergoes side chains rearrangement or intra-molecular condensation and such linkages in lignin failed to give aldehydes on oxidation.

The lowest yield of aldehyde obtained in case of ethanol as compared to other two (Table V) may be explained as the relatively condensed nature of ethanol lignin, since the condensed units do not give monomeric aldehydes.

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