The Structure of a Galactomannan from *Lotus corniculatus* L.

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Galactomannans are common water-soluble constituents of the ungerminated seeds of leguminous plants. They occur in the endosperm of the seeds and are considered as reserve polysaccharides. The galactomannans from the comparatively few species so far examined [1] all appear to have some structural features in common: D-Mannopyranose residues are united through 1,4-β-linkages to form a chain to which single α-D-galactopyranosyl units are attached at position 6 of the mannose residues.

Galactomannans of an entirely different structure have recently been isolated from several Dermatophytes.[2-4] These fungal galactomannans may be described [4] as built up around a basic chain formed predominantly by 1,6-linked D-mannopyranose units. The D-galactose is exclusively present as non-reducing terminal furanose units, and some terminal D-mannopyranosyl groups also create branching points on the basic mannan chain.

Although comparatively few leguminous galactomannans (from about 12 species) have been examined, significant structural deviations from the general pattern have been observed. Thus, the galactomannan from *Gymnocladus dioica* (Kentucky Coffee Bean) apparently contains some terminal mannopyranosyl units together with the expected terminal galactopyranosyl groups.[5] From recent work [6] on the galactomannan of *Gleditschia ferox* Desf. results have emerged which tend to indicate that some of the galactose units form non-terminal groups linked through positions 1 and 6.

It is therefore of interest to examine galactomannans from several other leguminous plants in order to determine to which extent galactomannans of the Leguminoseae are of uniform structure.

The present paper reports the results of an examination of *Lotus corniculatus* L, a plant of some agricultural interest (replacing clover species). Galactomannans from some clover species of agricultural importance have been examined.[6-8]

Already in 1903 [9] the seeds of *Lotus corniculatus* were shown to contain a polysaccharide, which consisted of galactose and mannose. In the present work the galactomannan was isolated from the water extract as its insoluble copper complex. This method has been shown to give a homogeneous galactomannan fraction.[4] Recovery of the polysaccharide from the copper complex yielded a product, $[\alpha]_D^{20}$ + 87°, consisting of only galactose and mannose in the molecular proportion 1:1.25. Isolation of crystalline derivatives confirmed the indentities of the sugars as the D-enantiomorphs. Enzyme experiments with galactosidases showed that the glycosidic linkages of the galactose are in the α-form. The galactomannan was oxidised with metaperiodate, which resulted in a periodate uptake corresponding to 1.30 mole of periodate per anhydrohexose unit, and a release of formic acid indicating that 42 % of the hexose units are present in terminal positions. The percentage of galactose in the polysaccharide is 44.5 %. The methylation experiment confirmed that all the galactose in the galactomannan is in terminal position, as 2,3,4,6-tetramethyl-D-galactopyranose was liberated in high yield by hydrolysis of the methylated product. This hydrolyzed product contained two other components which by paper chromatography were indistinguishable from 2,3,6-trimethyl-D-mannose and 2,3-dimethyl-D-mannose. This result is in agreement with the high periodate consumption of the polysaccharide, which showed that other sugar residues besides the galactose were oxidised by periodate and consequently possessed vicinal hydroxyl groups. The calculated value corresponding to complete oxidation of the galactomannan, is 1.42 mole periodate per anhydrohexose unit. The molecular proportion of the methylated sugars was found to be: dimethyl-mannose 4.07, trimethyl-mannose 1.00, tetramethyl-galactose 3.45.

The results of this investigation are in agreement with the general structure attributed to the majority of leguminous galactomannans examined; no significant deviation has been detected.

Experimental. Isolation of the galactomannan. The milled seeds (100 g) were extracted with cold water (1500 ml) with constant stirring at room temperature for 2 h. After centrifugation, the polysaccharide in the extract was fractionated by addition of Fehling's solution (250

ml) and purified further by ethanol precipitations.[7] The polysaccharide preparation was a white powder, yield 1.8 g; $[\alpha]_D^{20}$ + 87° (*c* 1.0, water) N, nil; sulfated ash, nil; the aqueous solution was neutral and gave no coloration with iodine. The product appeared to be homogeneous on examination by electrophoresis on glass fiber sheets in borate buffer and in sodium hydroxide (2 N).

Estimation and identification of constituent sugars. Hydrolysis of the galactomannan in N sulfuric acid in sealed tubes at 100° for 17 h yielded only galactose and mannose as shown by paper chromatography in different solvents. Mannose was characterized as D-mannose phenylhydrazone,[10] m.p. 197–198°, IR spectrum identical with that of a reference substance, $[\alpha]_D^{22}$ + 32 ± 3° (*c* 0.5, pyridine). The sugars were separated by paper chromatography (butanol-ethanol-water, 50:10:40, v/v) and the molar proportion was determined by periodate oxidation;[11] it was found to be 1.25 parts of mannose to 1 part of galactose as a mean value of replicate experiments.

Methylation and analysis of the hydrolyzed product. Methylation [7] of the polysaccharide (2.5 g) resulted in a product (1.2 g) with the following properties; white powder, $[\alpha]_D^{20}$ + 66.6° (*c* 1.2, chloroform); MeO 40.6 %. The completely hydrolyzed product was analyzed by paper chromatography (benzene-ethanol-water, 167:47:15, v/v and butanol-ethanol-water, 50:10:40, v/v). The three components moved exactly as the following reference mixture; (I) 2,3-dimethyl-D-mannose; (II) 2,3,6-trimethyl-D-mannose, and (III) 2,3,4,6-tetramethyl-D-galactose. The quantities of the sugars were determined by oxidation with hypoiodite[12] as adapted to methylated mannose derivatives.[7] The molecular ratios were as follows: (I) 4.07, (II) 1.00, (III) 3.45. Fraction III was isolated from chromatograms and identified as N-phenyl-2,3,4,6-tetramethyl-D-galactopyranosylamine, m.p. and mixed m.p. 198–199°, $[\alpha]_D^{26}$ −138° ± 2 (*c* 0.5, pyridine).

Table 1. Oxidation by periodate.

Time h	Moles of periodate consumed per $C_6H_{10}O_5$	Acid, equivalents per $C_6H_{10}O_5$
1	0.81	—
4	0.94	—
24	1.17	0.320
48	1.25	0.396
72	1.30	0.420

The IR spectrum was indistinguishable from that of an authentic specimen.

Periodate oxidations. Results from a typical series of experiments are given in Table 1. (a) *Periodate consumption.* The galactomannan (80 mg) was dissolved in water (45 ml), sodium metaperiodate (200 ml, 0.01 M) was added, and the total volume adjusted to 250 ml with water. Oxidation was allowed to proceed in the dark at room temperature. 25 ml samples were withdrawn at intervals and analyzed by the arsenite method.[13]

(b) *Formic acid liberated.* The galactomannan (242.2 mg) was dissolved in water (25 ml), potassium chloride (3 g) and sodium metaperiodate (25 ml, 0.36 M) were added. The oxidation mixture was constantly shaken and protected from light. The formation of acid was determined in 5 ml aliquots by titration with 0.01 N sodium hydroxide after treatment with ethylene glycol to destroy the excess periodate.

Hydrolysis by galactosidase. A solution of galactomannan (40 mg) and α- or β-galactosidase (2 mg) in 0.02 M acetate buffer of pH 4.8 (2.5 ml) was kept at 37°. After the elapse of 24, 48, and 72 h aliquots were withdrawn and examined by paper chromatography. Galactose was only liberated by α-galactosidase.

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