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Structure of oligosaccharides by hydrolytic studies from *Acrocarpus fraxinifolius* Wight. seeds polysaccharide

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ABSTRACT

Polysaccharide was extracted from *Acrocarpus fraxinifolius* Wight. seeds yielded a water soluble sugar as D-galactose and D-mannose in 1:3 molar ratio. Polysaccharide upon partial acid hydrolysis affords mainly 3 disaccharides and one trisaccharide as: (I) α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose, (II) α-D-mannopyranosyl-(1→6)-O-α-D-mannopyranose, (III) β-D-galactopyranosyl-(1→4)-O- β-D-mannopyranose and (IV) β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose. Oligosaccharides results corroborate the earlier proposed structure of seeds polysaccharide.

Key words: Oligosaccharides, *Acrocarpus fraxinifolius* Wight. seeds polysaccharide, hydrolytic studies.

INTRODUCTION

Acrocarpus fraxinifolius Wight.[1] plant commonly known as *Mandania* or *red cedar,* belong to the family. Fabaceae and Caesalpiniaceae. Its occurs in Asia, India, China, Indonesia, Nepal and Tropical Africa. In India it occurs in Northern Himalayas, Manipur, Nagaland and Southern Indian Forest. Seeds exhibited antimicrobial activities and also in Ayurvedic system of medicine while wood used for furniture etc. In earlier studies[2,3], polysaccharide from the seeds of *Acrocarpus fraxinifolius* Wight. was shown to be composed of D-galactose and D-mannose in 1:3 molar ratio. Methylation, periodate oxidation and Smith degradation studies showed that the D-galactopyranose units mostly occupy the terminal position in the main chain which consists predominantly of D-mannopyranose units. The present manuscript mainly deals with the isolation and characterization of various oligosaccharides obtained from the partial acid hydrolysis of galactomannan. The results reported provide additional information about the structure of the polysaccharide.

Partial acid hydrolysis of the polysaccharide followed by column chromatography over charcoal- celite and paper chromatography of the hydrolysate afforded three diasaccharides and one trisaccharides in pure state as the major components. Each oligosaccharide was purified separately and characterised by its optical rotation, formation of crystalline derivatives (disaccharide) degree of polymerisation, reduction with sodium borohydride and subsequent hydrolysis, complete acid hydrolysis and periodate oxidation studies. The oligosaccharides obtained were characterised as : α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose (I); α-D-mannopyranosyl-(1→6)-O-α-D-mannopyranose (II); α-D-galactopyranosyl-(1→4)-O-β-D-mannopyranose (III); β-D-

mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-β-D-mannopyranose (IV).

EXPERIMENTAL

Procedure

The paper chromatography[4] was performed on Whatman No. 1 filter paper sheet using upper phase of the solvent system (v/v): (A) *n*-butanol, acetic acid water (4:1:5)[5]; (B) ethyl acetate, acetic acid water (9:2:2)[6] and (C) ethyl acetate pyridine water (10:4:3)[7]. The spray reagent used was *p*-anisidine phosphate[8] for the detection of monosaccharides and oligosaccharides. Unless otherwise stated that the all evaporations were carried out at 40-50^{0}C under reduced pressure. The optical rotations are in equilibrium values and melting points are uncorrected. *R*-gal and *R*glu refer to the rate of movement of sugar relative to the D-galactose and D-glucose. Deionisation was done with freshly regenerated Amberlite IR-45 (OH^-) and IR-120 (H^+) ion exchange resins[9]. Sugar mixture were separated on column of charcoal-celite (1:1, W/W) using water, followed by 2.5, 5.0, 7.5 and 10.0% aqueous ethanol (v/v) as eluants and then it identified by paper chromatography Whatman No. 3MM filter paper sheet using solvent (B). The degree of polymerization (D.P.) was determined by Timell's method[10].

Partial acid hydrolysis and separation of oligosaccharides

After a long series of trial experiments the following procedure was carried out by partial acid hydrolysis[11] was found to give the maximum yield of oligosaccharides. Polysaccharide (30 gm) was dissolved in H_2SO_4 (1.5 N, 900 ml) then solution left (16 hrs) at room temperature and hydrolyzed in a boiling water-bath for 20 min. It was cooled in the same bath for 30 min, filtered, neutralized with barium carbonate and again filtered. The residue was washed well with water and washings were mixed with the filtrate and concentrated to a small volume (60 ml). It was added to ethanol (350 ml) with mechanical stirring when the degraded polysaccharide got precipitated as white course powder. It was filtered, washed with ethanol and dried. The filtrate left after the removal of degraded polysaccharide, upon concentration and subsequent chromatographic examination of the syrup (a) in solvent (A) showed the presence of D-galactose and D-mannose together with traces of lower oligosaccharides. Degraded polysaccharide was hydrolysed again by keeping it in sulphuric acid (1.5 N, 600 ml) at room temperature for 72 hr and then by heating in a boiling water-bath for 10 min followed by cooling in the same bath for 15 min. The hydrolysate was processed as above to a small volume which upon pouring into ethanol gave amount of the degraded polysaccharide. It was again filtered and residue washed with ethanol and rejected. Filtrate was concentrated to a syrup (b) and examined by chromatographically using solvent mixture (A). It showed the presence of D-galctose, D-mannose and a number of oligosaccharides. The two syrup (a and b) were mixed and purified by passing the aqueous solution through the column of freshly regenerated Amberlite ion exchange resin IR-45 (OH^-) and IR-120 (H^+) ions and the effluent was concentrated to a syrup.

The above sugar syrup was separated on charcoal celite (1:1, w/w) column (60x2.5 cm) employing the graded elution method[12]. The glass column was first eluted with 2 litre of water under 6 lbs/sq. inch pressure to remove monosaccharide and then successively with 2 litre each of 2.5, 5.0, 7.5 and 10.0% aq. ethanol (v/v) as eluants. Each fraction (50 ml) was concentrated and examined by paper chromatography using solvent mixture (A). It was found that the each sugars fraction was a mixture of 4 oligosaccharides and fraction were mixed then it concentrated to a thin syrup. Oligosaccharides were separated by paper chromatography on Whatman No. 3 MM filter paper sheet using solvent mixture (B). The different zones of oligosaccharides[13] were cut out with the help of guides pots and eluted with water according to Dent's method[14] then finally concentrated to a syrup. This led to the isolation of three disaccharides and one trisaccharide in authentic form which were identified and characterised as follows:

Fraction-I: α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose

Syrup (280 mg) dissolved in water-methanol (1;1, v/v) and filtered then *n*-butanol (5 ml) was added to the cold filtrate. Solution was evaporated on a water-bath to a slight turbidity. Upon cooling, the oligosaccharide was crystallised out

as cubes, yield (210 mg), m.p. 202-204^{0}C, Lit., m.p. 2031203^{0}C[15], αT_D^{24}+121.5^{0}C (H_2O), Lit. $[\alpha]_D$+120^{0}C[16]. *R*-gal 0.53 in solvent (B) and *R*-glu 0.45 in solvent (C).The degree of polymerisation (D.P.) was found to be 1.75 by Timell's method[10]. Upon acid hydrolysis with 1N H_2SO_4 afforded D-galactose and D-mannose in equimolecular proportion by phenol sulphuric acid method[17]. Disaccharide was reduced with sodium borohydride followed by acid hydrolysis gave D-galctose, indicating the reducing end to be D-mannose. Its osazone[18] derivative of disaccharide was prepared by usual manner had m.p, 174-175^{0}C, Lit. m.p. 175-176^{0}C[19].

Periodate oxidation[20] of the disaccharide showed the consumption of 5.86 moles of periodate with simultaneous liberation of 4.72 moles of formic acid per mole of disaccharide after 55 hrs.

Fraction-II: α-D-mannopyranosyl -(1→6)-O-α-D-mannopyranose

Syrup (260 mg) had *R* gal 0.58 in solvent (B) and *R* glu 0.41 in solvent (C) optical rotation $[\alpha]T_D^{24}$-11.8^{0}C (H_2O), Lit, $[\alpha]T_D$+12.4^{0}C[21] and $[\alpha]T_D^{24}$-20.6^{0}C (C_2H_5OH), Lit, $[\alpha]T_D$+21.0^{0}C[22], having m.p 155-157^{0}C. DP was found to be 2.14 indicating that the oligosaccharide was a disaccharide. Hydrolysis with sulphuric acid (1N), filtered and filtrate was neutralized with barium carbonate then concentrate to a thin syrup (hydrolysate) and on paper chromatogram, the hydrolysate showed the presence of D-mannose sugar only. Derivative of disaccharide (55 mg) was prepared by usual manner as O-α-D-mannopyranosyl-(1→6)-α-D-mannopyranose octa acetate, having m.p. 153-155^{0}C Lit m.p. 152-153^{0}C[23].

Periodate oxidation of the disaccharide syrup (60 mg) was carried out with sodium metaperiodate for 55 hrs at 4-8^{0}C in refrigerator. It consumed 5.16 moles of periodate as oxidant and liberation of 2.94 moles of formic acid per mole of disaccharide (55 hrs).

Fraction-III: β-D-galactopyranosyl -(1→4)-O- β-D-mannopyranose

Sugar syrup (240 mg) had *R* gal 0.58 in solvent (B) and *R* glu 0.52 in solvent (C), optical rotation $[\alpha]_D^{24}$+17.5^{0}C (H_2O), Lit, $[\alpha]_D$+17^{0}C[24]. D.P. was found to be 1.82 indicating that this oligosaccharide was a disaccharide. Acid hydrolysis with sulphuric acid (1N) and obtained hydrolysate by paper chromatography on Whatman No. 3 MM filter paper sheet showed the presence of D-galactose and D-mannose in equimolecular proportion. It showed the presence of D-galactose on paper chromatogram, indicating that the D-mannose sugars unit are at the reducing end position in the main polymer chain. The phneyl hydrazone derivative of disaccharide was prepared by usual manner having m.p. 195-197^{0}C Lit m.p. 194-195^{0}C[25]. It consumed 5.76 moles of periodate with simultaneous liberation of 3.62 moles of formic acid per mole of disaccharide by periodate oxidation studies after 55 hrs at 4-8^{0}C in refrigerator.

Fraction-IV: β-D-mannopyranosyl -(1→4)-O-β-D-mannopyranosyl-(1→4)-O-α-D-mannopyranose

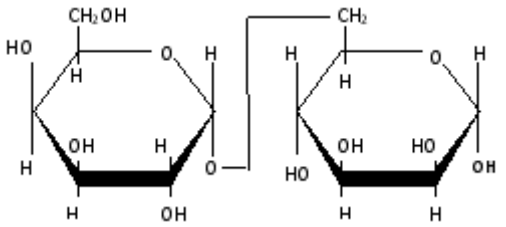
Sugar syrup (280 mg) had *R*-gal 0.33 in solvent (B) and *R*-glu 0.21 in solvent (C), $[\alpha]_D$-22.5°C (H_2O), Lit. $[\alpha]T_D$-22°C[26] and m.p. 167-169°C, Lit m.p. 169.5^{0}C[26]. Degree of polymerization was found to be 3.16 indicating that the oligosaccharide was a trisaccharide. On acid hydrolysis with sulphuric acid by usaual manner showed the presence of mannobiose and D-mannose sugars by paper chromatographic analysis on Whatman No. 3 MM paper sheert. Methylation of trisaccharide was carried out by Hakomari's method[27] yeilded 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6, tri-O-methyl-D-mannose which showed the presence of (1'!4)-α-type linkages by emulsion. Periodate oxidation studies by usual manner of trisaccharide, consumed 6.15 moles of periodate as oxidant and liberation of 3.72 moles of formic acid per mole of trisaccharide after 55 hrs at 4-8^{0}C in refrigerator.

RESULTS AND DISCUSSION

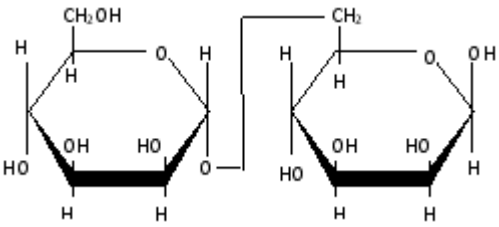
Acrocarpus fraxinifolius Wight. plant (Fabaceae) locally called *Mandania,* seeds yielded a water soluble sugar extracts as D-galactose and D-mannose in 1:3 molar ratio by paper chromatography. Partial acid hydrolysis of the purified seeds polysaccharide was carried out with sulphuric acid (1N) followed by charcoal-celite column chromatography and identified by paper chromatography on Whatman No. 3 MM filter paper sheet of the obtained hydrolysate which afforded

three diasaccharides and one trisaccharide. Oligosaccharides (Fig. 1) were identified as: α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose (I), α-D-mannopyranosyl-(1→6)-O-α-D-mannopyranose (II), β-D-galactopyranosyl-(1→4)-O-β-D-mannopyranose (III) and β-D-manno pyranosyl - (1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose (IV). Oligosaccharide was purified and characterised by optical rotation, degree of polymerisation, crystalline derivatives (disaccharides only), reduction with sodium borohydride, complete acid hydrolysis and periodate oxidation studies.

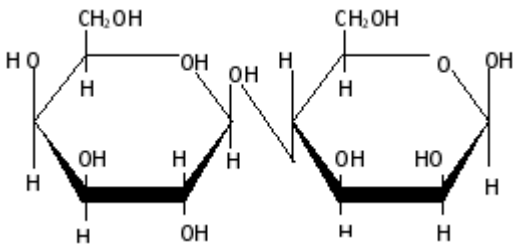
The isolation of oligosaccharide (III) as the main component and IV clearly indicates that the main chain of polysaccharide is made up of D-galactopyranose and D-mannopyranose units which are linked through (1→4)-β-type linkages. The isolation of (I) and (II) supports the fact that the branches in the main chain consist of double units of non-reducing D-galactopyranose and D-mannopyranose residues which are glycosidically attached by (1→6)-α-type with D-mannopyranose unit of the main chain and also (1→6)-α-type linkages with D-galactopyranose and D-mannopyranose residue at non-reducing terminal



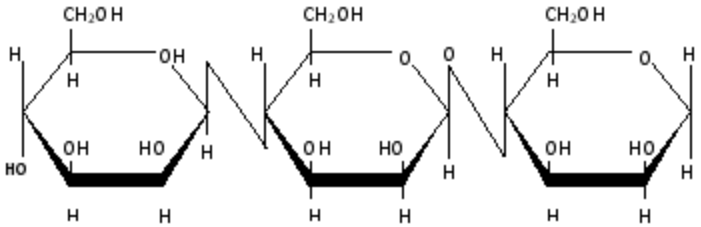
(A)α-D-galactopyranosyl-(1→6)-O-α-D-mannopyranose



(B) α-D-mannopyranosyl-(1→6)-O-α-D-mannopyranose



(C) β-D-galactopyranosyl-(1→4) -O-β-D-mannopyranose



(D) β-D-mannospyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose

Fig. 1: Structure of oligosaccharides from *Acrocarpus fraxinifloies* Weight. Seeds polysaccharides

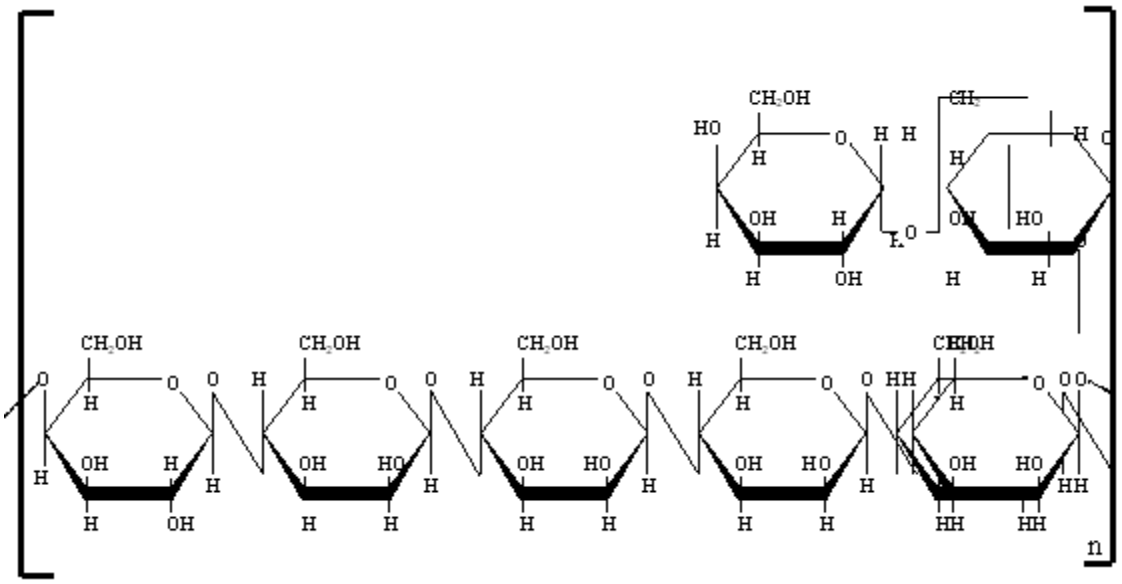


Fig. 2: Polysaccharide structure from *Acrocarpus fraxinifolius* Weight. seeds galactomannan

position of the backbone of the polysaccharide structure. Above oligosaccharide results are in the favour of the polysaccharide proposed structure for the galactomannon of *Acrocarpus fraxinifolius* Wight. seeds as shown in Fig. 2.

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