Carbohydrate Polymers 101 (2014) 438–443

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Structure of the oligosaccharides isolated from *Prosopis juliflora* (Sw.) DC. seed polysaccharide

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ARTICLE INFO

Article history:
Received 9 June 2013
Received in revised form 11 August 2013
Accepted 14 September 2013
Available online 23 September 2013

Keywords:
Prosopis juliflora (Sw.) DC.
Galactomannan
Polysaccharide
Oligosaccharide
Structure

ABSTRACT

A water soluble polysaccharide isolated from *Prosopis juliflora* seed was purified and major homogenous fraction obtained by GPC. Complete hydrolysis of the polysaccharide followed by paper chromatography and GLC analysis indicated the presence of D-galactose and D-mannose in the ratio 1:1.10, respectively. 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 and ^{1}H NMR spectral studies of oligosaccharides indicated the presence of (1 → 4) mannose units linked to (1 → 6) galactose units.

1. Introduction

The seed galactomannans (Dea & Morrison, 1975), commonly known as seed gums, are neutral polysaccharides which find widespread applications (Whistler, 1990) in various industries throughout the world. The growing industrial utility of these gums or modified derivatives in the field of paper, textile, petroleum recovery and pharmaceutical industries has resulted in an impetus in India for intensified research on new sources having varying galactose–mannose ratios and fine structures. They are non-toxic, inexpensive and eco-friendly and have been considered as GRAS (generally recognized as safe) (Hallagen, La Du, Pariza, Putnam, & Borzelleca, 1997; Mathur, Mathur, & Nagori, 1996). Seed galactomannans play important dual function to retain water by salvation thereby protecting embryo from desiccation and serve as food reserves for seed germination. Most of the galactomannans have a main core of β-(1 → 4) mannose residues with side chains of single galactose unit attached to the backbone through α-(1 → 6) linkage. They differ from each other in galactose/mannose ratio and distribution of galactose side stub along the main chain, thereby causing variation in solubility, viscosity and other properties. Structural study is a basic requirement for understanding the gum properties, particularly the rheological, gelling and interaction behaviors, which are considered to be directly related to the structural features.

Prosopis 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 9–12 m and a girth of 90 cm. Under favorable conditions the tree attains a height of 18 m (Tewari, 1995, chap. 1). The wood of *P. juliflora* was found to have strong screw and nail holding resistance, considerably stronger than that of teak (*Tectona grandis*), particularly when dry. The wood of *P. juliflora* is soluble to varying degrees in water, sodium hydroxide, alcohol and benzene, which means that it can be successfully pulped for the production of writing and printing papers, textile fibers, tyre cord or cellophane (Madan & Tandon, 1991).

The bark of the tree exudes gum in the form of nearly smooth light yellowish brown more or less opaque translucent tears. The gum forms adhesive mucilage and can be used as an emulsifying agent. It also finds use in confectionery and is also used in mending pottery. The gum is also used as an adulterant and substitute of gum Arabic and proves to be an excellent source of sugar. (Tewari et al., 2013). Pods are sweet and succulent. They are of high nutritive value. It provides good fodder for livestock and in some places more important is the usefulness of its pods as a fodder and food crop. The fodder value of the *prosopis* pods is roughly comparable to that of barley or corn. Pods are used to make bread, syrup, coffee, cocktails, refreshments, alua, brandy, flour and other products including algarobina a beverage highly appreciated in Peru and said to have strengthening and aphrodisiac effects and have great calcium, thiamine and riboflavin content. Rich and delicious flour can

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http://dx.doi.org/10.1016/j.carbpol.2013.09.039

be made from pulverized pods from which the seeds have been removed. This flour is used in the preparation of sweets (Habit & Saavedra, 1986, chap. 6).

P. juliflora pods are used in concentrated rations for dairy cows at the ratio of approximately 40–60%. *P. juliflora* fruit is used in the production of ethyl alcohol. Fruit extract and leaf ferment show strong antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The juice of new leaves is used for treating certain eye ailments. Roots of the plant yield coarse fiber. Tea made from *P. juliflora* pods is considered good for digestive disturbances and skin lesions (Habit & Saavedra, 1986, chap. 6). The present investigation was carried out to elucidate the detailed structure of oligosaccharides.

2. Experimental

2.1. General methods of analysis

Solutions were concentrated at or below 40 °C in a rotary evaporator under reduced pressure. All melting points are uncorrected. Optical rotation was determined on Autopol-II, automatic polarimeter (Rudolph Research, Flanders, New Jersey) at 589 nm, D-lines of sodium. Paper chromatography was carried on Whatman 1 and 3 mm filter paper sheets using the following solvent systems; n-butanol:ethanol:water (4:1:5, S_1) upper layer; n-butanol:pyridine:water (6:4:3, S_2) and ethyl acetate:acetic acid:n-butanol:water (4:3:2:2, S_3). Detection was effected with acetonical silver nitrate (R_1) and aniline phthalate spray (R_2). Detection with acetonical silver nitrate (solution of $AgNO_3$, water and acetone) was done by treatment with following agents: (a) to silver nitrate solution (12.5 g in 10 ml water), one-liter acetone was added with continuous shaking. Distilled water was added drop wise with stirring until the white precipitate completely dissolved to form a clear solution. (b) Sodium hydroxide (20 g) was dissolved in 400 ml of ethanol. (c) Aqueous ammonia solution.

The dried chromatograms were dipped and passed through reagent solution (a) for about 5 min, dried at room temperature and passed through reagent (b); when the dark brown spots were visualized the paper was dipped in reagent (c) for some time with shaking (5–10 min). Finally the chromatograms were washed with water and dried in air (Trevelyan, Proctor, & Harrison, 1950).

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 (V_o) 40 ml; total column volume (V_t) 140 ml]. The flow rate was maintained at 0.5 ml/min by using minipuls 2 peristaltic pumps and Gilson fraction collector (Model-202) programmed in time mode. Fractions (2 ml) each were collected and monitored with anthrone sulphuric acid reagent (McCready, Guggolz, Silviera, & Owens, 1950) at 660 nm using Chemito UV-Vis spectrophotometer UV-2500. The graph of the absorbance v/s fractions collected was plotted. Gas liquid chromatography of the sugar mixtures was carried out on a Shimadzu Gas Chromatograph GC-9A fitted with flame ionization detector (FID). The samples were analyzed in the form of their alditol acetates on ECNSS-M (3%) on Gas chrom Q (100–200 mesh) packed into $5' \times 1/8''$ stainless steel column under the following operating conditions: column temperature 170 °C, nitrogen flow rate 35–40 ml/min (C1). Nuclear Magnetic Resonance Spectroscopy (NMR) of oligosaccharides was done on BrukerAvance II 400 NMR spectrometer at 400 MHz at room temperature using D_2O as solvent.

2.2. Isolation of polysaccharide

The seeds of *P. 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%. Seed coats and germs were separated by impact grinding using high speed domestic grinder. Endosperm (20 g) was isolated by soaking the seed coats in cold water for 3 h and then separated from the swollen seed coats with the help of forceps. It was stirred vigorously in distilled water (1000 ml) for 5 h 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 (18.0 g) 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 72 h 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 °C to obtain finally the pure polysaccharide in the form of a white amorphous powder (16.1 g).

2.3. Complete hydrolysis

The pure polysaccharide (1.0 g) of *P. juliflora* seed endosperm was subjected to hydrolysis with sulphuric acid (2 N, 25 ml) for 18 h on a steam bath at 100 °C. The hydrolysate was cooled, neutralized with saturated solution of barium carbonate by drop wise addition till the pH of the solution reached at 7, filtered and the residue washed with water. The combined filtrate was concentrated.

2.4. Partial hydrolysis

Polysaccharide (5.0 g) was heated with sulphuric acid (0.05 N, 100 ml) on steam bath at 100 °C for 3 h. The hydrolysate was cooled, neutralized with saturated solution of barium carbonate till neutral pH and filtered. The combined filtrate was concentrated to syrup (4.73 g). The mixture of oligosaccharides and monosaccharide was resolved into its components by preparative chromatography on Whatman No.3 mm 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.

2.5. Methylation of oligosaccharides

Oligosaccharide-I (0.0283 g), oligosaccharide-II (0.0292 g), oligosaccharide-III (0.0321 g) was methylated completely by Hakomori method (Hakomori, 1964) using sodium hydride–dimethyl sulphoxide followed by the method of Purdie and 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%, 10 ml) for 1 h on steam bath at 100 °C, the solutions evaporated and treated with aqueous sulphuric acid (0.13 M, 15 ml) for 18 h 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 asoligo-I (0.0123 g), oligo-II (0.0132 g), oligo-III (0.0171 g).

2.6. Alditol acetates

Alditol acetates of the hydrolyzed material were prepared by the method of Jannson, Kenne, Liedgren, Lindberg, and Lonngren (1976). Sodium borohydride (0.020 g) was added to hydrolyzates, and the mixture was kept for 18 h at room temperature. The mixture was neutralized by slow addition of dilute acetic acid (6 ml), and concentrated to dryness in vacuum rotator at 40 °C. 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× 5 ml). The residue was treated with redistilled acetic anhydride and pyridine, 1:1 (4 ml) and refluxed for 6 h. Toluene (6 ml), 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 (6 ml) was added and the solution was concentrated to dryness. It was dissolved in water (10 ml) and the acetylated sugars separated by shaking with dichloromethane (4× 25 ml). Traces of water present in dichloromethane were removed by adding anhydrous sodium sulphate followed by filtration and washing with dichloromethane before concentration.

2.7. Periodate oxidation

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

Liberation of formic acid was determined by the methods reported earlier (Abdel-Akher & Smith, 1951; Bobbitt, 1956; Dayer, 1956; Halsall, Hirst, & Jones, 1947). To an aliquot (5 ml) of the periodate reaction mixture was added acid free ethylene glycol (0.5 ml), followed by an excess of potassium iodide (20%, 5 ml) after 10 min. To the above solution an excess of 0.01 N sodium thiosulphate was back titrated with 0.01 N iodine solution using starch as an indicator. A blank solution was titrated concurrently.

3. Result and discussion

The polysaccharide was isolated from the endosperm of seeds in 81% yield as detailed in Section 2. The homogeneity was checked by exclusion chromatography by passing it through sephadex G-150 (Fig. 1). The fractions of the major peak were pooled together, concentrated and polysaccharide precipitated by addition of ethanol.

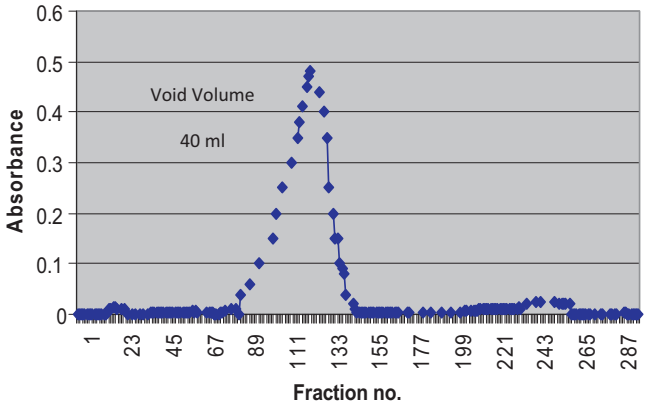

Fig. 1. GPC elution pattern of *P. juliflora* seed polysaccharide on sephadex G-150 [void volume (V_o) 40 ml; total column volume (V_t) 140 ml].

Chemical analysis of pure polysaccharide has shown specific rotation, [α] + 104° [c 0.05%, H_2O], ash content 0.1% (on dry basis), nitrogen 0.33%, pH ~ 6.90.

Complete hydrolysis of the polysaccharide followed by paper chromatography revealed the presence of two spots, corresponding to D-galactose and D-mannose respectively. For the quantitative estimation of sugar mixture, a part of the hydrolysates was converted into its alditol acetate by the method of Jannson et al. (1976) and analyzing it by GLC (Bishop, 1964) using ECNSS-M (3%) column employing conditions C_1. It showed that the retention time of the two peaks coincided with the retention time of D-galactopyranose and D-mannopyranose. On the basis of G.L.C results the molar ratio among D-galactose and D-mannose was determined as 1:1.10 respectively.

3.1. Partial hydrolysis

P. juliflora seed endosperm polysaccharide upon hydrolysis with dilute sulphuric acid (0.05 N, 3 h) 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 Whatman 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. The nature and sequence of glycosidic linkages was determined by methylation analysis, periodate oxidation and NMR spectral assignment of the oligosaccharides.

3.2. Structure of oligosaccharide-I

It was found to be a heptasaccharide [α] + 112° (c 0.05%, H_2O), m.p. 200–202 °C (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.

α –D- Galp α –D- Galp α –D- Galp

1 1 1

↓ ↓ ↓

6 6 6

β –D- Manp1→4- β –D- Manp1→4- β –D- Manp1→4- β –D- Manp

Fig. 2. Oligosaccharide-I.

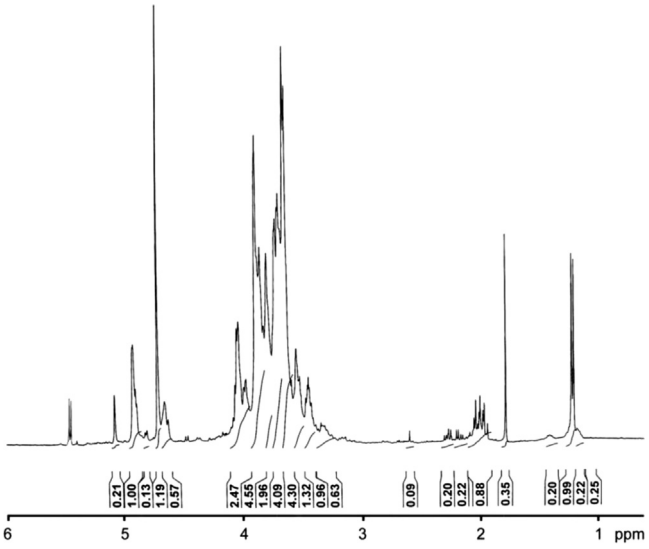

Fig. 3. ^{1}H NMR spectrum of oligosaccharide-I.

The oligosaccharide was completely methylated by Hakomori method (Hakomori, 1964) followed by the method of Purdie & Irvine (1904). Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590–3225 cm^{-1}). It was hydrolyzed and transformed into its alditol acetates according to the method of Jannson 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. On the basis of above discussion, the heptasaccharide may be assigned the following plausible structure (Fig. 2).

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the ^{1}H NMR spectroscopy. Resonances of the anomeric protons in ^{1}H NMR spectroscopy are well separated and identified. The ^{1}H NMR spectrum of the polysaccharide contained signals for H-1of Gal (δ5.4J1, 2–3.3 Hz) and Man (δ5.1) are compatible with the expected $^{4}C_1$ conformation of the anomeric α-D-galactopyranose and β-D-mannopyranose units respectively (Fig. 3) (Gupta, Jain, Bajpai, & Sharma, 1987).

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.

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the results of periodate oxidation (Abdel-Akher & Smith, 1951; Bobbitt, 1956; Dayer, 1956; Halsall et al., 1947; Malaprade, 1928; Rankin & Jeans, 1954). The oligosaccharide when subjected to oxidation with sodium metaperiodate, consumed 1.69 mol of periodates per anhydrohexose unit and released 0.69 mol of formic acid per anhydrohexose unit. As the oligosaccharide structure consists of 7 sugar residues, the above results can be interpreted in terms of the consumption of 11.83 mol of periodate with simultaneous liberation of 4.83 mol of formic acid by the repeating unit of oligosaccharide. Thus, periodate oxidation data of oligosaccharide as calculated on the basis of proposed structure and obtained experimentally are found to be in close agreement with each other. Therefore, above structure proposed on the basis of methylation analysis and NMR spectroscopic studies gets corroborated by the oxidation studies.

α –D- Galp α –D- Galp α –D- Galp

1 1 1

↓ ↓ ↓

6 6 6

β –D- Manp1→4- β –D- Manp1→4- β –D- Manp1→4- β –D- Manp1→4- β –D- Manp

Fig. 4. Oligosaccharide-II.

3.3. Structure of oligosaccharide-II

It was found to be an octasaccharide [α] + 86° (c 0.05%, H_2O), m.p. 218–220 °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 the method of Purdie & Irvine (1904). Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590–3225 cm^{-1}). It was hydrolysed and transformed into its alditol acetates according to the method of Jannson 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. On the basis of above discussion, the octasaccharide may be assigned the following plausible structure (Fig. 4).

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the ^{1}H NMR spectroscopy. Resonances of the anomeric protons in ^{1}H NMR spectroscopy are well separated and identified. The ^{1}H NMR spectrum of the polysaccharide contained signals for H-1 of Gal (δ5.20J1, 2–3.3 Hz)and Man (δ5.10) are compatible with the expected $^{4}C_1$ conformation of the anomeric α-D-galactopyranose and β-D-mannopyranose units respectively (Fig. 5) (Gupta et al., 1987).

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.

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the results of periodate oxidation (Abdel-Akher & Smith, 1951; Bobbitt, 1956; Dayer, 1956; Halsall et al., 1947; Malaprade, 1928; Rankin & Jeans, 1954). The oligosaccharide when subjected to oxidation

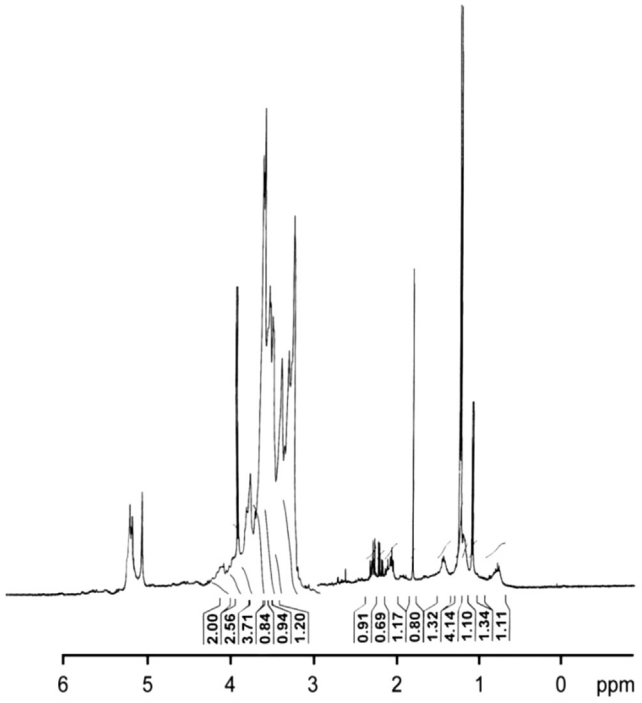

Fig. 5. ^{1}H NMR spectrum of oligosaccharide-II.

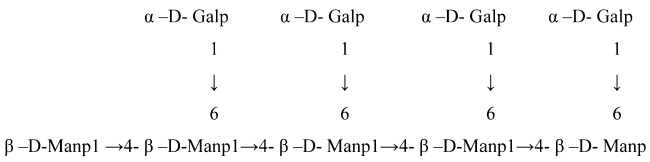

Fig. 6. Oligosaccharide-III.

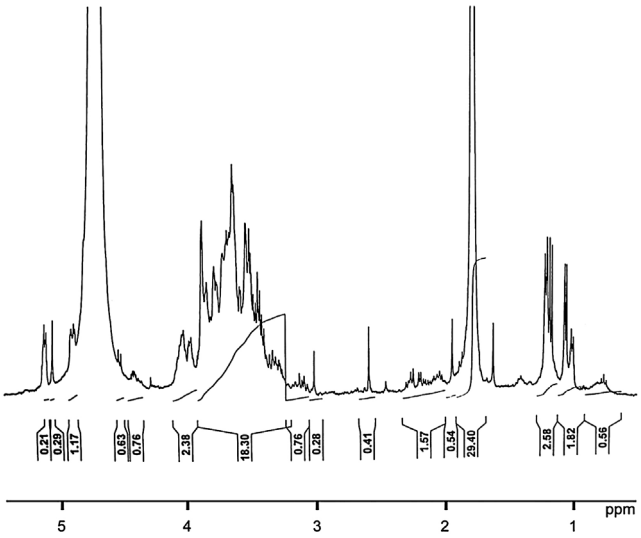

Fig. 7. ^{1}H NMR spectrum of oligosaccharide-III.

with sodium metaperiodate, consumed 1.60 mol of periodate per anhydrohexose unit and released 0.60 mol of formic acid per anhydrohexose unit. As the oligosaccharide structure consists of 8 sugar residues, the above results can be interpreted in terms of the consumption of 12.80 mol of periodate with simultaneous liberation of 4.80 mol of formic acid by the repeating unit of oligosaccharide. Thus, periodate oxidation data of oligosaccharide as calculated on the basis of proposed structure and obtained experimentally are found to be in close agreement with each other. Therefore, above structure proposed on the basis of methylation analysis and NMR spectroscopic studies gets corroborated by the oxidation studies.

3.4. Structure of oligosaccharide-III

It was found to be a nonasaccharide [α] + 129° (c 0.05%, H_2O), m.p. 240–242 °C (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 the method of Purdie & Irvine (1904). Complete methylation was confirmed by IR spectrum of the methylated heptasaccharide which showed complete absence of –OH band (3590–3225 cm^{-1}). It was hydrolyzed and transformed into its alditol acetates according to the method of Jannson 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. On the basis of above discussion, the nonasaccharide may be assigned the following plausible structure (Fig. 6).

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the ^{1}H NMR spectroscopy. Resonances of the anomeric protons in ^{1}H NMR spectroscopy are well separated and identified. The ^{1}H NMR spectrum of the polysaccharide contained signals for H-1 of Gal (δ5.20J1, 2–3.3 Hz) and Man (δ5.05) are compatible with the expected $^{4}C_1$ conformation of the anomeric α-D-galactopyranose and β-D-mannopyranose units respectively (Fig. 7) (Gupta et al., 1987).

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 residue.

Evidence supporting the presence of 1 → 4 and 1 → 6 linkages in the framework of oligosaccharide has been obtained from the results of periodate oxidation (Abdel-Akher & Smith, 1951; Bobbitt, 1956; Dayer, 1956; Halsall et al., 1947; Malaprade, 1928; Rankin & Jeans, 1954). The oligosaccharide when subjected to oxidation with sodium metaperiodate, consumed 1.64 mol of periodate per anhydrohexose unit and released 0.61 mol of formic acid per anhydrohexose unit. As the oligosaccharide structure consists of 9 sugar residues, the above results can be interpreted in terms of the consumption of 14.76 mol of periodate with simultaneous liberation of 5.49 mol of formic acid by the repeating unit of oligosaccharide. Thus, periodate oxidation data of oligosaccharide as calculated on the basis of proposed structure and obtained experimentally are found to be in close agreement with each other. Therefore, above structure proposed on the basis of methylation analysis and NMR spectroscopic studies gets corroborated by the oxidation studies.

Acknowledgements

This work is supported by Forest Research Institute (FRI), Dehradun. The authors are thankful to Director (FRI), Head, Chemistry Division, (FRI), Dehradun for providing laboratory facilities. The authors are also thankful to Director Punjab University, Chandigarh for N.M.R studies. Thanks are also due to Dr. Vikas Rana for support and discussion.

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