CHAPTER - V

ENZYMATIC AND NMR STUDIES OF GALACTOMANNAN FROM SESBANIA GRANDIFLORA SEEDS

INTRODUCTION :

Srivastava and his co-workers[28] determined the structure of galactomannan isolated from the Sesbania grandiflora (Family - Leguminoseae) seeds by chemical methods and suggested that, it contains regular arrangement of D-galactosyl residues in the side chains[1] (Fig.5.1). It was confirmed by structure of oligosaccharides.

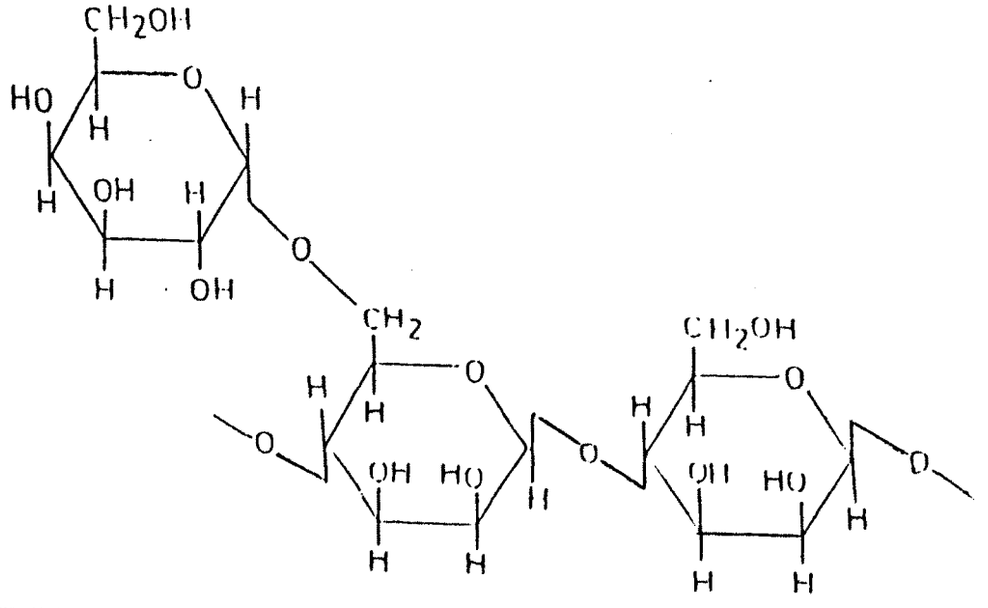

Fig.5.1

CHAPTER - V

ENZYMATIC AND NMR STUDIES OF GALACTOMANNAN FROM SESBANIA GRANDIFLORA SEEDS

However, the methylation analysis, the primary structural technique used is dependent on complete methylation and subsequent total hydrolysis of the methylated polymer and provides only information on the mole percentages of the various chain and branch point units. It gives no information about the relative position of these residues in the polymer. NMR spectroscopy provides a convenient technique complementary to methylation fragmentation analysis. It can disprove or confirm previous structural findings and provide new, independent structural information about nearest neighbour sequences and anomeric configurations[98]. We have carried out enzymatic hydrolysis and NMR spectroscopy to obtain the information on the fine structure of the galactomannan of Sesbania grandiflora seed.

EXPERIMENTAL :

The preparation of the purified and fractional Sesbania grandiflora seed polysaccharide was done by the method of Srivastava et, al[28]. The homogeneity of the polymer was established by paper electrophoresis. The elctrophoretic analysis showed a single spot. The enzymatic hydrolysis of the polysaccharide was carried out with α-D-galactosidase. Suspension (5 mg/ml), from coffee beans, Bochringer mannheim using sorensen phosphate buffer (pH = 6.5). The reaction mixture was dialysed and the released sugars are characterised by paper chromatography[64] by comparing with

standard sugars. The solvent system n-butanol-ethanol-water (4:1:5) upper layer, was used and the spraying reagents acetonic $AgNO_3$ and alcoholic NaOH were used.

The ^{1}H NMR and ^{13}C NMR spectra were recorded in the FT mode at 100 MHz and 25 MHz using the solutions of the polymer (2 mg/0.4 ml) and (25 mg/0.4 ml) in D_2O at 90°C and 95°C respectively with JEOL FX 100 spectometer. Chemical shifts are expressed relative to sodium 2,2,3,3-tetradeuterio-4,4-dimethyl, 4-silapentanoate (TDS) as an internal standard[99]. The deuterium resonance was used as a field frequency lock ^{13}C NMR spectrum, acquired by using 8000 data points and a spectral width of 5KHz. Free induction decay was accumulated with a 75° pulse and a repitition time of 0.8 sec spectrum in which the NOE removed were also measured, in order to ensure that relative peak areas represented relative abundances. A probe temperature 90°C (in ^{1}H NMR) and 95°C (in ^{13}C NMR) were used to diminish viscocity and, thereby, line width, peak areas were measured by planimetry. The C-4 signal of a D-mannopyranosyl unit was reconstructed in a computer by superposition of three lorentzian lines of equal width.