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A high-viscosity glycoglucuronomannan from the gum exudate of *Vochysia thyrsoidea*: Comparison with those of other *Vochysia* spp.

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Abstract

The polysaccharide (VTh) extracted with water from the gum exudate of the trunk *Vochysia thyrsoidea* gave a solution of high viscosity and was homogeneous, with M_w 92.5×10^3 and $dn/dc = 0.212$. Partial hydrolysis gave a main chain of →4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1→ repeating groups. Since VTh contained 24% GlcA, neither its monosaccharide ratio (GC–MS of alditol acetates) nor methylation results were accurate. It was therefore carbodiimide-reduced to CR_3VTh, which contained Ara, Xyl, Man, Gal, and Glc in a molar ratio of 28:4:29:19:25: methylation analysis showed mainly nonreducing end-units of Ara*f* (25%), Ara*p* (8%), and Gal*p* (8%) and side-chain units of 3-*O*- (6%) and 3,4-di-*O*-subst. Gal*p* (12%). Main-chain units of Man*p* were 2,3-di-*O*-substituted (17%), showing that they were substituted at O-3 by side chains, but there was less side-chain substitution of Glc*p* units, which were mainly 4-*O*-substituted (12%). Most Ara*f* units in VTh and CR_3VTh were single nonreducing end-units, and were present in groups of α-L-Ara*f*-(1 → 3)-D-Man*p* and α-L-Ara*f*-(1 → 3)-[α-L-Ara*f*-(1 → 4)]-β-D-Gal*p*-(1 → 3)-α-D-Man*p*. Other degradation products derived from VTh agreed with this structure. The degree of side-chain substitution of the same main chain was greatest with the gum exudate polysaccharide of *V. tucanorum*, and progressively less with those of *V. thyrsoidea*, and *V. lehmannii*. High-viscosity aqueous solutions were formed by the gum of *V. thyrsoidea*, in contrast with those of *V. lehmannii* and *V. tucanorum*.
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Keywords: *Vochysia thyrsoidea*; Gum glycoglucuronomannan; Structure; Chemotaxonomy

1. Introduction

Trees of the family Vochysiaceae are common in the sub-tropical and tropical regions of Brazil, often occurring in the transition zone between the savannah and Atlantic forest. Wagner et al. (2004) showed that the gum exudate, growing on the trunk of *Vochysia lehmannii*, contained a polysaccharide with a repeating →4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1→ group as its main chain, which was lightly *O*-substituted with complex side chains. The gum polysaccharide from *V. tucanorum* has a similar main chain, but with a much higher degree of *O*-substitution (Wagner et al., 2007). These structures are represented by their ^{13}C NMR spectra, that of *V. lehmannii* having predominant C-1 signals at δ 98.3 and 101.6 arising from the main chain (Fig. 1a). These were not evident in those of *V. tucanorum*, which were mostly from side chains (Fig. 1b), while those of the main chain were only revealed on partial hydrolysis. We now investigate the glycoglucuronomannan from the gum exudate of *V. thrysoidea*, which has been described by Almeida, Proença, Sano, & Ribeiro (1998). Its C-1 signals (Fig. 1c) indicated an intermediate degree of side-chain substitution.

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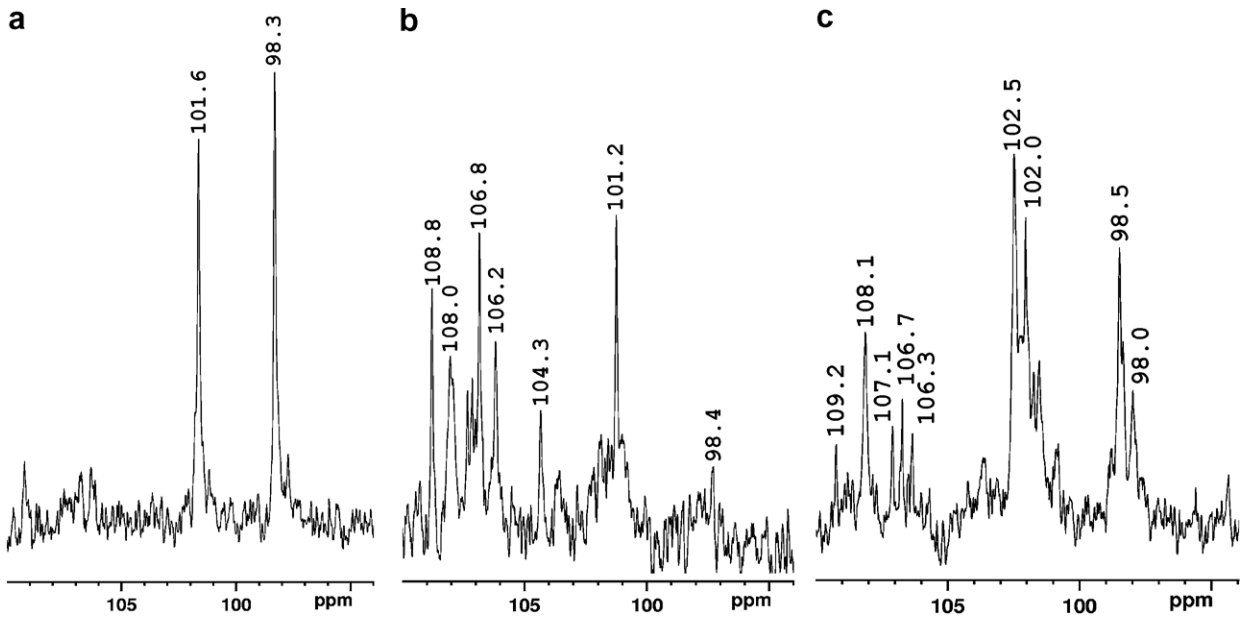


Fig. 1. C-1 regions of ^{13}C NMR spectra of polysaccharides from gum exudates of *V. lehmannii* (a), *V. tucanorum* (b), and *V. thyrsoidea* (c).

2. Experimental

2.1. Vochysia thyrsoidea gum

The gum was collected during January, 2005 from the trunk of a tree growing in the Ecological Reserve (RECOR) of the Brazilian Institute of Geography and Statistics, an affiliate of the Brazilian Institute of Geography and Statistics (IBGE). This is situated in a sub-tropical location 35 km to the south of the capital Brasília, in a region subject to frequent fires and dry periods, contributing to periodic tree stress.

2.2. Preparation of gum polysaccharide (VTh)

A sample of the original, dry gum was cleaned to remove manually charcoal arising from fires. A sample (110 g) dissolved almost completely in H_2O (1.0 L), and the viscous solution was evaporated to 450 mL, insoluble particles centrifuged off, and the supernatant added to EtOH (×3). The precipitated polysaccharide was dissolved in H_2O and the solution was dialyzed with a membrane having pores of 12,000–14,000, and then freeze-dried to give VTh (57 g).

2.3. Analytical methods

2.3.1. Monosaccharide composition of polysaccharides

The uronic acid contents of polysaccharides (1.0 mg) were determined using an *m*-hydroxydiphenyl colorimetric method, with which neutral, reducing sugars do not interfere (Filisetti-Cozzi & Carpita, 1991).

Samples (1.0 mg) were hydrolyzed with 2 M TFA for 8 h at 100 °C, and the products were reduced with $NaBD_4$ (Wolfrom & Thompson, 1963a) to give alditols, which were acetylated with Ac_2O–pyridine (Wolfrom & Thompson, 1963b). The resulting alditol acetate mixtures were analyzed by GC–MS (Jansson, Kenne, Liedgren, Lindberg, & Lönngren, 1970), 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.

The hydrolyzate of VTh was examined by silica gel TLC (solvent: *n*-PrOH–EtOAc–HOAc–H_2O, 2:4:2:1, spray:orcinol), to detect glucuronolactone with R_F 0.83. The mixture was treated with 0.5% aq. $NaHCO_3$ for 18 h at 25 °C to convert it to Na glucuronate, which does not afford glucitol on $NaBD_4$ reduction.

2.3.2. HPSEC analysis

The molecular weight distribution of VTh 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 ASTRA 4.70.07 computer software.

2.3.3. Methylation analysis of polysaccharides

Samples of polysaccharide (25 mg), when not soluble in Me_2SO, were partially *O*-methylated by the method of Haworth (1915), using Me_2SO_4/aq. NaOH, which rendered the product Me_2SO-soluble, and which was then per-*O*-

methylated with the Me_2SO–MeI–NaOH method of Ciucanu and Kerek (1984).

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 solutions were neutralized ($BaCO_3$), filtered, and the filtrates evaporated to residues of partially *O*-methylated aldoses, which were converted to their corresponding mono-deuterated *O*-methyl alditol acetates by successive treatments with $NaBD_4$ and Ac_2O–pyridine. GC–MS was carried out on the mixtures, as in Section 2.3.1, except that a maintained temperature of 185 °C was employed, and its components were identified by their typical retention times and e.i breakdown patterns. Comparison was carried out 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 separate experiments.

2.3.4. ^{13}C NMR spectroscopy

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

2.4. Polysaccharides derived from VTh

2.4.1. Partial hydrolysis of VTh to its main chain (Ph-VTh)

VTh (2.00 g) was partially hydrolyzed with 0.5 M TFA (300 mL) at 100 °C for 4 h and the solution then evaporated to 100 mL, which was added to EtOH (×3) to give a precipitate (0.67 g) of the main chain (Ph-VTh).

2.4.2. Carboxy-reductions

VTh (2.00 g) was submitted to three carboxy-reduction cycles, each according to Taylor and Conrad (1972), to give CR_3VTh (1.56 g). A single carboxy-reduction of Ph-VTh (200 mg) gave rise to Ph-VTh-CR (168 mg).

2.4.3. Controlled Smith degradation of CR_3VTh to S_1CR_3VTh

A controlled Smith degradation of CR_3VTh (880 mg) to give S_1CR_3VTh (126 mg) was carried out by successive $NaIO_4$ oxidation, $NaBH_4$ reduction, and partial hydrolysis to polysaccharide (Wagner et al., 2004).

3. Results

3.1. Preparation and preliminary examination of gum polysaccharide (VTh)

The gum exudate of *V. thyrsoidea* was dissolved in water to form a solution of high viscosity, unlike those of gums from *V. lehmannii* (Wagner et al., 2004) and *V. tucanorum* (Wagner et al., 2007), whose solutions were not viscous. Precipitation with excess ethanol provided polysaccharide (VTh; 52% yield), which was homogeneous on HPSEC with M_w 92.5×10^3 and $dn/dc = 0.212$ (Fig. 2), and was completely precipitated with Cetavlon at pH 7.0. VTh gave a ^{13}C NMR spectrum (Fig. 1c) showing an intermediate degree of side-chain substitution, when compared with polysaccharides of *V. lehmannii* (Fig. 1a) and *V. tucanorum* (Fig. 1b).

The ethanol supernatant from the above contained monosaccharides, mainly arabinose, but no free, reducing oligosaccharides were detected.

3.2. Preparation of polysaccharide fractions from VTh

VTh was subjected to a series of degradations necessary for its structural analysis (Fig. 3).

Partial hydrolysis revealed its main chain (Ph-VTh), and which was carboxy-reduced to form fraction Ph-VTh-CR.

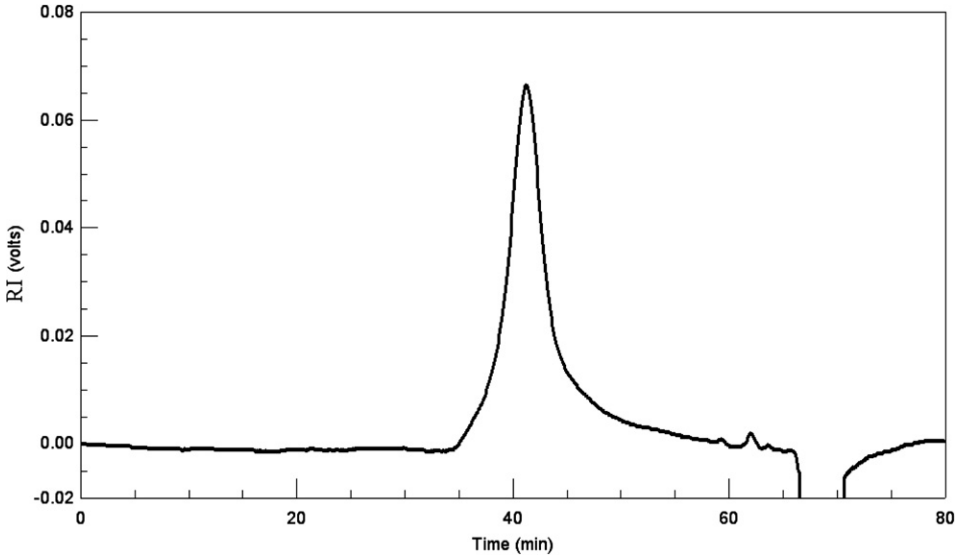


Fig. 2. HPSEC of gum polysaccharide of *V. thyrsoidea* (VTh), using a refractive index detector.

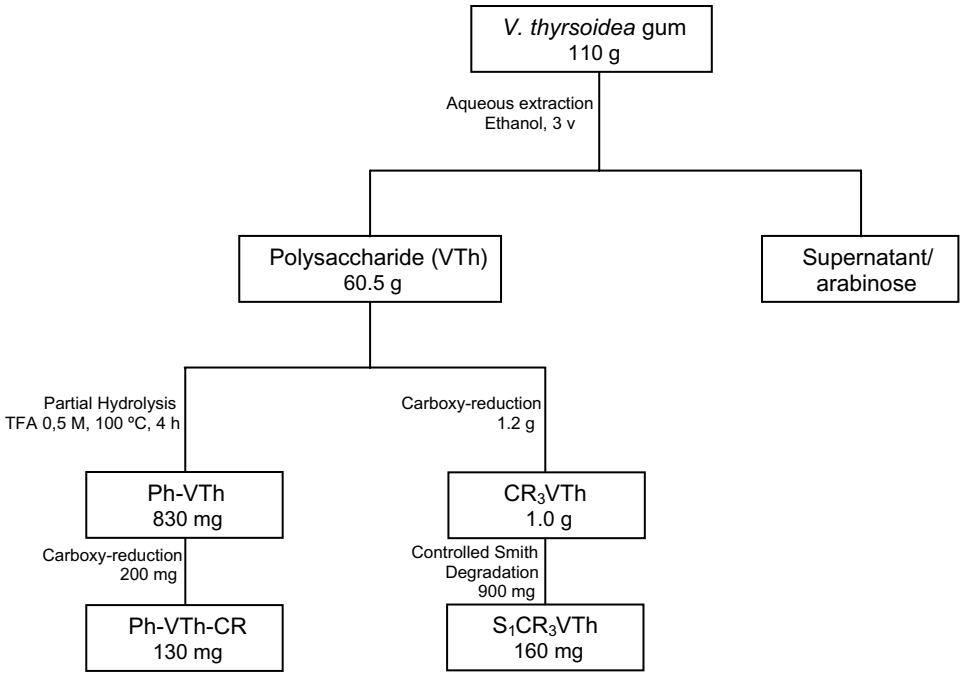


Fig. 3. Scheme of products derived from gum polysaccharide of *V. thyrsoidea* (VTh).

Three successive carboxy-reductions of VTh using carbodiimide converted it to CR_3VTh, most of its GlcA being converted to Glc units. The product was subjected to a controlled Smith degradation providing S_1CR_3VTh.

3.3. Analysis of polysaccharide fractions

3.3.1. Analysis of polysaccharide VTh

VTh contained 24% uronic acid, determined colorimetrically, but the ratio of 48:3:10:10:4 for Ara, Xyl, Man, Gal, and Glc (Table 1), obtained by GC–MS of alditol acetates, derived from the acid hydrolyzate, is inaccurate with a low mannose content, due to the resistance of resulting β-D-Glc*p*A-(1 → 2)-D-Man. Furthermore, any liberated glucuronic acid would be partially converted to glucuronolactone, which would be reduced to glucose on $NaBD_4$ reduction, as shown by GC–MS of derived alditol acetates, which gave an ion with *m*/*z* 141 from di-deuterated C-6. The hydrolyzate contained glucuronolactone, as shown by TLC, and was therefore treated with dilute aq. $NaHCO_3$, which converted it to sodium glucuronate. GC–MS of derived alditol acetates showed Ara, Xyl, Man, Gal, and Glc in a 24:48:11:10:1 molar ratio (Table 1). A trace of glucose was originally present in VTh, since the glucitol acetate furnished only ions with *m*/*z* 139 and 140.

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

Polysaccharide	Uronic acid	Ara	Xyl	Man[b]	Gal	Glc
VTh	24	48	3	10	10	4
VTh[c]	24	48	5	11	10	1
Ph-VTh	39	2	–	35	3	21
Ph-VTh-CR	–	–	–	51	5	44
CR_3VTh	4	28	4	29	10	25
S_1CR_3VTh	5	5	–	47	9	25

[a] Neutral monosaccharides were estimated by hydrolysis, followed by GC–MS of derived alditol acetates: 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 from relatively stable aldobiouronic acid.

[c] Values obtained following removal of glucurnolactone from hydrolyzate with aq. $NaHCO_3$.

Methylation analysis of VTh (Table 2) revealed only its neutral components, the principal ones being nonreducing end- of Ara*f* (23%), 2,3-di-*O*-substituted Man*p* (38%), and nonreducing end- (8%) and 3,4-di-*O*-substituted Gal*p* units (10%). The ^{13}C NMR spectrum of VTh (Fig. 1c) was extremely complex with at least 11 C-1 signals, which included 4 of α-L-Ara*f* units at δ 106.2–108.0.

3.3.2. Characterization of main-chain structure in polysaccharide VTh

Partial acid hydrolysis of VTh gave Ph-VTh (17% yield), which contained 39% uronic acid. Acid hydrolysis gave Man and Glc in a 35:21 molar ratio (Table 1), the latter being derived from glucuronolactone. Methylation analysis showed mainly 2-*O*- (76%) and 2,3-di-*O*-substituted Man*p* units (10%) (Table 2). The ^{13}C NMR spectrum of Ph-VTh contained 10 main signals (Fig. 4a), similar to that of the main chain of →4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1→ repeating groups (Wagner et al., 2007).

Ph-VTh was carboxy-reduced to give Ph-VTh-CR (65% yield), which contained Man, Gal, and Glc in a molar ratio

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

OMe alditol acetate[b]	% of total fragment area[a]					
	R_t[c]	VTh	Ph-VTh	Ph-VTh-CR	CR_3VTh	S_1CR_3VTh
2,3,5-Me_3-Ara	0.81	23	2	1	25	2
2,3,4-Me_3-Ara	0.83	3	–	–	8	–
2,3,4-Me_3-Xyl	0.85	3	–	–	4	–
2,5-Me_2-Ara	0.96	1	–	–	–	–
2,3,4,6-Me_4-Man	0.99	–	3	4	–	3
2,3,4,6-Me_4-Glc	1.00	–	2	4	–	1
2,3,4,6-Me_4Gal	1.05	8	4	2	8	5
5-Me-Ara	1.13	2	–	–	1	1
3,4,6-Me_3-Man	1.28	5	76	48	3	34
2,4,6-Me_3-Gal	1.35	4	1	5	6	14
2,3,6-Me_3-Glc	1.37	–	–	32	12	20
4,6-Me_2-Man	1.63	38	10	4	17	12
2,6-Me_2-Gal	1.70	10	–	–	12	3
2,6-Me_2Glc	1.75	–	–	–	4	3
2-Me-Gal	2.60	3	–	–	–	2

[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.

of 51:5:44 (Table 1). Methylation analysis showed the presence of 2-*O*-substituted Man*p* (48%) and 4-*O*-substituted Glc*p* units (32%) (Table 2). Its ^{13}C NMR spectrum contained 12 main signals (Fig. 4b), consistent with a →4)-β-D-Glc*p*-(1 → 2)-α-D-Man*p*-(1→ repeating group.

3.3.3. Analysis of carboxy-reduced CR_3VTh

For an accurate characterization of VTh, which was prejudiced by the presence of glucuronic acid, it was converted by three successive carboxy-reductions to CR_3VTh (93% yield), which contained only 4% uronic acid (Table 1). Its monosaccharides were Ara, Xyl, Man, Gal, and Glc in a 28:4:29:10:25 molar ratio (GC–MS of derived alditol acetates).

Methylation analysis of CR_3VTh (Table 2), in which Glc*p*A of VTh were reduced to Glc*p* units, indicated mainly nonreducing end-units of Ara*f* (25%) and Ara*p* (8%), nonreducing end- (8%) and 3,4-di-*O*-substituted Gal*p* units (12%), and 2,3-di-*O*-substituted Man*p* (17%) and 4-*O*-substituted Glc*p* (12%) from its main chain.

The ^{13}C NMR spectrum of CR_3VTh was complex, as expected, with 5 α-L-Ara*f* signals in the C-1 region at δ 106.3–109.2 (Fig. 5a).

A controlled Smith degradation of CR_3VTh formed S_1CR_3VTh (21% yield), which contained Ara, Man, Gal, Glc, and uronic acid in a 5:47:9:34:5 molar ratio (Table 1). Most of the arabinose-containing nonreducing end-units were thus removed, confirmed by methylation analysis, and of which only 2% remained (Table 2). Principal components were nonreducing end- (5%) and 3-*O*-substituted Gal*p* (14%), as well as main-chain components of 2-*O*- (34%) and 2,3-di-*O*-substituted Man*p* (12%), and 4-*O*-substituted Glc*p* units (20%). The ^{13}C NMR spectrum of S_1CR_3VT (Fig. 5b) contained mainly C-1 signals of the main chain at δ 99.4 and 101.5, with six minor ones, including an α-L-Ara*f* signal at δ 109.0. This indicated that the side chains contained internal Gal*p* *O*-substituted by single-unit Ara*f* units to form branches, similar to those occurring in the glycoglucuronomannan of the gum exudate isolated from *V. tucanorum* (Wagner et al., 2007).

4. Discussion

The polysaccharide VTh from the gum of *V. thyrsoidea* differed from those from *V. lehmannii* and *V. tucanorum*, as it dissolved completely in water, had a high viscosity, and was homogeneous on HPSEC. Each polysaccharide contained a main chain of →4)-β-D-Glc*p*A-(1 → 2)-α-D-Man*p*-(1→ repeating groups, although they were substituted by side chains in the order *V. tucanorum* > *V. thyrsoidea* > *V. lehmannii*, as evidenced by their respective ^{13}C NMR spectra (Fig. 1a–c, respectively) and other data.

Although detailed structural analyses were carried out on VTh and derived polysaccharides, an emphasis was placed on carbdiimide-reduced CR_3VTh, which contained only 4% of Glc*p*A units, as compared with the 24% in VTh, prejudicial in determining monosaccharide ratios and methylation-GC–MS analysis. CR_3VTh contained Ara, Xyl, Man, Gal, Glc, and GlcA in a 28:4:29:10:25:4 molar ratio (Table 1) mainly with side-chain units of nonreducing ends of Ara*f* (25%), Ara*p* (8%), and Gal*p* (8%), and internal 3-*O*- (6%), and 3,4-di-*O*-substituted Gal*p* units (12%). The main-chain units were 2,3-di-*O*-substituted Man*p* (17%), showing considerable side-chain substitution at O-3, in contrast with 4-*O*-substituted Glc*p* (12%) derived from original Glc*p*A units. Only 4% of the Glc*p* units were 3-*O*-substituted by side chains (Table 2).

The side chains of CR_3VTh were susceptible to a controlled Smith degradation with a survival of only 5% Ara (Table 1) and those of Gal*p* were principally nonreducing ends (5%) and 3-*O*-substituted units (14%). Most of the surviving Glc*p* units were 4-*O*- (12%) and those of Man*p*

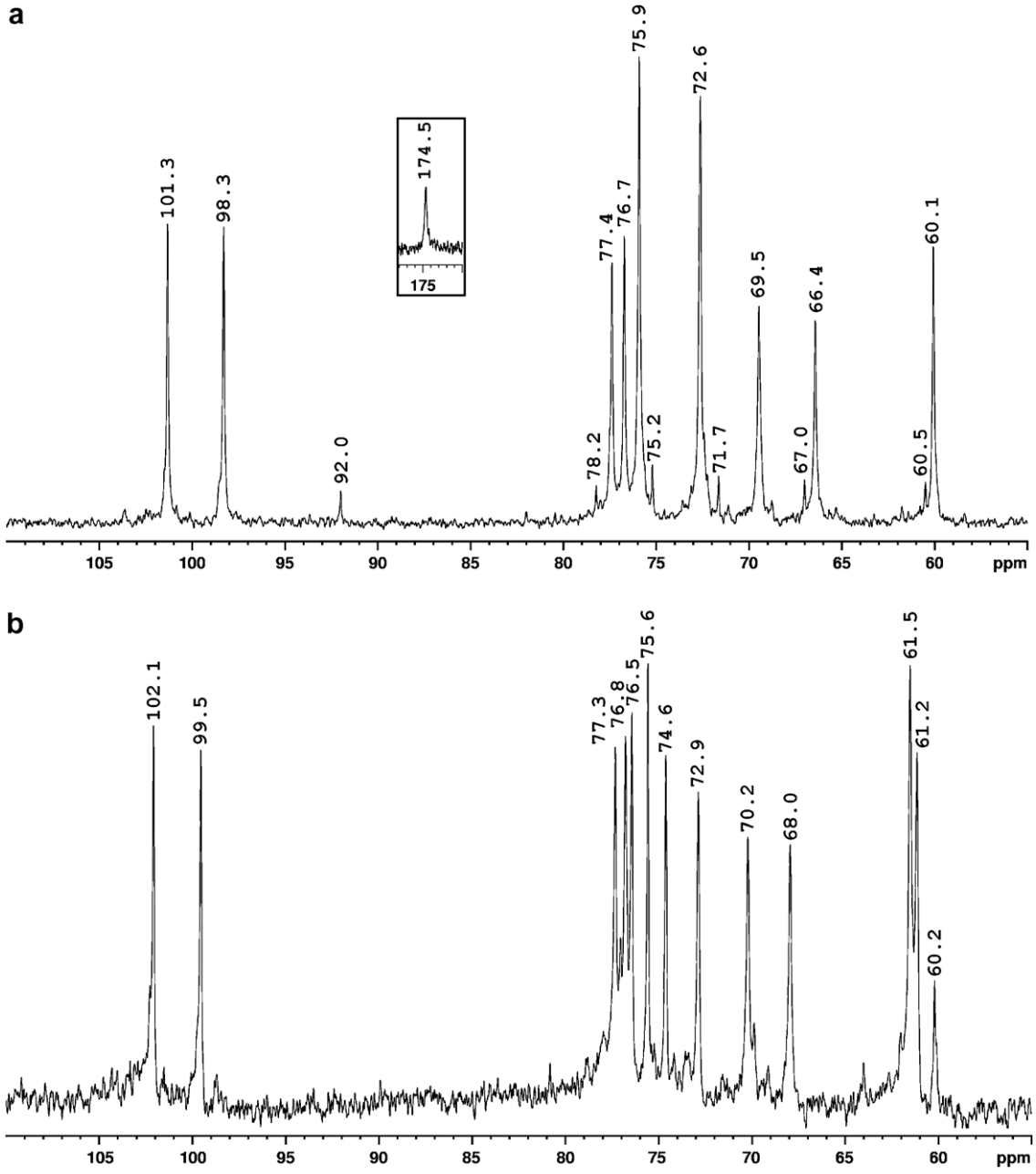
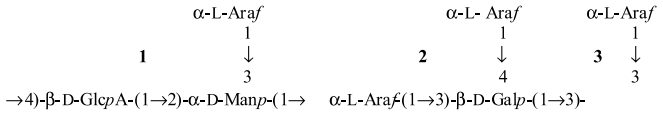


Fig. 4. ^{13}C NMR spectra of glucuronomannan (Ph-VTh; (a) obtained on partial hydrolysis of the gum polysaccharide of *V. thyrsoidea* (VTh) and glucomannan (Ph-VTh-CR; (b) obtained on carboxy-reduction of Ph-VTh.

were 2-*O*- (34%) and 2,3-di-*O*-substituted (12%), still with side chains at O-3.

The side-chain structures of the glycoglucuronomannan from *V. thyrsoidea* are related to those of *V. tucanorum* (Wagner et al., 2007), although the latter have much longer ones of Ara*f* units. Both have main-chain units substituted at O-3, with a difference of predominant structure **1** with single-unit side chains of Ara*f* of *V. thrysoidea*. Other such structures of *V. thyrsoidea* are **2** and **3**.



To date, we have found that three different *Vochysia* spp. growing in semi-tropical regions of Brazil, form gum exudate polysaccharides with a similar glucuronosyl-mannose main chain, although with different degrees of side-chain substitution. Di Fabio, Dutton, and Moyna (1982) found that the polysaccharide of the trunk gum exudate of the ornamental South American silk floss tree, *Chorisia speciosa* St. Hil., family *Bombaceae*, has a similar main chain and related side-chain structures. Only a few similar gum polysaccharides have so far been found, namely those isolated from gum ghatti (*Anogeissus latifolia*) from India and *Anogeissus leiocarpus* from Senegal. A possible chemotaxonomic significance, based on the continent of origin, has been raised (Wagner et al., 2007).

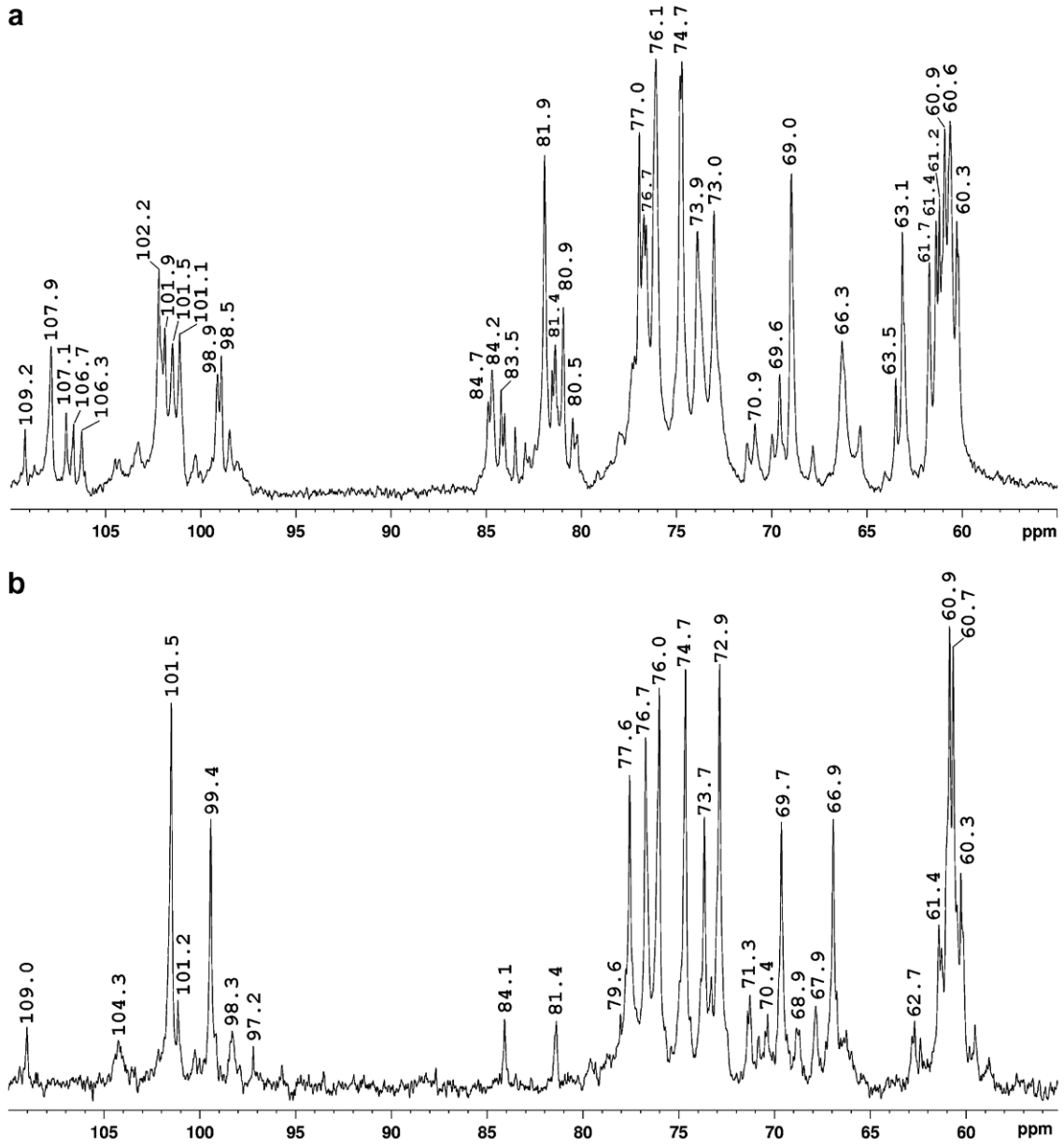


Fig. 5. ^{13}C spectra of product (CR_3VTh; (a) obtained by carboxy-reduction of the gum polysaccharide of *V. thyrsoidea* (VTh) and that from a controlled Smith degradation (S_1CR_3VTh; (b) of CR_3VTh.

It may be significant that other similar, branched glycoglucurononmannans have American origins, such as those isolated from cell cultures of tobacco (*Nicotinia tabacum*; Mori & Katō, 1981) and tuberose (*Polianthes tuberosa*), a flowering plant native to Mexico (Honda, Inoaka, Takei, Sugimura, & Otsuji, 1996).

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