Technology Section

Pak. j. sci. ind. res., vol. 33, no. 12, December 1990

SEPARATION AND ANALYSIS OF SUGARS FROM *PINUS ROXBURGHII* BARK

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(Received February 27, 1990; revised January 6, 1991)

Sugars were extracted from the bark of *Pinus roxburghii* by two methods. Their separation and identification were carried out by thin-layer and paper chromatography. The amount of sugars determined by UV method for different samples varied in the order, glucose 1.25 - 2.49%, fructose 1.2 - 2.9% and arabinose, 1.17 - 1.87%. The amounts of total sugar (4.9 - 6.8%) determined by titrimetric and UV method are comparable.

Key words: Sugar analysis, *Pinus roxburghii* bark, Carbohydrates.

Introduction

Carbohydrates form the main part of nontannins extracts from wood and bark of various species wattle [1,2], quebracho [3], hemlock [4] and pinus [5-7] etc. Black extract [8] consists of galactose, xylose, sucrose and arabinose. According to Milan Mladek [9], valonea contains sucrose and arabinose, chestnut extract: galactose, glucose, arabinose and xylose, spruce back. sucrose, glucose, fructose and arabinose, quebracho extract: sucrose, galactose, glucose and xylose. As sugars are present in significant amount and they do not participate in resin formation with formaldehyde, their presence reduce [10] the strength and water resistance of the glue joints. The results of sugars determined by different methods are being reported here.

Experimental

Preparation of sugar samples. Sugar samples were prepared by two methods:

(1) *E. Gulbaran method* [11]; Tannin was removed as lead tannate by using lead acetate. The filtrate was treated with sulfuric acid and filtered. H_2S was passed through the filtrate to remove the traces of lead. After drying the filtrate at 60°, sulfuric acid (2N) was added to it and then placed in an oven at 105° for 3 hrs. The precipitated phlobaphene material was separated by centrifugation and the pH was brought to 5-6 by $Ba(OH)_2.6H_2O$, Amberlite CG 120 was added to the centrifugate and then filtered. The volume of this solution was reduced to 5-7 ml. The use of chromed hide powder was excluded for the reason given elsewhere [11] and Amberlite resin CG 120 was used instead of Levatit-M-ion exchange resin.

(2) The 280 mg of powdered bark [12] (40/60 mesh) was treated with 2.5 ml of H_2SO_4 (72%) with thorough stirring for about 10 mins. It was placed in refrigerator for over night. 2.5 ml of H_2SO_4 (25%) was added to this solution and kept it for 2 hrs in a thermostate at a temperature of 50°. At the end it was diluted with 100 ml of distilled water and refluxed for 6 hrs. The hydrolysate was neutralized with 11.5 gm of $Ba(OH)_2.6H_2O$, then filtered and the volume of the filtrate was reduced to 7-8 ml.

Qualitative analysis of sugar. Sugar samples prepared by the methods mentioned above were used for qualitative and quantitative analysis. Fehling's solution test [13] was conducted to identify the reducing sugars.

Paper and thin-layer chromatography . Silica gel 60 GF254 was used for coating the plate of thin- layer chromatography. The following methods were used for both paper and thin-layer chromatography. A mixture of acetone and water [14] (9:1) was used as developing solvent and aniline diphenylamine as locating reagent. The chromatogram was heated at 85° for 10 mins. The sample sugar gave three spots of different colours, which were identified as yellowish brown (glucose), pink (fructose) and blue (arabinose) by comparison with the standard samples. Another solvent used was mixture of ethyl acetate, pyridine and water (24:14:4), while visualization was done by *p*-anisidine reagent. The chromatogram was heated at 100° for 15 mins. The colour of glucose was redish brown, fructose - dark yellow and arabinose - brown. The spots of sugars on the TLC plates were dissolved in acetone for further analysis by IR and UV methods.

IR study . IR spectra of the sample and model compounds- glucose and fructose were recorded to characterise the sugars from the bark of *Pinus roxburghii*. The absorption frequencies of functional groups in the model compounds, locating reagent *p*-anisidine and those in the sample sugars are reported in Table 1 for comparison. The spectra were recorded in KBr on a Pye-Unicam double beam spectrophotometer (SP_3-100).

Quantitative study of sugars. Quantitative study of sugars was carried out by two methods:(i) Titrimetric and (ii) U.V.

(i) Titrimetric method. All the solutions and reagents were prepared according to Brown *et al.* methods [15] in the usual manner. The amounts of total sugar obtained are shown in Table 2.

TABLE 1. ABSORPTION FREQUENCIES (cm^{-1}) OF ANISIDINE, GLUCOSEANISIDINE AND FRUCTOSEANISIDINE.

S.No.	Anisidine	Glucoseanisidine (sample)	Glucoseanisidine (model)	Fructoseanisidine (sample)	Fructoseanisidine (model)	Functional group
1.	3610	3610	–	3450	3450	N-H stretching
2.	3200	2860	2900	2820	2825	C-H methyl stretching
3.	3500	2940	2950	2870	2940	C-H aromatic stretching
4.	1620	1620	1620	1630	1640	C-C aromatic stretching
5.	1340	1260	1240	1230	1280	C-O aromatic stretching
6.	1130	1100	1120	1060	1080	C-O methylic stretching
7.	1280	–	–	–	–	C-N stretching
8.	920	960	970	920	920	C-N aromatic O.O.P bending
9.	1400	1395	1395	1400	1395	C-H methyl bending
10.	–	–	1460	1460	1460	C-H methylene bending
11.	1570	–	–	–	–	N-H bending
12.	1730	1740	1740	1740	1725	Para substitution
13.	–	1520	1520	–	–	HOC-H bending
14.	–	2680	2680	–	–	HOC-H stretching
15.	–	3410	3400	3400	3400	O-H stretch
16.	–	1090	1090	1110	1120	O-H bending
17.	–	1710	–	1700	1710	C-O stretching
18.	–	–	–	660	–	C-C aromatic

TABLE 2. QUANTITATIVE ANALYSIS OF SUGARS BY UV AND TITRIMETRIC METHODS.

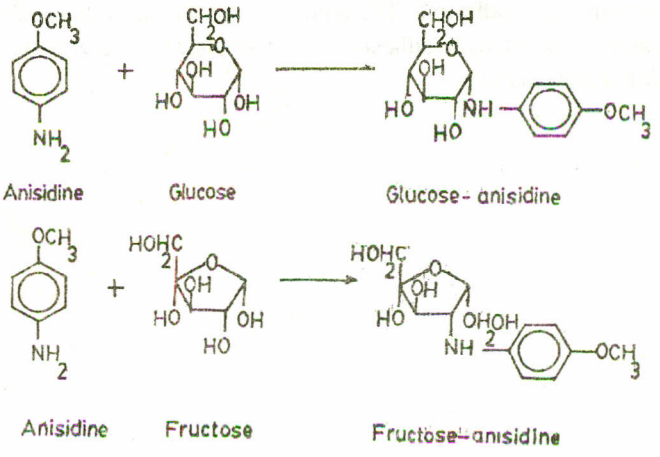
S. No.	Glucose %	Arabinose %	Fructose %	Total sugar in the sample %		Total sugar in tannin extract %	
	a	a	a	a	b	a	b
1.	2.40	1.75	1.20	5.35	6.802	18.407	23.404
2.	2.49	1.47	2.52	6.58	6.318	23.861	22.911
3.	1.25	1.17	2.50	4.92	6.689	17.813	24. 22
4.	1.26	1.87	2.90	5.23	5.544	18.843	19.974

a = Results obtained by UV method., b = Results obtained by titrimetric method.

(ii) UV method. The absorbance of sugar solution was recorded on an UV- visible spectrophotometer (DMS-200). The calibration curve of absorbance vs. concentration was prepared at 460 nm for each sugar. The concentration of sample sugars were determined from the respective calibration curves. The results are summarised in Table 2.

Discussion

In the extraction of sugar, acid hydrolysis is involved in both the methods. In the first method, tannins are removed as lead tannate and then the hydrolysis converts disaccharide [11] into monosaccharide; while in the second method hydrolysis causes degradation of biflavonid (Tannins) [16], leading to anthocyanidine and catechine (or sugar molecules) formation on further hydrolysis, disaccharides change into monosaccharides. The anthocyanidine and other material (phlobaphenes) formed are removed by filtration. The presence of sugar in the filtrate was determined by Fehling solution test. The separation and identification of glucose, fructose and arabinose were carried out by paper and thin-layer chromatography. Their presence was also confirmed by comparing the IR spectra of glucose anisidine and fructose anisidine of sample sugars with those of anisidine and model sugar-anisidine. Glucose and fructose anisidine complexes are formed according to following scheme [17].



Scheme

The carbohydrate anisidine complexes were identified by the presence of broad band of hydroxyl group [18] at 3400 cm^{-1} which is absent from the spectrum of pure anisidine. The absorbance of C=C aromatic stretching at 1620 cm^{-1}, N-H at 3610-3450 cm^{-1} and C- H methyl stretching at 2825-2900 cm^{-1} (Table 1) indicates that sugars are present as complexes of anisidine. The N-H bending band for anisidine at 1570 cm^{-1} is very weak in sugar complexes and this may be due to the reason that one hydrogen of amino group has been replaced by carbon atom of sugar molecule. The presence of stretching and bending bands of aldehyde group at 2620 and 1520 cm^{-1} of the sample and reference glucose-anisidine differentiate it from fructose-anisidine. The intensity of carbonyl group band at 1710 cm^{-1} is very weak which indicates that most of the sugar molecules are in the ring form.

The total sugar in the sample determined by titrimetric method in this study varied from 5.54 - 6.80% (Table 2), while the reported quantity for *Pinus brutia* and *Pinus silvestris* [11] is 5.59 and 4.29% respectively. The total sugar estimated by Abraham in Mimosa and Oak bark extracts [19], using Schroter method are 6.30 and 6.90% respectively while the Kohnstein method gave only 4.60 and 4.70%. Similarly the quantity of glucose determined in the present study by UV method ranges from 1.3 to 2.5% while the reported range for *Pinus silvestris* is from 1.80 - 4.40% and that for Mimosa [20] extract 1.80 - 1.90% respectively.

The effect of sugar on phenol formaldehyde adhesive has been investigated [16] by varying quantity of sucrose from 10-50%. The conclusion drawn from the study is that sugar reduces the shear strength and water resistance of the glue proportionally to the amount of sugar added. As tannin extract of *Pinus roxburghii* contains 17-24% sugar, it can be expected that unfortified *Pinus roxburghii* extract can only achieve about 83-76% of the performance shown by phenol-formaldehyde adhesive. The quality of the *Pinus roxburghii* tannin-formaldehyde adhesive can be improved by fortification of tannin extract.

Acknowledgement. We thank University Grants Commission, Islamabad (Pakistan) for financial support of this work.

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