

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

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SUMMARY

Dioxane, thio and soda lignin 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 reference and confirms that bamboo lignin is composed of guaiacyl syringyl and p-hydroxy-phenyl-propanes 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 nitration 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.¹

Many methods for the separation and estimation of the alkaline nitrobenzene oxidation products have been reviewed by Pepper and Sidiquellah².

Stone and Blundell³ separated vanillin (V), Syringaldehyde (S) and p-hydroxybenzaldehyde (P) found by alkaline nitrobenzene oxidation by paper chromatography.

Kratll and Puschmann⁴ separated V.S. and P by TLC using silica gel G as adsorbent and di-isomyl 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 Ruffini⁵.

Towere and Maass⁶ 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)

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

Bolkar & Co-workers⁷ 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-workers⁸ also carried out nitration of hydrochloric acid lignin from fir and isolated 4,6 - Dinitroguaiacol (DNG) as a byproduct.

EXPERIMENTAL

Dioxane, thio and soda lignins were isolated from bamboo. Dioxane lignin was isolated by the method of Pepper and Co-workers⁹. Thio and soda lignins were isolated by conventional methods.

Dioxane lignin was nitrated in carbon-tetrachloride and ether media by the method of shorygina and Co-workers⁸. In methanol medium, it was nitrated by the methods of Kurechner and Co-workers¹⁰.

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

(i) Reaction Conditions

The oxidation was carried out by the method followed by stone and Blundell³ under the following reaction conditions:—

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

(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 pH₃ and further extracted with ether to remove

the lignin oxidation products. The mixture of oxidation 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 standardized mixture of silica 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 GelG(0.3mm. 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.M., 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 were 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_f and R_v values) are recorded in tables I & II.

TABLE—I R_f AND R_v VALUES OF OXIDATION PRODUCTS OF LIGNIN USING THREE DEVELOPING SOLVENTS.

Value	Vanillin			Syringaldehyde			p hydroxy benzaldehyde		
R _f	BE	BA	MC	BE	BA	MC	BE	BA	MC
R _v	0.73	0.76	0.76	0.67	0.71	0.62	0.56	0.74	0.52
	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-Acetone (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	Diszotized p-nitroaniline followed by Na ₂ CO ₃ solution
Vanillin	Purple	Deep Orange	Violet
Syringaldehyde	Purple	Deep Orange	Light green
p-hydroxy benzaldehyde	Brown	Deep Orange	—
Vanillic acid	Light blue	—	Purple
Syringic acid	Light brown	—	Blue
Acetogua:acone	Light Purple	—	Violet

(e) Recovery from Plates

After detection, corresponding zones of absorbent 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—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
p-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)

TABLE—IV YIELD OF ALKALINE NITROBENZENE OXIDATION OF DIOXANE NITROLIGNINS

Products (%)	Dioxane nitrolignin (Methanol medium)	Dioxane nitrolignin (Carbontetra-chloride medium)	Dioxane nitrolignin (Ether medium)
Vanillin	1.40	1.15	0.96
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

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

4, 6 - Dinitroguaiacol (DNG) was isolated from the used reaction media i.e. acidic carbon-tetrachloride and ether media left after isolating nitrolignins.

The reaction medium was extracted with ether several times and the 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 syrupy 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 adsorbent⁷. 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.

All the isolated lignins gave vanillin, syringaldehyde and p-hydroxybenzaldehyde as the major products upon alkaline nitrobenzene (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 thin-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, vanillic acid and syringic acid. Identification of the same oxidation products from

TABLE-V YIELD OF 4,6-DINITROGUAICOL OBTAINED BY THE NITRATION OF ISOLATED LIGNINS

Type of Nitrolignin	Yield of 4, 6 Dinitro guaiacol %	
	In Ether	In CCl_4
Dioxane nitrolignin	0.28	0.33
Thio nitrolignin	0.19	0.4
Methylated thionitrolignin	Traces	Traces
Soda nitrolignin	0.22	0.25

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

(i) 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 substitutes in the benzene ring of the phenylpropane units of lignin pulping stones.

the three lignins indicated the close resemblance of these lignins.

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 production confirm that bamboo lignin is composed of guaiacyl, syringyl and p-hydroxyphenyl-propane building units. Higuchi¹¹ 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 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 and contains more syringal 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.

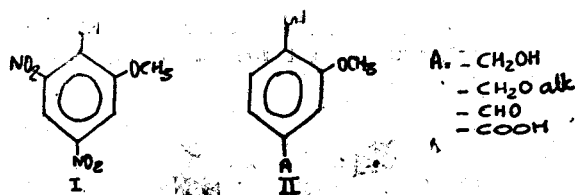
The greater loss in the yield of syringaldehyde as compared to vanillin shows that the loss of syringyl units is more than guaiacyl units on nitration. On the basis of studies carried out by Dence¹² on lignin like model compounds this is due to syringyl nucleus being more reactive towards electrophilic 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

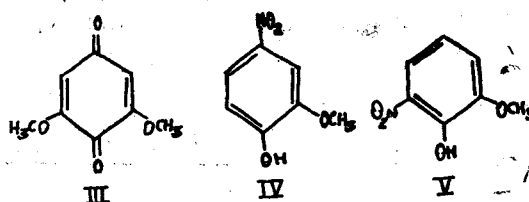
As a by-product, 4, 6-dinitroguaiacol (DNG) was isolated by STC from the carbon-tetra-chloride and other filtrates left after isolating nitrolignins. As is evident from Table V, the yield of DNG varied from 0.19-0.33 percent (% on weight of lignin taken).

In the nitration of lignin 4,6-DNG is now forced as a result of electrophilic displacement of the side chain by a nitrogroup⁸. The displacement of sidechain 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 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-workers⁸ 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 by Gustafsson and Anderson¹³. 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-Workers⁷ and from the R_f values of unknown compounds, it is anticipated that three of the unknowns were probably 4,4' dimethoxy-di-quinhydrone (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 (DMG) 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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