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Structure of a glycoglucuronomannan from the gum exudate of *Vochysia tucanorum* (family Vochysiaceae)

Ricardo Wagner [a], Fernanda F. Simas [a], Graciele C.Z. Pereira [a], Aline Angeli [b], José O. Brito [b], Sandra M. Woranovicz-Barreira [c], Cirene L. Delgobo [d], Guilherme L. Sassaki [a], Marcello Iacomini [a], Philip A.J. Gorin [a,*]

[a] *Departamento de Bioquímica, e Biologia Molecular, Universidade Federal do Paraná, CxP 19046, 81531-990 Curitiba, PR, Brazil*
[b] *Departamento de Engenharia Florestal, Escola Superior de Agricultura Luiz de Queiroz, CxP 9, 13418-900 Piracicaba, SP, Brazil*
[c] *Departamento de Farmácia, Universidade Federal do Paraná, 80210-170 Curitiba, PR, Brazil*
[d] *Departamento de Análises Clínicas, Universidade Federal de Santa Catarina, 88040-970 Florianópolis, SC, Brazil*

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Abstract

The polysaccharide (VTP) from the gum exudate of *Vochysia tucanorum* gave aqueous solutions of low viscosity. VTP contained two components, which on freeze–thawing, gave rise to soluble (S-VTP) and precipitated (P-VTP) fractions, with M_w 350,000 and 520,000 g/mol, and d*n*/d*c* 0.134 and 0.139, respectively. Acid hydrolysis of S-VTP provided Ara, Xyl, Man, Gal, Glc in a 43:1:5:18:2 molar ratio (GC–MS) with 31% uronic acid, whereas the values for P-VTP were 51:1:5:16:2 and 25%, respectively. Methylation analysis of S-VTP revealed 11 neutral, partially *O*-methylated alditol acetates, mainly from nonreducing end- (31%) and 5-*O*-substituted Ara*f* (9%), 3-*O*- (16%) and 3,4-di-*O*-substituted Gal*p* (9%), and 2,3-di-*O*-substituted Man*p* units (10%): P-VTP was similar, containing the same structures, consistent with their almost identical ^{13}C NMR spectra. Partial hydrolysis of P-VTP removed side chains giving rise to the main chain (PH4h) of -β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → 4)- repeating groups. A controlled Smith degradation of S-VTP gave a polysaccharide (S_1-SVTP), shown by methylation analysis to contain α-L-Ara*f*-(1 → 3)-β-D-Gal*p*-, α-L-Ara*f*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-, α-L-Ara*f*-(1 → 3)[α-L-Ara*f*-(1 → 4]-β-D-Gal*p*-, and α-L-Ara*f*-(1 → 2)-α-D-Man*p*- groups, with the Ara*f* region probably containing some additional, oxidized 5-*O*-substituted L-Ara*f* units. This agreed with the structure of a polysaccharide (PH30), formed by milder hydrolysis of P-VTP, sufficient to only remove Ara*f* groups. For a more reliable characterization and quantification of S-VTP structures, it was subjected to two carbodiimide reductions and methylation analysis of the product (CR_2-SVTP) showed the former Glc*p*A units to be 3,4-di-*O*-substituted. Minute amounts of a (1 → 4)-linked β-D-xylan were present in the gum, along with free reducing oligosaccharides. ESI-MS–MS, ^{13}C NMR, and methylation analysis showed Ara-Gal-Man side-chain sequences similar to those of the glycoglucuronomannan. Mainly present was Ara_4-Hex_2, whose daughter ions were Ara_3-Hex_2, and Ara_2. The oligosaccharide mixture contained nonreducing end-, 2-*O*- and 3-*O*-substituted Ara*f*, internal 3-*O*- and 3,4-di-*O*-substituted internal Gal*p*, and 2-*O*-substituted Man*p* reducing end-units. The glycoglucuronomannan of *Vochysia lehmannii* gum, although having the same main-chain, contained less side-chains.

Keywords: Glycoglucuronomannan; *Vochysia tucanorum*; Chemotaxonomy; Free oligosaccharides; β-D-Xylan

1. Introduction

The family Vochysiaceae comprises approximately 7 genera and 200 species, among which is *Vochysia tucanorum*. The tree grows in the transition zone between the Brazilian savannah and Atlantic forest. A natural gum exudate occurs on its trunk and is reported to be a food for monkeys (Almeida, Proença, Sano, & Ribeiro, 1998). When dissolved in water, it forms solutions with low viscosity, similar to the gum from *Vochysia lehmannii* (Wagner et al., 2004).

* Corresponding author. Tel.: +55 41 3361 1670; fax: +55 41 3266 2042.
E-mail address: cesarat@ufpr.br (P.A.J. Gorin).

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Many trees and shrubs produce gum when stressed naturally by temperature, lack of humidity, insect attack, and by manual cutting.

The gum from *V. lehmannii* contained a glycoglucuronomannan, with a repeating → 4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1→ group in its main chain, lightly substituted with complex side-chains (Wagner et al., 2004).

We now report structural analyses on the glycoglucuronomannan of *V. tucanorum* gum, and traces of free, reducing oligosaccharides and a β-D-xylan.

2. Experimental

2.1. Vochysia tucanorum gum

The gum was collected from the trunk of a tree growing in a sub-tropical region, near Piracicaba, State of São Paulo, Brazil: the tree was identified by Aline Angeli and José Otávio Brito, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba.

2.2. Preparation of VTP, S-VTP, and P-VTP polysaccharides

The gum (200 g) dissolved almost completely in H_2O (300 mL) and the remaining debris was removed by filtration, followed by centrifugation. The supernatant was added to excess EtOH (3×) to give a precipitate (VTP; 65 g) and a portion (50 g) was dissolved in H_2O (200 mL), which was frozen, thawed gently at 4 °C, and resulting insoluble material was removed by centrifugation and isolated to give P-VTP (18 g). The aqueous supernatant was treated with excess EtOH to give a precipitate of S-VTP (32 g). Both polysaccharide fractions were analyzed along with components of supernatant I (Fig. 1), which was partially evaporated to give a moist residue containing oligosaccharides and β-xylan (see Section 2.8).

2.3. Partial hydrolyses of P-VTP

P-VTP (400 mg) was partially hydrolyzed with 0.5 M TFA (300 mL) at 100 °C for 30 min. to remove the α-L-Ara*f* units and to reveal units of β-D-Gal*p* and α-D-Man*p*. The solution was partially evaporated and treated with excess EtOH to give precipitated polysaccharide PH30 (39% yield).

In order to prepare the main chain of P-VTP, it (3.0 g) was partially hydrolyzed with 0.5 M TFA (300 mL) at 100 °C for 4 h to give, using the same procedure, PH4h (yield 20%).

2.4. Monosaccharide composition of polysaccharides

Each sample (1 mg) was analyzed for uronic acid content using an *m*-hydroxydiphenyl colorimetric method, in which neutral sugars do not interfere (Filisetti-Cozzi & Carpita, 1991).

Other samples were hydrolyzed with 2 M TFA for 8 h at 100 °C, and the product was successively reduced with $NaBD_4$ and acetylated with Ac_2O-pyridine (1:1, v/v) at 100 °C for 1 h. The resulting alditol acetates mixtures were analyzed by GC–MS (Jansson, Kenne, Liedgren, Lindberg, & Lönngren, 1976), using a Saturn 2000 model installed with a capillary DB-225 column (30 m × 0.25 mm i.d.), programmed from 50 °C (1 min) at 40 °C/min to 220 °C (then hold), with He as carrier gas.

2.5. Controlled Smith degradation of S-VTP

The polysaccharide (5.0 g) was dissolved in H_2O (250 mL) containing $NaIO_4$ (2.7 g). After 72 h, ethylene glycol (3.0 mL) was added and the solution dialyzed for 2 days against tap H_2O. $NaBH_4$ (2.0 g) was then added and after 24 h the solution was acidified to pH 6.0 with HOAc and dialyzed. The solution was evaporated to 100 mL, adjusted to pH 2.0 with dil. H_2SO_4, and kept at 100 °C for 40 min (Gorin, Horitsu, & Spencer, 1965), neutralized ($BaCO_3$), filtered, and the filtrate evaporated to a small volume. Addition to excess EtOH gave S_1-SVTP (1.0 g).

2.6. Carboxy-reduction of S-VTP

S-VTP (350 mg) was submitted to two successive carboxy-reduction cycles according to Taylor & Conrad (1972) to give CR_1-SVTP (205 mg) and then CR_2-SVTP (190 mg). Another portion (10 mg) was successively carboxy-reduced using $NaBD_4$ and hydrolyzed with 2 M TFA at 100 °C for 8 h and the derived alditol acetates were analyzed by GC–MS to determine the deuteration patterns in the of glucitol and galactitol derivatives (Jansson et al., 1976).

2.7. Methylation analysis of polysaccharides

Samples (1 mg) of S-VTP, P-VTP, S_1-SVTP, CR_2-SVTP, PH30, PH4h, and β-xylan were each per-*O*-methylated by the method of Ciucanu & Kerek (1983). The products were then partially hydrolyzed with 50% H_2SO_4 (v/v, 1 mL) for 1 h at 4 °C (Saeman, Moore, Mitchell, & Millet, 1954), after which the solution was diluted to 1 M and maintained for 16 h at 100 °C for complete hydrolysis. The solution was neutralized ($BaCO_3$), filtered, and the filtrate evaporated to give a residue of partially *O*-methylated aldoses, which was converted to their corresponding monodeuterated *O*-methyl alditol acetates by successive treatments with $NaBD_4$ and Ac_2O-pyridine. GC–MS was carried out on the mixture, as in Section 2.4, and its components were identified by their typical retention times and e.i breakdown patterns, using standard samples containing all possible isomers, (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005), with the exception of 4,6-di-*O*-methyl-

galactitol acetate, which was examined in a separate experiment.

2.8. Isolation of EtOH-soluble oligosaccharides and β-D-xylan

The EtOH-soluble fraction obtained on precipitation of polysaccharide (Section 2.2) contained mainly arabinose and galactose (PC in *n*-BuOH-pyridine-H_2O, 5:3:3); developed with $AgNO_3$–NaOH (Trevelyan, Procter, & Harrison, 1950), and a trace of oligosaccharides. To obtain the latter, the mixture was applied to a charcoal-Celite column (Whistler & Durso, 1950). It was eluted with H_2O, followed by 5%, 10%, 20%, and 40% aq. EtOH. PC examination showed that the oligosaccharides were present in the 10% and 20% aq. EtOH eluates and these were combined and applied to a 25×3 cm i.d. Sephadex LH-20 gel permeation column (Amersham Bioscience—dead volume = 13 mL). Elution with H_2O gave four fractions (Fig. 5), the elution profile being followed by the phenol-H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using a micro-technique in 96-well plates. Fraction 2 contained β-D-xylan (5.0 mg) and fraction 4 oligosaccharides (9.0 mg), which were both further investigated.

2.9. ESI-MS and ESI-MS–MS of oligosaccharide mixture

ESI-MS examination of the oligosaccharide fraction 4 was carried out using a Micromass Quattro LC spectrometer. The sample (1 μg/mL) was dissolved in H_2O to which CH_3CN was added to give a 1:1 mixture. It was applied using a manual loop injector (10 μL volume) on to a flow rate of 20 μL/min of the 1:1 solvent. The cone voltage was 88 V and the capillary voltage 3.64 kV. The analysis was carried out in the +ve mode from *m*/*z* 100 to 1200. MS–MS was carried out using a collision energy of 139.9 eV.

2.10. ^{13}C NMR spectra

NMR spectra were obtained with a Bruker 400 MHz DRX Avance spectrometer from solutions in 99.9% D_2O at 50 °C (shifts are expressed as δ ppm, relative to external Me_4Si, $\delta = 0$).

2.11. HPSEC analysis

The molecular weight distribution of S-VTP and P-VTP was determined using Wyatt Technology equipment incorporating ultrahydrogel columns 2000, 500, 250, and 120, connected to a differential refractometer (model 2410, Waters) and a laser light scattering detector, at 632.8 nm (Dawn DSPF model). The eluant was aq. 0.1 M $NaNO_2$ + 0.2 g/L NaN_3, at a flow rate of 0.6 mL/min. The samples were dissolved in aq. $NaNO_2$ (1 mg/mL) and filtered through a cellulose membrane with an average pore diameter of 0.2 μm: a volume of 100 μL was injected into the apparatus. Results were provided directly with the aid of computer software ASTRA 4.70.07.

3. Results

3.1. Fractionation, M_w values, and analysis of polysaccharide components

The gum from *V. tucanorum* dissolved almost completely in water at room temperature and after removal of debris, EtOH precipitation gave rise to polysaccharide VTP (33%). This was subjected to fractionation, derivatization, and analysis procedures (Fig. 1).

HPSEC examination, using a refractive index detector, showed it to be heterogeneous (Fig. 2a). Fractionation by freeze–thawing of the aqueous solution gave soluble S-VTP (24%) and precipitated P-VTP (12%; yields based on original gum), and each was homogeneous by HPSEC with M_W 350,000 g/mol (d*n*/d*c* 0.134) and 520,000 (d*n*/d*c* 0.139), respectively (Fig. 2b). They had similar monosaccharide compositions and provided molar ratios of Ara, Xyl, Man, Gal, Glc, and uronic acid of 43:1:5:18:2:31 and 51:1:5:16:2:25, respectively (Table 1), and although the uronic acid contents were accurate, the percentages of the neutral monosaccharides depended on those liberated by hydrolysis followed by GC–MS of derived alditol acetates, the mannose contents being low due to the resistance of glucuronosyl-mannose to hydrolysis. The ^{13}C NMR spectra of S-VTP and P-VTP were also almost identical (Fig. 3a and b), with at least 5 high-frequency C-1 signals at δ 106.8–108.8 corresponding to α-L-Ara*f* units (Joseleau, Chambat, Vignon, & Barnoud, 1977).

In the following analyses, insoluble P-VTP was used for partial hydrolyses since it dissolved rapidly in the reaction medium, whereas soluble S-VTP was employed in the controlled Smith degradation and carbodiimide reduction, since complete dissolution would not occur rapidly.

3.2. Partial hydrolysis products from P-VTP

Partial hydrolysis of P-VTP with 0.5 M TFA at 100 °C for 30 min gave rise to a polysaccharide PH30 (39% yield). Complete hydrolysis gave Ara, Man, Gal, and Glc in a 2:23:45:5 molar ratio (GC–MS) with 26% uronic acid (Table 1); the presence of Glc is due to reduction of glucuronolactone in the preparation of alditol acetates. High frequency C-1 signals of α-L-Ara*f* units were absent in the ^{13}C NMR spectrum of PH30 (Fig. 4a) and the signal at δ 103.5 was from β-D-Gal*p* units, whose high frequency and $J = 164$ Hz (^{13}C, ^{1}H coupled spectrum, not shown) showed a β-configuration (Perlin & Casu, 1969). Another prominent signal at δ 98.3, $J = 174$ Hz was from α-D-Man*p* units.

Under stronger conditions of 0.5 M TFA at 100 °C, for 4 h, polysaccharide PH4h (20% yield) was formed. It provided Man, Gal, and Glc in a 39:4:18 molar ratio on acid

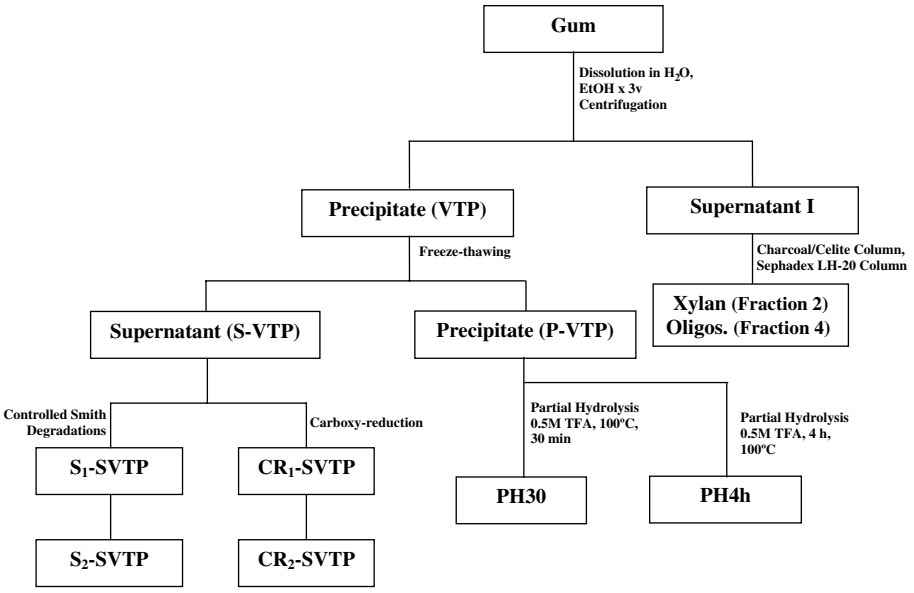


Fig. 1. Fractionation and isolation of polysaccharide components.

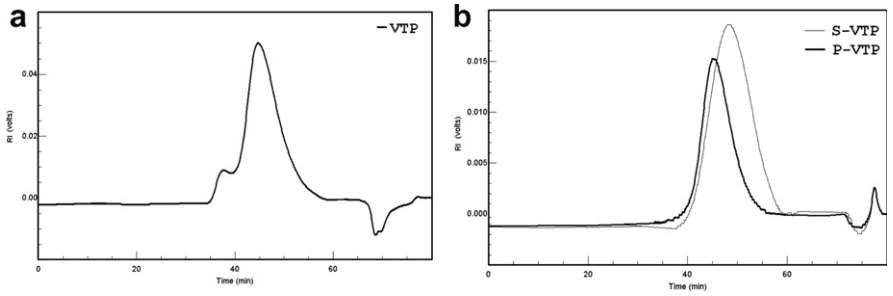


Fig. 2. HPSEC profiles, using a refractive index detector, of VTP (a), and both S-VTP and P-VTP (b).

Table 1
Monosaccharide composition of fractions obtained from gum of *V. tucanorum*[a]

Polysaccharide	Uronic acid	Ara	Xyl	Man[b]	Gal	Glc
S-VTP	31	43	1	5	18	2
P-VTP	25	51	1	5	16	2
PH30	26	2	–	23	45	5
PH4h	39	–	–	39	4	18
S_1-SVTP	19	21	1	9	46	4
CR_1-SVTP	9	43	1	11	28	8
CR_2-SVTP	4	43	1	13	29	10

[a] Neutral monosaccharides were estimated by hydrolysis, followed by GC–MS of derived alditol acetates, and the uronic acid content was determined colorimetrically.

[b] In the case of uronic-acid containing polymers, the proportion of mannose can be low, due to its incomplete liberation, with formation of aldobiouronic acid.

hydrolysis (GC–MS) and contained 39% uronic acid (Table 1). The ^{13}C NMR spectrum of PH4h contained 11 main signals showing a two-unit repeating group (Fig. 4b), with signals analogous to those of β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → 4)-β-D-Glc*p*A-(1 → 2)-D-Man, isolated via partial hydrolysis of the glycoglucuronomannan of *Vochysia lehmannii* (Wagner et al., 2004).

Assignment of the eleven signals arising from repeating groups of the -(1 → 4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-main-chain, with C-1 signals from β-D-Glc*p*A at δ 101.6 and α-D-Man*p* at δ 98.4, was carried out. The ^1H NMR spectrum of PH4h (not shown) contained two main H-1 signals, one at δ 5.44 with a J value too small to be detected (α-D-Man*p*), and the other at δ 4.53, $J = 7.6$ Hz (β-D-Glc*p*A). For assignment of other ^1H signals of β-D-Glc*p*A units, initial TOCSY and COSY approaches were used. The TOCSY spectrum (Fig. 4c) showed four correlations of H-1 of β-D-Glc*p*A until H-5, and COSY (not shown) identified these as follows: δ 3.44 (H-2), 3.73 (H-3), 3.57 (H-5), and 3.84 (H-4). The HMQC spectrum (Fig. 4d) was incomplete, with $^1H/^{13}C$ correlation of nuclei (N) of N-1 (δ 4.53/101.6), N-2 (δ 3.44/72.9), N-3 (δ 3.73/76.6), and N-4 (δ 3.84/77.1). That of N-5 did not appear, but it should be at δ 3.57/77.0, based on the unassigned ^{13}C signal. For assignment of the α-D-Man*p* signals, TOCSY showed correlation of H-1 at δ 5.44 up to H-2 at δ 4.18 (Fig. 4c), a limitation which also appeared in COSY. HMQC gave an N-1 at δ 5.44/98.4 and an N-2 signal at

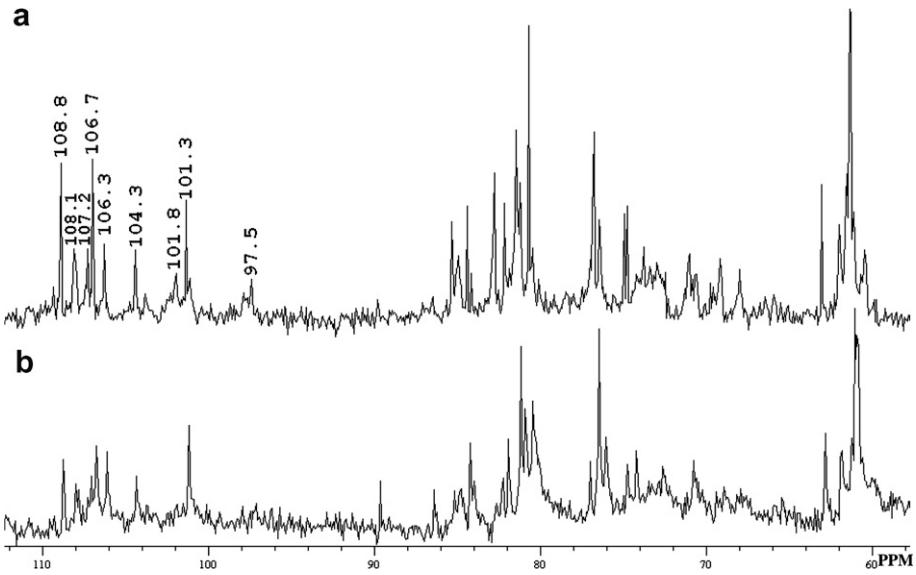


Fig. 3. ^{13}C NMR spectra of S-VTP (a) and P-VTP (b), obtained at 50 °C in D_2O: numerical values are in δ ppm.

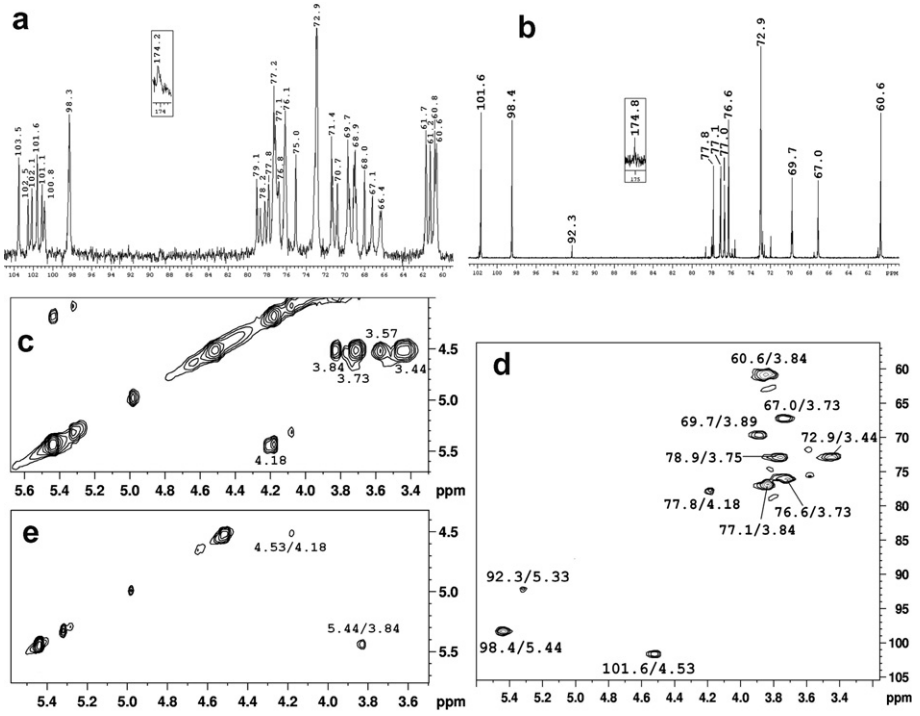


Fig. 4. ^{13}C NMR spectra of products formed by progressive partial hydrolysis of P-VTP to form PH30 (a) and then PH4h (b). TOCSY (c), HMQC (d), and ROESY spectra (e) of PH4h, obtained at 50 °C in D_2O: numerical values are in δ ppm.

δ 4.18/77.8 (Fig. 4d). The other ^{13}C signals were assigned by comparison with shifts of Me α-D-Man*p* and Me β-D-Glc*p*A (Gorin & Mazurek, 1975). The three signals at lowest frequencies (Fig. 4b) could only arise from α-D-Man*p* units and the assignments are δ 60.6 (C-6), 67.0 (C-4) and 69.7 (C-3). Consistent with the structure of PH4h, its ROESY (Fig. 4e) showed correlation of H-1 of β-D-Glc*p*A at δ 4.53 with H-2 of α-D-Man*p* at δ 4.18 and H-1 of α-D-Man*p* at δ 5.44 with H-4 of β-D-Glc*p*A at δ 3.84. The above assignments for PH4h spectra are summarized in Table 3.

3.3. Controlled Smith degradation of S-VTP

A controlled Smith degradation (Gorin et al., 1965) was carried out on S-VTP to give a polysaccharide (S_1-SVTP; 20% yield), which provided on hydrolysis Ara, Xyl, Man,

Gal, and Glc in a 21:1:9:46:4 molar ratio (GC–MS): 19% of uronic acid was present (Table 1). The overall analysis data showed that S-VTP (and P-VTP) had main chains consisting of -(1 → 4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*- repeating groups, substituted by branched side-chains.

3.4. Carboxy-reductions of S-VTP

In order to determine the *O*-substitution positions on the uronic acid, S-VTP was carboxy-reduced to give CR_1-SVTP, which provided on hydrolysis Ara, Xyl, Man, Gal, and Glc in a 43:1:11:28:8 molar ratio (GC–MS) with 9% uronic acid, and since the latter content was high, it was reduced again to give CR_2-SVTP with respective values of 43:1:13:29:10 and 4% (Table 1). Since there were increases of values for Gal (18–29%) and Glc (2–10%), when compared with S-VTP, each one could have arisen from their respective uronic acid, so S-VTP was reduced with a carbodiimide system incorporating $NaBD_4$. GC–MS of derived alditol acetates showed that glucitol acetate gave additional +2 Da ions with *m*/*z* 117, 129, 141, and 189 from its di-deuterated C-6, formed from GlcA units, whereas galactitol acetate had a non-deuterated C-6, showing that there were no GalA units.

3.5. Methylation analysis of selected polysaccharides

The ^{13}C NMR spectra of S-VTP (Fig. 3a) and P-VTP (Fig. 3b) were almost identical. In agreement, methylation analysis of S-VTP and P-VTP (Table 2) also gave rise to the same neutral, partially *O*-methylated derivatives, the main ones corresponding respectively to nonreducing end-units (31% and 31%), 3-*O*- (5% and 8%), and 5-*O*-substituted Ara*f* (9% and 10%), 3-*O*- (16% and 17%), and 3,4-di-*O*-substituted Gal*p* (9% and 9%), and. 2,3-di-*O*-substituted Man*p* units (10% and 17%), as in structure **1**.

Table 3
^{13}C and ^{1}H NMR assignments for the PH4h main chain, determined at 50 °C in D_2O, numerical values are in δ ppm

Nuclei	→2)-α-D-Man*p*-(1→	→4)-β-D-Glc*p*A-(1→
C-1/H-1	98.4/5.44	101.6/4.53
C-2/H-2	77.8/4.18	72.9/3.44
C-3/H-3	69.7/3.89	76.6/3.73
C-4/H-4	67.0/3.73	77.1/3.84
C-5/H-5	72.9/3.89	77.0/3.57[a]
C-6/H-6	60.6/3.84	174.8

[a] This resonance did not appear in the HMQC spectrum and the ^{13}C value was assigned by process of elimination.

The main-chain structure of the glycoglucuronomannan P-VTP was a repeating group of –β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → 4), as represented by its partial hydrolysis product PH4h. As expected on methylation analysis, GC–MS gave almost exclusively (88%) of the partially *O*-methylated alditol acetates arising from 2-*O*-substituted Man*p* units (Table 2).

A series of methylation analyses were carried out on the glycoglucuronomannan and selected derivatives to determine its side-chain structures (Table 2). Twice, carboxy-reduced CR_2-SVTP contained Glc*p*, instead of Glc*p*A units present in S-VTP and P-VTP, and a better characterization and quantification of each unit was possible. Mainly present were nonreducing end- (24%), 3-*O*- (6%) and 5-*O*-substituted Ara*f* (2%), 3-*O*- (18%) and 3,4-*O*-substituted Gal*p* (11%), 2,3-di-*O*-substituted Man*p* (18%), and 3,4-di-*O*-substituted Glc*p* (13%) units, the latter corresponding to Glc*p*A units in the former main chain.

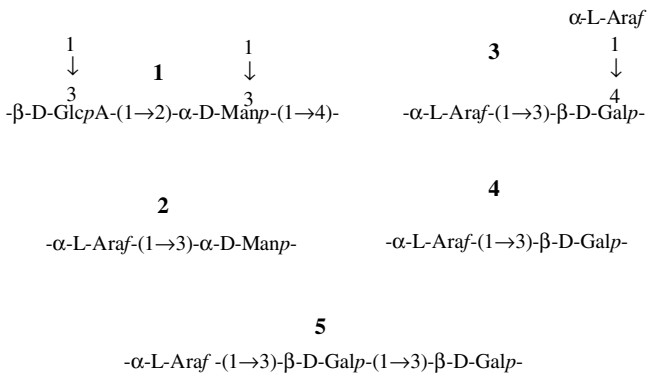
Table 2 shows that a controlled Smith degradation of S-VTP to give S_1-SVTP resulted in a decrease of nonreducing end-units of Ara*f* from 31% to 10%, with the complete elimination of internal Ara*f* units. The appearance of 2-*O*-substituted from 2,3-di-*O*-substituted Man*p* indicated

Table 2
Methylation analysis fractions from *V. tucanorum*: partially *O*-methylated, neutral alditol acetates obtained from per-*O*-methylated fractions

OMe alditol acetate[b]	% of total fragment area[a]						
	R_t[c]	S-VTP	P-VTP	S_1-SVTP	CR_2-SVTP	PH30	PH4h
2,3,5-Me_3-Ara	0.81	31	31	10	24	2	–
3,5-Me_2-Ara	0.93	3	2	–	1	–	–
2,5-Me_2-Ara	0.96	5	8	–	6	–	–
2,3,4,6-Me_4-Man	0.99	–	–	–	–	2	4
2,3-Me_2-Ara	1.03	9	10	–	2	–	–
2,3,4,6-Me_4-Gal	1.05	5	–	17	3	30	3
5-Me-Ara	1.13	3	5	–	3	–	–
3,4,6-Me_3-Man	1.28	3	1	12	2	19	88
2,4,6-Me_3-Gal	1.35	16	17	33	18	16	–
2,3,6-Me_3-Glc	1.37	3	–	–	5	–	–
4,6-Me_2-Man	1.63	10	17	24	18	31	5
2,6-Me_2-Gal	1.70	9	9	4	11	–	–
2,6-Me_2-Glc	1.75	–	–	–	13	–	–
2-Me-Gal	2.60	–	–	–	1	–	–

[a] % relative to total peak area; values <1% not included.
[b] *O*-Methyl alditol acetates analyzed by GC–MS.
[c] Retention time compared with that of 2,3,4,6-tetra-*O*-methylglucitol acetate.

the oxidation and removal of α-Ara*f* (structure **2**) and 5-*O*-linked α-Ara*f* units. As Gal*p* units were 3-O- and 3,4-di-*O*-substituted in the glycoglucuronomannan, the exposure of nonreducing end- (17%) and 3-*O*-substituted Gal*p*



1 **3**

2 **4**

-α-L-Ara*f*-(1→3)-α-D-Man*p*- -α-L-Ara*f*-(1→3)-β-D-Gal*p*-

5

-α-L-Ara*f* -(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-

units (33%), should have arisen via removal of nonreducing Ara*f* end-units, from structures **3**, **4**, and **5**. Some periodate-oxidized 5-*O*-substituted Ara*f* units could also be present.

The side chains of P-VTP were partially removed under mild hydrolytic conditions to give PH30. The Ara*f* units were removed increasing the proportions, when compared to glycoglucuronomannan, of nonreducing end- of Gal*p* (30%), and 2-*O*- (19%) and 2,3-di-*O*-substituted Man*p* units (31%), in agreement with the controlled Smith degradation data.

3.6. Free, reducing oligosaccharides of the gum and a β-D-xylan

Free, reducing oligosaccharides are often present in gum exudates and their structures had similarities to those of the polysaccharide side-chains (Delgobo, Gorin, Jones, & Iacomini, 1998; Delgobo, Gorin, Tischer, & Iacomini, 1999; Maurer-Menestrina, Sassaki, Simas, Gorin, & Iacomini, 2003; Menestrina, Iacomini, Jones, & Gorin, 1998; Tischer, Gorin, & Iacomini, 2002; Tischer, Iacomini, & Gorin, 2002; Tischer, Iacomini, Wagner, & Gorin, 2002), and appear to be byproducts of polysaccharide synthesis. As they lend themselves better to NMR and ESI-MS–MS analysis, those of *V. tucanorum* gum were examined to provide further structural information as to the polysaccharide structures.

Supernatant I (Fig. 1), obtained from ethanol precipitation of VTP, contained mainly arabinose and galactose, and a small amount of mixed oligosaccharides. The mixture was applied to a column of charcoal-Celite, which was eluted with water to remove monosaccharides, followed by increasing concentrations of aqueous ethanol from 5% to 40%. Oligosaccharides were eluted with 10% and 20% aqueous ethanol, and these were combined and subjected to gel-permeation column chromatography on a Sephadex LH-20 column. Four fractions were obtained (Fig. 5), of which fraction 2, a (1 → 4)-linked β-D-xylan, gave a ^{13}C NMR spectrum with 5 typical signals (Fig. 6a) (Ebringerová, Hromádková,

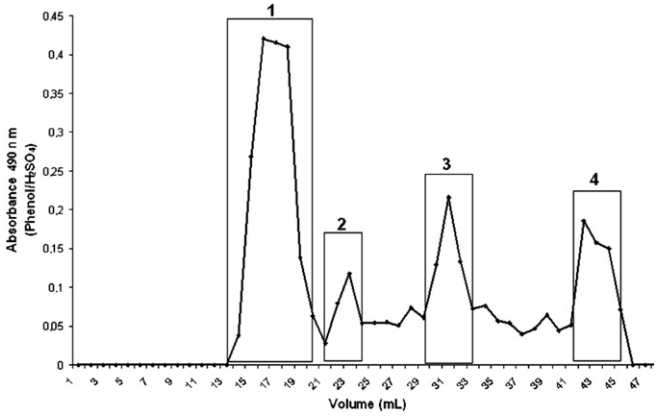


Fig. 5. Elution profile of Sephadex LH-20 column of the mixture, obtained by charcoal–Celite chromatography.

Petráková, & Hrincovini, 1990). The H-1 region of its ^{1}H NMR spectrum (Fig. 6b) contained a signal corresponding to (1 → 4)-linked β-D-Xyl*p* units with δ 5.07, $J = 7.7$ Hz, but with minor signals, which probably arise from β-D-Glc*p*A units of a hemicellulose.

Fraction 4 contained Ara, Man, and Gal in a molar ratio of 5:3:2 and was a mixture of oligosaccharides, as shown by ESI-MS. It gave main molecular ions (Na^+ forms) with *m*/*z* 893 (Ara_4-Hex_2) > 1025 (Ara_5-Hex_2) = 731 (Ara_4-Hex) (Fig. 7a). A very small ion appeared at *m*/*z* 1157, arising from Ara_6-Hex_2. MS–MS on the *m*/*z* 893 ion (Fig. 7b) sequentially removed the Ara units to give daughter ions with *m*/*z* 761 (-Ara), 629 (-Ara_2),

⌊α-L-Ara*f*-⌊α-L-Ara*f*-⌊α-L-Ara*f*-⌊α-L-Ara*f*-⌊-Hex*p*-(1→2)-⌊Man **6**
893 761 629 497 365 203

497 (-Ara_3), 365 (-Ara_4 = Hex_2), and 203 (a hexose). These correspond to structure **6** (the glycosidic configurations and linkage were shown by NMR and methylation data: see below). An Ara_2 ion at *m*/*z* 305 also appeared. The daughter ions of the *m*/*z* 1025 ion were not so well sequenced, although one at *m*/*z* 893 (-Ara) appeared, along with similar ions down to *m*/*z* 497, but no further, possibly indicating a branched structure.

Methylation analysis of the mixture showed the presence of nonreducing end- (21%), 2-*O*- (12%), and 3-*O*-substituted Ara*f* (10%), 3-*O*- (8%) and 3,4-di-*O*-substituted Gal*p* (12%), and 2-*O*-substituted Man*p* units (37%). This and the ESI-MS results showed longer side chains than those present in glycoglucuronomannans S-VTP and P-VTP, with a higher proportion of 2-*O*-substituted Ara*f* units. The oligosaccharide mixture gave a ^{13}C NMR spectrum with main C-1 signals at high frequencies of δ 106.0 > 107.5 (Fig. 6c), showing α-L-Ara*f* units. Those of reducing end-units were at δ 91.7 and 93.9 and can be assigned to 2-*O*-glycosylated α- and β-D-Man*p* units respectively, by the following comparison with β-shifts obtained with 2-*O*-methylated derivatives (Gorin, 1975), in which the C-1 resonance of α-D-Man*p*

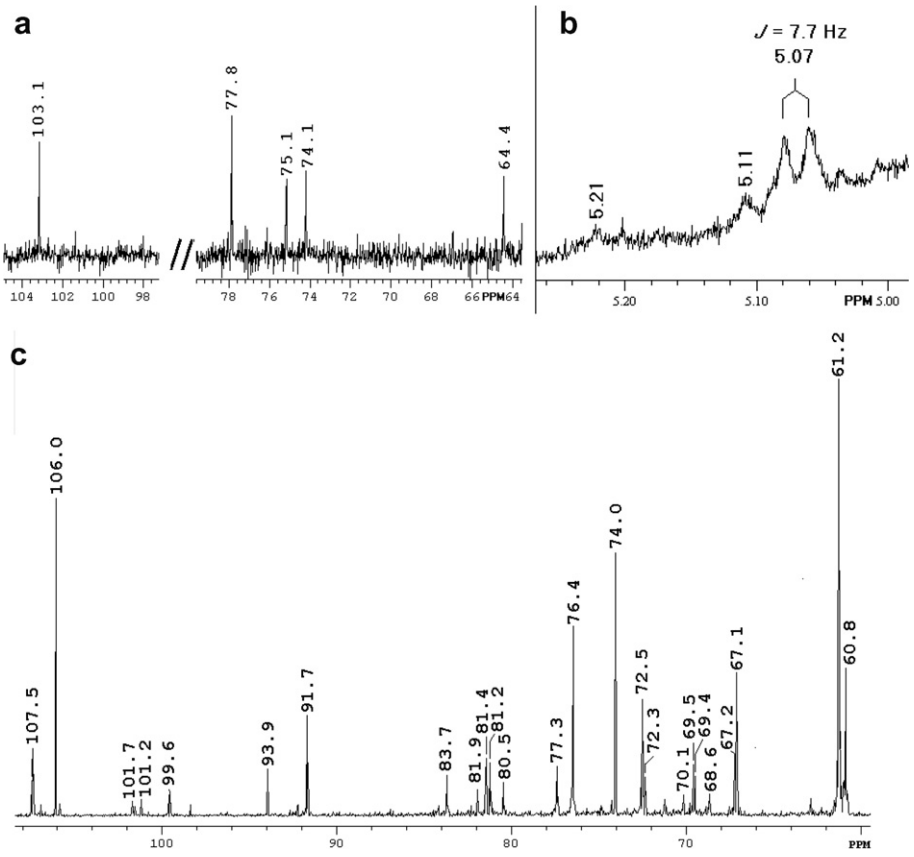


Fig. 6. ^{13}C NMR spectrum of (1 → 4)-linked β-D-xylan (a), the H-1 portion of its ^{1}H spectrum (b), and the ^{13}C NMR spectrum of oligosaccharide mixture (fraction 4, c), obtained at 50 °C in D_2O: numerical values are in δ ppm.

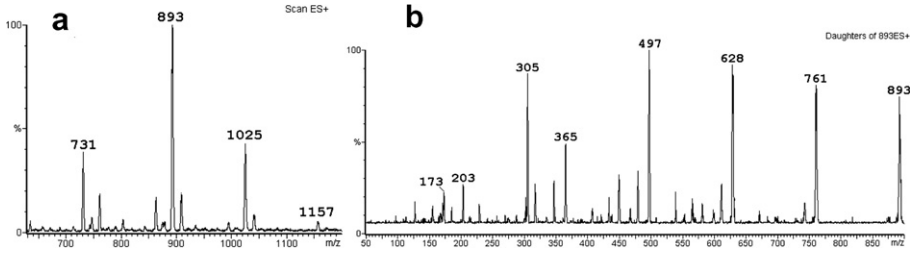


Fig. 7. ESI-MS profile of oligosaccharide mixture (fraction 4) isolated from Sephadex LH-20 column (a) and MS–MS of *m*/*z* 893 ion (b).

appeared at δ 94.1 and that of β-D-Man*p* at δ 93.7. So considering the β-shifts of C-1 found on 2-*O*-methylation of α- and β-mannose of −3.2 and +0.4 ppm, respectively, the present corresponding β-shifts would be −2.4 and +0.2 ppm, consistent with 2-*O*-glycosylation. These results indicate an arabinose–galactose–mannose sequence in oligosaccharide **6**. The oligosaccharide mixture could well contain both internal and nonreducing end-units of 2-*O*-substituted Man*p*, due to the high content of 34% found in the methylation analysis.

4. Discussion

Vochysia tucanorum gum polysaccharide contained a main chain of -β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1 → 4)-repeating groups, substituted with side-chains at O-3 of each unit (**1**). The side chains were branched, consisting mainly of α-L-Ara*f* linked to β-D-Gal*p*, as in structures **3**, **4**, and **5**, and also linked to α-D-Man*p* units (**2**). The gum exudate of *Vochysia lehmannii* contained a glycoglucuronomannan with a similar main-chain, but its ^{13}C NMR spectrum contained almost exclusively, two C-1 signals at δ101.5 and 98.3, arising from the main chain and very small signals from its side chains (Wagner et al., 2004). This contrasts with *V. tucanorum* polymers, whose many side-chain signals of C-1 are prominent (Fig. 3a and b).

Free, reducing oligosaccharides were present in minute quantities and had α-Araf_4-Hex$_2$ (**6**) and Ara$_2$ structures. Also found was a trace of a hemicellulose-like (1 → 4)-linked β-D-xylan, which could not be detected in the gum

of *V. lehmannii*. Such oligosaccharides could not be detected in the gum of *V. tucanorum* and suggests a connection between their biosynthesis and that of the glycoglucuronomannan.

The similarity of the structures of free, reducing oligosaccharides to the heteropolysaccharides of gums of *Anedanthera colubrina* (Delgobo et al., 1998), *Acacia senegal* (Tischer et al., 2002), and others, was used as an aid in determining those of the polysaccharides. In these acidic glycogalactans, the main chain consisted mainly of (1 → 3)-linked β-D-Gal*p* units, substituted at O-6 with a variety of complex side-chains. The free oligosaccharides had similar side-chain structures, except for 6-*O*-substituted reducing end-units of galactose. If the polysaccharides were biosynthesized via block-type intermediates, simultaneously forming the main and side chains, these reducing ends appeared to be equivalent to the 3,6-di-*O*-substituted branch points in the main chain of the polysaccharide, the oligosaccharides being byproducts from the intermediates by the action of water. These are primary byproducts, as defined by Kandler & Hopf (1980). Other possibilities could be *in situ* autohydrolysis, or by the action of *endo*- or *exo*-hydrolases, with emphasis on cleavage of the main chain.

In contrast, the mechanism for formation of free, reducing oligosaccharide mixture in the gum of *V. tucanorum* does not belong to any of these categories. Although they contained an arabinose–galactose–mannose sequence, as in **6**, with most of its side chain components structurally similar to those of the glycoglucuronomannan, it had a 2-*O*-substituted mannosyl reducing-end. However, the main chain of the polysaccharide already had Man*p* 2-*O*-substituted by Glc*p*A units. Perhaps, pre-formation of the main chain occurred with subsequent addition of side chains.

To date, the glucuronomannan main-chain has been demonstrated in only a few plant gum exudate polysaccharides, other than native, Brazilian *Vochysia* spp. (Wagner et al., 2004). These are from gum ghatti (*Anogeissus latifolia*; family Combretaceaea) (Aspinall, Auret, & Hirst, 1958; Aspinall & Christensen, 1965), occurring in the Indian subcontinent, and *Anogeissus leiocarpus*, occurring in Senegal (Aspinall & Carlyle, 1969; Aspinall, Carlyle, McNab, & Rudowski, 1969; Aspinall & Chaudhari, 1975; Aspinall & McNab, 1969). Species of this genus are of interest in terms of plant systematics and evolution, since one can speculate that a conserved common ancestry existed, considering that the three regions were present in the same land mass, before the occurrence of continental drift(s). In this vein, it would be interesting to search for similar, gum exudate polysaccharides in the Australian sub-continent.

A study is now continuing on the gum exudate of a third local species, *Vochysia thyrsoidea*, to see if the glycoglucuronomannan structure is maintained.

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