The Structure of a Galactomannan from White Clover Seeds (*Trifolium repens* L.)

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Galactomannans occur as water-soluble polysaccharides in the seeds of various leguminous plants. The polysaccharides from the comparatively few species so far examined, all appear to exhibit the same structural features.[1] D-Mannopyranose residues are united through 1,4-β-linkages to form a chain to which single α-D-galactopyranosyl units are attached. Each galactose unit appears to be linked to position 6 of a mannose residue, thus creating a highly branched polysaccharide structure where the degree of branching is indicated by the molecular proportion galactose: mannose.

-Manβ1-4Manβ1-4Manβ1-4Man-
6 6
| |
1αGal 1αGal

Formula I

Gal = D-Galactopyranose
Man = D-Mannopyranose

The galactomannan from one of the clover species of agricultural interest, *Trifolium pratense L.*, has previously been examined,[2] and the present paper reports the results of a comparative examination of the structure of the galactomannan from white clover, *Trifolium repens L.*

The purified galactomannan consisted of galactose and mannose of the molecular proportion 1:1.3. By methylation and subsequent hydrolysis all the galactose present was transformed to 2,3,4,6-tetramethyl-D-galactose, which may arise only from terminal galactopyranosyl groups. Determination of terminal groups by titration of the amount of formic acid liberated during oxidation of the polysaccharide by periodate, gave results accounting for 41.2 % of terminal hexose residues. The percentage of anhydrogalactose residues calculated is 43.5. The capacity of the polysaccharide to reduce periodate (1.1—1.2 mole per anhydrohexose unit) tends to indicate that also the mannose residues of the chains are extensively attacked by this reagent, and consequently possess vicinal hydroxyl groups. The methylated polysaccharide gave by hydrolysis also two products which by paper chromatography could not be distinguished from 2,3,6-trimethyl-D-mannose and 2,3-dimethyl-D-mannose. The molecular proportion of the methylated sugars was found to be: tetramethyl-galactose 3.38, dimethyl-mannose 3.00, trimethylmannose 1.00.

The results obtained are in agreement with the structure (Formula I) previously attributed to some galactomannans from leguminous seeds, and thus contribute to support the general character of this formula. The polysaccharides extracted from *Trifolium pratense L.*[2] and *Trifolium repens L.* even contain mannose and galactose in practically the same proportion (1.3:1) and also exhibit the same optical rotation. The identical composition of products from these closely related species should, however, be considered as accidental. The role of the galacto-mannans as reserve polysaccharides implies that the amount of galactose attached to the mannose chain may be submitted to considerable variation; thus the two hexoses have recently[3] been found to be present in nearly equimolecular proportion in a polysaccharide sample from the seeds of *Trifolium repens L.*

Experimental. Extraction of the galactomannan. Milled seeds (100 g) were extracted with cold water (750 ml). The polysaccharide present in the extract (after purification by filtration and centrifugation) was separated and further purified through its copper complex [2] to yield 1.6 g of galactomannan, slowly soluble in cold water: $[\alpha]_D^{20}$ + 77.8° (c 1.0, water) N, 0.15 % sulphated ash, 1.03 %; acid functions negligible.

Complete hydrolysis (in N sulphuric acid at 100°, 12 h) gave only the two sugars mannose and galactose, detected on chromatograms run in different solvent systems. Mannose was characterized [4] as phenylhydrazone, m.p. 188°. The molecular proportion of the sugars determined by periodate oxidation [5] after separation on chromatograms (solvent system: butanol-ethanol-water, 50:10:40, v/v), was 1.00 (galactose) to 1.30 (mannose). The figures are mean values of replicate analysis.

Oxidation by periodate. (a) *Formic acid liberated.* The polysaccharide (225.7 mg) was suspended in water (25 ml) and to the mixture potassium chloride (3 g) and sodium metaperiodate (0.36 N, 25 ml) were added. The

mixtnre was protected against light and frequently shaken. Aliquots of 5 ml were titrated by 0.01 N sodium hydroxide after reduction of the periodate by ethylene glycol; 48 h 5.22 ml; 92 h 5.74 ml (170 h, yellow-brownish colour of solution, side reactions). The amount of formic acid liberated (92 h) corresponds to 41.2 % terminal hexose units. (b) *Periodate uptake.* The galactomannan (80 mg) was dissolved in 45 ml of water, sodium meta-periodate (200 ml, 0.01 M) was added and water to make 250 ml. Aliquots of 25 ml were titrated by the arsenite method.[6] Results from a typical experiment given as moles of periodate reduced per anhydrohexose unit 0.67 (1 h); 1.03 (4 h) 1.06 (24 h) 1.11 (48 h); 1.14 (70 h).

Methylation and analysis of the methylated sugars. The polysaccharide (1.25 g) gave by methylation [2] a product (0.7 g) with the following properties; white, crisp powder, $[\alpha]_D^{24}$ + 74.4° (c 1.20 chloroform); MeO 43.55 % After complete hydrolysis the mixture of methyl derivates on paper chromatograms (benzene--ethanol-water, 167: 47: 15 v/v) behaved exactly as a mixture of the following reference substances; (I) 2,3-dimethyl-D-mannose (R_g 0.09), (II) 2,3,6-trimethyl-D-mannose (R_g 0.41), (III) 2,3,4,6-tetramethyl-D-galactose (R_g 0.88) (R_g = 1.00 for tetramethyl-D-glucopyranose). Their molecular proportion was determined to be (I) 3.00: (II) 1.00: (III) 3.38, respectively, as mean values of replicate analysis. The quantitative method used was oxidation with hypoiodite [7] as adapted to methylated mannose derivatives.[2] Fraction (III) was further characterized as the crystalline derivative N-phenyl--2,3,4,6-tetramethyl-D-galactopyranosylamine; m.p. 204°, $[\alpha]_D^{20}$ −142° (c 0.50, pyridine);[8] MeO calc. 39.81 %, found 37.8 %.

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Uridine Diphosphate Glucose in Cellulose Forming Cultures of *Acetobacter xylinum*

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Glaser [1] has shown that a particulate enzyme preparation from *Acetobacter xylinum* catalyses the synthesis of cellulose from uridine diphosphate glucose, UDPG. It is therefore of interest to show whether UDPG actually is present in cellulose forming cultures of *A. xylinum*. Colvin *et al.*[2,3] examined the extracellular medium of active glucose metabolising cells of *A. xylinum* and found no UDPG present. Weigl [4] examined the cellulose pellicles from cultures of *A. xylinum* on a *p*-aminobenzoic acid dependent medium and was unable to detect UDPG. This paper reports the presence of UDPG in cultures of *A. xylinum* with evident cellulose formation. The UDPG is found in a perchloric acid extract of the cellulose pellicles and also, in lower concentration, in the liquid phase of the cultures.

Culture conditions. The bacterium, *Acetobacter xylinum* ATCC 10245, was grown on a synthetic medium [4] containing 4 % glucose, 1 % ethanol, inorganic salts, and small amounts of *p*-aminobenzoic acid. The liquid phase of a three-day culture with evident cellulose formation served as inoculum. Each flask containing 100 ml medium was inoculated with 10 ml inoculum. The cultures were incubated quiescently at 29°C.

Isolation of UDPG. Four days after inoculation the pellicles from 5 cultures were withdrawn. The pellicles with included bacteria and substrate were treated in a Waring Blendor with 0.5 l distilled water. Perchloric acid was added at 0°C to give a concentration of 6 % w/w and the mixture was stirred for 30 min. After centrifugation the supernatant liquid was neutralized with KOH to pH 6.0. After removal of precipitated $KClO_4$ the solution was reduced in volume to 100 ml and precipitated $KClO_4$ again removed. Nucleotides were adsorbed on 0.5 g of norite and the norite washed with 100 ml of distilled water. UDPG and some pigments were then desorbed with 50 ml portions of 50 % ethanol. Each ethanol