Carbohydrate Polymers 75 (2009) 534–537

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Short communication

An *Escherichia coli* antimicrobial effect of arabinoglucomannan from fruit of *Bryonia lacinosa*

Vandana Singh [a,*], Tulika Malviya [a], Devendra Narayan Tripathi [a], Upma Naraian [b,c]

[a] *Department of Chemistry, University of Allahabad, Allahabad 211002, India*
[b] *Kamala Nehru Hospital, Allahabad 211002, India*
[c] *Tejas Microbiology Micro diagnostic, Allahabad 211002, India*

ARTICLE INFO

Article history:
Received 19 May 2007
Received in revised form 14 March 2008
Accepted 29 August 2008
Available online 12 September 2008

Keywords:
Bryonia lacinosa
Fruit polysaccharide
Escherichia coli active

ABSTRACT

Extraction of the pulp of ripe berries of *Bryonia lacinosa* with 1% aqueous acetic acid yielded a polysaccharide material, having D-glucose, D-mannose and L-arabinose in the molar ratio of. 5.00:3.01:4.00. Hydrolysis of the fully methylated polysaccharide furnished 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose and 2,3,5,-tri-*O*-methyl-L-arabinose in 1:4:2:1:4 molar ratio. Partial hydrolysis of the polysaccharide furnished; mannobiose, epicellobiose, 6-*O*-β-L-arabinofuranosyl-D-glucose, 6-*O*-α-mannopyranosyl-D-mannose and epimaltose along with the component monosaccharides. On metaperiodate oxidation studies, 100 g of the polysaccharide liberated 0.055 mol of HCOOH consuming 0.7127 mol of periodate, indicating about 8.33% of the end groups. On the basis of the above results, a structure for the repeating unit of the polysaccharide has been proposed. The polysaccharide was tested for the microbial activity and was found to be active against *Escherichia coli* with a minimum dose of 6.25 mg/mL.

1. Introduction

Bryonia lacinosa syn *Bryonopsis lacinosa* (N.O. Cucurbitaceae) plant is distributed throughout India and locally known as "Shivlingi" and "Gargumaru." It is 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 *B. lacinosa* is being used as trivial medicine since long in India, but not much work has been done on the plant except fatty acids from fruit, sugars and a glucomannan from seeds (Paul & Hem Raj, 1960; Singh & Malviya 2006) have been reported. In the present communication we report on the polysaccharide isolated from the mucilaginous contents of the *B. lacinosa* fruit berries and its activity against *Escherichia coli* and fungi were tested.

2. Materials and methods

Fruits of *B. 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 Nicolet 5700 of FTIR spectrophotometer using KBr pellet. 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. GLC was carried out using 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 is nitrogen. Viscosity of the polysaccharide solution was measured with Brookfield LVDVE viscometer at 30 °C using small sample adapter (spindle S-18). Endotracheal aspirates were obtained from critically ill patients of local tertiary care centre and the samples were processed for microscopy, culture and antibiogram. For microbial activity testing all solvents used were triply distilled and autoclaved prior to use. Bacterial samples were obtained from the chest of critically ill patients as per direction of the consultants. Hinton Agar (M173), Sabouraud Dextrose Agar (M 1313) and sterile disc (SD067) were purchased from the Hi Media Laboratory Pvt. Ltd., Mumbai, India.

* Corresponding author. Tel: +91 532 2461518; fax: +91 532 2540858.
E-mail address: singhvandanasingh@rediffmail.com (V. Singh).

0144-8617/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2008.08.025

Sigma Aldrich Disposable dialyzer (pore size 3,500 Da MWCO) was used for dialysis.

2.1. Isolation and purification

2.1.1. Extraction of the polysaccharide

Pulp (1 kg) of *B. lacinosa* berries was suspended in 1% aqueous AcOH overnight. The filtrate of the 1% solution was stirred well and precipitated with 4 volumes of 95% EtOH and the dissolution and precipitation was repeated six times to give a white amorphous product. The crude polysaccharide was collected, washed with ethanol and dried (yield 20 g/kg). The crude polysaccharide was purified through barium complexing (Singh, Srivastava, pandey, Sethi, & Sanghi, 2003) 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 50 mL of 1 M CH_3COOH, stirred for 8 h, centrifuged and precipitated with EtOH. It was washed with 70%, 80%, 90% and 95% ethanol. The sample was finally purified by dialysis using Spectra/Por® 3 Dialysis Membrane (MWCO 3500) and filtration through Standard MF-Millipore Membranes (0.45 μm) filters. The pure polysaccharide was a non-reducing, white, amorphous material with ash content 0.34% and $[\alpha]_D^{25}-30°$ (water).

2.2. Investigation of the structure of the polysaccharide

The pure polysaccharide was completely hydrolyzed with 1 M trifluoroacetic acid (4 h at 100 °C). Paper chromotography (solvent B) of the hydrolyzate revealed the presence of D-glucose (Smith & Montgomery, 1959a) (R_f 0.09 cm) and D-mannose (R_f 0.11 cm) and L-arabinose (R_f 0.23 (Solvent D). Identities and configurations of the monosaccharides were confirmed by co-chromatography with authentic samples and preparation of derivatives (Clarke, 1970); 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. L-arabinose, mp 160 °C, $[\alpha]_D^{30}$+170–190° (Clarke, 1970), phenylosazone, mp 156 °C .The ratio of the constituent monosaccharides was determined by GLC (Kapoor, Heneri, & Travel, 1995). The complete hydrolyzate of the polysaccharide 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), taking 2,3,4,6-tetra-*O*-methyl glucose as standard. The ratio of D-glucose, D-mannose and L-arabinose was found to be 5.00:3.01:4.00.

Graded acid hydrolysis of the polysaccharide (Smith & Montgomery, 1959b) 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 first 15 min, D-mannose and L-arabinose were detected only after 40 min. On metaperiodate oxidation studies 100 g of the polysaccharide liberated 0.055 mol of HCOOH consuming 0.7127 mol of periodate, indicating about 8.33% of the end groups.

The polysaccharide was first methylated by Haworth's method (Haworth, 1915) followed by Hakomori's method (Hakomori, 1964) to yield a fully methylated product $[\alpha]_D^{25}-12.2°$ (chloroform). The completely methylated polysaccharide 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 fractionated on Whatman No. 3 MM paper (Solvent A) to give following methylated sugars (1) 2,3,4,6-tetra-*O*-methyl-D-glucose; (2) 2,3-di-*O*-methyl-D-glucose; (3) 2,3,6-tri-*O*-methyl-D-mannose; (4) 2,3-di-*O*-methyl-D-mannose and (5) 2,3,5-tri-*O*-methyl-L-arabinose. GLC of the partially methylated alditol acetates (Kapoor et al. 1998), taking alditol acetate of 2,3,4,6-tetra-*O*-methyl-D-glucose as standard, revealed that 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose and 2,3,5-tri-O-methyl-L-arabinose are in 1:4:2:1:4 molar ratio.

The polysaccharide 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, L-arabinose and D-mannose along with the following oligosaccharides: (1) Mannobiose [βManp(1→4)-D-Manp], mp 203–205 °C, (from ethanol), $[\alpha]_D^{25}-9°$ (water) cf. literature (Aspinall, Rashbrook, & Kessler, 1958) values, mp 202–203°, $[\alpha]_D^{25}-5.2–8.2$; derivative phenyl osazone had mp 204 °C cf. literature (Srivastava & Singh, 1967) values mp 203–206 °C (2) epicellobiose mp 180–182 °C, $[\alpha]_D^{25}$+6.5° c.f. literature (Meier, 1960) values mp 179-182 °C, $[\alpha]_D^{25}$+6° (3) 6-*O*-β-L-arabinofuranosyl-D-glucose, (4) 6-*O*-α-mannopyranosyl-D-mannose, syrup, $[\alpha]_D^{25}$+71° c.f. literature (Jaroslav, Miloslav, & Josef, 1965, chap. XII) value 72° and (5) 4-*O*-α-glucopyranosyl-D-mannose (epimaltose) mp 212 °C, $[\alpha]_D^{25}$+100–115° c.f. literature (Jaroslav et al., 1965, chap. XII) value mp 215 °C, $[\alpha]_D^{25}$ +96–115°.

2.3. Microbial activity of the polysaccharide

The antibacterial susceptibility test was done by determining the zone of inhibition (Koneman, Allen, Janda, Schreckenberger, & Winn, 1997). The polysaccharide was dissolved in water for preparing stock solution (50 mg/mL) in sterile test tubes. Sterlized filter discs were socked with the stock solution and dried. Four different bacterial broth cultures (1 mL) namely *Staphylococcus aureus, Klebsiella aerogenes, Escherichia coli, Streptococcus pyogenes* were added in the M.H. plates and spread with the sterile spreader.

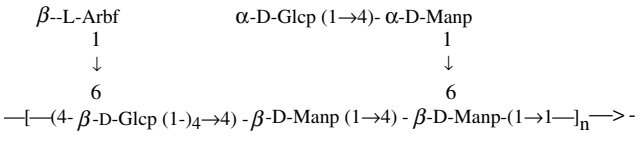

Fig. 1. Structure of the repeating unit of *B. lacinosa pulp* polysaccharide.

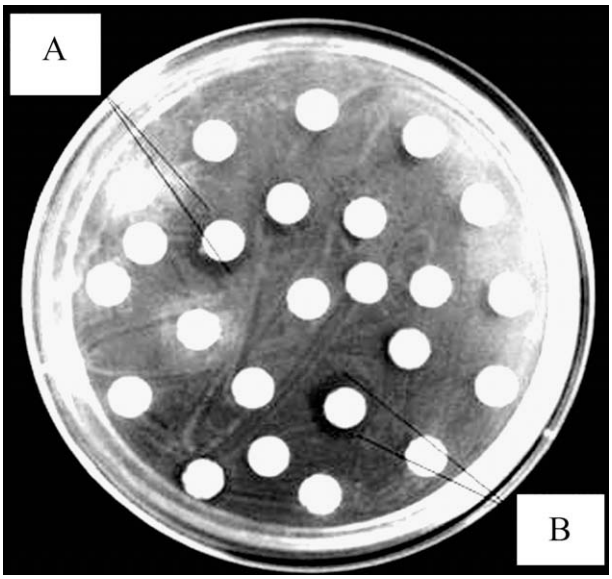

Fig. 2. Inhibition zone for *E.coli* growth by *B. lacinosa* fruit polysaccharide (A) and the control (B).

Charged sterile discs were placed aseptically over the inoculated plates using the sterile forceps. All the procedures were done under a Laminar flow. The plates were inoculated at 37 °C for 24 h and the zone of the inhibition was measured. Amoxycillin–clavulanic acid control was used for comparison (Bauer, Kirby. & Sherris, 1966) (Fig. 2).

For performing *in vitro* antifungal test, the solutions of the polysaccharide were prepared in water at initial concentration 25 mg/mL. Candida and its species were isolated from the patients having chest infection on sabouraud Dextrose Agar without antibiotics. A standardized inoculum was prepared by counting mitochondria. Cultures were grown on Sabouraud Dextrose Agar for 48 h at 37 °C. Sterile saline solution (0.85%) was added to the slant and cultures were gently swabbed with cotton tipped applicator to dislodge conidia from the hyphal mat. The suspension was transferred to a sterile tube and volume was adjusted to 5 mL with sterile saline solution. Sabouraud Dextrose Agar was poured to depth of 5 mm in 90 mm Petri dishes and stored at 4 °C. The plates were dried, standardized suspension was poured and uniformity spread by means of swab sticks. The excess inoculum was examined.

3. Results and discussion

The polysaccharide was isolated from defatted and decolorized pulp by extracting them with 1% aqueous AcOH by followed by precipitation with 4 volume of 95% EtOH in 2.0% yield. It was purified by barium complexing, dialysis and filtration through various Millipore membranes. The pure polysaccharide had $[\alpha]_D^{25} -30°$ (water), ash content 0.34% and negligible percentage of acetyl, methoxyl and uronic acid (Adams & Castagne, 1951; Schultz, 1965). Viscosity 1% solution was 125.4 cP (at 5 rpm 100 rpm at 20.9% Torque).

Complete acid hydrolysis yielded D-glucose, D-mannose and L-arabinose. The ratio of the constituent monosaccharides was found to be 5.00:3.01:4.00, respectively, by GLC. Graded hydrolysis resulted in the preferential release of D-glucose indicating their peripheral position as end groups. Fully methylated polysaccharide $[\alpha]_D^{25} -12.2°$ (chloroform) on hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-mannose, 2,3-di-*O*-methyl-D-mannose and 2,3,5-tri-O-methyl-L-arabinose. GLC of the alditol acetates of the methylated monosaccharides taking 2,3,4,6-tetra-*O*-methyl-D-glucose as standard showed them to be present in 1:4:2:1:4 molar ratios. On metaperiodate oxidation studies 100 g of the polysaccharide liberated 0.055 mol of HCOOH consuming 0.7127 mol of periodate, indicating about 8.33% of the end groups.

Acid catalyzed partial hydrolysis of the polysaccharide gave oligosaccharides (1) mannobiose (4-*O*-β-D-mannopyranosyl-D-mannopyranose) (2) epicellobiose (4-*O*-β-D-Glucopyranosyl-D-mannopyranose) (3) 6-*O*-β-L-arabinofuranosyl-D-glucose, (4) 6-*O*-α-mannopyranosyl-D- mannose, and (5) 4-*O*-α-glucopyranosyl-D-mannose (epimaltose) along with the component monosaccharides D-glucose and D-mannose and L-arabinose.

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

4. Microbial activity

The polysaccharide was tested for four different stains of bacteria for the antibacterial activity, namely *Staphylococcus aureus, K. aerogenes, S. pyogenes and E. coli*. It was found to show activity against *E. coli*. The polysaccharide was tested for four dilution, 1.25 mg/mL, 3.12 mg/mL, 6.25 mg/mL and 12.5 mg/mL and the activity was observed at minimum concentration of 6.25 mg/mL. (Table 1). While the control Amoxycillin–clavulanic acid was active over the concentration range studied. Zone of inhibition was measured to be 16–18 mm for both the BL as well as control. The polysaccharide was inactive at all the dilution for other bacterial strains studied. The antimicrobial activity of the polysaccharide can be attributed to its interaction with the proteins in the outer cell wall of the bacteria which largely consists of porins which coexist with lipopolysaccharide (Nogami & Mizushima, 1983; Ratledge and Dale, 1999). However a detailed study is required to fully understand the mechanistic steps of the interaction.

Table 1
Antibacterial testing for the fruit pulp polysaccharide of *B. lacinosa*

S.No	Bacteria	Serial dilution in mg/mL			
		12.5	6.25	3.12	1.25
1.	*S. aureus*	Nil	Nil	Nil	Nil
2.	*K. aerogene*	Nil	Nil	Nil	Nil
3.	*E. coli*	√	√	Nil	Nil
4.	*Sterptococcus pyogenes*	Nil	Nil	Nil	Nil
5.	Amoxycillin–clavulanic acid control	√	√	√	√

The polysaccharide also did not show any antifungal activity for the tested stains of the fungi.

Acknowledgements

The authors are thankful to the University Grants Commission, New Delhi, India for financial support to carry out this work. I.R. facility from I.I.T. Kanpur and G.L.C. facility from PAR lab, Allahabad, is also gratefully acknowledged.

References

Adams, G. A., & Castagne, A. E. (1951). Hemicelluloses of wheat straw. *Canadian Journal of Chemistry, 29*(2), 109–122.
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.
Aspinall, G. O., Rashbrook, R. B., & Kessler, G. (1958). The mannans of ivory nut (*Phytelephas macrocarpa*). Part II. the partial acid hydrolysis of mannans A and B. *Journal of the Chemical Society*, 215–221.
Bauer, A. W., Kirby, W. M. M., & Sherris, J. C. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*, 493–496.
Chopra, R. N., Chopra, S. L., & Chopra, I. C. (1956). *Glossary of Indian medicinal plant*. New Delhi, India: C.S.I.R..
Clarke, H. T. (1970). *A Handbook of organic analysis qualitative and quantitative*. London: Longman.
Koneman, Elmer W., Allen, Stephen D., Janda, William M., Schreckenberger, Paul C., & Winn, Washington C. Jr., (1997). *Lippincott, color Atlas and textbook of diagnostic microbiology* (5th ed.). New york: Philadelphia.
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., & 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.
Jaroslav, S., Miloslav, C., & Josef, P. (1965). Description and properties of oligosaccharides. In E. Ivan & H. Jaromir (Eds.), *The Oligosaccharides* (pp. 233–273). New York, London: Academic Press.
Kapoor, V. P., Heneri, Chanzy, & Travel, F. R. (1995). X-ray diffraction studies on some seed galactomannans from India. *Carbohydrate Polymer, 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 Polymers, 306*, 241.
Kirtikar, K. R., & Basu, B. D. (1987) 2nd ed.. In E. Blatter et al. (Eds.). *Indian medicinal plants* (vol. II, pp. 1158–1159). Allahabad: Lalit Mohan basu Press.
Meier, H. (1960). Studies on glucomannan from Norwegian Spruce-III partial hydrolysis. *Acta Chemica Scandinavica, 14*, 752.
Nogami, T., & Mizushima, S. (1983). Outer membrane porins are important in maintenance of the surface structure of *Escherichia coli* cells. *Journal of Bacteriology, 156*, 402–408.

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

Ratledge C., Dale J. (1999). Mycobacteria: Molecular Biology and Virulence. Berlin: Blackwell Publishing. 231.

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

Schultz, T. H. (1965). Determination of the degree of esterification of pectin (Determination of the ester methoxyl content of pectin by saponification and titration. Determination of the anhydrouronic acid content by decarboxylation and titration of the liberated carbondioxide). In R. L. Whistler (Ed.), *Methods in Carbohydrate Chemistry, (Vol. V)* (pp. 189–193). New York and London: Academic press.

Singh, V., & Malviya, T. (2006). *A non-ionic glucomannan from the seeds of an indigenous medicinal plant: Bryonia lacinosa, 64*, 481–483.

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 plants gums and mucilages* (pp. 90–91). New York: Reinhold.

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

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