

Glycosyl composition and linkages of polysaccharide from *Bauhinia malabarica*

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ABSTRACT

Polysaccharide from *Bauhinia malabarica* was isolated, purified, methylated, hydrolyzed, reduced and acetylated. The partially methylated alditol acetate (PMAA) derivative thus obtained was subjected to GC-MS studies. The following types of linkages were noticed: terminal-glucose, terminal galactose, terminal xylose 2-xylose, 4-glucose, and 4, 6-glucose.

Keywords: glycosyl linkage, partially methylated alditol acetate derivative, GC-MS studies, *Bauhinia malabarica*.

INTRODUCTION

Glycosyl composition of a polysaccharide from *Bauhinia malabarica* [1] (sub-family *caesalpinaceae*), a medium sized bushy deciduous tree occurring in sub-himalayan tracts in Bengal, Assam, South India and Burma contain about 19% gum which is the highest among all its species analysed. Furthermore the protein content is 15% and the weight per 100 seeds is 7.60 grams [2] which is economically attractive. Polysaccharides are often subjected to different chemical modifications in order to gain information on their sequences and possible configurations. Methylation analysis has proven to be valuable in following the outcome of such modifications. The present work involves determination of glycosyl linkages present in the polysaccharide and thereby suggesting the possible sequence of monosaccharides.

MATERIALS AND METHODS

Methylation, hydrolysis, reduction and acetylation

Standard procedure was followed for the isolation and purification of the polysaccharide from the aqueous extract of the dried seed endosperm of *Bauhinia malabarica* [3]. The polysaccharide was methylated using the NaOH/CH₃I method [4]. To a solution of the sample (5 mg) in dimethylsulphoxide (0.5 mL), finely powdered NaOH (20 mg) and methyl iodide (0.1 mL) were added. The mixture was stirred for 6 minutes in a closed tube at 25 °C. Water (1 mL) and chloroform (1 mL) were then added, and the chloroform layer was washed with water (3 x 10 mL) and dried over Na₂SO₄. To form free glycoses, the methylated polysaccharide was hydrolyzed by the following procedure [5]

Trifluoroacetic acid (TFA, 2 mol L⁻¹, 250 μL containing 25 μg myo-inositol as internal standard) is added to the sample. It is then placed in a heating block at 121 °C for 2 hours. TFA is then evaporated at 40 °C under a stream of air. When the tube appears dry, 250 μL of isopropyl alcohol is added and evaporated at room temperature. The evaporation of isopropyl alcohol results in complete removal of TFA. The resulting partially *O*-methylated glycoses

are reduced to the corresponding partially *O*-methylated alditols by dissolving them in 95% ethanol (220 μ L) and adding aqueous NaBD₄ (200 μ L of 10 mg mL⁻¹, solution in 1 mol L⁻¹, NH₄OH). The test tube is closed with a teflon-lined screw cap and kept for 1 hour at room temperature. Acetic acid (50 μ L) is then added to convert the excess borodeuteride into borate. Acetic acid/methanol (1:9 V/V, 200 μ L) is added to the tube, its contents are mixed, and the solvents are evaporated with air at room temperature. Three more evaporations of 1:9 (V/V) acetic acid/methanol (200 μ L) are followed by two evaporations of methanol (200 μ L).

The partially *O*-methylated alditols are *O*-acetylated as follows. Acetic anhydride (50 μ L) is added to the test tube containing partially *O*-methylated alditols. The tube is sealed and heated for 3 hours at 120 °C. The tube is then allowed to cool to room temperature and water (500 L) is added. Solid Na₂CO₃ is added in small amounts (25 mg at a time), until effervescence ceases. If all of the Na₂CO₃ does not dissolve, more water can be added. Dichloromethane (500 μ L) is then added to the tube, and the contents of the tube are mixed. The organic and aqueous phases are separated by low speed centrifugation. The methylene chloride phase is removed, transferred to a fresh tube and carefully evaporated. Great care must be taken in evaporating the methylene chloride to prevent loss of some of the more volatile partially *O*-acetylated, partially *O*-methylated alditols. The products are analyzed by gas chromatography-mass spectrometry (GC-MS) with a fused-silica 30-m column in the splitless mode (Supelco SP 2330, Quadrex, USA), and following the temperature programme: two minutes at an initial temperature of 80 °C, increased to 170 °C at 3 °C min⁻¹, then to 240 °C at 4 °C min⁻¹, and kept for 5 min at 240 °C. All chemicals used are from Merck (India). Mass spectra were continuously recorded by scanning from 70 to 300 *m/z*. The MS operating parameters were: ionization voltage 70 eV, scan rate 1100 amu s⁻¹, electron multiplier energy 1600 V, and ion source temperature 200 °C.

RESULTS AND DISCUSSION

The identity of most polysaccharides cannot be ascertained without determining the glycosyl linkage composition. Here the glycosyl linkage composition was determined by methylation analysis. The polysaccharide was difficult to dissolve in dimethylsulphoxide. Undermethylation is said to be very common in large polymers. Our first attempt at methylation analysis of the polysaccharide produced about 60% undermethylation resulting from some of the sample not dissolving well in the first stage of the polysaccharide permethylation. The positions of *O*-acetyl and *O*-methyl groups on partially methylated alditol acetate (PMAA) polysaccharide derivatives were determined by GC-MS.

The electron-impact fragmentation patterns of the mass spectra of PMAA derivatives are well known [5]. The retention times and the *m/z* values of the main fragments in GC-MS of PMAA of glycosyl residues from the *B. malabarica* polysaccharide are given.(eg,Table 1). The possible fragmentation of PMAA of glycosyl residues is shown (eg, Figure 1). It matches the *m/z* values in GC-MS suggesting all the given types of glycosyl linkages in the sample.

TABLE-1: Retention times and the main fragment in GC-MS of PMAA glycosyl residues from *B. malabarica*

Peak	tR	Characteristic ion peaks (m/e)	Interpretation	Mode of linkage
1	13.93	118,161,162,205	1,5-Di-O-acetyl-1-deuterio-2,3,4,6-tetra-O-methyl glucitol	T-Glcp
2	18.45	118,233	1,4,5-Tri-O-acetyl-1-deuterio-2,3,6-tri-O-methyl glucitol	4-Glc-p
3	21.81	118,261	1,4,5,6-Tetra-O-acetyl-1-deuterio-2,3-di-O-methyl glucitol	4,6-Glc-p
4	14.40	118,129,145,161,162,000	1,5-Di-O-acetyl-1-deuterio-2,3,4,6-tetra-O-methyl galactitol	T-Gal-p
5	12.24	118,233	1,5-Di-O-acetyl-1-deuterio-2,3,4-tri-O-methyl xylitol	T-Xyl-p
6	16.30	43,118,130,190	1,2,5-Tri-O-acetyl-1-deuterio-3,4-di-O-methyl-D-xylitol	2-Xyl-p
7	28.35	126,157,168,210,214,000	Myo-inositol	

Table 2. Glycosyl linkages in *B. malabarica*

Carbohydrate residue	%
Terminal glucose	4.5
Terminal xylose	27.1
Terminal galactose	9.2
2-Xylose	9.7
4-Glucose	13.1
4, 6-Glucose	36.3

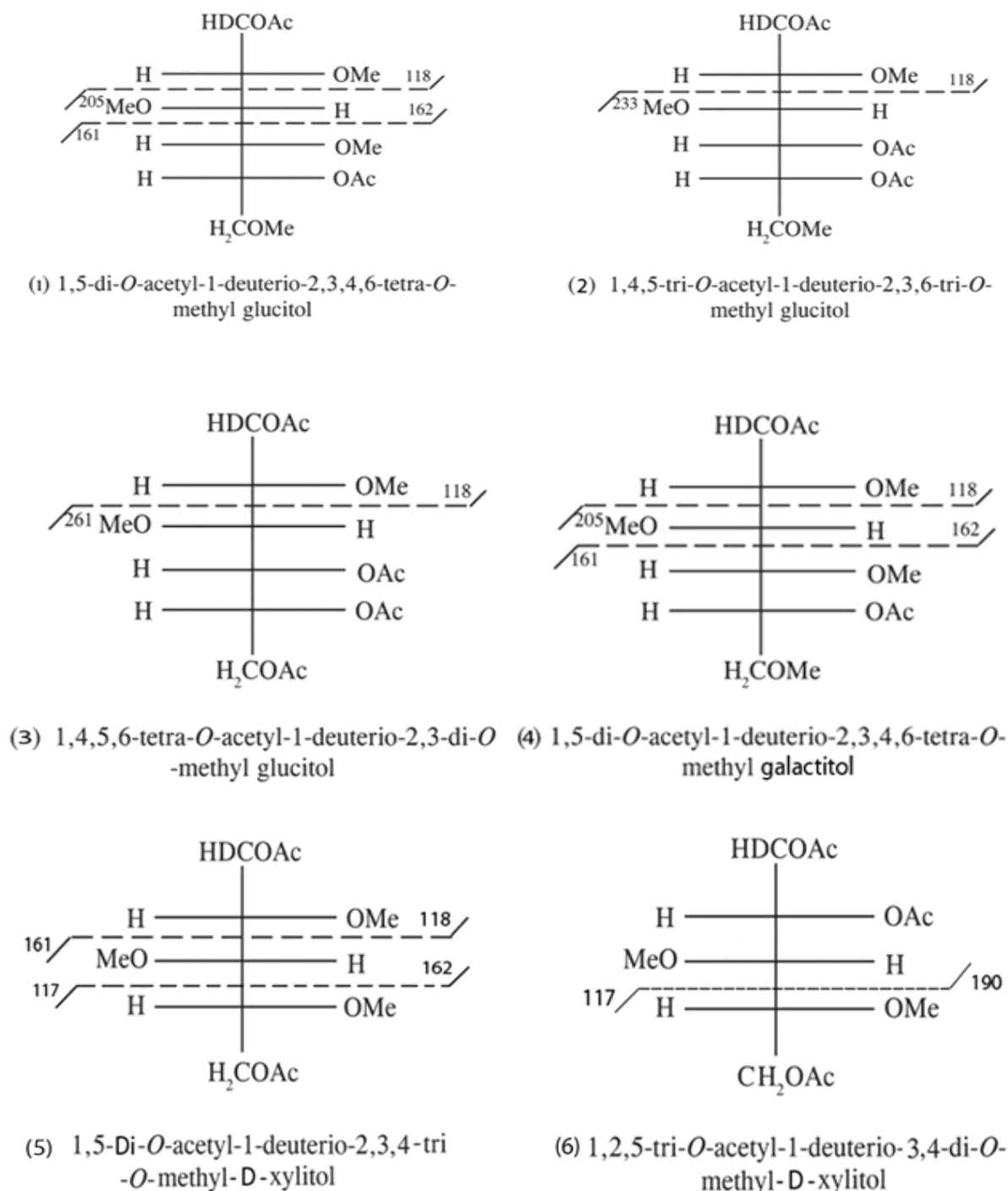


Fig. 1 Fragmentation of PMAA(1)*t*-Glc-p,(2)*t*-Xyl-p,(3)*t*-Gal-p,(4)*2*-Xyl-p,(5)*4*-Glc-p(6)*4,6*-Glc-p.
Glc-p=glucose-pyranose, *Xyl-p*=xylose-pyranose, *Gal-p*=galactose-pyranose.

The peak areas corresponding to each of the PMAA were divided by the appropriate response factor and the resulting quotients were normalized to 100%. Response factors were calculated by the effective carbon-response method [5]. The mole percentage of glycosyl linkages in *Bauhinia malabarica*. Glycosyl-composition analyses and glycosyl-linkage composition analyses were sufficient to determine the identity of the polysaccharide (eg, Table-2) However, they were not sufficient to determine the complete primary structure of the polysaccharide. This can be accomplished by other techniques [6, 7]

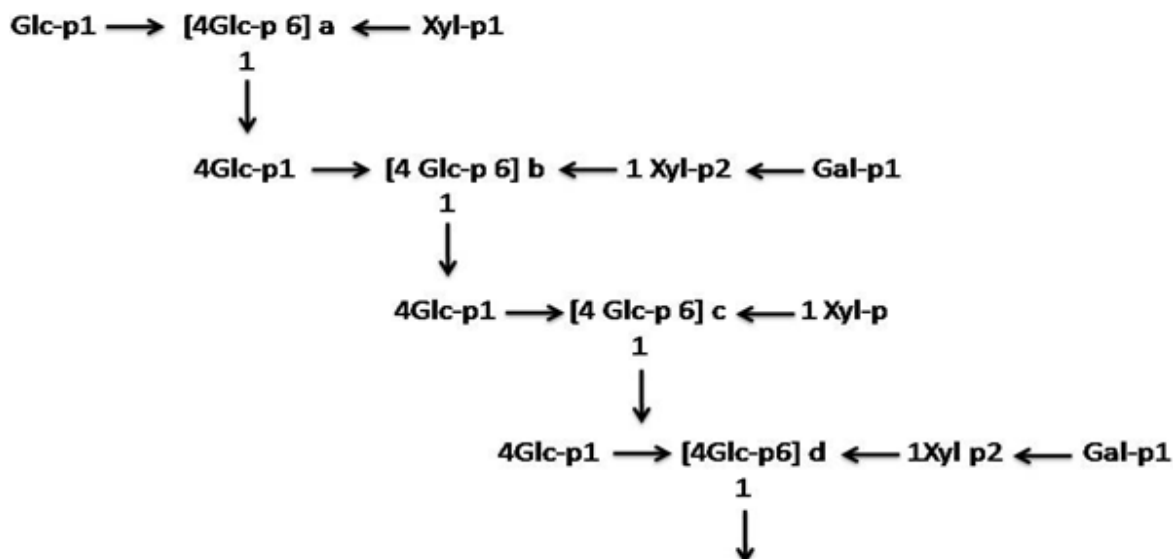


Fig.2. The proposed partial structural fragment for the polysaccharide
Glc-p=glucose pyranose, Xyl=xylose, Gal=galactose. a,b,c and d are numbers.

CONCLUSION

The present paper gives the glycosyl linkages of the polysaccharide from *Bauhinia malabarica*. Considering the results, the partial structural pattern of the repeating unit of the polysaccharide given in Fig. 2 may be suggested [8, 9].

Acknowledgements

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