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EXTRACTION, PURIFICATION AND CHARACTERIZATION OF A GALACTOMANNAN FROM *PROSOPIS JULIFLORA* (SW.) DC. SEED POLYSACCHARIDES

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Abstract: *Prosopis juliflora* (Sw.) DC. an Indian fast growing and spreading tree of which pods, leaves are extensively used for various applications. The seeds contain about 22% endosperm and possess the characteristics of becoming a potential source of seed gum. The structural aspects of the galactomannans have been determined for a better understanding of its properties. The purified seed polysaccharide has been characterized as a pure galactomannan having a mannose-galactose ratio of 1:1.4 and the average molecular weight (Mw) is 10.7 x 10^5 D. Partial hydrolysis of the polysaccharide furnished one hepta-(I), one octa-(II) and nona-(III) saccharides. Hydrolysis of oligosaccharide I, II and III followed by GLC analysis furnished D-galactose and D-mannose in the ratio 3:4, 3:5 and 5:4 respectively. Methylation analysis, periodate oxidation, smith degradation and NMR studies confirm that the gum has the basic structure of legume galactomannans with a main chain of (1→4) -linked β-D-mannopyranosyl units to which galactose units are attached at O-6.
Keywords: Prosopis juliflora (Sw.) DC; Galactomannan; Polysaccharide; Oligosaccharide; Structure; Galactopyranose; Mannopyranose.

1. Introduction

The seed galactomannans (Dea & Morrison, 1975), commonly known as seed gums belongs to very important class of neutral polysaccharides which find widespread applications in industries (Whistler, 1990) and interaction studies. These vegetable products are found in seeds of leguminous plants as cell wall storage component and energy reserve which is produced in large amounts for international consumption. They are used as such or as modified derivatives. In mixture with some other polysaccharides, they show very interesting rheological properties. They are used in the fields of paper, textile, pharmaceuticals, cosmetics, food and oil recovery industries. Current international trend demands the introduction of alternative source of seed gums. Galactomannans have the fundamental structure consisting of a main chain of β-(1→4) -D-mannopyranose units substituted by single α-D-galactopyranose units at O-6, although there are few deviations

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from this basic structure. They differ from each other in mannose: galactose ratio and fine structure regarding distribution of galactose branches on the main chain, thereby causing variations in solubility, rheology and other properties. *P. juliflora* commonly known as mesquite, vilayti babul, vilayti kikar belongs to family leguminosae sub family (mimosoidae). It is a large shrub to a small evergreen tree. Usually tree attains a height of 9-12m and a girth of 90cm. Under favourable conditions the tree attains a height of 18m (Tewari, 1995). The seeds of the plant are found as weed in Rajasthan. The seeds contain about 22% endosperm which is a rich source of galactomannan polysaccharide. In view of this high content of endosperm gum, it was subjected to structural characterization. However, few reports are there for galactose/mannose ratio in prosopis juliflora such as (Pinto Vieira et. al., 2007) reported NMR study of galactomannan from the seeds of prosopis juliflora, (Figueiredo, 1983) reported chemical structure from defatted flour, (Cruz Alcedo, 1999) reported production and characterization of prosopis seed galcatomannan and (Buckeridge et. al., 1995) reported galactose/mannose ratio in various seed galactomannans. This is the first report on the detailed structural elucidation of a galactomannan isolated from the seeds of *Prosopis juliflora* (Sw.) DC.

2. Results and Discussion

2.1. Isolation and Purification

The seeds of *Prosopis juliflora* were collected from Jodhpur, Rajasthan. The seeds were harvested in the month of May/ June. The moisture content of seeds was found to be 11%. The seeds were air dried in shade to moisture content of 0.09%. Endosperm (20 g) was isolated by removing seed coat and germ by impact grinding using high speed domestic grinder. Seed coat was soaked in cold water for 3h and endosperm was isolated from swollen seed coat by separating the seed coat with the help of forceps and was stirred vigorously in distilled water (1000 ml) for 5h at room temperature and centrifuged to remove water insoluble impurities. The supernatant solution was poured into three times its volume of ethanol with constant stirring. The polysaccharide was precipitated out in the form of a fluffy precipitate. The polysaccharide was purified by fractionation from 0.8% aqueous solution by increasing the concentration of ethanol stepwise; nearly 90% of the initial weight precipitation at an ethanol contraction between 22 and 26% (wt. of ethanol/wt. of solution); no significant precipitation was obtained at lower or higher concentration (upper limit 50%). It was filtered under vacuum and dried in vacuum desiccator at room temperature.

The polysaccharide (18.0g) so obtained was deionised by passing the aqueous solution successively through the columns of freshly regenerated cation [Dowex-50W-X8] and anion [Seralite-SRA-400] exchange resins in the ratio 1:2 (w/w Ratio of polysaccharide to cation /anion exchange resins). The columns were washed with distilled water until the washings showed a negative Molisch test for carbohydrates. The combined eluents were concentrated to small volume [1/4th] and subjected to further purification by dialysis. In the process, the concentrated product was transferred into a cellophane bag and dialyzed for 72h in running water. The dialyzed product was concentrated and re-precipitated with a large volume of ethanol to obtain finally the pure polysaccharide. It was kept overnight, alcohol was decanted off, and the precipitated polysaccharide was dried by treating with solvent ether, acetone and absolute ethanol thereby removing inorganic impurities. It was filtered and lyophilized at -40^{o}C to obtain finally the pure polysaccharide in the form of a white amorphous powder (16.1g).

2.2. Galactomannan Characterization

The purified galactomannan was a white, fibrous material. It appeared to be homogenous by exclusion chromatography by passing it through sephadex G-150 and had $[\alpha]_D 16+112^0$ (c 0.1%, H_2O), ash content 0.1%, nitrogen 0.33%, easily water soluble, pH 6.90, free from methoxyl, sulphur, uronic acid, and did not reduce Fehling solution. The molecular weight of polysaccharide was found 10.7×10^5D.The ratio of the constituent sugars was determined by complete hydrolysis of the polysaccharide with sulphuric acid (2N, 18h) followed by GLC Jannson et. al. (1976) (corresponding alditol acetate derivative) using column ECNSS-M (3%). The polysaccharide has been characterized as a pure galactomannan comprising D-galactopyranosyl and D-mannopyranosyl units.

Prosopis juliflora seed endosperm polysaccharide upon partial hydrolysis with dilute sulphuric acid (0.05N, 3h) furnished a mixture of oligosaccharides along with monosaccharides. Preliminary paper chromatographic examination of the hydrolyzates revealed the presence of three oligosaccharides along with 2 monosaccharides, D-galactose and D-mannose. The Rgal values of oligosaccharides were 0.31, 0.19 and 0.11 respectively in the solvent system S_2. From this mixture, the oligosaccharides were separated by preparative chromatography on whatmann No.3 mm sheets and each oligosaccharide eluted separately and the elutes combined to isolate pure polysaccharide. The homogeneity of the oligosaccharides was checked by paper chromatography using organic solvent systems S_1, S_2

and S_3 and spray reagents R_1 and R_2. The degree of polymerization of three oligosaccharides corresponds to one hepta, one octa and one nonasaccharides.

2.3. Structural Studies

The purified polysaccharide and oligosaccharides were converted into its fully methylated derivative using two successive Hakomori methylations (Hakomori, 1964) followed by two subsequent purdie (Purdie& Irvine, 1903) methylations for complete etherification. They were hydrolysed, converted to alditol acetate derivatives and analysed by GLC. Alditol acetates of the hydrolyzed material were prepared by the method of (Jannson et al., 1976). Sodium borohydride (0.020g) was added to hydrolyzates, and the mixture was kept for 18h at room temperature. The mixture was neutralized by slow addition of dilute acetic acid (6ml), and concentrated to dryness in vacuum rotator at 40^0C. Sodium was removed by passing it through cation exchange resin (Dowex-50 W-X8). Boric acid was removed by co distillations, in the vacuum rotator with methanol (3 × 5ml). The residue was treated with redistilled acetic anhydride and pyridine, 1:1 (4ml) and refluxed for 6h. Toluene (6ml), which gave an azeotrope with acetic anhydride, was added and the mixture was distilled as above, until the rate of distillation decreases. A new portion of toluene (6ml) was added and the solution was concentrated to dryness. It was dissolved in water (10ml) and the acetylated sugars separated by shaking with dichloromethane (4 × 25ml). Traces of water present in dichloromethane were removed by adding anhydrous sodium sulphate followed by filtration and washing with dichloromethane before concentration. The proportions of resultant sugars obtained are presented in Table-1.

Table-1: Relative retention time and Approximate molar proportion of the different partially methylated sugars obtained from *Prosopis Juliflora* methylated polysaccharide.

Sugars	Rt*	Approximate molar proportion	Nature of linkages
2, 3, 4, 6-tetra-O-methyl-D-galactose	1.20	10	$-C_1$
2, 3, 6-tri-O-methyl-D-mannose	2.20	4	$-C_1,-C_4$
2, 3-di-O-methyl-D-mannose	4.83	10	$-C_1,-C_4,-C_6$

* Retention Time of the methylated sugars is with respect to 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol.

Isolation of 2, 3, 6- tri-O-methyl- D- mannose (4moles) and 2, 3-di-O-methyl- D-mannose (10 moles) indicated that the main chain is composed of β- (1→4)- linkages and the

polymer is branched. The presence of 2, 3, 4, 6-tetra-O-methyl galactose (10moles) showed that the non-reducing single galactose units are attached to the branched mannose units through α-(1→6) - glycosidic linkages. On the basis of the methylation analysis, it can be concluded thatthe gum possesses the basic structure of a galactomannan having a main chain of (1→4)-linked mannopyranosyl units with single side chains of galactopyranosyl units attached to the main chain through (1→6)-linkages. By methylation analysis, the galactose: mannose ratio was found to be 1:1.4, which is in close agreement with the results of chemical analysis.

Evidence supporting the presence of 1→4 and 1→6 linkages in the framework of polysaccharide has been obtained from the results of periodate oxidation (Abdel-Akher& Smith, 1951; Bobbit, 1956; Dayer, 1956; Halsall et al. 1947; Malprade, 1928; Rankin & Jeanes, 1954).The gum consumed 1.43 mol of periodate per hexosyl unit, with concomitant liberation of 0.43 mol of formic acid per hexosyl unit. Completion of periodate reaction with reference to uptake of periodate required 8days as the reaction was carried out at4^0C to avoid overoxidation. Moreover, the oxidation of mannopyranosyl units is slow owing to hemiacetal formation (Painter et al. 1979; Ishak & Painter, 1973). The periodate oxidation results are in good agreement with the theoretical values of the proposed galactomannan structure based on methylation analysis. The IR spectrum of the purified polysaccharide absorption bands at 814 and 871cm^{-1} indicating the presence of α- linked D-galactopyranosyl and β- linked D mannopyranosyl units respectively (Barker et al. 1956).Resonances of the anomeric protons in ^{1} H NMR spectroscopy are well separated and identified. The doublet at δ 5.4 (H-1 of D-galactopyranosyl units) (J1,2~Hz) and the singlet at δ 5.1 (β-D mannopyranosyl units) (Gupta et. al., 1987) indicate that the D-galactopyranosyl and D-mannopyranosyl units in the polymer could have the $^4C_1$conformation (Kapoor & Chaubey, 2001) (Duss et. al., 2000) (Fig.1).

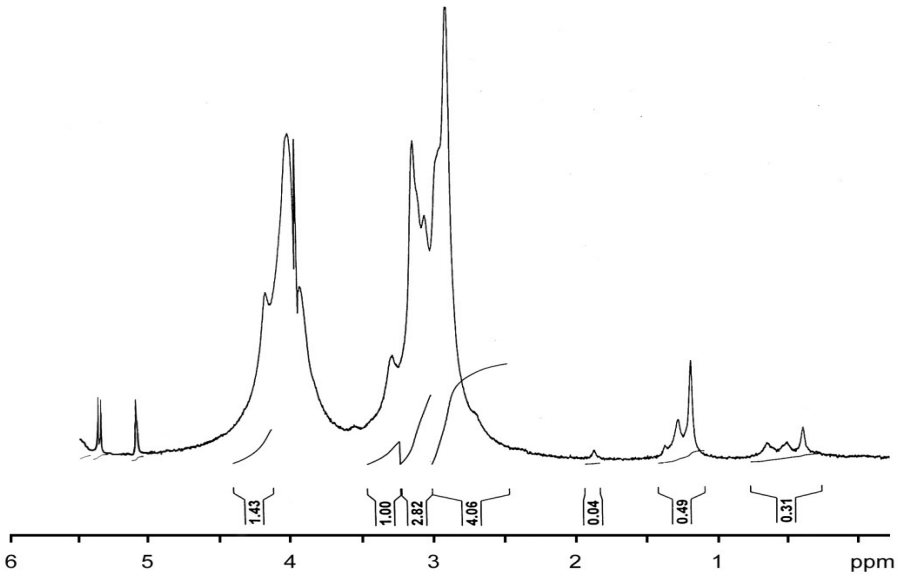


Fig.1 ^{1}H NMR Spectrum of Polysaccharide

Structural aspects of the galactomannans were also analysed by ^{13}C NMR spectroscopy (Fig.2). The gum forms viscous solutions and successful spectroscopy was possible only after sonication.

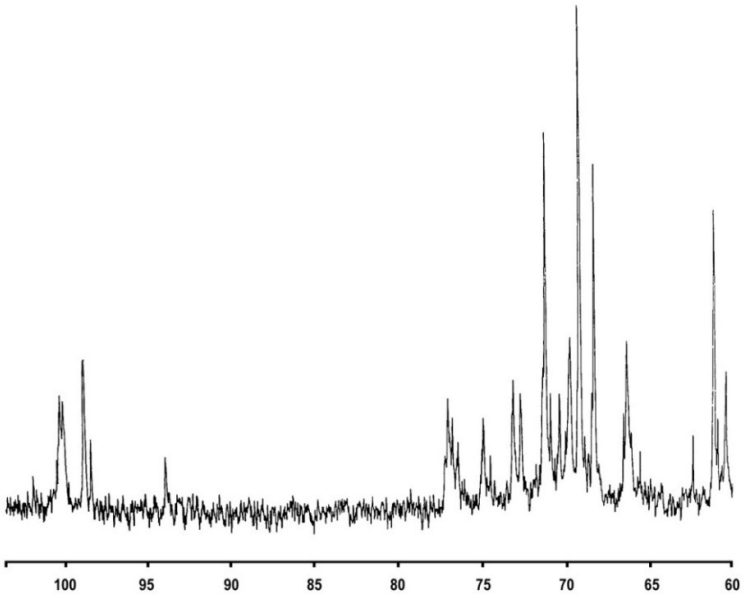


Fig.2 ^{13}C NMR Spectrum of Polysaccharide

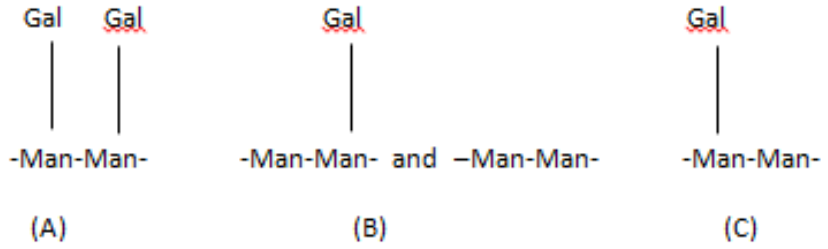
Resonances of all carbon atoms are fully resolved and well identified. Spectra are in close agreement with those reported for other galactomannans (Table-2).

Table-2: Assignments of Peaks in the ^{13}C-NMR spectrum of *Prosopis Juliflora* Polysaccharide

Type of unit	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Galactopyranosyl	98.6	68.3	68.7	69.2	70.9	61.1
β-D-Mannopyranosyl residue, unbranched at O-6	100.1	69.7	71.2	76.3 76.7	74.9	60.4
β-D-Mannopyranosyl residue, branched at O-6	99.9	69.7	71.2	76.7 76.9	73.1	66.6 66.4

The spectrum showed a signal at δ 98.6 for anomeric carbon of galactose residues (Gorin & Mazurek, 1975). The anomeric carbon of D-mannose units appeared as almost completely resolved two signals at δ 100.1 and δ 99.9 which correspond to non substituted and substituted residues, respectively (Gorin, 1975). Resonances of C-2, C-3and C-5 of galactopyranosyl and mannopyranosyl units are well resolved lines and readily identified. C-4 of mannopyranosyl units produced three resolved signals corresponding to branching patterns A, B and C. (Scheme 1).

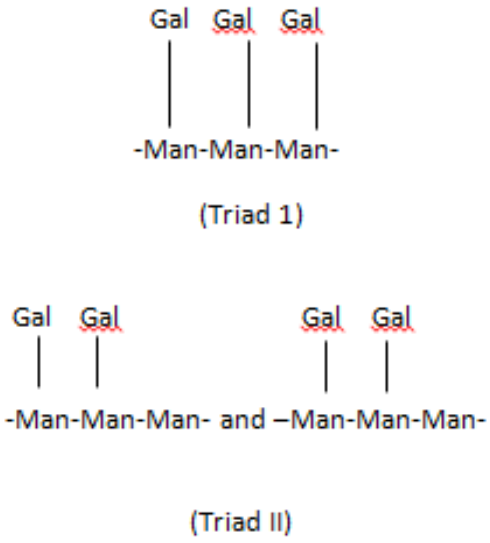
Branching pattern indicates the distribution of D-galactose on the mannan backbone. Signals in the triplet result from the three nearest neighbour probabilities of mannopyranosyl units in the main chain. In the spectrum, peak A (δ 76.9) is more prominent in comparison to B (δ 76.7) and A (δ 76.3).



Scheme 1 ^{13}C NMR spectral region of C-4 (mannose): A, B and C are probable diad interpretation.

It clearly indicates the larger proportion of the galactopyranosyl units in comparison to the mannopyranosyl units (Kapoor et al., 1989) (Grasdalen & Painter, 1990).It was observed that substituted mannose units are branched at O-6, the resonances of their C-6 were readily identified suggesting that peak at lowest field (δ 66.6 ppm) originates from the

C-6 resonance of the intermediate unit from groups of three contiguous, substituted mannosyl residues (Triad I, Scheme 2) and peak at (δ 66.4ppm) originates due to superposition of signals from triads wherein two contiguous mannosyl units are substituted (Triad II, Scheme 2) (Manzi et al., 1986).



Scheme 2 ^{13}C NMR spectral region of C-6 (mannose): Triad I and II are probable interpretation.

Structure of oligosaccharide-I

It was found to be a heptasaccharide [α] + 112^0 (c 0.05%, H_2O), m.p. 200-202^0C (d), that was homogenous and gave single spot [Rgal 0.31] using organic solvent S_2 and spray reagents R_1 and R_2 upon chromatographic examination. Upon complete acid hydrolysis, it gave D- galactose and D- mannose on paper chromatogram in solvent systems S_1, S_2 and S_3. The alditol acetates of the hydrolysates of oligosaccharide on GLC analysis under conditions C-1 showed two peaks corresponding to D-galactose and D- mannose in the molar ratio of 3:4.

The oligosaccharide was completely methylated by Hakomori method (Hakomori, 1964) followed by Purdie (Purdie & Irvine, 1904) for complete etherification. Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590-3225cm^{-1}). It was hydrolyzed and transformed into its alditol acetates according to the method of Jansson et al. (1976). GLC of the resulting alditol acetate under conditions C_1, furnished 2, 3, 4, 6- tetra-O-methyl-D-galactose, 2, 3-di-O-methyl-D-mannose, 2, 3, 4, 6-tetra-O-methyl-D-mannose in the molar ratio of 3:3:1, respectively.

On the basis of methylation study it was found that 2, 3-di-O-methyl-D-mannose and 2, 3, 4, 6- tetra-O-methyl-D-galactose indicate the presence of 1→4 and 1→6 linkages in this heptasaccharide. These results suggest that the oligosaccharide has the fundamental structural pattern having β- (1→4) main chain of D-mannose units and side chain of single α-(1→6)-D-galactose residues.

On the basis of above discussion, the heptasaccharide may be assigned the following plausible structure (Fig.3).

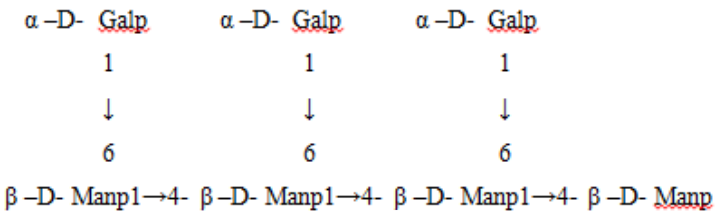


Fig. 3 Oligosaccharide- I

Structure of oligosaccharide-II

It was found to be an octasaccharide [α] + 86^0 (c 0.05%, H_2O), m.p. 218-220^{0}C (d), that was homogenous and gave single spot [Rgal 0.19] using organic solvent S_2 and spray reagents R_1 and R_2 upon chromatographic examination. Upon complete acid hydrolysis, it gave D- galactose and D- mannose on paper chromatogram in solvent systems S_1, S_2 and S_3. The alditol acetates of the hydrolysate of oligosaccharide on GLC analysis under conditions C-1 showed two peaks corresponding to D-galactose and D- mannose in the molar ratio of 3:5.

The oligosaccharide was completely methylated by Hakomori method (Hakomori, 1964) followed by Purdie (Purdie & Irvine, 1904) for complete etherification. Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590-3225cm^{-1}). It was hydrolysed and transformed into its alditol acetates according to the method of Jansson et al. (1976). GLC of the resulting alditol acetate under conditions C_1, furnished 2,3,4,6- tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-mannose, 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-mannose in the molar ratio of 3:3:1:1 respectively.

On the basis of methylation study it was found that 2, 3-di-O-methyl-D-mannose, 2, 3, 6-tri-O-methyl-D-mannose and 2, 3, 4, 6- tetra-O-methyl-D-galactose indicate the presence of

1→4 and 1→6 linkages in this octasaccharide. These results suggest that the oligosaccharide has the fundamental structural pattern having β- (1→4) main chain of D-mannose units and side chain of single α- (1→6)-D-galactose residues. On the basis of above discussion, the octasaccharide may be assigned the following plausible structure (Fig.4).

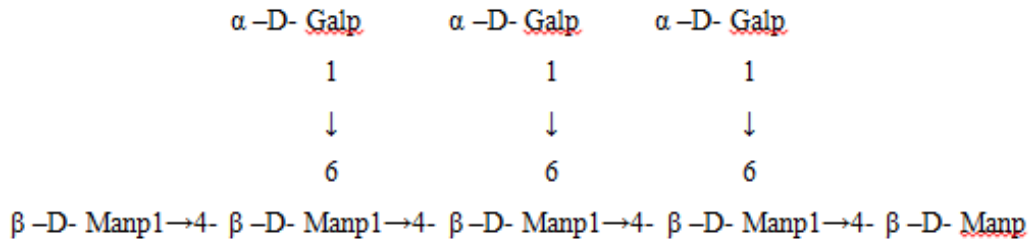


Fig.4 Oligosaccharide- II

Structure of oligosaccharide-III

It was found to be a nonasaccharide [α] + 129^0 (c 0.05%, H_2O), m.p. 240-242^0C (d), that was homogenous and gave single spot [Rgal 0.11] using organic solvent S_2 and spray reagents R_1 and R_2 upon chromatographic examination. Upon complete acid hydrolysis, it gave D- galactose and D- mannose on paper chromatogram in solvent systems S_1, S_2 and S_3. The alditol acetates of the hydrolysates of oligosaccharide on GLC analysis under conditions C-1 showed two peaks corresponding to D-galactose and D- mannose in the molar ratio of 5:4.

The oligosaccharide was completely methylated by Hakomori method (Hakomori, 1964) followed by Purdie (Purdie & Irvine, 1904) for complete etherification. Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590-3225cm^{-1}). It was hydrolyzed and transformed into its alditol acetates according to the method of Jansson et al. (1976). GLC of the resulting alditol acetate under conditions C_1, furnished 2,3,4,6- tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-mannose, 2,3,4,6-tetra-O-methyl-D-mannose in the molar ratio of 4:4:1 respectively.

On the basis of methylation study it was found that 2,3-di-O-methyl-D-mannose and 2,3,4,6- tetra-O-methyl-D-galactose indicate the presence of 1→4 and 1→6 linkages in this nonasaccharide. These results suggest that the oligosaccharide has the fundamental structural pattern having β- (1→4) main chain of D-mannose units and side chain of single α- (1→6)-D-galactose residues. On the basis of above discussion, the nonasaccharide may be assigned the following plausible structure (Fig.5).

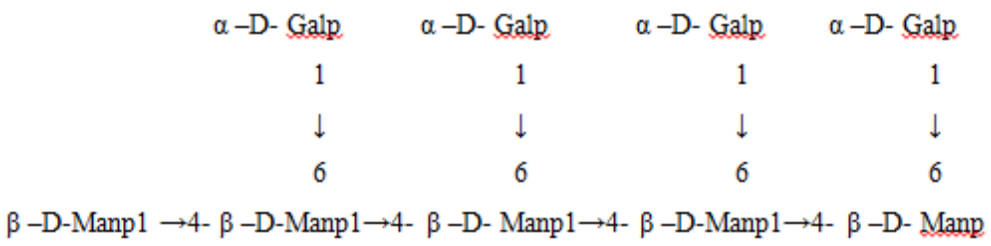


Fig.5 Oligosaccharide-III

3. Experimental

3.1. Analytical Methods

Evaporations were conducted under diminished pressure at < 45^0 (bath). Descending p.c. was done on whatman no. 1 and 3MM papers using 1-butanol-pyridine-water (6:4:3). Sugars were detected by aniline hydrogen phthalate (Patridge, 1949) and alkaline silver nitrate (Trevelyan, 1950). Constituent sugars were determined as the alditol acetates by GLC on ECNSS-M (3%) on Gas Chrom Q (100-120 mesh) packed into 5' X 1/8" stainless steel column in case of methylated sugars and BPX-70 capillary column with 0.22mm X 0.25µm in case of completely hydrolysed polysaccharides. The operating conditions employed for different sugar samples are listed below:

(C1) Column temperature 170^0C, nitrogen flow rate 35-40 ml/min

(C2) Column temperature 210^0C, nitrogen flow rate 2 ml/min

Authentic standards viz. 1, 5-di-O-acetyl-2, 3, 4, 6-tetra-O-methyl-D-glucitol, in the case of methylated sugars and a mixture of mannose and galactose in the case of alditol acetates of completely hydrolyzed oligosaccharides and polysaccharides were also run simultaneously under the same conditions to give the values of relative retention times Rt. N.M.R spectra were recorded on Bruker Avance II 400 instrument NMR spectrometer at 400 MHz. For ^{1}H-NMR, the galactomannan sample was firstly exchanged in D_2O by repeated evaporations of 1mg/ml solution and finally dissolved in high quality D_2O (99.96%D). Proton spectra were obtained at 80^0C under conditions of quantitative analysis. For ^{13}C-NMR spectra, 20mg/ml galactomannan was prepared inD_2O at 70^0C by continuous stirring for 6h followed by sonication for 10min. The solution was filtered and filled in NMR tube. Optical rotation was determined on Autopol-II, automatic polarimeter (Rudolph research, Flanders, New Jersey) at 589nm, D-lines of sodium.

3.2. Isolation and purification of the polysaccharide

The endosperm of the seeds of *Prosopis juliflora* contains the seed galactomannan. Cold water extraction of endosperm and continuous stirring with EtOH followed by centrifugation yielded a crude polysaccharide (~81%). Polysaccharide was purified by ion exchange followed by dialysis.

3.3. Investigation of the Polysaccharide

(a) Gel filtration: Gel permeation chromatography was performed on sephadex G-150 columns (superfine grade), Pharmacia column (56/70) using 0.02% solution of sodium azide as eluent [void volume (Vo) 40 ml; total column volume (Vt) 140 ml]. The flow rate was maintained at 0.5ml/min by using minipuls 2 peristaltic pumps and Gilson fraction collector (Model-202) programmed in time mode. Fractions (2ml) each were collected and monitored with anthrone sulphuric acid reagent (McCready et al., 1950) at 660nm using Chemito UV-Vis spectrophotometer UV-2500. The graph of the absorbance v/s fractions collected was plotted (Fig.6).

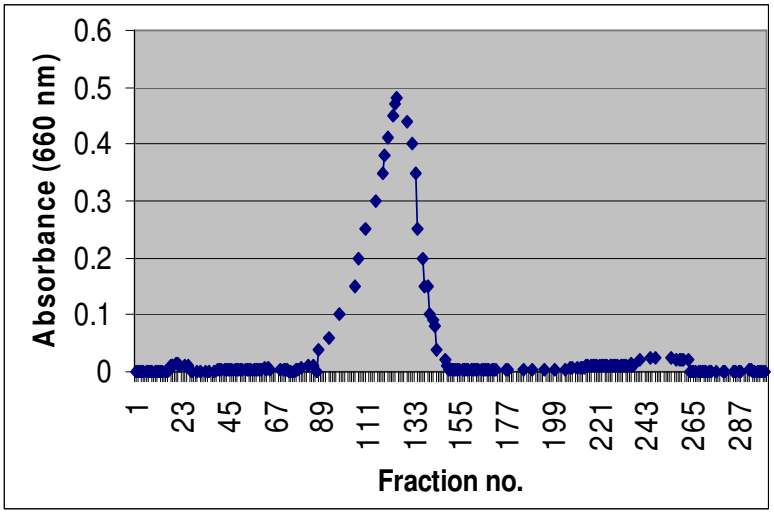


Fig.6 GPC elution pattern of *P.juliflora* seed polysaccharide on sephadex G-150 [void volume (V_o) 40 ml; total column volume (V_t) 140 ml]

(b) Sugar composition: The constituent sugar analysis was carried out after complete hydrolysis with 2N H_2SO_4 for 18h at 100^0C. The sugars were separated by descending paper (Whatmann No. 1) chromatography using 6:4:3 butanol- pyridine-water. The chromatograms were sprayed with aniline hydrogen phthalate and alkaline silver nitrate. Constituent sugars were determined as alditol acetates by GLC. Polysaccharide upon partial acid hydrolysis was

heated with sulphuric acid (0.05N, 100ml) on steam bath at 100^0C for 3h. The mixture of oligosaccharides and monosaccharide was resolved into its components by preparative chromatography on whatmann No.3mm filter paper sheets using solvent system S_2. The strips corresponding to individual oligosaccharides were eluted with water, elutes were concentrated separately, to obtain the three oligosaccharides. Homogeneity of the oligosaccharides was checked by paper chromatography in solvent system S_1, S_2, S_3 using R_1 and R_2 as spray reagent.

(c) Molecular Weight Determination: Polysaccharide solution (1 gm in 100 ml distilled water) was prepared in 100 ml volumetric flask. It's dilutions of 0.75, 0.50 and 0.25 in 50 ml volumetric flask were made. The flow time of solvent (deionized water) was determined by using an Ubbelohde viscometer. Viscometer was cleaned with suitable solvent and dry, clean, filtered air blowed through viscometer to remove traces of solvent. Sample was introduced into viscometer by pipetting ~10 ml. Viscometer was placed in constant-temperature bath (25^0C) and allow 10 minutes for viscometer to thermally equilibrate. Efflux time is measured by allowing the sample to flow freely. Efflux time was recorded in seconds to hundredths and repeated. Efflux time measurement at least three times was repeated.

(d) Methylation Analysis: The polysaccharide (0.0550 gm) was dissolved in distilled dimethyl sulphoxide (5ml) by magnetic stirring and warming at 45-50^0C in an inert atmosphere (N_2). The reaction mixture was stirred for 4 h till the evolution of hydrogen ceased. After evaporation of the chloroform extracts to dryness, the residues were hydrolyzed with formic acid (90%, 10ml) for 1h on steam bath at 100^oC, the solutions evaporated and treated with aqueous sulphuric acid (0.13 M, 15ml) for 18h on a steam bath. The partially methylated compound was subjected to Purdie's methylation by dissolving it in methyl iodide (5 ml) with stirring under inert atmosphere and silver oxide (0.50 g) was added periodically in 4 h and reaction was allowed for 6 h. This process was repeated two times on the successive days. The completely methylated polysaccharide processed further as above, and obtained in the syrup form with the yield as (0.0265g).The hydrolysate was neutralized ($BaCO_3$), concentrated, reduced (sodium borohydride) and transformed into alditol acetate. GLC of partially methylated sugars was done on ECNSS-M (3%) column under condition C_1.

Oligosaccharide-I (0.0283g), Oligosaccharide- II (0.0292g), Oligosaccharide- III (0.0321g) was methylated completely by Hakomori method (Hakomori, 1964) using sodium hydride- dimethyl sulphoxide followed by Purdie (Purdie & Irvine, 1904) for complete

etherification. Each methylated oligosaccharide was recovered by chloroform extraction. After evaporation of the chloroform extracts to dryness, the residues were hydrolyzed with formic acid (90%, 10ml) for 1h on steam bath at 100^{o}C, the solutions evaporated and treated with aqueous sulphuric acid (0.13 M, 15ml) for 18h on a steam bath. The partially methylated compound was subjected to Purdie's methylation by dissolving it in methyl iodide (5 ml) with stirring under inert atmosphere and silver oxide (0.50 g) was added periodically in 4 h and reaction was allowed for 6 h. This process was repeated two times on the successive days. The methylated oligosaccharide processed further as above, and obtained in the syrup form with the yield as oligo-I (0.0123g), oligo-II (0.0132g), oligo-III (0.0171g).

3.4 Periodate Oxidation: To a solution of galactomannan polysaccharide (0.0510 g in 25 ml) in water an aqueous solution of sodium metaperiodate (0.2g in 50ml) was added and the volume of the resultant solution was made up to 100ml. A blank solution of sodium metaperiodate (0.2g in 100 ml) was also prepared. These were kept in dark at room temperature (4^{0}C) for 192 h. To determine periodate consumed, an aliquot (5ml) of the periodate reaction mixture was added to a solution containing distilled water (20ml), potassium iodide (20%, 2ml) and sulphuric acid (0.5N, 3ml). The liberated iodine was immediately titrated with 0.1N sodium thiosulphate solution using starch as an indicator (Bobbitt, 1956; Malaprade, 1928; Rankin &Jeans, 1954).

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