ELSEVIER

Carbohydrate Polymers **24** (1994) 277–280

0144-8617/94/$07 00

Structural studies of a mucilage from *Abroma augusta* root bark

Nilufar Nahar,* Balai K. Hazra, M. Mosihuzzaman, M. Mahbubur Rahman

Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh

&

Rolf Andersson

Department of Chemistry, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

(Received 4 March 1994; revised version received 21 April 1994; accepted 3 May 1994)

The structure of an acidic polysaccharide isolated from *Abroma augusta* root bark was determined by sugar and methylation analyses and high resolution ^{1}H- and ^{13}C-NMR spectroscopy. The main chain of the polysaccharide was composed of 1,2-linked α-L-rhamnopyranose and 1,4- or 1,3-linked α-D-galacturonic acid residues. The terminal β-D-glucuronic acid residue was attached to the 3- and/or 4-position of the α-D-galacturonic acid residue.

INTRODUCTION

Abroma augusta (Devils's cotton; local name: ulat kambal) is a well known medicinal plant and is being traditionally used for various diseases (Kirtikar & Basu, 1935; Hakim, 1970). The extract of its root bark is reported to contain antifertility agent (Bingel & Farnsworth, 1987). Some secondary metabolites from the plant have been isolated (Mukherjee & Badruddoza, 1978), but the mucilage from the stem or root bark was not reported on earlier. However, the mucilage of the stem bark was studied in this laboratory (Nahar *et al.*, 1988). In the present study a pure polysaccharide was isolated from the mucilage of the root bark and structural studies were carried out with the help of high resolution NMR spectroscopy with COSY (COrrelated SpectroscopY), relayed COSY and C–H COSY experiments.

EXPERIMENTAL

Solutions were concentrated under reduced pressure at <40°C. For GLC, a Pye-Unicam 4500U instrument was used. Separations were performed on an OV-225 glass capillary or CP Sil 88 quartz column at 160 to 220°C (3°C/min). For GLC–MS, a Finnigan 4021 equipment was used. The absolute configuration of the sugars was determined according to the procedure of Gerwig *et al.* (1978, 1979). The uronic acid content was estimated by the carbazole method (Bitter & Muir, 1962). Sugar (Swardeker *et al.*, 1965) and methylation (Hakomori, 1960; Jansson *et al.*, 1976) analyses were carried out using standard procedures with the help of GLC and GLC–MS.

The NMR spectra were recorded with a Varian VXR 400 instrument: ^{1}H- at 400 MHz on solutions in D_2O at 85 or 60°C with internal sodium 3-trimethylsilylpropionate-d_4 as reference (for some spectra, a 180°-t_1-90° pulse sequence was used to suppress the water peak); ^{13}C- at 101 MHz for solutions (10 mg/ml) in D_2O at room temperature with the same internal standard (2·0 ppm). Two-dimensional (2D) NMR spectroscopy was performed with standard COSY, relayed COSY, double-relayed COSY and heteronuclear correlated C/H pulse sequences.

Roots of *A. augusta* plant were collected from the Dhaka University campus. The fresh root bark (320 g) was extracted with cold water (2·5, 1·0 and 0·5 litres, successively, for 16, 8 and 4 h, respectively). The extracts gave some precipitate on standing, which was separated by centrifugation, and the supernatant was freeze-dried to give three different mucilage fractions (Table 1).

*Author to whom correspondence should be addressed.

Table 1. Sugar composition of the different fractions of mucilage (relative %)

Fractions	Yield[a] (%)	Sugars in the hydrolysate					Uronic acid
		Rha	Ara	Man	Gal	Glc	
Mucilage 1	0·54	13·3	—	6·7	40·0	33·4	6·6
Mucilage 2	3·52	32·1	1·2	5·8	4·2	52·2	4·5
Mucilage 3	0·44	26·9	5·7	16·5	5·4	32·2	13·3
Precipitate 1	0·58	75·1	—	—	—	18·1	6·8
Precipitate 2	0·82	—	—	—	—	95·0	5·0
Precipitate 3	0·44	20·3	—	—	6·5	52·8	20·4

[a]Percentage of fresh root bark.

The major mucilage fraction (mucilage 2, Table 1, 900 mg) was extracted with aqueous 80% ethanol (3 × 100 ml, 30 min each time) followed by chloroform (3 × 100 ml, 30 min each time) and then the residue was dissolved in water (50 ml), dialysed and freeze-dried (300 mg, crude mucilage polysaccharide (CMPS)). The CMPS (250 mg) was deesterified (Aspinall *et al.*, 1970) (DMPS, 230 mg) and the DMPS (200 mg) was fractionated into neutral (NMPS, 16·5 g) and acidic (156 mg, AMPS) parts by ion-exchange chromatography using a DEAE Sephadex A-50 column (2·6 × 90 cm). The AMPS was carboxyl-reduced (CRAMPS) (Taylor & Conrad, 1972).

A solution of the AMPS (100 mg) in 0·1 M trifluoroacetic acid (5 ml) was heated at 96°C for 1 h. The solution was evaporated with added water until it was acid-free. The acid-free material was treated with sodium borohydride (30 mg, 2 h). After conventional work-up the product was fractionated by passing through a Bio-gel P-2 column. A fraction was eluted in the void volume region (PAMPS, 70 mg), followed by another fraction in the monosaccharide region (10 mg).

RESULTS AND DISCUSSION

The root bark of *A. augusta* contained about 20% of dry material. Three successive extractions with water gave a total of 32% (of the dry matter) of mucilage of which the major fraction was obtained in the second extraction (Table 1). Glucose, galactose, rhamnose and uronic acid were the main sugar constituents of the mucilages. The mucilages gave some precipitate on standing. Rhamnose, glucose and uronic acid (Table 1) were the main sugar constituents of the precipitates 1 and 3. Precipitate 2 contained mostly glucose (*c.* 95%). Since the second extraction gave the highest amount of mucilage, further work was done on that fraction. CMPS was isolated from the freeze-dried mucilage 2 (Table 1) by removing low-molecular weight substances by extraction with aqueous 80% ethanol followed by chloroform. Rhamnose, glucose and uronic acid were the sugar constituents of the crude polysaccharide and its deesterified form (DMPS). On fractionation by ion-exchange chromatography, DMPS gave a small amount of NMPS and one major acidic fraction (AMPS, 156·0 mg). L-Rhamnose was the only neutral sugar found in AMPS, but its carboxyl-reduced product (CRAMPS) was found to contain L-rhamnose (34·8%), D-glucose (30·4%) and D-galactose (34·8%) (Table 2), indicating that the AMPS contained D-glucuronic and D-galacturonic acids. The NMPS fraction, which was found to contain D-glucose and D-galactose, was not studied further.

Methylation analysis of AMPS gave only 3,4-di-*O*-methyl-L-rhamnose (Table 3) and, as expected CRAMPS gave 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6,-tri-*O*-methyl-D-galactose, 2,4,6-tri-*O*-methyl-D-galactose and 2,6-di-*O*-methyl-D-galactose, indicating that rhamnose was linked through position 2,· D-glucuronic acid was a terminal residue, and the D-galacturonic acid was linked through 3- and/or 4-positions.

The ^{1}H-NMR spectrum of PAMPS (Table 4) had, *inter alia*, three anomeric signals at 5·35 (1·0 H, $J_{1,2}$ n.r.), 5·08 (1·0 H, $J_{1,2}$ n.r.) and 4·71 (1·0 H, $J_{1,2} \sim 8{\cdot}2$ Hz) ppm

Table 2. Sugar composition of the different fractions of mucilage 2

Fractions	Yield[a] (%)	Constituent neutral sugars				Uronic acid
		Rha	Man	Gal	Glc	
CMPS	30·0	44·1	—	1·5	22·4	32·0
DMPS	—	45·7	—	Trade	22·2	32·1
NMPS	5·5	Trace	—	22·0	78·0	—
AMPS	52·0	30·8	—	1·3	1·6	66·3
CRAMPS	—	34·8	—	30·4	34·8	—
PAMPS	—	32·7	—	—	—	67·3
CRPAMPS	—	35·5	—	31·8	32·7	—

[a]Percentage of mucilage 2.

Table 3. Methylation analysis of the carboxyl-reduced acidic mucilage (CRAMPS)[a]

Partially methylated sugar	Relative moles (%)		Tentative linkage
	AMPS	CRAMPS	
3,4-Di-*O*-methylrhamnose	100	19·2	–2)Rha*p*(1–
2,3,4,6-Tetra-*O*-methylglucose	—	37·4	Glc*p*(1 –
2,3,6-Tri-*O*-methylgalactose	—	3·3	–4)Glc*p*(1–
2,4,6-Tri-*O*-methylgalactose	—	2·3	–3)Glc*p*(1
2,6-Di-*O*-methylgalactose	—	37·8	–3,4) Gal*p*(1–

[a]Determined by GLC and GLC–MS.

Table 4. ^{1}H–NMR chemical shifts (ppm) of the partially hydrolysed acidic polysaccharide of *Abroma augusta* root bark (PAMPS)

Sugar	H-1	H-2	H-3	H-4	H-5	H-6
GalA	5·08	4·08	4·19	4·65	4·81	–
GlcA	4·71	3·50	3·52	3·61	3·80	—
Rha	5·35	4·10	3·87	3·41	3·71	1·24

(Fig. 1), which were assigned to α-L-rhamnopyranose, α-D-galacturonic acid and β-D-glucuronic acid residues, respectively. The signal at 1·3 (3·0 H, $J_{5,6}$ 4·5 Hz) ppm was assigned to C-methyl protons of the α-L-rhamnopyranose residue. The ^{13}C-NMR spectrum showed two anomeric signals at 99·1 and 104·8 ppm of which the latter was assigned to β-D-glucuronic acid residue. The former signal was accounted for by the anomeric carbon of α-D-galacturonic acid and α-L-rhamnopyranose residues (overlapped).

Hydrolysis of AMPS with acid under mild conditions followed by reduction with sodium borohydride and fractionation on Bio-gel P-2, gave a low-molecular-(monosaccharide alditol) and a high-molecular-weight fraction (PAMPS). Sugar analysis of the PAMPS and that of the corresponding carboxyl-reduced material (CRPAMPS) (Table 1) showed that L-rhamnose was the only neutral sugar and D-glucuronic acid and D-galacturonic acids were the acidic sugars present in the partial hydrolysate. The methyne protons attached to each sugar residue of the partially hydrolysed product of AMPS were assigned by COSY, relayed and double relayed 2D spectra (Tables 4 and 5). Signals for each carbon of the three different sugar residues were also assigned by a C–H COSY experiment (Table 5). The C-2 signal of rhamnopyranose residue appeared downfield at 77·6 ppm, indicating that it was 1,2-linked. The C-3 and C-4 signals of D-galacturonic acid residue also appeared downfield at 79·6 and 78·4 ppm, respectively,

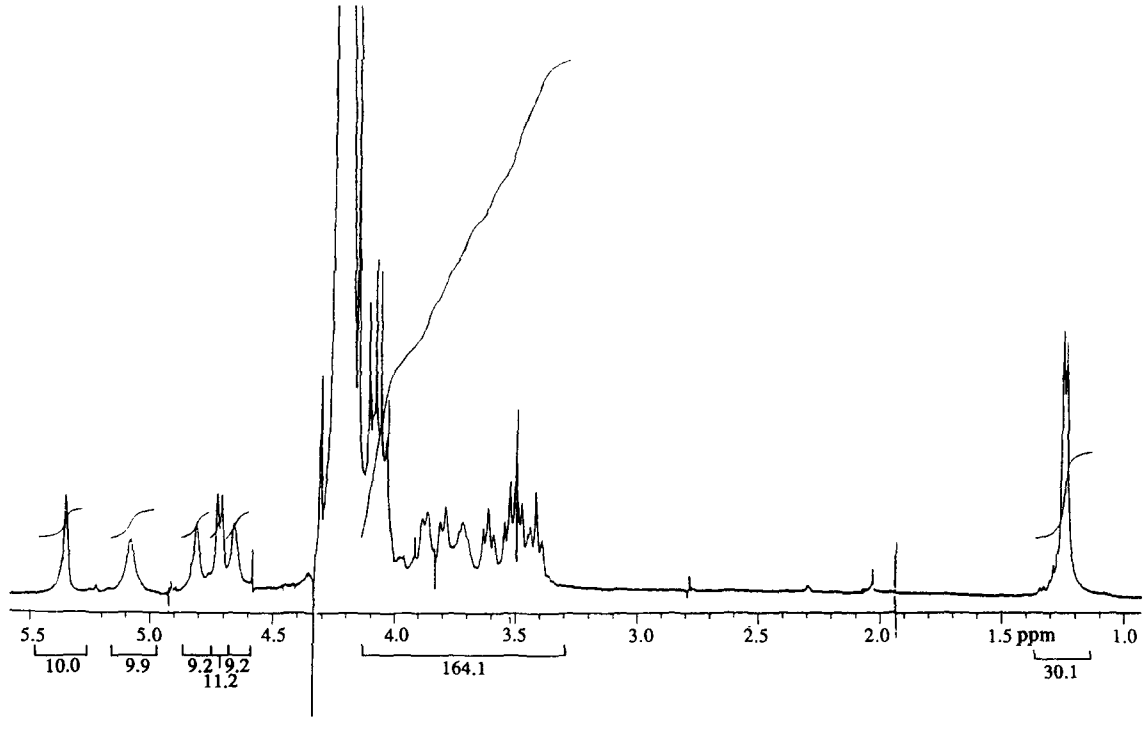
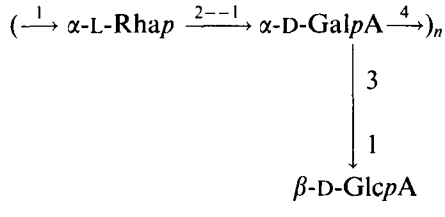


Fig. 1. ^{1}H-NMR spectrum of partially hydrolysed acidic polysaccharide (PAMPS) of *Abroma augusta* root bark.

Table 5. ^{13}C–NMR chemical shifts (ppm) of partially hydrolysed acidic polysaccharide of *Abroma augusta* root bark (PAMPS)

Sugar residue	C-1	C-2	C-3	C-4	C-5	C-6
GalA	99·1	68·2	79·6	78·4	74·6	175·0
GlcA	104·8	72·5	76·5	72·5	76·5	175·9
Rha	99·1	77·6	72·0	74·6	70·2	17·5

which showed that the D-galacturonic acid residue had linkages at positions C-3 and C-4. From the sugar and methylation analyses and from the NMR data it was concluded that the main chain of the acidic polysaccharide of the major mucilage fraction from *A. augusta* was composed of 1,2-linked α-L-rhamnopyronose and 1,4- or 1,3-linked α-D-galacturonic acid residues. Terminal β-D-glucuronic acid residues were attached to the 3- and/or 4-position of the α-D-galacturonic acid residues. On the basis of all the results it was concluded that the polysaccharide is built up of the following repeating unit:



A similar structural feature has been given for mucilagenous polysaccharides from Carya gum (Aspinall & Nasir-uddin, 1965).

REFERENCES

Aspinall, G.O., Cottrel, I.W., Molloy, J.O. & Uddin, M. (1970). *Can. J. Chem.*, **48**, 1290.

Aspinall, G.O. & Nasir-uddin, M. (1965). *J. Chem. Soc.*, 2710.

Bingel, A.S. & Farnsworth, N.R. (1987). *Proceedings of Princess Congress 1*, Vol. II, ed. Prof. Dr H.R.H. Princess Chulabhorn. Chulabhorn Foundation and Mahidol University, Bangkok, Thailand, p. 265.

Bitter, T. & Muir, M.H. (1962). *Anal. Biochem.*, **4**, 330.

Gerwig, G.J., Kamerling, J.P. & Vligenthart, J.F.G. (1978). *Carbohydr. Res.*, **62**, 259.

Gerwig, G.J., Kamerling, J.P. & Vligenthart, J.F.G. (1979). *Carbohydr. Res.*, **77**, 1–7.

Hakim, M.S. (1970). *Hamdard Pharmacopia of Eastern Medicine*, Hamdard National Foundation, Pakistan, p. 353.

Hakomori, S. (1960). *J. Biochem. Tokyo*, **55**, 205.

Jansson, P.E., Kenne, L., Lindberg, B., Liedgren, H. & Lonngren, J. (1976). *Chem. Commun. Univ. Stolckholm*, **8**, 1.

Kirtikar, K.R. & Basu, B.D. (1935). *Indian Medicinal Plants*, 2nd edn. Lalit Mohan Basu, Allahabad, India, p.380.

Mukherjee, K.S. & Badruddoza, S. (1978). *Curr. Sci.*, **47**, 301.

Nahar, N., Mosihuzzaman, M., Chowdhury, T.A. & Rahman, S. (1988). *J. Bangladesh Acad. Sci.*, **12**, 207.

Swardeker, J.S., Sloneker, J.H. & Jeanes, A.R. (1965). *Anal. Chem.*, **37**, 1602.

Taylor, R.L. & Conrad, H.E. (1972). *Biochemistry*, **11**, 1383.