Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2012, 4(6):2194-2197
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Isolation and identification of polysaccharides from Indian plantation white sugar- part -II

Bhoopendra Singh*, Azrabegam Afridi and Om Kumari

Department of Chemistry, K. K .P. G. College, Etawah, Uttar Pradesh (India).

ABSTARCT

The study has been carried out for the isolation and identification of polysaccharides from raw as well as refined sugars samples. The glycosidic linkages involved in the structure have been established, but their distributions are fine structures are not known. From the data, the arabinogalactan is envisaged as having a framework of (1-3) linked β-D- galactose residues of which two out of every three carry a galactosyl or aralunosyl side chain attached at O-6. The obtained results were further confirmed by chromatography technique.

Keywords: Polysaccharides, sugar crystal, Indian plantation white sugar, chromatography.

INTRODUCTION

In the last few decades increasing research has been directed at classifying and characterizing polysaccharides associated with cane sugar production. The objectives have been better under standing of their roles in processing sugarcane and their influence on raw sugar quality. Many different kind of polysaccharides are found in the sugarcane plant. These polysaccharides are all long chain molecules made up of simple carbohydrate units, the linkages between the units and the structure of the molecules, straight or branched can vary.

Difficulties in sugar manufacture and this effect is not easily diminished as very few of the processing steps remove them. Even during the refining operations these polysaccharides are only partially removed and remain occluded inside the sugar crystal.

The major proportions of polysaccharides entering the mill or the refinery are generated by the sugarcane plant. The remaining polysaccharides are formed by microbiological contamination, which can occur either in field or during processing. The production of sugar (sucrose) from sugarcane juice is based on the ability of sucrose to crystallize from thick syrup. It is generally accepted in the literature that polysaccharides have greater tendency to go preferentially into the sugar crystal and thus impact refined sugar quality [1,2]. These high molecular weight polysaccharides negatively effect sugar processing and have been implicated in the inclusion of colour in crystals, formation of color on storage processing problems and final product quality issues such as turbidity and acid beverage floc [3].

Polysaccharides present in cane juice adversely affect its processing and crystallization steps in the following ways- poor filterability, slow settling rate, increased viscosity of molasses, difficulties in low grade boiling, decrease in the rate of crystallization and centrifugation, less exhaustion and increased production of molasses, occlusion and distortion of sugar crystal, etc.

Sugarcane plant hosts many polysaccharides. Cellulose and hemicelluloses, which are components of cell wall, give structural strength to the standing cane plant. These are not soluble in water and consequently are not likely to affect

sugar manufacture. Starch which is involved in the metabolic activity of the growing plant largely occurs in sugarcane as insoluble granules. Because these granules can be solubilized during processing starch has an effect on the manufacturing process. Other soluble polysaccharides such such as indigenous sugarcane polysaccharides, Robertz glucan, galactomannan CP, dextran are also detected in sugarcane plant. The soluble polysaccharides of sugarcane account for a greater proportion of the organic non-sugars than any other group of compounds other than organic and amino acids [4].

In the early days of sugar manufacture many processing problems were attributed to the presence of starch [5]. The early investigation were hindered by the lack of an accurate starch analysis method, and by disagreement as to what starch content caused a problem [6]. There was also disagreement whether the problems were caused solely by starch, or by a combination of starch and other non-sugar impurities. It is now believed that the presence of starch in cane juice and in raw sugar has its greatest effect on the filterability of those materials [7].

As well as starch, other cane polysaccharides have a deleterious effect on processing, usually by affecting viscosities, polarization values and evaporation processes. The polysaccharides which are produced by microbiological contamination, whether in the field, factory or refinery also cause difficulties in sugar production [8]. The formation of dextrans, primarily by the bacteria, Leuconostoc mosenteroids, not only affects every stage in sugar manufacture but also represents a loss of sucrose. Refined cane sugar characteristically contains polysaccharides which precipitate from water solution on adding alcohol [9]. The resulting turbidity poses problems for distillers and wenders who produce cordial-type beverages which are high in both sugar and ethanol. The major polysaccharides are dextran, starch and indigenous sugar cane polysaccharides (ISP). The alcoholic component can also result in the precipitation of floc of any polysaccharides which may have escaped removal in refining.

In our country cane sugar is directly manufactured in a single step from cane juice by liming and sulphitation, whereas internationally, a two step process involving the manufacture of raw sugar followed by its purification to refined sugar is followed. There is hardly any study carried out to investigate the presence of any major polysaccharides other than starch or dextran in these granulated plantation white sugars. Such a study will on the one hand give information about the nature of polysaccharides if any present in our factory juices and on the other hand it will reveal the inadequacies of the present sugar manufacturing process and subsequently will guide to the means for its improvement. The success of this investigation may lead the sugar industry in our country to adopt improved clarification/refining techniques for the production of polysaccharide-free sugar, which will be most suitable for the needs of various industries.

MATERIALS AND METHODS

(I) Isolation of alcohol precipitable polysaccharide from plantation white cane sugar

To 200 gm sugar dissolved in 200 cm^3 water was added 5cm^3 of acetic acid and 600 cm^3 ethanol. The solution was filtered with suction through a ½ inch thick mat of 'Celite' analytical filter aid on a sintered glass funnel. The filter mat was washed with five 200 cm^3 portions of ethanol to remove sugars, then was washed again with two 200 cm^3 portions of boiling water to dissolve the precipitated polysaccharides and recovered again using ethanol to get the crude polysaccharide.

(ii) Purification

Polysaccharide (500 mg) dissolved in 50 ml of 0.02 M phosphate buffer (pH- 7.0) was incubated under a few drops of toluene for 20h at 38^0C with 0.3 ml of dextranase and 1.0 mg of α-amylase. After the incubation, ethanol (150ml) was added and the resulting precipitate was collected by centrifugation. The precipitate was dissolved in water and dialyzed against deionised water for 72 h. The water soluble portion dialyzed obtained as just described was divided into 3 fractions by precipitation with ethanol; insoluble's in 50% of aqueous ethanol, insolubles in the ethanol concentration range 50-67.5% and those soluble in 67.5% ethanol. The water soluble fraction 10.5% precipitated in ethanol in the concentration range 50-67.5%.The compositions of the fractions obtained are shown in table-I.

Table – I Proportions of neutral monosaccharides present in the various fractions produced during the isolation of polysaccharides from sugar crystal.

Sample origin	Proportion of neutral monosaccharides %						
	Rha	***Fruc.***	***Ara***	***Xyl***	***Man***	***Gal***	***Glu***
Crude plant extract (A)	4	2	36	5	8	34	10
1 vol. ethanol ppt. of A	1	1	25	10	13	31	20
2 vol. ethanol ppt. of A	2		42	4	01	43	07
Fractions not pptd. by 2 vols.	1	1	38	6	18	24	13
G.P.C. on Sepharose CL-6B	2		43	4		47	03
G.P.C. on Sephacryl S-300	2		43	4		51	

RESULTS AND DISCUSSION

The major fractions, the 0-50% ethanol precipitate representing 67% of the starting material, was 64% protein (from the relationship elemental N×6.25) and 2.2% carbohydrate (by phenol – sulphuric acid colorimetric against a reference of glucose).

Chromatography twice of the 50-67% ethanol fraction (0.44g) on sepharose CL – 6B in 7M area on a 90×2.5 cm column led to the recovery of 0.33 g of polysaccharides and a small proportion of material absorbing UV radiation at 278 nm.

STRUCTURAL ANALYSIS

Methylation was effected by the Hakomori method [10]. the methylated polysaccharides (2-10mg) was dissolved in 2ml of 1.5 m HCl in dry methanol and the mixture in sealed tube was kept for 22h at 85^0C. The mixture was made neutral with silver carbonate, filtered and the filtrate was made deionised with Amberlite IR – 120 (H^+ form) resins and evaporated to dryness under diminished pressure at 40^0C. The resuling methyl glycosides were then hydrolysed with 2 ml of 0.6 M sulphuric acid for 4 hours at 100^0C. The hydrolyzed was reduced with sodium borohydride and then actylated and the alditol acetates were analysed by G.L.C. and also by GC-MS. with a 063 Hitachi gas chromatograph connected to a RM – 50 GC Hitachi GC mass spectrometer. A glass WCOT column (0.25mm×30m) coated with PEG 20 M was used isothermally at 180^0C. The helium flow rate regulated at 1.0 ml/min with a split ratio of 30:1. The temperature on the ion source was 200^0C and the spectra were recorded at 70 eV. G.L.C. – M.S. analysis of the alditol acetates was carried out Methylation data is summarized in table II [11] and is based on separation shown in Fig. – 1.

Table II Methylation analysis of polysaccharide from cane sugar

Peak No[a].	Methylated sugar[b]	T (OV-225)[c]	T (ECNSSM)[c]	Mole (%)[d]
1	2,35 – Ara	0.50(0.41)	0.52(0.48)	35.6
2	3,5-Ara	0.81 (0.80)	0.93 (0.91)	1.5
3	2,3-Ara	1.06(1.07)	1.25(1.25)	8.8
4	2,3,4,6 –Gal	1.19(1.19)	1.25(1.25)	6.0
5	2,4,6- Gal	2.00 (2.03)	2.25(2.28)	13.1
6	2,3,4-Gal	2.74(2.89)	3.32(3.41)	4.5
7	2,4-Gal	5.00 (5.1)	6.4(6.35)	30.5

(a) As labelled in Fig. 3 (b) 2,3,5, Ara ≅1,4-di-O-acetyl-2,3,5-tri-O-methylarabinitol etc. (c) Retention times of the corresponding alditol acetate relative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol on OV – 225 at 170 and ECNSS-M at 150. Times were actually calculated from the retention time of 2,3,4,6- Gal. Figures in parentheses are retention time from the literature [12]. (d) Calculated from integrated areas measured on OV-225.

The glycosidic linkages involved in the structure have been established, but their distributions are fine structures are not known. From the data, the arabinogalactan is envisaged as having a framework of (1-3) linked β-D- galactose residues of which two out of every three carry a galactosyl or aralunosyl side chain attached at O-6.

The essential structural features of the arabinogalactan establihses it as a type widely distributed in plant world. The negative specific rotation ($[\alpha]^{20}_{589}$-56^0) indicates the β-configuration inD. galactose and α in L -arabinose.

The arabinose occurs only in furanose form. This conclusion is supported by data from partial acid hydrolysis and n.m.r spectroscopy [13]. Partial acid hydrolysis involved two procedures as described elsewhere [13, 14]. In the first instance the mild hydrolytic step released only arabinose but significant quantities were also present in total acid hyrolyzate. In the second procedure, all arabinose was cleaved in the mild acid stage, but some scission of galactose occurred as well. Oligosaccharides were also present when examined my paper chromatography fail to produce but any pink colour characteristic of pentoses when sprayed with p-anisidine hydrochloride.

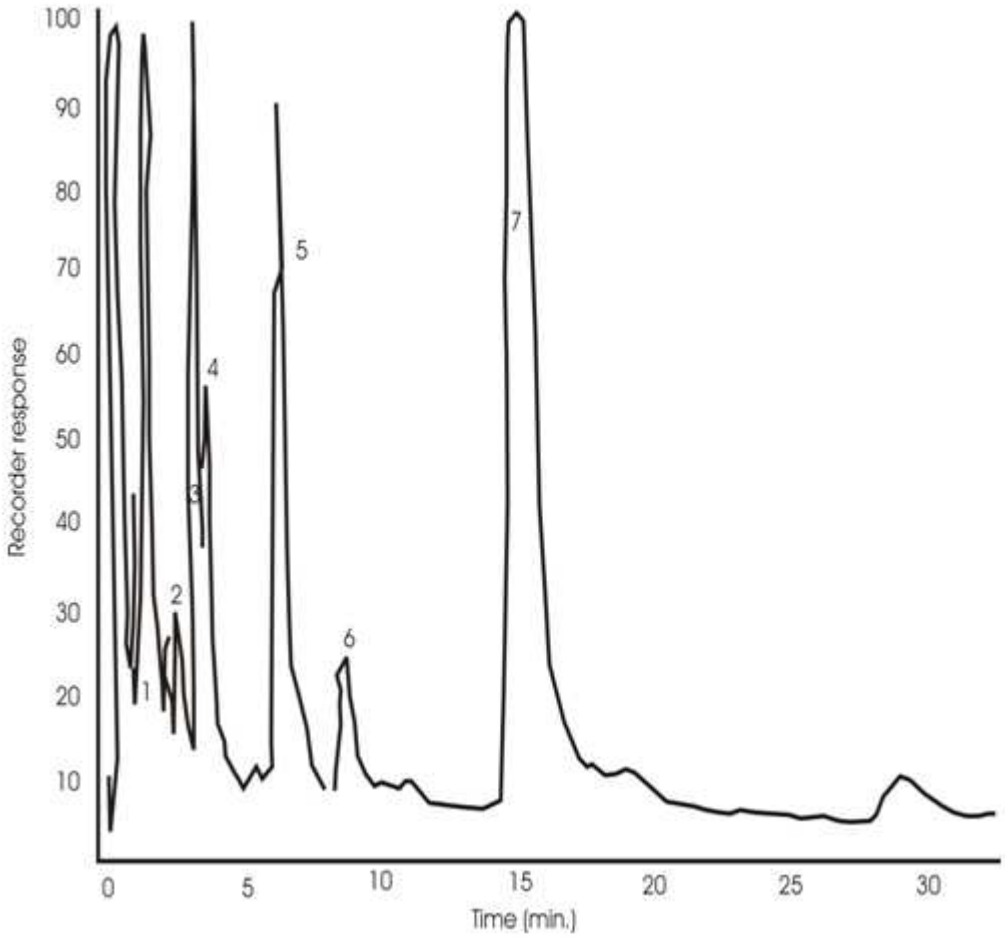

FIG 1. Gas chromatogram of methylation products of the polysaccharides from cane sugar on 3% OV/-225 of 170. Retention times of the numbered peaks are shown in table II

REFERENCES

[1] G E Sloane, *Sugar Journal*, **1977**, 40, 2, 9-12.
[2] M A Godshall, M A Clarke, C D Dooley and E J Roberts, Proc *Sugar Industry Technologists*, **1987**, 46, 193-211.
[3] F Donald Charles, *ISJ*, **1984**, 86, 1024.
[4] Roberts and Martin, *Proc. 6th. Tech, Sess. Bone. Char. Res. Prof*, **1961**, 67-88.
[5] J B Alxander and M Matic, *Proc. Tech. Sess. Cane Sugar Refining Res*, **1974**, 13-28.
[6] E C Vignes, *Proc. Intern. Soc. Sugar Cane Technologists*, **1974**, 15, 1288-1295.
[7] J P Murray, *Proc. Ann. Cong. S. African Sugar Tech. Assoc.* **1972**, 46, 116-132.
[8] J Bruijn, *International Sugar Journal*, **1970**, 72, 195-198.
[9] E J Roberts, J T Jackson and J H Vance, *Tech. Sess. Cane sugar Refining Res*. **1964,** 75-84.
[10] S Hakomori, *J Biochem* (70 Tokyo) **1964**, 55, 205-208.
[11] V Kovacik, K Linck and R Sandtnerova. *Carbohydrate Res*. **1974**, 38, 300-304.
[12] K R Hanson. *Proc. Sugar Ind. Tech*, **1980**, 39, 152-159.
[13] M L Walfrom and C C Christman, *J. Am. Chem. Soc*. **1931**, 53, 3413-3419.
[14] L Mester and G Vass, *Tetrahedron Lett*. **1968**, 5191-5193.