

# Thin-layer Chromatography of Oxidation & Nitration Products of Lignin from *Dendrocalamus strictus*

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*Dioxane, thio and soda lignins as well as dioxane nitrolignin prepared in different media (carbon-tetra-chloride, ether and methanol) were subjected to alkaline nitrobenzene oxidation. The oxidation products were separated by thin-layer chromatography (TLC) using benzene-ethanol (150:22) as the developing solvent and silicagel G as adsorbent. All the lignins gave vanillin, syringaldehyde and p-hydroxy benzaldehyde as the major oxidation products, which indicates their close resemblance and confirms that bamboo lignin is composed of guaiacyl syringyl and p-hydroxy-phenylpropane building units. The higher percentage of oxidation products from dioxane lignin confirms its higher purity than thio and soda lignins. The amount of oxidation products obtained from dioxane nitrolignins was very low, showing the degradation of lignin on nitration. From the spent nitrating liquor, 4,6-Dinitroguaiacol (DNG) was obtained indicating the substitution of nitro groups in 4 and 6 positions of the aromatic nucleus.*

## Introduction

Thin-layer chromatography is an important technique for rapid and good separations and is the method of choice (a) when the time factor is crucial (b) when separation on paper is difficult to achieve and (c) when only micro amounts of substances are available. TLC is best considered as a complementary technique to paper chromatography.

It was presumed that degradation reactions of lignin might throw some light on the mechanism of delignification of bamboo with nitric acid, so, in this paper some preliminary studies on the oxidation and nitration degradation products of lignin were carried out.

Lignin being phenolic in nature is susceptible to a wide variety of oxidants. Alkaline nitrobenzene oxidation was first of all introduced by Freudenberg.<sup>1</sup>

Many methods for the separation and estimation of the

alkaline nitrobenzene oxidation products have been reviewed by Pepper and Sidiquellah.<sup>2</sup>

Stone and Bludell<sup>3</sup> separated vanillin (V), Syringaldehyde (S) and p-hydroxybenzaldehyde (P) found by alkaline nitrobenzene oxidation by Paper chromatography.

Kratll and Puschmann<sup>4</sup> separated V, S and P by TLC using silica gel G as adsorbent and di-isoamyl ether (water-saturated)-n-butanol, (3:1) as the developing solvent.

The TLC separation of many aromatic aldehydes including P, V, and S has been reported by Rffini<sup>5</sup>.

Towers and Maass<sup>6</sup> have also described the separation of P,V,S on silica gel G.

In the present investigation, the following solvents were tried for the separation of oxidation products :-

- (i) Benzene—Acetone (3:2)
- (ii) Benzene—Ethanol (150:22)

- (iii) Methanol—Chloroform (3:7)

Out of these benzene-ethanol (150:22) gave the best separation therefore, it was used for the quantitative estimations.

Bolker & Co-workers<sup>7</sup> carried out studies on the reactions of simple model compounds (like guaiacol, veratrole, etc.), with nitrous acid in order to throw some light on the degradation reactions of lignin.

Shorygina & Co-workers<sup>8</sup> also carried out nitration of hydrochloric acid lignin from fir and isolated 4, 6-Dinitroguaiacol (DNG) as a by-product.

## Experimental

Dioxane, thio and soda lignins were isolated from bamboo, Dioxane-lignin was isolated by the method of Pepper and Co-workers<sup>9</sup>. Thio and soda lignins were isolated by conventional methods.

Dioxane lignin was nitrated in carbon-tetra-chloride and ether

media by the method of Shorygina and Co-Workers<sup>9</sup>. In methanol medium, it was nitrated by the methods of Kurschner and Co-Workers<sup>10</sup>.

The isolated lignins and nitro-lignins were subjected to alkaline nitrobenzene, oxidation.

#### (i) Reaction Conditions

The oxidation was carried out by the method followed by Stone and Blundell<sup>3</sup> under the following reaction conditions :-

Reaction temperature, °C = 170  
Time, hours = 2.5  
Nitrobenzene, ml. = 2  
Sodium hydroxide, ml. = 15  
(2 N)

#### (ii) Procedure

1 g. of lignin sample was weighed accurately and placed in a 100 ml. stainless steel bomb, 15 ml. of 2 N sodium hydroxide and 2 ml. of nitrobenzene were added to the bomb. The bomb was sealed, shaken vigorously and heated in an electrically operated digester for 2½ hours at 170°C.

#### (iii) Isolation of Reaction Products

After cooling, the contents of the bombs were transferred quantitatively to a separating funnel. The nitrobenzene was removed from the reaction mixture by vigorously shaking it with ether. After whole of the nitro-benzene was removed, the reaction mixture was acidified with glacial acetic acid to pH<sub>2</sub> and further extracted with ether to remove the lignin oxidation products. The mixture of oxida-

tion products was dissolved in 5 ml. of ethanol. These ethanol solutions were used for the chromatographic analysis and for the quantitative estimations.

#### (iv) Separation and Quantitative estimation of oxidation products of lignin (Vanillin, Syringaldehyde and p-hydroxy benzaldehyde) by TLC

##### (a) Adsorbent

The oxidation products of lignin were separated by means of TLC using silica Gel G (E. Merck A.G. Darmstadt, Germany-a standardized mixture of silicic acid and gypsum), as adsorbent.

##### (b) Preparation of Chromatoplates

Smooth glass plates (20×20×0.4 cm) were coated with a thin, even layer of silica Gel G (0.3 mm. thick) by spreading a well stirred mixture of 25 g. of Silica Gel G with 50 ml. of distilled water using Desaga applicator (C. Desaga G.M.B.H., Heidelberg, Germany). The plates were allowed to dry at room temperature and then, activated at 105°C for 50 minutes.

##### (c) Development of Chromatoplates

The chromatoplates were developed by the ascending technique by using benzene-ethanol (150:22) as the developing solvent.

Aliquots containing oxidation products were spotted on the chromatoplates at 2 cm. from the base line with a distance of 1.5 cm. in between. After drying, the chromatoplates were placed in glass jars, covered with ground glass plates and fitted with glass

racks to keep the chromatoplates up-right.

For development, the jars filled with a quantity of solvent mixture to wet the plates to a height of approximately 0.5 cm; shortly before the solvent front was about to reach the top, the development was interrupted. The development took 40-70 minutes.

After development, the chromatoplates were taken out of the jar and their front line was marked. After drying thoroughly, they were suitably sprayed and used for further investigation.

##### (d) Detection

The various spots were observed under U.V. lamp and they were further confirmed by spraying with 2,4-dinitro-phenyl hydrazine and diazotized p-nitroaniline followed by saturated solution of sodium carbonate. The observations (colours produced by different oxidation products and their R<sub>f</sub> and R<sub>v</sub> values) are recorded in Tables I & II.

##### (e) Recovery from Plates

After detection, corresponding zones of adsorbent were carefully scraped across the complete width of the plate and transferred to centrifuge tubes containing ethanol. The supernatant liquor containing oxidation products was decanted into further centrifuge tubes. Any precipitate (Silica gel) which was formed at this stage was centrifuged down. The clear supernatant liquor was evaporated to dryness. After weighing, the amounts of the individual aldehydes were determined quantitatively. The results are recorded in Tables III & IV.

TABLE I

$R_f$  and  $R_v$  values of Oxidation Products of Lignin using three developing Solvents

Value	Vanillin			Syringaldehyde			<i>p</i> -hydroxy benzaldehyde		
	BE	BA	MC	BE	BA	MC	BE	BA	MC
$R_f$	0.73	0.76	0.76	0.67	0.71	0.62	0.56	0.74	0.52
$R_v$	1.0	1.0	1.0	0.92	0.94	0.96	0.77	0.98	0.84

BE = Benzene-Ethanol (150:22)

BA = Benzene-Accone (3:2)

MC = Methanol-Chloroform (3:7)

$R_f$  = Distance travelled by respective compounds  
Distance travelled by the solvent

$R_v$  = Distance travelled by respective compounds  
Distance travelled by vanillin.

TABLE II

Compound	Under UV Lamp	2, 4 dinitrophenyl-hydrazine	Diazotized <i>p</i> -nitroaniline followed by $Na_2CO_3$ Solution
Vanillin	Purple	Deep Orange	Voilet
Syringaldehyde	Purple	Deep Orange	Light green
<i>p</i> -hydroxy benzaldehyde	Brown	Deep Orange	—
Vanillic acid	Light blue	—	Purple
Syringic acid	Light brown	—	Blue
Acetoguaiacone	Light Purple	—	Voilet

TABLE III

Yield of Alkaline Nitrobenzene Oxidation Products of Isolated Lignins

Products (%)	Dioxane lignin	Thio lignin	Soda lignin
Vanillin	5.4	5.1	4.8
Syringaldehyde	8.5	6.5	6.6
<i>p</i> -hydroxy benzaldehyde	2.3	1.5	1.9
Total yield	16.2	13.1	13.3
Molar ratio (S/V)	1.3	1.1	1.1

(S/V ratio=syringaldehyde to Vanillin ratio).

#### (v) 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 media left after isolating nitrolignins.

The reaction medium was extracted with ether several times and ethereal extract was further extracted with NaOH. The combined NaOH extract was acidified and again extracted with ether. From the ethereal extract, ether was evaporated and to the dark brown sprupy mass so obtained, ethanol was added. TLC of ethanol extract was carried out using a mixture of methanol: benzene acetic acid: petroleum ether (8:45:3.5:20) as the developing solvent and silica gel G as adsorbent<sup>7</sup>. The dark yellow zone at  $R_f$  0.47 was carefully scraped from the plate and extracted with methanol. When the methanol was evaporated under reduced pressure, a yellow residue of 4,6-Dinitroguaiacol (DNG) (M.Pt. 120°C) was obtained. The yields are reported in Table V.

Besides DNG, the chromatogram showed the presence of some distinct spots at the following  $R_f$

$R_f$  value

(i) 0.33

(ii) 0.52

(iii) 0.66

(iv) 0.72

These compounds could not be isolated as these were present in traces.

## Discussions

### (1) Oxidative Degradation Products of Lignin

The three isolated lignins (dioxane, thio and soda) were oxidized with alkaline nitrobenzene, which is a mild oxidant in order to determine the position of various substituents in the benzene ring of the phenylpropane units of lignin building stones.

All the isolated lignins gave vanillin, syringaldehyde and p-hydroxy-benzaldehyde as the major products upon alkaline nitrobenzene oxidation, which is true in case of monocotyledone (softwood lignins yield vanillin and hardwoods lignins yield vanillin and syringaldehyde upon alkaline nitrobenzene oxidation).

$R_f$  and  $R_v$  values of alkaline nitrobenzene oxidation products of isolated lignins obtained by using three different developing solvents are recorded in Table I. As is clear from these  $R_f$  and  $R_v$  values, the solvent benzene-ethanol (150:22) gave the best separation, therefore, it was selected for quantitative estimations.

The quantitative this-layer chromatography of ether-soluble nitrobenzene oxidation products of isolated lignins and their comparison with known reference compounds indicated the presence of vanillin, syringaldehyde, p-hydroxy-benzaldehyde, acetoguaiacone, vanillin acid and syringic acid. Identification of the some oxidation products from the three lignins indicates the close resemblance of these lignins.

TABLE IV

Yield of Alkaline Nitrobenzene Oxidation Products of Dioxane Nitrolignins

Products (%)	Dioxane nitro-lignin (Methanol medium)	Dioxane nitro-lignin Carbon-tetra-Chloride medium	Dioxane nitro-lignin (Ether medium)
Vanillin	1.40	1.15	0.95
Syringaldehyde	0.98	0.85	0.70
p-hydroxybenzaldehyde	0.20	Traces	Traces
Total yield	2.58	2.00	1.65
Molar ratio	0.58	0.62	0.62

TABLE V

Yield of 4, 6—Dinitroguaiacol obtained by the Nitration of Isolated Lignins

Type of Nitrolignin	Yield of 4, 6-Dinitro-guaiacol %	
	In Ether	In Col.
Dioxane nitrolignin	0.28	0.33
Thio nitrolignin	0.19	0.4
Methylated thio nitrolignin	Traces	Traces
Soda nitrolignin	0.22	0.25

Table II shows the colour of the various oxidation products under U.V. lamp and after spraying with 2, 4-dinitro-phenyl hydrazine and diazotized p-nitroaniline followed by a saturated solution of sodium carbonate.

Table III indicates the yield of nitrobenzene oxidation products of isolated lignins. The oxidation products confirm that bamboo lignin is composed of guaiacyl, syringyl and p-hydroxyphenyl-propane building units. Higuchi<sup>11</sup> obtained similar results for other species of bamboo. A perusal of Table VI reveals that the yields of syringaldehyde are higher as compared to that of vanillin.

This suggests that bamboo (*Dendrocalamus strictus*) contains more syringyl repeating units than guaiacyl repeating units. This is also evidenced by the high methoxyl values of bamboo lignins.

The ratio of syringaldehyde to vanillin (S/V ratio) is an important factor; higher the S/V ratio, lesser is the degradation of lignin. As is clear from Table VI, dioxane lignin has a somewhat higher molar S/V ratio and higher percentage of oxidation products as compared to the other two lignins (thio and soda). This confirms that it is least degraded & contains more syringyl units than soda and thio

lignins. This is further supported by the fact that dioxane lignin has a higher methoxyl value than thio and soda lignins.

A perusal of Table IV indicates that the amount of aromatic aldehydes obtained by the alkaline nitrobenzene oxidation of dioxane nitrolignins is very low as compared to the amount of aldehydes obtained by the oxidation of dioxane lignin. This is due to the degradation of lignin by nitration.

The greater loss in the yield of syringaldehyde as compared to vanillin shows that the loss of syringyl units is more than guaiacyl units on nitration. On the basis of studies carried out by Dence<sup>12</sup> on lignin like model compounds, this is due to syringyl nucleus being more reactive towards electrophilic reagents (like nitric acid) than guaiacyl nucleus. It is apparent that as syringyl units are more susceptible to nitration than the guaiacyl units; on oxidation the loss in the yield of syringaldehyde is more than vanillin.

## (ii) Nitration Products of Lignin.

As a by-product, 4, 6-dinitroguaiacol (DNG) was isolated by TLC from the carbon-tetra-chloride and ether filtrates left after isolating nitrolignins. As is evident from Table V, the yield of DNG varied from 0.19-0.33 percent (% on weight of lignin taken).

In the nitration of lignin 4, 6-DNG is formed as a result of electrophilic displacement of the side chain by a nitro group<sup>8</sup>. The displacement of side-chain occurs particularly readily in units with a free phenolic hydroxyl group in the para

position of the aromatic nucleus. The traces of DNG obtained in case of methylated thiolignin was due to the fact that the free phenolic hydroxyl groups in lignin were blocked as a result of methylation. Shorygina and Co-workers<sup>8</sup> carried out nitration of lignin model compounds and reported that the formation of 4, 6-DNG in the nitration of lignin is associated, in particular with the presence of structural elements containing a benzyl alcohol or benzyl ether group together with a free phenolic hydroxyl in the para position of the aromatic nucleus. This is further evidenced by the studies carried out by Gustafsson and Andersen. They concluded that DNG I is formed only when the compounds subjected to nitration are type II.

Besides DNG, the chromatogram revealed the presence of some other compounds, which could not be isolated as these were present in traces. Based on the studies carried out by Bolker and Co-Workers<sup>7</sup> and from the  $R_f$  values of unknown compounds, it is anticipated that three of the unknowns were probably 4, 4'-dimethoxy-diquinhydrone (III) ( $R_f=0.33$ ), 4-nitroguaiacol (iv) ( $R_f=0.52$ ) and 6-nitroguaiacol (v) ( $R_f=0.67$ ).

## Conclusions

- (i) The isolation of vanillin, syringaldehyde and p-hydroxybenzaldehyde by the oxidation of all the lignins confirms that bamboo lignin is composed of guaiacyl, syringyl and p-hydroxyphenyl propane building units.
- (ii) The higher yield of syringaldehyde than vanillin indicates that bamboo lignin contains more syringyl than guaiacyl repeating units.

(iii) The lower amount of aromatic aldehydes obtained by the oxidation of dioxane nitrolignin shows the degradation of lignin on nitration.

(iv) The greater loss in the yield of syringaldehyde than vanillin shows that the loss of syringyl units is more than guaiacyl units on nitration.

(v) The isolation of 4, 6-Dinitroguaiacol (DNG) from the spent nitrating liquor shows the substitution of nitro group in the 4 and 6 position of the aromatic nucleus.

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