Composition of non-cellulosic polysaccharide from sal wood (shorea robusta)

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SUMMARY

Commercially available wood of *Shorea robusta* (sal) was analysed for the composition of non-cellulosic wood polysaccharides commonly known as hemicelluloses. Holocellulose fraction was prepared and hemicelluloses were extracted under mild conditions The hemicellulose fraction was purified and identified as 4,0-Mee-D Glucuronexylan. The Chromotographic as well as spectrophotometric techniques were adopted during study. The methylation and periodate Oxidation reactions were carried out on purified hemicellulose fraction.

Shorea Robusta is a commercially available wood species in India[1]. It is already consumed as an admixture with other woods for pulp manufacture. Non cellulosic polysaccharides (N.C.P.) play an important role to improve or deteriorate the pulp properties depending on end use of pulp. Removal of N.C.P. from rayon grade pulp is desired with a view to attain desired viscosity with optimum chemical doses. Whereas, in case of paper grade pulp it is desirable to retain optimum amount of N.C.P. with the final bleached pulp. This improves the strength properties of paper. It appears interesting to establish chemical composition and nature of non-cellulosic wood polysaccharides (N.C.P.) for better understanding during their removal or retention from pulp. Therefore, it was found necessary to establish the composition of non-cellulosic polysaccharides present in **Shorea Robusta** wood.

Chemical composition of various wood components was summarised by Dickey[2] He found that hard wood generally contains more hemicelluloses than soft wood. Composition of soft wood vary considerably unlike hard woods. Hard wood hemicelluloses consist of mainly pentose sugar zylose. Both hard wood as well as soft wood species were found to contain branching groups of hexuronic acid such as galacturonic acid or glucuronic acid. Hemicelluloses are generally made up of five monosaccharides, namely three hexose sugar units such as glucose, galactose and mannose and two pentose sugar units namely xylose and arabinose. Xylose sugar monomer does not have a free-CH_2OH group. Two types of derivative groups present in wood hemicelluloses are methoxyl and acetyl groups. The methoxyl group is linked as an ether whereas, acetyl group is substituted as an acetic acid ester group.

Howely and Norman[3] have classified hemicelluloses based on Cross and Bevan cellulose preparation. Those which are retained with cellulose are called as cellulosans and the fraction which goes into solution is further divided into two groups. First group includes hemicellulose fraction not containing Uronic acid groups and second fraction contains Uronic acid group which are known as Polyuronides.

The advance theory about the role of hemicelluloses in wood suggests that in addition to imbibing water, they serve as matrix bonding substance between the cellulosic structural elements which build wood fiber. According to Spiegelberg[4], the hemicellulose in wood is important to intrinsic fiber strength, because they impart maximum stress distribution between cellulosic structural elements. This composition in structure makes wood a strong material.

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EXPERIMENTAL

40 mesh passed and 60 mesh retained wood dust was taken for study. Dust was analysed for proximate composition using latest Tappi standard procedures[5]. Results are recorded in Table-I.

TABLE—I

PROXIMATE CHEMICAL ANALYSIS OF SHOREA ROBUSTA DUST

	Particulars	Percentage based on O.D. weight of dust.
1.	Ash content	0.28
2.	Cold water solubility	0.87
3.	Hot water solubility	4.29
4.	Ether solubility	2.51
5.	Alcohol benzene solubility	3.95
6.	1% NaOH solubility	18.22
7.	Pentosan content	12.05
8.	Lignin content	25.30
9.	Holocellulose	78 88
10.	Alpha cellulose	49.19
11.	Hemicellulose	29.97
12.	Acetyl content	1.20
13.	Methoxyl content	3.51
14.	Uronic anhydride	3.41

During complete study, it was kept in mind to impart minimum modification as well as minimum degradation during isolation, fractionation and purification of wood polysaccharides.

Holocellulose, representing total Carbohydrate fraction, was prepared by chlorite method[6]. The hemicellulose was extracted from holocellulose using potassium hydroxide for extraction[7]. The hemicellulose fractions were purified using method proposed by Chanda and Coworker[8], and also by R.L. Whilstler[9]. Thus obtained purified hemicellulose fraction waa taken for chemical composition studies.

Paper chromotagraphy and gas liquid chromotagraphy were used for qualitative as well as quantitative determination of monosaccharides which was recorded as constituents of hemicellulose fraction.

E.P. Crowell et al[10] and Borchardt and Piper[11] suggested the procedure for hydrolysis of wood and pulp samples which was adopted in the present study. The alditol acetate derivatives of hydrolyzate were prepared from wood dust, holocellulose and purified hemicellulose. These samples were subjected to Gas liquid chromatographic examination for determining the sugar composition[12]. The precautions suggested by L.B. Barchardt and D.B. Earty[13] for better reproducibility of results were taken into consideration.

PROCEDURE FOR ALDITOL ACETATE DERIVATIVE

About 0.1 g of sample was treated with 1 ml of 72 percent Sulphuric Acid for one hour at 30°C while stirring. After half an hour 28 ml of distilled water was added to dilute to 3 percent acid concentration. The content was refluxed for one hour in an auto clave at 120°C corresponding to 15 p. s. i. The solution containing hydrolyzates was passed through regenerated IR 45 anion exchange resin, till 4.5 pH was attained. The resin column was later washed with 150 ml distilled water. 2 percent meso-inocital as an internal reference was added to the elute. The content was concentrated under reduced pressure at 40°C to one ml syrup. The syrup was diluted to 2ml and 0.065 g of sodium borohydride was added to reduce the sugar monomers. The solution was left for three hours The content was passed through IR 120 cation exchange resin to remove sodium ion. The resin was eluted five times with 3 ml of water : ethanol (1:1 by volume).

The solution thus obtained was evaporated to dryness at reduced pressure at 40°C to avoid charring of components

The concentrated syrup was evaporated three to four times with 10 ml of 99.9 percent methanol. The final syrup (0.08 ml) was dissolved in about 2 ml of chromatographic grade pyridire solvent, to which 2 ml of acetic anhydride was added. The content was refluxed for four hours at temperature ranging from 130 to 135°C, and brown concentra-

TABLE—II

SUGAR COMPOSITION OF SHOREA ROBUSTA

Name of Sugar Unit	Percentage	
	Dust	Holocellulose
L rhamnose	2.95	Absent
L—arabinose	15.74	1.5
D—xylose	13.57	14.50
D—Mannose	9.06	Absent
D—Glucose	57.40	83.91
D—Glactose	Trace	Absent.

ted syrup was obtained for gas chromatographic analysis.

The resolution of alditol acetate derivative was performed on Perkin Elmer Gas chromatograph (model 3920), equipped with differential flame ionisation detector and Perkin Elmer Recorder (Model-56). The stainless steel column (1.5 m length and 0.3 cm dia) filled with L phase 3 percent ECNNS-M on chromosorb-W (Gas chrom Q), was used, Nitrogen was used as a carrier gas with a flow rate of 30 ml/min. The temperature of injection and D-block portion was maintained at 225°C. The column temperature was maintained at 180°C. A suitable quantity of sample solution (0.5-1.0 ml) was injected to gas chromatograph.

The chromatographs obtained for shorea robusta dust, holocellulose as well as hemicellulose, are recorded in fig. 1, 2 and 3 respectively.

The standard methylation[14] and periodate oxidation[15] studies were carried out to establish the point of linkage and nature of bonding in purified hemicellulose fractions and 2-3-di-o-methyl-D-xylose was identified as main component. Spectrophotometric estimation of periodate ion was carried out[15] at 222.5 nm. It was found that 0.91 mole of periodate was consumed by one anhydrosugar.

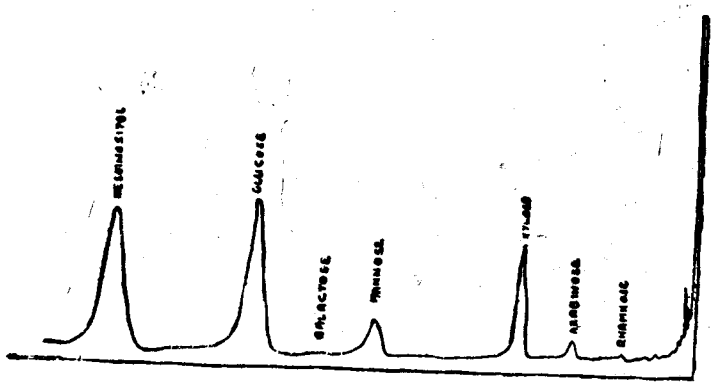


FIG.1 GAS CHROMATOGRAM OF ALDITOL ACETATE OF SHOREA-ROBUSTA- DUST

RESULTS AND DISCUSSIONS

The presence of L. rhamnose, D-xylose, D-mannose, D-galactose and D-glucose as identified by paper chromatography of wood dust, were further confirmed by gas chromatogram of dust (Fig.2) which shows resolution of these sugars. The percentage composition among these sugars are L-rhamnose, 2.9%, L-arabinase 15.2%, D-xylose 13.5%, D-Mannose 9.0%, D-glucose 57.4% and D-galactose in traces.

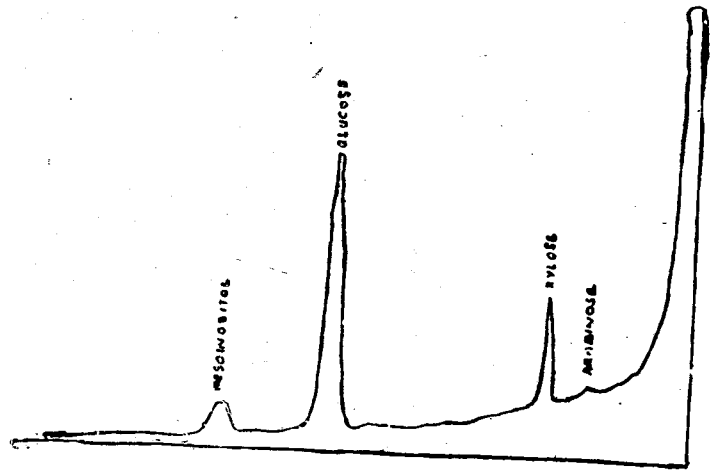


FIG 2 GAS CHROMATOGRAM OF ALDITOL ACETATE OF SHOREA ROBUSTA HOLOCELLULOSE

The L-arabinose, D-xylose and D-glucose were identified by paper chromatography hydrolyzates of holocellulose. The presence of these three sugars were confirmed by Gas chromatography as shown by resolution recorded in gas chromatogram Fig. 2. The percentage among these sugars are L-arabinose 1.5%, D-xylose 14.6% and D-glucose 83.9%.

Paper chromatography of hemicellulose fraction showed that it contains xylose in neutral fraction and glucuronic acid in acidic fraction. These findings were confirmed by Gas chromatography as only one peak (Fig-3) corressponding to xylose was observed with alditol acetate derivative of neutral fraction of purified hemicellulose.

Paper chromatograpy of methylated hemicellulose monomers showed the presence of 2, 3 di-o-methyl D-xylose as main component, with 3-0-methyl-D-xylose and 2, 3, 4-tri-0-methyl xylose in traces, from their R. G. values. Molar ratio among these sugars were determined by iodometric titration of chromalographic extract, which is as follows: 3-0-methyl D-xylose, 3.4, 2.3, di-o-methyl-D-xylose 172.5, 2, 3, 4, tri-o-methyl -D-Xylose 1.0, and 2, 3, 4-tri -O-methyl-D glucose 15.6. The 2, 3, 4 tri-o-methyl -D-glucose was obtained after reduction of methylated hexuronic acid with lithium aluminium hydride in tetrahydrofuram followed by hydrolysis which represents the amount of glucuronic acid present in purified hemicellulose fraction. The periodate oxydation studies showed that there are nine points for branching per hundred anhydroxylose sugar. This confirms the molar ratio obtained for 2, 3, di-o-methyl-D-xylose, 172.5 and 2, 3, 4, tri-O-methyl glucose 15.6, repre-

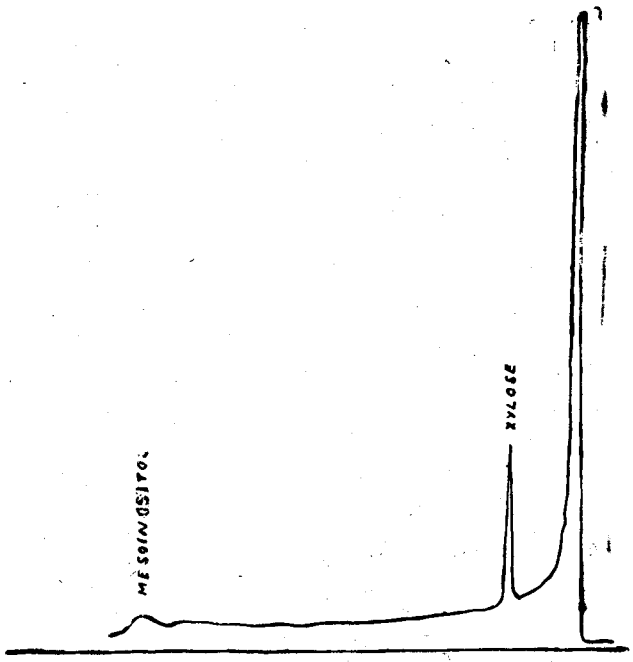
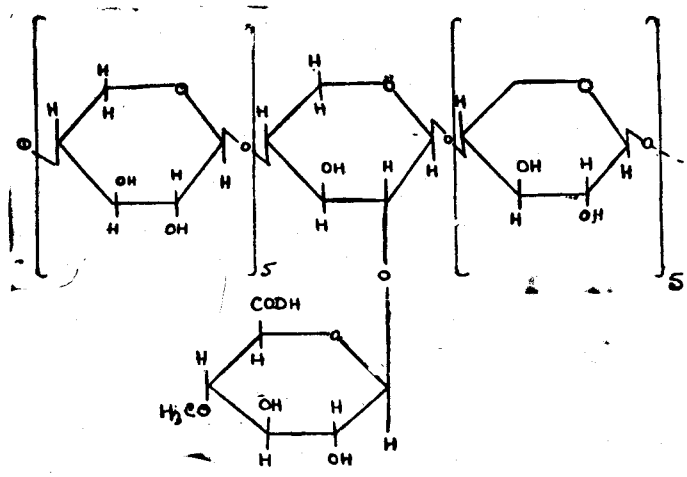


FIG. 3. GAS CHRAMATOGRAM OF ALDITOL ACETATE OF PURIFIED SHOREA ROBUSTA - HEMICELLULOSE.



Simplified structure of methyl glucorono xylan from **Shorea robusta** wood.

senting glucuronic acid is 172.5: 15.6, i. e. one glucuronic acid group is present per elevent anhydroxylose monomers,

1-4-glucosidic linkage is concluded due to presence of 2, 3 di-O-methyl D-xylose as major components. The C_1 and C_4 carbon in xylose are not methylated as they were involved in 1-4 glycosidic bonding.

Presence of 3-0-me thyl xylose, shows that branching occurs at C—2, Carbon of xylose with hexuronic acid group. The 2-0-methyl xylose were not recorded.

Presence of 2, 3, 4 tri-O-methyl-D-Xylose may be explained to originate from non-reducing terminal end monomer. From the facts mentioned here it has been concluded that methyl glucurono xylan is a basic unit present in **shorea robusta** hemicelluloses. The xylose units are bonded together with 1-4-glucosidic bond and glucuronic acid is linked to xylan-back bone by C-1, carbon to C-2, Carbon of xylose unit. It was also confirmed that one glucuronic acid is present per eleven xylose sugar units. Therefore, following simplified structure can be assigned for methyl glucuronoxylin present in non cellulosic polysaccharide of **Shorea robusta** wood.

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