

High Performance Liquid Chromatography of Lignin Oxidative Products

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SUMMARY

The paper reports the results of investigation obtained by HPLC method of oxidised lignin from different sources. The materials include different categories of lignin containing plants as well as lignin from industrial byproducts. The phenolic aldehydes obtained by alkaline nitrobenzene oxidation were characterised, and estimated by HPLC using μ -Bondapak C₁₈ bonded phase silica column.

INTRODUCTION

The nature of lignin varies from source to source and also gets altered during its process of isolation. The complex nature of lignin (Fig. 1) present in plant source has been widely studied adopting the oxidative degradation techniques in order to obtain^{1,2} information about its nature and type of aromatic fragments present in the lignin molecules. The identification and separation of

aromatic compounds so formed^{3,4} during mild oxidation were studied by paper chromatography and thin layer chromatography. The availability of modern techniques for identifying^{5,6,7} the organic compounds have now become more popular for the efficiency and accuracy. High performance liquid chromatography (H.P.L.C.) is one such tool available⁸ to the scientists.

The mild oxidative degradation of lignin leads to the formation of architecture of lignin molecules showing the lignin precursors. The experiment is aimed to obtain basic information and to identify the precursors involved in lignin built-up in different lignin containing plants as well as in the industrially isolated lignin from the onward utilisation point of view. The lignin built up (Fig. 2) of soft woods coniferous species is

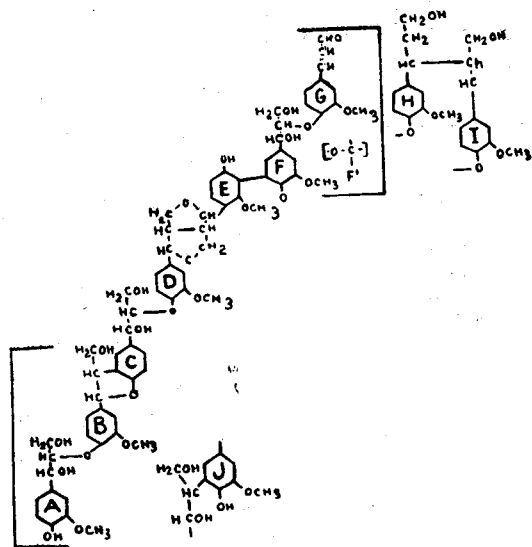


Fig. 1. Structure of Lignin

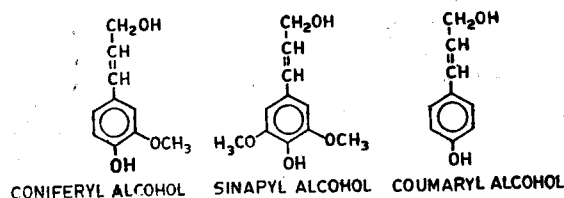


Fig 2 Lignin Precursors

generally derived from coniferyl alcohol (A) deciduous hard woods contain coniferyl and sinapyl alcohol (B) and grasses i.e. angiosperm mono cotyledonous contain p-coumaryl alcohol (C) in addition to coniferyl and sinapyl alcohol. Mild oxidative degradation studies of lignin from eight different sources, using H.P.L.C. for

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characterisation and quantitative analysis of the phenolic aldehyde bearing degradation products is reported in the paper.

MATERIAL AND METHODS

The materials taken for the study consisted *Cedrus deodara* a coniferous species extensively used as a commercial timber, *Salix alba* (willow) a hard wood dicotyledonous angiosperm and agricultural residues like rice straw, cotton stalk, and bamboo, besides, commercially available lignin from paper mills in the form of ligno sulphonates marketed under trade name Totanin FNA; alkaline black liquor and Celax (lignosulphonate from bamboo and hard wood species). The lignin from different sources may represent different classes of lignin that could be obtained. The alkaline nitrobenzene oxidation was adopted as a mild oxidation degradation method for the lignin complex molecules.

The woody material viz. *Cedrus deodara* and *Salix alba*, were taken in the form of saw dust. Rice straw, cotton stalk and bamboo were chopped into small pieces prior to oxidation reaction. Ligno-sulphonates available under the trade name Totanin FNA was a fine brown coloured powder, black sulphite liquor and Celax were thick syrupy liquids which were used as such. The lignin content of lignin containing materials and lignosulphonates was determined according Tappi standard as given in Table I & II.

TABLE—I CHEMICAL COMPOSITION OF LIGNIN MATERIAL

S. No.	Raw material	Extractive			Pentosan %	Lignin %	Holo-cellulose %	Alpha cellulose %	Ash %
		Ether soluble %	Alcohol/Benzene 2:1 %	Hot water soluble %					
1.	<i>Cedrus deodara</i> (Indian soft wood)	—	—	—	13.0	32	66.2	43.5	3.0
2.	<i>Salix alba</i> (Indian hard wood)	0.99	1.41	3.28	20.5	25	75.2	44.4	0.87
3.	Cotton Stalk	2.1	5.4	18.9	22.2	26.2	70.3	42.2	10.1
4.	Rice Straw	1.0	3.2	5.33	25.4	16.0	77.0	47.0	14.0
5.	Bamboo	2.2	2.2	6.9	16.7	24.9	67.5	58.8	3.9

TABLE II— CHEMICAL COMPOSITION OF TOTANIN FNA

S. No.	Constituents	Percentage
1.	Total solids	93.2
2.	Water solubility	100.0
3.	Volume weight kg/litre	0.71
4.	Lignin	61.0
5.	Total ligno sulphonates	76.0
6.	Methoxy content 10 CH ₃	10.0
7.	Reducing sugar	12.0
8.	Ash 700°C 3 hrs	20.0

A). OXIDATION OF LIGNIN MATERIAL : 450 g of the material on oven dry basis was taken and reacted with nitro benzene (250 ml) and 2.N NaOH (6 L) in a 10 litre autoclave. The reaction mixture was heated to 160° for 3 hours with continuous stirring. After the reaction, the reaction mixture was centrifuged to remove the solid residue, the unreacted nitrobenzene and its derivatives were removed by extracting at pH 13 with CHCl₃ (5+500 ml). The CHCl₃ extract was washed with 50 ml of 1 N. NaOH. The combined sodium hydroxide layer and original aqueous layer were acidified to pH—1 with 1 N. H₂SO₄ and filtered to remove the unreacted lignin. The filtrate was extracted with 3 litre of CHCl₃. The solvent fraction was dried over anhydrous sodium sulphate, reduced to a small volume (20 ml) by vacuum distillation; then transferred into 1 litre conical flask, where it was treated with 200 ml NaHSO₃ soln. (20%) The mixture was stirred mechanically for 1–2 hrs. The aqueous bisulphite soln. was decomposed with dil. H₂SO₄ and extracted with CHCl₃. when aromatic phenolic aldehydes were obtained after drying CHCl₃ extract over anhydrous Na₂SO₄ and distilled under vacuum.

B). OXIDATION OF LIGNO-SULPHONATES

—Lignin sulphonate (200 g) Nitrobenzene (250 ml) and 2. N NaOH (6 litres) were autoclaved in 10 litre autoclave at 160°C for 3 hrs. The reaction mixture was processed in a similar

manner as in case of lignin materials. The mixture of phenolic aromatic aldehydes thus obtained were subjected to HPLC. A known weight of 5 mg of each samples was dissolved in a known volume (5 ml) of solvent.

SEPARATION AND ESTIMATION OF REFERENCE MIXTURES OF PHENOLIC ALDEHYDES

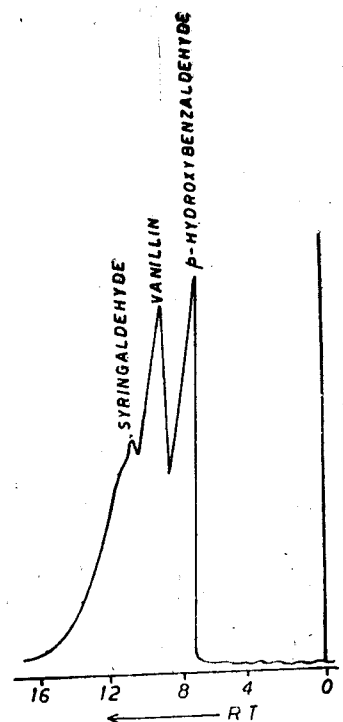
Mixture of p-hydrobenzaldehyde vanillin and syringaldehyde were separated by HPLC using high pump (Waters 6000 A) a loop injector, (Water

U6K) Wave length UV detector (Waters) set at 254nm with UV sensitivity 0.5 (Houston instrument omniscrite), chart speed 1.5 cm/min. Reversed phase chromatography was carried out using a steel column (3.9 mm×30 cm) containing μ -Bondapak C₁₈ bonded phase silica (Waters) isocrated solution with water+n-butanol+acetic acid (374:11:1 Solvent). The flow rate was 2 ml/min. and column pressure 3000 psi (Table-III).

RESULTS AND DISCUSSION

The major phenolic products of lignin oxidation with nitrobenzene in an alkaline medium are p-hydroxybenzaldehyde, vanillin and syringaldehyde and the other phenolic compounds were in trace amounts. HPLC has facilitated the resolution of all major and minor phenolic components of lignin oxidation by single analysis. The yield of aromatic compounds of 100 μ g per g lignin were detected by HPLC. The chromatogram of mixture of equimolecular standard aromatic aldehyde (Fig. 3) was used for comparing the chromatograms of oxidation products of lignin material and lignosulphonates (Fig. 4).

HPLC Chromatograms of eight samples was used for separation and estimation of phenolic aldehydes. The HPLC studies show that in Totanin and *Cedrus deodar* oxidation products contain vanillin as major product. In the case of *Salix alba* cotton stalks, rice straw, and black sulphite liquor besides vanillin contains syringaldehyde too.



H.P.L.C CHROMATOGRAM OF EQUIMOLAR MIXTURE OF AUTHENTIC SAMPLES

Fig. 3. H. P. L. C. Chromatogram of Standard Samples

TABLE - III

Sample	Amount mg.	Sensitivity A	Flow rate ml/min.	Retention time (RT) (Min)
1. P-Hydroxybenzaldehyde (P)	5	0.5	2.8	8.4
2. Vanillin (V)	5	0.5	2.8	9.6
3. Syringaldehyde (S)	10	0.5	2.0	10.8
4. Mixture (P.V.S.)	5	0.5	2.0	8.4, 9.6, 10.8
5. Totanin	40	0.5	2.0	6.0, 8.2, 9.8
6. Cedrus deodara	40	0.5	2.0	3.0, 3.4, 6.6, 9.6, 10.8
7. Salix alba (Willow)	50	0.5	2.0	9.6, 10.8
8. Black spent liquor	50	0.5	2.0	5.2, 8.4, 9.6, 10.8
9. Cotton stalk	40	0.5	2.0	8.4, 9.6, 10.8
10. Rice straw	50	0.2	2.0	5.2, 6.0, 8.4, 9.6, 10.8
11. Bamboo	40	0.5	2.0	8.4, 9.7, 10.8
12. Celax	50	0.5	2.0	3.0, 4.0, 6.1, 8.2, 9.6, 10.8

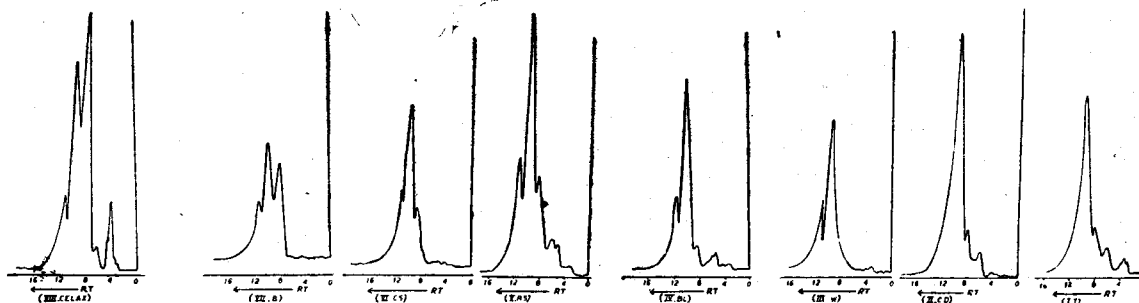


Fig. 4. HPLC. Chromatogram of Phenolic aldehydes from
(I) Totanin (II) Cedrus deodara (III) Willow (IV) Black liquor
(V) Rice straw (VI) Cotton stalk (VII) Bamboo and (VIII) Celax.

The bamboo and Celax was found to contain p-hydroxy benzaldehyde in addition to vanillin and syringaldehyde. The percentage of vanillin p-hydroxy benzaldehyde and syringaldehyde obtained from oxidation of various lignins are shown in Table-IV. The ratio of concentration of these phenolic aldehydes can be seen in (Fig. 5). HPLC here lends itself to an evaluation of chemical composition of lignin and permits evaluation of chemical change with lignin polymer brought by treatments.

ACKNOWLEDGEMENTS

The authors are indebted to Prof. C.K. Atal, Director for his interest and constant encouragement during the course of this investigation.

TABLE-IV PERCENTAGE OF MAJOR PHENOLIC OXIDATION PRODUCTS FROM VARIOUS LIGNIN

Lignin	Vanillin	Aromatic products Syringaldehyde	p-hydroxy benzaldehyde
1. Totanin	87.8	—	—
2. Cedrus deodar	70.8	—	—
3. Salix alba	61.4	33.2	5.2
4. Black spent liquor	69.0	22.0	5.5
5. Cotton stalk	45.3	33.7	21.0
6. Rice straw	40.6	34.6	15.2
7. Bamboo	44.6	27.8	27.9
8. Celax	41.2	17.2	32.6

REFERENCES

1. M.J. Reale Anal Biochem 13 (1965), 161.
2. J. KuG and O.E. Nelson Arch Biochem Biophys 105 (1954), 103.
3. P.F. Nelson and J.G. Smith Tappi 49 (1966), 215.
4. F.C. Dalba and K.G. Koeppl J. Chromatogr. Sci. 7 (1969), 665.
5. E.D. Pellizzari C.M. Chuang J. KuG and E. B Williams. J. Chromatogr. Sci. 40 (1969), 285.
6. N.F. Cymbaluk and T. S. Neudoeiffar J. Chromatogr. Sci. 51 (1970) 167.
7. Monties B. Chambat J.C. Journ. Etudo Prog. Recent method and Quil, Quan Stricture Polyphenels Asam Gen Group., 10 CLHP, 11 (Fr) 1976 C.A. 106159 (86) (1977).
8. Hartley, R.D. and H. Buchan, J. Chromatogr. Sci. 80 (1979), 139-143.

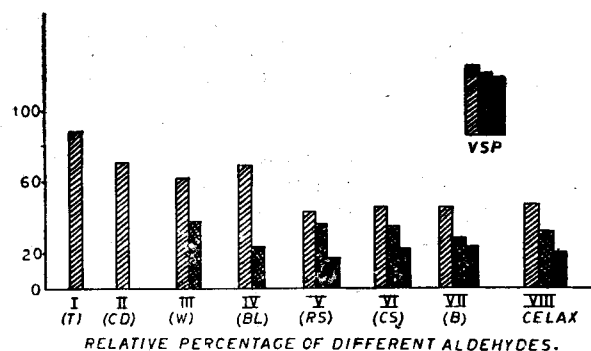


Fig. 5 Relative Percentage of Different aldehydes