SECTION - A

GENERAL INTRODUCTION

(a) POLYSACCHARIDES

(b) CARBOHYDRATE CONTAINING MUCILAGES AND GALACTOMANNANS

(c) MORPHOLOGY OF "CASSIA LAEVIGATA" SEED POLYSACCHARIDES

(d) GENERAL STRUCTURE AND NOMENCLATURE OF POLYSACCHARIDES
GENERAL INTRODUCTION

(a) POLYSACCHARIDES

Natural macromolecules containing carbohydrate units are of widespread occurrence and include (I) polysaccharides as exclusively carbohydrate polymers; (II) glycoproteins, proteoglycans, and peptidoglycans; (III) glycolipids and lipopolysaccharides; (IV) teichoic acids and related macromolecules containing phosphorodiester-linked oligosaccharide repeating units; and (V) nucleic acids.

Polysaccharides are essential constituents of almost all living organisms (I). Apart from their abundant occurrence in the higher order of plants, seaweeds and animals, they are also found in fungi lichens, capsules of microorganisms, cartilage, animal joint fluids, fluid cancer, skin and in mucosa.

The term "polysaccharide" is applied here to those carbohydrate polymers that contain periodically repeating structures in which the dominant, but not necessarily exclusive interunit linkages are of the O-glycosidic type. Polysaccharides fill diverse role in the physiology of plants, animals and microorganisms. The most important role of polysaccharides lies in constituting the structural material of plants (cellulose chitin) while others (starch glycogen) occur as reserve substances, readily convertible as and when required into energy. As surface material they partially
protect tissues from desiccation and as gums they are exuded from plants to seal and protect wounds. They are of importance in blood group specificity and in other immunological relations. Polysaccharides as found in the tissues of plants and animals are a complex admixture which may contain more than one variety of polysaccharides along with substances like proteins, fats and traces of other materials.

The commercial value of mucilages is due to their characteristic emulsifying, suspending and jellying properties. These are in use since ancient times in India and in other countries. The seaweed mucilages (2) were used as food and medicine by the natives of coastal regions of France, Wales, Ireland, Scotland and Scandinavia. In general, mucilages find use in medicine as demulcents, emollients and diuretics. They may be used as laxative because of their lubricant actions. In the food industry, the mucilages are used in preparation of salad dressings, soft cheeses, ice creams, flavour emulsions, jellies syrup and confectioneries and find use in printing, rubber latex, paper and textile industries.

Structural polysaccharides fall into two distinct classes. The fibrous polysaccharides, cellulose in higher plants and some algae, chitin in yeast and fungi, and less frequently 3-linked β-D-xylans and 4-linked β-D-mannans in some plants and algae, are relatively invariant in their respective structures and can adopt regular chain conformations. In contrast, the matrix polysaccharides are
characterised by their gel-forming capacity, which confers flexibility on the structural assembly. These gel-forming polysaccharides can adopt regular chain conformations for substantial parts of their structures but interruptions in regularity of structure permit the modifications of cell wall texture. Thus, subtle changes in structure cause marked difference in physical properties. The biosynthesis of matrix polysaccharides frequently involves structural modification after the basic skeleton has been assembled. In plant polysaccharides it is common to encounter branched structures in which linear chains of uniform linkage type carry variable proportions of rather short side chains e.g., arabinoxylans and galactomannans, in which there is little apparent regularity and branching suggesting that the attachment of side chains to the extent desired for the modification of properties occurs as a separate process after completion of the main chains.

Another type of structurally variable polysaccharide is encountered when initially laid down regular chains undergoes post-polymerisation modifications, alterations is the configuration of individual sugar residues, e.g. of β-D-mannuronic acid to α-L-guluronic acid in alginic acid result in changes in polysaccharide chain conformations.

Polysaccharides, as found in the tissues of plants and animals are a complex admixture which may contain more than one variety of polysaccharides along with the substances like proteins, fats and of other materials.
FIG. 1. SEGMENTS OF A POLYSACCHARIDE CHAIN SHOWING DEFINED CHAIN SENSE FROM NONREDUCING TERMINI (a), VIA CHAIN UNITS (b), POSSIBLY THROUGH BRANCH POINTS (c), TO "REDUCING" TERMINUS (d), WHICH MAY BE A REDUCING HEMIACETAL OR SOME OTHER HYDROXYLATED COMPOUND ACTING AS GLYCOSYL ACCEPTOR (OR AGLYCON). BRANCHING SUGAR RESIDUES SERVE AS "POLYVALENT" AGLYCONES.
Knowledge of the primary or covalent structure of polysaccharides forms the basis for their classification, for an understanding of their three dimensional structures in the solid state and in solution, and for an appreciation of the ways in which polysaccharides are synthesised and broken down naturally.

It is difficult to give an accurate definition of the polysaccharides, because the properties usually associated with them gradually reverts to those of the simple sugars as the molecular weight decreases. The term "Glycan" is the systematic generic name given to polysaccharides in which a large number of glucose (monosaccharide) residues are naturally joined by O-glycosidic linkages. Polysaccharides (Fig.1) may be regarded as condensation polymers in which each intersugar glycosidic linkage is formed from the glycosyl moiety of a hemiacetal or hemiketal and a hydroxyl group of another sugar unit acting as an acceptor molecule or aglycone. Since a given sugar residue may form only one glycosidic linkage with another sugar residue as aglycone but a sugar residue acting in the latter capacity carries several hydroxyl groups, one or more of which may be an acceptor of glycosyl substituents, polysaccharides may be linear or branched.

The present classification of the polysaccharides is proposed on the basis of chemical structural compositions and known as structural classification as shown in Table-1.
<table>
<thead>
<tr>
<th>POLYSACCHARIDE</th>
<th>(A) HOMOGLYCANS</th>
<th>(B) HETEROGLYCANS</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Derived from single monosaccharide residues)</td>
<td>(Derived from different monosaccharide residues)</td>
<td></td>
</tr>
<tr>
<td>I. Glucose Polymers, e.g., cellulose, lichenin, starch, glycogen bacterial and yeast dextran.</td>
<td>I. Hemicellulose and cell wall polysaccharide.</td>
<td></td>
</tr>
<tr>
<td>II. Fructose polymers – e.g., Inulin, levans.</td>
<td>II. Mucilage gums and gel forming substances.</td>
<td></td>
</tr>
<tr>
<td>III. Galacturonic acid polymers, e.g., pectin of fruits, berries and sugar beet etc.</td>
<td>III. Polysaccharides associated with proteins and/or microorganisms:</td>
<td></td>
</tr>
<tr>
<td>IV. Polymers of mannose, galactose, Xylose, L-arabinose, manno-uronic acid, e.g., mannans, galactans, xylans, arabinans, alginic acid.</td>
<td>(a) Mucopolysaccharide</td>
<td></td>
</tr>
<tr>
<td>V. Glucosamine polymers, e.g., chitin of crustacea, fungi and insects.</td>
<td>(b) Glycoproteins.</td>
<td></td>
</tr>
</tbody>
</table>

Table-1
Carbohydrate containing mucilages and galactomannans

Plant mucilages have been known for several thousand years. They constitute an important group of economic plant products and utilized in many ways. The mucilages are hydrophilic colloids of high molecular weight, some are completely soluble in water forming viscous solutions while others swell and absorb considerable amount of solvent without dissolving. These colloidal properties of mucilages and essential in manufacturing processes of various industries.

The term mucilage is applied to those substances which enormously swell up in water and produce a slimy liquid. The mucilages can exist either as secondary membrane thickening agents or as intercellular substances and they may therefore be classified as membrane mucilage or cell content mucilage. Membrane mucilages occur in the (Althaea), the cortex (cinnamomum), the stalk (Tragacanth), the leaves (Buccu), the flowers (Malvaceae, tillia), the endosperm (Trigonella) and in the seed shell (Coceea). In seaweeds the mucilage is intercellular Laminaria, Carrageenan). Cell content mucilage occurs in the succulants (Aloe, Euphorbiaceae), bulbs (Scilla) and in orchis (Salep).

The mucilages may be classified into three groups according to their chemical characteristics:

(i) **Neutral Mucilages** consisting of one or more kinds of sugar residues joined together through their reducing groups with the formation of substances of high molecular weight but having no uronic acid (e.g. seed mucilages).
(ii) **Acidic mucilages** resembling gums but generally possessing D-galacturonic acid as the uronic acid part (e.g. plant mucilages).

(iii) **Seaweed mucilages.** High molecular weight compounds consisting in the main of the salts of sulphate, esters, of sugar derivatives.

Galactomannans are branched heteropolysaccharide chains of uniform linkage type, composed of sugars, D-galactose and D-mannose. They are commonly found in the endosperm of legumes where they serve as reserve food. They are water soluble and form a highly viscous solution and hence are classified, at times, as plant mucilages. Because of their mucilaginous nature, some galactomannans are of industrial importance. To a lesser extent they are found in cocusnucifera, *Elaeis quinensis*, *Coffea arabia*, *Phoenix dactylifera*, African oil palm (*Elaeis quinensis*), *Borassus flabellifer* and in various micro-organisms.

Anderson (3) studied 163 species of legumes. Three-fourths of these were found to contain mucilage yielding endosperm upto 60 per cent of the total seed weight. These polysaccharides almost in all the cases, bear a chain of D-mannose residues to which the D-galactose residues are attached as numerous single unit side chains. Thus chemically they can be considered as substituted mannans (poly-galactomannan). Mostly in galactomannans the quantity of D-mannose units predominates (60-80 per cent) over D-galactose units.
(40-20 per cent). In case of alfalfa seed which is exception to the above generalisation consist approximately 60 per cent D-galactose and 30 per cent D-mannose residues.

Galactomannans from different sources are found to differ in quantitative ratio of D-galactose and D-mannose. The relative amount of these sugars in several seeds are shown in Table-2. Leaving aside the galactose, mannose ratio which varies in galactomannans isolated from different species, the galactomannans of almost all the seeds of various species of leguminosae appear to exhibit the same structural features and involve branched structure comprising of a backbone of $\text{B-}(\rightarrow 4)$ - linked $\text{D-mannopyranose}$ residues which carry, through position 6, $\alpha$-D-galactopyranose residues. The degree of branching is indicated by the molecular proportion of galactose and mannose.

Micro-organisms excrete extracellular polysaccharides in the form of slims into the medium. The cellwall of dermatophytes contain galactomannans together with other water soluble polysaccharides. The composition of the galactomannans isolated from some of these sources is given in Table-3.

It is interesting therefore to compare the chemical structure of these galactomannans and to search for common structural features which may account for the similarity in physical properties. Whereas the galactomannans possess similarities in structure it is becoming apparent that they
Table-2
Galactomannan composition isolated from endosperm of certain legumes

<table>
<thead>
<tr>
<th>Seed</th>
<th>D-galactose (%)</th>
<th>D-mannose (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indigofera hirsuta</td>
<td>22.9</td>
<td>72.0</td>
<td>[3]</td>
</tr>
<tr>
<td>Lotus predunculatus</td>
<td>49.0</td>
<td>51.0</td>
<td>[4]</td>
</tr>
<tr>
<td>Lotus corniculatus</td>
<td>44.5</td>
<td>55.5</td>
<td>[5]</td>
</tr>
<tr>
<td>Gleditsia amorphoides</td>
<td>28.6</td>
<td>71.4</td>
<td>[6]</td>
</tr>
<tr>
<td>Crotolaria intermedia</td>
<td>27.7</td>
<td>63.3</td>
<td>[3]</td>
</tr>
<tr>
<td>Crotolaria mucronata</td>
<td>30.5</td>
<td>69.4</td>
<td>[7]</td>
</tr>
<tr>
<td>Poinciana pulcherrima</td>
<td>34.1</td>
<td>65.9</td>
<td>[7]</td>
</tr>
<tr>
<td>Centroseina plumeri</td>
<td>92.0</td>
<td>8.0</td>
<td>[7]</td>
</tr>
<tr>
<td>Cyamopsis tetragonolobus(Guar)</td>
<td>16.0</td>
<td>84.0</td>
<td>[8, 9]</td>
</tr>
<tr>
<td></td>
<td>38.0</td>
<td>58.5</td>
<td>[3]</td>
</tr>
<tr>
<td></td>
<td>36.0</td>
<td>64.0</td>
<td>[10]</td>
</tr>
<tr>
<td>Kentucky Coffee bean</td>
<td>26.0</td>
<td>70.5</td>
<td>[3]</td>
</tr>
<tr>
<td>Gymnocladus dioica</td>
<td>20.0</td>
<td>80.0</td>
<td>[11]</td>
</tr>
<tr>
<td>Desmenthus illinoiensis</td>
<td>26.0</td>
<td>70.0</td>
<td>[3]</td>
</tr>
<tr>
<td>Medicago lupulina</td>
<td>47.5</td>
<td>52.5</td>
<td>[12]</td>
</tr>
<tr>
<td>Ipomea muricate</td>
<td>35.7</td>
<td>64.3</td>
<td>[13]</td>
</tr>
<tr>
<td>Cereidium torreyanum</td>
<td>21.6</td>
<td>73.0</td>
<td>[3]</td>
</tr>
<tr>
<td>Trigonella foenum graecum</td>
<td>45.0</td>
<td>55.0</td>
<td>[14]</td>
</tr>
<tr>
<td>Medicago sativa (Lucerne)</td>
<td>48.0</td>
<td>52.0</td>
<td>[16]</td>
</tr>
<tr>
<td>Cassia: Leptocarpa (Senna)</td>
<td>20.8</td>
<td>64.5</td>
<td>[3]</td>
</tr>
<tr>
<td>fistula</td>
<td>24.0</td>
<td>76.0</td>
<td>[7]</td>
</tr>
<tr>
<td>merilandica</td>
<td>21.0</td>
<td>79.0</td>
<td>[17]</td>
</tr>
<tr>
<td>Occidentalis</td>
<td>25.0</td>
<td>75.0</td>
<td>[17]</td>
</tr>
<tr>
<td>absus</td>
<td>25.0</td>
<td>75.0</td>
<td>[18]</td>
</tr>
<tr>
<td>Sesbenia grandifolia</td>
<td>33.3</td>
<td>66.7</td>
<td>[19]</td>
</tr>
<tr>
<td>Trifolium repens (white clover)</td>
<td>43.4</td>
<td>56.6</td>
<td>[20]</td>
</tr>
<tr>
<td>Glycin soja (Soyabean hulls)</td>
<td>29.85</td>
<td>70.14</td>
<td>[21]</td>
</tr>
<tr>
<td>Caesalpinia spinosa</td>
<td>26.3</td>
<td>70.9</td>
<td>[3]</td>
</tr>
</tbody>
</table>
must also have difference in structure as they display different physical properties, especially in their behaviour with water in electrophoretic studies and in molecular weight.

(c) MORPHOLOGY OF "CASSIA LAEVIGATA" SEED POLYSACCHARIDE

Cassia Linn (N.O. Leguminosae) is a large and predominantly tropical genus of over 150 species. Some twenty representatives of these herbs, shrubs and trees are found in India. Many of these plants have been known for their medicinal and tanning properties. The amount of endosperm found in them varies from zero to as much as 60 per cent of the total seed weight. The endosperm mucilage of the legumes contains galactomannan and is considered to be a 'Reserved Polysaccharide'.

Of these, Cassia Laevigata seed has been selected for structural studies as it is widely available in the country.

Table-3
Galactose, Mannose ratio from certain microorganisms Dermatophytes

<table>
<thead>
<tr>
<th>Name of Organism</th>
<th>D-mannose</th>
<th>D-galactose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton interdigitale</td>
<td>88.0</td>
<td>12.0</td>
<td>[22]</td>
</tr>
<tr>
<td>Trichophyton granulosum</td>
<td>92.5</td>
<td>7.5</td>
<td>[23]</td>
</tr>
<tr>
<td>Trichophyton Schonleinii</td>
<td>87.0</td>
<td>17.0</td>
<td>[23]</td>
</tr>
<tr>
<td>Microsporum quinckeanum</td>
<td>95.5</td>
<td>4.5</td>
<td>[23]</td>
</tr>
<tr>
<td>Trichosporon fermentans</td>
<td>66.7</td>
<td>33.3</td>
<td>[25]</td>
</tr>
<tr>
<td>Lipomyces starkeyi</td>
<td>60.0</td>
<td>40.0</td>
<td>[26]</td>
</tr>
</tbody>
</table>
and contains a large percentage of endosperm (60 per cent). It is herbaceous undershrub or small tree usually upto 3 to 4.5 meter or more high found in moist hilly places at 3000'. It is abundant in the coastal regions of Madras, and run wild in Nilgiri. It blooms in August-January. The flowers are yellow, the leaflet lanceolate to ovate, almost symmetrical 4-11.3 cm. long, 2-4 cm. wide. Pod subterets, brown, very shortly or not beaked, slowly dehiscent, 6.2-10 cm. long, 1-1.5 cm. in diameter, longitudinally and transversely obvate.

The potentialities of Cassia laevigata are promising. It finds industrial use in chemicals, pharmaceuticals, food-stuff preparation, dyes, and other industries. Our present study is confined to determination of its chemical structure.

(d) GENERAL STRUCTURE AND NOMENCLATURE OF POLYSACCHARIDES

Polysaccharides - containing only one kind of monosaccharide unit are homopolysaccharides or homoglycans, e.g., glucan, arabinan, and galacturonan. Although many sugars found as polysaccharide constituents occur in only one enantiomeric form, it is usual to insert enantiomeric prefixes (D or L), and, if known, anomeric prefixes (α or β) are added. At least for homopolysaccharides of uniform linkage type, the systematic name may also designate ring size (furanose or pyranose) and linkage type.

Those containing two or more kinds of monosaccharide unit are heteropolysaccharides or heteroglycans, e.g., arabinonoxyllans and galactomannans. Enantiomeric prefixes are
Fig. 2. OLIGOSACCHARIDE FROM GUAR GUM.
frequently added, but anomeric prefixes, although correct, are rarely appended.

Galactomannans: Leguminous Seeds.

**Guar (Cyamopsis Tetragonolobus) Structure**

The polysaccharide in guar contains 84 per cent D-mannose and 16 per cent D-galactose (8,9). The gum purified through copper complexing contains D-mannose and D-galactose in the ratio of 2:1 (3,27,28).

Partial hydrolysis of guar gum by heating a solution in 0.5 N hydrochloric acid for 3 hours affords 4-0-β-D-mannopyranosyl-β-D-mannose (29,30). Fig.2-A, 6-0-α-D-galactopyranosyl-β-D-mannose (29). Fig.2-B, and 4-0-(6-0-α-D-galactopyranosyl-β-D-mannopyranosyl)-β-D-mannose (31) Fig.2-C. The structure of mannobiose 'A' was proved by methylation and other studies. The structure of second disaccharide 'B' was determined by preparing phenyl osazone identical with that from melibiose and by periodate oxidation studies, which proves that the D-galactose moiety is linked through C₄ to C₆ of the D-mannose unit. The structure of trisaccharide 'C' is based upon periodate oxidation studies and partial hydrolysis.

Methylation of the galactomannan was done by Haworths method. Essential homogeneity of the methylated product was ascertained by fractional precipitation. Methylated gum was subjected to methanolysis and the cleavage fragments on separation leads to the isolation of equimolecular amounts
of 2, 3, 4, 6-tetra-O-methyl-D-galactose, 2, 3, 6-tri-O-methyl-D-mannopyranose and 2, 3-di-O-methyl-D-mannopyranose (9, 32). Methylation data suggests four average repeating units for guar gum. On periodate oxidation 4 moles of periodate were consumed with the concomitant liberation of one mole of formic acid (32, 33).

The above evidences show that galactomannan consists \(\beta-(1\rightarrow4)\) linked mannopyranosyl units in the main chain while \(\alpha-(1\rightarrow6)\) galactopyranosyl units constitute the terminal units. The proposed structure (Fig.3) is supported by eusymatic degradation (34) results.

\[
G\overset{\beta-(1\rightarrow4)}{\rightarrow}G\overset{\alpha-(1\rightarrow6)}{\rightarrow}G
\]

A structure similar to guar gum was reported by H.C. Srivastava et al from the seeds of Sesbania grandifolia (35). The specific rotation, galactose mannose ratio and methylation data of this galactomannan corresponds very closely with similar data from guar galactomannan, the two polysaccharides may have the same chemical structure.

**Fenugreek (Trigonella Foenum-Graecum) Structure**

The galactomannan isolated from the fenugreek seed contains D-galactose and D-mannose in the ratio of 1:1 (15), 5.6 (14). The polysaccharide was completely methylated as usual and hydrolysed. The cleavage fragments are found to
be 2, 3, 4, 6-tetra-O-methyl-D-galactose (5 parts), 2, 3, 6-tri-O-methyl-D-mannose (1 part) and 2, 3-di-O-methyl-D-mannose (5 parts)(14), together with traces of a mono-O-methyl hexose. Upon periodate oxidation 5 moles of formic acid are formed for every 11 hexose residues with the consumption of 16.4 moles of periodate. The yield of formic acid corresponds to the presence of 43.6 per cent of hexose units, occupying terminal position. Partial hydrolysis (36) of galactomannan afforded three oligosaccharides, which were identified as 4-O-\(-\)-D-mannopyranosyl-D-mannose, 6-O-\(\alpha\)-D-galactopyranosyl-D-mannose and 0-\(\beta\)-D-mannopyranosyl-(1\(\rightarrow\)4)-0-\(\beta\)-D-mannopyranosyl-(1\(\rightarrow\)4)-D-mannose.

The above data are consistent with a polymer structure involving a main chain of \(\beta-(1\rightarrow4)\) mannose units with \(\alpha\)-D-galactose units in attachment of various intervals through their reducing group to C-6 of mannose unit. The structure (Fig.4) appears to be highly branched but the basic linkages are the same as those in guar, carob and kentucky coffee bean gums.

\[
\begin{align*}
\mathbf{M}^\prime & \xrightarrow{\chi} \mathbf{M} \xrightarrow{\chi} \mathbf{M}^\prime \xrightarrow{\beta} \mathbf{M}^\prime \xrightarrow{\beta} \mathbf{M}^\prime \xrightarrow{\chi} \mathbf{M}^\prime \\
\mathbf{G} & \xrightarrow{\chi} \mathbf{G}^\prime \xrightarrow{\chi} \mathbf{G}^\prime
\end{align*}
\]

Fig.4: Structure of Fenugreek galactomannan
where, \(Y = 1\) and \((X + Z) = 3\).

\(M = D\)-mannopyranose, \(G = D\)-galactopyranose.
Galactomannans isolated from the seeds of Lucerne (Medicago Saliva) (36,37), Clover (37), Soyabean hulls (21,38), Lotus pendunculatus (4), White clover (35), Lotus corniculatus (5), have been structurally examined by different workers. Galactomannans from these on methylation and subsequent hydrolysis yielded 2, 3, 4, 6-tetra-0-methyl-D-galactose, 2, 3, 6-tri-0-methyl-D-mannose and 2, 3-di-0-methyl-D-mannose in various amounts. Periodate oxidation results are in agreement with methylation data. The structure of the galactomannan isolated from the seeds of Medicago lupulina (39) and Anthyllis vulneraria are determined on the basis of identification of oligosaccharides obtained during partial hydrolysis and periodate oxidation studies. Partial hydrolysis of these galactomannans resulted in isolation of four oligosaccharides. Fraction I and II are identified as 4-0-\(\beta\)-D-mannopyranosyl-D-mannose and 6-0-\(\alpha\)-D-galactopyranosyl-D-mannose, respectively. Fraction III is identified as a mixture of two trisaccharides \(0-\alpha\)-D-galactopyranosyl-(1→6)-0-\(\beta\)-D-mannopyranosyl-(1→4)-D-mannose and \(0-\beta\)-D-mannopyranosyl-(1→4)-0-\(\alpha\)-D-galactopyranosyl-(1→6)-\(\beta\)-D-mannose. Fraction IV is an unidentified one containing mixture of 3 tetra saccharides, representing one galactose unit (from side chain) along with three mannose units (from main chain).

Similar to fenugreek, these galactomannans are highly branched polymer containing 91 per cent to 63 per cent branched units in the main chain. Out of these, Medicago lupulina and Lotus pendunculatus are highly branched polysaccharides as compared to others as they contain 91 per cent
and 86 per cent branch chains. The galactomannan isolated from soyabean hulls contains only 63 per cent branch chains in the molecule. It is found that the galactomannans isolated from lucerne and lotus corniculatus have similar chemical structure and they contain 80 per cent branch units in the main chain. It is also interesting that the structures of white clover and anthyllis vulneraria seed galactomannans are identical and they contain 75 per cent branch chains in the polymer. The repeating unit of clover seed galactomannan has 7 branched units out of 9 hexose units of the main chain corresponding to 78 per cent branch chains.

Table-4
Suitable values of X + Z and Y for galactomannans Fig.4

<table>
<thead>
<tr>
<th>Source of galactomannan</th>
<th>X + Z</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicagolupulina</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Clover (trifolium pratense)</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Lotus pedunculatus</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Lotus corniculatus</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lucerne</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Anthyllis vulneraria</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>White clover</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Soyabean hulls</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

These data suggests that the structure of these galactomannan can be represented by general structural formula. Fig.4 already proposed for fenugreek by placing suitable value of X + Z and Y as given in Table-4.
Kentucky Coffee Bean (Gymnocladus Dioica) Structure

The Kentucky Coffee bean seed contains a galactomannan gum which forms the hard vitreous inner layer of the seed coat. The composition of the Kentucky bean varies. In one case it is reported to be 20 per cent galactose and 80 per cent mannose, while in another it is 70.5 per cent D-mannose and 26 per cent D-galactose (3).

The ratio of galactose to mannose end groups was found to be 6:1. On periodate oxidation the gum consumed 6 moles of periodate for every five sugar residue with the liberation of one mole of formic acid (arising from terminal non-reducing galactose and mannose residues). Hydrolysis of the methylated polysaccharide gives 2, 3, 4, 6-tetra-O-methyl-D-galactose (1 mole), 2, 3, 6-tri-O-methyl-D-mannose (3 moles), 2, 3-di-O-methyl-D-mannose (1 mole) and 2, 3, 4, 6-tetra-O-methyl-D-mannose (0.17 mole).

The presence of the 2, 3, 4, 6-tetra-O-methyl derivatives of D-galactose and D-mannose shows that these must have arisen from D-galactose and D-mannose units which are end groups in the side chains of the molecule. For each side
chain galactose unit there are four mannose residues, one of which affords the 2, 3-di-O-methyl-D-mannose cleavage fragment of the methylated gum and hence represents the branching point in the molecule, i.e., at C\textsubscript{1}, C\textsubscript{4} and C\textsubscript{6}; the other three mannose units occupy positions in a chain of residues joined by 1→4 glycosidic bonds.

**Coconut (Cocos nucifera) Structure**

A water soluble galactomannan isolated from the Kernel of coconut (40) had [\(\alpha\)]\textsubscript{D}\textsuperscript{25} = 85° and contained D-galactose (1 mole) and D-mannose (2 moles), methylation and hydrolysis yielded 2, 3, 4, 6-tetra-O-methyl-D-galactose, 2, 3, 4, 6-tetra-O-methyl-D-mannose, 2, 3, 6-tri-O-methyl-D-mannose, 2, 3, 6-tri-O-methyl-D-galactose and 3, 6-di-O-methyl-D-galactose in the molar ratio of 0.5:0.51:5.52:1.51:1, respectively. On periodate oxidation it consumed 1.03 moles of periodate liberating 1 mole of formic acid from 8 hexose units. When the periodate oxidised compound was reduced, hydrolysed, glycerol, erythritol and galactose were obtained in the ratio of 1:6.8:1.9:0.9 respectively.

On partial acid hydrolysis with 0.4N sulphuric acid, it gave 4-O-\(\beta\)-D-mannopyranosyl-D-mannose, 4-O-\(\beta\)-D-mannopyranosyl-D-mannose, 2-O-\(\beta\)-D-mannopyranosyl-D-galactose, 2-O-\(\beta\)-D-galactopyranosyl-D-galactose. The simplest structure, which can accommodate the above experimental facts is shown in Fig.6.
Manp 1 $\rightarrow$ 4 Manp 1 $\rightarrow$ 4 Galp 1 $\rightarrow$ 4 Manp 1 $\rightarrow$ 4 Galp 1 $\rightarrow$ 4 Manp 1 $\rightarrow$ 4

\[
\text{Galp} \quad \beta_{1,2}^{1n} \\
\text{Manp 1} \rightarrow 4 \text{Galp 1} \rightarrow 4 \text{Manp 1} \rightarrow 4 \text{Manp 1} \rightarrow 4 \text{Manp 1} \rightarrow 4 \text{Galp 1} \rightarrow 4 \text{Manp 1}
\]

$\rightarrow 4 \text{Manp 1} \rightarrow 4 \text{Manp 1} \rightarrow 4 \text{Galp 1}$ $\left[ \text{Manp} \right]_n$

Galp = D-galactopyranose

Manp = D-mannopyranose

Fig 6 Structure of Coconut (Cocos Nucifera) Galactomannan
The galactomannan isolated from the kernel of coconut is structurally different from the galactomannan isolated from the other sources. The latter ones generally possess 1,4 linked D-mannopyranose residues as main chain with D-galactopyranose residues as nonreducing terminal units joined through C₆ of certain of mannose units of the main chain. In the case of coconut galactomannan, both D-galactopyranose and D-mannopyranose residues are present as nonreducing terminal units as well as in the main chain. Some of the D-galactopyranose residues are present as branch points connected through C₁, C₂ and C₄.

**Gleditsia Amorphoides and Carob Bean Structure**

The galactomannan isolated from Gleditsia amorphoides, seeds (6) contains D-galactose 26% and D-mannose 59% in the ratio 1:2.7. From methylation and periodate results, the general structure proposed consists of (1→4)-D-mannose units in the main chain and to position 6 of one out of three mannose units -D-galactose units are attached as shown in Fig.7 where n = 2. It is of interest to note

\[
\begin{array}{c}
\text{G} \\
\uparrow \quad \beta \\
\downarrow \\
\text{M}_n \\
\end{array}
\]

\[G = \text{D-galactopyranose unit}
\]

\[M = \text{D-mannopyranose unit}\]

Fig.7: Structure of Carob and Gleditsia amorphoides galactomannan.

that the general structural features of this galactomannan are very much similar to those of Carob bean gum (41,42) as shown in Fig.7, where n = 2 to 4.
Cassia Occidentalis Structure

The water soluble galactomannan isolated from Cassia Occidentalis (43) seed contains D-galactose and D-mannose in the ratio of 1:3.1.

Fully methylated product, upon hydrolysis, produced 2, 3, 4, 6-tetra-O-methyl-D-galactose, 2, 3, 6-tri-O-methyl-D-mannose and 2, 3-di-O-methyl-D-mannose in the molar ratio of 1.02:2.2:1.0 respectively.

On periodate oxidation the polysaccharide consumed 4.84 moles of periodate with the concomitant liberation of 1.14 moles of formic acid for every 4 hexose units. The periodate oxidised polysaccharide upon Smith degradation produced glycerol (1.0 mole), erythritol (2.85 moles) and mannose traces. These results are well in agreement with the methylation studies.

The following structure Fig. 8 has been proposed for galactomannan:

\[
\begin{align*}
\text{G} & \xrightarrow{\alpha} \text{G} \\
\text{M} & \xrightarrow{\beta} \text{M} \\
\text{M} & \xrightarrow{\gamma} \text{M} \\
\text{M} & \xrightarrow{\delta} \text{M}
\end{align*}
\]

Fig. 8: Structure of Cassia Occidentalis Galactomannan.

This structure is further confirmed by partial acid hydrolysis of the polysaccharide and by identifying the oligosaccharides so obtained.
Cassia Absus (Chaksu) Structure

The galactomannan isolated from the seeds of Cassia absus (18) by water contains galactose, mannose, xylose in the ratio of 1:3:0.17.

Polysaccharide was methylated, followed by hydrolysis produced 2, 3, 4, 6-tetra-0-methyl-D-galactose (2 moles), 2, 3, 6,-tri-0-methyl-D-mannose (4.2 moles), 2, 3-di-0-methyl-D-mannose (1 mole) and 4, 6-di-0-methyl-D-mannose (1 mole). Periodate consumption was 1.09 moles for each hexose unit with concomitant liberation of 0.26 moles of formic acid. The periodate oxidised and reduced galactomannan afforded on hydrolysis : glycerol (1.94 moles), erythritol (5 moles) and D-mannose (1.08 moles). These results are well agreement with the methylation studies. The following structure Fig.9 has been proposed for galactomannan.

![Structure](image)

Fig.9: Structure of Cassia absus galactomannan.

This structure is further confirmed by partial acid hydrolysis of the polysaccharide and by identifying the oligosaccharides so obtained.
Cassia Renigera Structure

The galactomannan isolated from the endosperm of Cassia renigera seeds (44), by extraction with cold water. Galactomannan "A" was recovered as an acid soluble, major fraction from the barium complex of the polysaccharide.

Complete acid hydrolysis of the purified polysaccharide "A" afforded D-galactose, D-mannose and xylose in the molar ratio of 1:2.6:0.07.

Methylation of polysaccharide "A" afforded 2, 3, 6-tri-O-methyl-D-mannose (3.2 mole), 2, 3-di-O-methyl-D-mannose (2 mole) and 2, 3, 4, 6-tetra-O-methyl-D-galactose (2.12 mole), together with traces of 2, 3, 4, 6-tetra-O-methyl-D-mannose in the main fraction of the hydrolyzate. The presence of 2, 3, 4-tri-O-methyl-D-galactose (2 mole) was also reported by Gupta et.al (45).

The molar ratio of the methylated sugars suggests that the average repeating unit of the polysaccharide must be seven sugar residues, containing two galactose for every five mannose residues Fig.10.

![fig10](image_url)

Fig.10: Structure of Cassia renigera galactomannan.
Polysaccharide consumed 1.32 mole of periodate with the liberation of 0.26 mole of formic acid. The periodate oxidised and reduced polysaccharide afforded on hydrolysis: glycerol (2 mole), erythritol (4.88 mole), and traces of mannose from mannose residues that has escaped periodate oxidation.

The structure is further confirmed by i.r. spectrum of polysaccharide which showed bands at 817 and 874 cm\(^{-1}\), indicating the presence of \(\alpha\)-linked D-galactopyranose and \(\beta\)-linked-D-mannopyranose residues respectively.