Carbohydrate Polymers 90 (2012) 827–832

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Isolation and structural characterization of the water-extractable polysaccharides from *Cassia obtusifolia* seeds

Mingsheng Shang, Xiaoman Zhang, Qun Dong*, Jian Yao, Qin Liu, Kan Ding**

Glycochemistry and Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 14 February 2012
Received in revised form 1 June 2012
Accepted 7 June 2012
Available online 15 June 2012

Keywords:
Cassia obtusifolia
Seeds
Structure
Galactomannan
Polygalacturonic acid

ABSTRACT

The seed of *Cassia obtusifolia* is a food or herbal medicine used for improving eyesight, treating constipation and other disorders, and polysaccharides have been implicated in these pharmacological activities. The endosperm of the seeds, *Cassia* gum, is a commercial thickening or gelling agent, composed mainly of galactomannans. However, the whole seeds of *C. obtusifolia*, rather than the endosperm, are used in folk medicine or food, which might contain more complex constituents of polysaccharides. In this study, the whole seeds of *C. obtusifolia* were extracted with boiling water, and from the water extract, three homogeneous fractions were isolated, designated CFAA-1, CFAA-3, and CFBB2, respectively, after treatment with Fehling solution followed by anion-exchange and gel permeation chromatography. Using chemical and spectroscopic methods, CFAA-1, and CFAA-3 were elucidated to be both branched galactomannans with different molecular weights, consisting of 1,4-linked β-D-mannopyranosyl backbone with single-unit α-D-galactopyranosyl branches attached to O-6 of mannose, while CFBB2 was shown to be a linear (1 → 4)-α-polygalacturonic acid.

1. Introduction

Cassia obtusifolia L. is an annual herb (family: Leguminosae), whose dried whole seeds are used in China as a drink or a folk medicine. The seeds are reported to have the effects of improving eyesight, alleviating constipation, and lowering hypertension and hyperlipidemia (Hao, Sang, & Zhao, 2001). In recent years, there are many studies on its chemical constituents and pharmacological activities, particularly on the anthraquinones and their neuroprotective effects (Drever et al., 2008; Ju et al., 2010; Kim et al., 2011; Li, Xiao, Li, Zhang, & Pang, 2009; Wu et al., 2011). The endosperm flour of *C. obtusifolia* or *Cassia tora* seeds, after removing the husk and germ, is *Cassia* gum, which is used widely as thickener or gelling agent. It was reported that the gum contains more than 75% of galactomannans (Hallagan, La Du, Pariza, Putnam, & Borzelleca, 1997). Wu and Abbott (2005) reported that gum content and monosaccharide composition varied with particle size after fine grinding and sieving. The galactomannan can be chemically modified for hair conditioning (Staudigel et al., 2007). However, the galactomannan from the endosperm probably could not account for all the polysaccharides in *C. obtusifolia* seeds used in a traditional Chinese medicine since the whole seeds rather than the husk-removed ones are used. Therefore, it is possible that the polysaccharides other than galactomannan are responsible for the pharmacological effects of the seeds. In order to get a full understanding of the types and structural features of the polysaccharides in *C. obtusifolia* seeds, we isolated and characterized the water-extractable polysaccharides from the whole seeds of *C. obtusifolia*.

2. Experimental

2.1. Material

The dried whole seeds of *C. obtusifolia* were purchased as unprocessed crude drug from Shanghai Xuhui TCM Slices Co. Ltd., and the original plants were collected from Anhui Province. DEAE-cellulose 32 was purchased from Whatman International Ltd. (Bradstone, England), Sephacryl S-300 HR from Amersham Biosciences AB (Uppsala, Sweden), and Bio-Gel P-2 from Bio-Rad Laboratories, Inc. (Hercules, USA). L-Arabinose, D-galactose, D-mannose, D-galacturonic acid, sodium borohydride, iodomethane, and N-cyclohexyl-N′-(2-morpholino-ethyl)-carbodiimidemethyl-*p*-toluene-sulfonate (CMC) were obtained from Fluka Chemie GmbH (Buchs, Switzland). Trifluoroacetic acid (TFA), dimethyl sulfoxide (DMSO) and PEI-cellulose precoated TLC plate were obtained from Merck KGaA (Damstardt, Germany). Dialysis tubes (Membra-cei^{TM}, MWCO 3500) were purchased from Sino-American Biotechnology Co. (SABC, Shanghai). Other reagents are

---

* Corresponding author. Tel.: +86 21 50806600x3203; fax: +86 21 50806928.
** Corresponding author. Tel: +86 21 50806928; fax: +86 21 50806928.
E-mail addresses: qdong@mail.shcnc.ac.cn (Q. Dong), kding@mail.shcnc.ac.cn (K. Ding).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.06.007

all analytical grade from Shanghai Chemical Reagent Company unless claimed otherwise.

2.2. General methods

IR spectra were determined with a Perkin-Elmer 591B spectrophotometer as KBr pellets. Optical rotations were determined with a Perkin-Elmer 241M digital polarimeter. NMR spectra were recorded on a Varian Mercury 400 NMR spectrometer. DEPT experiments were carried out using a polarization transfer pulse of 135°. Gas chromatography (GC) was performed with a Shimazu-14B apparatus equipped with a 3% OV-225/AW-DMCS-Chromosorb W column (2.5 m × 3 mm), with N_2 as the carrier gas at a flow rate of 25 ml/min. The column temperature was kept at 210 °C for sugar composition analysis and at 190 °C for methylation analysis. GC–MS was performed on a Finnigan Trace/DSQ instrument, equipped with an HP-5 capillary column. The initial temperature was 120 °C, increased to 250 °C at the gradient of 1.5 °C/min, and kept for 5 min. The neutral carbohydrate and uronic acid were determined with phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and *m*-hydroxyl diphenyl method (Blumencrantz & Asboe-Hansen, 1973), using D-glucose and D-glucuronic acid as the standards with appropriate correction by response factors, respectively. Protein was determined with Lowry method (Bensadoun & Weinstein, 1976).

2.3. Isolation and purification of polysaccharides

The dried seeds of *C. obtusifolia* (2.0 kg) were defatted twice by soaking in 95% ethanol (10 l) for 5 days at room temperature (25 °C). After filtration, the residue was dried at ambient atmosphere, and then extracted 5 times with boiling water, each for 5 h. The extracts were concentrated, and dialyzed against running water for 2 days. The retentate was centrifuged, and to the supernatant were added 3 volumes of 95% ethanol under vigorous stirring. After standing overnight at 4 °C, the precipitate was obtained by centrifugation, washed successively with absolute ethanol and acetone, and dried in vacuum, to give the water-extracted crude polysaccharide COA (152 g, 7.6%).

Treatment with Fehling solution was performed as described (Jones, 1965). Briefly, to the solution of COA (2 g in 100 ml H_2O), Fehling solution was added dropwise with rigorous stirring until no more precipitate formed. The suspension was stirred at room temperature for 4 h, and then centrifuged. The precipitate was washed with deionized water, then macerated for 0.5 h with 5% (v/v) hydrochloric acid in ethanol and filtrated on a sintered glass funnel, washed with ethanol until the filtrate was free of chloride ion. The pellet was dried in vacuum to give CFA (0.66 g, yield 33%). The supernatant was neutralized with HOAc, dialyzed, concentrated, and precipitated with 3 volumes of ethanol, to give CFB (0.68 g, yield 34%).

CFA (10.1 g) was fractionated by anion-exchange chromatography on a DEAE-cellulose column (5 cm × 60 cm, Cl^- form), which was eluted stepwise with water, 0.2, 0.4, and 0.8 M NaCl, and monitored with phenol-sulfuric acid method, to give 3 fractions, designated CFAA (6.03 g, 59.8%), CFAB1 (112 mg, 1.1%), and CFAB2 (78 mg, 0.8%) from water, 0.2, and 0.4 M NaCl elution, respectively. No carbohydrate was detected in 0.8 M NaCl eluate. CFAA (1.06 g) was purified on a Sephacryl S-300 column, equilibrated and eluted with 0.2 M NaCl, giving CFAA-1 (166 mg, 15.7%), CFAA-2 (153 mg, 14.4%), and CFAA-3 (168 mg, 15.9%). CFB (5 g) was also fractionated into three fractions by using the DEAE-cellulose column as described above, designated as CFBA (397 mg, 7.9%), CFBB1 (276 mg, 5.5%), and CFBB2 (890 mg, 17.8%).

2.4. Homogeneity and molecular weight

The homogeneity and molecular weight of polysaccharides were estimated by HPGPC method (Alsop & Vlachoogiannis, 1982; Wei & Fang, 1989). The HPLC instrument was equipped with series-connected Ultrahydrogel™ 2000 and Ultrahydrogel™ 500 columns, eluted with 0.045 M phosphate buffer at a flow rate of 0.5 ml/min. The column was calibrated by molecular weight-known standard Dextrans (T-700, 580, 300, 110, 80, 70, 40, 9.3 and 4, Pharmacia). The column temperature was kept at 30.0 ± 0.1 °C. All samples were prepared as 0.2% (w/v) solutions, and 20 μl of solution was analyzed in each run.

2.5. Intrinsic viscosity [η] and viscosity average molecular weight (Mv)

The intrinsic viscosity was measured at 25 °C by using an Ubbelohde capillary viscometer. The polysaccharide was dissolved in distilled water in an original concentration of 2 mg/ml. The kinetic energy correction was negligible. [η] was estimated using the Huggins and Kraemer equations by extrapolation to infinite dilution as follows:

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c$$

$$\ln \eta \frac{r}{c} = [\eta] - k''[\eta]^2 c$$

where η_{sp}/c is the reduced viscosity; $\ln \eta r/c$, the inherent viscosity; c, sample concentration; k' and k'', constants for a given polymer under given condition in a given solvent.

Viscosity average molecular weights, Mv, were calculated using the established Mark–Houwink relationship for galactomannans, taking into account the ratios of M/G in galactomannans (Cerqueira et al., 2009).

$$[\eta] = 11.55 \times 10^{-6}[(1-\alpha) \times \mathrm{Mv}]^{0.98}$$

where $\alpha = 1/[(\mathrm{M/G}) + 1]$ and $[\eta]$ is expressed in dl/g.

2.6. Glycosyl composition analysis (Blakeney, Harris, Henry, & Stone, 1983)

The polysaccharide sample (2 mg) was hydrolyzed with 2 M TFA (2 ml) at 110 °C for 2 h in a sealed test tube. TFA was removed under reduced pressure by repeated evaporation with methanol. An aliquot of the hydrolysate was analyzed by TLC on a Pei-cellulose plate, developed with EtOAc–pyridine–HOAc–H_2O (5:5:1:3, v/v). The other part was dissolved in 2 ml of H_2O, and reduced with 25 mg of $NaBH_4$ at room temperature for 2 h. The boric acid was removed by repeated evaporation under reduced pressure with methanol after neutralization with acetic acid. After acetylation with acetic anhydride, the alditol acetates was extracted with chloroform and analyzed by GC.

For uronic acid-containing polysaccharides, the carboxyl reduction of uronic acid was performed according to Conrad procedure (Taylor & Conrad, 1972) before acid hydrolysis and derivatization.

2.7. Methylation analysis

The polysaccharides were methylated thrice with the modified method of Ciucanu as described by Needs and Selvendran (1993). The permethylated polysaccharide was depolymerized with 90% formic acid at 100 °C for 4 h, followed by hydrolysis with 2 M TFA at 100 °C for 4 h. The hydrolyzate was then converted into the partially methylated alditol acetates and analyzed by GC–MS (Sweet, Shaprio, & Albersheim, 1975).

Table 1
Yields, properties and glycosyl composition of the polysaccharides isolated from water-extract of *C. obtusifolia* seeds.

Fractions	Yields (%)	[η] (ml/g)	Mw (kDa)	Mv (kDa)	UA[b] (%)	Glycosyl compositions (mol%)			
						Xyl	Man	Gal	GalA
CFAA-1	3.1	96	81.8	133	–	–	78.3	21.7	–
CFAA-3	3.1[a]	29	19.0	38.2	–	–	80.3	19.7	–
CFBB2	6.1[a]	21	18.5		85.4	2.6	–		97.4

[a] Yields in relative to COA.
[b] UA: uronic acid content determined by *m*-hydroxyl diphenyl method.

For polysaccharides not soluble in DMSO (CFAA-1 and CFAA-3), the samples were first methylated with Haworth procedure (Cerezo, 1973), and the resulting partially methylated polysaccharide got readily soluble in DMSO, and could be methylated as described above.

2.8. Partial acid hydrolysis

CFAA-1 (100 mg) was dissolved in 0.2 M TFA (20 ml) and hydrolyzed at 100 °C for 1 h. After evaporation with methanol to remove TFA, the residue was dialyzed against deionized water (2 l × 4). The retentate was freeze-dried to give the degraded polysaccharide, FA1-D (50 mg). CFAA-3 (100 mg) was partially depolymerized as described above, to give the degraded polysaccharide FA3-D (50 mg).

2.9. NMR analysis

The polysaccharides (30 mg) were deuterium-exchanged and dissolved in 0.5 ml of D_2O (99.9% D). The ^{13}C, ^{1}H NMR, heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra were measured at room temperature, with acetone as the internal standard at 31.50 ppm.

3. Results and discussion

3.1. Isolation and purification of polysaccharides

From 2 kg of dried seeds of *C. obtusifolia*, COA (152 g, 7.6% of the material) was obtained as the water-extractable crude polysaccharide. At first, we tried to fractionate COA by anion-exchange chromatography on a DEAE-cellulose column; however this did not afford a successful separation of different polysaccharides, according to the sugar analyses of COA and its sub-fractions from anion-exchange chromatography (data not shown). It was probable that other types of negatively charged polysaccharides might associate with galactomannan and could be eluted by NaCl elution during anion-exchange chromatography. The tendency of galactomannan to associate easily with other types of polysaccharides, such as xanthan, carrageenan, has been reported (Bresolin, Milas, Rinaudo, & Ganter, 1998). In order to overcome this difficulty, we used Fehling solution to selectively precipitate the galactomannan in COA (Jones, 1965). After treatment with Fehling solution, the resulting CFA and CFB were fractionated separately by chromatography on DEAE-cellulose and Sephacryl S-300 columns. As a result, CFAA-1 and CFAA-3 were obtained from CFA, while CFBB2 from CFB, as the major fractions.

3.2. Structural investigation of the galactomannans CFAA-1 and CFAA-3

CFAA-1 and CFAA-3 were both purified by gel permeation chromatography from CFAA, the water-eluted fraction from CFA upon anion-exchange chromatography. HPGPC analysis showed both CFAA-1 and CFAA-3 gave one symmetrical peak, indicating their homogeneity in molecular weight distribution. Their average molecular weights were estimated to be 81.8 and 19 kDa, and the specific rotation $[\alpha]_D^{23}$ were +14.2° and +10.6° (c 0.5, H_2O), respectively. They were both free of protein, as indicated by a negative Lowry reaction. Their intrinsic viscosity was determined and given in Table 1. Compare to literature value (8–12 dl/g) for similar galactomannans from other sources (Cerqueira et al., 2009), they have lower viscosity, which could be accounted for by their lower molecular weights. Their IR spectra showed similar absorptive pattern, and thus only the IR of CFAA-1 was given (Fig. 1), with characteristic absorptions for a polysaccharide, at 3405, 2927, 1379, and 1028 cm^{-1}, originating from O-H, C-H, out-plane and in-plane C—O stretching vibrations, respectively.

After complete hydrolysis with 2 M TFA, TLC analysis of the hydrolysate of CFAA-1 detected no uronic acid. GC analysis as alditol acetates showed that CFAA-1 contains Man and Gal, in the molar ratio of 3.6:1.0, suggesting that CFAA-1 is a galactomannan, which has been known to be the main component of *Cassia* gum. Similarly, CFAA-3 contains Man, Gal, in the ratio of 4.1:1.0, and no uronic acid, suggesting that CFAA-3 is also a neutral galactomannan. Both CFAA-1 and CFAA-3 showed absorption at 890 cm^{-1} in their IR spectra, which was characteristic for the pyranosyl ring vibration of β-Man*p* (Jiang, 1994). The results above suggested a high similarity between the structures of CFAA-1 and CFAA-3, and this is appreciable considering the fact that they were both separated from CFAA by gel permeation chromatography on a Sephacryl S-300 column. As a consequence, they have different molecular weights, and showed a slight difference in Gal/Man ratios.

Linkage analysis for CFAA-1 showed that galactosyl residues are present exclusively at nonreducing terminals, while mannosyl residues are 1,4-, or 1,4,6-linked (Table 2). It could thus be proposed that the backbone of CFAA-1 contains 1,4-linked mannopyranosyl residues, with galactosyl branches attached to O-6 of the backbone. For CFAA-3, methylation analysis identified terminal Gal, 1,4-, 1,4,6-linked Man, in molar ratio of 1.0:2.8:1.0. Partial acid hydrolysis of CFAA-1 and CFAA-3 gave FA1-D and FA3-D, respectively, as the degraded polysaccharides. Methylation analysis of FA1-D and FA3-D showed that the amount of terminal Gal and 1,4,6-linked Man decreased, while that of 1,4-linked Man increased, indicating

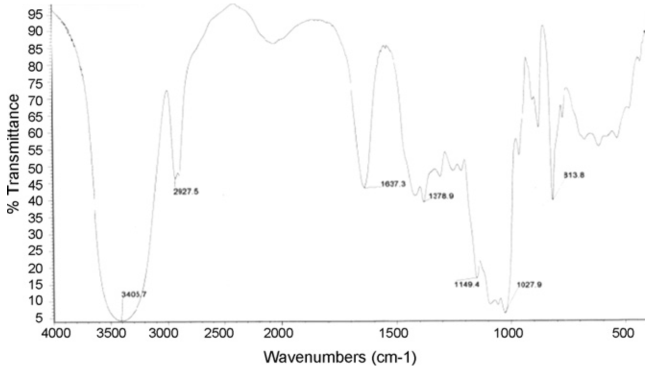


Fig. 1. IR spectrum of CFAA-1.

Table 2
Linkage analysis of CFAA-1, CFAA-3 and the degraded polysaccharides derived from partial hydrolysis.

Methylated sugars	Linkages	Molar ratios (%)			
		CFAA-1	FA1-D	CFAA-3	FA3-D
2,3,4,6-Me_4-Gal	Terminal	24.1	17.9	20.5	15.9
2,3,6-Me_3-Man	1,4-Man*p*	51.0	63.8	58.7	65.3
2,3-Me_2-Man	1,4,6-Man*p*	24.9	18.3	20.8	18.8

that Gal is attached to O-6 of Man. Therefore, CFAA-1 and CFAA-3 proved to have typical galactomannan structure, that is, 1,4-linked mannosyl backbone and single-unit galactosyl branches attached to O-6 of mannose.

The ^{1}H and ^{13}C NMR spectra were assigned according to the single-bond H/C correlation in HSQC (Fig. 2) and literature values (Ishrud, Zahid, Zhou, & Pan, 2001). The two anomeric protons at δ 5.08 and 4.79 ppm could be assigned to α-Gal*p* and Man*p*, respectively. In the ^{13}C NMR spectrum, the two anomeric resonances at δ 101.43 and 101.22 ppm corresponded to 1,4- and 1,4,6-linked Man*p*, respectively, whereas the resonance at δ 99.99 ppm was assigned to terminal α-Gal*p*. Gated decoupling experiment was performed to determine the $^1J_{C-H}$, and the result showed mannose of CFAA-1 has a $^1J_{C-H}$ 159.3 Hz, corresponding to β-Man ($^1J_{C-H}$ 160 Hz). The reversed resonances at δ 61.79 ppm and δ 67.65 ppm in DEPT spectrum (not shown) undoubtedly arose from C-6 of 1,4- and 1,4,6-linked Man*p*, while δ 62.51 ppm from C-6 of terminal α-Gal*p*. In HMBC (Fig. 3), the cross peak at δ 5.08/δ 67.65 showed that H1 of Gal is correlated to C6 of Man, indicating that Gal is attached at O-6 of 1,4,6-linked Man residues. The cross peak at δ 4.81/δ 77.92 showed that H1 of Man residues is correlated to C4 of neighboring Man residues, indicating a 1,4-linked mannopyranosyl backbone.

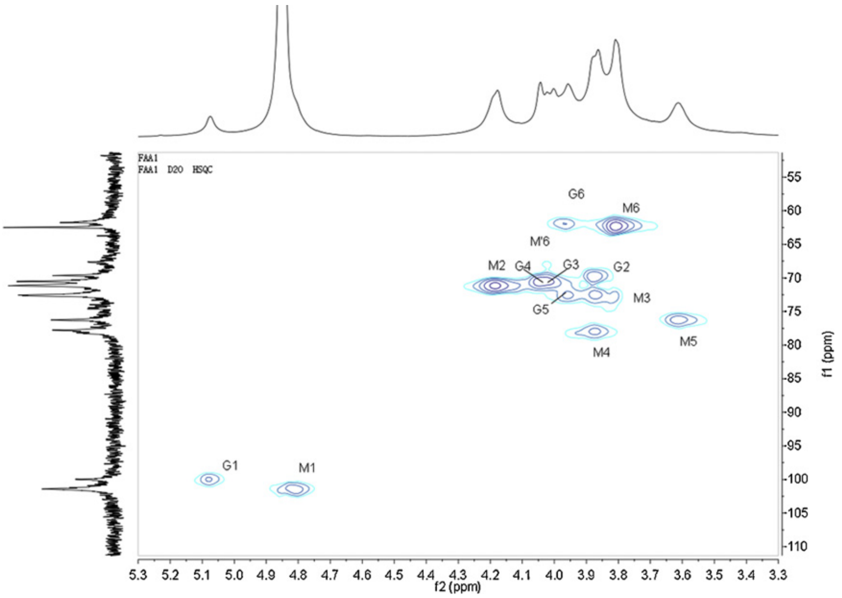


Fig. 2. HSQC spectrum of CFAA-1.

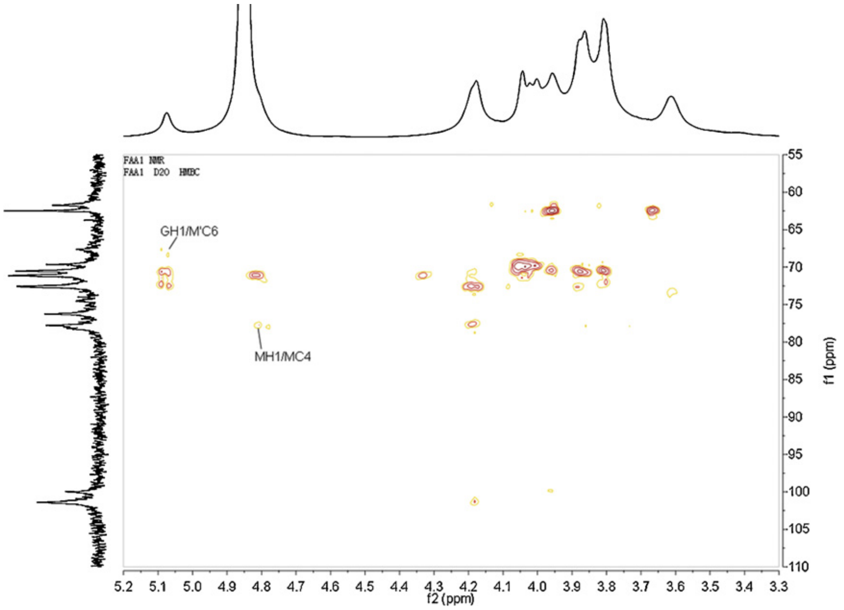
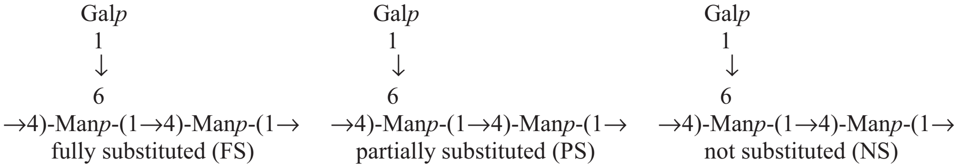


Fig. 3. HMBC spectrum of CFAA-1.

Other resonances were assigned as shown in Fig. 2. The NMR spectrum of CFAA-3 was almost the same to that of CFAA-1, and thus the data are not given here.

Taken together, both CFAA-1 and CFAA-3 were characterized to be galactomannans, containing 1,4-β-D-Man*p* backbone, and at O-6 are attached single unit α-D-Gal*p* branches. CFAA-1 showed the galactose/mannose ratio at 1.0:3.6, with approximately 1/3 of the backbone residues being substituted. In contrast, CFAA-3 has higher mannose content, less branches, and lower molecular weight. Actually, the galactose units are randomly distributed along the 1,4-linked mannan backbone, and the mannose dyads (units including two neighboring mannose residues) could be fully, partially, or not substituted (as shown below), with an approximate molar ratio of 1.0:1.5:3.0 for FS, PS, and NS in CFAA-1, as quantitated by the C-4 resonances in ^{13}C NMR (Fig. S1). Previous reports described the gum of *C. obtusifolia* contains Gal and Man in the ratio of 1:5 and a molecular weight ranging 100–300 kDa (Hallagan et al., 1997). In comparison, CFAA-1 and CFAA-3 both have relatively higher galactose proportion and lower molecular weights. The boiling water extraction and subsequent treatment with Fehling solution might lead to partial depolymerization and hence lower molecular weights.



$$\begin{array}{ccc} \text{Gal}p & \text{Gal}p & \text{Gal}p \\ 1 & 1 & 1 \\ \downarrow & \downarrow & \downarrow \\ 6 & 6 & 6 \\ \rightarrow 4)\text{-Man}p\text{-}(1\rightarrow 4)\text{-Man}p\text{-}(1\rightarrow & \rightarrow 4)\text{-Man}p\text{-}(1\rightarrow 4)\text{-Man}p\text{-}(1\rightarrow & \rightarrow 4)\text{-Man}p\text{-}(1\rightarrow 4)\text{-Man}p\text{-}(1\rightarrow \\ \text{fully substituted (FS)} & \text{partially substituted (PS)} & \text{not substituted (NS)} \end{array}$$

3.3. Structural investigation of CFBB2

CFBB2 was isolated from the supernatant CFB after Fehling reagent treatment by using anion-exchange chromatography, as 0.4 M NaCl-eluted fraction. Its homogeneity was estimated by HPGPC as shown by one symmetrical peak, corresponding to an average MW of 18.5 kDa. CFBB2 has a specific rotation $[\alpha]_D^{22}$ +192.0° (*c* 0.5, H_2O). IR spectrum (Fig. 4) shows characteristic absorption for polysaccharide at ν 3430.7, 2940.9, 1417.4, and 1101.2 cm^{-1} from O—H, C—H, C—O stretching. A shouldered peak at 1730 cm^{-1} from C=O stretching and the strong absorption at 1616 cm^{-1} indicated the presence of abundant uronic acid, which was further confirmed by TLC analysis after complete acid hydrolysis. The uronic acid content was 85.4% as determined by *m*-hydroxyl diphenyl method. Monosaccharide analysis showed that CFBB2 contains xylose and galacturonic acid, in the molar ratio of 2.6:97.4.

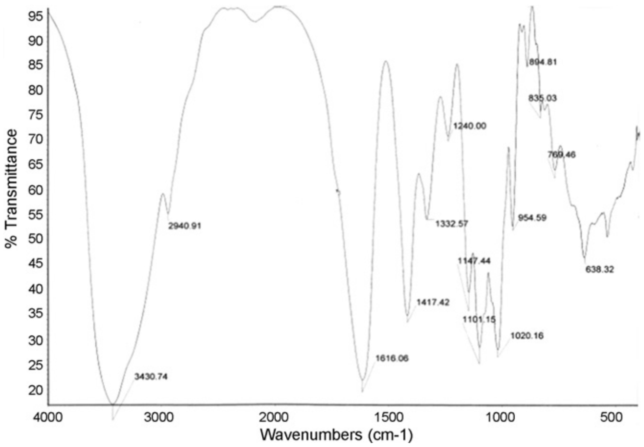


Fig. 4. IR spectrum of CFBB2.

The ^{1}H NMR spectrum CFBB2 showed only one strong anomeric signal at δ 5.06 ppm, corresponding to α-D-galacturonic acid. The α-anomeric assignment was further confirmed by the anomeric signal at δ 101.21 ppm in ^{13}C NMR (Fig. 5). Moreover, the strong carboxyl carbon resonance at 176.67 ppm and the absence of methylene signal as reversed peak in DEPT spectrum (data not shown) indicated that the polysaccharide is composed almost exclusively of galacturonic acid. The glycosyl linkage was revealed by the substituted C4 resonance at 79.09 ppm. Other carbon signals were assigned as shown in Fig. 5. HMBC spectrum (Fig. 6) showed that H1 (δ 5.06) is transglycosidically correlated to C4 (δ 79.09) and C1 (δ 101.21) is correlated to H4 (δ 4.40). These suggested that CFBB2 is a (1 → 4)-α-polygalacturonic acid, which is a typical pectic polysaccharide, also named homogalacturonan (HG). This is the first time HG was isolated from the seeds of *C. obtusifolia*. The galacturonosyl residues in HGs are often reported to be partially methyl esterified or O-acetylated (Mohnen, 2008), but no O-methyl or O-acetyl group was observed in CFFB2. However, whether the homogalacturan in the seeds of *C. obtusifolia*, in native state, is methyl esterified or acetylated remained to be determined since CFFB2 was isolated after treatment with Fehling solution, and the O-methyl or O-acetyl groups might be removed under the alkaline condition.

3.4. Conclusion

In this study, three homogeneous polysaccharide fractions were isolated from the water extract of the whole seeds of *C. obtusifolia* and characterized, among which, two are neutral galactomannans, and the other one is a polygalacturonic acid. As typical galactomannans, CFAA-1 and CFAA-3 both contain a 1,4-linked β-D-Man*p* backbone and single-unit α-D-Gal*p* branches substituted at O-6. They showed minor difference in structural features, such as glycosyl composition, molecular weight, and degree of branching.

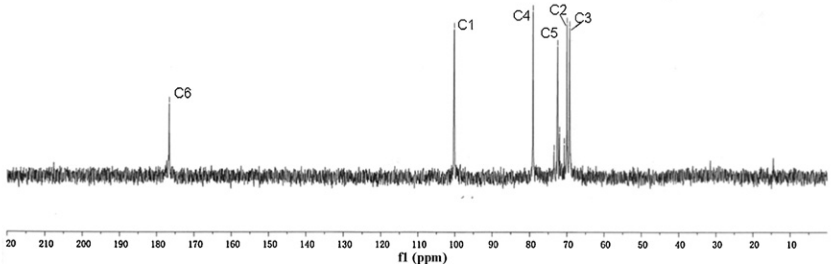


Fig. 5. ^{13}C NMR spectrum of CFBB2.

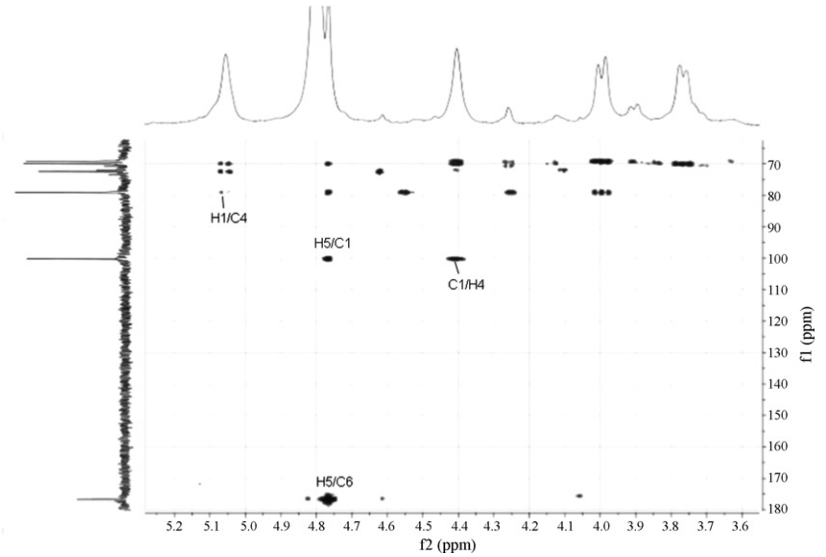


Fig. 6. HMBC spectrum of CFBB2.

Compared to the galactomannans (1:5 for Gal/Man) from *Cassia* gum in previous report (Hallagan et al., 1997), CFAA-1 and CFAA-3 showed higher degree of galactosyl substitution and lower molecular weight. CFBB2 is a polygalacturonic acid, which, as a typical pectic polysaccharide, might be a component of the husk of the seeds. To the best of our knowledge, HG has not been reported from *C. obtusifolia*. Its isolation and characterization indicated that the whole seed, used as the material in folk medicine and drink, does contain other types of polysaccharides other than the galactomannan reported previously for *Cassia* gum.

Acknowledgements

The authors are grateful to Ms Hongying Jin, Mr. Songshan Shi and Prof. Shunchun Wang (Key Laboratory of Standardization of Chinese Medicines Ministry of Education) for their help with the GC–MS tests and for valuable suggestions. This research was supported by the National Science Foundation (30870551) and Key New Drug Creation and Manufacturing Program (2009ZX09501-011, 2009ZX09103-071, and 2009ZX09301-001).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.06.007.

References

Alsop, R. M., & Vlachoogiannis, G. J. (1982). Determination of the molecular weight of clinical dextran by gel chromatography on TSK PW type columns. *Journal of Chromatography*, *246*, 227–240.

Bensadoun, A., & Weinstein, D. (1976). Assay of proteins in the presence of interfering materials. *Analytical Biochemistry*, *70*, 241–250.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*, 291–299.

Blumencrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Bresolin, T. M. B., Milas, M., Rinaudo, M., & Ganter, J. L. M. S. (1998). Xanthan–galactomannan interactions as related to xanthan conformations. *International Journal of Biological Macromolecules*, *23*, 263–275.

Cerezo, A. S. (1973). The carrageenans of *Gigartina skottsbergiis*. *Carbohydrate Research*, *26*, 335–340.

Cerqueira, M. A., Pinheiro, A. C., Souza, B. W. S., Lima, A. M. P., Ribeiro, C., Miranda, C., et al. (2009). Extraction, purification and characterization of galactomannans from non-traditional sources. *Carbohydrate Polymers*, *75*, 408–414.

Drever, B. D., Anderson, W. G., Riedel, G., Kim, D. H., Ryu, J. H., Choi, D. Y., et al. (2008). The seed extract of *Cassia obtusifolia* offers neuroprotection to mouse hippocampal cultures. *Journal of Pharmacological Sciences*, *107*, 380–392.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Hallagan, J. B., La Du, B. N., Pariza, M. W., Putnam, J. M., & Borzelleca, J. F. (1997). Assessment of *Cassia* gum. *Food and Chemical Toxicology*, *35*, 625–632.

Hao, Y. J., Sang, Y. L., & Zhao, Y. Q. (2001). The advancement of the studies on the seeds of *Cassia obtusifolia*. *Chinese Traditional Herbal Drugs*, *32*, 858–859.

Ishrud, O., Zahid, M., Zhou, H., & Pan, Y. J. (2001). A water-soluble galactomannan from the seeds of *Pheonix dactylifera* L. *Carbohydrate Research*, *335*, 297–301.

Jiang, T. D. (1994). Spectroscopic methods for structural analysis of glycans. In W. J. Zhang (Ed.), *Biochemical techniques in glycoconjugates* (pp. 193–201). Hangzhou: Zhejiang University Press.

Jones, J. K. L. (1965). Fractionation with copper complexes. *Methods in Carbohydrate Chemistry*, *5*, 36–38.

Ju, M. S., Kim, H. G., Choi, J. G., Ryu, J. H., Hur, J., Kim, Y. J., et al. (2010). Cassiae semen, a seed of *Cassia obtusifolia*, has neuroprotective effects in Parkinson's disease models. *Food and Chemical Toxicology*, *48*, 2037–2044.

Kim, S. J., Kim, K. W., Kim, M. C., Kim, M. C., Jeon, Y. D., Kim, S. G., et al. (2011). The protective effect of *Cassia obtusifolia* on DSS-induced colitis. *American Journal of Chinese Medicine*, *39*, 565–577.

Li, G., Xiao, Y., Li, L., Zhang, C., & Pang, Z. (2009). Studies on chemical constituents of roasted seeds of *Cassia obtusifolia*. *China Journal of Chinese Materia Medica*, *34*, 54–56.

Mohnen, D. (2008). Pectin structure and biosynthesis. *Current Opinion in Plant Biology*, *11*, 266–277.

Needs, P. W., & Selvendran, R. R. (1993). Avoiding oxidative degradation during sodium hydroxyl/dimethyl-iodide mediated carbohydrate methylation in dimethyl sulfoxide. *Carbohydrate Research*, *245*, 1–10.

Staudigel, J. A., Bunasky, K., Gamsky, C. J., Wagner, M. S., Stump, K. J., Baker, J. M., et al. (2007). Use of quaternized cassia galactomannan for hair conditioning. *Journal of Cosmetic Science*, *58*, 637–650.

Sweet, D. P., Shaprio, R. H., & Albersheim, P. (1975). Quantitative analysis by various g.l.c. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Research*, *40*, 217–225.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of the carbodiimide-activated carboxyl groups. *Biochemistry*, *11*, 1383–1388.

Wei, Y. A., & Fang, J. N. (1989). Determination of homogeneity and molecular weights of polysaccharides using high performance permeation chromatography. *Acta Pharmaceutica Sinica*, *24*, 532–536.

Wu, Y. V., & Abbott, T. P. (2005). Gum and protein enrichment from sicklepod (*Cassia obtusifolia*) seed by fine grinding and sieving. *Industrial Crops and Products*, *21*, 387–390.

Wu, X. H., Cai, J. J., Ruan, J. L., Lou, J. S., Duan, H. Q., Zhang, J., et al. (2011). Acetylated anthraquinone glycosides from *Cassia obtusifolia*. *Journal of Asian Natural Products Research*, *13*, 486–491.