The polysaccharide under study was isolated from the defatted seeds of Prunus armeniaca by extraction with water at 60°C followed by ethanol precipitation. The crude polysaccharide was purified sequentially by dialysis, copper complex formation and gel permeation chromatography on a column of sepharose –CL-2B. It eluted as two peaks – one major and the other minor with respective molecular weights 458000 Da and 79500 Da. Only major polysaccharide fraction (mol wt. 458000) was chosen for the structural elucidation. It was free from ash, nitrogen, and protein and was fairly soluble in hot water, soluble in DMSO on heating and freely soluble in dilute NaOH (5%). The specific rotation, $[\alpha]_D^{20}=35^\circ$. The observed peaks in the FT-IR spectrum of the polysaccharide (Fig.13) and their assignments are given below in Table-II.
### TABLE-II

**FT-IR peaks of the native polysaccharide and their assignment**

<table>
<thead>
<tr>
<th>Peak position (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>3444</td>
<td>OH stretching (hydrogen bonded)</td>
</tr>
<tr>
<td>2988 and 2917</td>
<td>CH stretching</td>
</tr>
<tr>
<td>1647</td>
<td>C=O stretching of carboxylic acid</td>
</tr>
<tr>
<td>1429</td>
<td>C-O stretching of acids</td>
</tr>
<tr>
<td>1251</td>
<td>CH deformation</td>
</tr>
<tr>
<td>1043</td>
<td>OH deformation</td>
</tr>
</tbody>
</table>

This spectrum shows commonly encountered peaks in acidic polysaccharides, i.e., -OH, -C=O and -CH stretching and deformation vibrations. This may also be noted that the characteristic frequencies due to carboxylate group (1550-1600 cm\(^{-1}\)) and acetamide group (-CH\(_3\)CONH\(_2\); ~3300 and 1550 cm\(^{-1}\)) were absent. The strong band at 1647 cm\(^{-1}\) in conjunction with a series of weak bands between 2800 &
2000 cm\(^{-1}\) and at 1251 cm\(^{-1}\) were indicative of the presence of COOH groups.

**HYDROLYSIS**

The purified polysaccharide on acid hydrolysis with 2N sulphuric acid under reflux for 12 hrs. followed by paper chromatographic examination in solvent systems (S\(_1\)) & (S\(_2\)) furnished a distinct spot corresponding to glucose and other less distinct spots probably corresponding to mannose and glucuronic acid. Using 4N TFA for hydrolysis followed by paper chromatography in solvent systems (S\(_1\)), (S\(_2\)) and (S\(_4\)) gave the same result. Vigorous hydrolysis was then performed with cold conc. H\(_2\)SO\(_4\) (70%) overnight, diluted to 2 M and heated at 100°C in a sealed tube for 2 hrs. The paper chromatographic examination of the hydrolysate in solvent system (S\(_3\)) furnished three distinct spots corresponding to glucuronic acid, glucose and mannose (Fig.5). TLC of the hydrolysate in n-butanol: acetic acid: ether: water (9:6:3:1) system gave the same results (Fig.6).

Spectrophotometric determinations of the hydrolysate by Phenol-Sulphuric acid\(^90\) and Carbazole methods\(^91\) estimated the hexose and uronic acid contents to be 86.9% and 13.1% respectively. The hexose: uronic acid molar ratio is, therefore, 7.13:1.
Fig. 5: Paper chromatogram of Polysaccharide hydrolysate

Fig. 6: TLC of Polysaccharide hydrolysate
The sugar analysis as alditol acetates by GLC (column a) showed only two peaks corresponding to hexoses -mannose and glucose which gives a molar ratio of 1.3:1 (Fig.22). Uronic acid alditol acetates are not volatile enough to be detected by GLC. This analysis of sugar constituents might have underestimated the neutral sugars to which glucuronic acid is bound because of the resistance of glycosiduronic acid linkages to complete hydrolysis by acid. The polysaccharide was, therefore, methanolysed with methanol and dry HCl, converted to TMS sugar derivatives and subjected to GLC analysis (column b). The chromatogram is shown in Fig.32 whereby six peaks emerged corresponding to mannose, glucose and glucuronic acid (two peaks for each). The relative retention times with respect to the standard mannitol TMS ether and the relative proportions of the anomeric sugar peaks presented in Table-III. Based on their peak areas and response factors the molar ratios of three sugar constituents were calculated as 4.1:2.9:1. The molar ratio of the sugars, thus, correlates well with that obtained from spectrophotometric and GLC analysis of their alditol acetates.
TABLE-III

Retention times and molar ratios of trimethylsilyl glycosides obtained after methanolysis of the native polysaccharide

<table>
<thead>
<tr>
<th>Peak</th>
<th>tR</th>
<th>Peak area proportion</th>
<th>Molar ratio</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.45</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.87</td>
<td>65</td>
<td>12.5</td>
<td>Glucuronic acid</td>
</tr>
<tr>
<td>2</td>
<td>0.60</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>9</td>
<td>51.25</td>
<td>Mannose</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.86</td>
<td>27</td>
<td>36.25</td>
<td>Glucose</td>
</tr>
</tbody>
</table>

Retention times (tR) is relative to mannitol
METHYLATION STUDIES

Methylation of the polysaccharide was carried out by Hakomori method followed by Purdie method. The methylated product did not show in the IR spectrum any band due to $-\text{OH} \ (3444 \text{ cm}^{-1})$ and Carboxylic group ($1647 \text{ cm}^{-1}$) but showed distinct peaks due to methyl ether ($1125 \text{ cm}^{-1}$) and ester carbonyl group ($1740 \text{ cm}^{-1}$). This completely methylated product was reduced with superdeuteride (lithium triethyl borodeuteride). Superdeuteride was preferred over lithium aluminium hydride for two reasons— one, it was much more effective than lithium aluminium hydride requiring only ambient temperature for complete reduction (lithium aluminium hydride required refluxing for several hrs. and a tedious workup) and second it incorporated isotopic hydrogen (deuterium) in the reduced group ($\text{CD}_2\text{OH}$) thereby allowing a distinguishable mass fragmentation pattern from the one produced by a neutral sugar.

The reduced product exhibited in its IR spectrum a weak $-\text{OH}$ stretching band ($3487 \text{ cm}^{-1}$) and disappearance of the ester carbonyl peak ($1740 \text{ cm}^{-1}$) indicating quantitative reduction of ester group (Fig.16). The reduced methylated polysaccharide was hydrolysed by the usual method and converted to alditol acetates. The partially methylated alditol acetates were subjected to GC-MS analysis. The
gas chromatogram (Fig. 23) along with mass fragmentation pattern are shown in (Fig. 24-29) and the assignment of the various peaks are presented in Table-IV. The mode of cleavages of various methylated alditol acetates are shown in (Fig. 24a and 24b)

As indicated above, these results were obtained from the methylated and reduced polysaccharide which was not remethylated. It is expected, therefore, that glucuronic acid residues would give rise to a neutral methylated glucose with a free -OH at 6-position. Further, the mass ion comprising the reduced primary hydroxyl group would exhibit an isotopic shift of two mass units (m/e) in its mass spectrum due to incorporation of two deuterium atoms by superdeuteride reduction (CD$_2$OH). The identification of Tetra-O-acetyl 2,3-di-O-methyl glucitol (peak 6) along with a mass ion at 263 m/e (which is two units higher than the mass of neutral fragment) in the mass spectrum indicates that glucuronic acid unit in the polysaccharide is linked through C-4. The molar ratio of glucuronic acid peak to glucose and mannose peaks also correlated well with that obtained from spectrophotometric and methanolysis composition analysis. This was further confirmed by the remethylation of the polysaccharide after methylation and reduction. The GC-MS analysis of the partially methylated alditol acetates from the remethylated
TABLE-IV
Retention times and molar ratios of partially methylated alditol acetates of methylated and reduced polysaccharide

<table>
<thead>
<tr>
<th>Peak</th>
<th>tR</th>
<th>Molar ratio</th>
<th>Characteristic ion peaks (m/e)</th>
<th>Interpretation</th>
<th>Mode of linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.00</td>
<td>102, 118, 129, 145, 161, 162, 205</td>
<td>1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol</td>
<td>Terminal non-reducing</td>
</tr>
<tr>
<td>2</td>
<td>1.42</td>
<td>1.98</td>
<td>118, 161, 233</td>
<td>1,3,5-Tri-o-acetyl-2,4,6-tri-o-methyl mannitol</td>
<td>1→3</td>
</tr>
<tr>
<td>3</td>
<td>1.47</td>
<td>0.88</td>
<td>118, 233</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl mannitol</td>
<td>1→4</td>
</tr>
<tr>
<td>4</td>
<td>1.56</td>
<td>2.10</td>
<td>118, 233</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl glucitol</td>
<td>1→4</td>
</tr>
<tr>
<td>5</td>
<td>1.74</td>
<td>1.00</td>
<td>118, 261</td>
<td>1,3,4,5-Tetra-o-acetyl-2,6-di-o-methyl mannitol</td>
<td>1→3 and 1→4</td>
</tr>
<tr>
<td>6</td>
<td>2.00</td>
<td>0.98</td>
<td>118, 263</td>
<td>1,4,5,6-Tetra-o-acetyl-2,3-di-o-methyl glucitol</td>
<td>1→4 of glucuronic acid</td>
</tr>
</tbody>
</table>

(a.) Retention time, tR, is relative to tR of 1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol as 1 on a OV-225 column.
(b.) Molar ratios are calculated taking the amount of 1,3,4,5-Tetra-o-acetyl-2, 6-di-o-methyl-mannositol as 1 and corrected by using molar response factors reported by Albersheim et al. 71
# TABLE-V

Retention times and molar ratios of partially methylated alditol acetates of methylated, reduced and remethylated polysaccharide

<table>
<thead>
<tr>
<th>Peak</th>
<th>t&lt;sub&gt;R&lt;/sub&gt;</th>
<th>Molar ratio</th>
<th>Characteristic ion peaks (m/e)</th>
<th>Interpretation</th>
<th>Mode of linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.90</td>
<td>102,118,129, 145, 161,162,205</td>
<td>1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol</td>
<td>Terminal non-reducing</td>
</tr>
<tr>
<td>2</td>
<td>1.42</td>
<td>1.95</td>
<td>118, 161, 233,</td>
<td>1,3,5-Tri-o-acetyl-2,4,6-tri-o-methyl mannositol</td>
<td>1→3</td>
</tr>
<tr>
<td>3</td>
<td>1.47</td>
<td>0.94</td>
<td>118, 233</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl mannositol</td>
<td>1→4</td>
</tr>
<tr>
<td>4</td>
<td>1.56</td>
<td>3.09</td>
<td>118, 233,235</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl glucitol</td>
<td>1→4 glucose and glucuronic acid</td>
</tr>
<tr>
<td>5</td>
<td>1.74</td>
<td>1.00</td>
<td>118,261</td>
<td>1,3,4,5-Tetra-o-acetyl-2,6-di-o-methyl mannositol</td>
<td>1→3 and 1→4</td>
</tr>
</tbody>
</table>

(a.) Retention time, t<sub>R</sub>, is relative to t<sub>R</sub> of 1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol as 1 on a OV-225 column.

(b.) Molar ratios are calculated taking the amount of 1,3,4,5-Tetra-o-acetyl-2, 6-di-o-methyl-mannositol as 1 and corrected by using molar response factors reported by Albersheim et al. 71
polysaccharide (Fig. 30 and Table-V) showed only five peaks – one less than that obtained from the methylated and reduced polysaccharide because the peak due to 2,3-di-O-methyl glucose was replaced by an equimolar amount of 2,3,6-tri-O-methyl glucose which was originally present in the methylated and reduced polysaccharide (without remethylation).

Appearance of the peaks due to 2,3,4,6-tetra-O-methyl glucose, 2,3,6-tri-O-methyl glucose, 2,3,6 and 2,4,6-tri-O-methyl mannose and finally 2,6-di-O-methyl mannose in their respective molar ratios suggest the following linkages for the possible polysaccharide repeating unit:

1) One unit of glucose is present at the non-reducing terminal end.

2) Glucuronic acid units were not present at the non-reducing terminal end.

3) Two units of 1→4 linked glucose are also present.

4) There is branching through one mannose unit only.

5) Mannose units are joined in the polysaccharide chain by 1→3 as well as by 1→4 linkages and there are at least two (1→3) linked and one (1→4) linked mannose units.
However, the fourth mannose unit which was involved in branching could be either \((1\rightarrow3)\) linked or \((1\rightarrow4)\) linked.

The above facts point towards a branched polysaccharide chain which is composed of a straight chain of either mannose only or both mannose and glucose units. However, the branching was indicated only through mannose units. It was also established that glucuronic acid units were not present at the non-reducing terminal end. Had there been a glucuronic acid unit at the terminal end it would have yielded a peak due to 2,3,4-tri-O-methyl glucose from the methylated and reduced polysaccharide as well as a peak due to 2,3,4,6-tetra-O-methyl glucose from the remethylated sample with the respective isotopic mass ion shifts in their mass spectrum.

**PARTIAL ACID HYDROLYSIS**

Although the methylation experiment provided certain useful structural information but left some important questions unanswered i.e., (1) which sugar units constitute the main chain? (II) Glucuronic acid is bound to which sugar residue- mannose or glucose? and (III) what is the linkage of the fourth mannose unit which was involved in
branching? In several cases partial depolymerisation of the polysaccharides has been reported to provide monosaccharides and oligosaccharides. Terminal residues are generally more acid-sensitive and, therefore, such sugar residues may be easily removed by mild hydrolysis which does not split the internal linkages of the main chain. In such cases the residual degraded polysaccharide can provide useful structural information including the position of side chain. More vigorous hydrolysis of the above degraded polysaccharide may liberate oligosaccharides from the internal chain and for an acidic polysaccharide it may yield acidic oligosaccharides specially an aldobiouronic acid which is very resistant to hydrolysis.

The polysaccharide was hydrolysed with 5mM H$_2$SO$_4$ at 95°C for 9 hrs. in a sealed tube, centrifuged and the residue was washed with water. The combined washings and the supernatant were neutralized, concentrated, and paper chromatographic examination of the concentrate in the solvent system (S$_2$) furnished a distinct spot corresponding to glucose only. Isolation of glucose under mild condition of hydrolysis placed it at the side positions.

The residue left after partial acid hydrolysis of the native polysaccharide was divided into two parts. One part was further subjected to hydrolysis with 0.1 M TFA for 1 hr. at 120°C in a sealed
tube. Any polymeric material from the hydrolysate was removed by ethanol precipitation and the supernatant was concentrated and adjusted to pH 4 with sodium hydroxide. It was then applied to a column of Amberlite 400 resin (acetate form) to separate neutral and acidic fraction. The acidic fraction on paper chromatographic examination in solvent system (S<sub>6</sub>) revealed mainly an aldobiouronic acid (I) along with minor components. The aldobiouronic acid was separated by gel chromatography on Bio Gel column (1.5X 3 cm). Hakomori methylation of (I), subsequent reduction of the permethylated aldobiouronic with lithium aluminium hydride, hydrolysis followed by reduction and acetylation yielded equimolar amounts of 2,3,4-Tri-O-methyl glucose and 2,3,6 -Tri-O-methyl mannose as their alditol acetates. Compound (I) is thus 4-O (D-glucopyranosyluronic acid)-D- mannopyranose.

**GlupA-(1→4)-Man** (I)

Another part of the residue was subjected to methylation reduction, hydrolysis, reduction and acetylation followed by GC-MS analysis of the alditol acetates. Only three peaks (Table-VI) corresponding to 2,3,6-tri-o-methyl
TABLE VI
Retention times and molar ratios of partially methylated alditol acetates of degraded polysaccharide (methylated and reduced) obtained after partial hydrolysis

<table>
<thead>
<tr>
<th>Peak</th>
<th>$t_R$</th>
<th>Molar ratio</th>
<th>Characteristics ion peak (m/e)</th>
<th>Interpretation</th>
<th>Mode of linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.42</td>
<td>2.98</td>
<td>118, 161, 233</td>
<td>1,3,5-Tri-o-acetyl-2,4,6 tri-o-methyl mannitol</td>
<td>1→3</td>
</tr>
<tr>
<td>2</td>
<td>1.47</td>
<td>1.00</td>
<td>118, 233</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl mannitol</td>
<td>1→4</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>1.11</td>
<td>118, 263</td>
<td>1,4,5,6-Tetra-o-acetyl-2,3-di-o-methyl glucitol</td>
<td>1→4 of glucuronic acid</td>
</tr>
</tbody>
</table>

(a.) Retention time, $t_R$, is relative to $t_R$ of 1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol as 1 on a OV-225 column.
(b.) Molar ratios are calculated taking the amount of 1,4,5-Tri-o-acetyl-2,3, 6-tri-o-methyl-mannositol as 1 and corrected by using molar response factor reported by Albersheim et al.71

mannose; 2,4,6-tri-O-methyl mannose and 2,3-di-O-methyl glucose were observed in the gas chromatogram. The absence of peak due to 2,6-di-O-methyl mannose coupled with a corresponding increase in the amount of 2,4,6-tri-O-methyl mannose indicates that the side chain was linked to the 4-position of the mannose in the original
polysaccharide- the 3-position being involved in the main chain linkage. Thus, the fourth mannose unit involved in branching was bound by a 1→3 linkage- the total 1→3 linked mannose units being three. These results indicate a polysaccharide backbone of mannose while glucose residues are bound as side chain. Furthermore, the glucuronic acid unit was interspersed in the mannose backbone bound to it by 1→4 linkages.

The partial hydrolysis experiment thus proves that all the three glucose residues are bound as a single side chain linked to main chain by a 1→4 linkages which are splitted under conditions of mild hydrolysis. The branch point could be any one of the 1→3 linked mannose residues. The aldobiouronic acid residues obtained under more vigorous conditions are present in the main chain. It also answers the main question that glucuronic acid is bound directly to mannose residues in the main chain.

The results of methylation studies and partial hydrolysis provided much useful structural information which helped in arriving at the following tentative structure of the probable octasaccharide repeating unit.
Periodate Oxidation

The above postulated polysaccharide framework has been confirmed by the results of periodate oxidation and studies on the smith degraded product. The polysaccharide on oxidation with 0.05N periodate consumed 4.2 moles per repeating unit after 72 hrs and liberated 1.2 moles of formic acid. The oxidized polysaccharide was dialyzed against distilled water, centrifuged, reduced with NaBH₄ and subjected to Smith degradation with 1N H₂SO₄. The residue was centrifuged and the supernatant after neutralization on paper chromatographic and TLC examination detected erythritol and glycerol only. The residue left was divided into two parts. One part was hydrolysed under vigorous condition and the hydrolysate on paper chromatographic examination in solvent system (S₃) gave spots corresponding to glucuronic acid and mannose (Fig.10).
Fig.10: Paper chromatogram of Smith degraded Polysaccharide hydrolysate
These results clearly indicate that mannose and glucuronic acid units resisted oxidation by periodate as these were recovered intact after hydrolysis. The resistance of mannose units to periodate oxidation may be attributed mainly to 1→3 linkages whereas 1→4 linked mannose residues have also been reported in many cases to be resistant to periodate oxidation. This has been explained due to formation of cyclic acetals. In case of glucuronic acid the resistance may be due to the electrostatic repulsion between the uronate ion and the attacking periodate ion as discussed in chapter I. However glucose is present as a trisaccharide unit in the side chain as glucopyranosyl (1→4)-O-glucopyranosyl (1→4)-O-glucopyranose which yields glycerol and erythritol on Smith degradation. This is supported by the consumption of about 4 moles of periodate per repeating unit which could be accounted for by these trisaccharides glucose units only.

The second part of the residue (periodate oxidized and Smith degraded) was subjected to methylation studies. The permethylated product on reduction with superdeuteride, hydrolysis, NaBD₄ reduction and acetylation followed by GC-MS analysis furnished
peaks (Table-VII) corresponding to 2,4,6-tri-O-methyl mannose, 2,3,6-tri-O-methyl mannose and 2,3-di-O-methyl glucose in the molar ratios of 2.95:1:0.99. The absence of the peak due to 2,6-di-O-methyl mannose as obtained by the methylation experiment and a

**TABLE-VII**

Retention times and molar ratios of partially methylated alditol acetates of periodate oxidized and smith degraded polysaccharide (methylated and reduced)

<table>
<thead>
<tr>
<th>Peak</th>
<th>t&lt;sub&gt;R&lt;/sub&gt;</th>
<th>Molar ratio</th>
<th>Characteristic ion peaks (m/e)</th>
<th>Interpretation</th>
<th>Mode of linkage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.42</td>
<td>2.95</td>
<td>118, 161, 233</td>
<td>1,3,5-Tri-o-acetyl-2,4,6-tri-o-methyl mannositol</td>
<td>1→3</td>
</tr>
<tr>
<td>2</td>
<td>1.47</td>
<td>1.00</td>
<td>118, 233</td>
<td>1,4,5-Tri-o-acetyl-2,3,6-tri-o-methyl mannositol</td>
<td>1→4</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>0.99</td>
<td>118, 263</td>
<td>1,4,5,6-Tetra-o-acetyl-2,3-di-o-methyl glucitol</td>
<td>1→4 of glucuronic acid</td>
</tr>
</tbody>
</table>

(a.) Retention time, t<sub>R</sub>, is relative to t<sub>R</sub> of 1,5-Di-o-acetyl-2, 3,4,6-tetra-o-methyl glucitol as 1 on a OV-225 column.

(b.) Molar ratios are calculated taking the amount of 1,4,5-Tri-o-acetyl-2,3, 6-tri-o-methyl-mannositol as 1 and corrected by using molar response factor reported by Albersheim et al. 71
corresponding increase of the peak due to 2,4,6 tri-O-methyl mannose confirms the oxidation of glucose side chain bound to main chain through O-4 of mannose residues.

The periodate oxidation results, thus, support the above tentative structure of the repeating unit which is a glucuronic acid interspersed (1→3) linked mannan core branched with a (1→4) linked trisaccharide of glucose.

**NMR SPECTROSCOPIC STUDIES**

Information regarding the nature of anomeric configuration of constituent sugars was drawn from the $^1$H-NMR spectrum (Fig. 31a & b) of the native polysaccharide recorded on a solution of the polysaccharide in DMSO-d$_6$ at 348K with 300MHz Bruker instrument. The anomeric resonances in $^1$H spectra are well separated from signals produced by hydrogen nuclei at other positions. This fact greatly helps in determining the different monosaccharide residues and estimating their relative proportions in a polysaccharide. Further, the axial and equatorial hydrogens resonate at different positions- at δ4.5-4.8 and δ5.3-5.8 respectively$^{114}$. This also helps in deciding the nature of anomeric linkages for different sugars as different sugar
anomers produce their characteristic signals. For example, an equatorial H-1 bond of an α-gluco or α-galacto residue normally produces a signal in the region of δ5.00-5.50 whereas axial H-1 of the β-anomer normally resonates upfield closer to δ4.50.

The 1H-NMR spectrum showed a complex anomeric region but when the spectrum was recorded at 300 MHz in DMSO-d6 it indicated seven anomeric signals out of which three were β-linked at δ4.68, δ4.71 and δ4.89 and four were α-linked at δ5.05, δ5.18, δ5.28 and δ5.29. The signals between δ3.50 to δ4.30 were assigned to C-2—C-6 protons which were complex and difficult to interpret.

The above seven anomeric signals were assigned to eight sugars in the polysaccharide repeating unit with the number of integral protons belonging to each signal shown in Table -VIII. The signals at δ4.68 & δ4.71 (2 protons approx.) were assigned to two β-glucose units and that at δ5.05 (1proton approx.) to α-glucose (terminal) unit74. α-Glucuronic acid has been reported to exhibit signals at δ5.17–δ5.1849. Therefore, the signal at δ5.18 (1 proton) was assigned to α-glucuronic acid. The remaining signals could only be assigned to mannose units.
### TABLE-VIII

1H-NMR chemical shifts and their assignments

<table>
<thead>
<tr>
<th>Chemical shifts of the anomeric protons</th>
<th>Assignment</th>
<th>Integral protons (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.29</td>
<td>α-man</td>
<td>2</td>
</tr>
<tr>
<td>5.28</td>
<td>α-man</td>
<td>1</td>
</tr>
<tr>
<td>5.18</td>
<td>α-GluA</td>
<td>1</td>
</tr>
<tr>
<td>5.05</td>
<td>Terminal α-glucuronic acid</td>
<td>1</td>
</tr>
<tr>
<td>4.89</td>
<td>β-man</td>
<td>1</td>
</tr>
<tr>
<td>4.71</td>
<td>β-glu</td>
<td>1</td>
</tr>
<tr>
<td>4.68</td>
<td>β-glu</td>
<td>1</td>
</tr>
</tbody>
</table>

Considering the usually reported positions of α and β-anomers of mannose, the signals at δ5.28 (1 proton) & δ5.29 (2 protons) were assigned to 3 α-mannose units. The remaining signal at δ4.89 (1 proton)
could only be assigned to the β- mannose residue bound to the C-4 position of glucuronic acid. Thus, the NMR spectrum is in full conformity with the proposed structure of the repeating unit and also gives a complete picture of the point & nature of anomeric linkages. Based on the foregoing discussions, the final detailed structure of the repeating unit can be represented by the following diagram (conformational):

![Diagram of the repeating unit of the Prunus armeniaca polysaccharide]

Repeating unit of the Prunus armeniaca polysaccharide

Aldobiouronic acid moieties of 4-O-β-D (glucuronopyranosyl uronic acid)-D-mannose type have a common occurrence among plant seed polysaccharide whereas the polysaccharide studied has a 4-O-α-D (glucuronopyranosyl uronic acid)-D-mannose moiety. However, it is interesting to note that such type of structural feature containing glucose, mannose & glucuronic acid resembles to some extent with the polysaccharide isolated from aerobacter aerogens. It has a
branched structure with an average repeating unit of about 40 sugar units containing D-glucose, D-mannose and glucuronic acid. The D-glucose residues are linked mainly 1:4 but few are linked 1:3. Non-reducing end groups of α-glucuronic acid are linked 1:4 to D-mannose, which in turn are linked to the remaining sugar units through 3-position.

The polysaccharide thus possesses a unique structural feature, which is uncommon among the uronic acid containing polysaccharides distributed in plant kingdom including Prunus species.
SAMPLE ANALYSIS

Column used:
Sephacryl CL-2B
Column capacity - 170 ml
Eluant used - Distilled water
Elution rate - 18 ml/h
Fraction volume: 3 ml

Standards used:
Dextran -
T-70 (-70000 Da)
T-150 (-150000 Da)
T-500 (-500000 Da)
T-2000 (-2000000 Da)

Sample dissolved in 0.1 N NaOH

Standard graph:

Fig. 11: Calibration Curve for the GPC
Sample:

Showed two peaks, one major and the other minor. Based on the standard graph, the molecular weight of the major peak corresponded to ~468000 Da, whereas that of minor peak, ~79,500 Da. The major peak also seems to be heterogeneous.

Fig.12: Gel Permeation Chromatogram of native polysaccharide
FIG. 13: IR Spectrum of native polysaccharide
FIG. 14: IR Spectrum of methylated polysaccharide (by Hakomori method)
FIG.15: IR Spectrum of remethylated polysaccharide (by Purdie method)
FIG. 16: IR Spectrum of methylated and reduced polysaccharide
FIG. 17: IR Spectrum of methylated polysaccharide (periodate oxidized and Smith degraded) by Hakomori method
FIG. 18: IR Spectrum of methylated polysaccharide (periodate oxidized and Smith degraded) by Purdie method
FIG. 19: IR Spectrum of methylated and reduced polysaccharide (periodate oxidized and Smith degraded)
FIG. 20: IR Spectrum of methylated degraded polysaccharide (by Hakomori method)
FIG. 21: IR Spectrum of reduced methylated degraded polysaccharide
Fig.22: GLC of alditol acetates of sugars on OV-225 Capillary column obtained from hydrolysis of polysaccharide
FIG. 23: Gas chromatogram of the partially methylated alditol acetates (from methylated and reduced polysaccharide)
FIG. 24: Electron impact mass spectrum of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol
MODE OF CLEAVAGES IN THE MASS SPECTRUM OF PARTIALLY METHYLATED ALDITOL ACETATES

Primary Fragmentation

\[
\begin{align*}
\text{CHDOAC} & \quad 118 \\
\text{H} & \quad \text{O}\text{Me} \\
\text{MeO} & \quad \text{H} \quad 162 \\
\text{H} & \quad \text{O}\text{Me} \quad 161 \\
\text{H} & \quad \text{OAc} \\
\text{CH}_2\text{OMe} & \quad 45
\end{align*}
\]

Secondary Fragmentation

\[
\begin{align*}
\text{CH} = \text{O}\text{Me} + & \quad \text{CH} = \text{O}\text{Me} + \text{CHDOAC} + \text{CHD} \\
\text{H-C-OMe} & \quad \text{C-OMe} \quad \text{MeO-C-H - ACOH} \quad \text{C-OMe} \\
\text{H-C-OAC} & \quad \text{CH} \quad \text{MeO=C-H} \quad \text{CH} = \text{O}\text{Me} \\
\text{CH}_2\text{OMe} & \quad \text{CH}_2\text{OMe}
\end{align*}
\]

\[
\begin{align*}
\text{m/e 205} & \quad \text{m/e 145} \quad \text{m/e=162} \quad \text{m/e-102}
\end{align*}
\]

\[
\begin{align*}
\text{CH}=\text{O}\text{Me} + & \quad \text{CH}=\text{O}\text{Me} + \text{CH}=\text{O}\text{Me} + \\
\text{CH} & \quad \text{H-C-OAC} - \text{OMe} \quad \text{C} = \text{O} \quad \text{CH}_3 \\
\text{H-C-OMe} & \quad \text{CH}_2\text{OMe} \quad \text{H}_2\text{C} \quad \text{CH}_2 \quad \text{C} = \text{O} \quad \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{m/e 101} & \quad \text{m/e 161} \quad \text{m/e=129} \quad \text{m/e-87}
\end{align*}
\]

Fig. 24. Mass fragment ions from 1,5-di-O-acetyl-2,3,4,6-tetra-O- methyl-D-glucitol-1-d

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Fig. 29. Mass fragment ions from 1,4,65-tetra-O-acetyl-2,3-di-O-methyl-D-glucitol-1-d,6-2d
FIG. 25: Electron impact mass spectrum of 1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-D-mannositol
FIG. 26: Electron impact mass spectrum of 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-mannositol
FIG. 27: Electron impact mass spectrum of 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl-D-glucitol
FIG. 28: Electron impact mass spectrum of 1,3,4,5-tetra-O-acetyl-2,6-di-O-methyl-D-mannositol
FIG. 29: Electron impact mass spectrum of 1,4,5,6-tetra-O-acetyl-2,3-di-O-methyl-D-glucitol
FIG. 30: Gas chromatogram of the partially methylated alditol acetates (from reduced and remethylated polysaccharide)
Fig. 31(a): 1H-NMR Spectra of the native polysaccharide
Fig. 31(b): 1H-NMR Spectra of the native polysaccharide (magnified)
FIG. 32: Gas Chromatogram of methyl trimethylsilylated glycosides obtained from the methanolysis of the native polysaccharide