CHAPTER - IV

HYDROLYTIC STUDY OF POLYSACCHARIDE FROM SESBANIA GRANDIFLORA SEEDS

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A) Complete acid hydrolysis of Sesbania seed polysaccharide and characterisation of the Sugar Moieties :

Acid hydrolysis of the polysaccharide (1 g) with 1N sulphuric acid (50 ml) in a boiling water bath for 15 hours caused complete breakdown of the polysaccharide into a mixture of neutral sugars. Preliminary chromatographic examination of the mixture revealed that the presence of two spots corresponding to D-galactose and D-mannose (Fig.4.1). The constituent sugars were quantitatively separated on whatman No.3 paper by Dent process.

Characterisation of Sugars

Sugar	Rf in solvent S_1	M.P. (in ^oC)	$[\alpha]_D^{24}$ in water
D-galactose	0.07	163	+81.2
D-mannose	0.11	131	+16.1

Paper Chromatography :

Partition chromatography on the filter paper sheets was carried out by descending method[94-95]. For separation of small quantities of sugars whatman No.1 chromatographic

papers were used and solvent was allowed to flow along the line direction as indicated on the paper sheets. For the separation of large quantity (upto 150 mg) Whatman filter paper No.3 were used. The following solvent system (V/V) was employed for the partition chromatography.

S_1 : n Butanol - ethanol and water (4:1:5) upper layer[96]

Following spraying reagents were used :

R_1 : Acetonic silver nitrate and Alcoholic sodium hydroxide[97].

Preparation of Spraying Reagents :

1) Acetonic Silver nitrate :

In 2 ml distilled water saturated with the silver nitrate solution was prepared and transferred in dark coloured bottle, followed by the addition of 400 ml acetone. Some part of silver nitrate separated out as a solid. Then small amount of distilled water was added till the solid dissolves. This was used as spraying reagent.

2) Alcoholic Sodium hydroxide :

4 gm. of Sodium hydroxide dissolved in minimum quantity of distilled water to which 100 ml distilled ethanol was added, to get to alcoholic Sodium hydroxide.

Development of Chromatogram :

The paper was kept in a descending chromatography chamber for three days dried and dipped in acetonic silver

nitrate which was taken in a tray. Paper was dried and alcoholic sodium hydroxide solution was sprayed over it. This paper was then dipped in an ammonium hydroxide solution and washed with distilled water and dried. The spots of D-galactose and D-mannose were observed which were compared with standard D-galactose and D-mannose (Fig.4.1).

Preparative Paper Chromatography :

The mixture of D-galactose and D-mannose was obtained after complete hydrolysis of Sesbania seed polysaccharide. The mixture was neutralised by a paste of barium carbonate, filtered and concentrated at 50°C in water bath to get about 1 ml volume. The syrup was spread on the base line of whatman filter paper no.3 as shown in the figure (Fig.4.2). By descending paper chromatography paper was run for 3 days in a chromatography chamber using the solvent, Butanol : Ethanol : water in the proportion (4:1:5) where the upper layer was used. Lower layer was kept in a 1000 ml beaker in the chamber at the bottom. After 3 days paper was removed from the chromatographic chamber and dried in air. Strips L,M,S (Fig.4.2) were cut down. The strips were developed with acetonic silver nitrate and alcoholic sodium hydroxide solution. The strips were washed with ammonium hydroxide solution and then with distilled water. The strips were further dried and developed strips were attached as shown in the Fig.4.3.

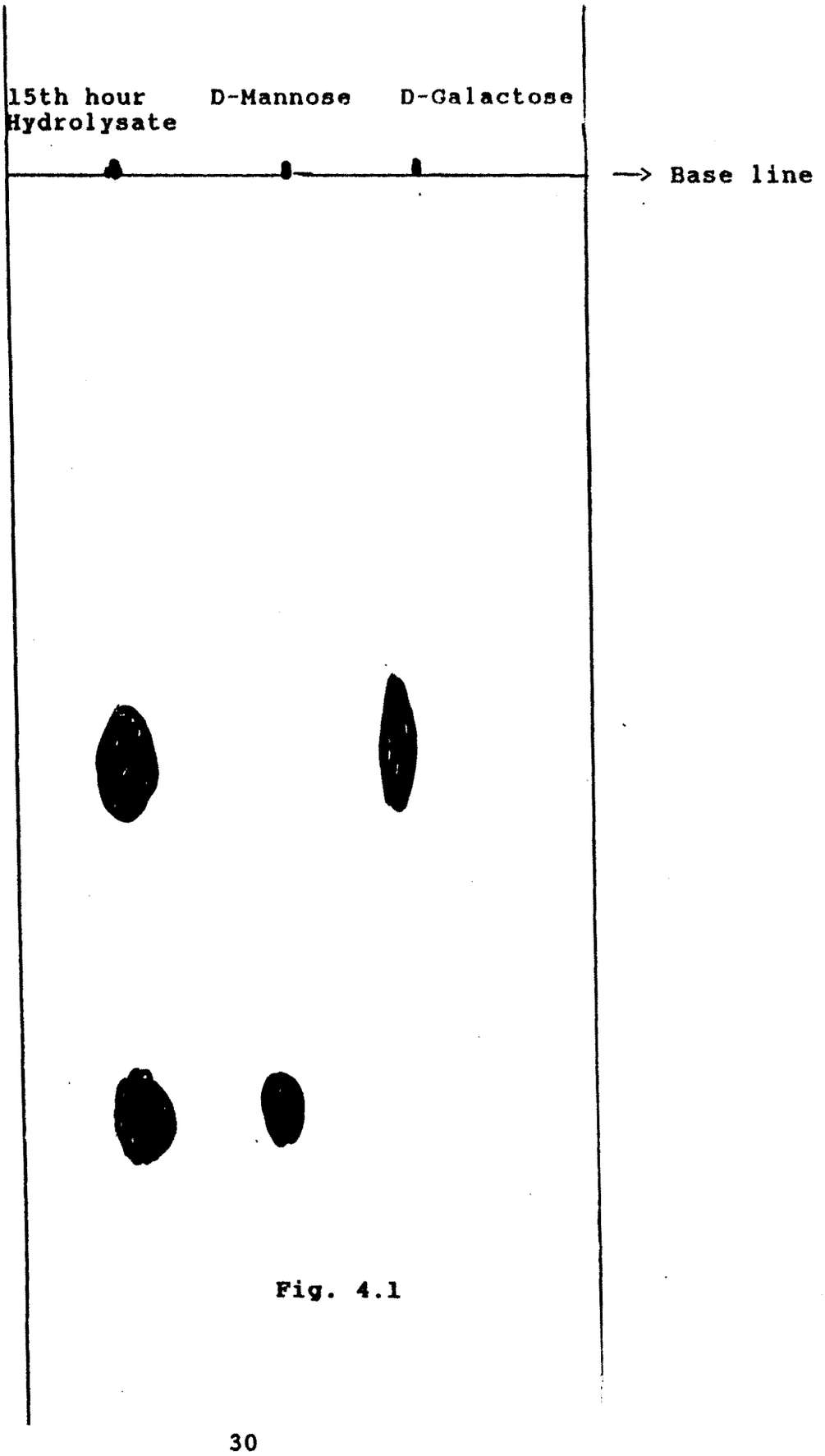

Fig. 4.1

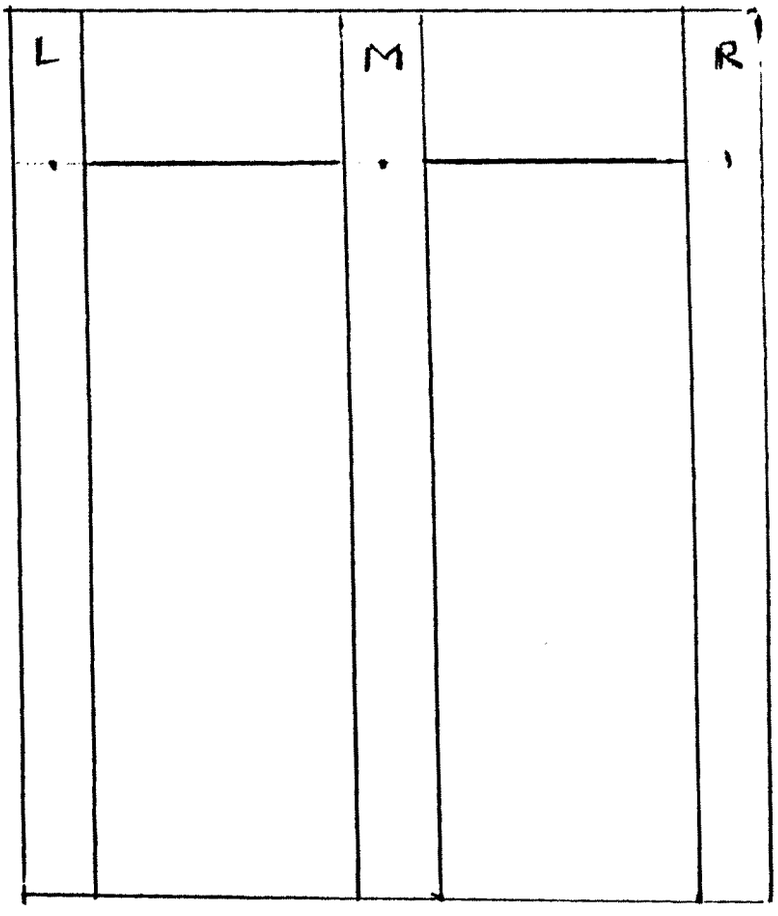

Fig.4.2 : Chromagraphy Paper for Descending Paper Chromatography (Preparative)

Spots given on L, M, R Strips.

Syrup spread on middle portion of the Base Line

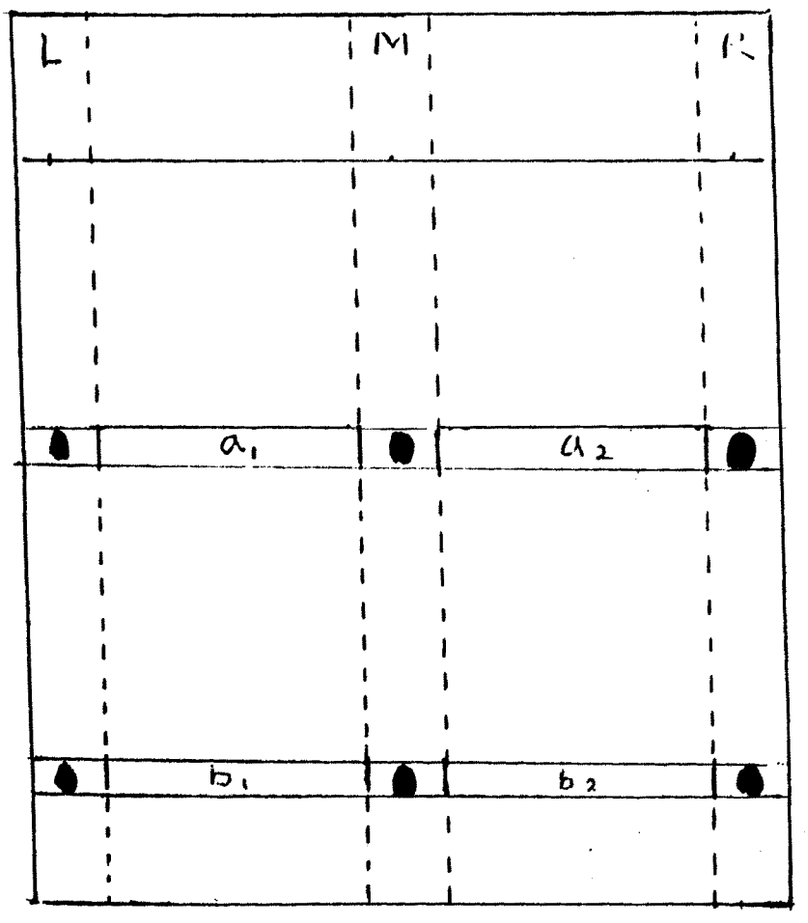

Fig.4.3 : L, M, R - Developed strips with Galactose Mannose spots

$a_1 a_2$, $b_1 b_2$ - Undeveloped strips used in Dent Process (Fig.4.4)

Strips a_1, a_2, b_1, b_2 were cut down. D-Galactose was obtained from strips a_1 and a_2. D-mannose was obtained from strips b_1 and b_2 by Dent process.

Dent Process :

A petri dish was filled with distilled water. The strip was pinched between two glass plates and rested in the petri dish as shown in the Fig.4.4. Water in the dish raised by capillary action between the plates and liquid eventually dropped off at the end of the paper into a little beaker. Evaporation was prevented by the larger outer vessel. In this way D-galactose solution was obtained from the strips b_1 and b_2. The sugar solutions were concentrated under reduced pressure at $50^{o}C$ to get D-galactose and D-mannose.

B) GRADED ACID HYDROLYSIS OF SESBANIA SEED POLYSACCHARIDE :

Polysaccharide (2 g) was subjected to the hydrolysis with dilute 0.05 N sulphuric acid (80 ml) in a boiling water bath. An aliquot sample (5 ml) was withdrawn at hourly intervals and examined as under.

The sample was cooled, neutralised with a paste of barium carbonate tested with litmus and filtered. Filtrate of each sample was concentrated to a syrupy mass under reduced pressure. The paper chromatography of a concentrated hourly sample using solvent S_1 : Butanol + Ethanol + Water (4:1:5) upper layer and spraying reagents R_1 : (Acetonic silver nitrate, alcoholic sodium hydroxide) was carried out.

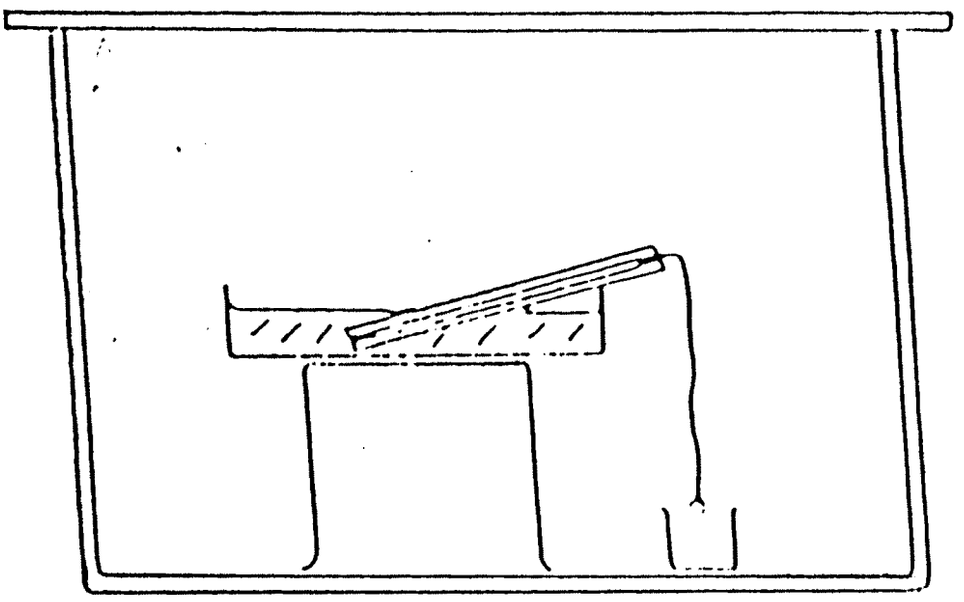

Fig.4.4 : Dent Process

During the hydrolysis the galactose appeared first after 2 hours followed by mannose after 3 hours. The spots of four oligosaccharides started appearing between 5 to 9 hours. After 12 hours of hydrolysis process some oligosaccharides were hydrolysed. Therefore, after 10^{th} hour hydrolysate (30 ml) was concentrated, neutralised and used for preparative paper chromatography to separate the oligosaccharides. Solvent was run for 312 hours to get good separation of 4 oligosaccharides (Table 4.1. Fig.4.5, Fig.4.6).

TABLE 4.1

CHARACTERISATION OF OLIGOSACCHARIDES OBTAINED FROM SESBANIA GRANDIFLORA SEED POLYSACCHARIDE

Sugar	Specific Rotation $[\alpha]_D^{26}$	P.C. Mobility relative to D-galactose (R-galactose) Solvent S_1	M.P. ^oC	Products of complete Acid hydrolysis of the oligo-saccharide
Disaccharide (A)	-7.5	0.81	193-94	D-mannose
Disaccharide (B)	+122	0.65	200-01	D-galactose & D-mannose
Trisaccharide (C)	+36.6	0.43	228	D-galactose & D-mannose
Trisaccharide (D)	-21.8	0.17	224	D-mannose

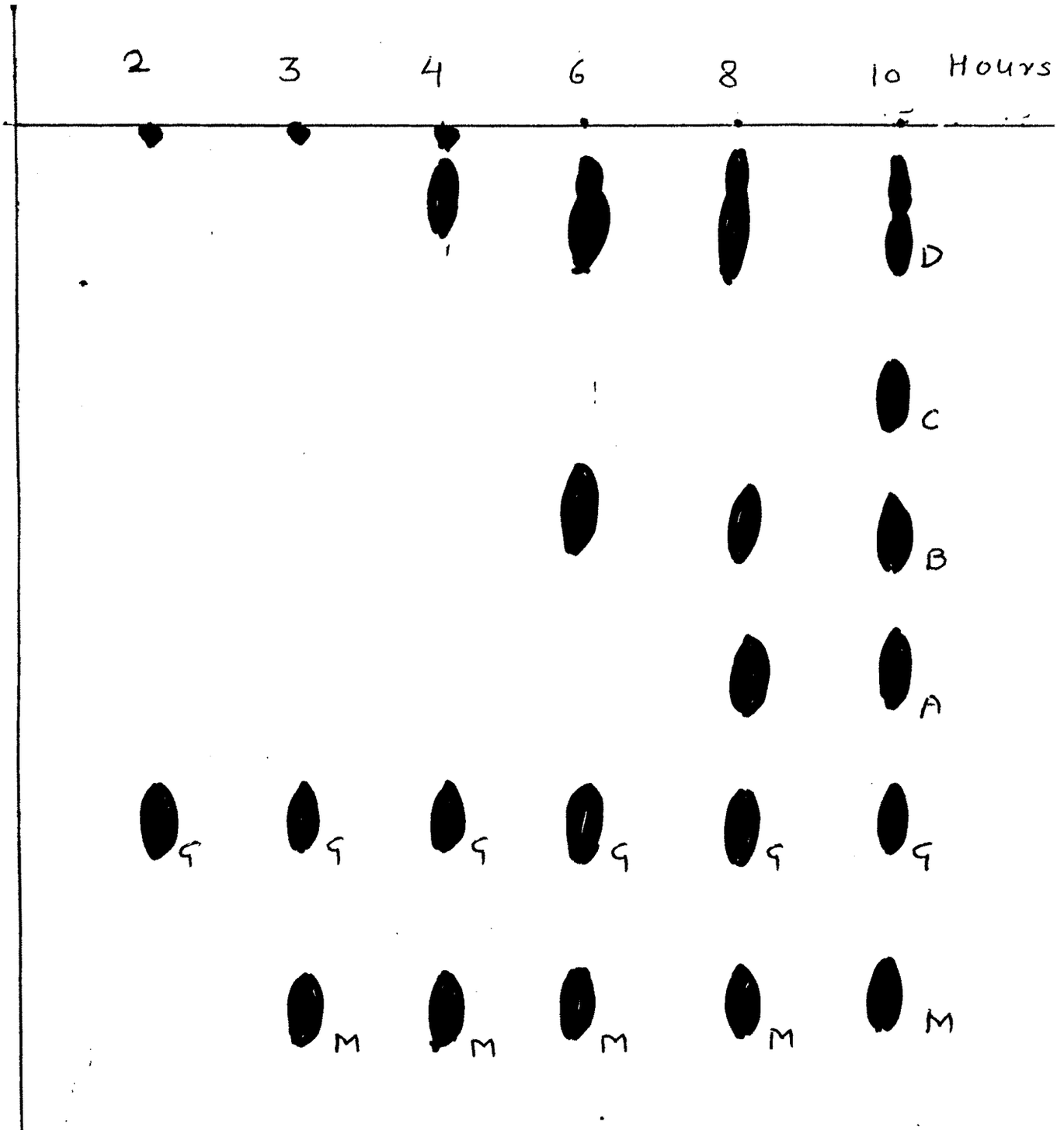

Fig.4.5 : Nature of Chromatogram of graded hydrolysis from 2 hours to 10 hours. Running period 7 days in Descending paper chromatography.

G = Galactose **M = Mannose**

A,B,C,D = Oligosaccharides

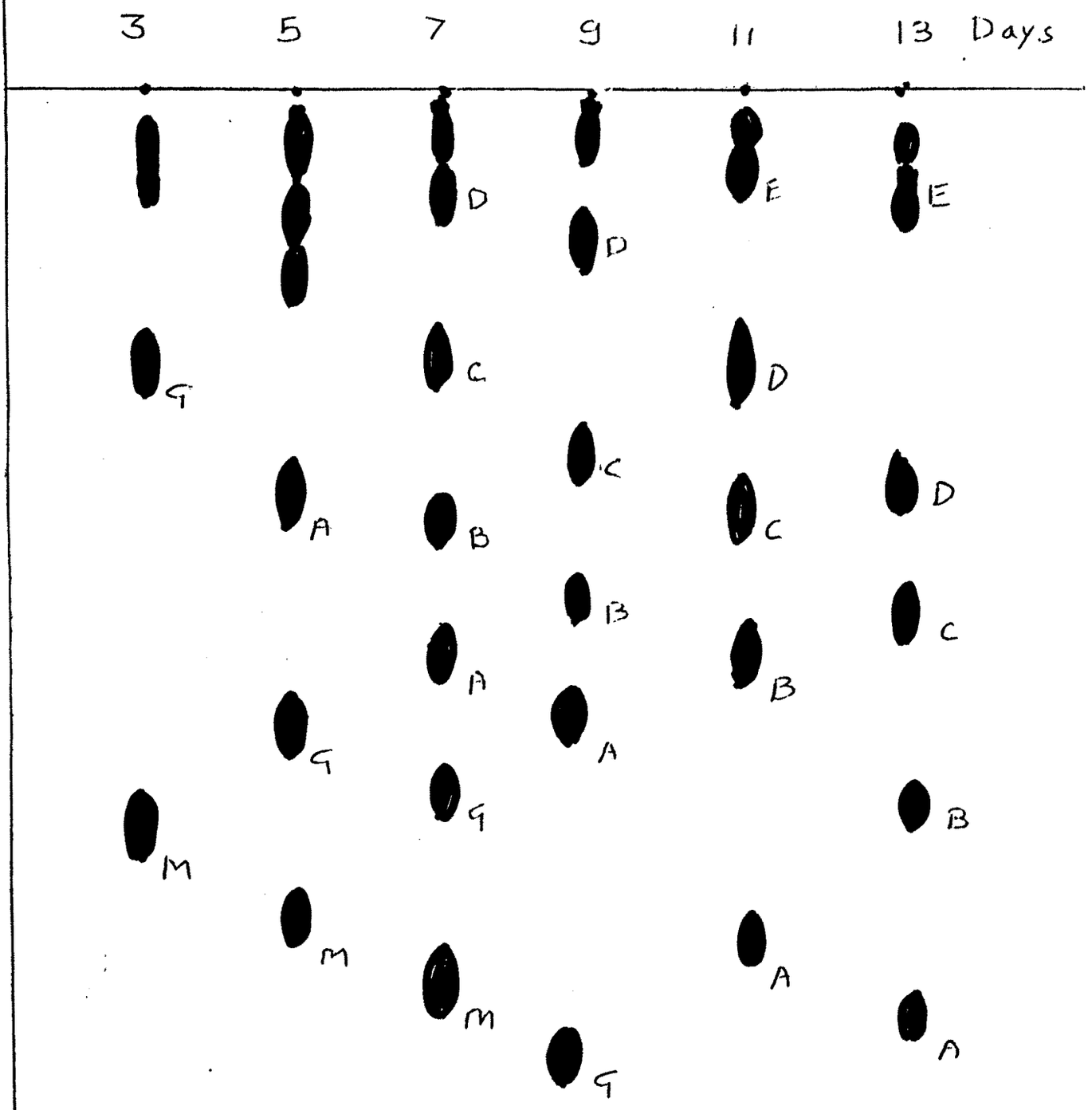

Fig.4.6 : Nature of Chromatogram hydrolysate after 10 hours by changing the running period from 3 days to 13 days.

G = Galactose M = Mannose

A,B,C,D = Oligosaccharides

Characterisation of Neutral Oligosaccharides :

1) Identification of Disaccharide (A) :

Disaccharide (A) $[\alpha]_D^{26}$ - 7.5° (C, 1.2% in water). M.P. 193-94°C, gave a single spot paper chromatographically R-galactose was found to be 0.81 in solvent S_1 and spraying reagent R_1.

The compound (0.046 g) was heated on a steam bath for 6 hours with sulphuric acid (1 N, 16 ml). The hydrolysate was cooled, neutralised and filtered. The filtrate was concentrated to a syrup. Paper chromatographic analysis of the syrup showed a spot corresponding to D-mannose only. All these results indicated that compound (A) may be designated as 4-O-ß-D-mannopyranosyl-D-mannose. The $[\alpha]_D$,-7.5 and M.P. 193-94°C of disaccharide (A) are in close agreement with the values reported by Bhattacharya[53] et al, and by Henderson[80], Hough and Painter, respectively (Fig.4.7).

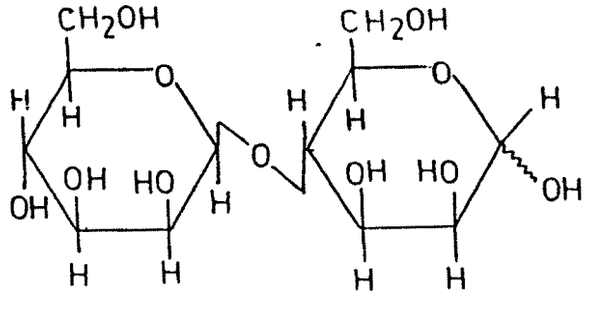

Fig.4.5 : 4-O-ß-D-mannopyranosyl-D-mannose

(Disaccharide A)

2) Identification of Disaccharide (B) :

Disaccharide (B) $[\alpha]_D^{26}$ + 122° (C, 0.9% in water), M.P. 200 to 201°C upon chromatographic examination furnished a single spot, i.e., R-galactose was found to be 0.65 in solvent S_1 and spraying reagent R_1. The disachharide (0.05 g) was hydrolysed with sulphuric acid (1 N, 10 ml) on a steam bath for 6 hours. The usual working up of the hydrolysate and subsequent paper chromatographic analysis gave rise to a mixture of D-galactose and D-mannose. All these results indicated that compound (B) may be designated as 6-O-α-D-galactopyranosyl-D-mannose. The $[\alpha]_D$ and M.P.values of B are in close agreement with the corresponding values reported in literature[53,80] (Fig.4.8)

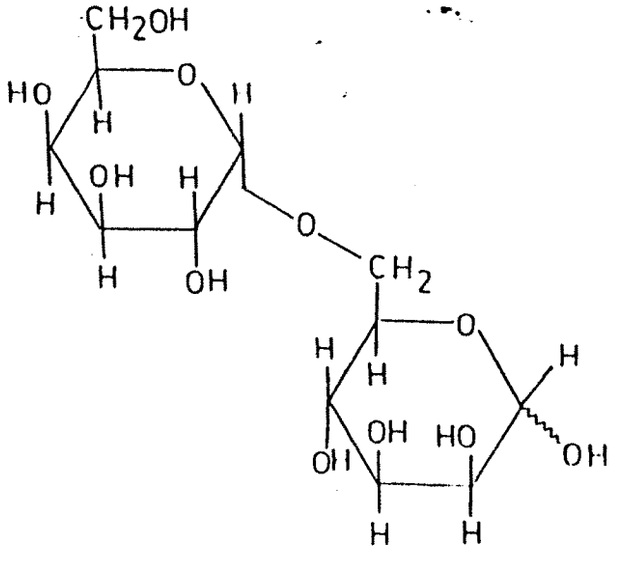

Fig.4.8 : 6-O-α-D-galactopyranosyl-D-mannose (Disaccharide B)

3) Identification of Trisaccharide (C) :

Trisaccharide (C), $[\alpha]_D^{26}$ + 36.6 (C, 1.02% in water) showed a single spot on a paper chromatogram, M.P. 228°C R-galactose of trisachharide (C) was 0.43, in solvent S_1 and spraying reagent R_1. Acid hydrolysis of the compound (0.062 g) with sulphuric acid (1N, 10 ml) on steam bath for 7 hours revealed a mixture of D-galactose and D-mannose identified by paper chromatography.

Trisaccharide (C) is therefore assigned a structure 0-α-D-galactopyranosyl (1 -> 6) 0-ß-D-mannopyranosyl-(1 -> 4) D-mannose. The same trisaccharide having identical characteristics earlier isolated by Bhattacharya and his co-workers (Fig.4.9)

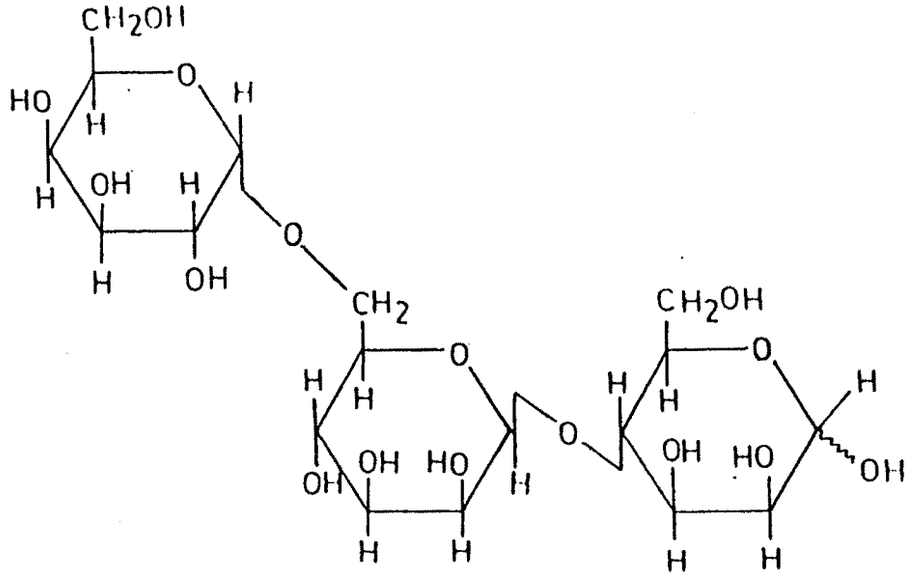

Fig.4.9 : 0-α-D-galactopyranosyl (1 -> 6)-0-ß-D-mannopyranosyl-(1 -> 4)-D-mannose

(Trisaccharide C)

4) Identification of Trisaccharide (D) :

Trisaccharide (D) $[\alpha]_D^{26}$ - 21.8°C (C 0.94% in water) showed a single spot on paper chromatogram. R-galactose was found to be 0.17 in solvent S_1 and spraying reagent R_1 and M.P.224°. Acid hydrolysis of the trisaccharide (0.04 g) with sulphuric acid (1 N, 5 ml) on steam bath for 7 hours produced D-mannose only, which was identified paper chromatographically.

On the basis of above findings the trisaccharide (D) may be designated as 0-ß-D-mannopyranosyl (1 -> 4)-0-ß-D-mannopyranosyl (1 -> 4)-D-mannose. The same trisaccharide having identical characteristics was also isolated earlier by Bhattacharya[53] et al. from other plant sources (Fig.4.10)

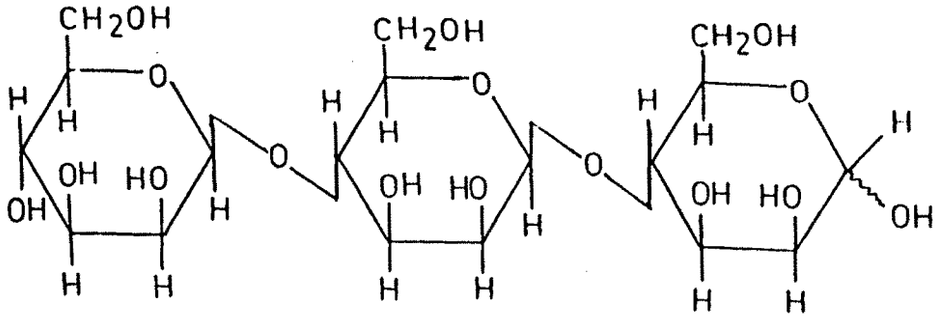

Fig.4.10 : 0-ß-D-mannopyranosyl (1 -> 4)-0-ß-D-mannopyranosyl (1 -> 4)-D-mannose

Gohar Khan and M.I.H.Farooqui[101]; studied the galactomannans obtained from Casia siamealam seeds. They have carried out graded acid hydrolysis and the oligosaccharides so formed were separated by paper chromatography, using the solvent system S_2 i.e. ethyl acetate-acetic acid-butanol-water in the proportion (8:6:4:4) with spraying reagent silver nitrate and sodium hydroxide. Thus, it was observed that the presence of four oligosaccharides in addition to galactose and mannose (Table 4.2).

TABLE 4.2

Results of Partial acid hydrolysis

Sugar	M.P. ^{o}C	P.C.Mobility relative to D-galactose (Solvent S_2)
Disaccharide (A)	203-5	0.66
Disaccharide (B)	200-2	0.52
Trisaccharide (C)	228-30	0.40
Trisaccharide (D)	224-26	0.28
Oligosaccharide (E)	Unidentified	-

The structures of oligosaccharides suggested by Gohar Khan and M.I.H.Farooqui[101] are the same which we have suggested for the oligosaccharides obtained from Sesbania

grandiflora seeds. From the present results obtained, so far a simple symmetrical structure I was proposed for the polysaccharide obtained from Sesbania grandiflora (Fig.4.11).

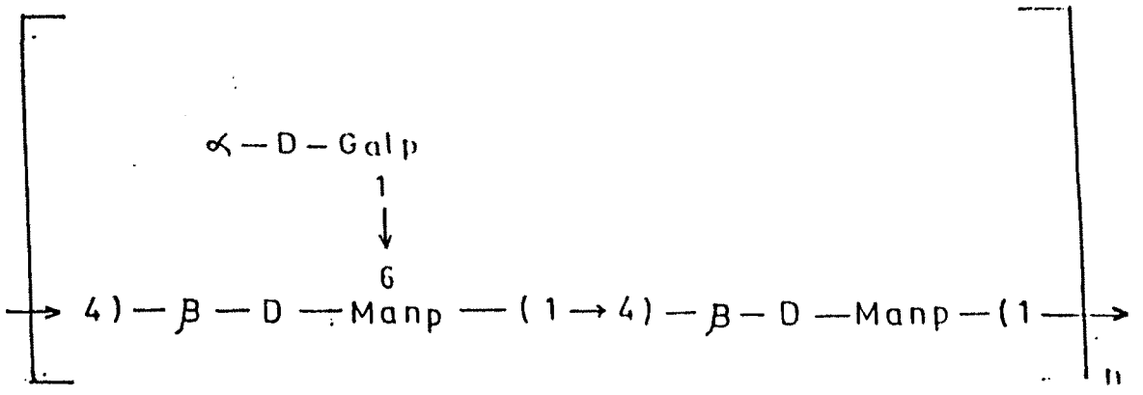

Fig.4.11 : Polysaccharide from seed of Sesbania grandiflora