PART II

SECTION (B)

CHEMICAL INVESTIGATIONS OF THE *Adenanthera pavonina* SEED POLYSACCHARIDE

CHAPTER I

ISOLATION AND PURIFICATION OF THE POLYSACCHARIDE OCCURRING IN THE SEEDS OF A. pavonina.

Matured Adenanthera pavonina seeds were cleaved in a low-speed grinder in order to obtain the endosperm together with the seed coat broken-off from the cotyledons. After removal of husk, the finely powdered, dried, and defatted seeds were extracted with water at 50°C, filtered and centrifuged to furnish a clear solution. The clear aqueous extract, after being acidified with acetic acid, was centrifuged and filtered through Kiesulghur bed in order to remove turbidity from the aqueous extract. The resulting solution was poured slowly into large excess of ethanol when a creamy white precipitate of crude polysaccharide was obtained (Chart-1.2) This was further purified by cation exchange resin treatment followed by dialysis and also fractionated through copper complex formation (Chart-1.3). The white fibrous material thus obtained, showed ash content (0.12%). It dissolves slowly in water to form an almost neutral (pH, 6.6) solution.

Homogeneity of A. pavonina seed polysaccharide :

Purified polysaccharide may be either homogeneous or heterogeneous. In elucidation of fine structure of polysaccharide, the purity and homogeneity of the starting material are of primary importance. With the help of modern analytical techniques, it is now possible to separate and identify components present to the extent of 0.5 percent or even less in the

ISOLATION OF ADENANTHERA PAVONINA SEED POLYSACCHARIDE

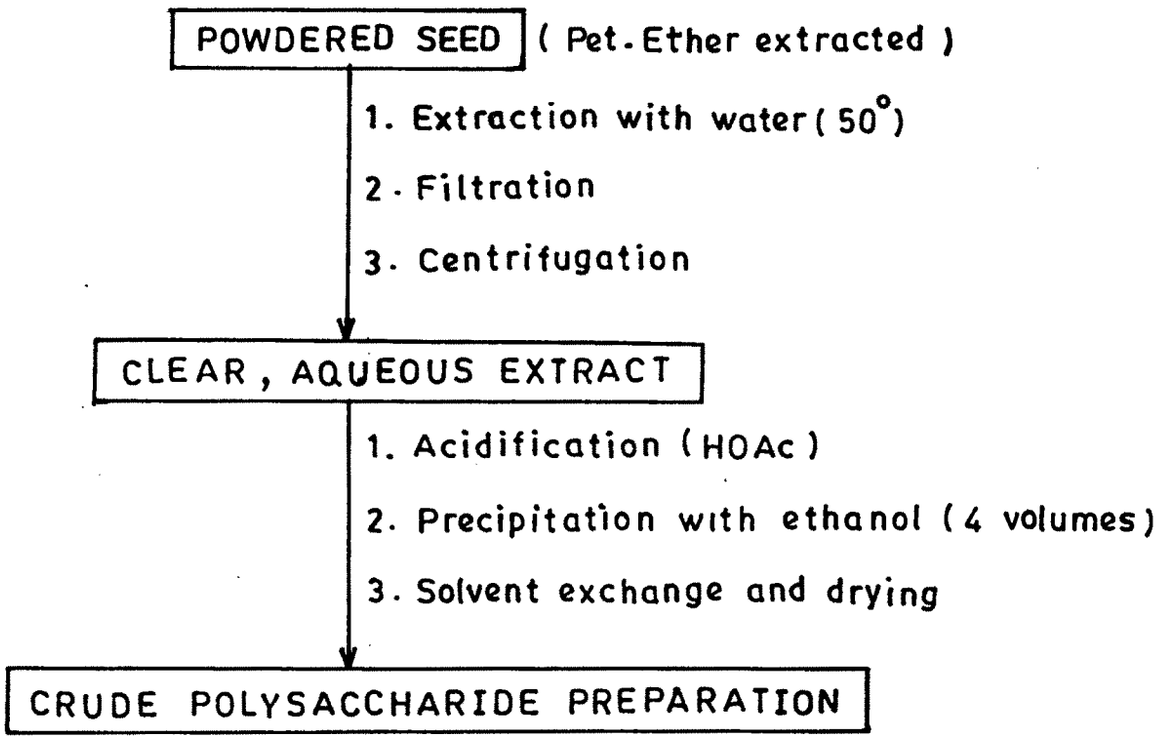


CHART-1·2

PURIFICATION AND FRACTIONATION OF A. PAVONINA SEED POLYSACCHARIDE PREPARATION

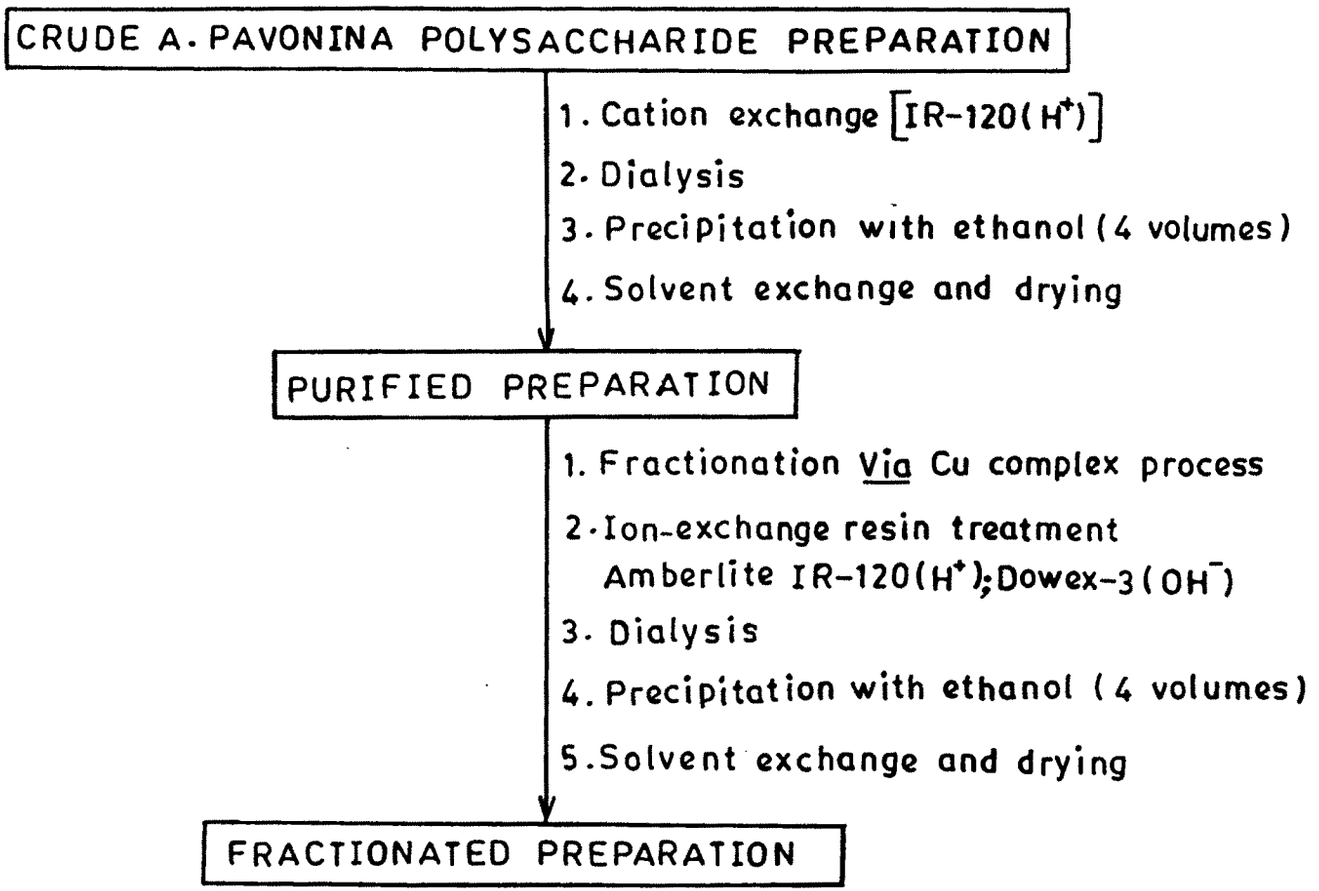


CHART 1·3

hydrolysate of polysaccharides and their methyl derivatives. Whether or not these compounds of low yield are of structural significance, depends mainly upon the purity of the original polysaccharide. If the minor components arise from the polysaccharide material itself, they may be helpful for the deduction of structure, but if they are derived from impurities present in the polysaccharide, then any structural elucidation based upon them will obviously be erroneous. It is therefore, of utmost importance to isolate the polysaccharide as a homogeneous entity before attempting to characterise it. A number of methods are available for establishing the homogeneity of the polysaccharide such as :

(1) Fractional precipitation by using organic solvents[4-6], salts[7], complexing agents[8,9] and subsequent analysis of different fractions.

(2) Selective precipitation of polysaccharide may also be effected with certain proteins such as antisera[10-13] or concanavalin[14] as developed by Heidelberger and coworkers.

(3) Deproteinization of the crude product of polysaccharide with proteolytic enzymes such as Pronase P[15].

(4) Fractionation by Gel permeation or molecular sieve chromatography[16,17] and ion-exchangers such as DEAE-Cellulose[18,19], ECTEOLA-Cellulose or resins[20,21].

(5) Electrophoresis in a Tiselius cell[22,23] using supporting matrix as filter paper[24] or silicagel or single cellulose fibre[25] or glass fibre sheets[26-28] or preparative zone electrophoresis on glass powder column[29].

(6) Displacement chromatography by using cellulose[30,31] or charcoal column[32].

(7) Ultracentrifugal analysis[33,34].

In the present investigation, the homogeneity of A. pavonina seed polysaccharide has been established by paper electrophoresis.

Paper electrophoresis

Electrophoresis, a device where the molecule migrates under the influence of an applied electric field, has found wide application for judging the homogeneity of a polysaccharide. A 1.0% solution of the purified and fractionated polysaccharide in 0.05 M sodium tetraborate decahydrate buffer (pH, 9.2) was examined on Laboratorium Felzercisk model DE-201 apparatus, using Whatman No.1 MM filter paper sheet. After spraying with staining reagent, the electrophoretic pattern (Fig. 1.3) shows only single spot, thereby establishing the homogeneity of the sample.

Preliminary Analysis of A. pavonina Seed Polysaccharide :

Purified and fractionated polysaccharide, $[\alpha]_D^{30}$ + 71.02° (C, 0.09 in water) was free from starch as indicated by the absence of blue colour with iodine solution and did not reduce Fehling's solution or Tollen's reagent. The usual tests showed that nitrogen, sulphur, halogens, methoxyl, pentoses and anhydrouronic acid groups were absent. The infrared spectrum (Fig.1.4) of the polysaccharide in KBr pellets showed a broad band for -OH (3400 cm^{-1}) along with other absorption bands at 2920, 1640, 1155, 1070, 1030, 875 and 817 cm^{-1}. The last two absorption bands at 817 and 875 cm^{-1} are indicative of the presence of α-linked-D-galactopyranose and ß-linked-

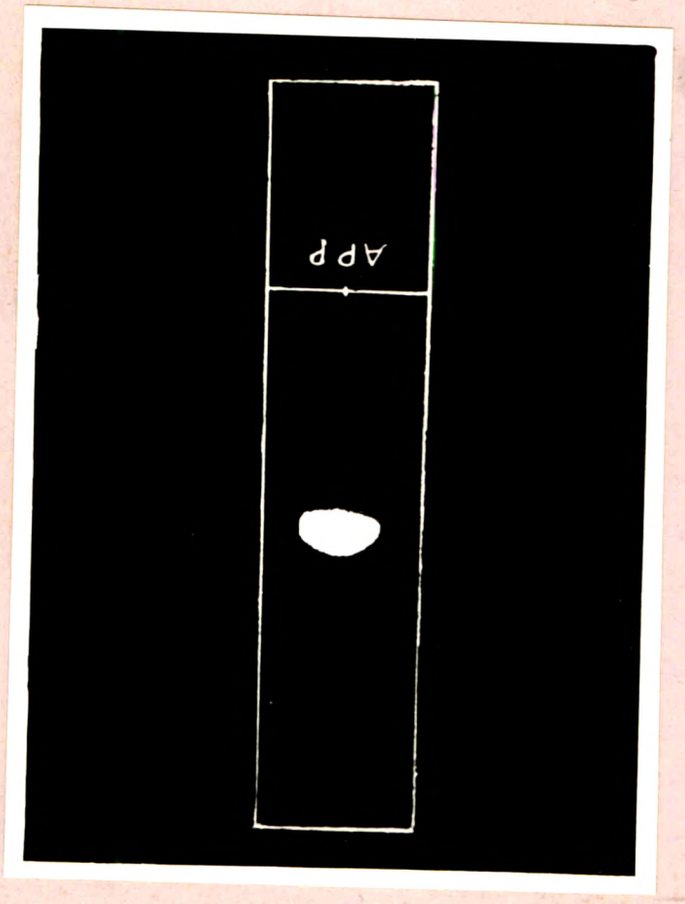
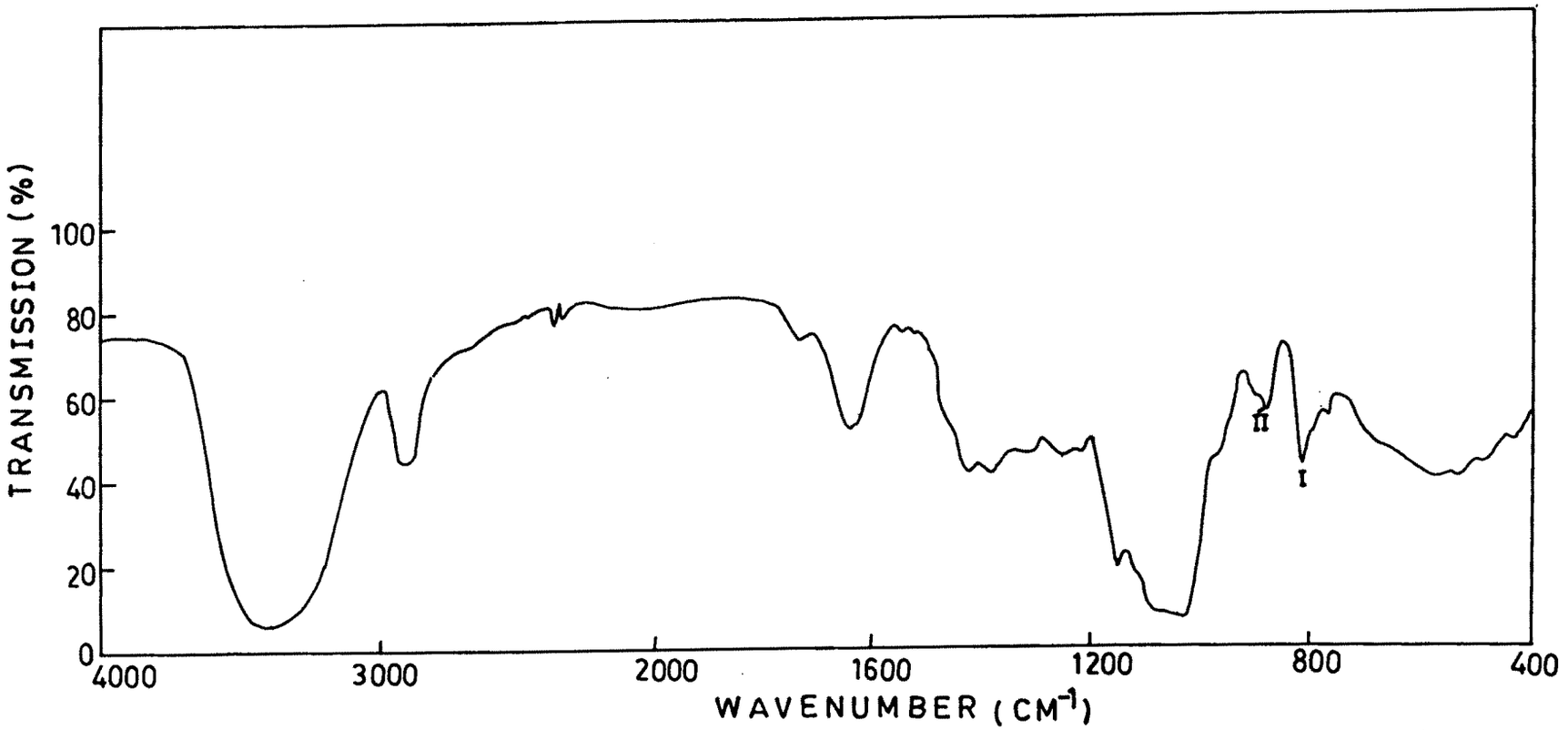


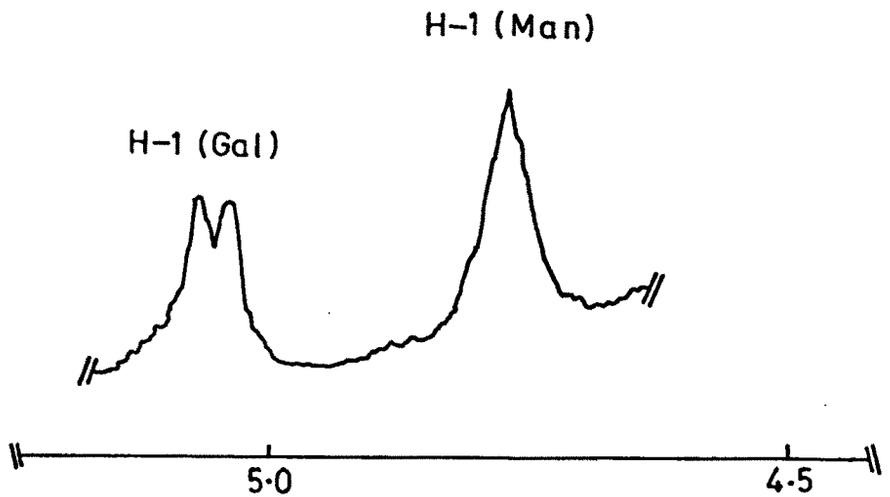
FIG.1.3



I.R. spectrum of A. pavonina seed polysaccharide (KBr pellets).

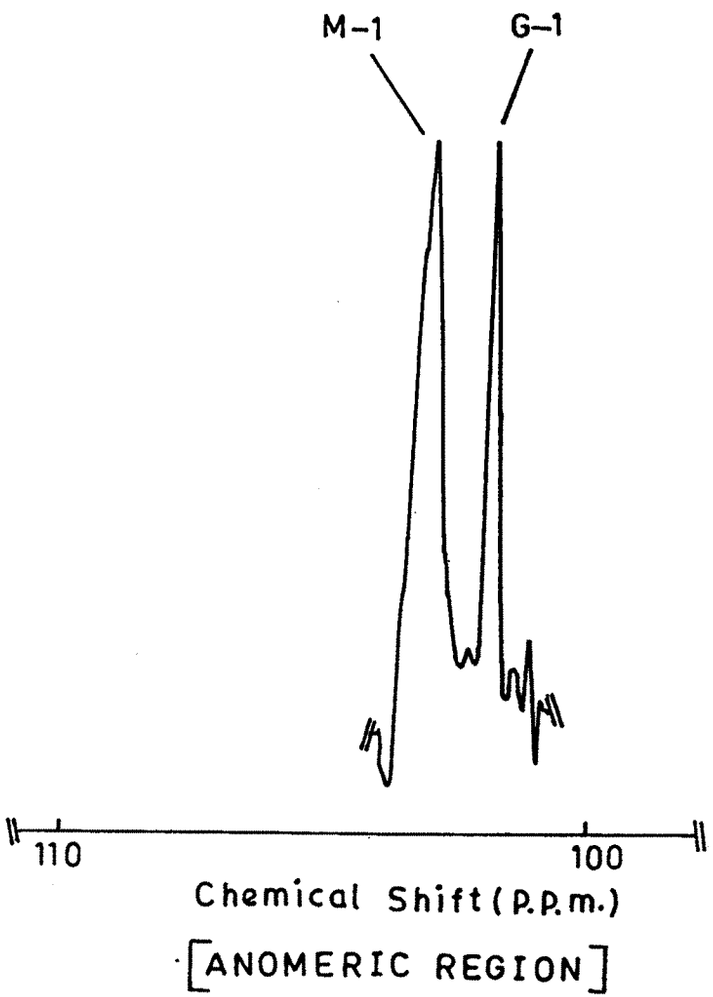
I. α-linked D-Galp, II. β-linked D-Manp

FIG. 1·4



^{1}H-N.m.r. spectrum (100 MHz) of solution (10 mg/ 0·4 ml) in D_2O (at 90°) of A. pavonina seed polysaccharide

FIG. 1·5



^{13}C – N.m.r. spectrum (25 MHz) of solution (32 mg/0·4 ml) in D_2O (at 90°) of A. pavonina seed polysaccharide

FIG. 1·6

-D-mannopyranose residues respectively[35]. The ^{1}H- and ^{13}C-N.m.r. analysis are now-a-days frequently used for ascertaining the anomeric configuration of the constituent sugar units of a polysaccharide. Accordingly ^{1}H- and ^{13}C-N.m.r. analysis of polysaccharide was carried out. In ^{1}H-N.m.r. spectrum of A. pavonina seed polysaccharide (Fig. 1.5), the resonances of the anomeric protons are well separated. The doublet at δ 5.0 p.p.m., from H-1 (Gal) having $J_{1,2} \sim 3.0$ Hz was observed for monomeric α-D-galactopyranose residues[36,37]. The signal at δ 4.8 p.p.m., from H-1 (Man) having $J_{1,2} \sim 1.0$ Hz was also observed for monomeric ß-D-mannopyranose residues[36,37]. In ^{13}C-N.m.r. spectrum of the polysaccharide (Fig. 1.6), two C-1 signals at low field values at δ 101.8 and 103.0 could be ascribed to the presence of α-D-galactopyranosyl and ß-D-mannopyranosyl units, respectively. Such C-1 signals for α-D-galactopyranosyl and ß-D-mannopyranosyl moieties have been observed earlier by Gupta et al.[37] and Grasdalen and his coworkers[36].

****