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Short communication

A non-ionic glucomannan from the seeds of an indigenous medicinal plant: *Bryonia lacinosa*

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Abstract

Extraction of defatted and decolorized seeds of *Bryonia lacinosa* with 1% aqueous acetic acid yielded a polysaccharide material, having D-glucose and D-mannose in the molar ratio of 1.00:1.01. Hydrolysis of the fully methylated seed gum furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose in equimolar ratio. Partial hydrolysis of the polysaccharide furnished three oligosaccharides namely; epigentiobiose, mannobiose, and mannotriose along with the component monosaccharides. Periodate oxidation indicated about 49.75% of the end groups in the seed gum, which was in close agreement with the end groups calculated from methylation analysis, i.e. 49.55%.

On the basis of the above results, a structure for the repeating unit of the polysaccharide has been proposed in which the main chain consists of β (1→4) linked mannopyranosyl units to which D-glucopyranosyl units are linked by α (1→6) linkages.

Keywords: *Bryonia lacinosa*; Glucomannan; Polysaccharide

1. Introduction

Bryonia lacinosa syn *Bryonopsis lacinosa* (N.O. Cucurbitaceae) plant locally known as 'Shivlingi' and 'Gargumaru' is distributed throughout India, an annual climber with bright red fruits and is reported to be highly medicinal (Kirtikar & Basu, 1987). Locally in India its seeds are being used for promoting conception in women. Plant as a whole is bitter, tonic and mild laxative. Its leaves are used on inflammations. Roots with roots of *Michelia champaca* is given against asthma and promotes conception. Plant is also used against snake-bite (part not specified). From leaves a bitter principle, bryonin, has been reported (Chopra, Chopra, & Chopra, 1956). *Bryonia alba* is well-established homeopathic medicine, while *Bryonia lacinosa* is being used as trivial medicine since long in India, but no work has been done except few fatty acids and sugars are reported (Vishwa Paul & Hem Raj, 1960) to be present in the seeds. Due to tremendous medicinal importance of the seeds, the seed mucilage from *B. lacinosa* was subjected to phytochemical investigation.

2. Material and methods

Seeds of *Bryonia lacinosa* (N.O. Cucurbitaceae) were locally collected in Allahabad, India, and were identified at Botanical Survey of India, Allahabad. All the solutions were concentrated under reduced pressure, melting points are uncorrected and all the optical rotations are equilibrium values, infra red (IR) spectra were recorded on a Brucker Vector-22 Infrared spectrophotometer using KBr pellets. Paper chromatography was done using following solvent systems. (A) 5:1:4 1-butanol:ethanol:water (Hirst & Jones, 1949); (B) 11:6:3 1-butanol:isopropanol:water (Rizvi, Gupta, & Kaul, 1971); (C) 10:4:3 ethylacetate:pyridine:water (Aspinall, Begbie, & Mackay, 1962); (D) 2:1:2 ethylacetate:pyridine:water (Meier, 1960). Spots were located with the help of aniline hydrogen phathalate.

3. Isolation and purification

3.1. Extraction of the polysaccharide

Seeds (1 kg) of *B. lacinosa* were extracted (Tiwari, Singh, & Gupta, 2005) with light petroleum and EtOH and then suspended in 1% aqueous AcOH overnight. The filtrate of the 1% solution was precipitated with 95% EtOH and this was repeated six times to give a white amorphous product. The crude gum was collected, washed with ethanol and dried

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(yield 20 g/kg). The crude gum was purified through barium complexing (Tiwari et al., 2005) by preparing 2.5% (w/v) solution of the gum by continuous stirring for 12 h at 60 °C and precipitating with saturated barium hydroxide solution. The complex was separated by centrifugation and taken in 1 M CH_3COOH, stirred for 8 h, centrifuged and precipitated with EtOH. It was washed with 70, 80, 90, 95% ethanol. The sample was finally purified by dialysis and filtration through millipore membranes. The pure seed gum was a non-reducing, white, amorphous material with ash content 0.28% and $[\alpha]_D^{25}+70°$ (water).

3.2. Investigation of the structure of the polysaccharide

The pure seed gum was completely hydrolyzed with 1 M trifluoroacetic acid (4 h at 100 °C) (Singh et al., 2003). Paper chromotography (solvent B) of the hydrolyzate revealed the presence of D-glucose (Smith & Montgomery, 1959a,b) (R_f 0.09 cm) and D-mannose (R_f 0.11 cm). Identities and configurations of the monosaccharides were confirmed by co-chromatography with authentic samples and preparation of derivatives (Clarke, 1926); D-glucose, mp 148 °C, $[\alpha]_D^{30}+53.1°$ (water); D-glucosazone, mp 205 °C; D-mannose, mp 131 °C $[\alpha]_D^{30}+14°$ (water); D-mannose phenyl hydrazone, mp 198 °C. The ratio of the constituent monosaccharides was determined by GLC (Kapoor, Chanzy Heneri, & Travel, 1995). The complete hydrolyzate of the seed gum was evaporated, the residue was reduced with sodium borohydride and the products acetylated with pyridine–acetic anhydride (1:1 v/v, 1 h at 100 °C). The resulting alditol acetates were analyzed by GLC (Kapoor et al., 1995) using a model Neukon 5700 Gas Chromatograph equipped with flame ionization detector at 190 °C with a Superleco SP 2380 column (3.0×0.53 mm), the carrier gas being nitrogen. The ratio of D-glucose and D-mannose was found to be 1.00:1.01. Graded acid hydrolysis of the polysaccharide (Smith & Montgomery, 1959a,b) was done with 25 mM H_2SO_4 at 100 °C for 6 h and the sequential paper chromatographic separation of the hydrolyzate at various time intervals indicated that D-glucose was released after 45 min, while D-mannose could be detected only after 90 min. Metaperiodate oxidation studies (Singh, Srivastava, Pandey, Sethi & Sanghi, 2003) revealed that 0.9262 mol of metaperiodate were consumed with the liberation of 0.3060 mol of formic acid per 100 g of the polysaccharide indicating 49.75% of the end groups.

The seed gum was first methylated by Haworth's method (Haworth, 1915) followed by Hakomori's method (Hakomori, 1964) to yield a fully methylated product $[\alpha]_D^{25}+50°$ (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 °C. The products were fractioned on Whatman No. 3 MM paper (Solvent A) to give following methylated sugars (1) 2,3,4,6-tetra-*O*-methyl-D-glucose and (2) 2,3-di-*O*-methyl-D-mannose.

GLC of the partially methylated alditol acetates (Kapoor et al., 1998) obtained by reduction with $NaBH_4$ and acetylation of the hydrolyzate of methylated seed gum showed that 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose are present in equimolar ratio. The seed gum was partially hydrolyzed with 50 mM H_2SO_4 for 12 h at 100 °C and the hydrolyzate was subjected to paper chromatography (solvent-D). Elution of different fractions with distilled water gave D-glucose and D-mannose along with the following oligosaccharides: (1) Epigentiobiose, [α-D-Glup (1→6)-D-Manp] mp 140 °C, $[\alpha]_D^{25}$ −12° (C1, water), cf. literature values, mp 137–138 °C, $[\alpha]_D^{25}$ −11° (water), (Bailey, 1965), derivative phenylosazone had mp 165° cf. literature 166–170° (Bailey, 1965, Chap. 8) (2) Mannobiose [βManp(1→4)-D-Manp], mp 203–205 °C; (from ethanol), $[\alpha]_D^{25}$ −9° (water), cf. literature (Aspinall et al., 1958) values, mp 202–203°, $[\alpha]_D^{25}$ −5.2 to 8.2; derivative phenyl osazone had mp 204 °C cf. literature (Srivastava & Singh, 1967) values mp 203–206 °C; (3) Mannotriose [β-D-mannopyranosyl(1→4)-β-D-mannopyranosyl(1→4)-D-mannopyranose] mp 163–167 °C, $[\alpha]_D^{25}$ −18° (water), cf. literature (Jaroslav, Miloslav, & Josef, 1965) values mp 169.5 °C $[\alpha]_D^{25}$ −15° to (−)16° (water).

4. Results and discussions

The polysaccharide was isolated from defatted and decolorized seeds by extracting them with 1% aqueous AcOH followed by precipitation with 95% EtOH in 2.0% yield. It was purified by barium complexing, dialysis and filtration through various Millipore membranes. The pure polysaccharide had $[\alpha]_D^{25}+70^{\circ}$ (water), ash content 0.28% and negligible percentage of acetyl, methoxyl and uronic acid. Complete acid hydrolysis yielded D-glucose and D-mannose. The ratio of the constituent monosaccharides was found to be 1.00:1.01 by GLC. Graded hydrolysis resulted in the preferential release of D-glucose indicating its peripheral position as end groups. Fully methylated seed gum $[\alpha]_D^{25}+50°$ (chloroform) on hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-mannose. GLC of the alditol acetates of the methylated monosaccharides showed them to be present in 1:1 molar ratios. On oxidation with sodium metaperiodate seed gum consumed 0.9262 mol of metaperiodate with the liberation of 0.3060 mol of the formic acid per 100 g of the seed gum, which corresponded to 49.75% of the end groups. Acid catalyzed partial hydrolysis of the seed gum gave oligosaccharides (1) epigentiobiose (2) mannobiose and (3) mannotriose along with the component monosaccharides D-glucose and D-mannose.

Above results suggest the following structural pattern for the repeating unit of the seed gum (Fig. 1).

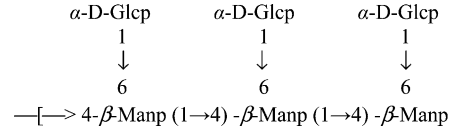

Fig. 1. Structure of the repeating unit of *B. lacinosa* seed polysaccharide.

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References

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

Aspinall, G. O., Rashbrook, R. B., & Kessler, G. J. (1958). The mannans of Ivory nut (*Phytelephas macrocarpa*) part II. The partial acid hydrolysis of Mannans A & B. *Journal of the Chemical Society*, 215–221.

Bailey, R. W. (1965). *Oligosaccharides heterogeneous reducing oligosaccharides containing simple hexoses only, international series of monographs on pure and applied biology* (Vol. 4) (p. 100). Oxford: Pergamon Press.

Chopra, R. N., Chopra, S. L., & Chopra, I. C. (1956). *Glossary of Indian medicinal plant* (p. 42). New Delhi, India: CSIR.

Clarke, H. T. (1926). *A handbook of organic analysis qualitative and quantitative* (4th ed.) (pp. 162–163). London: Longmans.

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 the Chemical Society*, *107*, 8–12.

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

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

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

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.

Kirtikar, K. R., & Basu, B. D. (1987). In E. Blatter, et al. (Eds), *Indian medicinal plants*, Vol. II, (2nd ed.) (pp. 1158–1159).

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

Paul, V., & Hem Raj, K. K. (1960). Chemical investigation of *Bryonopsis lacinosa* fruit oil. *Proceedings of the National Academy of Sciences of the USA*, A29(Pt 3), 218–221.

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

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. (1959a). In W. A. Harmor (Ed.), *The chemistry of plant gums and mucilages* (p. 134). New York: Reinhold.

Smith, F., & Montgomery, R. (1959b). In W. A. Harmor (Ed.), *The chemistry of plant gums and mucilages* (pp. 90–91). New York: Reinhold.

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

Tiwari, K., Singh, V., & Gupta, P. C. (2005). A non-ionic water soluble seed-gum from *Parkinsonia aculeata*. *Carbohydrate Polymers*, *9*, 393–396.