

Note

The Chemical Structure of Galactomannan Isolated from Seeds of *Delonix regia**

Yukihiro TAMAKI, Takeshi TERUYA, and Masakuni TAKO†

Department of Subtropical Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan

Received December 16, 2009; Accepted January 18, 2010; Online Publication, May 7, 2010

[doi:10.1271/bbb.90935]

A galactomannan isolated from seeds of *Delonix regia* was investigated. The specific rotation of the galactomannan was +15° at 25°C. The molecular mass of the galactomannan was estimated to be 2.5×10^5 . The ratio of D-mannose to D-galactose was estimated approximately to be 4.0:1.0 by HPLC and ¹H-NMR. NMR (¹H and ¹³C) spectra, and methylation analysis of the galactomannan indicated that it was composed of 1,4-linked β-D-mannose, 1,4,6-linked β-D-mannose, and terminal α-D-galactose. It was composed of pentasaccharide repeating-units, the chemical structure of which is proposed below.

Key words: galactomannan; *Delonix regia*; chemical structure

Galactomannans are included as storage polysaccharides in the seeds of legume plants.¹⁾ Since they have high viscosity¹⁾ and synergistic interactions with certain polysaccharides, including xanthan,^{2–7)} κ-carrageenan⁸⁾ and agarose,⁹⁾ they are often used as thickening and stabilizing agents in the food industry, and they and their derivatives are utilized in other industries.¹⁰⁾ Guar gum (*Cyamopsis tetragonolobus*) and locust bean gum (*Ceratonia siliqua*) are utilized mainly in the food industry.¹⁾ Galactomannans are based on a linear (1→4)-linked β-D-mannose residue backbone substituted partially with α-D-galactose residues linked to O-6. The degree of substitution of galactose differs in the galactomannans extracted from various plants. The differences of the degree in substitution greatly affect solution properties, including water solubility, thickening ability and synergistic interactions.¹⁰⁾ Thus the degree of substitution of galactomannans obtained from various plants has been studied.^{1,7,10–13)}

Delonix regia is a legume plant widely grown in tropical and sub-tropical regions. The tree is cultivated as an ornamental or a boulevard tree due to its beautiful red flowers and its dense foliage, providing shade. Here we report a structural characterization of a galactomannan from seeds of *D. regia* growing in Okinawa, Japan.

Seeds were collected from husks of *D. regia*. After the endosperm was separated from the seeds by hand, it was crushed to a powder with a mixer. The powder was soaked in benzen-ethanol solution (1:1) overnight to remove lipids, and then it was dried *in vacuo*. The

endosperm powder (10 g) was suspended in H₂O (100 ml) at 100°C for 2 h, and then the suspension was autoclaved at 140°C for 30 min. After filtration, ethanol (2 vols) was added to the filtrate. The precipitate obtained was washed with ethanol and then dried *in vacuo*. The dried precipitate was dissolved in H₂O at 90°C, and then the solution was filtered. Ethanol (2 vols) was added to the filtrate and the polysaccharide was prepared as described above.

The content of total carbohydrate of the polysaccharide was determined by the phenol-sulfuric acid method¹⁴⁾ using D-mannose as standard. The polysaccharide was dissolved in H₂O (20 ml), and sulfuric acid was added to a final concentration of 1M. The solution was heated at 100°C for 2 h. The hydrolyzate was neutralized with BaCO₃ and filtered. The hydrolyzate was analyzed by high-performance anion exchange chromatography (HPLC) on DX 500 liquid chromatography (Dionex, Sunnyvale, CA) fitted with a column of CarboPac PA1 (4 × 250 mm) and a pulsed amperometric detector. The column was eluted at a flow rate of 1 ml/min at 35°C with 15 mM NaOH.

The molecular mass of the polysaccharide was determined by HPLC using a chromatograph (LC-6A, Shimadzu, Kyoto, Japan) on a column of Superdex 200 with refractive index detection (RID-6A, Shimadzu, Kyoto, Japan). HPLC was performed at room temperature at a flow rate of 0.5 ml/min. The column was conditioned with 0.15M sodium chloride in 0.05M sodium phosphate buffer (pH 7.2), and elution was carried out with the same buffer. Standard pullulans (Showa Denko, Tokyo) were used as molecular mass markers.

The specific rotation of the galactomannan (0.2%) was measured at 589 nm on a polarimeter (DIP-180, Jasco International, Tokyo) with a cell 5 cm in length at 25°C. The infrared spectrum was recorded with an FT-IR spectrophotometer (FT-IR 8000, Jasco International, Tokyo) in transmittance mode from 4600 to 400 cm⁻¹ in KBr disc. The KBr disc was prepared by dispersing a solid sample in salt.

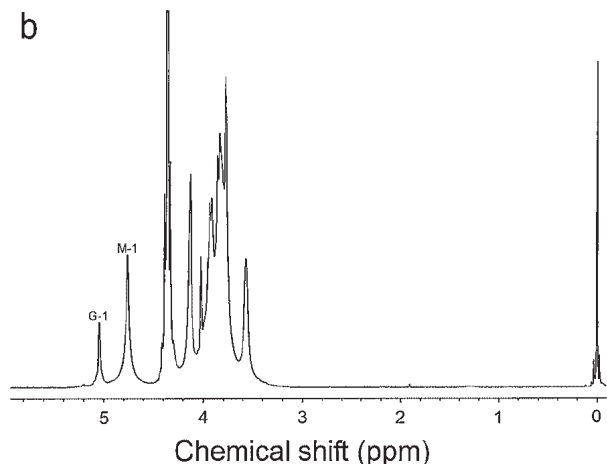
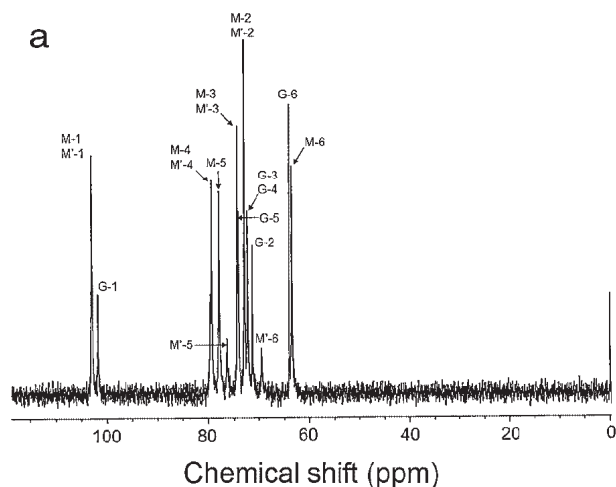
The polysaccharides were dissolved in D₂O and then freeze-dried. The dried sample (2%) was redissolved in D₂O, and the solution was examined in 5-mm o.d. tubes. Chemical shifts in the ¹H and ¹³C NMR spectra were expressed in δ (ppm), relative to the resonance of sodium 3-(trimethylsilyl) propionic-2,2,3,3,-d₄ acid

* Part of this paper presented at the 235th American Chemical Society National Meeting and Exposition, New Orleans, LA, April 6–10, 2008.

† To whom correspondence should be addressed. Tel/Fax: 81-98-905-9914. E-mail: tako@cc.ryukyus.ac.jp

Table 1. Chemical Shifts of the ^{13}C -NMR Spectrum of Galactomannan from Endosperm of *D. regia*

Type of unit	C-1	C-2	C-3	C-4	C-5	C-6
α -D-Galactopyranosyl (G)	101.7	71.3	72.3	72.2	74.1	64.0
β -D-Mannopyranosyl, unbranched at HO-6 (M)	103.0	72.9	74.3	79.3	77.9	63.4
β -D-Mannopyranosyl, branched at HO-6 (M')	103.3	72.9	74.3	79.4	76.3	69.6

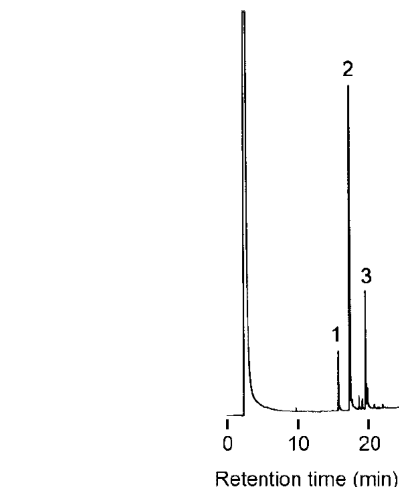
**Fig. 1.** ^{13}C (a) and ^1H (b) -NMR Spectra of the Galactomannan from *D. regia*.

(a) M, mannose; G, galactose. Numerals designate numbers of hydrogen atoms in the residues. (b) M, mannose unbranched at HO-6; M', mannose branched at HO-6; G, galactose. Numerals designate numbers of carbon atoms in the residues.

(TSP, 0.00 ppm) as internal standard. The ^1H and ^{13}C NMR spectra were recorded on an FT-NMR spectrometer at 500.00 and 125.65 MHz (JNM α 500, JEOL, Tokyo) at 70°C.

For methylation analysis, polysaccharides dissolved in DMSO were methylated by the procedure of Hakomori.¹⁵⁾ The methylated polysaccharides was hydrolyzed using 2M TFA for 3 h at 120°C. The partially methylated monosaccharides were reduced with 20 mg NaBH_4 in 1M NH_4OH at room temperature for 4 h, and were acetylated with a solution of pyridine and acetic anhydride (1:1) at 100°C for 1 h.

The partially methylated alditol acetates were analyzed with a gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector using a capillary column (30 m \times 0.25 mm, DB-1, I & W Scientific, Folsom, CA). The injector and detector

**Fig. 2.** Gas Chromatogram of Partially Methylated Alditol Acetate of the Galactomannan.

1, 1,5-di-*O*-acetyl-2,3,4,6,-tetra-*O*-methyl-galactitol; 2, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-mannitol; 3, 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-mannitol.

temperatures were 210 and 270°C respectively. After injection, the oven temperature was maintained at 150°C for 5 min, then raised by 5°C/min to 250°C, and kept there for 5 min. The identities of the peaks were confirmed with GC-MS (GCMS-QP 1000EX, Shimadzu, Kyoto, Japan) using same column.

Endosperm was obtained from the seeds at a yield of 28%. Polysaccharide was extracted from the endosperm by hot water and autoclave extraction. The yield was 73% on an endosperm basis.

The total carbohydrate content of the polysaccharide was determined to be 90.0% by the phenol-sulfuric acid method. By HPLC analysis of the hydrolyzate of the polysaccharide, D-mannose and D-galactose were identified, and the molar ratio was estimated to be 4.0:1.0. Thus the polysaccharide isolated from *D. regia* was identified as a galactomannan.

The molecular mass of the galactomannan was measured by gel chromatography, and was estimated to be approximately 2.5×10^5 . Its specific rotation was +15° at 25°C. This value is a little lower than that for locust bean gum (+22°).^{2,4,6)}

The infrared spectra of the galactomannan and locust bean gum were measured (data not shown). A major absorption around 3,400 cm^{-1} was attributed to stretching of the hydroxyl groups, and an absorption at 2,900 cm^{-1} was due to C-H stretching.¹⁶⁾ Absorption at 873 and 814 cm^{-1} was attributed to β -D-mannopyranose and α -D-galactopyranose respectively.¹⁷⁾ Absorption common to galactomannans was observed at 1,145 cm^{-1} , and was assigned to C-O bending vibration due to the pyranose ring, at 952 cm^{-1} was assigned to deformation of the axial C-OH at C-4, and between 150-950 cm^{-1} was characterized by C-OH bending.¹⁷⁾

The spectrum of the galactomannan was consistent with that of locust bean gum over wide ranges of wave numbers.

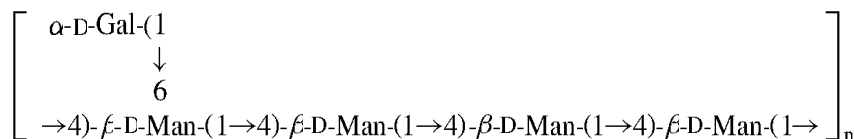
The ^{13}C -NMR spectrum of the galactomannan is shown in Fig. 1a. Well-resolved signals were observed, and they corresponded to β -D-mannopyranose (anomeric carbon, 103.0 ppm), β -D-mannopyranose branched at HO-6 (103.3), and α -D-galactopyranose (101.7) residue. The chemical shifts of the galactomannan summarized in Table 1 are in accordance with those reported in the literature.^{13,18)}

The ^1H -NMR spectrum of the galactomannan is shown in Fig. 1b. In the anomeric region, two distinct signals were observed. The signal at 5.03 ppm arose from the H-1 of α -D-galactopyranose ring, and the signal at 4.75 ppm arose from H-1 of β -D-mannopyranose.^{13,18)} The ratio of mannose to galactose can be also obtained directly from the relative areas of the signals for H-1 for both of them, and the ratio was 3.9:1.0.

Figure 2 shows a gas chromatogram of partially

methylated alditol acetate of the galactomannan. According to GC and GC-MS analyses of the partially methylated alditol acetates, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-mannitol, and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-mannitol were identified, indicating that they were terminal D-galactose, 1,4-linked mannose, and 1,4,6-linked mannose respectively. The molar ratio of terminal galactose, 1,4-linked mannose, and 1,4,6-linked mannose was estimated to be 0.8:2.7:1.0. The different proportions of terminal galactose and 1,4,6-linked mannose were probably to be attributed to their different behavior as to volatilization and degradation. Based on HPLC and methylation analysis, molar ratio of D-mannose to D-galactose was 4.0:1.0, similar to that of locust-bean gum.^{2,4-6,19)}

In conclusion, the chemical structure of the galactomannan from *D. regia* is proposed. Synergistic co-gelation characteristics between the galactomannan from *D. regia* and xanthan is now on progress.



References

- Maier H, Anderson M, Karl C, Magnuson K, and Whistler RL, "Industrial Gums," eds. Whistler RL and BeMiller JN, Academic press, California, pp. 181–226 (1993).
- Tako M, Asato A, and Nakamura S, *Agric. Biol. Chem.*, **48**, 2995–3000 (1984).
- Tako M and Nakamura S, *Carbohydr. Res.*, **138**, 207–213 (1985).
- Tako M and Nakamura S, *FEBS Lett.*, **204**, 33–36 (1986).
- Tako M, *J. Carbohydr. Chem.*, **10**, 619–633 (1991).
- Tako M, *Colloids Surf. B Biointerface.*, **1**, 125–131 (1993).
- Pakdee P, Tako M, Yokohari T, Kinjyo K, Hongo H, and Yaga S, *J. Appl. Glycosci.*, **42**, 105–113 (1995).
- Tako M and Nakamura S, *Agric. Biol. Chem.*, **50**, 2817–2822 (1986).
- Tako M and Nakamura S, *Agric. Biol. Chem.*, **52**, 1071–1072 (1988).
- Izydorczyk M, Cui SW, and Wang Q, "Food Carbohydrates: Chemistry, Physical Properties, and Applications," eds. Cui SW and Wang Q, CRC Press, Boca Raton, pp. 263–307 (2005).
- Buckeridge MS, Panegassi VR, Rocha DC, and Dietrich SMC, *Phytochem.*, **38**, 871–875 (1995).
- Ganter JLMS and Reicher F, *Bioresour. Technol.*, **68**, 55–62 (1999).
- Vieira ÍGP, Mendes FNP, Gallço MI, and Brito ES, *Food Chem.*, **101**, 70–73 (2007).
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, and Smith F, *Anal. Chem.*, **28**, 350–356 (1956).
- Hakomori S, *J. Biochem.*, **55**, 205–208 (1964).
- Singh V, Tiwari A, Shukla P, Singh SP, and Sanghi R, *React. Funct. Polym.*, **66**, 1306–1318 (2006).
- Figueiró SD, Góes JC, Moreira RA, and Sombra ASB, *Carbohydr. Polym.*, **56**, 313–320 (2004).
- Rakhmanberdyeva RK and Shashkov AS, *Chem. Nat. Compd.*, **41**, 14–16 (2005).
- Tako M and Nakamura S, *Agric. Biol. Chem.*, **47**, 2987–2993 (1984).