Carbohydrate Polymers 79 (2010) 975–980

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Structural elucidation of an acidic galactan from the eggs of mollusc *Pomacea lineata*

Ana Katarina Menezes Cruz [a,1], Giulianna Paiva Viana Andrade [a], Suely Ferreira Chavante [a], Cláudio L. de Vasconcelos [b], Rosângela Balaban Garcia [b], Edda Lisboa Leite [a], Ana Paula Valente [c], Mauricio Pereira Sales [a], Fernanda Wanderley Oliveira [a,*]

[a] *Departamento de Bioquímica, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*
[b] *Departamento de Química, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil*
[c] *Centro Nacional de Ressonância Magnética de Macromoléculas, Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Brazil*

ARTICLE INFO

Article history:
Received 12 May 2009
Received in revised form 25 September 2009
Accepted 13 October 2009
Available online 17 October 2009

Keywords:
Acidic galactan
Mollusc *Pomacea lineta*
NMR
Viscosity
Piruvylated-galactan

ABSTRACT

A polysaccharide fraction containing acidic galactan (AG) was obtained from eggs of the mollusc *Pomacea lineata* by precipitation with acetone. Its structure was investigated using a combination of chemical analysis, intrinsic viscosity and NMR spectroscopy methods, including 1D and 2D, COSY, TOCSY and HSQC experiments. The chemistry analysis showed that the acidic galactan did not possess neither sulfate nor uronic acid. The intrinsic and relative viscosities were 0.44 ± 0.05 and 1.744 ± 0.07 dL.g^{-1}, respectively. The NMR confirmed that the acidic galactan had a backbone containing β-D-Gal is a predominant compound and presence of the β-D-GlcNAc in less proportion. In addition, were found pyruvate groups forming six-membered cyclic ketals as 4,6-*O*-(1′carboxy)-ethylidene-β-D-galactose residues with configuration *R* in some β-D-Gal*p*.

1. Introduction

The polysaccharides are a complex group of macromolecules with a great structural heterogeneity and can display several biological activities. In marine organisms (algae and invertebrate) the sulfated polysaccharide are found predominantly as fucoidans, sulfated galactans and glycosaminoglycans. The fucoidans are composed by a variety of sulfated L-fucans with anticoagulant activities (Boisson-Vidal et al., 1995; Nader, Lopes, Rocha, Santos, & Dietrich, 2004) and other important pharmacological properties (Haroun-Bouhedja et al., 2002; Rocha et al., 2001). While the sulfated galactans from red algae also known as carrageenans or agarans, are composed by alternating 3-linked β-galactose and 4-linked α-galactose units bearing sulfate substitutions in different positions (Lahaye, 2001). In addition, besides the sulfate hemi-esters, pyruvate acetal and/or methyl ethers can also substitute the hydroxyl group of galactans. The pyruvate groups were present, forming 3,4-*O*-(1′carboxy)-ethylidene-β-D-galactopyranose generally located at noreducing terminals of the chairs of galatctan (Bilan, Vinogradova, Shashkov, & Usov, 2007; Farias et al., 2008). Differently from algae, invertebrate galactans are composed only by units of galactoses, which present a pattern of sulfation and the position of the glycosidic linkage vary among the different species. For example, the sea urchin *Echinometra lucunter* and the tunicate *Herdmania monus* present similar polysaccharides containing 2-*O*-sulfated, 3-linked α-L-galactose (Alves, Mulloy, Diniz, & Mourão, 1997) and 3-*O*-sulfated, 4-linked α-L-galactose, respectively. In other species of tunicates, the sulfated L-galactans have more complex and branched structures (Albano, Pavão, Mourão, & Mulloy, 1990; Mourao & Perlin, 1987; Pavao, Albano, Lawson, & Mourao, 1989; Santos, Mulloy, & Mourão, 1992). Sulfated polysaccharides, such as glycosaminoglycans (GAG), are found in vertebrate tissues (Nader et al., 1999) and posses the ability to create and fill spaces, organizing and modifying the extracellular matrix (ECM). Also other roles were related to its ability in the signalization of proliferative and/or migratory responses through more than one cell surface receptor (Lee & Spicer, 2000; Wrenshall, Stevens, Cerra, & Platt, 1999).

In recent years, studies using the mollusc *Pomacea* sp. as a model, showed that one non-sulfated acidic galactan (AG) was synthesized during morphogenesis of mollusc eggs (Nader, Jerônimo, Porcionatto, & Dietrich, 1985; Nader et al., 1996). This polysaccharide (AG) was topically used in rats intestinal wounds, having an effective action on inflammatory response as granulation tissue, collagen synthesis and extracellular matrix remodeling, which are important events in the wound healing (Cruz et al., 2004).

* Corresponding author. Address: Universidade Federal do Rio Grande do Norte, Centro de Biociências, Departamento de Bioquímica, Av. Senador Salgado Filho 3000, Lagoa Nova, CP: 59072-970 Natal, RN, Brazil. Tel.: +55 84 3215 3416; fax: +55 84 3215 9208.
E-mail address: fernanda@cb.ufrn.br0 (F.W. Oliveira).
[1] Centro de Ciências da Saúde – Programa de Pós-graduação CCS.

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.10.028

Thus, to understand better the potential of scarring the wounds from AG of mollusc *Pomacea* sp. became of considerable interest, to determine its structural composition. In the present work, the chemical characterization was performed to confirm the chemical identity of the polysaccharide and provides data concerning the full assignment of ^{1}H and ^{13}C NMR spectrum of AG which have not yet been reported in the literature.

2. Materials and methods

2.1. Isolation and purification

The mollusc *Pomacea lineata* was collected from the Extremoz lagoon, Natal, RN, Brazil. After capture, animals were maintained in a glass aquarium at room temperature. After oviposition, the eggs were separated from the main egg mass, and the polysaccharides were extracted. Around 80 g of 7 days old eggs after oviposition were collected and homogenized in 160 mL of 0.02 M sodium acetate buffer, pH 5.0. Superase was added to homogenate and then incubated during 18 h at 60 °C. Trichloroacetic acid solution was added to the mixture to a final concentration of 8.2% and maintained at 5 °C during 15 min. To the sobrenadant was added ethanol e maintained for 18 h at 4 °C. The precipitate obtained was fractionated by precipitation with increasing volumes of acetone (1:0.5, 1:0.7, 1:0.9, 1:1.5 and 1:2.0 v/v of sample and acetone). The polysaccharides were identified by agarose gel eletrophoresis as previously describe by Cruz et al. (2004) and the purified polysaccharides in fraction 1:2.0 were submitted to dialysis against distillated water and lyophilized for chemical analysis.

2.2. Characterization methods

2.2.1. Chemical analysis

The content of neutral sugars was determined by antrone reaction using galactose as standard (Roe, 1955). The uronic acid was estimated by the carbazole reaction (Dische, 1962a, 1962b). The amount of total sulfate was performed for the method of the jelly-barium (Dodgson & Price, 1962) and pyruvate was determined in acidic galactan after hydrolysis in 0.08 M oxalic acid for 6 h at 100 °C by the enzymatic method (KIT sigma). The protein was measured by the method of Bradford (Spector, 1978) using bovine serum albumin as the standard (Bio Rad protein assay).

2.2.2. Intrinsic viscosity

AG solution (1%) was prepared in duplicate using distilled water and filtered through a Millipore Millex 41 μm filter and the resultant dispersion was additionally stirred during 1 h at room temperature, before analysis. Intrinsic viscosity $[\eta]$ of acidic galactan solution was obtained using an Ubbelohde viscometer size 0B. The efflux time, in these measurements, was never shorter than 100 s. All these experiments were conducted at a temperature $T = 25.00 \pm 0.05$ °C. Two simultaneous linearization of equations: (1) $\eta_{red} = [\eta] + k'[\eta]^2 c$ and (2) $\eta_{inh} = [\eta] + (k' - 1/2)[\eta]^2 c$ were used, where η_{red} and η_{inh} are reduced and inherent viscosity numbers, respectively (De Vasconcelos et al., 2006). The intrinsic viscosity $dL.g^{-1}$ was showed in deciliters per gram or $dL.g^{-1}$.

2.2.3. NMR spectroscopy

1D ^{1}H and ^{13}C spectra of the AG were recorded using a Bruker DRX 400 MHz spectrometer (Pomin, Valente, Pereira, & Mourão, 2005). The AG (10 mg/mL) was dissolved in 0.5 mL of 99.9% deuterium dioxide (D_2O) at 60 °C. 2D NMR experiments were performed using a Bruker DRX 600 spectrometer. All spectra were recorded at 60 °C with HOD suppression by pre saturation. The two-dimensional homonuclear correlation spectroscopy (COSY), the total correlation spectroscopy (TOCSY) and $^{1}H/^{13}C$ heteronuclear correlation (HSQC) spectra were recorded using states–TPPI (states–time proportion phase incrementation) for quadrature detection in the indirect dimension. TOCSY spectra were run with 4096 · 400 points with a spin-lock field of about 10 kHz and a mixing time of 80 ms. HSQC were run with 1024, 256 points and GARP (globally optimized alternating phase rectangular pulses) for decoupling.

3. Results and discussion

3.1. Purification and chemical analyses of the acidic galactan

The identification of the acidic polysaccharides was performed by electrophoretic behavior in diaminopropane acetate buffer system. The results showed that the fraction 1:2.0 had the same electrophoretic migration to AG previously isolated in our laboratory (Cruz et al., 2004).

The chemical analysis of the acidic galactan showed that it contains galactose as the predominant sugar, small amounts of acid piruvic and the level of proteins was determined as very low at the concentration of approximately 0.2 mg/mL. None uronic acid and sulfate were detected (Table 1). These results are similar to that found by Cruz et al. (2004) demonstrating the effectiveness of the present extraction method.

3.2. Intrinsic viscosity

The value for $[\eta]$ obtained from η_{red} when extrapolated to zero concentration (Fig. 1) demonstrates that the intrinsic viscosity varies greatly depending on the molecular mass, data also obtained by other workers (Burkus & Temelli, 2005; Hokputsa, Jumel, Alexander, & Harding, 2003). The AG described possesses a molecular mass (M_w) in the order of 10^6 (Cruz et al., 2004) and its value of η_{red}: 1.744 ± 0.07 $dL.g^{-1}$ and $dL.g^{-1}$ 0.44 ± 0.05 $dL.g^{-1}$ could suggest that the high molecular mass adopts a more extended conformation which is typical for polyanionic polysaccharides (Tombs & Harding, 1998). Then viscosity presented by the AG could contribute in the process of wound healing (Cruz et al., 2004), once the viscosity can promote the perfect acid medium for the migration and cellular differentiation of tissue.

3.3. Structure of the acidic galactan

The structural analysis of the acidic galactan (AG) purified from eggs of the mollusc *Pomacea lineata* was accomplished using one-dimensional and two-dimensional NMR spectroscopy. The ^{13}C spectrum of the intact AG is shown in Fig. 2a. Mostly signals at the anomeric carbon region were seen around 101.1–107.0 ppm, other signals of carbon linked to oxygen (62.8–84.8 ppm), two peaks of C–CH_3 groups (24.6–26.2 ppm), and carboxyl group around 177.1 ppm. The ^{1}H spectrum as shown in Fig. 2b, reveals that the prevalent anomeric signals are present around 4.47–4.91 ppm, indicating that the configuration of the polysaccharide is β type. The absence of lower-field signals ($\delta > 5$) is usual in polysaccharides which contain β-pyranoses only, as recently described by Bilan et al. (2007). A prominent signal at ~1.47 ppm indicates the presence of a methyl group and the other signal in 2.11 ppm

Table 1
Chemical composition of eggs *Pomacea* sp. acidic galactan.

Polysaccharide	Neutral sugar (%)	Uronic acid (%)	Total sulfate (%)	Proteins (%)	Pyruvic acid (%)
Acidic galactan	86	<0.1	<0.1	<0.2	0.5

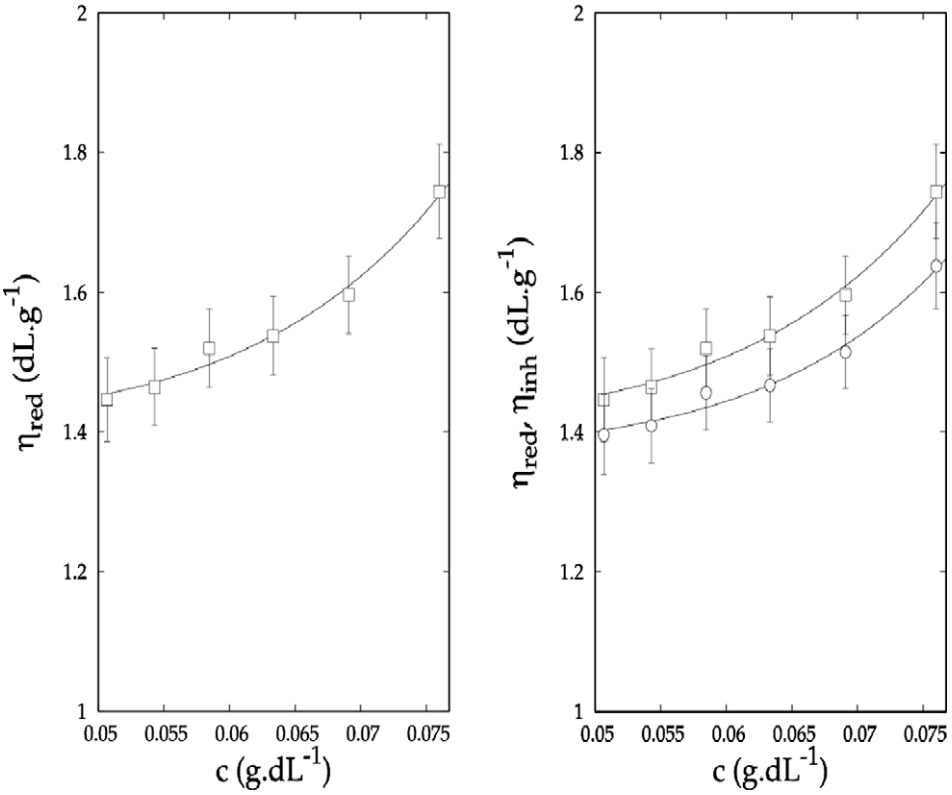

Fig. 1. Reduced viscosity (η_{red}/c) as a function of concentration (c) for acidic galactan in water at 25 °C is the 1.744 ± 0.07 dL.g^{-1} and intrinsic viscosities [η]: 0.44 ± 0.05 dL.g^{-1}.

a *N*-acetyl group, which was also evident in the ^{1}H spectrum of the AG (Fig. 2b).

In addition, 2D homonuclear COSY, TOCSY and heteronuclear HSQC experiments which were required to sign the main signals of this polysaccharide (Figs. 3a,b and 4), showed three preponderantly system of signals, denoted by A, A′, B and C.

The system traced for the AG was more complex due to the greater heterogeneity of the polymer. However, we identified two systems, denoted A and A′ (Table 2). According to the literature data, signals of anomeric protons belonging to the 6-linked galactose residues appeared in the region of δ_H 4.41–4.50 .The analysis of 2D spectra revealed the system A is β-D-Gal*p*-(1 → 6, obtaining values around δ_H/δ_c 3.79/63.0 ppm attributed to the unsubstituted C6 (Bilan et al., 2007; Ali, Weintraub, & Widmalm, 2007).

Moreover others data reveal the existence of another system 6)-β-D-Gal*p*-(1 → 6 denominated A′. The anomeric proton is present in the 4.7 ppm and correlation among the peaks δ_H/δ_c 4.05/70.5 of C6 reveling presence of bound β(1 → 6). The signal of H4 exhibited around 3.94 ppm is characteristic of non-substituted residues at the position 3 of the galactose (Fig. 3a and b). This 6)-β-D-Gal*p*-(1 → 6 exhibits several other signals (C2–C5) which occur around the condensed region in 73.4–76.0 ppm. Table 2 reports the chemical shifts based on interpretation of the COSY, TOCSY and HSQC spectra.

The ^{13}C RMN spectrum possesses an anomeric signal around 107.0 ppm possibly indicating the presence of another galactose, identified in the two-dimensional spectra as signal system B (Figs. 3a,b, 4 and Table 2). Literature data show values of anomeric signals similar to Gal*p* (B), corresponding to β-piranosidic structure described in rizom of *Polygonatum sibiricum,* (Dong & Fang, 2001; Liu, Dong, Dong, Fang, & Ding, 2007). The presence of a signal at 4.64 ppm of the residue of Gal*p* (B) indicates β(1 → 3) chemistry linkage. Bilan et al. (2007) showed that anomeric protons in the 3-*O*-linked residues are located in the region of 4.61–4.72 ppm. The signal value of H4 of 3-*O*-linked residue was found in the lower-field region (δ_H 4.23) and the C6 around 70.0 ppm. These values suggested that pyruvic acid is an acetal-linked to *O*-4 and *O*-6 of Gal (B) in the polysaccharide.

The pyruvate occurrence at the galactose residues (B) of AG is suggested by the presence of signals attributed to the CH_3 around 26.2 ppm and COOH groups at 177.5 ppm (Fig. 2a,b and Table 3). The identified chemistry values both groups CH_3 and COOH are identical to values describe at the literature for a 4,6-*O*-(carboxyethylidene)-β-D-galactopyranoside (Chiovitti et al., 1997; Garegg, Lindberg, & Kvarnström, 1979; Gorin, Mazurek, Duarte, Iacomini, & Duarte, 1982 and Farias et al., 2008). Furthermore, the low-field protons and carbon methyl group (δ_H/δ_c 1.47/26.2) signals also indicate that the presence of pyruvic acid in the AG may contain a 6-member cyclic acetal structure (Table 3) (Bilan et al., 2007). A correlation between the configuration and the CH_3 resonance values show that methyl-galactopyranoses derivated 6-member acetal groups possess signals around 24.0–26.0, when in a equatorial position (*R*), since the methyl values for axial configuration occur around 15.0–18.0 ppm (Gonçalves, Ducatti, Eugênia, Duarte, & Noseda, 2002; Gorin et al., 1982).

Generally the acetal carbon of the pyruvic acid can occur around δ_c 107.0–109.5 for 5-members or δ_c 100.5–102.4 for 6-member, the exact value depends on the configuration. The occurrence of pyruvate containing 5 (*O-3* and *O-4*) or 6 (*O-4* and *O-6*) acetal cyclic members is common in sugars, mainly in α and θ-carragenans 3-linked galactoses (Bilan et al., 2007; Van de Velde, Knutsen, Usov, Rollema, & Cerezo, 2002) and is also present in the albumen of the *Pomacea lineata* as demonstrated by Gorin et al. (1982).

A minor anomeric signal at 101.1 ppm was identified in ^{13}C spectrum to the D-GlcNAc (C) (Fig. 2a), then it was identified in

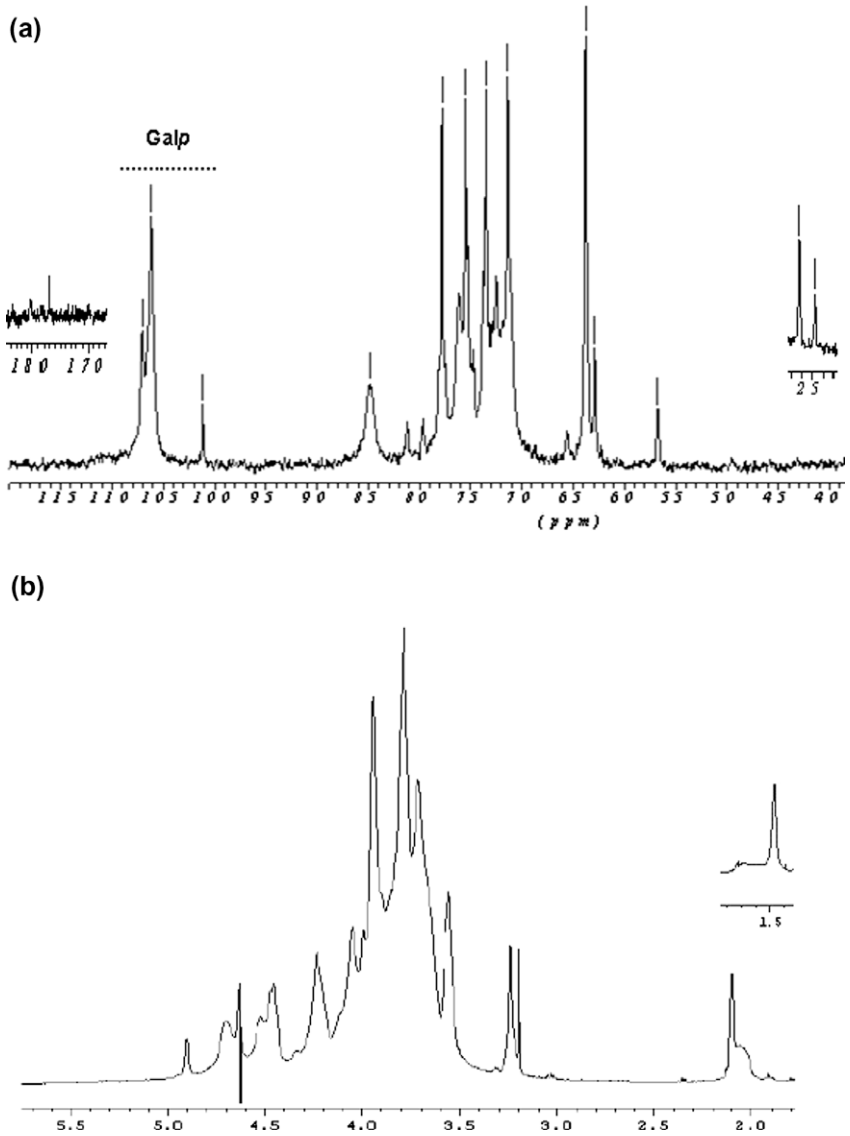

Fig. 2. ^{13}C and ^{1}H NMR spectrum at 400 MHz of the acidic galactan. Chemical shifts of carbon (a) and proton (b).

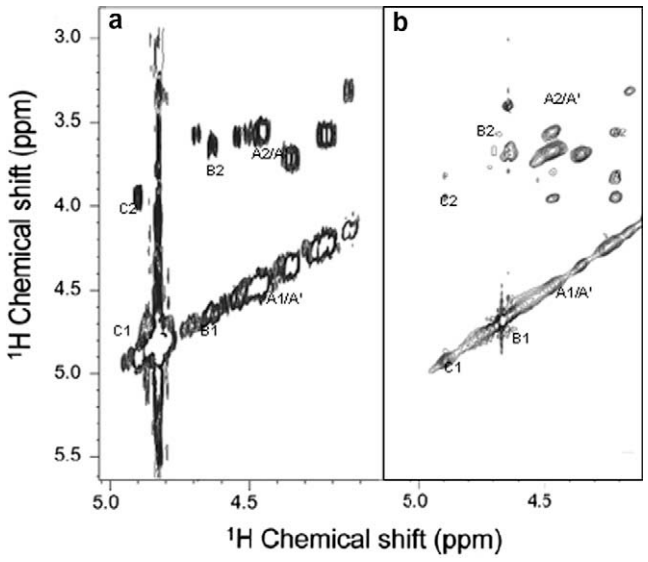

Fig. 3. Strips of the anomeric regions (expansions from 5.0 to 4.0 ppm) from the COSY (a) and TOCSY (b) spectra of the acidic galactan. The spin systems are denoted by A, A′ for 6-linked and by B and C for 3-linked β-galactose units.

the two-dimensional spectra as signal system C (Figs. 3a,b, 4 and Table 2). The HSQC spectrum showed anomeric values around δ_H/δ_c 4.91/101.1 ppm and one nitrogen-bearing carbon (C2 of GlcNAc) at 3.94/56.7 ppm, these data are similar to those presented by Shashkov et al. (2000) and Zhao et al. (2007), confirming the presence of an glucosamine *N*-acetylated in the structure of AG. The presence of this aminosugar in the structure of AG was previously suggested through monosaccaride composition revealing that relationship between glucosamine and galactose was in the ratio 1:14 (Jeronimo, Dietrich, & Nader, 1989). The assignment of the chemical shifts of the *N*-acetyl group to the aminosugar is based on chemical shift similarities of the corresponding monosaccharide (Table 2).

In synthesis, these results indicate, for the acidic galactan from *Pomacea lineata*, a preponderant constitution of the β-D-Gal containing at least two types of the glycosidic linkages, (1 → 6) and (1 → 3) in galactopyranose residues and *N*-acetyl-D-glucosamine. In addition, it was identified pyruvate group forming six-membered cycle ketals as 4,6-*O*-(carboxyethylidene)-β-D-galactopyranoside residues with configuration *R* in some β-D-Gal*p*. Taken together, these data show a great structural heterogeneity of the polysaccharide AG.

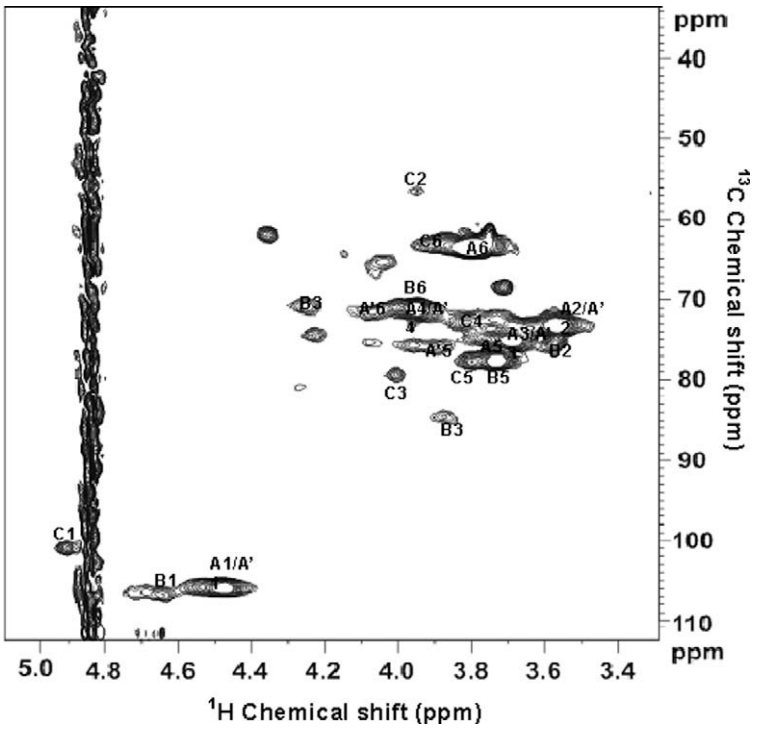

Fig. 4. The HSQC of the acidic galactan. The signals are denoted by A for 6-linked and B for 3-linked β-D-galactopyranosyl units. The peaks denoted A′ correspond to 6)-β-D-Gal*p*-(1 → 6 and the peaks C to β-D-GlcNAc.

Table 2
Proton and carbon chemical shifts for residues in acidic galactan[a].

Unit	^{1}H/^{13}C chemical shifts						Ref.
	H1 C1	H2 C2	H3 C3	H4 C4	H5 C5	H6 C6	
β-D-Gal*p*-(1 → 6 (A)	4.47	3.58	3.69	3.95	3.74	3.79	a
	106.1	73.4	75.4	71.3	77.8	63.7	a
β-D-Gal*p*-(1 → 6	4.43	3.53	3.63	3.92	3.7	3.78	b
	104.9	72.2	74.0	69.9	76.4	62.2	b
6)-β-D-Gal*p*-(1 → 6 (A′)	4.47	3.58	3.69	3.94	3.89	4.06	a
	106.1	73.4	75.4	71.3	76.0	70.0	a
6)-β-D-Gal*p*-(1 → 6	4.47	3.57	3.69	3.97	3.92	4.04	b
	104.8	72.2	73.9	69.9	74.8	70.5	b
β-D-Gal*p*-(1 → 3 (B)	4.64	3.62	3.89	4.23	3.62	3.90	a
	107.0	75.0	84.8	70.5	77.8	70.0	a
β-D-Gal*p*-(1 → 3	4.61	3.61	3.68	3.93	3.69	3.79	b
	105.7	72.5	73.9	70.0	76.5	62.3	b
β-D-GlcNAc (C)	4.91	3.94	4.05	3.82	3.78	3.95	a
	101.1	56.7	79.5	72.5	78.0	62.8	a
β-D-GlcNAc *p*-(1 → 3	4.91	3.77	3.83	3.50	3.39	3.91	c
	102.5	56.0	82.8	69.9	76.1	62.4	c
β-D-GlcNAc *p*-(1 → 3	4.88	3.79	3.71	3.67	3.42	3.85	d
	101.1	55.7	81.0	72.1	77.0	62.0	d

[a] Present paper.
[b] Bilan et al. (2007).
[c] Shashkov et al. (2000).
[d] Zhao et al. (2007).

Table 3
^{13}C chemical shift of pyruvate involved in cyclic ketals in galactoypranoses.

Compound	CH_3	O–C–O	COOH	Ref.
Six-membered ring (*O*-4 and *O*-6 substituted)	26.2	–	177.1	a
	26.9	102.3	177.3	Gorin et al. (1982)
	26.4	102.0	177.2	Bilan et al. (2007)
	25.0	–	175.9	Gonçalves et al. (2002)

[a] Present paper.

Acknowledgements

This work was supported by grants from Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES). We are grateful to Ana Paula Valente for the help with the NMR spectra.

References

Albano, R. M., Pavão, M. S. G., Mourão, P. A. S., & Mulloy, B. (1990). Structural studies of a sulfated-galactan from Styela plicata (Tunicate): Analysis of the Smith-degraded polysaccharide. *Carbohydrate Research, 208*, 163–174.

Ali, T., Weintraub, A., & Widmalm, G. (2007). Structural determination of the O-antigenic polysaccharide from *Escherichia coli* O166. *Carbohydrate Research, 342*, 274–278.

Alves, A. P., Mulloy, B., Diniz, J. A., & Mourão, P. A. S. (1997). Sulfated polysaccharides from the egg jelly layer are species-specific inducers of acrosomal reaction in sperms of sea urchins. *The Journal of Biological Chemistry, 272*, 6965–6971.

Bilan, M. I., Vinogradova, E. V., Shashkov, A. S., & Usov, A. I. (2007). Structure of a highly pyruvylated galactan sulfate from the Pacific green alga Codium yezoense (Bryopsidales, Chlorophyta). *Carbohydrate Research, 342*, 586–596.

Boisson-Vidal, C., Haroun-Bouhedja, F., Ellouali, M., Blondin, C., Fischer, A. M., Agostini, A., et al. (1995). Biological activities of polysaccharides from marine algae. *Drugs of the Future, 20*, 1237–1249.

Burkus, Z., & Temelli, F. (2005). Rheological properties of barley b-glucan. *Carbohydrate Polymers, 59*, 459–465.

Chiovitti, A., Bacic, A., Craik, D. J., Munro, S. L. A., Kraft, G. T., & Liao, M. L. (1997). Cellwall polysaccharides from Austalian red algae of the family *Solieriaceae* (Gigartinales, Rhodophyta): Novel, highly pyruvated carrageenans from the genus Callophycus. *Carbohydrate Research, 299*, 229–243.

Cruz, A. K. M., Pereira, W. O., Santos, E. A., Carvalho, M. G. F., Medeiros, A. C., & Oliveira, F. W. (2004). Comparative study between the effects of hyaluronic acid and acid galactan purified from eggs of the mollusk *Pomacea* sp. in wound healing. *Acta Cirúrgica Brasileira, 19*(1), 13–17.

De Vasconcelos, C. L., Bezerril, P. M., Santos, D. E. S., Dantas, T. N. C., Pereira, M. R., & Fonseca, J. L. C. (2006). Effect of molecular weight and ionic strength on the formation of polyelectrolyte complexes based on poly(methacrylic acid) and chitosan. *Biomacromolecules, 7*, 1245–1252.

Dische, Z. (1962a). Color reactions of 6-deoxi and 3–6-dideoxioses. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (Vol. 1, pp. 501–503).

Dische, Z. (1962b). Color reactions of pentoses. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (Vol. 1, pp. 484–488).

Dodgson, K. S., & Price, R. G. (1962). A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochemistry Journal, 84*, 106–110.

Dong, Q., & Fang, J. N. (2001). Structural elucidation of a new arabinogalactan from the leaves of Nerium indicum. *Carbohydrate Research, 332*, 109–114.

Farias, E. H. C., Pomin, V. H., Valente, A. P., Nader, H. B., Rocha, H. A. O., & Mourão, P. A. S. (2008). A preponderantly 4-sulfated, 3-linked galactan from the green alga *Codium isthmocladum*. *Glycobiology, 18*, 250–259.

Garegg, P. J., Lindberg, B., & Kvarnström, I. (1979). Preparation and studies of pyruvic acid and N.M.R related acetals of pyranosides: Configuration at the acetal carbon atoms. *Carbohydrate Research, 77*(1), 71–78.

Gonçalves, A. G., Ducatti, D. R. B., Eugênia, M., Duarte, R., & Noseda, M. D. (2002). Sulfated and pyruvalated disaccharide alditols obtained from a red seaweed galactan: ESIMS and NMR approaches. *Carbohydrate Research, 337*, 2443–2453.

Gorin, P. A. J., Mazurek, M., Duarte, H. S., Iacomini, M., & Duarte, J. H. (1982). Properties of ^{13}C-N.M.R. spectra of *O*-(1-carboxyethylidene) derivatives of methyl β-D-galactopyranoside: Models for determination of pyruvic acid acetal structures in polysaccharides. *Carbohydrate Research, 100*, 1–15.

Haroun-Bouhedja, F., Lindenmayer, F., Lu, H., Soria, C., Jozefonvicz, J., & Boisson-Vidal, C. (2002). In vitro effects of fucans on MDA-MB231 tumor cell adhesion and invasion. *Anticancer Research, 22*, 2285–2292.

Hokputsa, S., Jumel, K., Alexander, C., & Harding, S. E. (2003). Hydrodynamic characterisation of chemically degraded hyaluronic acid. *Carbohydrate Polymers, 52*, 111–117.

Jeronimo, S. M. B., Dietrich, C. P., & Nader, H. B. (1989). Struture of sulfated glycosaminoglicans synthesized during the ontogeny of the mollusc *Pomacea* sp. *Comparative Biochemistry and Physiology, 93B*, 899–903.

Lahaye, M. (2001). Development on gelling algal galactans, their structure and physico-chemistry. *Journal of Applied Phycology, 13*, 173–184.

Lee, J. Y., & Spicer, A. P. (2000). Hyaluronan: A multifunctional, megaDalton, stealth molecule. *Current Opinion in Cell Biology, 12*, 581–586.

Liu, L., Dong, Q., Dong, X., Fang, J., & Ding, K. (2007). Structural investigation of two neutral polysaccharides isolated from rhizome of *Polygonatum sibiricum*. *Carbohydrate Polymers, 70*, 304–309.

Mourao, P. A. S., & Perlin, A. S. (1987). Structural features of sulfated glycans from the tunic of Styela plicata (Chordata-Tunicata) A unique occurrence of L-galactose in sulfated polysaccharides. *European Journal Biochemistry, 166*, 431–436.

Nader, H. B., Chavante, S. F., Santos, E. A., Oliveira, F. W., Paiva, J. F., Jeronimo, S. J., et al. (1999). Heparan sulfates and heparins: Similar compounds performing the same functions in vertebrates and invertebrates. *Brazilian Journal of Medical Biology Research, 32*(5), 529–538.

Nader, H. B., Jerônimo, S. M. B., Porcionatto, M. A., & Dietrich, C. P. (1985). Biosynthesis of an acid galactan and sulfate glycosaminoglicans during embryonic development of the mollusc *Pomacea* sp. *Biochimica et Biophysica Acta, 840*, 187–192.

Nader, H. B., Lopes, C. C., Rocha, H. A. O., Santos, E. A., & Dietrich, C. P. (2004). Heparins and heparinoids: Occurrence, structure and mechanism of antithrombotic and hemorrhagic activities. *Current Pharmaceutical Design, 10*(9), 951–966.

Nader, H. B., Oliveira, F. W., Jerônimo, S. M. B., Chavante, S. F., Sampaio, L. O., & Dietrich, C. P. (1996). Synchronized order of appearance of hyaluronic acid (or acidic galactan) → chondroitin C-6 sulfate → chondroitin C-4/C-6 sulfate, heparan sulfate, dermatan sulfate → heparin during morphogenesis, differentiation and development. *Brazilian Journal of Medical Biology Research, 9*(29), 1221–1226.

Pavao, M. S., Albano, R. M., Lawson, A. M., & Mourao, P. A. (1989). Structural heterogeneity among unique sulfated L-galactans from different species of ascidians (tunicates). *Journal of Biological Chemistry, 264*, 9972–9979.

Pomin, V. H., Valente, A. P., Pereira, M. S., & Mourão, P. A. (2005). Mild acid hydrolysis of sulfated fucans: A selective 2-desulfation reaction and an alternative approach for preparing tailored sulfated oligosaccharides. *Glycobiology, 15*, 1376–1385.

Rocha, H. A., Franco, C. R., Trindade, E. S., Carvalho, L. C., Veiga, S. S., Leite, E. L., et al. (2001). A fucan from the brown seaweed Spatoglossum schröederi inhibits Chinese hamster ovary cell adhesion to several extracellular matrix proteins. *Brazilian Journal of Medical Biology Research, 34*, 621–626.

Roe, J. (1955). The determination of sugar in blood and spinal fluid with anthrone reagent. *Journal of Biological Chemistry, 212*, 335–343.

Santos, J. A., Mulloy, B., & Mourão, P. A. S. (1992). Structural diversity among sulfated α-L-galactans from ascidians (tunicates) Studies on the species Ciona intestinalis and Herdmania monus. *European Journal of Biochemistry, 204*(2), 669–677.

Shashkov, A. S., Senchenkova, S. N., Vinogradov, E. V., Zatonsky, G. V., Knirel, Y. A., Literacka, E., et al. (2000). Full structure of the O-specific polysaccharide of Proteus mirabilis O24 containing 3,4-*O*-[(*S*)-1-carboxyethylidene]-D-galactose. *Carbohydrate Research, 329*(2), 453–457.

Spector, J. (1978). Refinement of the coomassie blue method of protein qualification. A simple and linear spectrofotometric assay of 0.5 to 50 μg of protein. *Analytical Biochemistry, 86*, 142–146.

Tombs, M., Harding, S. E. (1998). An introduction to polysaccharide. In *Biotechnology*. London: Taylor and Francis.

Van de Velde, F., Knutsen, S. H., Usov, A. I., Rollema, H. S., & Cerezo, A. S. (2002). ^{1}H and ^{13}C high resolution NMR spectroscopy of carrageenans: Application in research and industry. *Trends in Food Science & Technology, 13*, 73–92.

Wrenshall, L. E., Stevens, R. B., Cerra, F. B., & Platt, J. L. (1999). Modulation of macrophage and B cell function by glycosaminoglycans. *Journal of Leukocyte Biology, 66*, 391–400.

Zhao, G., Perepelov, A. V., Senchenkova, S. N., Shashkov, A. S., Feng, L., Li, X., et al. (2007). Structural relation of the antigenic polysaccharides of *Escherichia coli* O40, Shigella dysenteriae type 9, and *E coli* K47. *Carbohydrate Research, 342*(9), 1275–1279.