

Studies on Isolation and Nitration of Isolated Lignins from *Dendrocalamus Strictus*

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Introduction

A survey of literatures reveals that very little work has been done on bamboo lignins.^{1,2} For further understanding of the chemistry of bamboo lignin: Dioxane lignin, soda lignin and Thio lignin were isolated from bamboo (*Dendrocalamus strictus*) and characterized. These lignins were nitrated under various conditions of nitrating agents, temperature and time. The nitrolignins thus produced in aqueous as well as non-aqueous media were characterized.

Experimental

(1) Preparation of extractive-free dust

Dendrocalamus strictus culms were chipped on a chipper. The chips were converted into dust (63-80 mesh). The dust was exhaustively extracted with a mixture of 95 percent ethyl alcohol and benzene (1 : 2). The extracted dust was washed with hot water and dried.

(ii) Isolation of Lignins

Dioxane, soda and thio lignins were isolated from bamboo. The

Dioxane, thio and soda lignins from Dendrocalamus strictus were nitrated in aqueous as well as in non-aqueous media. The isolated lignins and nitro-lignins were evaluated for their elements, functional groups and infra-red spectra. The yield of nitrolignins was higher in non-aqueous medium and increased with increase in time, temperature and amount of addition of sodium nitrite. The nitration was accompanied by a decrease in methoxyl content of nitrolignins. The higher yield of nitrolignins, the higher nitrogen substitution and the lesser methoxyl loss of nitrolignins in non-aqueous than in aqueous medium indicates the lesser drastic action of nitric acid in non-aqueous medium.

yield of isolated lignins are recorded in Table 1.

(a) Isolation of dioxane lignin

Dioxane lignin was isolated by the method of Pepper and co-workers³. Pre-extracted dust (200g.) was extracted with dioxane water (9 : 1, 1600 ml.) mixture containing 0.2N hydrochloric acid at 85°C for 2 hours in an atmosphere of nitrogen and the resulting extract was poured in cold water to precipitate dioxane lignin. The precipitate was filtered, washed and vacuum dried.

(b) Isolation of soda lignin

Dendrocalamus strictus chips were digested by soda process in an autoclave using 18 percent chemicals as Na₂O, keeping the material to liquor ratio 1 : 4 at 162°C for 4½ hours. The black liquor thus obtained was acidified with hydrochloric acid to precipitate soda lignin. The precipitate was washed free of acid and purified by Ahlm's method⁴. In this the method crude lignin was dissolved in anhydrous dioxane and concentrated under reduced pressure.

Table-I
Yield of Isolated Lignins

Lignin preparation	Yield (crude) %	Yield (Pure)* %	Yield (Pure)** %
Thio	18.8	15.5	62.6
Dioxane	11.5	9.8	39.6
Soda	16.0	13.4	54.1

* expressed on the basis of bamboo

** expressed on the basis of Klason lignin.

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The dioxane solution was centrifuged, and the clear solution was diluted with dioxane to give a 10% solution. Soda lignin was isolated by adding the dioxane solution dropwise to vigorously stirred anhydrous ether. The precipitate was washed with ether and vacuum dried.

(c) Isolation of Thiolignin

Thiolignin was isolated under similar conditions as used for the isolation of soda lignin, keeping the sulphidity as 25%.

(iii) (a) Analysis of Isolated lignings

The isolated lignings were analysed for their elements⁵, Klason lignin⁶ and methoxyl groups⁷ by standard methods of organic chemistry. Total hydroxyl and phenolic hydroxyl groups of dioxane lignins were also determined. The results are recorded in Table II.

(b) Determination of Infra-red (I.R.) Spectra

Infra-red Spectra of isolated lignins were determined by using perkin Elmer Infra-red spectrophotometer. Lignin samples were examined in clear discs containing 1.8 mg. of the sample in 100 mg. of potassium bromide. The I.R. Spectra are shown in Fig. I, II, III, and IV.

(iv) Nitration of Isolated Lignins in Aqueous Medium

Nitration of isolated lignings (dioxane, soda and thio) in aqueous medium was carried out by treating 2 g. (o.d. basis) of each lignin with nitric acid (sp. gr. 1.4) and sodium nitrite (2% on the

weight of material), keeping the material to liquor ratio as 1:2; at 65°C for 2 hours. The solution was filtered and from the filtrate, acid-soluble nitrolignin (A) was precipitated on cooling. The precipitate was filtered, washed and vacuum dried. The residue was extracted with 10% sodium hydroxide at 85°C for 2 hours keeping the material to liquor ratio as 1:20. The alkaline filtrate was acidified with hydro chloric acid to precipitate nitro lignin (B). The precipitate was filtered, washed and vacuum dried. The yields are recorded in Table III.

(v) Nitration of Isolated Lignins in Non-Aqueous Media

(a) In Carbon-tetra Chloride Medium

Nitration of isolated lignins in carbon-tetra-chloride medium was carried out by the method of Shorygina and Co-Workers⁸. 2 g. (o.d. basis) of the lignin was taken in a three necked flask fitted with a stirrer and a dropping funnel. A solution of nitric acid (sp. gr. 1.40) in carbon-tetra. chloride (15 ml. HNO₃ in 35 ml. CCl₄) was added dropwise with constant shaking through the funnel. The nitration was carried out at room temperature (20°C) for 2 hours. The nitrolignin was filtered off and transferred into ice-water. The precipitate was again filtered off, washed free of acid and vacuum dried. The yields are recorded in Table IV.

Table-II
Analysis of Isolated Lignins

Contents	Dioxane lignin	Thio lignin	Soda lignin
Carbon	58.8	53.6	55.8
Hydrogen	6.3	5.6	6.4
Oxygen	34.9	37.0	37.8
Methoxyl	17.3	14.8	15.5
Total hydroxyl	10.9	9.8	14.1
Phenolic hydroxyl	2.3	—	—
Ash	0.59	0.36	2.26
Klason Lignin	84.3	78.6	81.7

Table-III
Expirical Formulae of Isolated Lignins

Preparation	Expirical Formulae
Dioxane lignin	$C_9H_{9.4}O_{3.3} (OCH_3)_{1.1} (OH)_{1.03} \text{ (aliphatic)} (OH)_{0.27} \text{ (Phenolic)}$
Thio lignin	$C_9H_{9.6}O_{4.2} (OCH_3)_{0.98}$
Soda lignin	$C_9H_{11.0}O_{4.2} (OCH_3)_{0.86}$

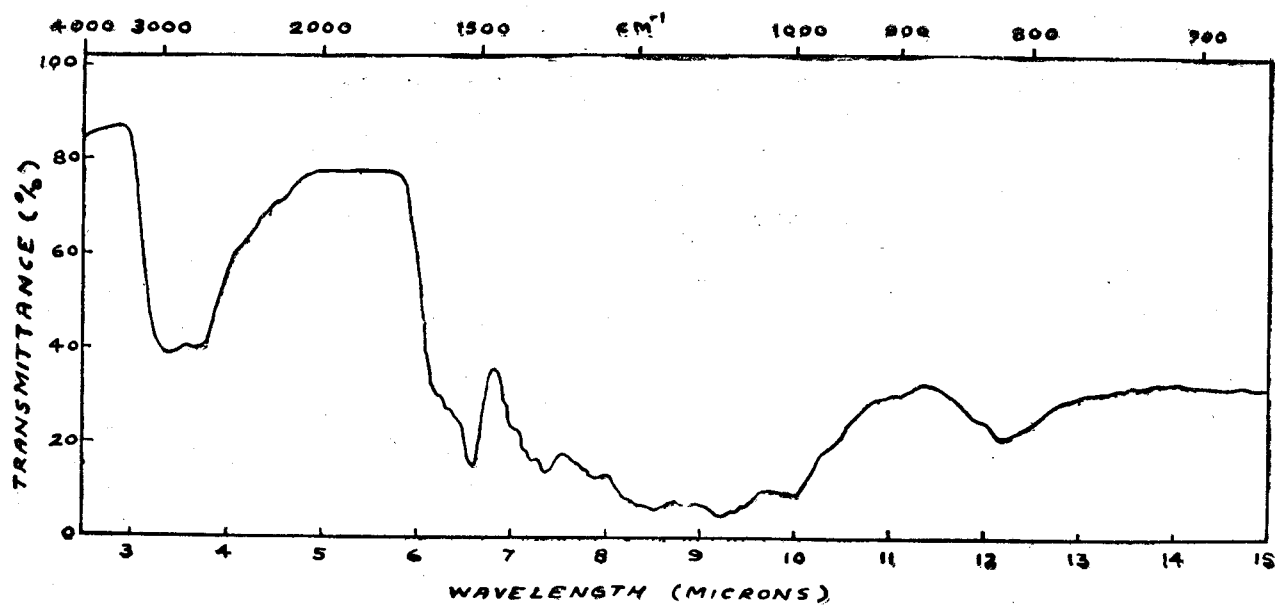


Fig. I-Dioxane Lignin

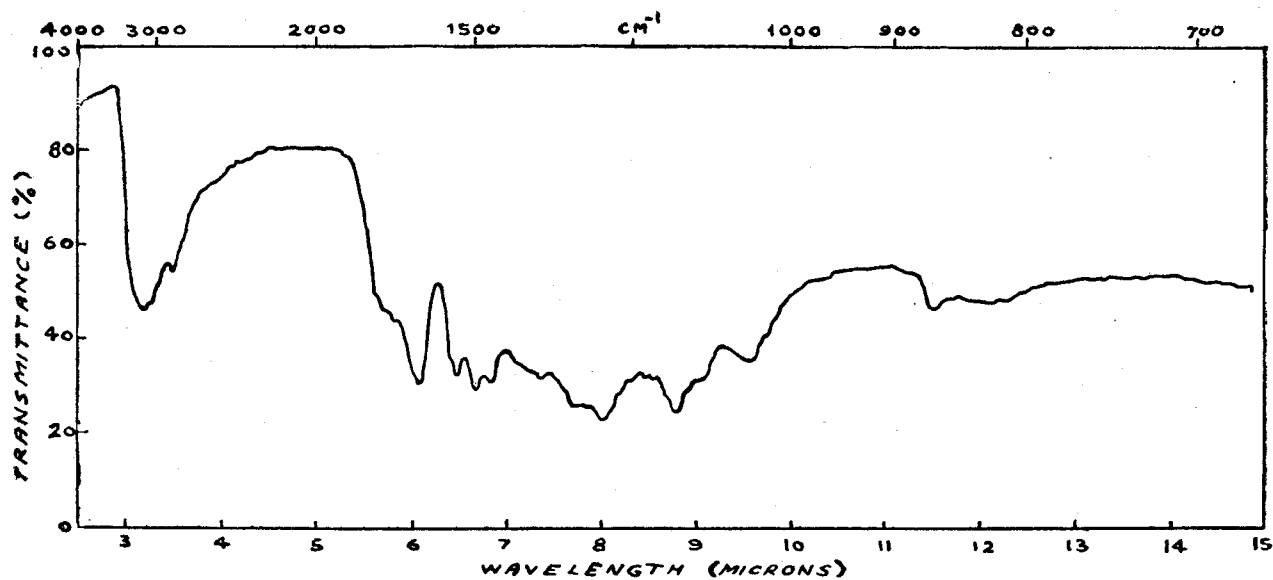


Fig. II-Soda Lignin

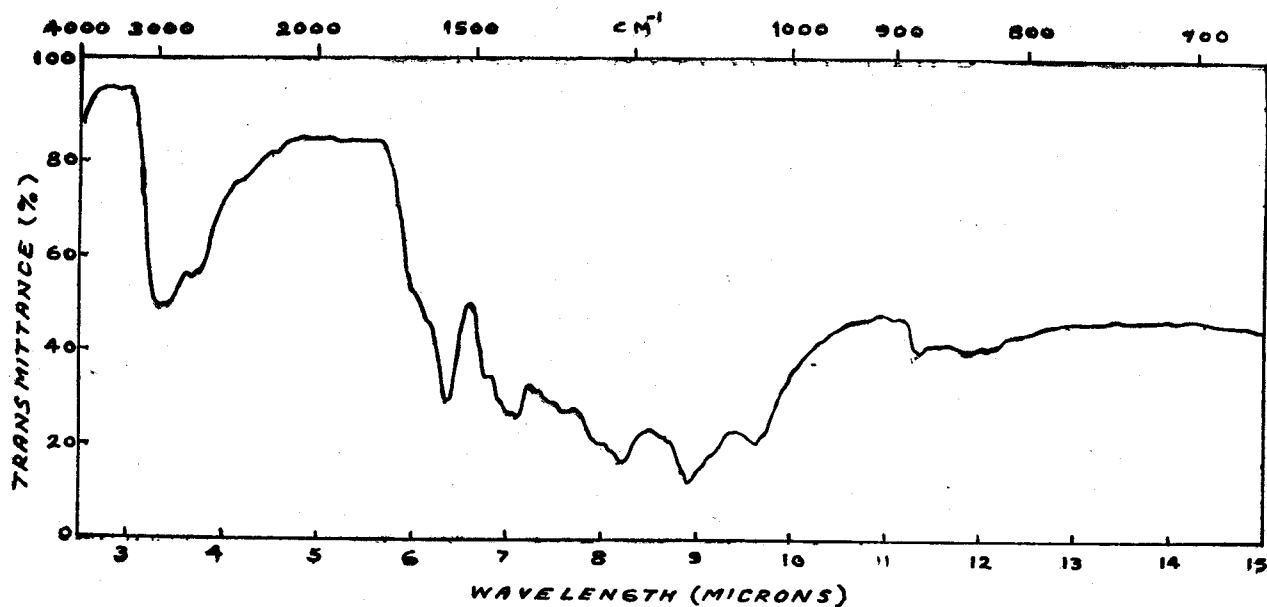


Fig. III-Thiolignin

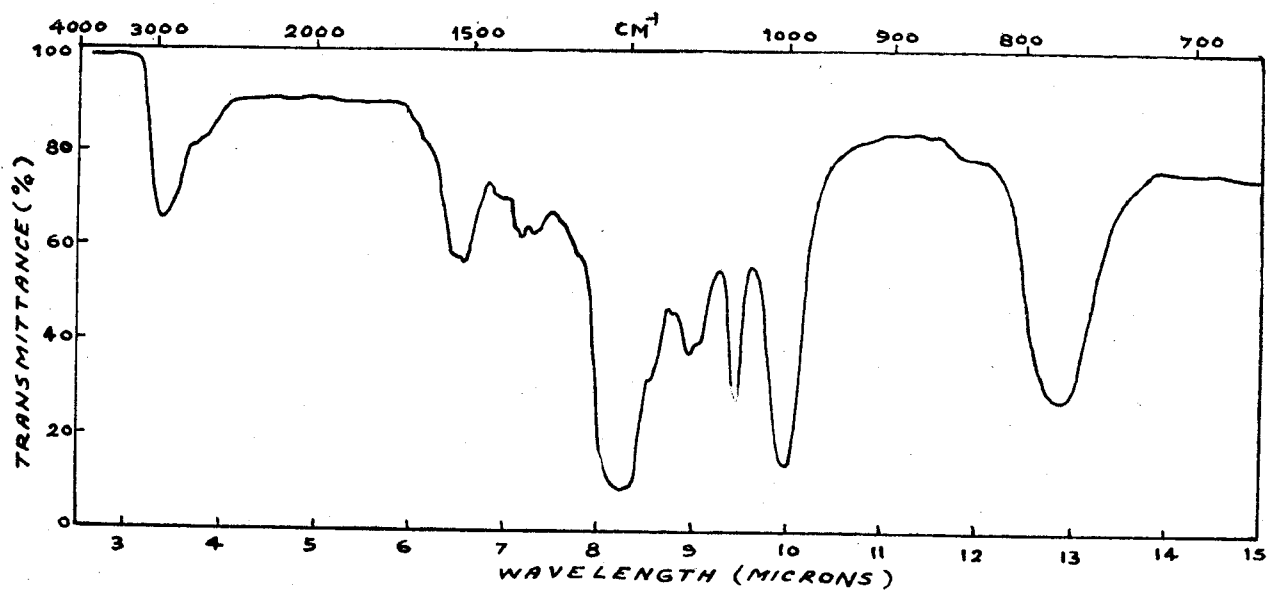


Fig. IV-Methylated Thiolignin (25% Sulphidity)

Table—IV
Yield & Analysis of Isolated Nitrolignins (Aqueous medium)

Type of Lignin	Treat-ment	Yield of* Nitrolignin %	Carbon %	Hydrogen %	Nitrogen %	Nitro %	Methoxyl %
Dioxane	A	8.0	—	—	2.3	—	5.2
	B	33.8	51.5	6.4	3.35	9.4	9.3
Thio	A	2.1	—	—	1.85	—	5.8
	B	26.2	47.8	5.4	2.70	8.2	8.6
Soda	A	5.6	—	—	1.92	—	6.5
	B	39.5	49.3	5.2	2.90	8.6	7.2

*expressed on the weight of lignin taken.

(b) In Ether Medium

In ether medium nitration was conducted by the method of Shorygina and co-workers². The procedure is similar to the nitration in carbon-tetra chloride medium. The yields are recorded in Table V.

(c) In Methanol Medium

Nitration in methanol medium was carried out by the method followed by Kurschner and Co-workers⁹. 2 g (o.d. basis) of each isolated lignin was treated with a methanol solution of nitric acid (sp. gr. 1.40) (5 ml. HNO₃ in 20 ml. methanol) at 45°C for 2 hours. The red-yellow solution was filtered and from the filtrate nitrolignin was precipitated by the addition of water. The precipitate was filtered, washed and vacuum dried. The yields are recorded in Table VI.

(vi) (a) Analysis of Nitrolignins

Isolated nitrolignins were analysed for Carbon, Hydrogen⁵, nitrogen¹⁰, nitro¹¹ and methoxyl¹⁷ contents by standard methods of organic chemistry. Results of chemical composition of nitrolignins are recorded in Table III to VI.

(b) Determination of Infrared Spectra

Infrared spectra of nitrolignins were taken by the method as described earlier and are shown in Figs. V and VI.

(vii) Methylation of Thioliignin Nitration of Methylated Thioliignin

Thioliignin was methylated by the method of Phillips and Goss¹². In this method, the lignin sample was methylated with dimethyl sulphate and sodium hydroxide solution at 60°C with continuous stirring for one and a half hour. The reaction product was filtered, washed with distilled water and vacuum dried. Methylated thio-lignin was nitrated in carbon-tetra-chloride, ether and methanol media by the methods already described. Results are recorded in Tables, IV, V & VI.

(viii) Isolation of 4,6-Dinitroguaiacol (DNG)

4,6-Dinitroguaiacol (DNG) was isolated from the used reaction media i.e. acidic carbon-tetra-chloride and ether solutions left after isolating nitrolignins⁸. The

used reaction medium was extracted with ether several times and the ethereal extract was further extracted with NaoH. The NaoH extract was acidified and again extracted with ether. From the ethereal extract, ether was evaporated and to the dark brown-Syrupy mass so obtained, ethanol was added. Thin-layer chromatography of ethanol extract was carried out¹³. The dark yellow residue of DNG at R_f 0.47 was obtained. The yields are reported in Tables V and VI.

Discussion

(i) Yield of the Lignins

The yields of isolated lignins recorded in Table I indicate that the yield of dioxane lignin (9.8%) is less than thio and soda lignin (15.5% and 13.4% respectively). This is due to the higher purity of dioxane lignin which is less associated with carbohydrates. The higher yield of thioliignin than soda lignin under identical conditions of isolation is in agreement with the finding¹⁴ that the rate of delignification is higher in the kraft process than in the soda process.

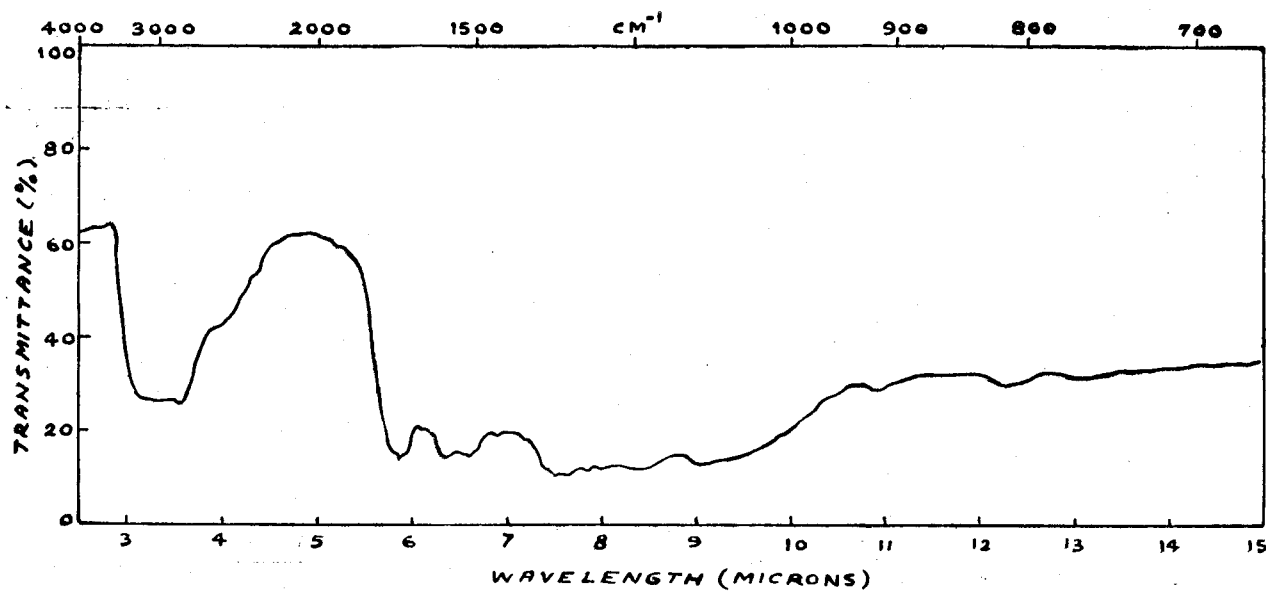


Fig. V-Dioxane Nitrolignin (Carbon-Tetra Chloride Medium)

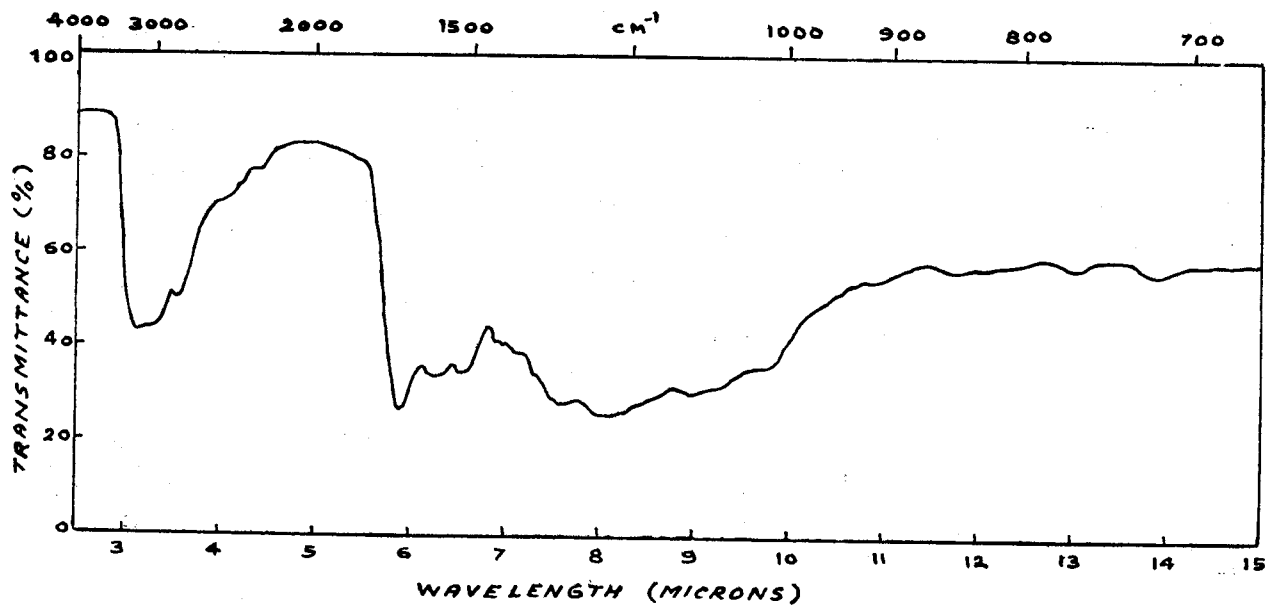


Fig. VI-Dioxane Nitrolignin (Methyl Alcohol Medium)

Table—V
Yield & Analysis of Isolated Nitrolignins (Carbon—tetra—chloride—medium).

Type of Nitro-lignin.	Yield of* nitro-lignin %	Carbon %	Hydrogen %	Nitrogen %	Nitro %	Methoxyl %	Yield of 4, 6* dinitro-guai-acol (DNG) %
Dioxane	105.0	52.6	4.8	6.4	13.8	12.3	0.33
Thio	92.5	48.7	3.5	5.8	12.3	10.1	0.21
Methylated thio	110.7	50.3	3.2	3.4	9.8	13.7	Traces
Soda	112.5	52.2	4.1	6.1	13.5	10.3	0.25

*Expressed on the weight of lignin taken.

Table—VI
Yield & Analysis of Isolated Nitrolignins (Ether Medium)

Type of Nitro-lignin	Yield of* Nitro-lignin %	Carbon %	Hydrogen %	Nitrogen %	Nitro %	Methoxyl %	*Yield of 4,6 dinitro guaiacol (DNG) %
Dioxane	102.0	55.0	4.8	4.1	10.5	12.1	0.28
Thio	88.5	51.2	4.5	3.5	10.0	9.8	0.19
Methylated thio	95.7	52.5	4.2	2.8	8.6	14.2	Traces
Soda	105.0	52.8	4.6	3.9	10.2	10.1	0.22

*expressed on the weight of lignin taken.

The results recorded in Tables IV-VII indicate that the yield of nitrolignins are higher in non-aqueous medium as compared to aqueous medium, indicating the lesser drastic action of nitric acid in non-aqueous medium. In aqueous medium, the nitrolignin was obtained in two fractions, the acid soluble (fraction A) and the acid-insoluble (fraction B). The low yield of nitrolignin A indicates the poor solubility of lignin in nitric acid.

(ii) Chemical Composition of Isolated Lignins

A perusal of Table II indicates that lignin consists of only carbon

hydrogen and oxygen. The higher carbon content of isolated lignins indicates their aromatic nature. The higher methoxyl value of dioxane lignin (17.3%) than thio-lignin (14.8%) and soda lignin (15.5%) indicates its higher purity than the other two lignins.

As lignin macromolecule is built up of monomers of phenylpropane (C_6-C_3) type, the results of the elementary analysis and functional group determination are, therefore, calculated on C_9 basis, excluding all methoxyl carbon. Lignin contains hydroxyl groups, a part of which is phenolic and the remainder are aliphatic

in character. Dioxane lignin has 10.9 percent hydroxyl groups out of which 2.3 percent are phenolic in nature. This value corresponds to 1.03 aliphatic hydroxyl groups and 0.27 phenolic hydroxyl groups per phenylpropane monomer. The empirical formulae calculated for isolated lignins on C_9 basis are given in Table III.

(iii) Changes in the Lignin Macromolecule On Nitration

(a) Substitution of Nitrogen in Nitrolignins

Tables IV—VII indicate the lower value of nitrogen substitution in aqueous medium as compared to

non-aqueous medium. This is due to the side reactions including oxidation, which occur predominantly suppressing nitration and resulting in the formation of acids and gaseous products containing nitrogen. In non-aqueous medium, the occurrence of these side reactions is minimized. The nitrogen estimated by Kjeldahl's method occurs mainly in the form of aromatic nitro groups^{15,16} & 17. Kee¹⁸ observed that 76 to 80% of the total nitrogen was present in the form of nitro groups. The nitro content varied proportionally to the amount of nitrogen present in the nitrolignin.

(b) Methoxyl loss During Nitration

Tables IV-VII indicate that nitration of lignin is accompanied by a decrease in its methoxyl content^{17,18} & 19. Methoxyl loss of 47 and 33 percent respectively was observed in aqueous and non-aqueous media-Methylated Thiolignin was nitrated in non-aqueous medium and the results indicated that by nitration about 46 percent methoxyl groups were split off.

According to Shorygina the demethylation occurs primarily at those units with a free phenolic hydroxyl group ortho to the methoxyl group (i.e. guaiacyl units).

(c) Isolation of 4, 6-Dinitroguaiacol (DNG)

As a by-product 4, 6-Dinitroguaiacol (DNG) was isolated from the used carbon-tetra-chloride and ether filtrates left after isolating nitrolignins. As is clear from Tables V and VI; the yield of DNG varied from 0.19 - 0.33 percent (% on weight of lignin taken). Only traces of DNG were obtained when methylated thiolignin was nitrated. This is due to the fact that the free phenolic hydroxyl groups in lignin were blocked as a result of methylation.

(iv) Infra-Red Spectroscopic Studies of Lignins

The infra-red spectra of isolated lignins and nitrolignins show a number of absorption bands, which were assigned to various functional groups present accord-

ing to the standard tables given by Sarkanen and Ludwig²⁰. The bands in the region 3200-3050 cm^{-1} are characteristic of hydroxyl groups. The bands in the vicinity of 2850-2800 cm^{-1} are due to methoxyl groups. The bands in the region 1700-1650 cm^{-1} arise from the C=O groups. The I.R. spectra of all the nitrolignins show bands in the region 1600-1500 cm^{-1} , which are characteristic of aromatic nitro groups.

Conclusions

- (i) The higher yield, methoxyl content and Klason lignin content of dioxane lignin than those of thio and soda lignins indicates its higher purity than the other two lignins.
- (ii) The yield of nitrolignins are higher in non-aqueous medium as compared to aqueous medium indicating the lesser drastic action of nitric acid in non-aqueous medium.
- (iii) During nitration, nitrogen is substituted mainly in the form of aromatic nitro groups.

Table-VII
Yield & Analysis of Isolated Nitrolignins (Methanol Medium)

Type of Nitrolignin	Yield of* nitrolignin %	Carbon %	Hydrogen %	Nitrogen %	Nitro %	Methoxyl %
Dioxane	56.5	53.5	4.2	6.3	12.8	14.5
Thio	64.2	54.2	5.0	5.2	11.9	15.4
Methylated						
Thio	50.5	54.6	5.2	3.6	9.8	21.2
Soda	53.0	52.8	3.8	5.8	12.2	14.8

*expressed on the weight of lignin taken.

The substitution of nitrogen is higher in non-aqueous than in aqueous medium.

(iv) The nitro content varies proportionally to the amount of nitrogen present in the nitro-lignin.

(v) Nitration is accompanied by a loss in methoxyl content of nitrolignins. The methoxyl loss is more in aqueous than in non-aqueous medium which further confirms the lesser drastic action of nitric acid in non-aqueous medium.

(vi) The isolation of 4, 6—Dinitroguaiacol (DNG) from the spent nitrating liquor indicates the substitution of the nitro group in the 4 and 6 positions of the aromatic nucleus.

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