CHAPTER II

HYDROLYTIC STUDIES WITH *Adenanthera pavonina* SEED POLYSACCHARIDE.

The purpose of acid catalysed cleavage of the glycosidic bonds in a polysaccharide is (1) to achieve maximum depolymerisation with minimum decomposition of monosaccharide units (2) to effect a measure of selectivity in the fragmentation of polysaccharide having more than one type of linkages. Four important factors which control the stability of linkages are (1) their nature (i.e., whether, 1→6, 1→5, 1→4, 1→3, 1→2, 1→1), (2) the vicinity of branch points, (3) ring structure of the sugar units, (4) the presence of substituent groups on the sugar ring. In unsubstituted aldohexopyranose polymers, the 1→6 linkages are generally the most stable to mineral acid and the 1→2 linkages are found to be less stable. It has also been shown that furanosidic residues are highly acid labile. The linkage involving the reducing carbon atom of a furanoside is more labile than similar pyranoside linkage and glycosidic bonds involving uronic acids are highly resistant to acid hydrolysis.

The hydrolysis of the polysaccharide upto the full reducing strength indicates complete break down of the polymer into monosaccharide moieties Important advances have been made in the development of highly sensitive techniques in the automated analysis of saccharides. The hydrolytic conditions required to cleave the glycosidic linkages are such that the liberated sugars are destroyed to a greater or lesser extent, resulting in

incomplete recovery. Methanolysis overcomes the problem, but introduces the complication of multiple, anomeric products. Exclusion of oxygen and choice of hydrolyting agent can be extremely important factors in minimising destruction of sugars during hydrolysis. The hydrolytic studies are generally carried out with mineral acids. Hough et al.[38] have compared the survival of neutral sugars during the hydrolysis of the macromolecules under three different acids conditions individually i.e. sulphuric acid, hydrochloric acid and trifluoroacetic acid, and have observed that sulphuric acid and trifluoroacetic acid are less destructive than hydrochloric acid. It has been further shown that the loss of sugar also occurs during neutralisation by ion exchange resin treatment and barium carbonate. It is advisable therefore to conduct the neutralisation with N,N-Dioctylmethylamine. Now-a-days, trifluoroacetic acid has been preferentially used for the hydrolytic study of the polysaccharide due to its volatile nature. Due to this nature, the hydrolysis of the polymer with TFA has been recommended in the sealed tubes. The step of neutralisation can therefore be avoided to obtain maximum recovery of the sugars from the macromolecule.

In the classical method, the separation of the constituent sugars from its mixture is performed by chromatographic techniques i.e. paper chromatography, column chromatography and thin layer chromatography. The absolute configuration of the sugar moieties is determined from its melting points, optical rotation and preparation of characteristic crystalline derivative. These operations are however, time consuming. In recent years, g.l.c. and g.l.c.-m.s. analytical techniques are being followed for rapid analysis of sugar constituents. Most carbohydrates are not sufficiently

volatile to be used for gas-liquid chromatography. It is, therefore, necessary to convert sugars into volatile derivatives. Different workers had prepared various types of volatile derivatives in order to provide rapid analysis by g.l.c. and g.l.c.-m.s. methods. Methyl ethers were the first volatile derivatives of carbohydrates used for gas-liquid chromatography by Kircher[39] and Evtushenko[40]. Consumption of much time for derivative preparation and lack of good resolution in g.l.c. has restricted their general use. Sweeley and coworkers[41] used trimethylsilyl derivatives of sugars for g.l.c. analysis. With trimethylsilyl derivatives, there exists the possibility of the formation of anomeric and isomeric products which often give rise to multiple peaks in the gas-liquid chromatogram. It has been further shown that, when the crystalline monosaccharides are trimethylsilylated in pyridine, the rate of reaction becomes much faster than the rate of mutarotation with the result that the essentially only one derivative is formed providing a single peak on the chromatogram. The acetates of monosaccharides are sufficiently volatile and may be used for gas-liquid chromatography but they are less readily formed than the trimethylsilyl derivatives and still present the problem of anomeric derivatives. Much effort has therefore been expended in seeking carbohydrate derivatives suitable for analysis in which the anomeric center is eliminated. This may be conveniently accomplished by oxidation to acid or lactone, by conversion into nitrile via oxime or by reduction to alditol. The last method is simpler than oxidation and is being frequently used now-a-days for rapid analysis by g.l.c. and g.l.c.-m.s. techniques.

In order to get relevant informations about the nature of sugar constituents present in A. pavonina seed polysaccharide, the compound has

been subjected to complete acid hydrolysis with 2N sulphuric acid. The complete fragmentation (the constituent sugars) of the neutral polysaccharide molecule under the above condition is depicted in Chart - 1.4.

Complete Acid Hydrolysis of Adenanthera pavonina Seed Polysaccharide and Characterisation of the Sugar Moieties

Acid hydrolysis of the polysaccharide with 2N sulphuric acid on a boiling water bath for 12 hours caused complete breakdown of the polysaccharide into a mixture of neutral sugars. Preliminary chromatographic examination of the mixture revealed the presence of two spots corresponding to D-galactose and D-mannose (Fig. 1.7). Fractionation of the mixture on cellulose column[42,43] using n-butanol half saturated with water as eluant[44] resulted in the isolation of two individual sugars which were characterised as D-galactose (50) and D-mannose (51) from their melting points, specific rotations and melting points of their characteristic derivatives. In order to estimate the molar ratio of D-galactose and D-mannose in the polysaccharide, the compound had been subjected to quantitative hydrolysis with 2N sulphuric acid for 12 hours in a sealed tube. The constituent sugars were quantitatively separated on Whatman No. 3MM filter and estimated by periodate oxidation[45] and phenol-sulphuric acid method[46]. It was observed that D-galactose and D-mannose were present in the approximate molar ratio of 1:1.05. These results are summarised in Table 1.2.

HYDROLYTIC STUDIES OF A. PAVONINA SEED POLYSACCHARIDE

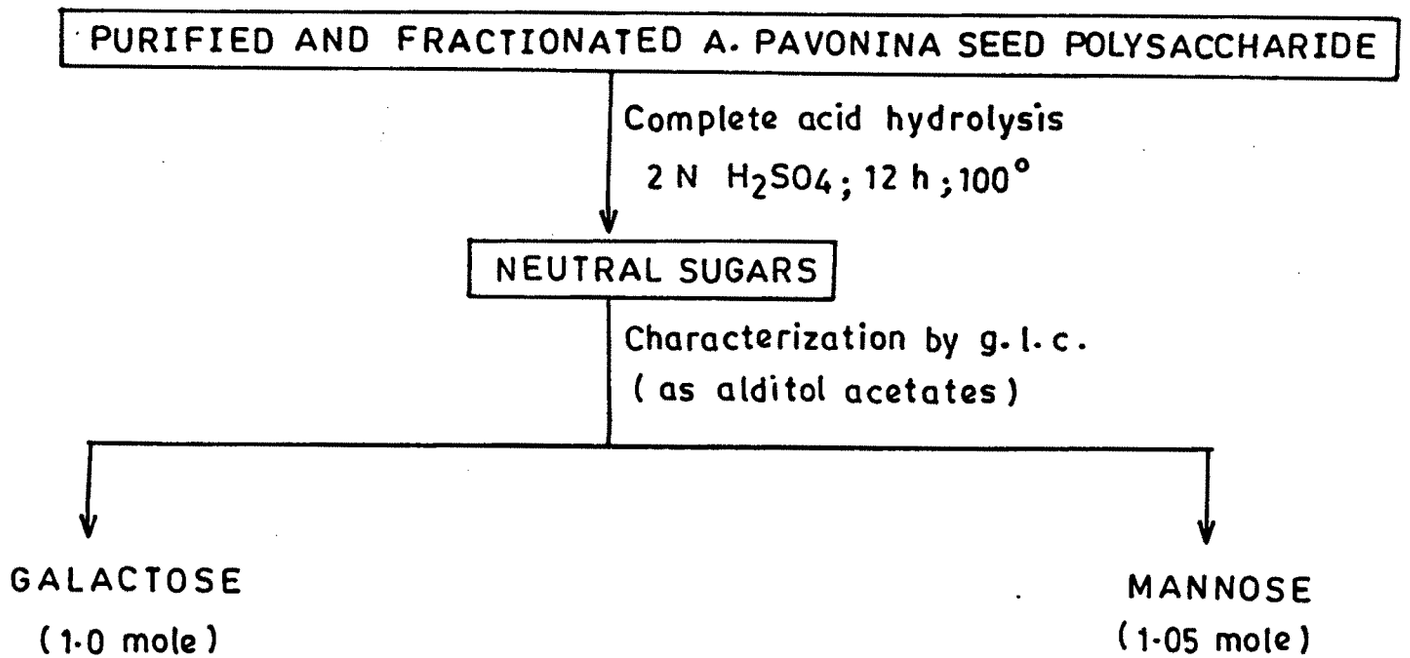


CHART – 1·4

FIG. 1.7

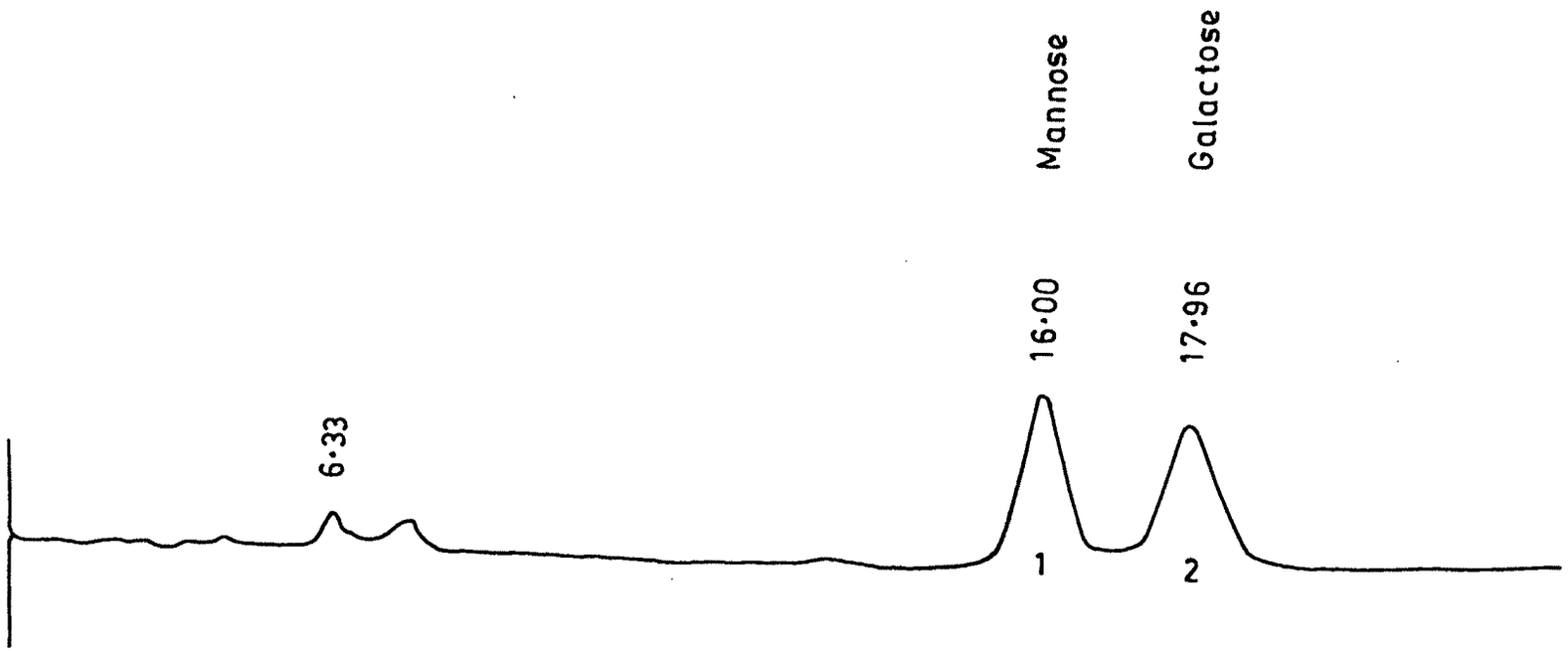
Table 1.2

Characterisation of Neutral Sugars (from the hydrolysate of the polysaccharide) by Classical Methods.

Sugar	R_f (in solvent S_1)	m.p. and m.m.p. (in ^oc)	$[\alpha]_D^{24}$ (in water)	Molar ratio	Derivative
D-galactose	0.07	165-66	+ 81.02	1.000	p-nitro-N-phenyl-D-galactosylamine, m.p. and m.m.p., 213-214^oC
D-mannose	0.11	131-32	+ 16.1	1.046	p-nitro-N-phenyl-D-mannosylamine, m.p. and m.m.p., 217-18^oC

In addition to the classical method, the separation and identification of the individual sugars from sugar mixture was also carried out by g.l.c. analysis under column condition C_1 (p. 97). The g.l.c. part is shown in Fig. 1.8. A mixture of known sugars was simultaneously subjected to g.l.c. analysis under column condition C_1 to determine the retention times (Table 1.4). A comparison of the retention times of the constituent sugars confirmed their identity as galactose (50) and mannose (51). The results

OV – 351 ; 209°C ; N_2 , 30 mL/Min.



Gas chromatogram of the alditol acetates of the sugars derived from A. pavonina seed polysaccharide

FIG. 1·8

as given in Table 1.3 showed that galactose and mannose were present in a molar ratio of 1:1.05.

Table 1.3

Characterisation of Sugars (as alditol acetates) from the Hydrolysate of the Polysaccharide by g.l.c. Analysis under Condition C_1 (OV-351).

Peak No.	Retention Time (RT*)	Molar ratio**	Alditol acetates
1	16.00	1.05	Mannose
2	17.96	1.00	Galactose

* Retention times of neutral sugars are identical with those of standard sugars.

** Relative to galactose.

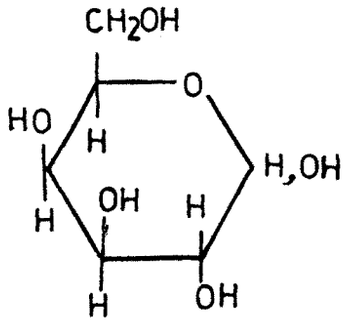
Table 1.4

G.l.c. Analysis Under Condition C_1 (OV-351) of the Alditol acetates Derived from Known Sugars

Peak No.	Retention Time (RT)	Alditol acetates
1	15.86	Mannose
2	17.85	Galactose

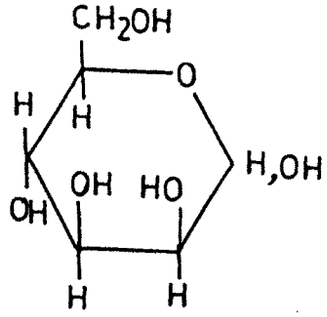
Nature of the Building Units of A. pavonina Seed Polysaccharide

A study of the complete acid hydrolysis products, clearly indicates that A. pavonina seed polysaccharide essentially composed of the following building units as shown below.



D-GALACTOSE

(50)



D-MANNOSE

(51)

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