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Polysaccharide structure of degraded glucomannan from *Abrus precatorius* Linn. seeds

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Abstract: *Degraded glucomannan was isolated from Abrus precatorius Linn. seed polysaccharide (Papilionaceae). Acid hydrolysis and methylation studies produced certain degraded methyl sugars as, 2, 3, 6-tri-O-methyl-D-glucose and 2, 3, 6-tri-O-methyl-D-mannose in 1:4 molar ratio. On the basis of hydrolysis and methylation experiments, a polysaccharide structure has been assigned to the degraded glucomannan and to the parent glucomannan of Abrus precatorius Linn. seed.*

Key words: *Degraded methyl sugars, Abrus precatorius, Polysaccharide*

Introduction

Abrus precatorius Linn. plant (Chadha, 1988), belongs to the family-Leguminosae, sub family- Papilionaceae, is a native of India and East and West Indies. It is known as Ratti or Gumchi in Hindi. Plant is used in Ayurvedic system of medicine, like leaves extracts for leucoderma, seed abrin as purgative and abortive and root extract in cough. Seeds are used as weights by Indian gold smiths since ancient times. Seed contains a water soluble sugar extract as D-glucose and D-mannose in 2 : 5 molar ratio. Preliminary investigation on the nature of constituent glucomannan (Singh *et al*., 2004a), methylation, periodate oxidation (Singh *et al*., 2004b), determination of polyalcohols by Smith degradation method (Singh *et al*., 2004c) and structure elucidation of oligosaccharides (Singh and Shelley, 2003) have been reported for the parent polysaccharide structure. Present manuscript mainly deals with the isolation of degraded glucomannan and methylation studies for proposing a possible structure of degraded *Abrus precatorius* Linn. seeds glucomannan.

The commercial use of glucomannan are in the various industries linked with the food items are in sugar, textile, pudding, pastry, ice-cream industry *etc.* Seeds glucomannan will also be explored for their air pollution minimising capacity in the environment.

Materials and Methods

The experiments stated that all evaporation were carried out at 45-50°C under reduced pressure. Optical rotations are measured for equilibrated solutions and melting points are not corrected. Paper chromatography were carried out by descending technique (Partridge, 1946) on whatman no. 1 and 3 mm paper for the detection of degraded methyl sugars, using upper phase of the following solvent mixture (v/v) : (S_1) *n*-butanol, acetic acid and water in 4 : 1: 5 (Partridge and Westall, 1948); (S_2) *n*-butanol, ethanol and water in 4 : 1 : 5 (Hirst and Jones, 1949), (S_3) benzene, ethanol and, water in 169 : 4 : 45 (Andrews *et al*., 1952) and (S_4) butanone, ethyl acetate, water and ammonia in 80:20:8:1 (Kapoor and Mukherjee, 1969). The spray reagent (R) *p*-anisidine phosphate (Mukherjee and Srivastava, 1952) was used for detection of methyl degraded sugars. The R_{gal} and R_{glu} refer to the rate of movement of sugars relative to D-galactose and D-glucose. Degree of polymerization (DP) was determined by Timell's method (Timell, 1960).

Isolation of degraded glucomannan : Glucomannan (14 gm) was hydrolysed (Parikh and Jones, 1966) with H_2SO_4 (1.5 N, 350 ml) on water bath (48 hr) under controlled conditions. Hydrolysate was filtered neutralized ($BaCO_3$) then concentrated to syrup (50 ml). Ethanol (250 ml) was poured into the solution to precipitate out the degraded sugars filtered and residue washed with ethanol, acetone then dried to yield (9.5 gm), a mixture of D-glucose and D-mannose. Degraded glucomannan washed several times with ethanol (15%) to remove the traces of oligosaccharides. Residue dissolved in water (100 ml) and added ethanol (25 ml) to produce a precipitate which was centrifuged out. Degraded glucomannan was precipitated out from centrifugate by adding ethanol (80 ml), filtered and washed with ethanol and acetone then dried. It was obtained as amorphous powder, yield (8.1 gm) and DP was found to be 18.

Hydrolysis of degraded glucomannan: Degraded glucomannan (0.8 gm) was completely hydrolysed (Parikh and Jones, 1966) with H_2SO_4 (1 N, 16 ml) on water bath (30 hr) at 100°C in a sealed tube. The obtained hydrolysate was neutralized with $BaCO_3$, filtered and filtrate concentrated to a thin syrup. It revealed D-glucose and D-mannose by paper chromatography on whatman no. 1 paper in solvent (S_1).

Quantitative estimation of degraded sugars: Degraded sugar mixture were quantitatively (Hirst and Jones, 1949) separated by paper chromatography on whatman no. 3 mm paper in solvent (S_2) and sugars component were estimated by alkaline hypoiodite method (Hirst *et al*., 1949). These degraded sugars showed that D-glucose and D-mannose were present in 1 : 4 molar ratio.

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Methylation of degraded glucomannan: Degraded glucomannan (3.5 gm) was methylated by Hakomari's method (Hakomari, 1964) with dimethyl sulphate (170 ml) and sodium hydroxide solution (45%, 140 ml) by three successive treatments to afford the partially methylated light yellow compound. It was further remethylated by dimethyl sulphoxide method (Srivastava *et al*., 1964) on ice bath. Methylated glucomannan was extracted with $CHCl_3$ then it evaporated to a glassy solid mass (2.35 gm). It found -OCH_3 group 43.2% (Belcher *et al*., 1944), which showed a slight absorption band at 3500-3600 cm^{-1} of hydroxyl group in IR-spectra. It was further remethylated by Purdie's reagent (Purdie and Irvine, 1903) with acetone, methyl iodide and silver oxide to furnish a fully methylated degraded product (1.9 gm). It found -OCH_3 group 42.6%, which did not show any peak of hydroxyl group at 3500-3600 cm^{-1} region in IR-spectra (Rao, 1963).

Formation of degraded methyl glucomannan: Degraded methyl product (2 gm) was extracted with petroleum ether (40-60°C) in 70 ml and chloroform (30 ml) containing increasing proportion of the latter solvent. At every stage the degraded residue was gently refluxed with solvent mixture (3 hr). It obtained four sugar fractions, out of them 1-2 are oily while 3-4 are crispy solids and physical contents of each fraction are given in Table 1.

Hydrolysis of degraded methyl glucomannan: Degraded methyl product obtained from Table 1 (Fr. 3-4) was hydrolysed (Whistler, 1965) with sulphuric acid (72%, 20 ml) at room temperature (2 hr). Content were diluted with distilled water to make up a 12% concentration of H_2SO_4 and left over night then concentrated to a thin syrup. Paper chromatographic analysis of syrup on whatman no. 1 paper in solvent (S_3) and used (R) as spray reagent to revealed the presence of two degraded methyl sugars which were identified as: (I) 2, 3, 6-tri-O-methyl-D-glucose and (II) 2, 3, 6-tri-O-methyl-D-mannose.

Mixture of degraded methyl sugars were separated by paper chromatography on whatman no. 3 mm paper in solvent (S_2) and corresponding methyl sugar strips were cut out with the help of guide spots and eluted with water (Dent, 1947). These fractions were isolated in the form of syrup which dissolved in water and decolourised with animal charcoal afterwards it evaporated to obtain colour less syrup and methyl sugar fractions were identified as follows.

2, 3, 6-tri-O-methyl-D-glucose: Syrup (450 mg) gave a single spot on paper chromatogram in solvent (S_1), $[\alpha]_D^{27}$+ 56°C (H_2O), R_g 1.00 (S_4) and R_f 0.82 (S_1). Product showed the presence of D-glucose by paper chromatography on whatman no. 1 paper on demethylation. It was converted into 2, 3, 6-tri-O-methyl-D-glucose-γ-lactone derivative having m.p. 146-147°C.

2, 3, 6-tri-O-methyl-D-mannose: Syrup (350 mg) had $[\alpha]_D^{27}$+ 69°C (H_2O), R_g 0.96 (S_4) and R_f 0.62 (S_1). Demethylation of product gave D-mannose on paper chromatography. Fraction was converted into 2, 3, 6-tri-O-methyl-D-mannose phenyl hydrazone derivative having m.p. 131-133°C Lit. m.p. 130-132°C (Unrau, 1961).

Quantitative estimation: Degraded methyl sugar mixture was quantitatively (Hirst *et al*., 1949) separated by paper chromatography on whatman no. 3 mm paper. Each methyl sugar fraction were estimated by alkaline hypoiodite method (Hirst and Jones, 1949). It was found that 2, 3, 6-tri-O-methyl-D-glucose and 2, 3, 6-tri-O-methyl-D-mannose were present in 1 : 4 molar ratio.

Results and Discussion

Water soluble degraded glucomannan was isolated from *Abrus precatorius* Linn. seeds after partial acid hydrolysis studies. It composed of D-glucose and D-mannose in 1 : 4 molar ratio by paper chromatography. Degree of polymerisation of glucomannan showed that the molecule was composed of 18 anhydrohexose sugar units. Upon acid hydrolysis of fully degraded methyl glucomannan yielded 2, 3, 6-tri-O-methyl-D-glucose and 2, 3, 6-tri-O-methyl-D-mannose in 1 : 4 molar ratio. From the parent methylated glucomannan also composed of methyl sugars as: 2, 3, 4, 6-tetra-O-methyl-D-glucose; 2, 3, 6-tri-O-methyl-D-glucose; 2, 3, 6-tri-O-methyl-D-mannose and 2, 3-di-O-methyl-D-mannose were quantitatively present in 1 : 1 : 4 : 1 molar ratio. The main chain length or backbone of degraded polymers smaller (DP 18). Ratio of terminal non reducing D-glucose unit to that in the main chain is not negligible in composition to the terminal D-mannose unit. This is also confirmed by the presence of 4-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose (Singh and Shelly, 2003) in oligosaccharide studies of the parent glucomannan.

This suggests that the degraded glucomannan is represented by a chain of D-glucose and D-mannose units in the main polymer chain. After every ten repeating unit, D-glucose is present as non reducing unit in a terminal position on degraded glucomannan. The D-glucose and D-mannose units are linked through (1→4)-β-type linkages with repeating unit (1 : 4). Specific

Table - 1: Fractionation of degraded methyl glucomannan from *Abrus precatorius* Linn

Fr. no.	State of methyl sugar	Solvent composition (%)		Yield (gm)	-OCH_3 (%)	$[\alpha]_D^{27}$ (H_2O)
		Pet. ether	$CHCl_3$			
1.	Oily liquid	100	00	0.0846	-	-
2.	Oily liquid	90	10	0.8424	-	-
3.	Crispy solid	80	20	1.2628	48.6	+69°
4.	Crispy solid	70	30	0.9842	42.8	+56°

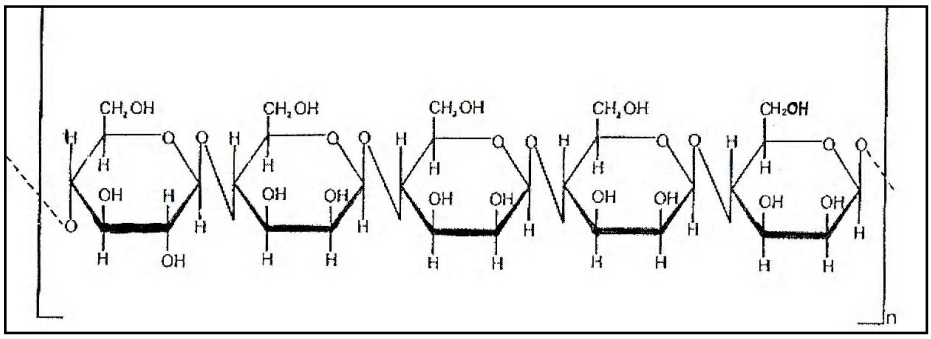

Fig. 1: Polysaccharide structure of *Abrus precatorius* Linn. seeds degraded glucomannan

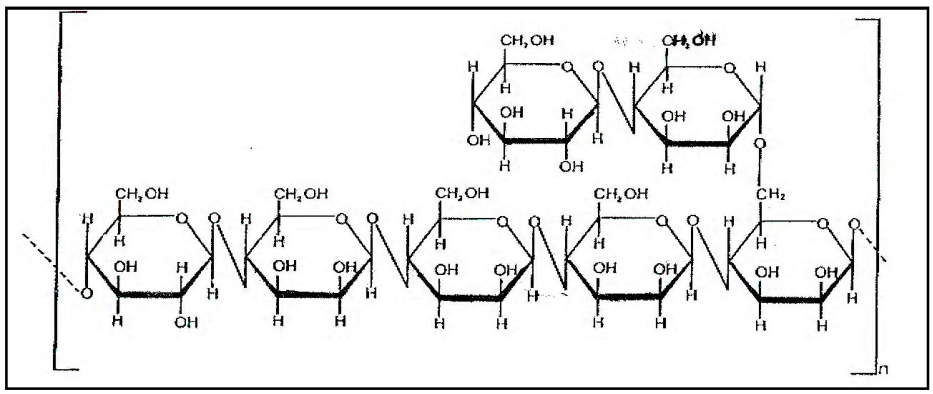

Fig. 2: Polysaccharide structure of *Abrus precatorius* Linn. seeds glucomannan

rotations of methyl derivatives showed that the interglycosidic linkages are of β-type.

The structure of degraded glucomannan from *Abrus precatorius* Linn. seed may be proposed on the basis of above finding methylation results (Fig. 1). In accordance with the previous data (Singh *et al*., 2004; Singh and Shelley, 2003) and analogy with the structure of oligosaccharides and degraded glucomannan. It is proposed that the parent glucomannan is composed of anhydrohexose sugar unit of which D-mannose is in the main chain while D-glucose at reducing end of the chain. On the basis of the above finding methylation results, a tentative polysaccharide structure of *Abrus precatorius* Linn. seed glucomannan has been confirmed (Fig. 2).

Glucomannan of *Abrus precatorius* Linn. are recognised as being beneficial for reducing heart disease by lowering cholesterol and reducing the glycemic response and commercially used to modify food textile and as fat substitute. It is used as a thickener for sauces, to prevent ice crystal formation in ice-cream and as a low calorie substitute for fat. Polysaccharides are capable of producing relatively stable low density or high density aqueous micro-environments. Polysacchande structure making salts concentrate is less structured aqueous phase and decrease the compatibility between hydropholic polymers, whilst structure breaking salts concentrate in more structured environments and increase such compatibility.

Glucomannan is used as a hunger suppressant because it produces a feeling of fullness by creating very viscous solution that retards absorption of the nutrients in food. The hydrolysis of acetate groups favour the formation of inter-molecular hydrogen bonds that are responsible for the gelling action.

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