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Methylation study of polysaccharides from seeds of *Bauhinia purpurea*

B.K. SRIVASTAVA[1], GIRISH CHANDRA[2], DEEPTI SAXENA and AKHILESH KUMAR

[1]Department of Chemistry, Narain P.G. College, Shikohabad - 205 135 (India).
[2]Department of Chemistry, Janta PG. College, Bakewar, Etawah (India).

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ABSTRACT

Methylation study of the polysaccharides showed three methylated components namely-2,3,di-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-mannose and 2,3,4,6-tetra-O-methyl-Dgalactose in the molar ratio of 1:2:1:. Thus, the polysaccharide consists of a main chain of D-mannanopyranose units and D-galactopyranose unit is present in the side chain.

Key words: *Bauhinia purpurea,* polysaccharides sides, methylation.

INTRODUCTION

Bauhinia Linn[1-2] (family-Leguminoceae) are the genus of 250 species. Nearly 40 species are found in India. Bauhinia purpurea is distributed sparingly throughout India and often cultivated in gardens and hedge because of ornamental value. The plant has medicinal value and economical value.

In the present communication we are reporting the methylation analysis of seed polysaccharide from Bauhinia purpurea for structural determination of the polysaccharide.

Methylation analysis[3] has been an important technique in the structural determination of polysaccharide. Recently with the introduction of micro-techniques of methylation and subsequent analysis by GLC and Mass spectra[4], the utility of this method has been greatly enhanced. Briefly the method involves complete methylation of a polysaccharide, hydrolysis of the methylated product to a mixture of partially methylated monosaccharides followed by reduction and acetylation giving an alditol acetae derivative for GLC study. The identification and quantitative estimation is done by chromatographic method and titrimetry. Since the method of Purdie and Irvine[5] many recent methods have been introduced for methylation Hakomori method[6] is the latest method.

This study indicates the position of glycosidic linkages between monosaccharide units of the polysaccharide and at the same time information regarding the presence of non-reducing terminal is also obtained. These data indicate that which monosaccharide unit constitutes the main chain and which are present in the side chain of the polysaccharide.

EXPERIMENTAL

The polysaccharide from the seeds of Bauhinia purpurea was completely methylated by Haworth method[3] followed by Hakomori method.

5gm of pure polysaccharide was dissolved in water and then treated with 50 ml NaOH (30%) and 25ml dimethyl sulphate in small quantities in a period of six hours with constant stirring in an inert atmosphere of nitrogen. After removing excess of dimethyl sulphate, the partially methylated

polysaccharide was extracted with $CHCl_3$ several times giving a glassy mass.

Partially methylated derivative was completely methylated by Hakomori method, by dissolving in dimethyl sulphoxide (DMSO) and then treated with sodium hydride (4gm) and methyl iodide (20 ml) with continuous stirring at 40°C in an inert atmosphere of nitrogen. Three batches of sodium hydride (25 mg) and methyl iodide (10 ml) were further added on successive days with continuous stirring. Fully methylated product were extracted with $CHCl_3$, (Yield 3.2gm). The completeness of methylation of the polysaccharide has been checked by I.R. giving no peak in the region of 3300-3450 cm^{-1}.

1.5 gm methylated polysaccharide was hydrolysed with 8.5 formic acid (100 ml) and then with 0.25 M H_2SO_4 (100 ml) by refluxing on a boiling water bath. After neutralization with $BaCO_3$ and filtration, the hydrolysate was concentrated to a syrup. The hydrolysate was examined qualitatively and quantialitively by paper chromatography, Alditol acetate derivative was also prepared for GLC analysis.

The hydrolysate was chromatographed in solvent system - butanol: ethanol: water (5:1:4) and the spots were visualized by spraying with aniline hydrogen phthalate.

RESULTS AND DISCUSSION

Paper chromatographic examination showed the presence of 2, 3-di-O-methyl-D-mannose [R_G 0.55, $[\alpha]_D^{25°}$ -15.6° R_G 0.54, $[\alpha]_D^{25°}$ - 15.5° (H_2O), 1,4,6-tri-O-p-nitrobenzoate derivative m.p. 192.8°C (Lit. - 192-194°], 2,3,6-tri-O-methyl-D-mannose [R_G 0.81, $[\alpha]_D^{25°}$ - 5.8° (Lit.[8]-R_G 0.81. $[\alpha]_D^{25°}$ - 5.60° (H_2O), m.p. of phenyl-D-mannanosylamine derivative 126.5-127.2°C (Lit. 127-128°C] and 2,3,4,6-tetra-O-methyl-D-galactose [R_G 0.89, $[\alpha]_D^{25°}$ + 110° (Lit[9] - R_G 0.88, $[\alpha]_D^{25°}$ + 109.5° (H_2O), m.p. of phenyl-D-galactosylamine derivative 192-192.5°C (Lit, - 192°C). The presence of the three methylated components were also confirmed by GLC.

Quantitative estimation of the methylation components by modified hypoiodide method[10] showed 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 were present in the molar ratio of 1.08: 2.18: 1.12 ie. 1:2:1. The quantitative estimation by GLC was found to be in the molar ratio of 1:1.98: 1.1 by measurement of retention time. The result are given in the table 1 and 2.

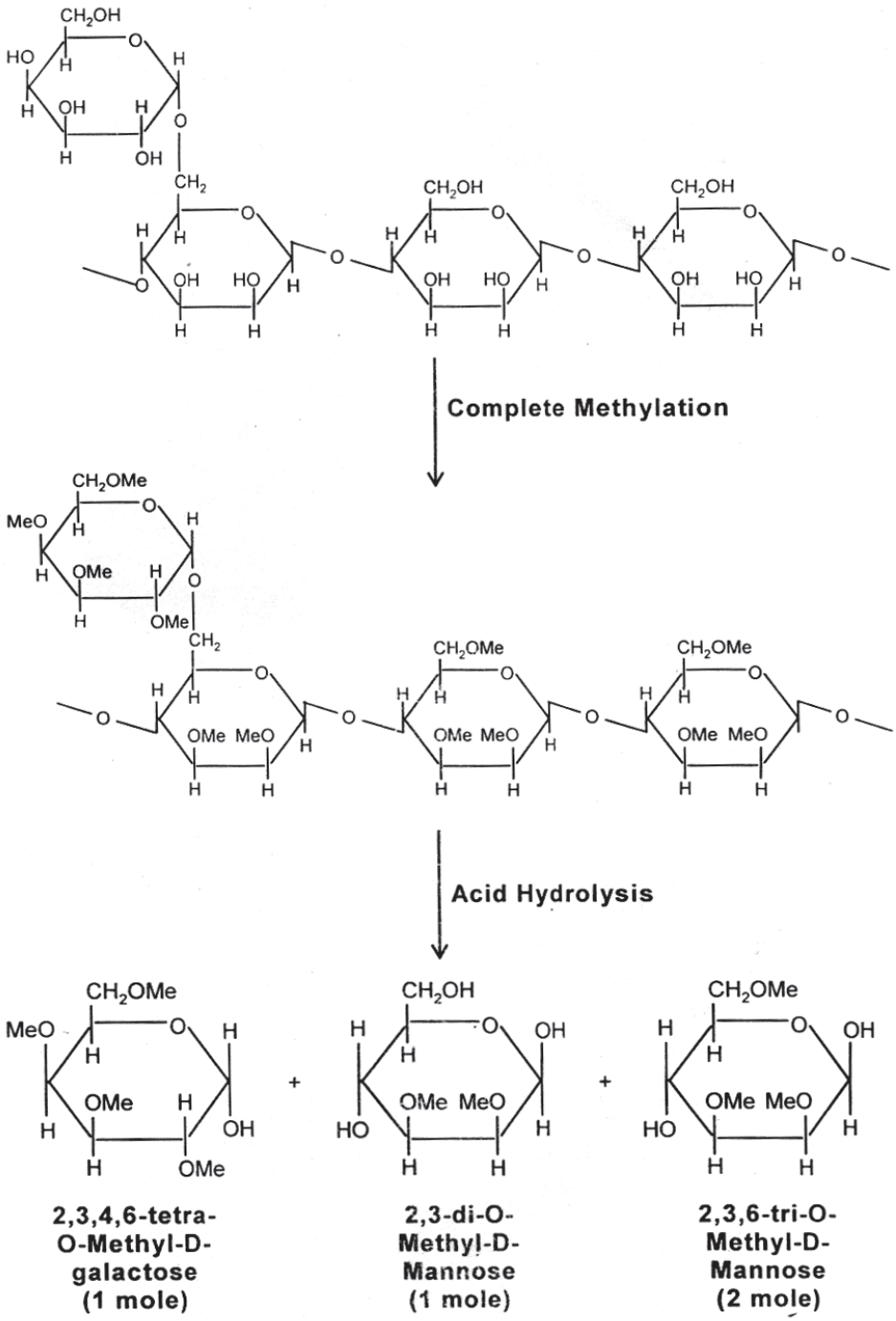
The ratio of tetra of dimethyl sugar components concludes that the repeating unit of

Table 1.

S. No.	Fractions	Methylated sugars	Iodine equivalent to hypo (ml)	Excess of I_2 equivalent to hypo (ml)	I_2 consumed in terms of hypo (ml)
1	-	Blank	9.92	-	-
2	I	2,3-di-O-methyl-D-mannose	9.92	8.84	1.08
3	II	2,3,6-tri-O-methyl-D-mannose	9.92	7.74	2.18
4	III	2,3,4,6,-tetra-O-methyl-D-galactose	9.92	8.92	1.12

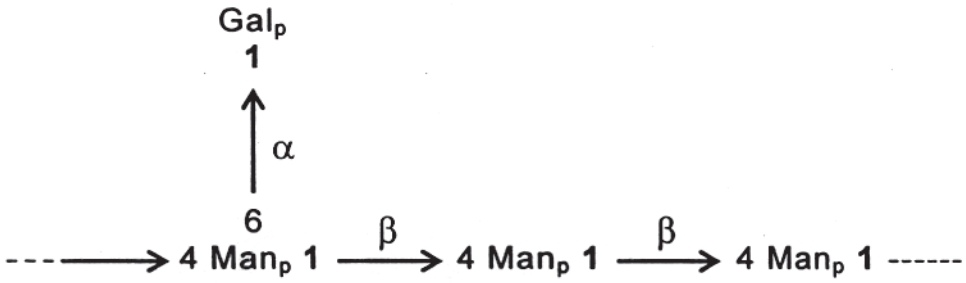
Table 2.

Peak	O-Methyl sugar as Alditol acetate	RT		Molar ratio
		Observed	Reported	
1	2,3-di-O-methyl-D-mannose	1.23	1.25	1
2	2,3,6-tri-O-methyl-D-mannose	2.19	2.20	1.98
3	2,3,4,6,-tetra-O-methyl-D-galactose 4.12	4.15	1.1	


Complete Methylation
Acid Hydrolysis
2,3,4,6-tetra-O-Methyl-D-galactose (1 mole)
2,3-di-O-Methyl-D-Mannose (1 mole)
2,3,6-tri-O-Methyl-D-Mannose (2 mole)

Scheme 1.

polysaccharide is composed of three mannose units of which one is branched and linked to single galactose unit. D-mannanopyranose units of the main chain have 1→4 linkage and D-galactopyranose unitis linked by 1→6 linkage in the side chain. This study showed that the polysaccharide from the seeds of Bauhinia purpurea may be named as 'Galactomnnan' and a tentative structure may be proposed as follows:



Scheme 2.

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