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Structural analysis of galactomannans by NMR spectroscopy

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ABSTRACT

The structure of naturally occurring galactomannans was characterized by high resolution NMR spectroscopy involving two-dimensional (2D) NMR measurements of the field gradient DQF-COSY, HMQC, HMBC, and ROESY experiments. Four galactomannans with different proportions of galactose (G) and mannose (M), from fenugreek gum (FG), guar gum (GG), tara gum (TG), and locust bean gum (LG), were investigated. Because these galactomannans had very high molecular weights, hydrolysis by dilute H_2SO_4 was carried out to give the corresponding low molecular weight galactomannans, the structural identities of which were established by comparison of the specific rotations, shape of the GPC profiles, and NMR spectra with those of higher molecular weight galactomannans. The correlation signals GH1–GC4, –GC5, and –MC6 in HMBC and GH1–GH6 in ROESY spectra of FG showed that more than two galactopyranose units with the $1 \rightarrow 4$ linkage were connected at C6 of the mannopyranose main chain. The coupling constant ($J_{H1,2}$) of galactose was 3.4 Hz, indicating that galactose has an α-linkage. The main chain mannose was found to connect through the $1 \rightarrow 4$ linkage, because of the appearance of the correlation signals MH1–MC4, and MC1–MH4 in the HMBC spectrum due to the long-range correlation signals between two neighboring mannopyranose residues through the M4–O–M1 bond. Although the main chain mannose $J_{H1,2}$ was not observed, probably because of the high molecular weight, the specific rotation of LG with a higher proportion of mannose was low, $[\alpha]_D^{25} = +10.8^\circ$, compared with that of FG with a lower proportion of mannose, $[\alpha]_D^{25} = +90.5^\circ$, suggesting that the mannose in the main chain had a α-linkage. These results suggest that the galactomannans comprise a $(1 \rightarrow 4)$-β-mannopyranosidic main chain connected with more than two $(1 \rightarrow 4)$-α-galactopyranosidic side chains, in addition to the single galactopyranose side chain, at C6 of the mannopyranose main chain.

1. Introduction

Galactomannans are naturally occurring branched polysaccharides consisting of a mannopyranose main chain with galactopyranose side chains that are distributed widely in the seeds of the leguminous plants. Galactomannans are used as stabilizing, thickening, binding, and gelling agents in the food industry (Srivastava & Kapoor, 2005). The proportions of galactose and mannose in the galactomannans are dependent on the source. The fundamental structure reported in the literature was mainly a $(1 \rightarrow 4)$-β-D-mannopyranosidic main chain with various proportions of single $(1 \rightarrow 4)$- or $(1 \rightarrow 6)$-α-D-galactopyranosyl side chains, which was determined by methylation analysis (McDougall, Morrison, Stewart, & Hillman, 1996). In general, naturally occurring galactomannans are difficult to dissolve in water because they have high molecular weights of more than 100×10^4 and high viscosity in aqueous solution. In addition, galactomannans show lyotropic and thermotropic liquid crystallinities at a certain concentrations in water (Hatakeyama, Naoi, & Hatakeyama, 2004; Tanaka, Hatakeyama, & Hatakeyama, 2004); thus, they are one of the important naturally occurring groups of branched polysaccharides.

We have reported the synthesis of branched polysaccharides by the ring-opening polymerization of anhydro disaccharide and trisaccharide monomers and have investigated the relationship between the structure and biological activities, such as the blood anticoagulation and anti-HIV activities of sulfated polysaccharides after sulfation of the synthetic and natural polysaccharides (Yoshida, 2001, 2005). For example, a new oligosaccharide-branched polysaccharide, 3-*O*-(β-D-lactosyl)-$(1 \rightarrow 5)$-α-D-ribofuranan, was obtained for the first time by the ring-opening polymerization of a trisaccharide monomer, 1, 4-anhydro-2-*O*-benzyl-3-*O*-(2′, 3′, 6′, 2″, 3″, 4″, 6″-hepta-*O*-benzyl-β-D-lactosyl)-α-D-ribopyranose, using BF3OEt2 as a catalyst into perbenzylated 3-*O*-(β-D-lactosyl)-α-D-ribofuranan and subsequent removal of the protective benzyl groups (Han, Kanematsu, Hattori, Nakashima, & Yoshida, 2009). The structure was identified by high-resolution NMR measurements. Similarly, the polysaccharides in lacquer tree sap are acidic branched polysaccharides with specific biological activities such as promoting blood coagulation and antitumor activities (Lu et al., 2000) and have complex structures (Lu et al., 1999;

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Lu, Yoshida, & Uryu, 1999). We carried out structural analysis of Asian lacquer polysaccharides from Vietnam, Myanmar, Cambodia, Taiwan, and Japan in comparison with a Chinese lacquer polysaccharide, revealing that the structures of polysaccharides in China and Japan, Taiwan and Vietnam, Myanmar and Cambodia, were similar to each other, and the polysaccharides in Myanmar and Cambodia had larger amounts of L-arabinose and L-rhamnose than those in other Asian lacquer polysaccharides (Lu & Yoshida, 2003).

After sulfation, branched polysaccharides are expected to have potent and specific anti-HIV, anti-influenza virus, and blood anticoagulant activities (Tegshi et al., 2011; Yoshida et al., 1994). Therefore, determination of the structure without destruction of the molecules of galactomannans is quite important not only to know the structural details but also to reveal the relationship between the structures and their biological activities. Several kinds of galactomannans with various proportions of galactose branches depending upon the botanical source are known. However, to our knowledge, there are few reports on the precise structural analysis by high resolution NMR spectroscopy because they have poor solubility due to their high molecular weights. In this paper, we report for the first time structural comparisons and determinations of galactomannans with different proportions of galactose and mannose residues obtained from different sources by NMR spectroscopy including 2D measurements after acid hydrolysis to decrease the molecular weight.

2. Experimental

2.1. Galactomannans

Four kinds of galactomannans with different proportions of galactose and mannose were used. Fenugreek gum (FG) from the seeds of *Trigonella foenum-graecum* was purchased from Air Green Co., Ltd., Japan, guar gum (GG) from the seeds of *Cyamopsis tetragonolobus* and locust bean gum (LG) from the seeds of *Ceratonia siliqua* were obtained from Sigma–Aldrich, Japan, and tara gum (TG) from the seeds of *Caesalpinia spinosa* was provided by Iwate Chemical Co., Japan, respectively.

2.2. Hydrolysis of galactomannans by dilute H_2SO_4 aqueous solution

A typical procedure for the hydrolysis of galactomannans was as follows: the galactomannan from FG (0.5 g) was added to 20% H_2SO_4 aqueous solution and stirred for 30 min at 50 °C. After cooling in a water bath, the reaction mixture was neutralized by saturated $NaHCO_3$ solution, and then dialyzed against deionized water overnight. The dialyzate was filtered to remove the water-insoluble precipitate and then freeze-dried to give 0.37 g of a low molecular weight galactomannan. The specific rotation was $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1) and the molecular weight was $\bar{M}_n = 2.7 \times 10^4$.

2.3. Measurements

The ^1H NMR and ^{13}C NMR spectra were recorded with a JEOL ECM-400 or with a JEOL α-500 spectrometer at 400 MHz and 100 MHz or 500 MHz and 125 MHz, respectively, for solutions in D_2O at 50 °C with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as an internal standard or in DMSO-d_6 at 60 °C. The assignment of the proton and carbon signals was carried out by a combination of the double-quantum filtered correlation spectroscopy (DQF-COSY) and heteronuclear single-quantum correlation (HSQC) 2D NMR measurements. The DQF-COSY was carried out with a 1024 × 256 data matrix, and four transients were acquired for each t1 value. The spectral width was 1870.58 Hz in both dimensions and the pulse delay was 1.5 s. The HSQC was carried out with a 4096 × 512 data matrix, and four transients were acquired for each t1 value. The proton and carbon spectral widths were 1870.87 Hz and 8544.65 Hz, respectively. The heteronuclear multiple-bond correlation (HMBC) measurement was carried out with a 4096 × 512 data matrix. The proton and carbon spectral width were 1870.87 Hz and 10000.52 Hz, respectively. The evolution time for the HMBC experiments was set to 62.5 ms. The 2D rotating-frame Overhauser effect spectroscopy (ROESY) experiment was recorded with a 4096 × 512 data matrix, with a 200 ms mixing time. The spectral width was 1869.76 Hz in both dimensions.

Infrared spectra were taken on a Perkin Elmer Spectrum One FT-IR spectrometer using a KBr pellet method. The molecular weight of polymers was determined by an aqueous phase GPC (column; Tosoh TSK-gel G2500PW_{XL}, G3000PW_{XL}, and G4000PW_{XL}, 7.6 mm × 300 mm × 3 eluted with 66.7 mM of phosphate buffer, pH = 6. 86) with a Tosoh RI detector using pullulan as a standard. Optical rotation was measured by using a JASCO DIP-140 digital polarimeter in H_2O at 25 °C in a water-jacketed 10 ml quartz cell.

3. Results and discussion

3.1. Acid degradation of galactomannans and structural identity

Galactomannans were extracted from the leguminous plants and included fenugreek gum (FG) with the approximate proportions of galactose and mannose of 1.0:1.04–1.20 (Andrews, Hough, & Jones, 1952; Daoud, 1932; Ramesh, Yamaki, Ono, & Tsushida, 2001), guar gum (GG) with 1.0:1.54–1.80 (Anderson, 1949; Dea & Morrison, 1975; Stéphane, Debon, & Tester, 2001), tara gum (TG) with 1.0:2.50 (Anderson, 1949), and locust bean gum (LG) with 1.0:3.0–3.75 (Anderson, 1949; Vieira & Gil, 2005), respectively. Because the molecular weights of the galactomannans were very high, more than 100×10^4, the galactomannans were difficult to dissolve in water and molecular weights were difficult to measure by GPC. Some studies on structural analysis of galactomannans have been reported, but there are few reports on precise structural determinations by high resolution NMR spectroscopies using 1D and 2D NMR. Therefore, in this work, the galactomannans were hydrolyzed in a dilute aqueous H_2SO_4 solution to give low molecular weight galactomannans, structural identities of which were established by the specific rotations, shape of the GPC profiles, and NMR measurement, respectively. Table 1 shows the results of the hydrolysis. When FG was hydrolyzed with 5% H_2SO_4 for 45 min at 50 °C, the molecular weight decreased to $\bar{M}_n = 30.0 \times 10^4$ and the specific rotation was $[\alpha]_D^{25} = +89.0^\circ$. The molecular weight was further decreased to $\bar{M}_n = 2.7 \times 10^4$ by increasing the concentration of H_2SO_4 to 20%, and the specific rotation was almost the same, $[\alpha]_D^{25} = +90.5^\circ$. The other galactomannans shown in Table 1 were hydrolyzed in the same manner to give low molecular weight galactomannans. There was little difference in the specific rotations between high and low molecular weights. Fig. 1 exhibits the GPC profiles after hydrolysis, in which (A) is the original FG and its hydrolyzates with the number-average molecular weights ($\bar{M}_n$) of (B) 30.0×10^4, (C) 16.3×10^4, and (D) 2.7×10^4, respectively. The molecular weight of the original FG was difficult to measure by GPC because substances with molecular weights higher than 100 × 104 are difficult to dissolve in water, as shown in Fig. 1A. Hydrolysis decreased the molecular weight of FG, but the shapes of the GPC profiles were unchanged, and the molecular weight distributions are almost the same in Fig. 1B–D. After hydrolysis, the FGs with the lower molecular weights were easily dissolved in water. In the ^{13}C and ^1H NMR spectra, the original galactomannans had low peak resolutions and gave a noisy spectrum in the highly viscous solution due to its high molecular weight. The peak resolutions were found

Table 1
Hydrolysis of galactomannans by dilute sulfuric acid.[a]

	Galactomannan[b]		H_2SO_4 (%)	Time (min)	Yield (g)	$\bar{M}_n$ [c] ($\times 10^4$)	$\bar{M}_w/\bar{M}_n$	[d]$[\alpha]_D^{25}$ (°)
	Mole ratio of G	Mole ratio of M						
FG			5	45	0.47	30.0	2.0	+89.0
			10	30	0.38	16.3	1.6	+87.2
	1.0	0.83	20	30	0.37	2.7	2.2	+90.5
GG			5	60	0.46	16.0	1.8	+56.3
			10	30	0.35	12.1	1.9	+58.9
	1.0	1.64	20	30	0.29	1.4	2.2	+58.9
TG			5	60	0.47	7.3	2.0	+21.9
			10	60	0.37	4.4	2.2	+21.2
			15	30	0.37	1.7	2.5	+20.7
	1.0	2.88	20	30	0.38	1.3	2.2	+20.0
LG			5	45	0.46	20.4	1.6	+12.8
			10	30	0.44	8.5	1.9	+10.3
			15	30	0.34	2.6	2.2	+11.5
	1.0	3.33	20	30	0.30	0.9	2.2	+10.8

[a] Galactomannan, 0.5 g; sulfuric acid, 50 ml; and temp., 50 °C.
[b] The mole ratio of galactose (G) and mannose (M) was determined by the integral values of the H1 signals. FG, Fenugreek gum; GG, guar gum; TG, tara gum; and LG, locust bean gum.
[c] Calculated by GPC using pullulan standards.
[d] Measured in H_2O at 25 °C (c 1).

to increase with decreasing molecular weight, and the intensity of each signal was not changed much by the decrease in molecular weights. Taking into account the specific rotations, shapes of the GPC profiles and NMR spectra, the structural identity was retained after hydrolysis. The signals were assigned by the correlation signals of the DQF-COSY and HSQC spectra, are represented in Fig. 4.

Fig. 2 shows the ^{1}H NMR spectrum of galactomannans with molecular weights of $\bar{M}_n = 2.7 \times 10^4$ from FG and $\bar{M}_n = 0.9 \times 10^4$ from LG, respectively. The galactomannans were found to have different proportions of galactose and mannose residues by the integrated ratios of the H1 protons as exhibited in Table 1. Using the coupling constant $J_{H1,2}$ of galactose in the side chain, $J_{H1,2} < 3.4$ Hz, the galactose residues were found to be glycosylated by an α-linkage. The integrated ratio of the H1 signals of the ^{1}H NMR spectra showed that the low molecular weight FG consisted of galactose and mannose in the proportions of 1.0 and 0.83, GG in 1.0 and 1.64, TG in 1.0 and 2.88, and LG in 1.0 and 3.33, respectively, proportions that were almost the same as reported previously. The ^{13}C NMR spectra of galactomannans with low molecular weights are shown in Fig. 3.

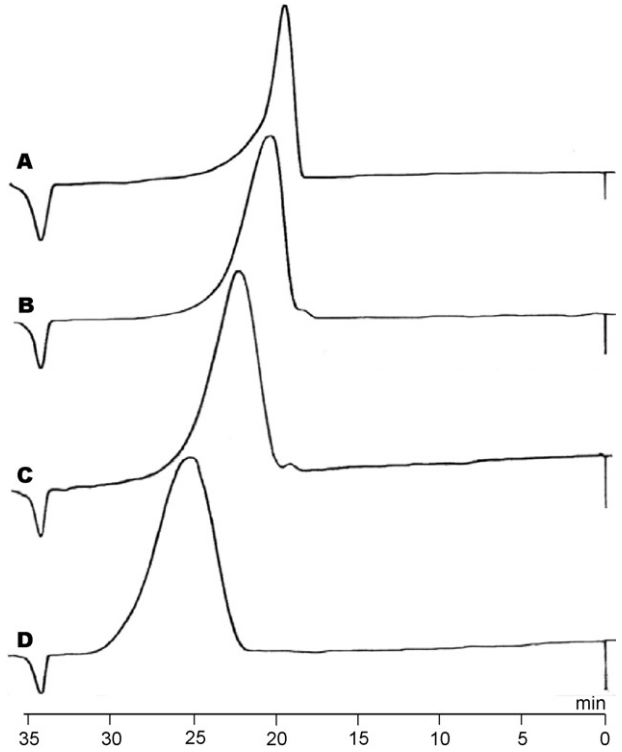

Fig. 1. Aqueous GPC profiles of galactomannan from fenugreek gum. (A) Original galactomannan with the molecular weight of $\bar{M}_n > 100 \times 10^4$, (B) with $\bar{M}_n = 30.0 \times 10^4$, and $[\alpha]_D^{25} = +89.0°$ (H_2O, c 1), (C) $\bar{M}_n = 16.3 \times 10^4$ and $[\alpha]_D^{25} = +87.2°$ (H_2O, c 1), and (D) $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5°$ (H_2O, c 1).

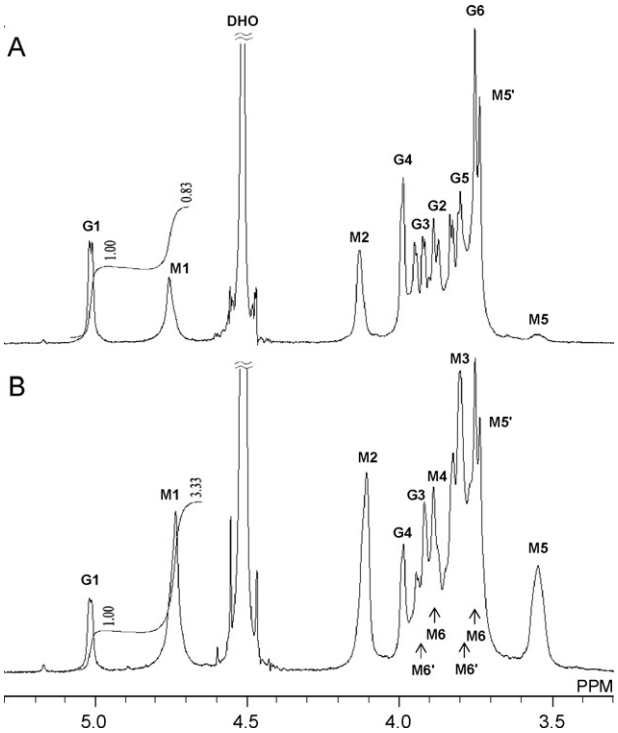

Fig. 2. 400 MHz ^{1}H NMR spectra of galactomannans (A) with $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5°$ (H_2O, c 1) from fenugreek gum and (B) with $\bar{M}_n = 0.9 \times 10^4$ and $[\alpha]_D^{25} = +20.0°$ (H_2O, c 1) from locust bean gum in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

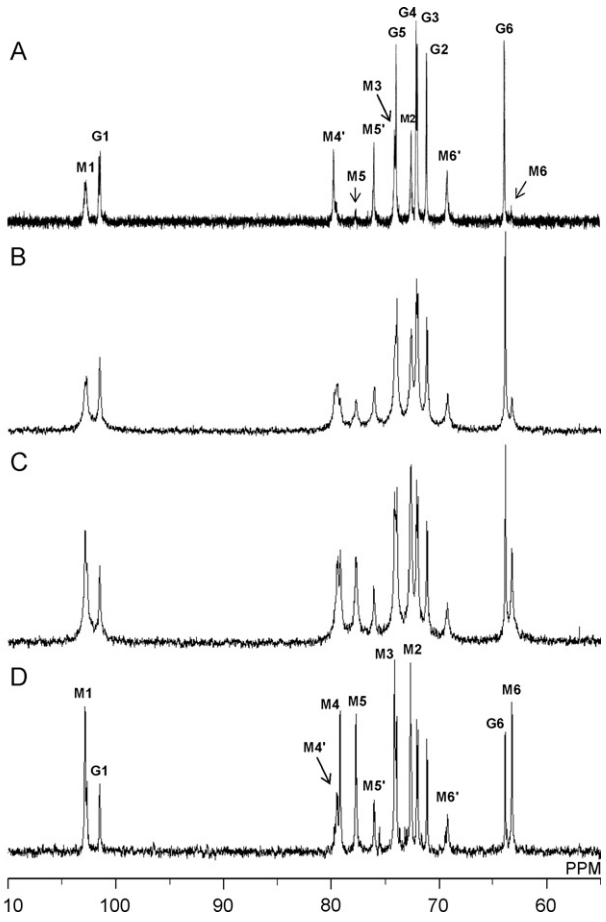

Fig. 3. 125 MHz and 100 MHz ^{13}C NMR spectra of galactomannans (A) with $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1) from fenugreek gum, (B) with $\bar{M}_n = 1.4 \times 10^4$ and $[\alpha]_D^{25} = +58.9^\circ$ (H_2O, c 1) from guar gum, (C) with $\bar{M}_n = 1.3 \times 10^4$ and $[\alpha]_D^{25} = +20.0^\circ$ (H_2O, c 1) from tara gum, and (D) with $\bar{M}_n = 0.9 \times 10^4$ and $[\alpha]_D^{25} = +10.8^\circ$ (H_2O, c 1) from locust bean gum, respectively, in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

The carbon signals of the galactomannans appeared at the same chemical shift points, and only the signal intensities were different, suggesting that the galactomannans used here had the same structure, and the only proportions of galactose and mannose residues were different. The intensity of the signal at 63 ppm increased with increasing proportions of mannose, and the signals at 63 and 69 ppm were assigned to the C6 mannose by the DQF-COSY and HSQC spectra as described in the next section. The signal at 63 ppm was assigned to C6 without an attached galactose side chain. The signal at 69 ppm, with almost the same intensity in Fig. 3A–D, was due to the C6 with an attached galactose side chain.

3.2. Assignment of proton and carbon signals by DQF-COSY and HSQC NMR measurements

Low molecular weight galactomannans were measured by the DQF-COSY and HSQC spectra in D_2O to assign the signals. Fig. 4 exhibits the 2D spectra of galactomannan from fenugreek gum. In the DQF-COSY spectrum (Fig. 4A), the H1 signals due to galactose (GH1) and mannose (MH1) residues appeared at 5.03 and 4.77 ppm, respectively. The correlation signal of the GH1–GH2 was observed to assign the GH2 signal to 3.84 ppm. The GH3 signal was exhibited at 3.93 and 3.95 ppm assigned by correlation signals of GH2–GH3. GH4 and GH5 signals were determined in a similar manner, as exhibited in Fig. 4A. The GH6 signal was

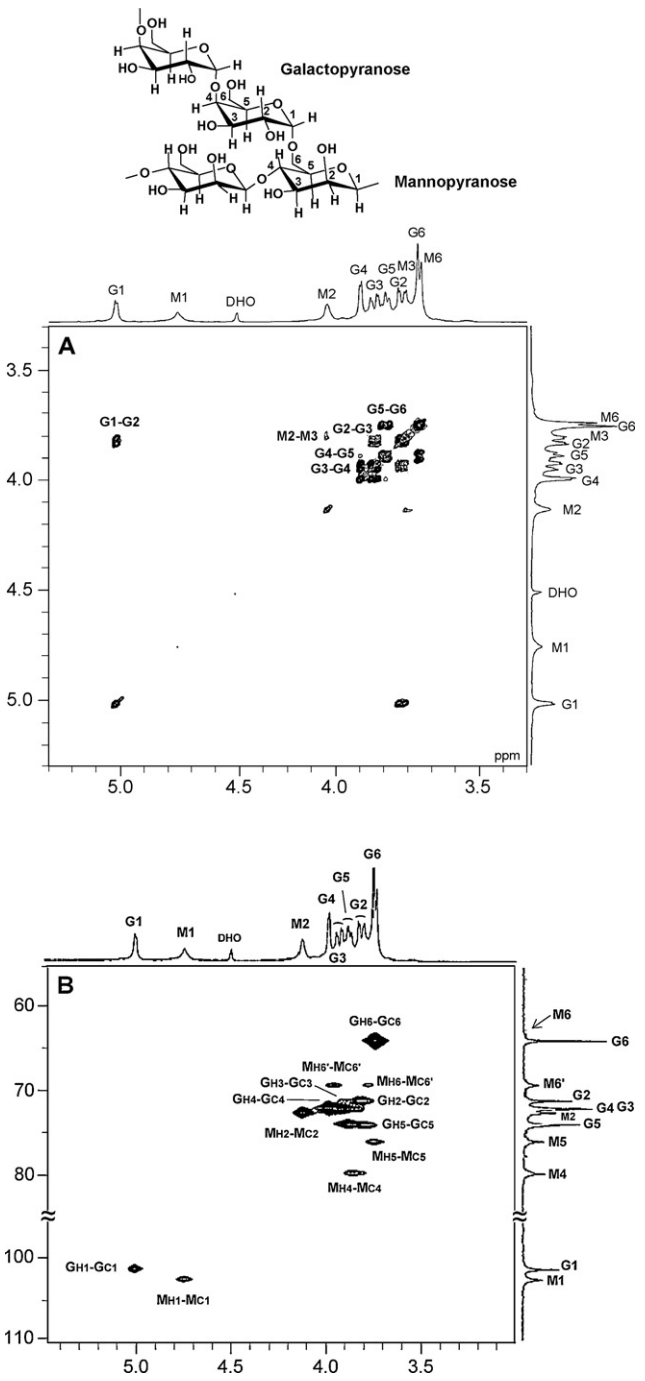

Fig. 4. DQF-COSY (A) and HSQC (B) spectra of low molecular weight galactomannans with $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1) from fenugreek gum in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

assigned by the correlation of the GH6 and GC6 signals in the HSQC spectrum (Fig. 4B). Although the MH2 and MH3 signals were found to appear at 4.14 and 3.81 ppm, respectively, by the correlation of signals of MH1–MH2 and MH2–MH3, other mannose proton signals were difficult to assign by only the DQF-COSY spectrum because of the complex peak overlapping. In the HSQC spectrum shown in Fig. 4B, the carbon signals were assigned from the corresponding proton signals. The MC6 at 63 and 69 ppm were separated into two correlation signals, respectively, of the corresponding two MH6 and MH6′ protons at 3.96 and 3.78 ppm and 3.91 and 3.75 ppm, respectively, due to the presence and absence of attached galactose side chains, as mentioned in Section 3.1. The

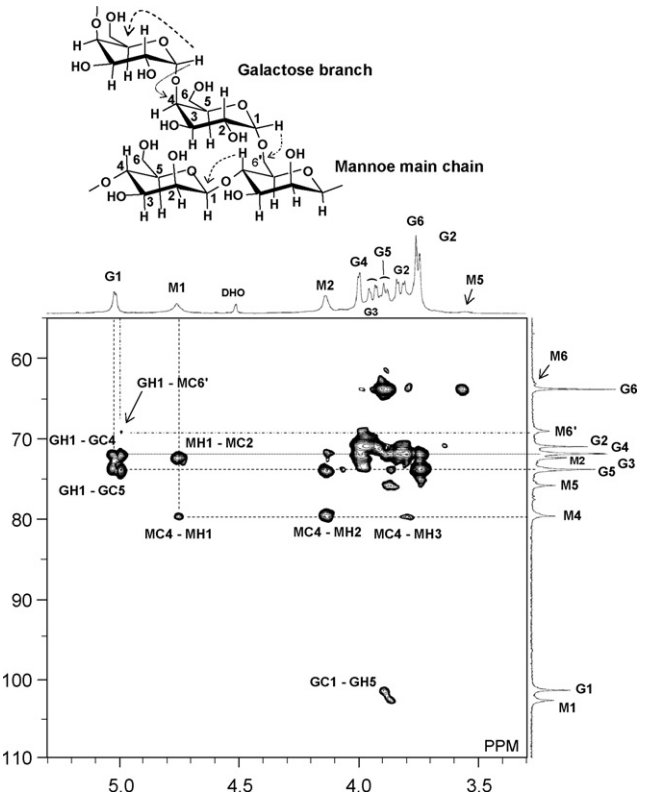

Fig. 5. HMBC spectrum of low molecular weight galactomannans with $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1) from fenugreek gum in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

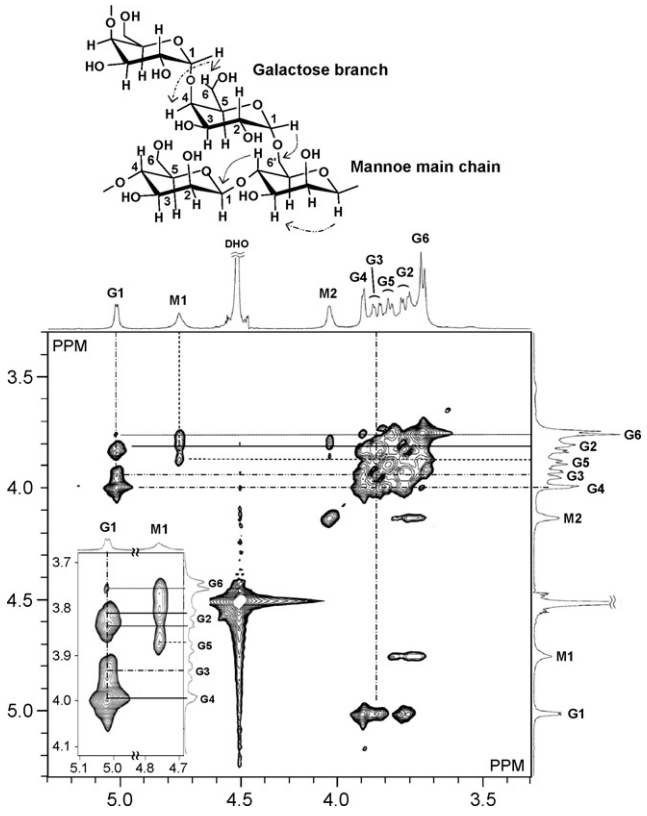

Fig. 6. ROESY spectrum of low molecular weight galactomannans with $\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1) from fenugreek gum in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

MC4 and MC5 signals were correlated to the MH4 and MH5 signals at 3.86 and 3.75 ppm in the DQF-COSY and HSQC spectra of FG, respectively. Although the galactomannan from FG gave complex ^1H and ^{13}C spectra, all signals were assigned by the decreasing molecular weight. The proton and carbon signals of other galactomannans with low molecular weights were also assigned on the basis of the FG data and by the measurements of the 2D NMR spectra.

3.3. Structural analysis of galactomannans by HMBC and ROESY NMR measurements

Because most proton and carbon signals of NMR spectra were assigned by the DQF-COSY and HSQC measurements, the whole structure of the galactomannans was elucidated by the HMBC and ROESY spectra. HMBC measurement is suitable for the determination of multiple bond connectivity and ROESY for the analysis of protons that locate near each other by use of NOE. Fig. 5 gives the HMBC spectrum of galactomannan ($\bar{M}_n = 2.7 \times 10^4$ and $[\alpha]_D^{25} = +90.5^\circ$ (H_2O, c 1)) from FG. The correlation signals between GH1–GC5 through the G1–O–G5 oxygen and GH1–GC3 and GC1–GH5 in the one galactopyranose residue, and the GH1–GC4 due to the two neighboring galactopyranose residues through the G1–O–G4 oxygen appeared successively on the GH1 and GC1 tracks, respectively. The appearance of the correlation signals between two galactose residues revealed that more than two galactose residues were glycosylated from the main chain mannose were glycosylated in addition to the single galactopyranose side chains. In addition, the long-range correlation signals between GH1 and MC6 through the G1–O–M6 oxygen appeared on the GH1 track, suggesting that galactose branches were attached at the C6 carbon of the main chain mannose.

The correlation signals between MH1–MC4, and MC1–MH4 on the MH1 and MC1 tracks appeared clearly, respectively, due to the long-ranged correlation signals through the M4–O–M1 oxygen between two neighboring mannopyranose residues, indicating that mannose has a 1 → 4-linked pyranoside structure. In addition, the specific rotations of galactomannans decreased with increasing proportions of mannose residues, as demonstrated in Table 1, revealing that mannopyranose residues were glycosylated by the β-linkage in the main chain.

Further structural analysis of galactomannans was carried out by ROESY, and the spectra are represented in Figs. 6(FG) and 7(LG), in which the proportions of galactose and mannose in FG were 1.0 and 0.83 and LG were 1.0 and 3.33, respectively, according to the results of the ^1H NMR spectra as shown in Fig. 2. In Fig. 6, the NOE correlation signals between GH1–GH2, –GH3, –GH4, and –GH6 of FG appeared clearly on the GH1 track; however, the correlation signal due to GH1–GH5 was not observed because the distance between the two protons was long, according to the pyranose structure. On the other hand, correlation signal between GH1–GH6 protons due to the neighboring two galactose residues appeared, suggesting that these two protons were located closely. From the HMBC and ROESY spectra of FG galactomannan, galactose was attached at the C6 of the main chain mannopyranose with the α-linkage. In the ROESY spectrum (Fig. 7) of LG, which has a large proportion of mannose residues, NOE correlation signals between MH1–MH2, –MH3, and –MH5, and MH2–MH3 and –MH5, and MH5–HM6′ in the one mannose residue, and between MH1–MH4 between the neighboring two mannose residues were observed. However, NOE correlation signals between MH1–MH6 did not appear clearly because the two protons were spatially very distant. These results of the 2D NMR also suggest that the mannopyranose in the main chain was glycosylated by the (1 → 4)-β-linkage.

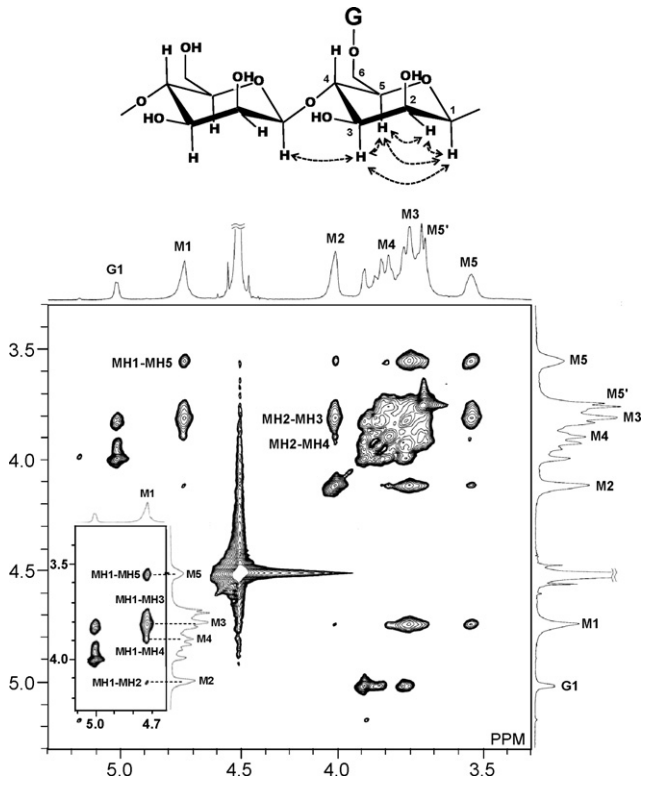

Fig. 7. ROESY spectrum of low molecular weight galactomannans with $\bar{M}_n = 0.9 \times 10^4$ and $[\alpha]_D^{25} = +10.8^\circ$ (H_2O, c 1) from locust bean gum in D_2O at 50 °C. M, mannose; G, galactose; ′, mannose or galactose attached sugar signal.

In conclusions, the NMR signals of galactomannans with different proportions of galactose and mannose residues from several seeds were assigned by the DQF-COSY and HSQC 2D NMR measurements. The linkages and whole structures of the galactomannans were revealed by the combination of HMBC and ROESY spectroscopies using the long-range and NOE correlations. Appearance of galactose side chain GH1–O–GC4 and GH1–O–MC6 correlation signals in HMBC spectrum and the coupling constant $J_{GH1,2} = 3.4$ Hz suggested that at least two galactose residues were glycosylated by a α-linkage from the C6 position of main chain mannose. This result was supported by the NOE correlation (ROESY) signals. Main chain mannose should have β-linkage because of the appearance of MH1–O–MH4 NOE correlation signal between two mannose residues and decrease of specific rotations with decreasing proportion of galactose side chains. After sulfation of the galactomannans, the relationship between the branched structure and biological activities, such as antiviral and blood anticoagulant activities, is under investigation.

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References

Anderson, E. (1949). Endosperm mucilages of legumes. *Industrial and Engineering Chemistry*, *41*, 2887–2890.
Andrews, P., Hough, L. & Jones, J. K. N. (1952). Mannose-containing polysaccharides. Part II. The galactomannan of fenugreek seed (*Trigonella foenum-graecum*). *Journal of the Chemical Society*, 2744–2750.
Daoud, K. M. (1932). The reserve polysaccharide of the seeds of fenugreek: Its digestibility and its fate during germination. *Biochemical Journal*, *26*, 255–263.
Dea, I. C. M. & Morrison, A. (1975). Chemistry and interactions of seed galactomannans. *Advances in Carbohydrate Chemistry and Biochemistry*, *31*, 241–312.
Han, S., Kanematsu, Y., Hattori, K., Nakashima, H. & Yoshida, T. (2009). Ring-opening polymerization of benzylated 1,6-anhydro-β-D-lactose and specific biological activities of sulfated (1 → 6)-α-D-lactopyranans. *Journal of Polymer Science. Part A. Polymer Chemistry*, *47*, 913–924.
Hatakeyama, T., Naoi, S. & Hatakeyama, H. (2004). Liquid crystallization of glassy guar gum with water. *Thermochimica Acta*, *416*, 121–127.
Lu, R., Hattori, K., Yoshida, T., Nakashima, H., Premanathan, M., Aragaki, R., et al. (2000). Specific biological activities of Chinese lacquer polysaccharides. *Carbohydrate Polymers*, *43*, 47–54.
Lu, R., Hattori, K., Yoshida, T., Yang, J., Du, Y., Miyakoshi, T., et al. (1999). Structural analysis of polysaccharide in Chinese lacquer by high resolution NMR spectroscopy. *Sen'i Gakkaishi*, *55*, 47–56.
Lu, R., Yoshida, T. & Uryu, T. (1999). Viscosity behaviors of Chinese urushi polysaccharide in aqueous solutions. *Sen'i Gakkaishi*, *55*, 402–407.
Lu, R. & Yoshida, T. (2003). Specific structure and molecular weight changing of Asian lacquer polysaccharides. *Carbohydrate Polymers*, *54*, 419–424.
McDougall, G. J., Morrison, I. M., Stewart, D. & Hillman, J. R. (1996). Plant cell walls as dietary fiber: Range, structure, processing and function. *Journal of the Science of Food and Agriculture*, *70*, 133–150.
Ramesh, H. P., Yamaki, K., Ono, H. & Tsushida, T. (2001). Two-dimensional NMR spectroscopic studies of fenugreek (*Trigonella foenum-graecum* L.) galactomannan without chemical fragmentation. *Carbohydrate Polymers*, *45*, 69–77.
Srivastava, M. & Kapoor, V. P. (2005). Seed galactomannans: An overview. *Chemical Biodiversity*, *2*, 295–317.
Stéphane, J., Debon, J. & Tester, R. F. (2001). In vitro binding of calcium, iron and zinc by non-starch polysaccharides. *Food Chemistry*, *73*, 401–410.
Tanaka, R., Hatakeyama, T. & Hatakeyama, H. (2004). Formation of locust bean gum hydrogel by freezing–thawing. *Polymer International*, *45*, 118–126.
Tegshi, M., Han, S., Kanamoto, T., Nakashima, H. & Yoshida, T. (2011). Synthesis and specific influenza A virus-adsorptive functionality of alkyl curdlan sulfate-coated membrane filter. *Journal of Polymer Science. Part A: Polymer Chemistry*, *49*, 3241–3247.
Vieira, M. C. & Gil, A. M. (2005). A solid state NMR study of locust bean gum galactomannan and Konjac glucomannan gels. *Carbohydrate Polymers*, *60*, 439–448.
Yoshida, T. (2001). Synthesis of polysaccharides having specific biological activities. *Progress in Polymer Science*, *26*, 379–441.
Yoshida, T. (2005). Synthetic and natural polysaccharides having specific biological activities in polysaccharides. In S. Dumitriu (Ed.), *Structural diversity and functional versatility*. New York: Marcel Dekker Inc.
Yoshida, T., Yasuda, Y., Uryu, T., Nakashima, H., Yamamoto, N., Mimura, T., et al. (1994). Synthesis and in vitro inhibitory effect of L-glycosyl-branched curdlan sulfates on AIDS virus infection. *Macromolecules*, *27*, 6271–6276.