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# STRUCTURE OF GALACTOMANNAN FROM *Gleditsia delavayi* SEEDS

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*Galactomannan with a galactose:mannose ratio 1:1.1 and molecular weight 79,000 was obtained from* Gleditsia delavayi *seeds by fractional precipitation of the water-soluble polysaccharides. Methylation, oxidation by chromic acid and periodate, and partial acid hydrolysis established that the principal galactomannan macromolecule consisted of* β*-1.4 mannopyranose units substituted at C-6 by* α*-galactopyranoses.*

**Key words:** *Gleditsia*, oligosaccharides, galactomannan, structure.

In continuation of the study of *Gleditsia* polysaccharides from *G. delavayi* seeds, water extraction isolated a heterogeneous polysaccharide consisting of galactose and mannose in a 1:1.12 ratio in 11.1% yield with $[\alpha]_D^{20}$ +8.8 (*c* 0.5, water) [1].

The water-soluble polysaccharides (WSPS) were fractionally precipitated with alcohol to produce a homogeneous fraction. Three fractions were obtained (Table 1).

Total acid hydrolysis of fractions **1**-**3** formed only galactose and mannose according to paper chromatography (PC). The isolated fractions differ in the ratio and molecular weight (MW). It should be noted that glucose was identified in fraction **3**, in contrast with **1** and **2**. Glucogalactomannans in addition to galactomannans probably accumlate in *G. delavayi* seeds.

Sedimentation analysis has shown that **2** is homogeneous with MW 79,000. Total acid hydrolysis produced galactose and mannose in a 1:1.1 ratio. Therefore, **2** is a galactomannan designated by us as GMD.

GMD is a white amorphous powder that is soluble in water. Its IR spectrum contains absorption bands ($cm^{-1}$) at 880 (*β*-glycoside bond) and 817 (pyranose ring). Observation of an absorption band at 720 indicates that an *α*-glycoside bond is present. This hypothesis was confirmed by total oxidation of acetylated GMD by chromic anhydride [2], the products of which contained only galactose according to PC.

Structural information for GMD was obtained using exhaustive methylation by the Hakomori method [3]. The reaction produced the fully methylated derivative with $[\alpha]_D^{20}$ +10 (*c* 0.1, $CHCl_3$). The completeness of the methylation was checked by IR spectroscopy. Hydrolysis of the compound led to 2, 3, 4,6-tetra-O-Me-D-mannose, 2,3,4,6-tetra-O-Me-D-galactose, 2,3,6-tri-O-Me-D-mannose, and traces of di-O-Me-hexose. These data are consistent with the results of periodate oxidation.

It was found that oxidation of GMD with sodium periodate consumed 1.1 mole per anhydro unit. The yield of formic acid was 0.3 moles. The products of Smith degradation contained according to PC mainly erythrite, indicating that there was a 1→4 bond between the hexose units in GMD, and glycerine, indicating that there were either 1→6 or 1→2 type bonds.

The sequence of monosaccharides was determined by partial cleavage of GMD. PC with authentic specimens identified galactose, mannose, and the oligosaccharides Man*p*-1→4-Man*p*, Man*p*-1→4-Gal*p* or Gal*p*-1→4-Man*p*, Man*p*-1→4-Man*p*-1→4-Man*p*, and Man*p*-1→4-Man*p*-1→4-Man*p*-1-4-Man*p* with $R_{gal}$ of 0.7, 0.5, 0.35, and 0.01, respectively. Since galactose forms the side chains, it is cleaved by this partial hydrolysis and only galactosylmannose (mannosylgalactose) is identified among the oligosaccharides.

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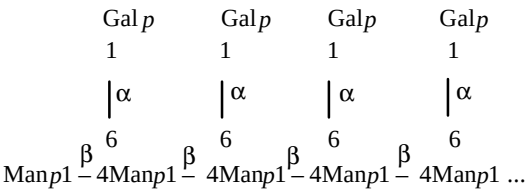
TABLE 1. Physicochemical Properties of *G. delavayi* Fractions

| Fraction | Yield, % | MW | Monosaccharide ratio | | |
|---|---|---|---|---|---|
| | | | Gal | Glc | Man |
| 1 | 54 | 100800 | 1.0 | - | 2.0 |
| 2 | 30 | 79000 | 1.0 | - | 1.12 |
| 3 | 2.0 | - | 1.5 | 1.0 | 2.0 |

*G. delavayi* galactomannans have much in common with those from other species of plants from the *Gleditsia* genus [5-8]. We present a scheme for the structure of one of the possible fragments in the *G. delavayi* galactomannan chain:




## EXPERIMENTAL

TLC was performed on Silufol UV-254 plates using the following solvent systems (by vol): $CHCl_3$:$(CH_3)_2CO$ (30:1) and benzene:acetone (2:1).

PC used Filtrak FN-12 and -16 (Germany) paper and the solvent system *n*-butanol:pyridine:water (6:4:3).

Compounds were detected by spraying with the developers anilinium acid phthalate and $KIO_4$:$KMnO_4$:benzidine.

GC was carried out on a Chrom-5 chromatograph with a flame-ionization detector, stainless-steel column (200 × 0.2 mm), Silicone XE-60 (5%) on Chromaton NAW (0.200-0.250 mm), 200°C, and $N_2$ carrier gas at 60 mL/min.

IR spectra were recorded on a Perkin—Elmer Model 2000 spectrometer in pressed KBr disks with 100 scans. Ultracentrifugation was carried out on a MOM-3170 instrument (5000 rpm, 20°C) for 30 min. An aqueous solution (1%) of GMD gave one narrow peak in the scan. The molecular weight of GMD was 79,000 ± 10%.

Specific rotation was measured on a Zeiss polarimeter in a 1-dm tube of 10-mL volume at 20°C.

**Fractional Precipitation of WSPS by Alcohol.** A solution of WSPS (0.5 g) in water (100 mL) was treated dropwise with vigorous stirring with alcohol (25 mL). The resulting precipitate was separated by centrifugation and washed with alcohol and acetone. Yield, 0.27 g (fraction 1). The supernatant solution was treated with another portion of alcohol (25 mL). The precipitate was worked up analogously. Yield, 0.15 g (fraction 2). Fraction 3 (yield, 0.01 g) was obtained by adding another portion of alcohol (50 mL) to the supernatant.

**Total Acid Hydrolysis of the Fractions.** Samples of **1**-**3** were hydrolyzed by $H_2SO_4$ (2 N) at 100°C for 8 h. The hydrolysates were worked up as before [1].

**Methylation of GMD.** GMD (0.04 g) was dissolved in DMSO (1 mL) and methylated by the Hakomori method [3]. Yield of permethylate, 0.048 g, $[\alpha]_D^{20}$ +10° (*c* 0.5, $CHCl_3$).

**Formolysis and Hydrolysis of GMD Permethylate.** GMD permethylate (0.01 g) was boiled with formic acid (1 mL, 85%) for 1 h, cooled, and evaporated. The solid was dissolved in $H_2SO_4$ (2.5 mL, 0.5 N) and hydrolyzed for 16 h at 100°C. The hydrolysate was worked up as usual. The product was studied by TLC (systems **1** and **2**, developer **1**).

**Periodate Oxidation.** GMD (0.03 g) was dissolved in water (10 mL) and treated with $NaIO_4$ (10 mL, 0.05 M). The oxidation was carried out at 5°C for 17 days. The consumption of $NaIO_4$ and yield of $HCO_2H$ were determined by titration using the literature method [4].

PC (system **3**, developers **1** and **2**) detected glycerine and erythrite.

**Chromic Oxidation.** GMD (0.01 g) was dissolved in formamide (5 mL) and treated with pyridine (2 mL) and dropwise with acetic anhydride (2 mL) with stirring for 7 d. The acetate was precipitated in glacial acetic acid. The yield of acetylated GMD was 0.018 g. This was oxidized by chromic anhydride in acetic acid as before [2]. PC (system **3**, developer **1**) of the reaction products identified galactose.

**Partial Hydrolysis of GMD.** GMD (0.05 g) was dissolved in water (5 mL), treated with $H_2SO_4$ (5 mL, 2 N), and boiled for 1 h. The hydrolysate was neutralized with $BaCO_3$ and deionized with cation-exchanger KU-2 ($H^+$). PC (system **3**, developer **1**) identified oligosaccharides with $R_{gal}$ 0.7, 0.55, 0.35, and 0.01.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