PART I

SECTION (A) INTRODUCTION

SECTION (B) A RESUME' ON THE STRUCTURES OF THE RECENTLY INVESTIGATED GALACTOMANNANS OCCURRING IN THE SEEDS OF THE DIFFERENT PLANTS

PART I

SECTION (A)

INTRODUCTION

INTRODUCTION

Polysaccharides are important industrial products. The major sources in India are large number of plants bearing polysaccharide materials in the form of gums, mucilages and hemicelluloses. The plant gums are the substances which are obtained as exudations from fruit, trunks or branches of trees spontaneously or after mechanical injury of the plant by incision of the bark or by removal of a branch. The mucilages which are hetero-polysaccharides, occur either in the endosperm of the seeds or in the stem of the plant. The hemicelluloses are non-starch polysaccharides found in cereal endosperms. They also occur as plant cell-wall polysaccharides in close association with cellulose, especially in lignified tissues.

Structurally, the polysaccharides may be defined as condensation polymers in which the monosaccharides (or their derivatives such as uronic acids or amino sugars) have been glyosidically joined, with the elimination of water. The classification of plant polysaccharides is based on their chemical compositions and structures[1]. In such a classification, the poly-saccharides hydrolysing to only a single monosaccharides type would be placed in a group which may be termed as homoglycans, while the poly-saccharides with two or more than two types of monosaccharide units would be placed in a separate group termed as heteroglycans. A systematic and broad classification of natural polysaccharides is given below (Chart - 1.1).

Classification of Natural Polysaccharides

Natural Polysaccharides

- Homoglycans
  - Nitrogenous polysaccharides
    - (i) Glucosamine polymers
  - Neutral polysaccharides
    - (i) Glucans
    - (ii) Mannans
    - (iii) Galactans
    - (iv) Fructans
  - Acidic polysaccharides
    - (i) Galacturonans
    - (ii) Mannuronans
    - (iii) Gluronans
- Heteroglycans
  - Nitrogenous polysaccharides
    - (i) Mucopoly-saccharides
    - (ii) Glycoproteins
  - Neutral polysaccharides
    - (i) Hemicelluloses
    - (ii) Galactomannans
    - (iii) Glucomannans
    - (iv) Arabinogalactans
    - (v) Arabinoxylans
    - (vi) Xyloarabinans
  - Acidic polysaccharides
    - (i) Gums
    - (ii) Mucilages

CHART - 1.1

Natural polysaccharides may further be classified depending upon whether they possess branched or linear chains. Linear polysaccharides of which cellulose is the prime example, are excellent structural material due to many intermolecular secondary valance attachments which make the structure strong, rigid and insoluble. Branched polysaccharides are easily soluble in water and have immense thickening power. Starch, the most important food material is a mixture of linear and branched polysaccharides with the latter generally predominating.

The medicinal values[2] of plant gums and mucilages was known to the practitioners of indigenous system of medicines since antiquity. They are often used in curing ringworm bacillary dysentry, urethritis, infantile diarrhoea, enlargement of liver and chronic cough etc. Mucilages are also used as a discutient, emollient and as poultice or plaster for swellings, as an external application for scalds and ulcers, sore throat etc. Recently the polysaccharides[3] extracted from natural sources have been reported to possess high drug potentiality against cancerous and tumorous growths. The degree of antitumor action of these polysaccharides was observed to be dependent upon the nature and mode of glycosidic linkages present in the molecular structure of polymer concerned. The $(1 \rightarrow 3)$-ß-D-glucans and ß-D-glucans having a preponderance of, or, perhaps, long stretches of $(1 \rightarrow 3)$ linkages in the main chain are active antitumor agents, particularly if they are mainly linear without excessively long branches and if they are not quickly hydrolysed by humoral D-glucanases. The effectiveness of the polysaccharides was tested in mice bearing Sarcoma 180, Ehrlich carcinoma, Sarcoma 37, Krebs-2 carcinoma, Hepatoma 134, P-288 and B_{16} Melanoma.

Various groups of research workers have also observed that the polysaccharides from several botanical sources i.e. wheat straw, bagasse, bamboo leaves, rice stalks and corn stalks are highly active in inhibiting the growth and inducing regression of sarcoma 180 subcutaneously implanted in mice. Certain polysaccharides from higher plants have been shown to inhibit the growth of transplanted tumors. The polysaccharides[4] also play the role of an antibiotic during covering and filling of bone defects at the time of insertion of dental implants.

The polysaccharides, especially galactomannans are substances of industrial importance. The galactomannans[5] isolated from the seed mucilage of the family, Leguminoseae are used as thickening and gelling agents in food industry, as binding agents in pharmaceutical industry, as clarifying agents in surgarcane juice in sugar industry, as sizing materials for textiles in textile industry, and as additives in insecticidal and herbicidal compositions. Natural gums[6] containing galactomannose (0.01-10 parts) as main component when added to 100 parts $CaSO_4$, 0.5 H_2O plasters improves its water retention properties and prevents hardening defects, such as cracking.

Due to the medicinal and industrial applications, the structure of the polysaccharide is always a subject matter of interest to Organic Chemist. The knowledge of structure of polysaccharide helps to understand its properties which do not depend much upon the actual building units but upon its over all molecular frame work. Investigation of structure of plant polysaccharides may enlighten the pathway of their biogenesis- the mechanism by which simple sugars are transformed into other hexoses, pentoses, uronic acids, and methyl pentoses. In recent years, it has been observed that some bacterial polysaccharides of immunological importance are structurally related to the plant polysaccharides. Hence researches on the structure of plant polysaccharides might lead to the development of a new group of physiologically active compounds for combating the action of various micro-organisms. The recent use of polysaccharides in cancer chemotherapy has stimulated further the interest of Organic Chemists to understand the structure activity relationship of these compounds.

In recent years, gums occurring in the plant seeds are attracting the attention of Phyto Chemists since one can utilize them as substitutes of plant gums whose supply has become limited due to their ever increasing demand in the world market. Seed gums are considered superior to plant gums due to the fact that their production can be easily increased by cultivating large area with crops of plants having gum bearing seeds.

A systematic chemical investigation of the plant polysaccharides is continuing for more than thirty years in various research laboratories of India. The following Table (1.1) shows some plant sources from which the polysaccharides have been isolated for the purpose of their structural studies.

Table 1.1

A List of Plant Material Investigated at Research Laboratories of India

Sl. No.	Name of the plant	Natural order,	Nature of the polysaccharide	References
1.	Azadirachta indica	Meliaceae	Gum	7-8
2.	Odina wodeir	Anacardiaceae	Gum	9-11
3.	Acacia sundra	Leguminoseae	Gum	12-13
4.	Salvia aegyptiaca	Labiatae	Mucilage	14
5.	Feronia elephantum	Rutaceae	Gum	15
6.	Saccharum officinarum	Gramineae	Hemicellulose	16-17
7.	Chloroxylan swietenia	Rutaceae	Gum	18
8.	Commiphora mukul	Burseraceae	Gum	19-20
9.	Salmalia malabarica	Bombacaceae	Gum	21
10.	Anacardium occidentale	Anacardiaceae	Gum	22-23
11.	Hibiscus-ficulneus	Malvaceae	Mucilage	24
12.	Anacardium occidentale shell	Anacardiaceae	Shell polysaccharide	25-27

Table 1.1 contd.

Sl. No.	Name of the plant	Natural order	Nature of the polysaccharide	References
13.	Sesbania grandiflora	Leguminoseae	Seed polysaccharide	28
14.	Cassia absus	Leguminoseae	Seed polysaccharide	29-32
15.	Phoenix dactylifera	Palmae	Seed polysaccharide	33-34
16.	Cassia fistula	Leguminoseae	Seed polysaccharide	35-36
17.	Cassia occidentalis	Leguminoseae	Seed polysaccharide	37-38
18.	Ocimum basilicum	Labiatae	Seed polysaccharide	39-40
19.	Aegle marmelos	Rutaceae	Gum	41-42
20.	Cassia grandis	Leguminoseae	Seed polysaccharide	43-44
21.	Crotalaria juncea	Leguminoseae	Seed polysaccharide	45-46
22.	Cassia multijuga	Leguminoseae	Seed polysaccharide	47
23.	Strychnos potatorum	Loganiaceae	Seed polysaccharide	48
24.	Aloe vera	Liliaceae	Mucilage	49
25.	Ipomoea fistulosa syn. I. carnea	Convolvulaceae	Seed polysaccharide	50
26.	Wood fordia fruiticosa	Lythraceae	Gum	51
27.	Sesbania speciosa	Leguminoseae	Seed polysaccharide	52
28.	Sesbania aegyptiaca	Leguminoseae	Seed polysaccharide	53
29.	Cassia corymbosa	Leguminoseae	Seed polysaccharide	54
30.	Cassia renigera	Leguminoseae	Seed polysaccharide	55-56
31.	Cassia laevigata	Leguminoseae	Seed polysaccharide	57
32.	Teramnus labialis	Leguminoseae	Seed polysaccharide	58
33.	Melilotus indica	Leguminoseae	Seed polysaccharide	59
34.	Indigofera tinctoria	Leguminoseae	Seed polysaccharide	60

Table 1.1 contd.

Sl. No.	Name of the plant	Natural order	Nature of the polysaccharide	References
35.	Cassia sophera	Leguminoseae	Seed polysaccharide	61
36.	Acacia leucophloea	Leguminoseae	Seed polysaccharide	62
37.	Linum usitatissimum	Linaceae	Seed polysaccharide	63
38.	Cassia alata	Leguminoseae	Seed polysaccharide	64-65
39.	Melilotus officinalis	Leguminoseae	Seed polysaccharide	66
40.	Crotalaria verrucosa	Leguminoseae	Seed polysaccharide	67
41.	Cassia siamea	Leguminoseae	Seed polysaccharide	68
42.	Sesbania bispinosa	Leguminoseae	Seed polysaccharide	69
43.	Ipomoea palmata	Leguminoseae	Seed polysaccharide	70
44.	Cassia ovata	Leguminoseae	Seed polysaccharide	71

A survey of literature shows that a large number of the plants belonging to Leguminoseae family have been chemically screened so far. Thus Anderson[72] has examined 163 Leguminoseae plants while Faroogi et. al.[73] have reported the screening of seeds of 237 Leguminoseae plants. Both the investigators have shown that the plants belonging to Leguminoseae family produce seeds rich in mucilagenous matter. Various species of legumes have been examined so far for seed mucilage in which endosperm is present in amounts varying between 0 to 60%. The survey of literature also shows that the polysaccharides occurring in the seeds of the plants of Leguminoseae family are generally of galactomannan type. Galactomannans are the common water soluble constituent of endosperm and are considered as reserve polysaccharides. Hence it was decided that it would be of interest to examine galactomannans from other seeds in order to determine their relationship with the known galactomannans. A literature survey to date revealed that the seeds of the plants of certain genus namely Adenanthera belonging to Leguminoseae family have remained almost uninvestigated for carbohydrates. No attempt has been made so far to search the polysaccharide material, if any, and determine the structure of the polysaccharide occurring in the seeds of Adenanthera pavonina[74] which is easily available in India. A chemical investigation (partial analysis) of the polysaccharide content of A. pavonina has, therefore, been taken up and this constitutes the subject matter of the present dissertation. Since appreciable volume of literature has accumulated on the structure of galactomannans during the past few years, it was thought desirable to prepare an upto-date resume' of work done so far on the polysaccharides isolated from the plants belonging to Leguminoseae and other families. This is being presented in the next section.

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PART I

SECTION (B)

A RESUME' ON THE STRUCTURES OF THE RECENTLY INVESTIGATED GALACTOMANNANS OCCURRING IN THE SEEDS OF THE DIFFERENT PLANTS

A RESUME' ON THE STRUCTURES OF THE RECENTLY INVESTIGATED GALACTOMANNANS OCCURRING IN THE SEEDS OF THE VARIOUS PLANTS.

1. Medicago sativa. (Lucerne or Alfalfa).

The galactomannan extracted from the seeds of Medicago sativa (Fam., Leguminoseae) with 10% potassium hydroxide[75] was found quite different in structure from that extracted with water. The polysaccharide contained galactose and mannose in the molar ratio of 2:1.

The fully methylated galactomannan was resistant to hydrolysis by methanolic hydrogen chloride but hydrolyzed by the use of a mixture of hydrochloric acid and acetic acid. The cleavage fragments were not identified completely but included 2,3,4,6-tetra-O-methyl-D-galactose, 2,4,6-tri-O-methyl-D-galactose and 3,4-di-O-methyl-D-mannose in the molar proportion of 1:1:1. At least one-third of the tri-O-methyl hexose was shown to be 2,4,6-tri-O-methyl-D-galactose and at least one third of the di-O-methyl-D-hexose was 3,4-di-O-methyl-D-mannose. This indicated that half of the galactose form all the end groups, the remaining galactose being attached through positions 1 and 3 and the branching points are at C_2 or C_6 on the mannose residues, whereas in the other galactomannans, the branching points are on mannose residues at C_4 and C_6.

Whistler et al. have also investigated the structure of hot water extractable galactomannan from Alfalfa[76] seed through partial acid hydrolysis, periodate oxidation and methylation. The galactomannan, which contained 52% anhydromannose and 48% anhydrogalactose, on partial acid hydrolysis gave four oligosaccharides (1-4) which are characterised as follows.

4-O-ß-D-mannopyranosyl-D-mannose (1)

6-O-α-D-galactopyranosyl-D-mannose (2)

4-O-(6-O-α-D-galactopyranosyl-ß-D-mannopyranosyl)-D-mannose (3)

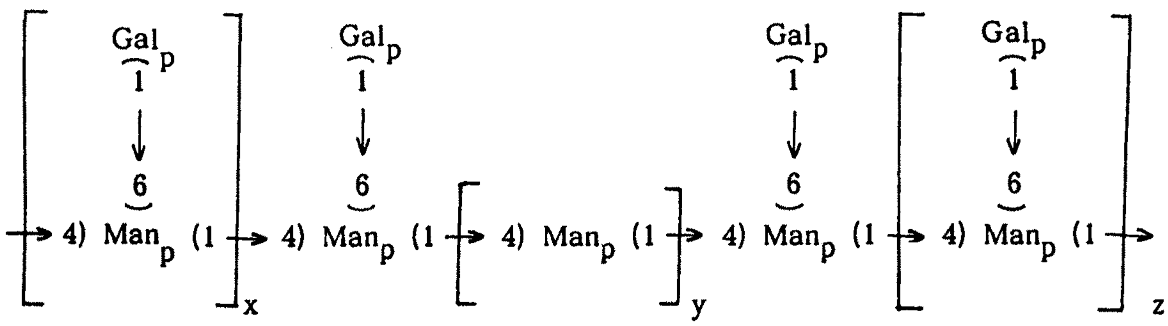
4-O-(4-O-ß-D-mannopyranosyl-ß-D-mannopyranosyl)-D-mannose (4)

Hough[77] had also studied the structure of galactomannan isolated from hot water extract of Lucerne seeds at 70-80^{o}. It was composed of galactose and mannose in the molar proportion of 1:1.23 and 1:1.27. On partial acid hydrolysis[78], it afforded three identified and one unidentified oligosaccharides. The three identified oligosaccharides have been shown to have the structures (1-2,4). The unidentified oligosaccharide was found to be composed of galactose and mannose in the molar ratio of 1:2, respectively.

Methylation analysis of the polysaccharide by Hough et al.[77] showed the presence of 2,3,4,6-tetra-O-methyl-D-galactose (44.5%) 2,3,6-tri-O-methyl-D-mannose (11.0%) and 2,3-di-O-methyl-D-mannose (44.5%) which showed that more than 40% of the D-mannopyranose in the ß-(1→4) linear chain contained, D-galactopyranosyl units attached to them by α-(1→6)-linkages. This was also confirmed from periodate oxidation results.

2. Trifolium pratense

A galactomannan[77] from the seeds of Trifolium pratense (Fam., Leguminoseae; subfam., Papilionaceae), after purification by copper complex formation, was shown to be composed of D-mannose and D-galactose in a molar ratio of 9 : 7. Hydrolysis of the fully methylated polysaccharide furnished 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 a molar ratio of 7:2:7. To accomodate these data, the following tentative structure (5) has been proposed for T. pratense seed galactomannan, here y = 2 and x + z = 5, support for which is found in the periodate oxidation of the polysaccharide when 7 moles of formic acid are produced for every 16 hexose residues. The completely periodate oxidised polysaccharide is found to contain no trace of D-galactose or D-mannose residues.



(5)

3. Trigonella foenum-graecum Linn.

The galactomannan from the seeds of T. foenum-graecum (Fam., Leguminoseae; Subfam., Papilonaceae), had been reported to be composed of D-galactose and D-mannose in a molar proportion of 1:1 by Raoud[79] while Andrews[80] and his coworkers showed it to be 1:1.2. Partial acid hydrolysis[78] of the compound resulted in the formation of three oligosaccharides having structures (1-2,4).

4-O-β-D-mannopyranosyl-D-mannose (1)

6-O-α-D-galactopyranosyl-D-mannose (2)

4-O-(4-O-β-D-mannopyranosyl-β-D-mannopyranosyl)-D-mannose (4)

Hydrolysis of the fully methylated polysaccharide furnished 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 5:1:5 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 16.39 moles of periodate. It is evident that the galactomannan is profusely branched structure as it contains branched chains to the extent of 63.4%. After Smith's degradation of the periodate-oxidised material and its subsequent hydrolysis, the product neither gives galactose nor mannose. Thus it had been concluded that the galactomannan is highly branched with all the galactose units occupying the terminal positions. The points of branching are situated on the mannose units which are linked through positions 1,4 and 6. These data suggested that the structure of Fenugreek seed galactomannan[81] is similar to that from Clover seed and can be represented by the structure (5) where y=1 and x+z=3.

4. Trifolium repens L.

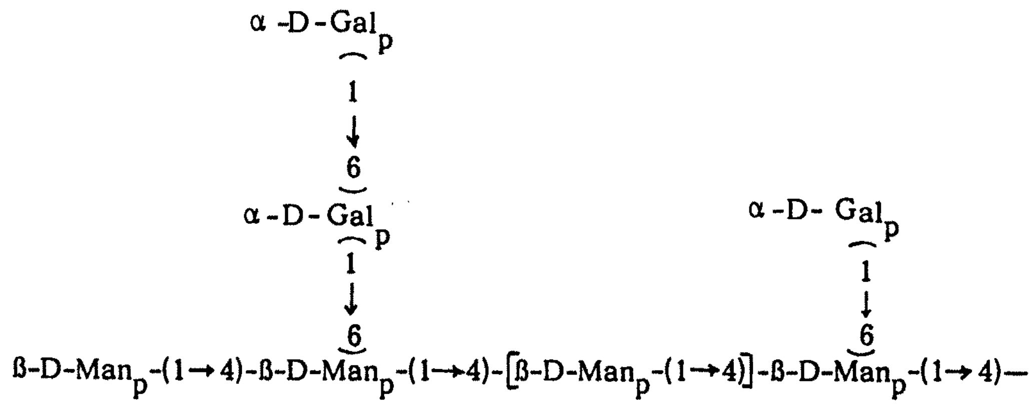
The water soluble galactomannan isolated from the seeds of Trifolium repens L. (Fam., Leguminoseae) by Horvei and his coworkers[82], is composed of D-galactose and D-mannose in a molar proportion of 1:1.3.

Fully methylated polysaccharide on hydrolysis, gave 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 3.38:1:3. Determination of terminal groups by titration of the amount of formic acid liberated during oxidation of the polysaccharide by periodate, gave indication of the presence of 41.2% terminal hexose residues which is slightly lower than the values calculated from methylation results. The capacity of the polysaccharide to reduce periodate (1.1-1.2 moles per anhydrohexose unit) tends to indicate that the mannose residues of the chains are also extensively attacked by the oxidant. The presence of approximately same molar proportion of 2,3,4,6-tetra-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose indicates that the main chain contains (1 → 4) linked D-mannan backbone to which are attached single α-D-galactosyl stubs at C-6 of each D-mannose residues, thus creating a highly branched polysaccharide structure (6).

1α Gal　　1α Gal
↓　　　　↓
6　　　　6
— Man β 1 → 4 Man β 1 → 4 Man β 1 → 4 Man—

(6)

Courtois and Ledizet[83] reinvestigated the same galactomannan. Hydrolysis of the fully methylated polysaccharide gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,3,4-tri-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose moieties. The occurrence of 2,3,4-tri-O-methyl-D-galactose indicates the presence of infrequent, short chains of α-D-(1→6)-linked-D-galactosyl residues attached to the main chain. Hence the revised structure (7) has been suggested for the galactomannan of Trifolium repens.

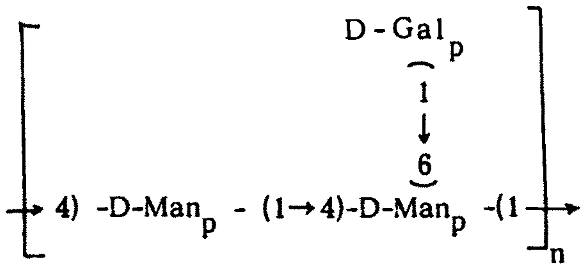


(7)

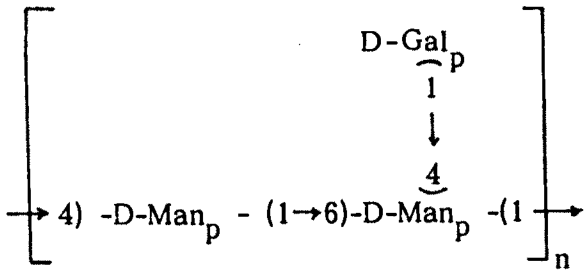
5. Sesbania grandiflora

The galactomannan[28] from the seeds of Sesbania grandiflora (Fam., Leguminoseae; Subfam., Papilionaceae) has been shown to be a neutral polysaccharide containing two major sugars D-galactose and D-mannose in a molar ratio of 1:2. The hydrolysis of the fully methylated compound furnished 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 a molar proportion of 1:1:1. Upon periodate oxidation, the polysaccharide consumed 1.2 moles of periodate with simultaneous liberation of 0.34 mole of formic acid per mole of anhydrohexose unit. On Smith's degradation of periodate-oxidised material, it furnished glycerol and erythritol in a molar proportion of 1:1.8.

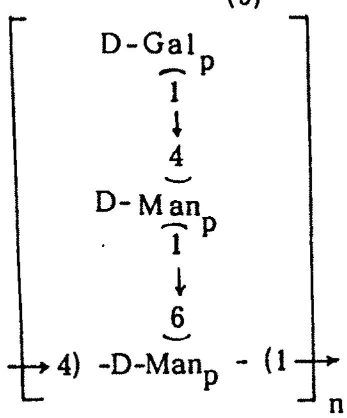
Based on the results of methylation and periodate oxidation and Smith's degradation studies, Srivastava and his coworkers have proposed four possible structures (8-11) for the galactomannan, none of which could be confirmed.


D-Galp
1
6
→4) -D-Manp - (1→4)-D-Manp -(1→
n

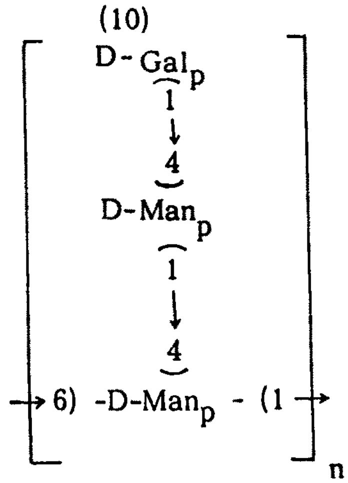
(8)


D-Galp
1
4
→4) -D-Manp - (1→6)-D-Manp -(1→
n

(9)


D-Galp
1
4
D-Manp
1
6
→4) -D-Manp - (1→
n

(10)


D-Galp
1
4
D-Manp
1
4
→6) -D-Manp - (1→
n

(11)

6. Cassia absus Linn.

Barium complex of the galactomannan isolated from the powdered seeds of Cassia absus (Fam., Leguminoseae), on fractionation with acetic acid gave rise to two polysaccharides (A) and (B). Polysaccharide (A)[29,30] which is soluble in acetic acid is composed of D-galactose, D-mannose and D-xylose in molar proportion of 1:3:0.17. Graded acid hydrolysis[31] of (A) furnished seven oligosaccharides, out of which five have been isolated in pure state and their structure have been deduced as follows (12 and 1-4)

2-O-α-D-galactopyranosyl-D-mannose	(12)
4-O-β-D-mannopyranosyl-D-mannose	(1)
6-O-α-D-galactopyranosyl-D-mannose	(2)
4-O-(6-O-α-D-galoctopyranosyl-β-D-manno pyranosyl)-D-mannose	(3)
4-O-(4-O- β-D-mannopyranosyl-β-D-manno pyranosyl)-D-mannose	(4)

$Gal_p\ (1 \rightarrow 2)\ Man_p$

(12)

$Man_p\ (1 \rightarrow 4)\ Man_p$

(1)

$Gal_p\ (1 \rightarrow 6)\ Man_p$

(2)

Gal_p

1

↓

6

$Man_p\ (1 \rightarrow 4)\ Man_p$

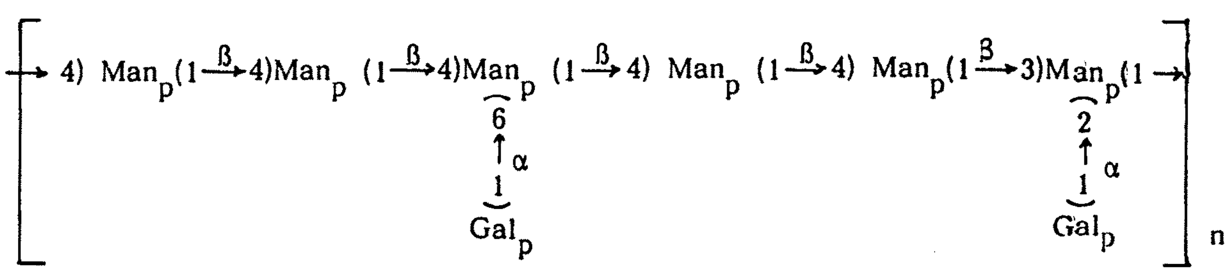
(3)

$Man_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Man_p$

(4)

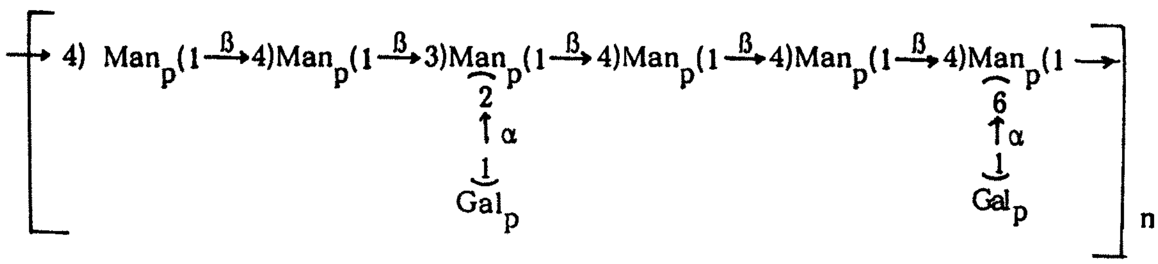
Fully methylated polysaccharide[30], on acid hydrolysis, gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,3-di-O-methyl-D-mannose and 4,6-di-O-methyl-D-mannose in the molar ratio of 2:4:1:1. Since 2,3,6-tri-O-methyl-D-mannose is a major component, it is suggested that the main chain is mostly composed of (1 ⟶ 4) linked mannose units. The isolation of 2,3-di-O-methyl and 4,6-di-O-methyl-D-mannose indicates the presence of two types of triply linked mannose units in the polysaccharide - one linked through C_1, C_4 and C_6 and the other linked through C_1, C_2 and C_3.

To accommodate all these linkages, the following tentative structure (13) has been suggested for the galactomannan (A). This structure has been further confirmed by periodate oxidation & Smith's degradation studies of the oxopolysaccharide. Upon periodate oxidation, galactomannan (A) consumed 1.09 moles of periodate with simultaneous liberation of 0.26 mole of formic acid per mole of anhydrohexose unit. The periodate oxidised polysaccharide on Smith's degradation followed by hydrolysis gave glycerol and erythritol in the molar proportion of 1.94:5.0 along with traces of D-galactose. Structure (A) explains satisfactory the graded hydrolysis products but fails to explain the occurrence of the periodate resistant galactose unit.



(13)

Acetic acid insoluble polysaccharide (B) is madeup of D-galactose, D-mannose and D-xylose in a molar proportion of 1:3:0.13. Methanolysis of the fully methylated compound[32] afforded 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,3-di-O-methyl-D-mannose and 4,6-di-O-methyl-D-mannose in the molar proportion of 2:4:1:1. Polysaccharide (B) was also subjected to periodate and Smith's degradated reactions. From the results of all these studies, structure (14) has been proposed for polysaccharide (B).



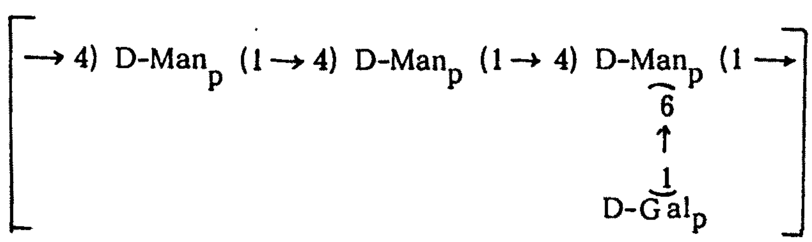
(14)

It may be mentioned here that the structures of two galactomannans (13) and (14) are more or less same, differing only in the placement of galactose residues in (1→4)- linked mannose main chain.

7. <u>Caesalpinia pulcherima Linn.</u>

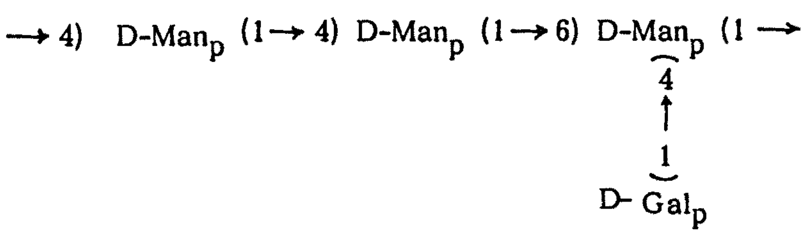
The galactomannan[84] from the seeds of <u>Caesalpinia pulcherima</u> (Fam., <u>Leguminoseae</u>), purified by repeated complex formation with copper acetate was shown to be composed of D-mannose and D-galactose in a molar ratio of 3:1. Fully methylated polysaccharide, on acid hydrolysis, furnished 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,4,6-tri-O-methyl-D-mannose, 3,4,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in a molar proportion of

1:27:60:4:2:32. Ordinary and sequential (Smith) periodate degradation indicated that the (1→6)-linked-D-galactose units occupied single unit branch points and the mannose units to the extent of 5% were resistant to periodate attack. The formation of equimolar quantities of 2-O-ß-D-mannopyranosyl-D-erythritol and O-ß-D-mannopyranosyl-(1→3)-O-ß-D-mannopyranosyl-(1→2)-D-erythritol in a low yield indicates a low frequency of isolated (1→3)-linked and consecutively (1→3)-linked bonds. Isolation of 3,4,6-tri-O-methyl-D-mannose is indicative of the presence of (1→2)-linkage in the polysaccharide. All the D-galactose units occupy terminal non-reducing positions, since no other methylated galactose other than 2,3,4,6-tetra-O-methyl-D-galactose could be isolated after methylation analysis. A relatively simple structure (15) which accomodates broadly the experimental results have been tentatively assigned to the galactomannan. This structure, however, does not show the presence of (1→2)-linked mannose units.



or

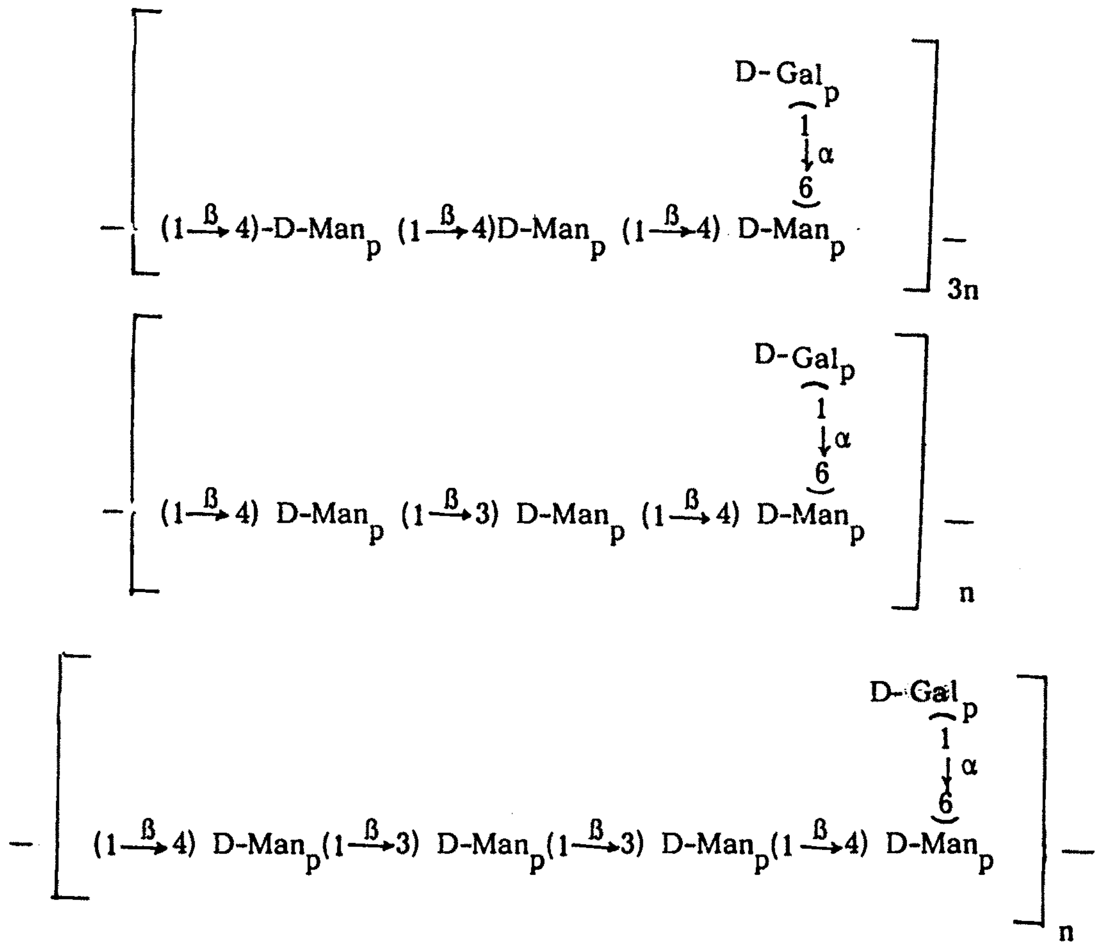
(1→ 4) to a D-mannose unit linked at C-6 e.g.



(15)

8. Crotalaria mucronata Desv.

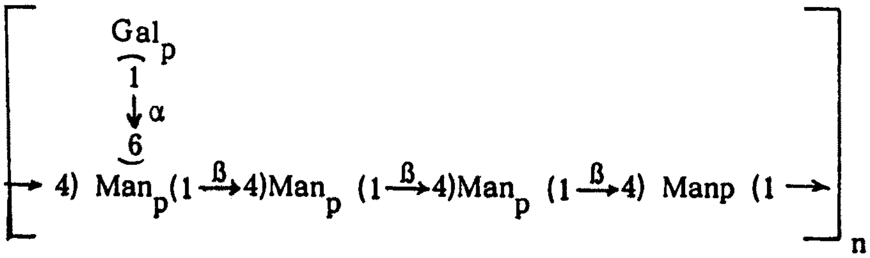
The galactomannan[85] from C. mucronata (Fam., Leguminoseae, Subfam., Papilionaceae), purified by repeated complex formation with copper acetate, was shown to be a neutral polysaccharide containing D-mannose and D-galactose in a molar ratio of 3:1. Methanolysis of the fully methylated compound yielded 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose, 2,4,6-tri-O-methyl-D-mannose, 3,4,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in a molar ratio of 1:23:28:11:4:26. Periodate oxidation studies indicated that the galactose residues occupy single unit branch point linked through (1 ⟶ 6). One in every six mannose units was resistant to periodate attack, further suggesting the presence of (1 ⟶ 3)-linkages. An approximately equimolar occurrence of 2-O-ß-D-mannopyranosyl-D-erythritol and O-ß-D-mannopyranosyl (1 ⟶ 3)-(2-O-ß-D-mannopyranosyl-D-erythritol) pointed out a significant frequency of isolated and consecutive (1 ⟶ 3)-linkages in the galactomannan molecule. The structure (16) which has been assigned tentatively for the galactomannan, is consistent with the methylation and periodate oxidation results. The low occurrence of (1 ⟶ 2) linkage in the main mannan chain (4 in 70) is not included in the following structural representation due to the complexity of the structure.



(16)

9. Cassia fistula Linn.

The galactomannan isolated from the seeds of Cassia fistula (Fam., Leguminoseae) by Mukherjee and Kelkar[35], has been shown to be a neutral polymer containing two major sugars, D-galactose and D-mannose in a molar ratio of 1:4. Acid hydrolysis of the fully methylated polysaccharide furnished 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 a molar proportion of 1:3:1. From methylation results it was proposed that the repeating unit of the galactomannan consisted of five hexose units only, out of which four (1→4)-linked mannose units constitute the main chain. The remaining (1→6)-linked galactose unit occurs as a side chain. The periodate oxidation results are also in good agreement with those expected from the structure (17).



(17)

Lal and his coworker[36] have also investigated the structure of C. fistula seed polysaccharide and reported the presence of galactose and mannose in the molar ratio of 1:3.38 which is slightly lower than the value reported earlier. Though they have not suggested any structure for the polysaccharide, methylation analysis shows the presence of units of singly linked galactose (8 moles) and mannose (2 moles), doubly linked mannose (15 moles) and triply linked mannose (10 moles) units which constitute the branch point.

10. Cassia occidentalis Linn.

A neutral galactomannan isolated from the powdered seeds of Cassia occidentalis (Fam., Leguminoseae), is composed of D-galactose and D-mannose in a molar ratio of 1:3.1 along with traces of xylose[37]. The partial acid hydrolysis of the galactomannan[38] afforded four oligosaccharides having structures (1-4).

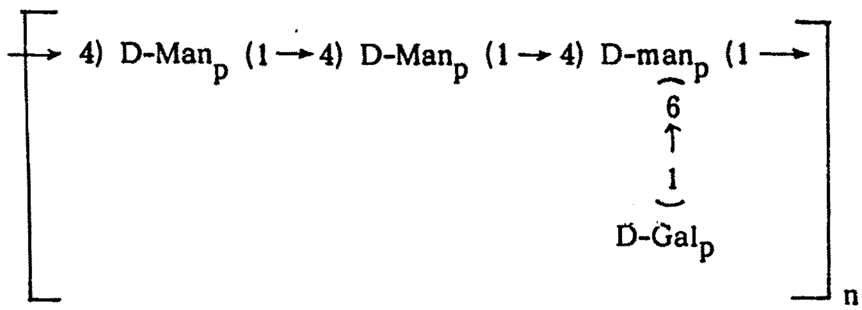
4-O-ß-D-mannopyranosyl-D-mannose (1)

6-O-α-D-galactopyranosyl-D-mannose (2)

4-O-(6-O-α-D-galactopyranosyl-ß-D-mannopyranosyl-D-mannose) (3)

4-O-(4-O-ß-D-mannopyranosyl-ß-D-mannopyranosyl)-D-mannose (4)

Fully methylated polysaccharide, upon methanolysis[37], 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 a molar proportion of 1.02:2.2:1.0. Isolation of 2,3,6-tri-O-methyl-D-mannose as a major component clearly indicated that the main chain was composed of (1→4)-linked mannose units. The branched character of the polysaccharide followed from the isolation of 2,3-di-O-methyl-D-mannose which is an indicative of mannose unit linked through C_1, C_4 and C_6. Based on the results of methylation and graded hydrolysis, the structure (18) has been proposed for the repeating unit present in the galactomannan. The structure was also confirmed from periodate results. On periodate oxidation, it liberated 0.28 mole of formic acid with concomitant consumption of 1.21 moles followed by acid hydrolysis furnished glycerol and erythritol in a molar ratio of 1:2.85. These results are in close agreement with those expected from the structure (18).



(18)

11. <u>Cassia grandis L.F.</u>

The polysaccharide isolated from the seeds of <u>Cassia grandis</u> L.F. (Fam., <u>Leguminoseae</u>), contains D-galactose, D-mannose and D-xylose in the molar proportion of 7:5:1. Graded hydrolysis of the polysaccharide[43] gives rise to a mixture of five oligosaccharides (19-23), which are designated as follows.

3-O-α-D-galactopyranosyl-D-galactose (19)

4-O-β-D-galactopyranosyl-D-galactose (20)

4-O-β-D-galactopyranosyl-D-mannose (21)

O-β-D-galactopyranosyl (1→4)-O-β-D-galacto pyranosyl (1→4)-D-mannopyranose (22)

O-β-D-mannopyranosyl (1→4)-O-β-D-galactopyranosyl(1→4)-O-β-D-galactopyranosyl (1→4)-D-mannose (23)

Gal_p (1→3) Gal_p

(19)

Gal_p(1→4)Gal_p

(20)

Gal_p(1→4)Man_p

(21)

Gal_p(1→4)Gal_p(1→4)Man_p

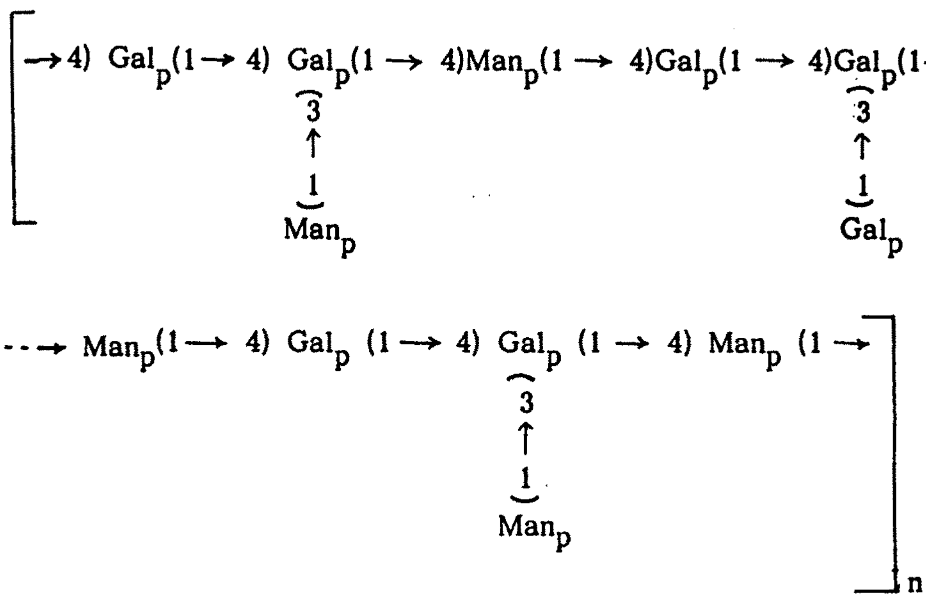
(22)

Man_p (1→4) Gal_p (1→4) Gal_p (1→4) Man_p

(23)

Acid hydrolysis of the fully methylated polysaccharide[44] yielded 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-galactose, 2,6-di-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-mannose and 2,3,6-tri-O-methyl-D-mannose in the molar ratio of 1:3:3:2:3. Upon periodate oxidation of the polymer, the polymer consumed 1.033 moles of periodate with simultaneous liberation of 0.276 mole of formic acid per mole of anhydrohexose unit. Smith's degradation of the periodate oxidised polysaccharide, furnished D-galactose, glycerol and erythritol in a molar ratio of 1:1.02:2.24, respectively.

To accomodate all these results, a tentative structure (24) consisting of three end groups in a repeating unit of the twelve numbers has been suggested for C. grandis seed galactomannan.



(24)

12. Crotalaria juncea Linn.

Water soluble galactomannan[45] occurring in the seeds of Crotalaria juncea (Fam., Leguminoseae; Sub fam., Papilionaceae) is composed of D-galactose (1 mole), D-mannose (2 moles) and D-xylose in traces. The polysaccharide when subjected to a graded hydrolysis gives rise to a mixture of four oligosaccharides (2,21 and 25-26) whose structures have been deduced from their physical properties, hydrolytic pattern and also from the results of methylation, periodate oxidation and Smith's degradation studies.

6-O-α-D-galactopyranosyl-D-mannopyranose	(2)
4-O-β-D-galactopyranosyl-D-mannopyranose	(21)
O-β-D-galactopyranosyl (1→4)-O-β-D-mannopyranosyl (1→4)-D-mannopyranose	(25)
O-β-D-galactopyranosyl (1→4)-O-β-D-mannopyranosyl (1→4)-O-β-D-mannopyranosyl (1→4)-D-galactopyranose	(26)

$Gal_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Man_p$

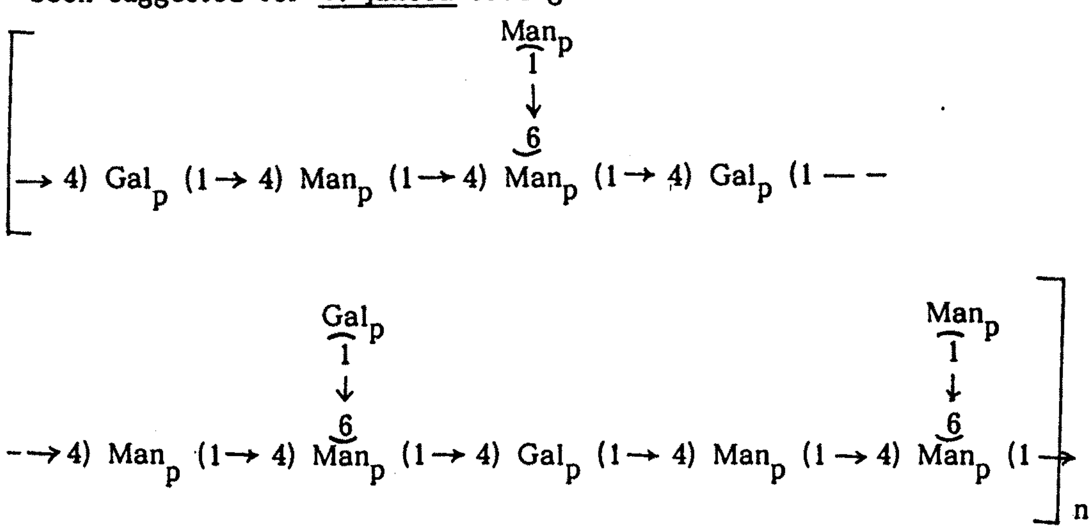
(25)

$Gal_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Gal_p$

(26)

Fully methylated polysaccharide, on acid hydrolysis[46], produced 2,3,4 6-tetra-O-methyl-D-mannose (2 moles), 2,3,4,6,-tetra-O-methyl-D-galactose (1 mole), 2,3,6-tri-O-methyl-D-mannose (3 moles), 2,3,6-tri-O-methyl-D-galactose (3 moles) and 2,3-di-O-methyl-D-mannose (3 moles). Upon oxidation with sodium metaperiodate, the polysaccharide consumed 1.17 moles of periodate with simultaneous liberation of 0.249 mole of formic acid per mole of anhydrohexose unit. Periodate oxdised polysaccharide on Smith's degradation furnished glycerol (1 mole) and erythritol (2.96 moles). The repeating unit consists of 12 hexopyranose units only, out of which 6 mannose and 3 galactose residues joined by (1→4)-linkages constitute the main galactomannan chain. The remaining 2 mannose and 1 galactose units occur as side chains joined by (1→6) glycosidic bonds. The molecular weight of the polymer shows that the repeating unit is repeated 19 times in the full structure of the polysaccharide.

Based on the above data, a following tentative structure (27) has been suggested for <u>C. juncea</u> seed galactomannan.



(27)

13. Cassia multijuga

A galactomannan[47] isolated from the seeds of Cassia multijuga (Fam., Leguminoseae), is made up of D-galactose, D-mannose and D-xylose in the molar ratio of 5:1:2. Partial acid hydrolysis of the polysaccharide yielded the four oligosaccharides having the following structures (2 and 28-30).

6-O-α-D-galactopyranosyl-D-mannose	(2)
6-O-α-D-galactopyranosyl-D-galactose	(28)
4-O-β-D-galactopyranosyl-D-xylose	(29)
3-O-β-D-xylopyranosyl-D-xylose	(30)

Gal_p (1 → 6) Gal_p

(28)

Gal_p (1 → 4) Xyl_p

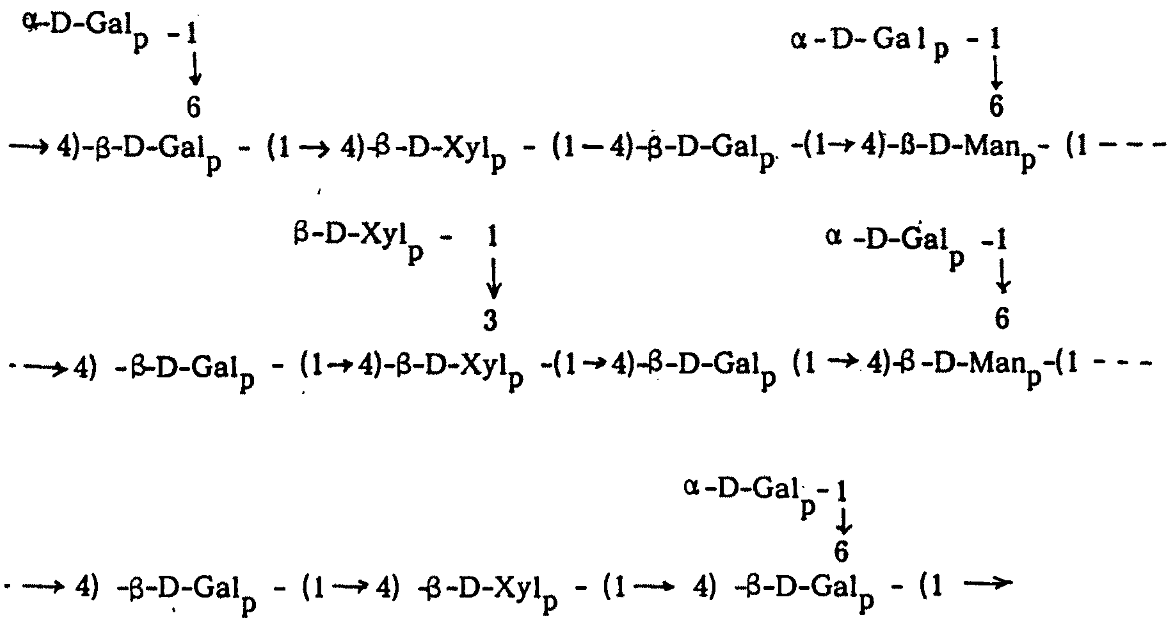
(29)

Xyl_p (1 → 3) Xyl_p

(30)

Hydrolysis of the fully methylated polysaccharide afforded 2,3-di-O-methyl-D-galactose, 2-O-methyl-D-xylose, 2,3-di-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-galactose, 2,3,4,6-tetra-O-methyl-D-galactose, 2,3-di-O-methyl-D-xylose and 2,3,4-tri-O-methyl-D-xylose in the molar proportion of 2:1:2:4:4:2:1. Periodate oxidation of the polysaccharide indicated 32.4% of end groups and the methylation studies indicated 31.2%. The foregoing data are consistent with the simplest repeating unit of the polysaccharide containing 16 sugar units of which 1 xylosyl and 4 galactosyl units (31.2% of the total repeating unit) are nonreducing terminal groups.

Based on the above results, it has been concluded that the main chain of the polysaccharide consists of (1⟶4)-linked residues of β-D-galactose, β-D-mannose and β-D-xylose to which α-D-galactosyl groups and β-D-xylosyl group are attached as branch points by (1⟶6) and (1⟶3) linkages, respectively. The periodate oxidation of polysaccharide consumes 19.8 moles of periodate with simultaneous liberation of 4.95 moles of formic acid. The structure (31) tentatively suggested, agrees well with the results of periodate oxidation.



(31)

14. Ipomoea fistulosa (Syn. Ipomoea carnea)

The water soluble galactomannan[50] extracted from the seeds of Ipomoea fistulosa (Fam., Convolvulaceae), is composed of D-galactose and D-mannose in the molar proportion of 3:10. Graded hydrolysis of the galactomannan yielded five oligosaccharides, having the structures (1-4 & 32).

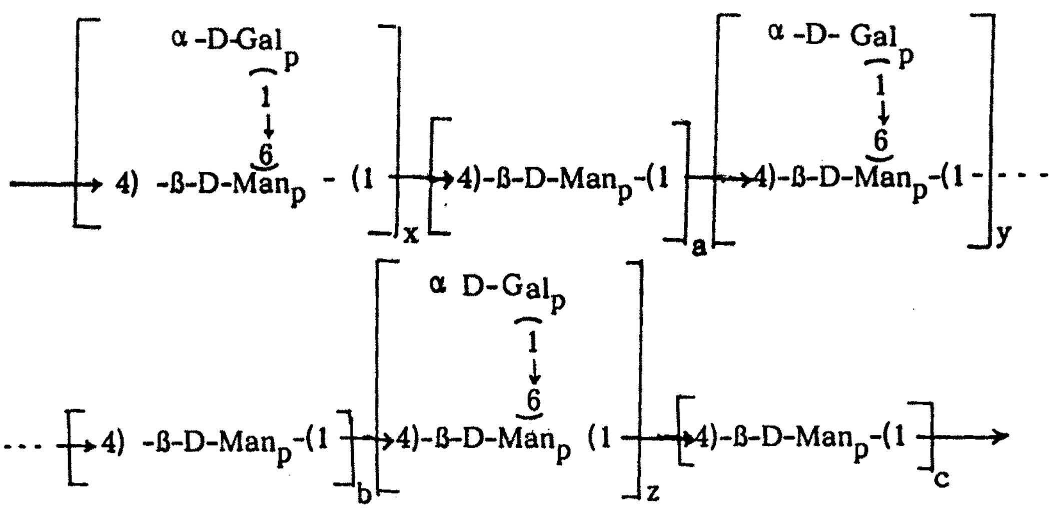
Mannobiose	(1)
Epimelibiose	(2)
Galactosyl mannobiose	(3)
Mannotriose	(4)
Mannotetrose	(32)

$$\{\beta\text{-D-Man}_p - (1 \rightarrow [4) - \beta\text{-D-Man}_p - (1]_2 \rightarrow 4) - \text{D- Man}_p\}$$

(32)

The fully methylated polymer, on acid hydrolysis, furnished 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in the molar proportion of 3:7:3. Periodate oxidation of the polysaccharide and subsequent estimation of formic acid liberated corresponded to 22.1% of hexosyl end groups. From the foregoing results, it is postulated that the galactomannan consists of a linear chain of (1→4)-linked D-mannopyranosyl residues to which D-galactopyranosyl groups are attached by (1→6)-linkages.

To accomodate all these results, the following tentative structure (33) has been suggested for the galactomannan of <u>Ipomoea fistulosa</u>.



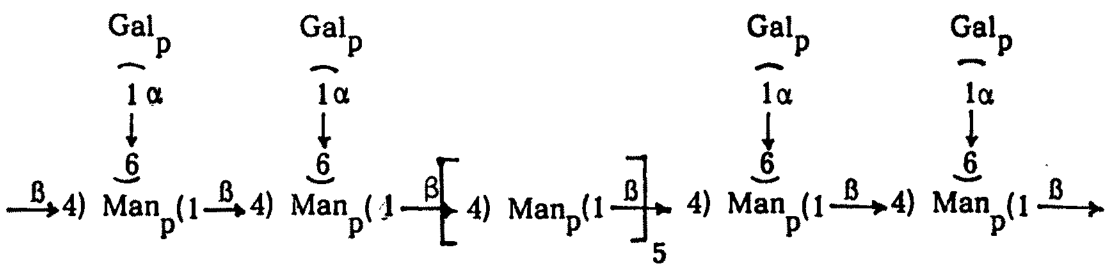
x + y + z = 3, a + b + c = 7 and possibly a = b = 3 and c = 1

(33)

15. Sesbania speciosa

Purified galactomannan[52] from the seeds of Sesbania speciosa (Fam., Leguminoseae; subfam., Papilionaceae), was shown to be composed of D-galactose and D-mannose in a molar ratio of 1:2.2. The hydrolysis of the fully methylated polysaccharide furnished 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 a molar ratio of 1:1:1. On Smith's degradation of periodate-oxidised material, it yielded glycerol and erythritol in a molar proportion of 1:2.06.

Based on methylation, periodate oxidation and Smith's degradation studies, the structure (34) has been advanced for Sesbania speciosa seed galactomannan.



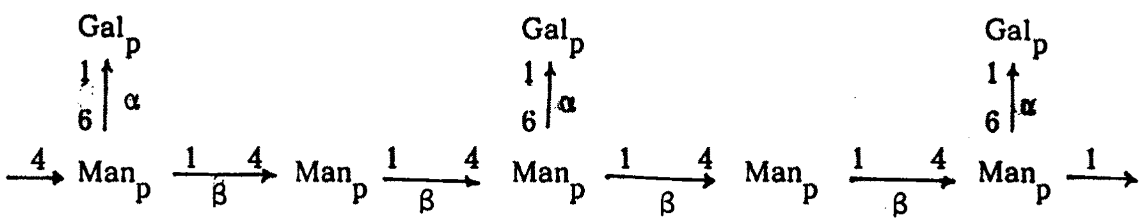
(34)

16. Sesbania aegyptiaca

A water-soluble galactomannan[53] isolated from the seeds of Sesbania aegyptiaca (Fam., Leguminoseae; subfam., Papilionaceae), contains D-galactose and D-mannose in the molar ratio of ca 1:1.67. Controlled acid hydrolysis of the galactomannan afforded four oligosaccharides (1-4)

4-O-β-D-mannopyranosyl-D-mannose	(1)
6-O-α-D-galactopyranosyl-D-mannose	(2)
6-O-α-D-galactopyranosyl-4-O-β-D-mannopyranosyl-D-mannose	(3)
4-O-β-D-mannopyranosyl-4-O-β-D-mannopyranosyl-D-mannose	(4)

The fully methylated polysaccharide, on acid hydrolysis, gave 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 proportion of 3:2:3. Periodate oxidation of the polysaccharide, followed by reduction and hydrolysis, furnished glycerol and erythritol in a molar ratio of 1:1.6. Based on these results, structure (35) has been proposed for Sesbania aegyptiaca seed galactomannan.



(35)

17. Cassia renigera

Barium complex of the galactomannan[55] isolated from the seeds of Cassia renigera (Fam.,Leguminoseae), on fractionation with acetic acid gave rise to two polysaccharides (A) and (B). Polysaccharide (A) is a major fraction from Barium complex of the polymer, soluble in acetic acid and composed of D-galactose, D-mannose and xylose in the molar ratio of 1:2.6 : 0.07. Though they have not suggested any structure for the polysaccharide, hydrolysis of the fully methylated polysaccharide furnished 2,3,6-tri-O-methyl-D-mannose (3.2 moles), 2,3-di-O-methyl-D-mannose (2 moles), and 2,3,4,6-tetra-O-methyl-D-galactose (2.12 moles), together with traces of 2,3,4,6-tetra-O-methyl-D-mannose. The tetra-O-methyl-D-galactose indicates a terminal, nonreducing D-galactopyranosyl group. As 2,3,6-tri-O-methyl-D-mannose is a major component, the main chain must be composed of (1$\rightarrow$4)-linked D-mannopyranose residues. The occurrence of 2,3-di-O-methyl-D-mannose suggests branching at either O-4 or O-6 of the main chain. Traces of tetra-O-methyl-D-mannose indicate that some mannose groups also occupy terminal, non-reducing positions. Upon periodate oxidation, the polysaccharide consumed 1.32 moles of periodate with the liberation of 0.26 mole of formic acid. On Smith's degradation of periodate-oxidised material, it yielded glycerol (2 moles) and erythritol (4.88 moles) and traces of mannose residues that had escaped periodate oxidation.

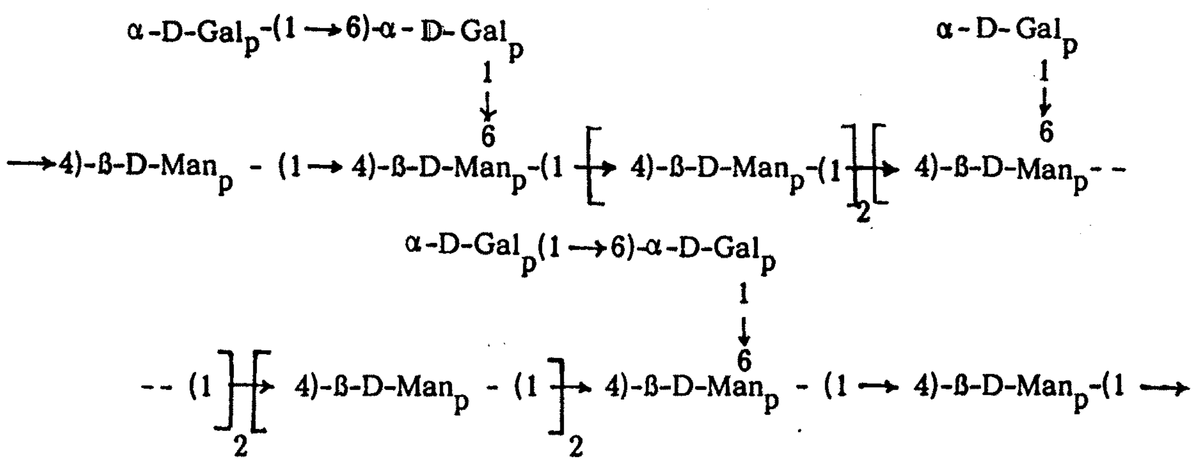
Gupta and coworkers[56] have also investigated the structure of C.renigera seed polysaccharide. The galactomannan from the seeds of C. renigera, purified by complex formation with Fehling solution was shown to be composed of D-galactose and D-mannose in the molar ratio of 3:5 which differs from the ratio reported earlier[55]. The partial acid hydrolysis of the galactomannan afforded five oligosaccharides having structures (1-4, 28 and 36).

Mannosylmannose	(1)
Galactosyl-mannose	(2)
Galactosylgalactose	(28)
Galactosylmannobiose	(3)
Mannotriose	(4)
Galactosyl epimelibiose	(36)

$$\alpha\text{-D-Gal}_p - (1\rightarrow 6) - \alpha\text{-D-Gal}_p - (1\rightarrow 6)\text{-D-Man}_p$$

(36)

Fully methylated polysaccharide, on acid hydrolysis gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose and 2,3,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in the molar proportion of 4:2:6:4. From the results of all these studies, structure (37) has been advanced for C. renigera seed galactomannan.



(37)

It may be mentioned here that the structure (37) differs from the structural features as reported earlier[55], not only in D-galactose-D-mannose ratio, but also due to presence of a galactobiose moiety not reported by Seth et al.[55].

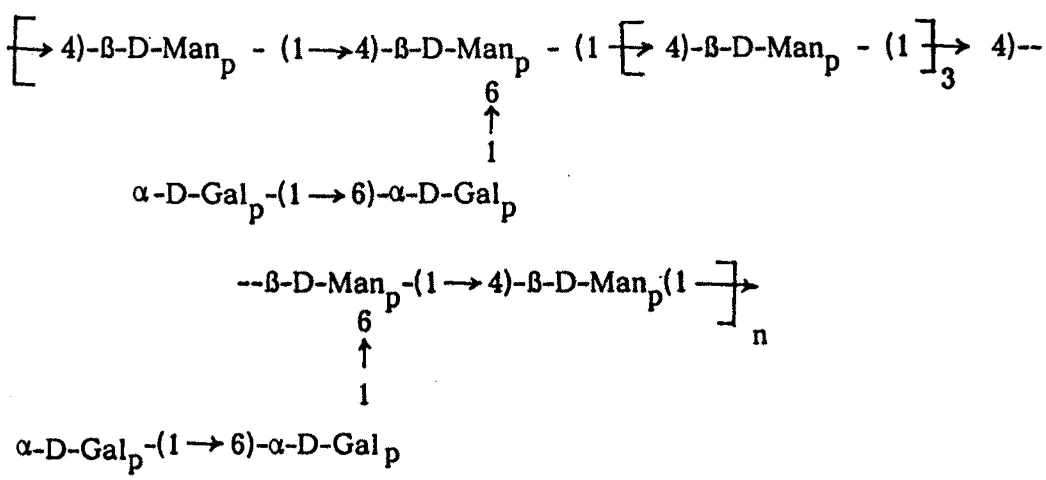
18. Cassia corymbosa

The galactomannan[54] extracted from the seeds of Cassia corymbosa (Fam., Leguminoseae) with cold, acidulated water, is made up of D-galactose and D-mannose in the molar ratio of 4:7. The polysaccharide which was subjected to a graded hydrolysis yielded five oligosaccharides, having the structures (1-2, 28, 4 and 36).

Mannobiose	(1)
Epimelibiose	(2)
Swietenose	(28)
Mannotriose	(4)
Galactosyl epimelibiose	(36)

Fully methylated compound, on acid hydrolysis gave 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in the molar proportion of 2:2:5:2. Determination of end (terminal) groups by titration of the amount of formic acid liberated during oxidation of the polysaccharide by periodate, gave indication of the presence of 36.4% terminal hexose residues which is in agreement with the value calculated from methylation results.

To accomodate all these results, the following structure (38) has been proposed for the galactomannan of <u>Cassia corymbosa</u>.



(38)

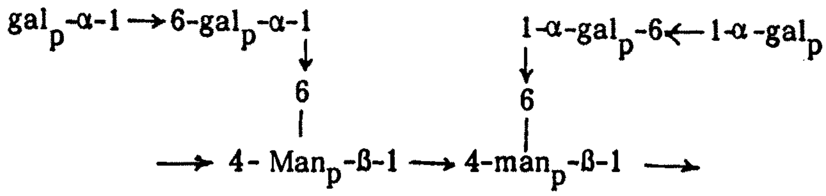
19. Cassia laevigata

A water soluble galactomannan[57] isolated from the seeds of Cassia laevigata (Fam., Leguminoseae) contains D-galactose and D-mannose in the molar proportion of 2:1. Acid catalysed partial hydrolysis of the galactomannan furnished four oligosaccharides having structures (1-2, 28 and 36).

Mannobiose	(1)
Epimelibiose	(2)
Galactobiose/Swietenose	(28)
Galactosyl epimelibiose	(36)

Hydrolysis of the fully methylated polysaccharide afforded 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose and 2,3-di-O-methyl-D-mannose in equimolar proportion. The occurrence of 2,3,4-tri-O-methyl-D-galactose indicates the presence of short chains of -D-(1 ⟶ 6)-linked-D-galactosyl residues attached to the main chain. Periodate oxidation of the polysaccharide liberated 0.413 mole of formic acid per 100 g of polysaccharide, which indicated 66.9% end group per repeating unit, suported by methylation result 66.6%. Borohydride reduction of the periodate-oxidized polysaccharide followed by acid hydrolysis gave glycerol and erythritol. On the basis of above facts, the repeating unit of the polysaccharide consists of six monosaccharide units out of which all the four galactose units occupy as end group and two mannose units themselves form the main chain.

To accommodate all the data, the structure (39) has been suggested for the repeating unit of C. laevigata seed galactomannan.



(39)

20. Teramnus labialis

A neutral galactomannan[58] occurring in the seeds of Teramnus labialis (Fam. Leguminoseae), is composed of D-galactose (2 moles) and D-mannose (3 moles). Partial acid hydrolysis of the polysaccharide afforded four oligosaccharides, having structures (1-4).

Mannobiose	(1)
Epimelibiose	(2)
Galactosylmannobiose	(3)
Mannotriose	(4)

Methylation analysis of the polysaccharide showed the presence of 2,3,4,6-tetra-O-methyl-D-galactose (2 moles), 2,3,6-tri-O-methyl-D-mannose (1 mole) and 2,3-di-O-methyl-D-mannose (2 moles). Periodate oxidation of the polysaccharide and subsequent estimation of formic acid liberated corresponded to 39.8% hexosyl end groups, as supported by methylation results 40%. The oxopolysaccharide, on reduction and hydrolysis, furnished glycerol and erythritol.

Based on the above results, a structure (40) consisting of two galactose units as nonreducing end-groups and three mannose residues in the main chain has been suggested for the galactomannan of T. labialis.

```
           α-D-Galp                                  α-D-Galp
              1                                         1
              ↓                                         ↓
              6                                         6
→ 4)-β-D-Manp-(1 → 4)-β-D-Manp - (1 → 4)-β-D-Manp-(1 →
```

(40)

21. Melilotus indica All

A D-galacto-D-mannan[59] isolated from the seeds of Melilotus indica All., syn. M. parviflora Desf. (Fam., Leguminoseae) was shown to be composed of D-galactose and D-mannose in the molar ratio of 1:1.14. Partial acid hydrolysis of the polysaccharide yielded five oligosaccharides having structures (41, 1-2, 42 and 3).

O-β-D-mannopyranosyl-(1 → 2)-D-mannopyranose	(41)
O-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose	(1)
O-α-D-galactopyranosyl-(1 → 6)-D-mannopyranose	(2)
O-α-D-galactopyranosyl-(1 → 4)-D-galactopyranose	(42)
O-α-D-galactopyranosyl-(1 → 6)-O-β-D-mannopyranosyl-(1 → 4)-D-mannopyranose	(3)

Man_p (1 → 2) Man_p

(41)

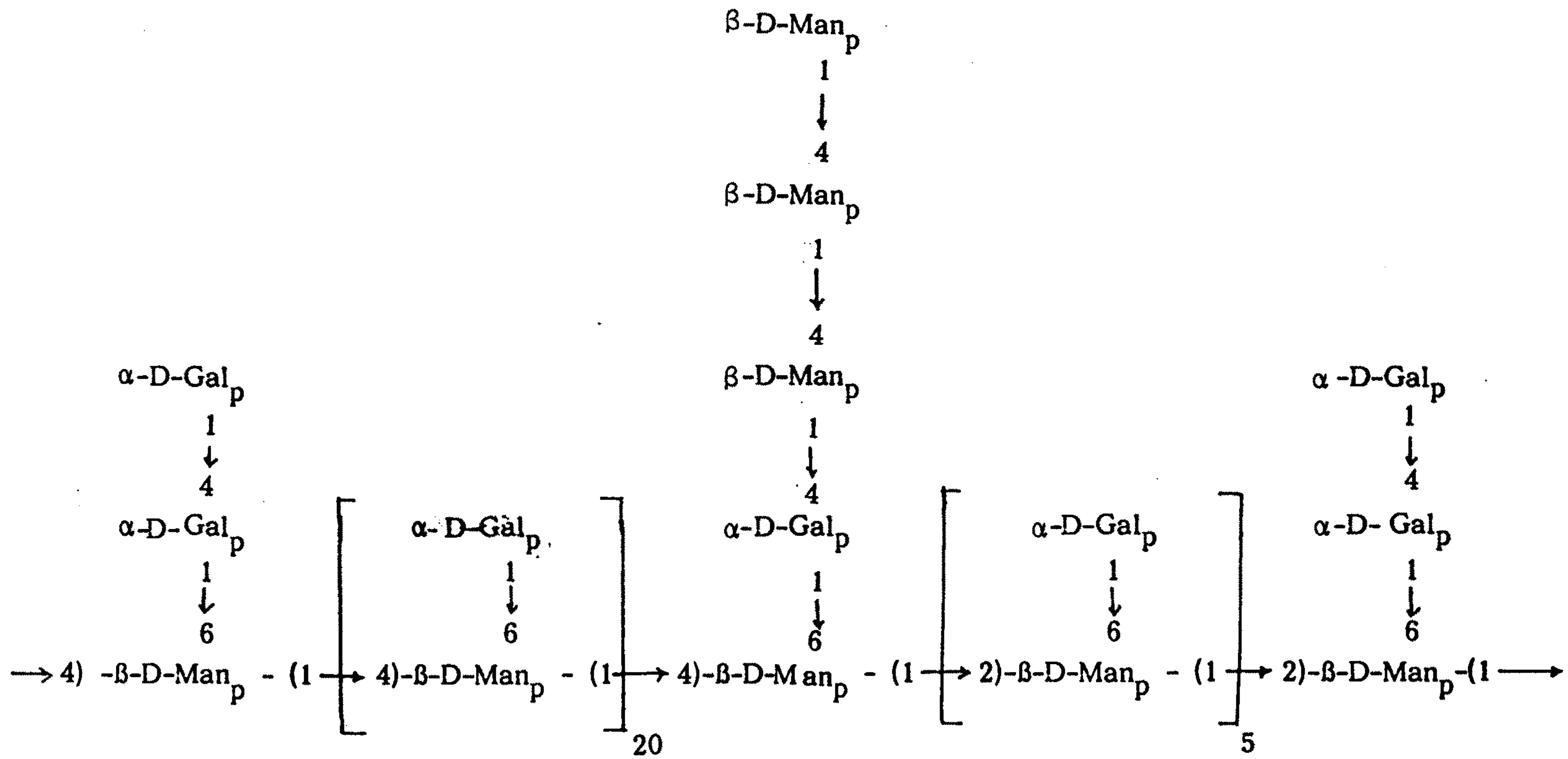
Methylation of the galactomannan, followed by hydrolysis, afforded 2,3,4,6-tetra-, 2,3,6-tri-, 2,3-di-, and 3,4-di-O-methyl-D-mannose, and 2,3,4, 6-tetra- and 2,3,6-tri-O-methyl-D-galactose in the molar proportion of 1:2:22:6:27:3. Upon periodate oxidation, the galactomannan consumed 1.44 moles of sodium metaperiodate, with concomitant liberation of 0.43 mole of formic acid, per "anhydrohexose" unit. The periodate-oxidised polysaccharide on Smith's degradation gave erythritol and glycerol in the molar ratio of 1:1.24. None of the augar units in the galactomannan survived periodate oxidation.

Based on the above findings, a tentative structure (43) showing one of the possible arrangements of the sugar residues was assigned for the average repeating unit of the galactomannan. The molecular weight of the compound shows that the repeating unit (consists of 61 hexosyl residues) is repeated five times in the full structure of M. indica galactomannan.

The characteristic features of M. indica galactomannan, as shown in proposed structure (43), indicate a mannan backbone composed of 36% of (1 ⟶ 4) - and 10% of (1 ⟶ 2)-linked ß-D-mannopyranosyl residues. Infrequent, short chains of (1 ⟶ 4)-linked α-D-galactopyranosyl residues (composed of two galactose units) are attached to the D-mannan backbone, in addition to single α-D-galactopyranosyl groups attached by an (1 ⟶ 6) linkages. The galactomannan also carries one short chain of (1 ⟶ 4)-linked ß-D-mannopyranosyl units (consisting of three mannose residues) attached at 0-4 of an α-D-galactosyl residue.

22. Indigofera tinctoria Linn.

The polysaccharide[60] extracted with hot water, from the seeds of Indigofera tinctoria (Fam., Leguminoseae), purified by gel-permeation chromatography on Sephadex G-200, has been reported to be composed of D-galactose and D-mannose in a molar ratio of 1:1.52. Graded hydrolysis of the polysaccharide gave four oligosaccharides in homogeneous form as separated by paper chromatography; whose structures (1-4) have been deduced from their physical properties, hydrolytic pattern and also from the results of methylation. A mixture of tetrasaccharides was also obtained during graded hydrolysis of the polymer, but they could not be separated in homogeneous form to characterize properly.



(43)

4-O-β-D-mannopyranosyl-D-mannose	(1)
6-O-α-D-galactopyranosyl-D-mannose	(2)
4-O-(6-O-α-D-galactopyranosyl-β-D-manno-pyranosyl)-D-mannose	(3)
4-O-(4-O-β-D-mannopyranosyl-β-D-manno-pyranosyl)-D-mannose	(4)

Hydrolysis of the fully methylated polysaccharide furnished 2,3,4,6-tetra-O-methyl-mannose (in traces), 2,3,4,6-tetra-O-methyl-galactose (2 moles), 2,3,6-tri-O-methyl-mannose (1 mole) and 2,3-di-O-methyl-mannose (2 moles). Periodate oxidation of the polysaccharide consumed 0.84 mol of periodate per mol of hexose residues. The periodate oxidised polysaccharide, on reduction followed by hydrolysis, yielded glycerol and mannose. These results show that only galactose was oxidized by periodate and almost all the mannose protected, probably by hemiacetal formation. Further, periodate oxidation of the polyol consumed 0.86 mol of periodate per mol of hexose residues. This led to the conclusion that ∿ 90% of total mannose units were oxidised.

Based on the above results, it was concluded that the galactomannan consists a β-(1 ⟶ 4)-linked D-mannose backbone with 67% of the mannose units being substituted by single α-D-galactose units through O-6. The first periodate oxidation indicated the protection of almost all of the mannose residues by hemiacetal formation, which reveals that the galacto-pyranosyl residues are linked to O-6 of mannopyranosyl residues randomly rather than in blocks. They have not suggested any particular structure for I. tinctoria galactomannan.

23. Cassia sophera

The galactomannan[61] extracted from the seeds of Cassia sophera (Fam., Leguminoseae) with cold, acidulated water is composed of D-galactose and D-mannose in the molar proportion of 1:3. Partial acid hydrolysis of the polysaccharide afforded three oligosaccharides (1-2 and 4).

Mannobiose	(1)
Epimelibiose	(2)
Mannotriose	(4)

Fully methylated compound, on acid hydrolysis gave 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 1:2:1. Periodate oxidation of the polysaccharide with liberation of formic acid indicated 25.2% of end groups and the methylation studies indicated 25% of end groups. The oxopolysaccharide (obtained after 84h) showed the absence of D-galactose but a small proportion of D-mannose was detected. Prolonged oxidation of the polysaccharide (for 96h) decomposed both the hexoses. These results indicated the presence of D-galactopyranosyl end groups and these protected some of the D-mannopyranosyl units from oxidation.

Based on the above data, a following tentative structure (44) has been suggested for the repeating unit of C. sophera seed galactomannan.

$$-\left[\rightarrow 4)\text{-}\beta\text{-D-Man}_p\text{-}(1-\left[\rightarrow 4)\text{-}\beta\text{-D-Man}_p\text{-}(1-\right]_2 \rightarrow\right]_n$$

6
↑
1
α-D-Gal$_p$

(44)

24. Cassia alata

A galactomannan[64] extracted with water at 40-50° from the seeds of Cassia alata (Fam., Leguminoseae), has been shown to be composed of D-galactose (26.6%) and D-mannose (71.8%). The fully methylated polysaccharide, on acid hydrolysis, gave 2,3,4,6-tetra-O-methyl-galactose, 2,3,6-tri-O-methyl-mannose and 2,3-di-O-methyl-mannose in the molar ratio of 0.89:1.50:1.00. Periodate-oxidised polysaccharide (obtained after 100h, 140h and 236h) on Smith's degradation showed the absence of galactose but survival of 46%, 20% and 15% residual mannose, respectively in the polymer. These results indicated that periodate oxidation of the backbone was hindered by the side chains. Methylation analysis of the periodate-oxidised (as obtained after 236h) and borohydride-reduced polysaccharide gave 2,3,6-tri- and 2,3-di-O-methyl mannoses, and showed that, not only the branch-point mannose but also some unsubstituted mannose escaped oxidation. The release of glycerol and erythritol on acid hydrolysis of the polyalcohol corroborates the results of methylation studies.

^{13}C-n.m.r. spectrum of the polysaccharide clearly differentiated the three structural units of the polymer, namely, O-6-substituted and unsubstituted internal (1 → 4) linked ß-D-mannopyranosyl units of the backbone and terminal (non-reducing) α-D-galactopyranosyl units. Though they have neither suggested any structure for the polysaccharide nor determined the distribution of the D-galactose residues in the side chains, the above findings concluded that C. alata seed galactomannan consists of a backbone of (1 → 4)-linked ß-D-mannopyranosyl residues to which α-D-galactopyranosyl groups are attached by (1 → 6)-linkages (one D-galactose residue per ∿ 2.5 D-mannose residues).

Sen et al.[65] have also studied the structure of galactomannan, isolated from hot water extract of <u>Cassia alata</u> seeds at 80°. It was composed of D-galactose and D-mannose in the molar ratio of 1:3.3 which is slightly higher than the value reported earlier[64]. On partial acid hydrolysis, it afforded five oligosaccharides having structures (1-4 and 32).

4-O-β-D-mannopyranosyl-D-mannose	(1)
6-O-α-D-galactopyranosyl-D-mannose	(2)
4-O-(6-O-α-D-galactopyranosyl-β-D-mannopyranosyl)-D-mannose	(3)
4-O-(4-O-β-D-mannopyranosyl-β-D-mannopyranosyl)-D-mannose	(4)
4-O-(4-O-β-D-mannopyranosyl-β-D-mannopyranosyl-β-D-mannopyranosyl)-D-mannose	(32)

$$Man_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Man_p\ (1 \rightarrow 4)\ Man_p$$

(32)

Methylation analysis of the polysaccharide showed the presence of 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 proportion of 1:2.3:1. Periodate oxidation of the polysaccharide (for 70h) consumed 0.9 mol of periodate of per mole of anhydrohexose. This oxidized product, on reduction followed by hydrolysis, revealed glycerol, erythritol and mannose as quantitatively analysed. These results concluded that all the galactose and 54.2% of total mannose were oxidized by periodate. The remaining mannopyranosyl units escaped periodate oxidation due to hemiacetal formation. A second periodate oxidation, on the polyol in the similar manner, oxidized all the remaining 45.8% mannose residues. Though they have also not suggested any structure for the galactomannan, from the above results, it was concluded that C. alata seed galactomannan had a ß-(1 → 4) linked D-mannan backbone with 23% of the mannopyranosyl residues being substituted by single α-D-galactopyranosyl groups through O-6.

25. Melilotus officinalis Lam

The galactomannan[66] isolated from the seeds of Melilotus officinalis (Fam., Leguminoseae) contains D-galactose and D-mannose in the molar ratio of 1.00 : 1.09. Graded hydrolysis of the polysaccharide furnished four oligosaccharides having structures (1-4).

O-β-D-mannopyranosyl- (1 → 4)-D-mannose (1)

O-α-D-galactopyranosyl- (1 → 6)-D-mannose (2)

O-α-D-galactopyranosyl- (1 → 6)-O-β-D-mannopyranosyl - (1 → 4)-D-mannose (3)

O-β-D-mannopyranosyl- (1 → 4)-O-β-D-mannopyranosyl - (1 → 4)-D-mannose (4)

Hydrolysis of the permethylated polysaccharide furnished 2,3,4,6-tetra-O-methyl-D-galactose (11.4 moles), 2,3,6-tri-O-methyl-D-mannose (1.0 mole) and 2,3,-di-O-methyl-D-mannose (11.2 moles). Upon periodate oxidation, the polysaccharide consumed 1.46 moles of sodium metaperiodate per mole, with the liberation of 465 mmole of formic acid for hexosyl unit. The hydrolysis of polyalcohol obtained by reduction of the periodate-oxidised polysaccharide, revealed the presence of glycerol (1.00 mole) and erythritol (1.09 moles). ^{13}C-n.m.r. spectrum of the polymer clearly differentiated and identified the unsubstituted (1 → 4)-linked β-D-mannopyranosyl units of the mannan backbone, as well as dominating the 6-O-substituted, (1 → 4)-linked β-D-mannopyranosyl units. The results of graded hydrolysis, periodate oxidation and n.m.r. analysis supported the methylation analysis of the polysaccharide.

The foregoing findings suggested that the structure of M. officinalis seed galactomannan is similar to that from Fenugreek seed and can be representated by the structure (5) where y = 1 and x + z = 9, for its average repeating unit.

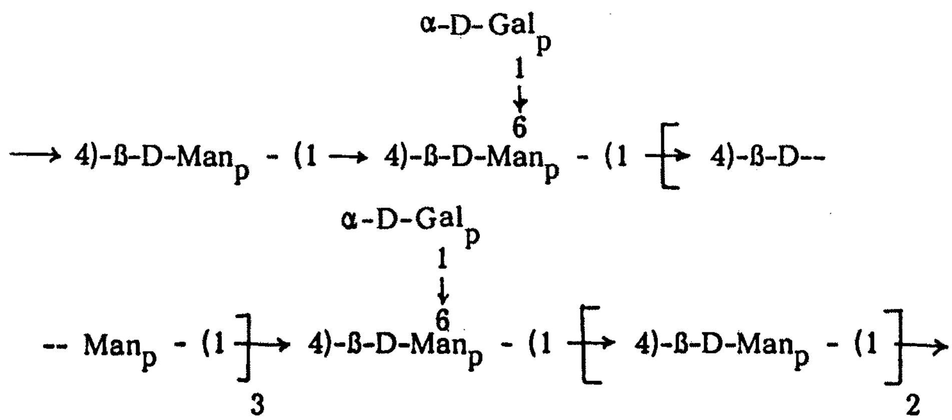
26. Crotalaria verrucosa

A neutral galactomannan[67] occurring in the seeds of Crotalaria verrucosa (Fam., Leguminoseae) is composed of D-galactose and D-mannose in the molar ratio of 1:4. Partial acid hydrolysis of the polysaccharide yielded five oligosaccharides having structures (1-4 and 32).

Mannobiose	(1)
Epimelibiose	(2)
Galactosyl mannobiose	(3)
Mannotriose	(4)
Mannotetrose	(32)

The fully methylated polysaccharide, on acid hydrolysis gave 2,3,4,6-tetra-O-methyl-D-galactose (2 mol%), 2,3,6-tri-O-methyl-D-mannose (6 mol %) and 2,3-di-O-methyl-D-mannose (2 mol%). Periodate oxidation of the polysaccharide and subsequent estimation of formic acid liberated corresponded to ∿ 20% hexosyl end groups per repeating unit, as supported by methylation results. The oxopolysaccharide, on reduction and hydrolysis, furnished glycerol and erythritol.

Based on the above findings, a tentative structure (45) has been assigned to the statistical units of the galactomannan from C. verrucosa seeds.



(45)

27. Cassia siamea Lam.

The galactomannan[68] from the seeds of Cassia siamea (Fam., Leguminoseae; Subfam., Caesalpinioideae) has been shown to be a neutral polysaccharide containing D-galactose and D-mannose in a molar ratio of 1:3.07. The hydrolysis of the fully methylated compound gave 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 a molar ratio of 1.0:1.97:1.1. Upon oxidation with sodium metaperiodate, the polysaccharide consumed 1.26 moles of oxidant with the concomitant liberation of 0.25 mole of formic acid per mole of hexose unit. On Smith's degradation of periodate-oxidised material, it yielded glycerol and erythritol in a molar proportion of 1.0:3.05. None of monosaccharides survived the oxidation.

Based on the results of methylation and periodate oxidation studies, Khan and other researchers have proposed the structure (18) for C. siamea seed galactomannan is similar to that from Cassia occidentalis seeds.

28) Sesbania bispinosa

A neutral galactomannan[69] occurring in the seeds of Sesbania bispinosa (Jacq) W.F. White, syn. S. aculeata Pers. (Fam., Leguminoseae; Subfam., Papilionoideae) is composed of D-galactose and D-mannose in the molar proportion of 1:1.9.

Methylation of the galactomannan, followed by hydrolysis afforded 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 1.0:0.8:1.0. From methylation results it was proposed that the repeating unit of the galactomannan consisted of three hexose units only, out of which two (1 → 4)-linked mannose units constitute the main chain. The remaining (1 → 6)-linked galactose unit occurs as a side chain. ^{13}C-n.m.r. and X-ray diffraction of the polymer also supported the linkage pattern as suggested from the methylation results.

Based on the above findings, a structure (8) was proposed for the repeating unit of S. bispinosa seed galactomannan which is similar to that (suggested as one the possible structures) from Sesbania grandiflora seeds. The periodate oxidation and Smith's degradation results are also in close agreement with those expected from the structure (8) as advanced for S. bispinosa seed galactomannan.

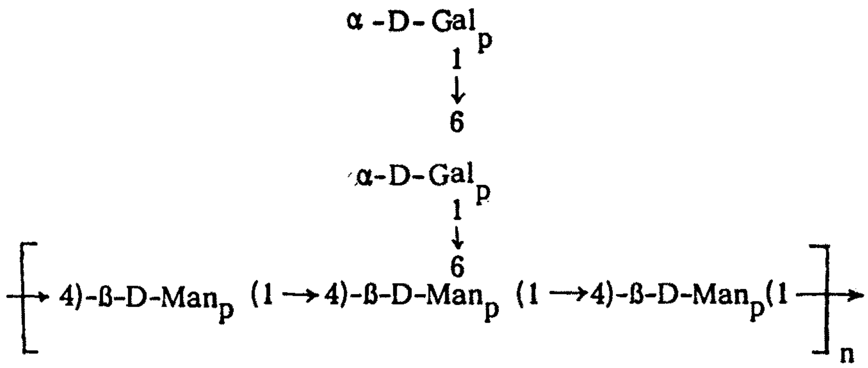
29. Ipomoea palmata

A water soluble galactomannan[70] extracted from the seeds of Ipomoea palmata (Fam., Convolvulaceae) is composed of D-galactose and D-mannose in the molar ratio of 2:3. The polysaccharide was subjected to a graded hydrolysis which yielded five oligosaccharides having the structures (1-2, 28, 4 and 36).

Mannobiose	(1)
Epimelibiose	(2)
Swietenose	(28)
Mannotriose	(4)
Galactosylepimelibiose	(36)

Fully methylated compound, on acid hydrolysis furnished 2,3,4,6-tetra-O-methyl-D-galactose, 2,3,4-tri-O-methyl-D-galactose, 2,3,6-tri-O-methyl-D-mannose and 2,3-di-O-methyl-D-mannose in the molar proportion of 1:1:2:1. The occurrence of 2,3,4-tri-O-methyl-D-galactose indicates the presence of infrequent, short chains of α-D-(1 $\rightarrow$ 6)-linked-D-galactosyl residues attached to the main chain. Isolation of 2,3,6-tri-O-methyl-D-mannose as a major component clearly indicated that the main chain was composed of (1 $\rightarrow$ 4)-linked mannose units. The branched character of the polysaccharide followed from the isolation of 2,3-di-O-methyl-D-mannose which is an indicative of mannose unit linked through C_1, C_4 and C_6. Periodate oxidation of the polysaccharide and subsequent estimation of formic acid liberated corresponded to 39.7% hexosyl end groups which are in close agreement with the value calculated from methylation results.

To accomodate all these results, the following structure (46) has been suggested for the galactomannan of Ipomoea palmata.



(46)

30. Crotalaria medicaginea

A galactomannan[86] occurring in the seeds of Crotalaria medicaginea (Fam., Leguminoseae) contains D-galactose and D-mannose in the molar ratio of 1:3.1. Partial acid hydrolysis of the polysaccharide yielded five oligosaccharides (1, 47 and 2-4) which are characterised as follows.

4-O-β-D-mannopyranosyl-D-mannose (1)

3-O-α-D-galactofuranosyl-D-mannose (47)

6-O-α-D-galactopyranosyl-D-mannose (2)

4-O-(6-O-α-D-galactopyranosyl-β-D-mannopyranosyl)-D-mannose (3)

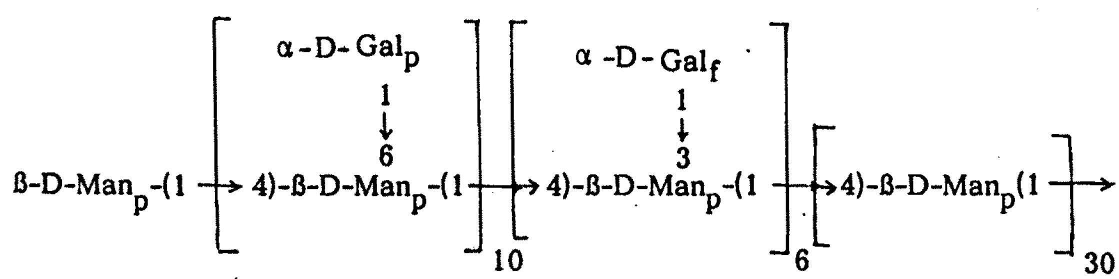
4-O-(4-O-β-D-mannopyranosyl-β-D-mannopyranosyl)-D-mannose (6)

$$Gal_f\ (1 \longrightarrow 3)\ Man_p$$

(47)

Methylation of the galactomannan, followed by hydrolysis afforded 2,3,4,6-tetra-O-methyl-mannose, 2,3,5,6-tetra-O-methyl-galactose, 2,3,4,6-tetra-O-methyl-galactose, 2,3,6-tri-O-methyl-mannose, 2,6-di-O-methyl-mannose and 2,3-di-O-methyl-mannose in the molar proportion of 1.00:6.00:10.20:30.06:6.06:9.96. On the basis of methylation studies, it has been concluded that the heteroglycan is a branched galactomannan, containing a basic chain of (1→4)-linked mannose residues. The terminal, non-reducing groups are mannopyranose, galactofuranose and galactopyranose residues. On the basis of ^{13}C-n.m.r. spectral data, mild hydrolysis with oxalic acid and chromic oxide oxidation of the polymer, the galactofuranose and galacto pyranose residues are assigned the α-D-configuration whereas the manno-pyranosyl residues appear to be of the ß-D-type.

Based upon the above findings, the structure (48) proposed for C. medicaginea galactomannan is unique due to the presence of (1→3)-linked α-D-Gal_f units.



(48)

31. Cassia ovata

A water-soluble galactomannan[71] having a D-galactose-D-mannose ratio of 2:5, has been isolated from the seeds of Cassia ovata (Fam., Leguminoseae). Graded hydrolysis of the polysaccharide gave four oligosaccharides (1-4).

Mannobiose (1)

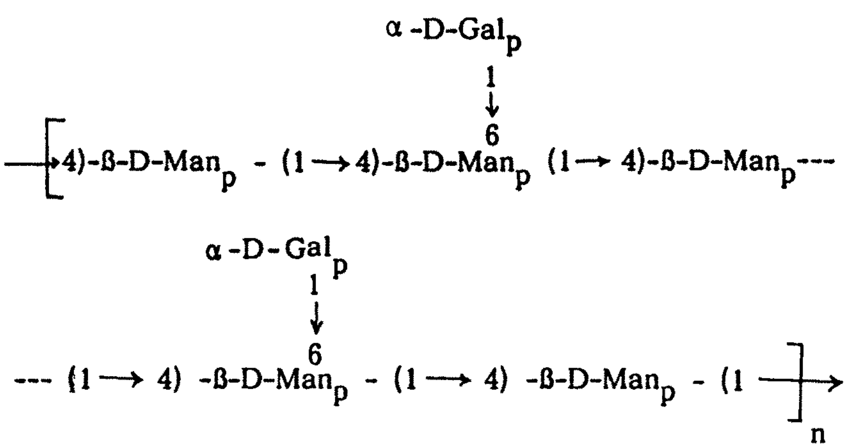
Epimelibiose (2)

Galactosyl-mannobiose (3)

Mannotriose (4)

Hydrolysis of the permethylated polysaccharide gave 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 2:3:2. Periodate oxidation of the polysaccharide indicated 28.7% of end groups which was supported by methylation study. The foregoing data is consistent with the simplest repeating unit of the polysaccharide containing 7 sugar units of which 2 galactosyl units (28.6% of total repeating unit) are non-reducing terminal groups.

Based on the above results, it has been concluded that the main chain of the polysaccharide consists of (1 → 4)-linked residues of ß-D-manno pyranosyl units to which α-D-galactosyl groups are attached as branch points by (1 → 6) linkages. The structure (48) tentatively suggested for repeating unit of <u>C. ovata</u> galactomannan, agrees well with the results of graded hydrolysis, methylation, and periodate oxidation.



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REFERENCES

PART I

REFERENCES

1. Danishefsky, I., Whistler, R.L. and Bettleheim, F.A., "The Carbohydrate", (Eds) Pigman, W. and Horton, D., (Academic Press Inc., New York), 2A (1970), 377.

2. Nadkarni, A.K., "Indian Materia Medica", (1954).

3. Tipson, R.S. and Horton, D., "Advances in Carbohydrate Chemistry and Biochemistry". 32 (1976), 258-59.

4. Scheicher, H., Chem. Abs., 89 (1978), 135886b.

5. Tipson, R.S. and Horton, D., "Advances in Carbohydrate Chemistry and Biochemistry", 31 (1975), 309-12.

6. Fukuka, K., Ogushi, Y., Otsuka, Y., Yasunori, Y., Chem. Abs., 89 (1978), 151570z.

7. Mukherjee, S. and Srivastava, H.C., Curr. Sci., 20 (1951), 127.

8. Mukherjee, S. and Srivastava, H.C., J.Am.Chem.Soc., 77 (1955), 422.

9. Mathur, G.P. and Mukherjee, S., J.Sci.Industr.Res., 11B (1952), 544; ibid 13B (1954), 452.

10. Gupta, A.K. and Mukherjee, S., Proc. 8th International Symposium on the Chemistry of Natural Products, New Delhi, (1972), 360.

11. Gupta, A.K. and Mukherjee, S., Indian J. Chem., 11(7) (1973), 648-52.

12. Mukherjee, S. and Srivastava, A.N., J. Sci. Industr. Res., 16B (1957), 566.

13. Mukherjee, S. and Srivastava, A.N., J. Am. Chem. Soc., 80 (1958), 2536.

14. Chaterjee, A.K. and Mukherjee, S., J. Am. Chem. Soc., 80 (1958), 2538.

15. Dhar, P.K. and Mukherjee, S., J. Sci. Industr. Res., 18B (1959), 219.

16. Bose, S., Mukherjee, S. and Banarjee, B.C., Sarkara, 3 (1960), 125.

17. Bose, S., Mukherjee, S. and Banarjee, B.C., Science and Culture, 27 (1961), 498-9.

18. Bose, S., Mody, N.N. and Mukherjee, S., Indian J. Chem., 1 (1963), 324.

19. Gupta, K.C. and Bose, S., Sci. and Cult., 29 (1963), 107-8.

20. Gupta, K.C. and Bose, S., Indian J. Chem., 2(2) (1964), 57-60.

21. Bose, S. and Dutta, A.S., J. Indian Chem. Soc., 40 (1963), 257-62; ibid, 42 (1965), 367-72.

22. Bose, S. and Biswas (Miss) M., Sci. and Cult., 32 (1966), 134-5.

23. Bose, S. and Biswas (Miss) M., Indian J. Biochem., 7(11)(1970), 68-72

24. Bajpai, K.S. and Mukherjee, S., Indian J. Chem., (1966), 545; ibid, 8(6) (1970), 490-2.

25. Bose, S. and Menon, P.A.A., J. Indian Chem. Soc., 44 (1967), 1092.

26. Bose, S. and Soni, P.L., J. Indian Chem. Soc., 48 (1971) 467; ibid, 49(6) (1972), 593.

27. Bose, S. and Soni, P.L., Indian J. Chem., 11(10) (1973), 996-99.

28. Srivastava, H.C., Singh, P.P. and Rao, P.V.S., Carbohydr. Res., 6 (1968), 361.

29. Kapoor, V.P. and Mukherjee, S., Curr. Sci., 38 (1969), 38.

30. Kapoor, V.P. and Mukherjee, S., Can. J. Chem., 47 (1969), 2883-7.

31. Kapoor, V.P. and Mukherjee, S., Phytochem., 10(2) (1971), 1955-9.

32. Kapoor, V.P. and Mukherjee, S., Indian J. Chem., 10(2) (1972), 155-8

33. Jindal, V.K. and Mukherjee, S., Curr. Sci., 38(9) (1969), 459.

34. Jindal, V.K. and Mukherjee, S., Indian J. Chem., 8(5) (1970), 417-9.

35. Kelker, P.S. and Mukherjee, S., Indian J. Chem., 9 (1971), 1085-7.

36. Lal, J. and Gupta, P.C., Planta. Med., 22 (1972), 71-7.

37. Gupta, D.S. and Mukherjee, S., Indian J. Chem., 11 (1973), 1134-7.

38. Gupta, D.S. and Mukherjee, S., Indian J. Chem., 13 (1975), 1152-4.

39. Tharanathan, R.N. and Anjaneyalu, Y.V., Indian J. Chem., 12 (1974), 1164-5.

40. Anjaneyalu, Y.V. and Gowda, D.C., Carbohydr. Res., 75 (1979), 251-6

41. Roy, A., Mukherjee, A.K. and Rao, C.V.N., Carbohydr. Res., 41 (1975), 219-26.

42. Roy, A., Mukherjee, A.K. and Rao, C.V.N., Carbohydr. Res., 54 (1977), 115-24.

43. Bose, S. and Srivastava, H.C., Indian J. Chem., 16B (1978), 966-9.

44. Bose, S. and Srivastava, H.C., J. Indian Chem. Soc., 56 (1978), 1216-8.

45. Bose, S. and Singh, L., Indian J. Chem., 18B(3) (1979), 222-5.

46. Bose, S. and Singh, L., Indian J. Chem., 18B(1) (1979), 59-61.

47. Dubey, P. and Gupta, P.C., Carbohydr. Res., 72 (1979), 151-5.

48. Rao, E.V. and Rao, M.V., Planta Med., 35(1), (1979), 66-70.

49. Gowda, D.C., Neelisiddaiah, B. and Anjaneyalu, Y.V., Carbohydr. Res., 72 (1979), 201-5.

50. Gupta, O.C.D., Gupta, R., Srivastava, V.P. and Gupta, P.C., Carbohydr. Res., 73 (1979), 145-50.

51. Bose, S. and Tomar, V.B.S., J. Indian Chem. Soc., 56 (1979), 647-8.

52. Rao, V.M., Rao, S.P.V. and Rao, V.E., Indian J. Chem., 19 B (1980), 48-50.

53. Bhattacharyya, S.B., Das, A.K., Banerji, N., and Farooqi, M.I.H., Phytochem., 22(1) (1983), 161-4.

54. Tiwari, K., Khare, N., Singh, V. and Gupta, P.C., Carbohydr. Res., 135(1) (1984), 141-6.

55. Seth, R.P., Mukherjee, S. and Verma, S.D., Carbohydr. Res., 125(2) (1984), 336-9.

56. Gupta, R., Khare, N., Singh, V. and Gupta, P.C., Carbohydr. Res., 159(2) (1987), 336-40.

57. Alam, N., Srivastava, A.K. and Gupta, P.C., J. Indian Chem. Soc., 62 (10) (1985), 768-70.

58. Alam, N. and Gupta, P.C., Carbohydr. Res., 153 (1986), 334-8.

59. Gupta, A.K. and Bose, S., Carbohydr. Res., 153 (1986), 69-77.

60. Sen, A.K., Banerjee, N. and Farooqi, M.I.H., Carbohydr. Res., 157 (1986), 251-6.

61. Khare, N., Singh, V., Gupta, M.K. and Gupta, P.C., J. Indian Chem. Soc., 63(7) (1986), 685-7.

62. Soni, S.K. and Bose, S., Indian J. Chem., 25B (1986), 123-6.

63. Muralikrishna, G., Salimath, P.V. and Tharanathan, R.N., Carbohydr. Res., 161 (1987), 265-71.

64. Gupta, D.S., John, B., Bajpai, K.S. and Sharma, S.C., Carbohydr. Res., 162(2) (1987), 271-6.

65. Sen, A.K., Sarkar, K.K., Banerjee, N. and Farooqi, M.I.H., Indian J. Chem. Sect. B., 26B(1) (1987), 21-5.

66. Gupta, A.K. and Grasdalen, H., Carbohydr. Res., 173 (1988), 159-68.

67. Gupta, R. and Gupta, P.C., Carbohydr. Res., 181 (1988), 287-92.

68. Khan, G., Kapoor, V.P., Farooqi, M.I.H., Sen, A.K. and Banerjee, N., Indian J. Chem. Sect. B., 27(B) (1988), 821-4.

69. Kapoor, V.P., Sen, A.K. and Farooqi, M.I.H., Indian J. Chem. Sect. B., 28(B)(11) (1989), 928-33.

70. Tiwari, R., Mishra, U.C., Mathur, N., Dixit, A.K. and Gupta, P.C., J. Indian Chem. Soc., 66(2) (1989), 128-30.

71. Kumar, P., Singh, V., Mishra, U.C. and Gupta, P.C., Carbohydr. Res., 198(2) (1990), 384-6.

72. Anderson, E., Ind. Eng. Chem., 41 (1949), 2887.

73. Farooqi, M.I.H., "A Search for New sources of Industrial Seed Gums" National Botanical Gardens, (C.S.I.R., Lucknow), (1975), 1-59.

74. The Wealth of India, "Raw Materials", 1 (1948), 31.

75. Hirst, E.L., Jones, J.K.N. and Walder, O.W., J. Chem., Soc., (1947), 1443.

76. Whistler, R.L. and McCredie, R.J., Dissertation Abstract, 19 (1958), 432.

77. Andrews, P., Hough, L. and Jones, J.K.N., J. Am. Chem. Soc., 74 (1952), 4029-32.

78. Henderson, M.E., Hough, L. and Painter, T.J., J. Chem. Soc.,(1958), 3519-22.

79. Raoud, K.M., Biochem. J., 26 (1932), 255.

80. Andrews, P., Hough, L. and Jones, J.K.N., J. Chem. Soc., (1952), 2744.

81. Smith, F. and Montgomery R., "The Chemistry of Plant Gums and Mucilages" (Reinhold Publishing Co., New York), 1959.

82. Horvei, F. and Wickstrom, A., Acta Chem. Scand.,18 (1964), 833-5.

83. Courtois, J.E. and Ledizet, P., Carbohydr. Res., 3 (1966),141-51.

84. Unrau, A.M. and Choy, Y.M., Carbohydr. Res., 14(2) (1970), 151-8.

85. Unrau, A.M. and Choy, Y.M., Can. J. Chem., 48(7) (1970), 1123-8.

86. Gupta, A.K. and BeMiller, J.N., Phytochem., 29(3) (1990), 853-5.

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