Available online at www.sciencedirect.com

SCIENCE DIRECT®

Carbohydrate Polymers 59 (2005) 393–396

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Short communication

A non-ionic water soluble seed-gum from *Parkinsonia aculeata*

Kailash Tewari[a], Vandana Singh[b,*], Purna Chandra Gupta[b]

[a]*Bipin Behari P.G. College, Jhansi, India*
[b]*Department of Chemistry, University of Allahabad, Allahabad-211002, India*

Received 11 September 2002; revised 8 June 2004; accepted 1 July 2004
Available online 9 December 2004

Abstract

A nonionic water-soluble galactomannan, having a galactose and mannose in 1:2.66 molar ratio was isolated from endosperm of the seeds. Acid catalyzed fragmentation, periodate oxidation, methylation and enzymatic hydrolysis showed that seed gum has a branched structure consisting of a linear chain of β (1→4) linked mannopyranosyl units with D-galactose side chains attached through α (1→ 6) linkage to the main chain. Hydrolysis of the fully methylated seed gum gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-Di-*O*-methyl-D-mannose in 1.00:1.66:1.01 molar ratios. Both the methylation and periodate oxidation studies indicated about 26.5% of the end groups. The significance of these results together with the findings of partial hydrolysis by acid is discussed in relation to ascertaining the structure of the repeating unit of the polysaccharide.

Keywords: Parkinsonia aculeata; Seed-gum; Galactomannan

1. Introduction

Parkinsonia aculeata (N.O.leguminoseae) is reported to be highly medicinal, whole plant is used as antipyretic, and leaves are considered to be diaphoretic and abortifacient (Hussain & Prasad, 1966). The leaves, stems and flowers contain alkaloids and steroids. The seeds are edible and contain albumin and glutelin as principal proteins. Seeds are mucilaginous and reported to contain golden colored fatty oil (Waston & Fowden, 1973) and neutral sugars (Tookey, Lohman, & Wolff, 1962).

2. Materials and methods

The seeds were collected locally and identified at the Botanical Survey of India, Allahabad, India. Solutions were concentrated at diminished pressure at 60–62°. Paper chromatography was carried out at room temperature with solvent system A, 1 butanol–ethanol–water (Hirst & Jones, 1949) (5:1:4); B, 1 butanol–2 propanol–water (Rizvi, Gupta, & Kaul, 1971) (11:6:3); C, ethyl acetate–pyridine–water (Aspinall, Begbie, & Mackay, 1962) (10:4:3); D, water–pyridine–water (Meier, 1960) (2:1:2), with detection using aniline hydrogen phthalate. I.R spectra were recorded by Perkin–Elmer model 457 infra red spectrophotometer.

2.1. Isolation of the seed-gum

Dried crushed seeds were extracted successively with light petroleum and ethanol to defat and decolorize respectively, then extracted with 1% aqueous acetic acid and extract was added slowly, with stirring to large excess of ethanol. The crude gum was collected, washed with ethanol and dried (yield 2.5/100 g).

2.2. Purification of the seed gum

The crude polysaccharide was purified through barium complexing by preparing 2.5% (w/v) solution of the gum by continuous stirring for 12 h at 60° and precipitating with saturated barium hydroxide solution. The complex was separated by centrifugation and taken in 1 M acetic acid, stirred for 8 h, centrifuged and precipitated with ethanol. It was washed with 70, 80, 90, 95% ethanol. The sample was

---

* Corresponding author.
E-mail address: singhvandanasingh@rediffmail.com (V. Singh).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.07.008

Table 1
Constituent monosaccahrides and methylated monosaccharides identified

Sl. no.	Sugar/Derivative	Constant	Observed	Reported	Reference
1.	D-Galactose	Rf (Solvent-B)	0.15	0.16	Wolform and Thompson (1962)
		m.p	163°	162–165°	Wolform and Thompson (1962)
		$[\alpha]_{\mathrm{D}}^{30}$	+80° (Water)	80° (Water)	Wolform and Thompson (1962)
	Phenylhydrazone	m.p	153°	155°	Mestler, 1963
2.	D-Mannose	Rf	0.21	0.22	Isbell and Frush (1962)
		m.p.	131°	130°	Isbell and Frush (1962)
		$[\alpha]_{\mathrm{D}}^{25}$	+14° (water)		Isbell and Frush (1962)
	Phenylhydrazone	m.p.	198°	199–200°	Isbell and Frush (1962)
3.	2,3,4,6-tetra-*O*-methyl-D-galactose	m.p.	72–73°	74°	Robertson (1934)
		$[\alpha]_{\mathrm{D}}^{32}$	+120° (*C1*,water)	+121° (water)	Robertson (1934)
	N-phenyl-galactosamine	m.p.	191°	190–194°	Smith and Montgomery (1959)
		$[\alpha]_{\mathrm{D}}^{28}$	−42° (acetone)	−77–(+)3.7°	Smith and Montgomery (1959)
4.	2,3-di-*O*-methyl-D-mannose	m.p.	107–108°	106°	Robertson (1934)
		$[\alpha]_{\mathrm{D}}^{25}$	−16° (C 1.5, water)	−15.8° (water)	Robertson (1934)
	Anilide	m.p.	136°		Hirst and Jones (1949)
5.	2,3,6-tri-*O*-methyl-D-mannose	m.p.	Syrup	Syrup	Hirst et al. (1949)
		$[\alpha]_{\mathrm{D}}^{25}$	−11° (water)	−10°(water)	Hirst, Hough, and Jones (1949)
	Hydrazide	m.p	121–131°	129–130°	Hirst and Jones (1949)

finally purified by dialysis and filtration through various Millipore membranes. The pure seed gum was non-reducing, white, amorphous material with ash content 0.44% and $[\alpha]_{\mathrm{D}}^{25}+65.5^\circ$ (water).

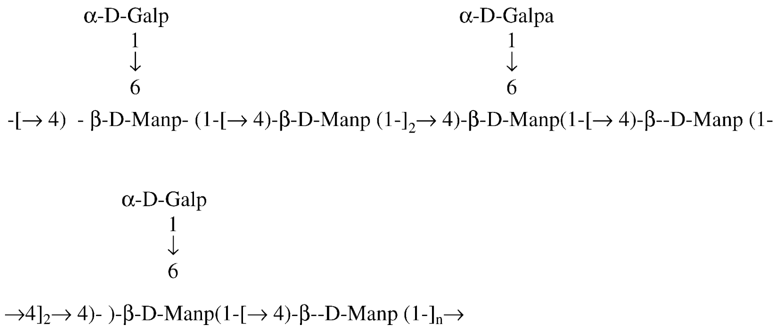
2.3. Investigation of the structure of the polysaccharide

The pure seed gum was completely hydrolyzed with 1 M trifluoroacetic acid (4 h, at 100 °C), identities and configurations of the constituent monosaccharides were confirmed by Co-chromatography with authentic samples and preparations of derivatives. (Table 1) The ratio of the constituent monosaccharides was determined by GLC (Kapoor, Chanzy Heneri & Travel, 1995). The seed gum hydrolysate was concentrated by evaporation, the residue reduced with sodium borohydride and the products acetylated with pyridine–acetic anhydride (1:1 v/v, 1 h at 100°). The resulting alditol acetates were analyzed by GLC using a model Neukon 5700 Gas Chromatograph equipped with flame ionization detector, at 190° with a Superleco S P 2380 column (3.0×0.53 mm), the carrier gas being nitrogen. The graded hydrolysis (Smith & Montgomery, 1959) of the polysaccharide was done with 25 mM H_2SO_4 at 100° for 6 h and was monitored by Paper chromatography. Metaperiodate oxidation studies (Singh, Mishra, Khare, & Gupta, 1997) revealed that 0.7830 mole of metaperiodate were consumed with the liberation of 0.1660 moles of formic acid per 100 g of the polysaccharide.

The seed gum was first methylated by Haworth's method (Haworth, 1915) followed by Hakomori method (Hakomori, 1964) to yield a fully methylated product, $[\alpha\}_{\mathrm{D}}^{25}+44^\circ$ (Chloroform). The completely methylated seed gum having no absorption at 3600–3400 cm^{-1} was boiled under reflux with 90% aqueous HCOOH for 6 h then with 1 M H_2SO_4 for 14 h at 100°. The products were fractionated on Whatman no 3 MM paper (solvent-A) to give methylated monosaccharides which were identified by their m.p, optical rotation and by making their crystalline derivatives (Table 1). GLC of the partially methylated alditol acetates (Kapoor et al., 1995), obtained by reduction with $NaBH_4$ and acetylation of the hydrolysate of methylated seed gum, showed that 2,3,4,6-tetra-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose

Table 2
Oligosaccharides identified

S.No.	Sugar/derivative	Constant	Observed	Reported	Reference
1.	Epimelibiose	m.p	199°	200°	Bailey (1965)
		$[\alpha]_{\mathrm{D}}^{32}$	(+)120.5° (C, 1 water)	(+)121° (Water	Bailey (1965)
	osazone	m.p.	172–173°	175–176°	Whistler and Durso (1951)
2.	Mannobiose	m.p.	203–205°	202–203°	Jaroslav, Miloslav, and Josef (1965)
		$[\alpha]_{\mathrm{D}}^{25}$	−9°	−8–(−)7°	Jaroslav et al. (1965)
	Phenylosazone	m.p	204°	203–206°	Jaroslav et al. (1965)
3.	Galactosylmannobiose	m.p.	227°	228-229°	Bailey (1965)
		$[\alpha]_{\mathrm{D}}^{25}$	+92–93°(C, 1 Water)	+93.3–94.4°	Bailey (1965)
4.	Mannotriose	m.p.	164°	165°	Jaroslav et al. (1965)
		$[\alpha]_{\mathrm{D}}^{25}$	−18.8° (C1.2,Water)	−16°	Jaroslav et al. (1965)



$$-[\rightarrow 4)\text{ - }\overset{\substack{\alpha\text{-D-Galp}\\ 1\\ \downarrow\\ 6}}{\beta\text{-D-Manp}}\text{- }(1\text{-}[\rightarrow 4)\text{-}\beta\text{-D-Manp }(1\text{-}]_2\rightarrow 4)\text{-}\overset{\substack{\alpha\text{-D-Galpa}\\ 1\\ \downarrow\\ 6}}{\beta\text{-D-Manp}}(1\text{-}[\rightarrow 4)\text{-}\beta\text{--D-Manp }(1\text{-}$$

$$\rightarrow 4]_2\rightarrow 4)\text{- })\text{-}\overset{\substack{\alpha\text{-D-Galp}\\ 1\\ \downarrow\\ 6}}{\beta\text{-D-Manp}}(1\text{-}[\rightarrow 4)\text{-}\beta\text{--D-Manp }(1\text{-}]_n\rightarrow$$

Fig. 1. Seed gum from *Parkinsonia aculeata*.

and 2,3,6-tri-*O*-methyl-D-mannose are present in 1.00:1.01:1.66 molar ratio. The seed gum was partially hydrolyzed with 50 mM H_2SO_4 for 12 h at 100° and hydrolysate was subjected to paper chromatography (solvent-D). Elution of different fractions with distilled water gave D-galactose and D-mannose along with oligosaccharides. The oligosaccharides were separated (Singh, Srivastava,, pandey, Sethi, & Sanghi, 2003) and identified (Table 2).

3. Results and discussions

The pure isolated polysaccharide had $[\alpha]_D^{25} + 65.5°$ (water), ash content 0.44% and negligible percentage of acetyl, methoxyl and uronic acid. Complete acid hydrolysis revealed that the constituent monosaccharide units of the seed gum are D-galactose and D-mannose. Identities of the monosaccharides were determined by co-chromatography with authentic samples, m.p, optical rotation and by preparing derivatives (Table 1). The ratio of the constituent monosaccharides was found to be 1.0:2.66 by the GLC. Graded hydrolysis resulted in to the preferential release of D-galactose indicating it is probably linked by weaker *α*-linkage at peripheral position as end groups. Fully methylated seed gum $[\alpha]_D^{25} + 44°$ (Chloroform) on hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-Galactose, 2,3,6-tri-*O*-methyl-D-mannose and 2,3-di-*O*-methyl-D-mannose. The presence of dimethyl sugar as one of the cleavage fragment indicated that the polysaccharide had a ramified structure. 2,3,4,6-tetra-*O*-methyl-D-mannose might have arisen from D-galactose units, which are present as non-reducing end groups in the side chain confirming the earlier conclusion from the graded acid hydrolysis. 2,3,6-tri-*O*-methyl-D-mannose indicates the presence of non-terminal unbranched D-mannopyranosyl residues linked (1→4) in the molecule forming backbone of the polysaccharide. Methylated monosaccharides were separated and identified (Table 1). GLC of the alditol acetates of the methylated monosaccharides showed them to be present in 1.00:1.01:1.66 molar ratios respectively. On oxidation with sodium metaperiodate, seed gum consumed 0.7830 moles of the oxidant and liberated 0.1660 moles of formic acid per 100 g of the seed gum, which corresponded to 26.8% of the end groups. Following acid catalyzed partial hydrolysis of the seed gum, mannobiose, mannotriose, epimelibiose, and galactosylmannobiose were separated and identified along with the component monosaccharides. (Table 2) indicating the anomeric configuration of the glycosidic linkages, the type of branching and the sequence of sugar units in the seed polysaccharide (Fig. 1).

The results suggest following structural pattern for the seed gum.

References

Aspinall, G. O., Begbie, R., & Mackay, J. E. (1962). The hemicelluloses of European Larch (Larix deciduas) Part II. The glucomannan component. *Journal of Chemical Society* , 214–219.

Bailey, R. W. (1965). *Oligosaccharides*, Vol. 4 pp. 97–101.

Hakomori, S. I. (1964). A rapid permethylation of glycolipid, and polysaccharide catalyzed by methylsulfinyl carbanion in dimethylsulfoxide. *Journal of Biochemistry*, *55*, 205–208.

Haworth, W. N. (1915). A new method for preparing alkylated sugars. *Journal of Chemical Society*, *107*, 8–12.

Hirst, E. L., Hough, L., & Jones, J. K. N. (1949). Quantitative analysis of mixtures of sugars by the method of partition chromatography Part-II. The separation and determination of methylated aldoses. *Journal of Chemical Society* , 928–932.

Hirst, E. L., & Jones, J. K. N. (1949). The application of partition chromatography to the separation of the sugars and their derivatives. *Discussion of Faraday Society*, *7*, 268–271.

Hussain, S., & Prasad, B. (1966). In R. N. Chopra, et al., *Wealth of India* (Vol. 7). New Delhi: Publication and Information Directorate, C.S.I.R..

Isbell, H. S., & Frush, H. L. (1962). Methods in carbohydrate chemistry. In R. L. Whistler, & M. L. Wolform (Eds.), (p. 145). New York, London: Academic press.

Jaroslav, S., Miloslav, C., & Josef, P. (1965). In E. Ivan, & H. Jaromir (Eds.), *The oligosaccharides*, pp. 233–273.

Kapoor, V. P., Francois, R. T., Joseleau, J. P., Milas, M., Chanzy, H., & Rinaudo, M. (1998). Cassia spectabilis DC seed galactomannan: structural, crystallographical and rheological studies. *Carbohydrate Research*, *306*, 241.

Kapoor, V. P., Heneri, C., & Travel, F. R. (1995). X Ray diffraction studies on some seed galactomannans from India. *Carbohydrate Polymer*, *27*, 229–233.

Meier, H. (1960). Studies on glucomannan from Norwegian Spruce-III partial hydrolysis. *Acta Chemica Scandinavica*, *14*, 752.

Mestler, L. (1963). In R. L. Whistler, & M. L. Wolform (Eds.), *Methods in carbohydrate chemistry*, pp. 1170.

Rizvi, S. A. I., Gupta, P. C., & Kaul, R. K. (1971). Nodososide in Cassia nodoa. *Planta Medica*, *20*, 24–28.

Robertson, G. J. (1934). The condensation of α-methylmannoside with benzaldehyde. *Journal of Chemical Society* , 330.

Singh, V., & Mishra, U. C. (1997). A neutral seed gum from Abutilon indicum. *Carbohydrate Polymer*, *33*, 203–205.

Singh, V., Srivastava, V., pandey, M., Sethi, R., & Sanghi, R. (2003). Ipomoea turpethum seeds: A potential source of commercial gum. *Carbohydrate polymers*, *51*, 357–359.

Smith, F., & Montgomery, R. (1959). The chemistry of plants gums and mucilages. In W. A. Harmor (Ed.), (p. 134). New York: Reinhold.

Srivastava, H. C., & Singh, P. P. (1967). Structure of polysaccharide from Tamarind kernal. *Carbohydrate Research*, *4*, 326–331.

Waston, R., & Fowden, L. (1973). Aminoacids of Ceaselpinea tinctoria and some allied. *Phytochemistry*, *12*, 617.

Whistler, R. L., & Durso, D. F. (1951). Separation of sugars by charcoal. *Journal of American Chemical Society*, *73*, 4189.

Wolform, M. L., & Thompson (1962). D-Galactose. In R. L. Whistler, & M. L. Wolform, *Methods in carbohydrate chemistry* (Vol. 1) (p. 121). New York: Academic press.

Tookey, H. L., Lohman, K. L., & Wolff, I. A. (1962). Evaluation of seed galactomannan from legumes as paper sizers. *Phytochemistry*, *10*, 131.