Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 71 (2008) 218–228
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Comparison of structure of gum exudate polysaccharides from the trunk and fruit of the peach tree (*Prunus persica*)

Fernanda F. Simas [a], Philip A.J. Gorin [a], Ricardo Wagner [a], Guilherme L. Sassaki [a], Antonio Bonkerner [b], Marcello Iacomini [a,*]

[a] *Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, C.P. 19046, CEP 81531-990, Curitiba, PR, Brazil*
[b] *Instituto Agronômico do Paraná (IAPAR), C.P. 282, CEP 84670-000, Palmas, PR, Brazil*

Received 12 February 2007; received in revised form 17 May 2007; accepted 24 May 2007
Available online 2 June 2007

Abstract

The peach tree (*Prunus persica*) produces gum exudates on its trunk and fruit. The former was extracted with water to give polysaccharide (PPN) and residual material was extracted with alkali giving structurally similar PPNA (NMR examination), which was formed in a slightly higher yield (42% compared with 37%). PPNA consisted of Ara, Xyl, Man, Gal, and uronic acids in a 36:7:2:42:13 molar ratio and was homogeneous on HSPEC with M_w 5.61(±0.22) × 10^6 g mol^{-1}. Methylation analysis showed mainly nonreducing end- (20%), and 3-*O*- (6%) and 4-*O*-substituted Ara*p* and/or 5-*O*-substituted Ara*f* units (14%) and nonreducing end-units of Xyl*p* (13%). The core Gal*p* units were mainly 3,6-di-*O*- (19%) and 3,4,6-tri-*O*-substituted (14%). The ^{13}C NMR spectrum of PPNA confirmed its complexity with 5 C-1 signals from δ 107.5–109.5 from α-L-Ara*f* and a main one at δ 103.2 from the β-D-Gal*p* units. A controlled Smith degradation of PPNA gave 19% polymer (PPNAS), which was almost completely degraded, in a very low yield, to an inner core of PPNAS2 after a second cycle. This contained mainly β-D-Gal*p* units, that were nonreducing end- (25%), 3-*O*- (34%), and 2,3-di-*O*-substituted units (21%), with no evidence of 6-*O*-substitution. Partial hydrolysis of PPNAS with 0.1 M TFA at 100 °C removed Ara*f* units, to give polymeric PPNAS60 (20% yield), which had Gal as its major component (85%). Methylation analysis showed a branched structure mainly nonreducing end- (18%), 3-*O*- (15%), 6-*O*- (45%), and 3,6-di-*O*-substituted Gal*p* units (9%): its ^{13}C NMR spectrum had a C-1 main signal at δ 103.8 from β-D-linked units. Under stronger hydrolysis conditions β-D-Glc*p*A-(1 → 6)-αβ-D-Gal and β-D-Glc*p*A-(1 → 2)-αβ-D-Man were formed. The fruit gum polysaccharide (PPNF) was homogeneous with M_w 6.43(±0.64) × 10^6 g mol^{-1} and according to ^{13}C NMR and HMQC, and TOCSY H-1 correlations, showing few structural differences. These were not apparent on monosaccharide composition and methylation analyses, which only revealed quantitative variations.

Keywords: Peach tree; *Prunus persica*; Arabinogalactan; Gum exudates; Trunk; Fruit

1. Introduction

The genus *Prunus* belongs from family Rosaceae and includes several fruit-bearing trees including the peach (*P. persica*), damson (*P. insitia*), egg plum (*P. domestica*), cherry (*P. cerasus* and *P. virginiana*), and almond (*P. amygdalus*). These species produce copious gum exudates, which is caused by a disease (gummosis) on their fruit and trunk, especially after mechanical injury followed by microbial attack. In large peach tree orchards, trunk gummosis commonly occurs, because of enhanced fungal infection due to continuous pruning. The polysaccharide components of these gums belong to the arabinogalactan group (Stephen, 1983) and can be composed of Ara, Xyl, Gal, Glc*p*A, 4-Me-Glc*p*A, with smaller amounts of Rha and Man. Structural variation is based on the proportion of monosaccharides and glycosidic linkages. The first detailed analysis of the polysaccharide isolated from the

* Corresponding author. Tel.: +55 41 3361 1655; fax: +55 41 3266 2042.
E-mail address: iacomini@ufpr.br (M. Iacomini).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.05.032

trunk gum exudate of *P. persica* was by Jones (1950), who identified its monosaccharide components and Glc*p*A-(1 → 6)-Gal formed on acid hydrolysis. This β-Glc*p*A isomer, β-L-Ara*p*-(1 → 3)-L-Ara and β-D-Xyl*p*-(1 → 4)-L-Ara were characterized later (Andrews, Ball, & Jones, 1953; Rosík, Kubala, Kardošová, & Kovácik, 1973).

Further studies were carried out, presumably also on the polysaccharide from the trunk gum exudates. Its structure was investigated using methylation analysis and polarimetry of native and degraded polysaccharide and a β-(1 → 6)-linked Gal*p* main-chain was assigned (Rosík, Bruteničová-Sósková, Zitko, & Kubala, 1966). Partial acid hydrolysis of the polysaccharide gave rise to β-D-Glc*p*A-(1 → 6)-D-Gal, 4-Me-β-D-Glc*p*A-(1 → 6)-D-Gal, and β-D-Glc*p*A-(1 → 2)-D-Man (Rosík, Kardošová, & Kubala, 1967). Examination of neutral oligosaccharides obtained by partial acid hydrolysis or enzymolysis showed that the structures β-L-Ara*f*-(1 → 3)-L-Ara*f*-(1→, α-D-Man*p*-(1 → 3)-D-Gal*p*-(1→, β-D-Gal*p*-(1 → 6)-β-D-Gal-(1→, β-L-Ara*p*-(1 → 3)-L-Ara*p*-(1→, β-D-Gal*p*-(1 → 3)-β-D-Gal*p*-(1 → 6)-β-D-Gal-(1→, β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 6)-β-D-Gal-(1→, β-D-Gal*p*-(1 → 6)-β-D-Gal*p*-(1 → 3)-β-D-Gal-(1 → 6)-β-D-Gal-(1→, were present (Kardošová, Rosík, & Kubala, 1978; Kardošová, Rosík, Kubala, & Kovácik, 1979; Kubala & Rosík, 1977; Rosík et al., 1973), which are consistent with (1 → 3)-linked β-D-Gal*p* units in the main-chain. We now study the fine structure of the gum exudate polysaccharide from the trunk of the peach tree using modern techniques, such as GC–MS, ^{13}C NMR and HMQC spectroscopy, and ESI-MS and that from the fruit for comparative purposes.

2. Materials and methods

2.1. Collection of trunk and fruit gum exudates and isolation of their polysaccharides (PPN and PPNF, respectively)

The gum exudates of *P. persica* were collected in Palmas (State of Paraná, Brazil) at the Agronomic Institute of Paraná (IAPAR) (trunk of var. junegold) and from a private property (fruit of var. aerogil). These formed gels in the presence of water at concentrations higher than 3% (w/v), so it was necessary to resort to a dilute solution (1% w/v) for aqueous extraction.

A trunk gum sample (12.0 g) was stirred overnight in H_2O (1.3 L) to give a dispersion containing insoluble fragments, which were removed by passage through a fine cloth. The filtrate was added to excess EtOH (×3), to give a precipitate (PPN; 37% yield). The retained material was submitted to 1% aq. KOH extraction, in the presence of $NaBH_4$ at 25 °C, and the extract was neutralized (HOAc) and then added to EtOH (×3) to give a precipitate (PPNA; 42% yield). PPN and PPNA were each redispersed in H_2O, dialyzed against tap water (24 h), and then freeze-dried. Further ^{13}C NMR examination of these fractions showed that both had an almost identical structure. So, further characterization studies were carried out only with PPNA because its slightly better yield.

A moist sample of the fruit gum exudate (2.3 g) was submitted to the same method of extraction of trunk gum and yield PPNF (0.20 g) and PPNFA (0.14 g) from aqueous and alkaline extraction, respectively. These two fractions also had the same structure (^{13}C NMR) and further chemical studies were carried out only with the PPNF fraction.

2.2. Controlled Smith degradations of PPNA to give PPNAS and then PPNAS2

PPNA (1.0 g) was dissolved in H_2O (100 mL) and oxidized with 0.1 M $NaIO_4$ (100 mL) for 72 h in the dark, ethylene glycol (20 mL) then being added. The solution was dialyzed (48 h) and treated with $NaBH_4$ (to pH 10.0) for 16 h, and then neutralized (HOAc) and dialyzed (48 h). The volume of solution was concentrated to 20 mL and a partial acid hydrolysis of the reduced product at pH 2.0 (TFA), at 100 °C for 40 min was carried out. The resulting solution was adjusted with 1 M NaOH to pH 5.0 and added to excess EtOH (4:1 v/v) to give a precipitate (PPNAS, 189 mg; 19% yield).

PPNA (2.0 g) was also submitted to two successive controlled Smith degradations under the latter conditions to produce fraction PPNAS2 (9 mg; 0.45% yield).

2.3. Partial acid hydrolyses of PPNAS to give PPNAS45 and PPNAS60

PPNAS was partially hydrolyzed with 0.1 M TFA at 100 °C for 45 and 60 min to give PPNAS45 (58% yield) and PPNAS60 (20% yield), respectively. 1 M NaOH was then added to pH 5.0 and PPNAS45 and PPNAS60 were obtained by ethanol precipitation (4:1 v/v).

2.4. Determination of homogeneity and molar mass (M_w)

HPSEC analysis of polysaccharide fractions was carried out using a Waters size exclusion chromatography apparatus. Four columns of Waters Ultrahydrogel 2000/500/250/120 were connected in series and coupled to a multidetection system. Refractive index increments were determined by using a Waters 2410 detector. The value of d*n*/d*c* (differential refractive index increment of the solvent–solute solution, with respect to change in solute concentration) was calculated because it is important for the measurement of molecular mass. It was 0.143, 0.135, and 0.141 for PPNA, PPNF, and PPNAS60, respectively. Samples (1.0 mg/mL) were filtered through a 0.22 μm filter (Millipore) and then injected (200 or 100 μL loop). A 0.1 M $NaNO_2$ solution, containing NaN_3 (0.5 g L^{-1}), was used as solvent.

2.5. Production of acidic oligosaccharides from PPNA

PPNA (2.0 g) in 100 mL of M TFA was kept at 100 °C for 4 h, the solution then evaporated to dryness and the res-

idue dissolved in a small volume of H_2O. This was added to excess EtOH, the resulting precipitate centrifuged off, and the supernatant evaporated to dryness. The residue was applied to Whatman 3MM preparative paper using *n*-BuOH–Pyr–H_2O, 5:3:3 (v/v/v) as eluant and fractions were isolated with R_{Lac} 0.30, 0.78, and 0.96. These were named oligo-0.30 and oligo-0.96, respectively.

2.6. General methods

Polysaccharide fractions were hydrolyzed with 1 M TFA for 8 h at 100 °C to give monosaccharide mixtures. Part of the residue obtained after evaporation was examined using Whatman No. 1 filter paper [solvent: *n*-BuOH–Pyr–H_2O, 5:3:3 (v/v/v)] and the products were detected by the acetone–$AgNO_3$ dip reagent (Trevelyan, Procter, & Harrison, 1950). The other part of hydrolyzed polysaccharide was reduced with $NaBH_4$, acetylated with Ac_2O–Pyr (1:1, v/v) for 18 h at 25 °C and the resulting alditol acetates were examined by GC–MS. This was performed with a Varian model 3300 gas chromatograph coupled to a Finnigan Ion-Trap (model 810 R-12) mass spectrometer using a DB-225 capillary column (30 m × 0.25 mm i.d.) held at 50 °C during injection and then programmed at 40 °C min^{-1} to 220 °C (constant). He was the carrier gas.

The protein and uronic acid content of polysaccharide fractions were determined by the colorimetric methods described by Hartree (1972) and Filisetti-Cozzi and Carpita (1991), respectively. Carboxy-reduction of PPNA to PPNA-CR was carried out by the 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide method (Taylor & Conrad, 1972), $NaBH_4$ being used as the reducing agent.

2.7. Methylation analysis

Polysaccharide fractions PPNA, PPNAS, PPNAS45, PPNAS60, and PPNAS2 (~10 mg) were methylated according to Ciucanu & Kerek (1984), by dissolution in Me_2SO followed by addition of powdered NaOH and MeI. Each mixture was agitated strongly for 30 min and then left for 18 h. The per-*O*-methylated products were extracted with $CHCl_3$ from aq. solutions and were hydrolyzed with 50% v/v H_2SO_4 (0.5 mL) at 0 °C for 1 h, followed by dilution to 5.5% v/v. The solution was maintained at 100 °C for 16 h (Saeman, Moore, Mitchell, & Millet, 1954), and successively neutralized ($BaCO_3$), reduced with $NaBD_4$, and acetylated as described above to give a mixture of partially *O*-methylated alditol acetates, which were analyzed by GC–MS. These were identified by their typical retention times and electron impact spectra (Sassaki, Gorin, Souza, Czelusniak, & Iacomini, 2005).

2.8. ^{13}C nuclear magnetic resonance spectroscopy

^{13}C NMR, ^{1}H NMR, ^{1}H (obs.), ^{13}C HMQC, COSY, and TOCSY spectra were obtained using a 400 MHz Bruker model DRX Avance spectrometer equipped with a 5 mm inverse probe. ^{13}C NMR (100.6 MHz) analyses were performed at 60 °C in D_2O or Me_2SO-d_6 solutions. The ^{1}H NMR, ^{1}H (obs.), ^{13}C HMQC, COSY, and TOCSY analyses were performed at 60 or 70 °C in D_2O. Chemical shifts of the samples are expressed in PPM (δ) relative to external standard of acetone (δ 30.2) or Me_2SO-d_6 (δ 39.5).

3. Results

3.1. Homogeneity, monosaccharide content, methylation analysis, and ^{13}C NMR spectra of polysaccharides and structure of acidic oligosaccharides formed on partial hydrolysis

Polysaccharides obtained from the gum exudates of the trunk and fruit of *P. persica* (PPNA and PPNF, respectively) were homogeneous, as determined by HPSEC, and had M_w $5.61(\pm0.22)\times10^6$ and $6.43(\pm0.64)\times10^6$ g mol^{-1}, respectively (Fig. 1). They contained <1% protein and were composed of Ara, Xyl, Man, Gal, and uronic acids in a 36:7:2:42:13 and 32:13:2:33:20 molar ratio, respectively (Table 1). The uronic acids in PPNA were characterized by GC–MS of carboxy-reduced polysaccharide (PPNA-CR), which showed the presence of Glc and 4-Me-Glc (2:1 ratio; Table 1), which arose from Glc*p*A and 4-Me-Glc*p*A units, respectively.

Methylation–GC–MS analysis of PPNA (Table 2) showed nonreducing end- (20%) and 3-*O*-substituted Ara*f* units (6%), 4-*O*-substituted Ara*p* and/or 5-*O*-substituted Ara*f* units (14%), and nonreducing end-units of Xyl*p* (13%). Gal*p* units were from the highly branched core, with 3,6-di-*O*- (19%) and 3,4,6-tri-*O*-substitutions (14%). Also present were small amounts of 3-*O*- (7%) and 6-*O*- (1%), 2,3-di-*O*- (2%), and 3,4-di-*O*-substituted Gal*p* units (4%). The methylation data for PPNF (Table 2) showed a higher proportion of 3,6-di-*O*- (26%) and a lower one of 3,4,6-tri-*O*-substituted Gal*p* units (6%), when compared with PPNA. As with the monosaccharide compositions, it showed indistinguishable structures for the trunk and fruit gum polysaccharides, but in different proportions.

The ^{13}C NMR spectrum of PPNA (Fig. 2a) contained at least 10 signals in the anomeric region, in accord with the complex structure. The signals at δ 107.5–109.8 were from α-L-Ara*f* units (Joseleau, Chambat, Vignon, & Barnoud, 1977). The main signal at δ 103.2 can be assigned to C-1 of β-D-Gal*p* units (Tischer, Gorin, & Iacomini, 2002).

Comparison of the ^{13}C NMR spectrum of PPNA (Fig. 2a) with that PPNF (Fig. 2b) showed only very minor differences. This was also true of HMQC (Fig. 3a, b) and TOCSY examination (Fig. 4a, b), thus agreeing with the small quantitative differences in monosaccharide composition and methylation data.

The complex HMQC spectra of PPNA and PPNF (Fig. 3a, b) contained a group of 4 α-L-Ara*f* C-1 signals, which were at high frequency of δ 107.2–109.2. Although β-D-Xyl*p*, β-D-Gal*p*, and β-Glc*p*A units were components of PPNA, according to the literature, only one representa-

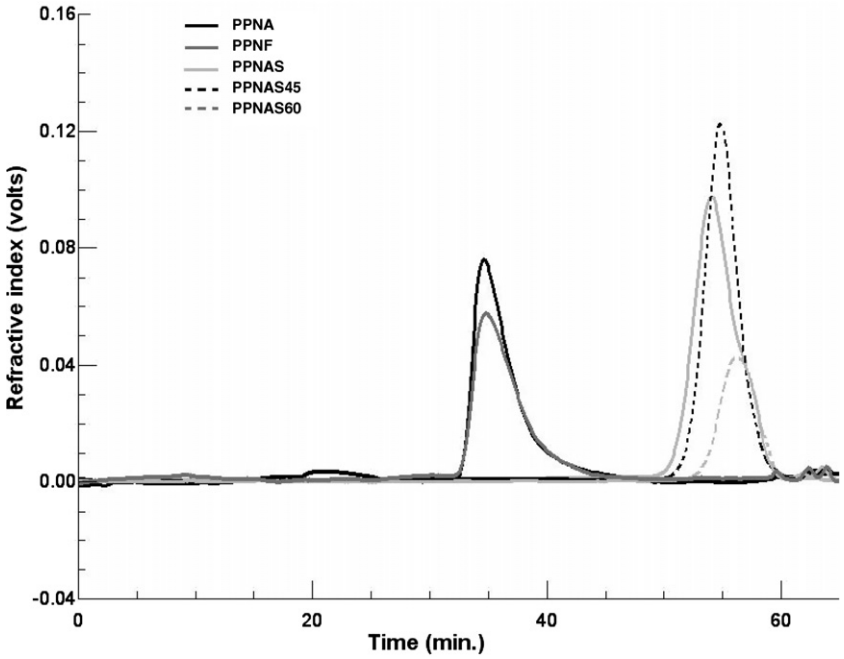

Fig. 1. Elution profiles of PPNA, PPNF, PPNAS, PPNAS45, and PPNAS60 using HPSEC, obtained with a refractive index detector.

Table 1
Monosaccharide composition of polysaccharides PPNA, PPNF, PPNA-CR, PPNAS, PPNAS45, PPNAS60, and PPNS2

Polysaccharide fraction	Monosaccharide composition (%)[a]						
	Ara	Xyl	Man	4-Me-Glc	Gal	Glc	Uronic acids[b]
PPNA	36	7	2	–	42	–	13
PPNF	32	13	2	–	33	–	20
PPNA-CR	38	8	3	2	45	4	8
PPNAS	19	–	9	–	70	–	2
PPNAS45	13	–	10	–	75	–	2
PPNAS60	–	–	12	–	85	–	3
PPNS2	–	–	29	–	71	–	–

[a] Analyzed on a DB-225 column by GC–MS after total hydrolysis, reduction, and acetylation.
[b] Determined by the colorimetric method of Filisetti-Cozzi and Carpita (1991).

Table 2
Partially *O*-methylalditol acetates formed on methylation analysis of polysaccharide fractions

Partially *O*-methylated alditol acetates	Parent linkage	R_t[a]	Polysaccharide fraction (%)					
			PPNA	PPNF	PPNAS	PPNAS45	PPNAS60	PPNS2
2,3,5-Me_3-Ara	Ara*f*-(1→	7:42	20	16	10	5	–	–
2,3,4-Me_2-Xyl	Xyl*p*-(1→	8:07	13	16	–	–	–	–
2,5-Me_2-Ara	→3)-Ara*f*-(1→	8:57	6	3	–	–	–	–
2,3-Me_2-Ara	→5)-Ara*f*-(1→, →4)-Ara*p*-(1→	9:26	14	12	–	–	–	–
2,3,4,6-Me_4-Gal	Gal*p*-(1→	9:31	–	3	10	11	18	25
2,4,6-Me_3-Man	→3)-Man*p*-(1→	11:04	–	–	5	4	7	17
2,4,6-Me_3-Gal	→3)-Gal*p*-(1→	11:21	7	7	16	17	15	34
2,3,4-Me_3-Gal	→6)-Gal*p*-(1→	12:46	1	4	31	34	45	–
4,6-Me_2-Gal	→2,3)-Gal*p*-(1→	12:58	2	4	5	8	6	21
2,6-Me_2-Gal	→3,4)-Gal*p*-(1→	13:19	4	3	–	–	–	–
2,4-Me_2-Gal	→3,6)-Gal*p*-(1→	16:27	19	26	23	20	9	3
2-Me-Gal	→3,4,6)-Gal*p*-(1→	18:29	14	6	–		–	–

[a] Retention times (s) obtained with a DB-225 column at 215 °C.

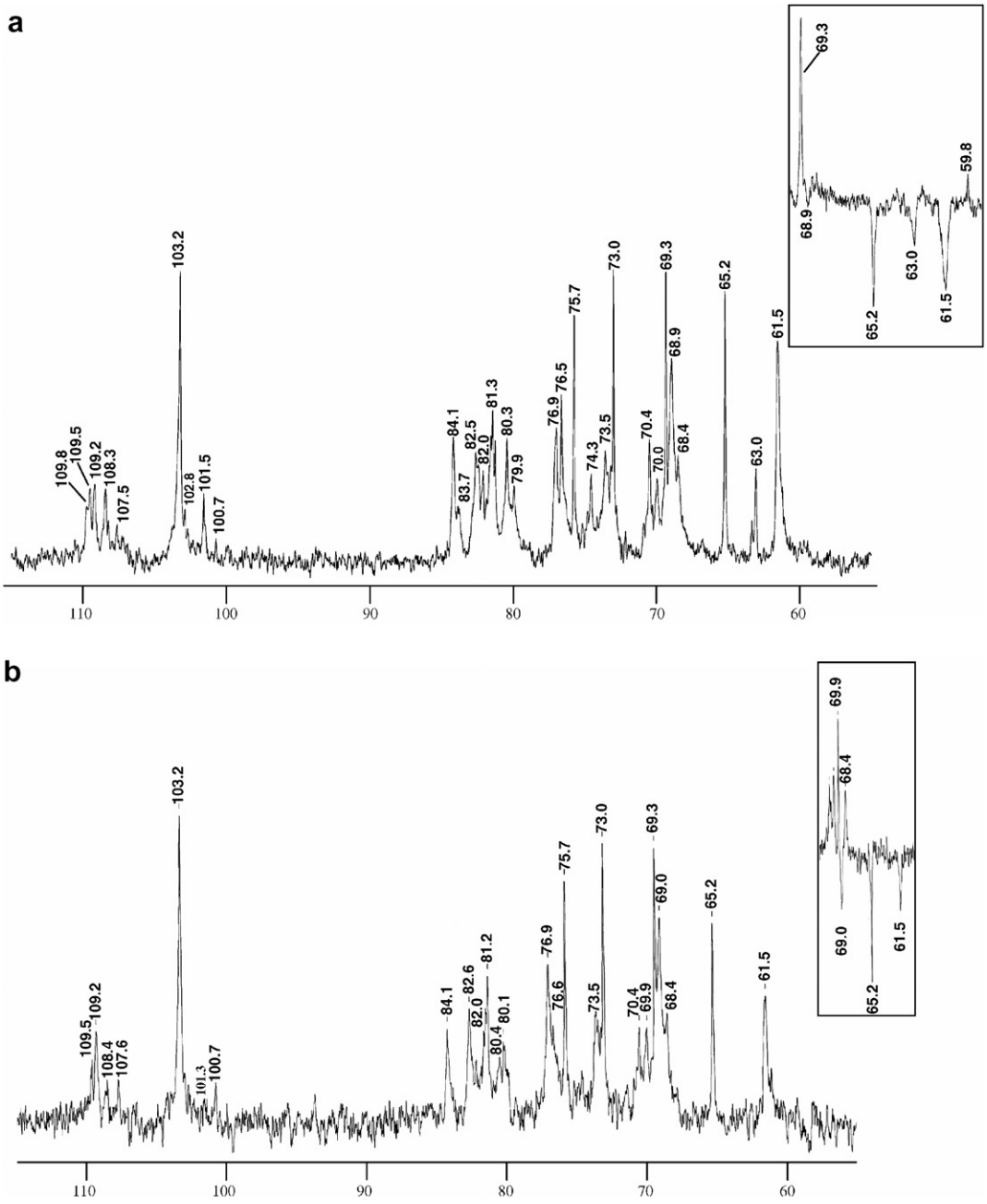

Fig. 2. ^{13}C NMR spectra of PPNA (a) and PPNF (b) from trunk and fruit gum exudates, respectively. Solvent: D_2O at 60 °C with numerical values in δ (PPM). Inserts: inverted signals in a ^{13}C NMR-DEPT spectra.

tive C-1 signal at high (δ 103.2) and low H-1 frequencies (δ 4.476) was present. The β-D-pyranosyl configuration was confirmed by a coupled HMQC spectrum of PPNA (Fig. 5) which contained a typical signal with $J_{C\text{-}1/H\text{-}1} = 161$ Hz (Perlin & Casu, 1969). Three other signals were from δ 99.9 to 101.5 (Fig. 3a, b) with $J_{C\text{-}1/H\text{-}1} = 173$–181 Hz, which could be from α-D-Man*p* and β-L-Ara*p* units.

As HMQC often gives signals that are not quantitative and sometimes absent, fractions of acidic oligosaccharides were obtained following partial hydrolysis of PPNA. These had R_{Lac} 0.30 and 0.96 and were named oligo-0.30 and 0.96, respectively. Oligo-0.96 gave a ^{13}C NMR spectrum (Fig. 6a) identical with that of β-D-Glc*p*A-(1 → 2)-αβ-D-Man*p* (Wagner et al., 2004). Its HMQC spectrum (not shown) contained main anomeric signals at δ 101.7/4.57 (N-1′) and 92.2/5.37 (N_α-1). Oligo-0.30 was mainly β-D-Glc*p*A-(1 → 6)-αβ-D-Gal, according to its ^{13}C NMR spectrum (Fig. 6b) (Delgobo, Gorin, Jones, & Iacomini, 1998): its DEPT spectrum had an inverted CH_2-6 arising from 6-*O*-substitution. The HMQC spectrum of oligo-0.30 (not shown) contained main anomeric signals at δ 102.9/4.53 (N-1′), 96.9/4.63 (N_β-1), and 92.8/5.31 (N_α-1). ESI-MS (negative mode) (not shown) gave a corresponding

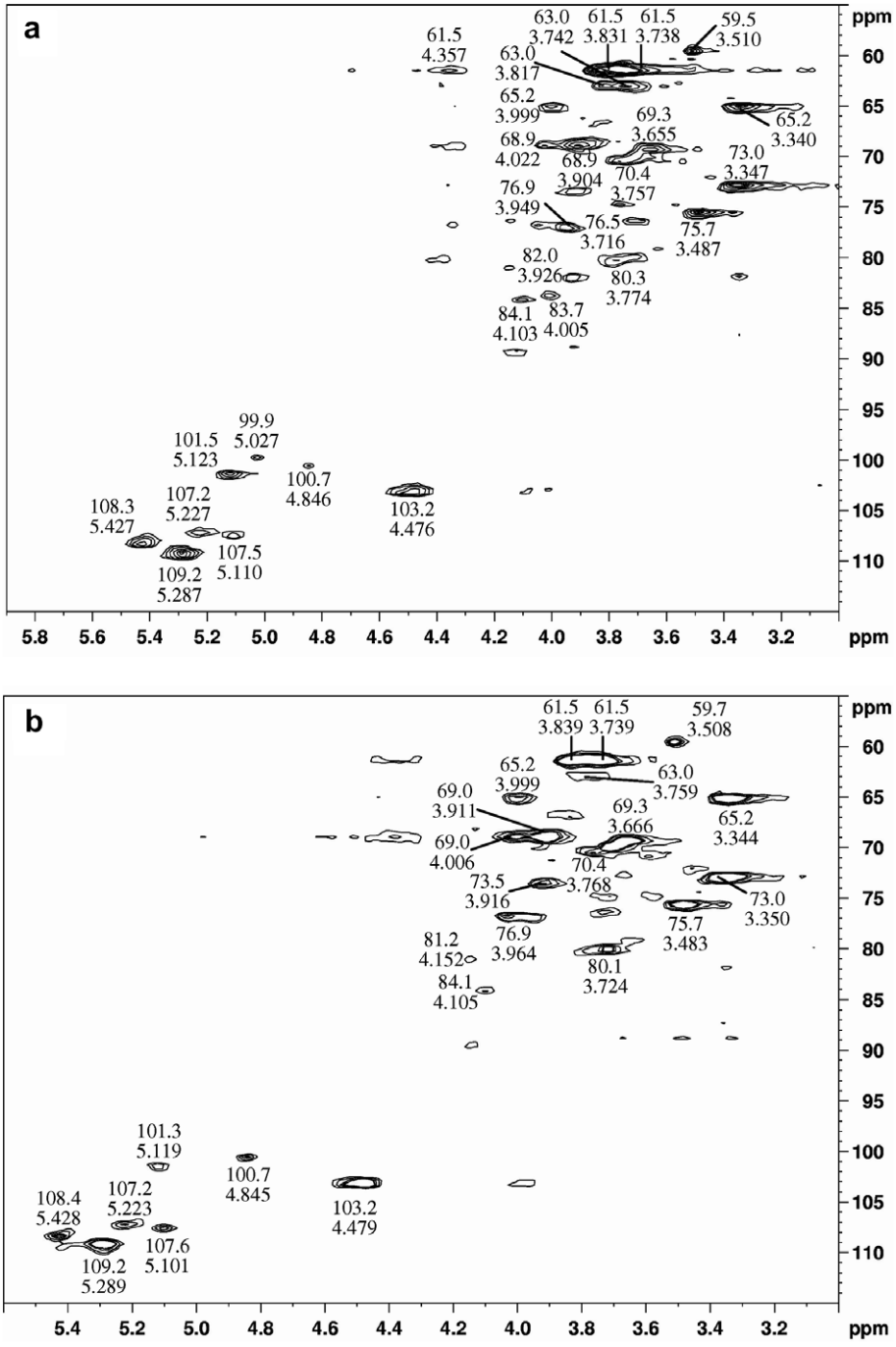

Fig. 3. HMQC spectra of PPNA (a) and PPNF (b), obtained from trunk and fruit gum exudates, respectively. Solvent: D_2O at 70 °C with numerical values in δ (PPM).

molecular ion at *m*/*z* 355 with a much smaller one of its 4Me-Glc*p*A derivative at *m*/*z* 369.

3.2. Successive, controlled Smith degradations of PPNA

A controlled Smith degradation of PPNA produced a polysaccharide (PPNAS; 19% yield) that was homogeneous when analyzed by HPSEC (Fig. 1) and was composed of Ara, Man, Gal, and uronic acids in a 19:9:70:2 molar ratio (Table 1). Methylation data (Table 2) showed that remaining Ara*f* units were present only as nonreducing end-units (10%) with nonreducing end- (10%), 3-*O*- (16%), 6-*O*- (31%), 2,3-di-*O*- (5%), and 3,6-di-*O*-substituted Gal*p* units (23%), showing a still complex structure. The ^{13}C NMR spectrum of PPNAS (Fig. 7a) was less complex than that of PPNA, with four signals in the anomeric region. The signal at δ 109.2 belongs to nonreducing end-units of residual α-L-Ara*f* units, which survived the degradation, and which were 3-*O*-substituted. The signals at δ 104.4 and 103.7 were assigned to nonreducing end-units and 3-*O*- and/or 6-*O*-

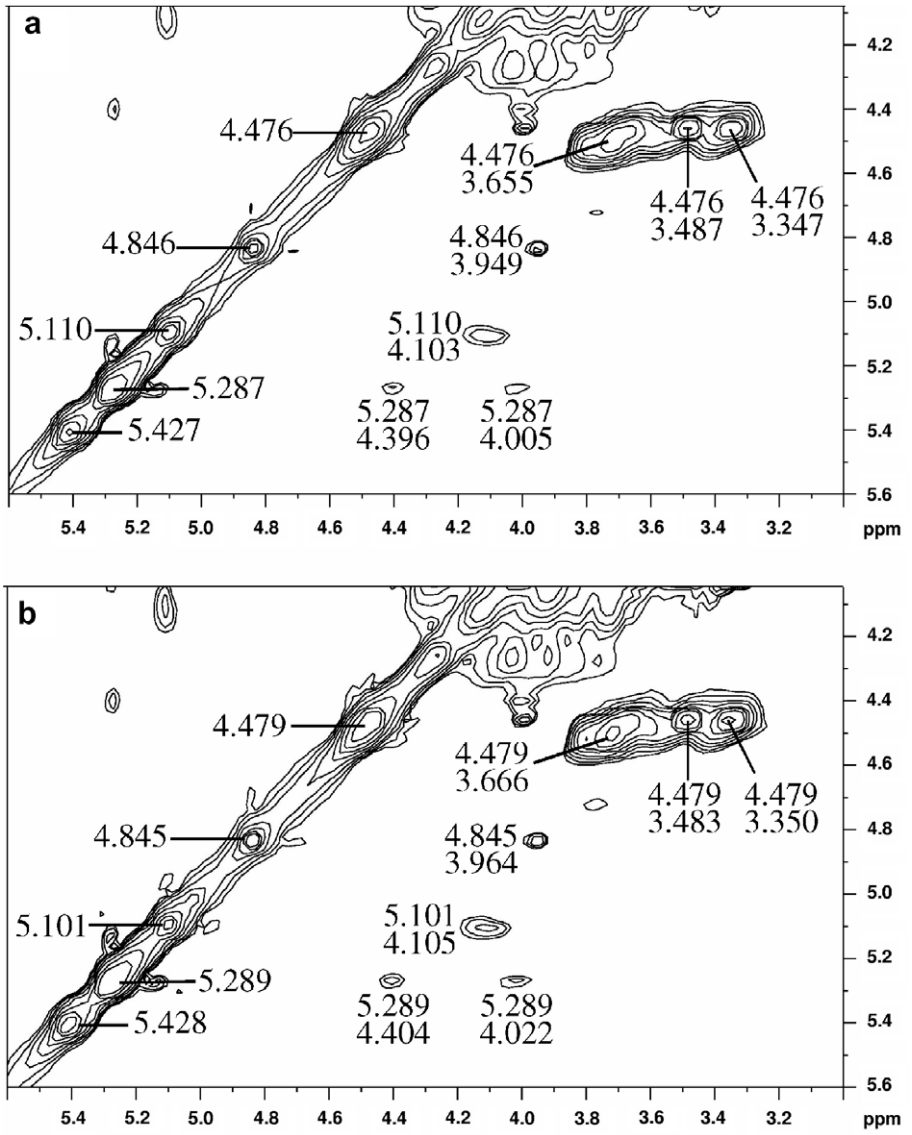

Fig. 4. H-1 correlations in TOCSY spectra of PPNA (a) and PPNF (b) from trunk and fruit gum exudates, respectively. Solvent: D_2O at 70 °C with numerical values in δ (PPM).

substituted β-D-Gal*p* units (Gorin & Mazurek, 1975; Tischer et al., 2002). The smaller signal at δ 100.8 could be from 3-*O*-substituted α-D-Man*p* units (Wagner et al., 2004). At lower frequency there were signals at δ 68.1, 61.8, 60.7, and 60.5, inverted in a ^{13}C NMR DEPT spectrum (Fig. 7a, insert), characterizing 6-*O*-substituted β-D-Gal*p* core units, C-5 of α-L-Ara*f* units, and free C-6 of β-D-Gal*p* and α-D-Man*p* units, respectively (Delgobo, Gorin, Tischer, & Iacomini, 1999; Sims & Furneaux, 2003; Tischer et al., 2002).

A second successive controlled Smith degradation on PPNA gave polymeric PPNAS2 in a 0.45% overall yield, low since PPNAS contained many free *vic*-hydroxyl groups, by virtue of the (1 → 6)-linked Gal*p* units. It was composed of Gal and Man in a 71:29 molar ratio (Table 1). Methylation analysis (Table 2) suggested a core component of 3-*O*-substituted Gal*p* units (34%) with some of these being substituted at O-2 (21%) by side chains of 3-*O*-substituted Man*p* (17%) and at O-6 (3%) by nonreducing end-units of Gal*p* (25%). These data indicated the presence of chains of Man*p* (1 → 3)-linked to Gal*p* units and suggested that this type of linkage does not alternate with (1 → 6)-linkage at main-chain Gal*p* units. The anomeric region of the ^{13}C NMR spectrum of PPNS2 (Fig. 8) had signals at δ 104.5–104.8 and 103.7 from nonreducing end-units and 3-*O*-substituted β-D-Gal*p* units respectively (Delgobo et al., 1999; Gorin & Mazurek, 1975) and at δ 99.2–100.6 from α-D-Man*p* units (Wagner et al., 2004).

3.3. Partial acid hydrolyses of Smith degraded PPNAS

In order to investigate further the core structure of PPNA, a partial acid hydrolysis of the Smith degraded product (PPNAS) was carried out with 0.1 M TFA at

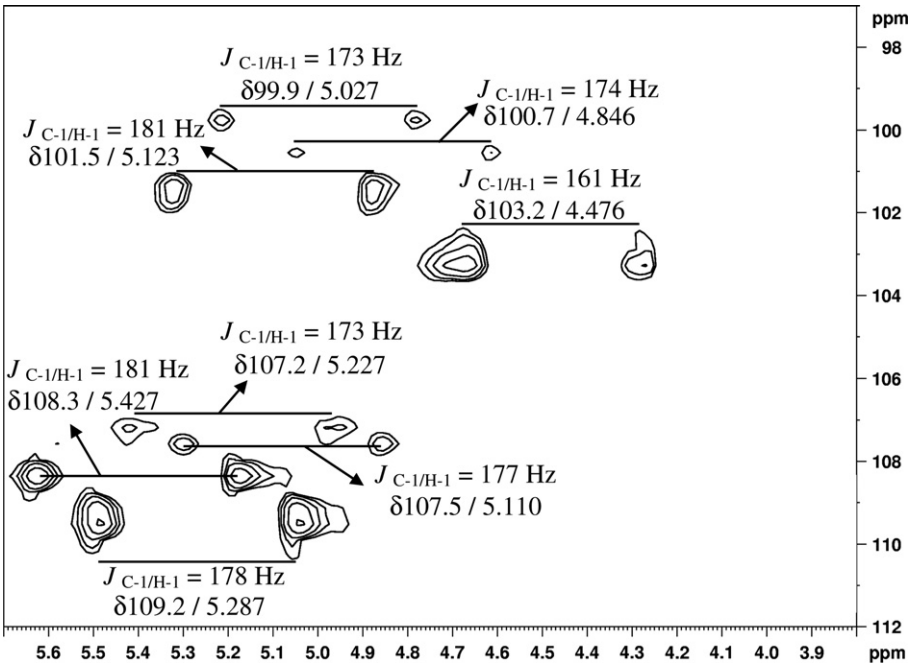

Fig. 5. $^{13}C/^{1}H$ coupled HMQC spectrum of anomeric region of PPNA in D_2O, at 70 °C.

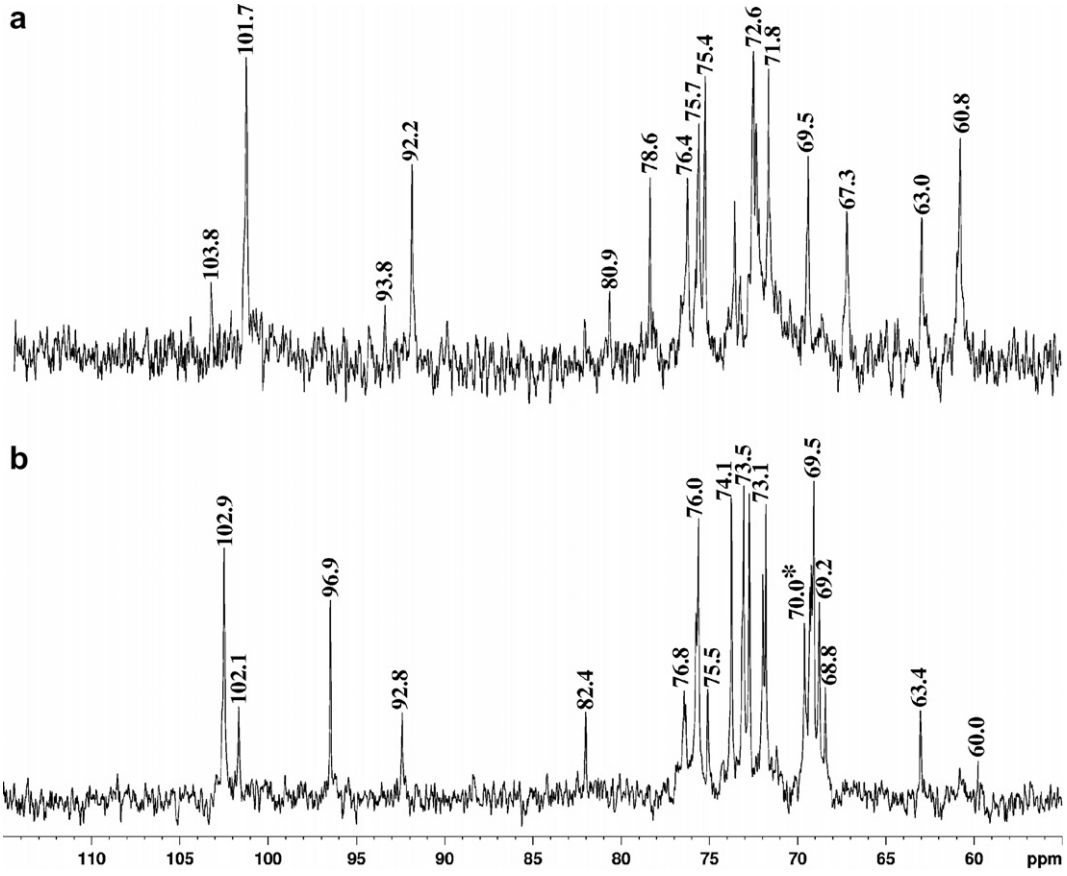

Fig. 6. ^{13}C NMR of aldobiouronic acids oligo-0.96 (a) and oligo-0.30 (b). Solvent: D_2O at 60 °C with numerical values in δ (PPM). *Signal from 6-*O*-substitution that inverted on DEPT-^{13}C NMR.

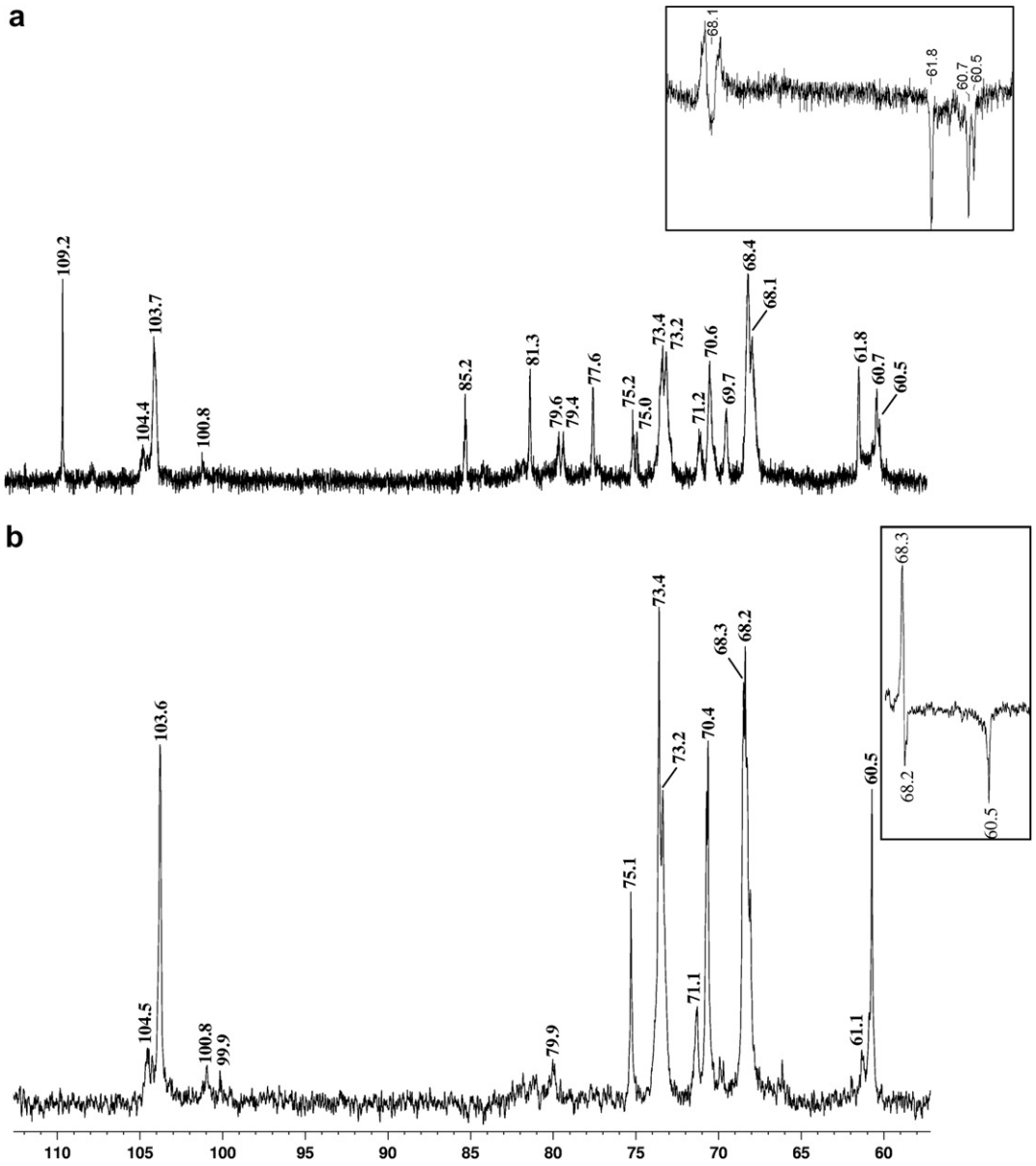

Fig. 7. ^{13}C NMR spectra of PPNAS (a) and PPNAS60 (b). Solvent: Me_2SO-d_6 at 60 °C with numerical values in δ (PPM). Inserts: inverted signals on DEPT ^{13}C NMR.

100 °C for 45 and 60 min, to yield PPNAS45 (58% yield) and PPNAS60 (20% yield), respectively.

PPNAS45 was homogeneous on HPSEC (Fig. 1) and was composed of Ara, Man, Gal, and uronic acids in a 13:10:75:2 molar ratio (Table 1). Methylation data (Table 2) showed that the 45 min hydrolysis was not sufficient to remove residual Ara*f* units, which appeared as nonreducing end-units (5%) in PPNAS45, the other derivatives being the same as those encountered in PPNAS. PPNAS60 was also homogeneous HPSEC (Fig. 1) and had M_w $1.26(\pm 0.19) \times 10^4$ g mol^{-1}. It was composed of Man, Gal, and uronic acids in a 12:85:3 molar ratio (Table 1) and methylation data showed a marked decrease of 3,6-di-*O*-substituted (9%) with a concomitant increase of 6-*O*-substituted Gal*p* units (45%) (Table 2), which showed that the core contained a high proportion of the latter structure.

The ^{13}C NMR spectrum of PPNAS45 (not shown) contained the same signals as PPNAS, although that of α-L-Ara*f* units at δ 109.2 was less intense. On the other hand, the ^{13}C NMR spectrum of PPNAS60 (Fig. 7b) had one main C-1 signal at δ 103.6 from the mono- and di-*O*-substituted β-D-Gal*p* units. The other signals at δ 104.5 and 100.8 can perhaps be assigned to nonreducing end-units of β-D-Gal*p* (Tischer et al., 2002) and to α-D-Man*p* units, respectively (Wagner et al., 2004). Those at δ 68.2 and 60.5, which were inverted in a ^{13}C NMR DEPT spectrum (Fig. 7b, insert) were from *O*-substituted and non-substituted C-6 of β-D-Gal*p* units, respectively. At higher frequency, the signals at δ 75.1, 73.4, 70.4, 68.3 were from to C-5, C-3, C-2, and C-4, respectively, of β-D-Gal*p* core units (Gorin & Mazurek, 1975).

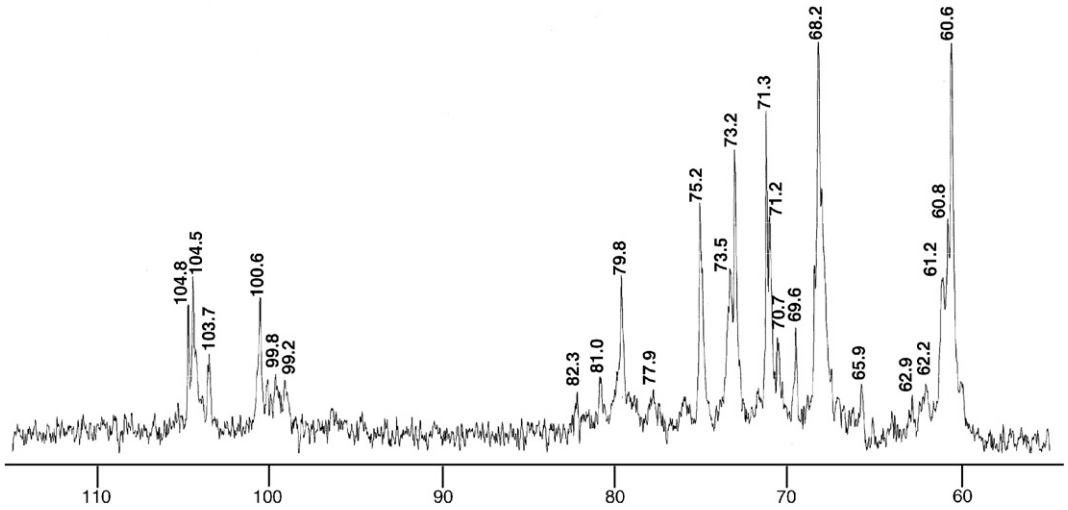

Fig. 8. ^{13}C NMR spectrum of PPNS2 obtained after two successive controlled Smith degradations of PPNA. Solvent: Me_2SO-d_6 at 60 °C with numerical values in δ (PPM).

4. Conclusions

NMR and ESI-MS analytical techniques have now been employed, in addition to those previously used to study the structure of the polysaccharides of *P. persica* gum exudates. Previous studies, starting with methylation analyses by Rosík et al. (1966), led to a proposal that the acidic arabinogalactan had a (1 → 6)-linked Gal*p* main-chain. Partial acid hydrolysis of the polysaccharide had given rise to β-D-Glc*p*A-(1 → 6)-D-Gal, 4-Me-β-D-Glc*p*A-(1 → 6)-D-Gal, β-D-Glc*p*A-(1 → 2)-D-Man (Rosík et al., 1967), β-L-Ara*p*-(1 → 3)-L-Ara, β-D-Xyl*p*-(1 → 4)-L-Ara (Andrews et al., 1953; Rosík et al., 1973), and [β-Gal*p*-(1 → 6)]$_{1\text{-}2}$-Gal (Kubala & Rosík, 1977), while enzymolysis furnished β-Gal*p*-(1 → 6)-Gal, β-Gal*p*-(1 → 3)-β-Gal*p*-(1 → 6)-Gal (Kardošová et al., 1978), and β-Gal*p*-(1 → 6)-β-Gal*p*-(1 → 3)-β-Gal*p*-(1 → 6)-Gal (Kardošová et al., 1979). However, none of these fragments contained more than two successive (1 → 6)-linkages, so that the main-chain structure is still in doubt.

We now find PPNA to be homogeneous and to contain Ara, Xyl, Man, Gal, and uronic acids in a 36:7:2:42:13 ratio, with a ~2:1 ratio between GlcA and its 4-*O*-methyl derivative. Its main components were nonreducing end-units of β-D-Xyl*p* and α-L-Ara*f*, 2-*O*-, 4-*O*-substituted Ara*p* and/or 5-*O*-substituted Ara*f* units, and 3,6-di- and 3,4,6-tri-*O*-substituted β-D-Gal*p* units. Attempts to show a uniform main-chain structure were only partially successful, the most predominant link being 45% of (1 → 6)-links after one controlled Smith degradation, the second serving to almost completely open the structure, although an inner core was formed in very low yield. This contained mainly β-D-Gal*p* units, which were nonreducing end- (25%), 3-*O*- (34%), and 2,3-di-*O*-substituted units (21%) with only 3% of 3,6-di-*O*-substituted units, unlike an exclusive (1 → 6)-linked main-chain (Rosík et al., 1966). PPNA could thus have a core with mixed (1 → 3)- and (1 → 6)-linked Gal*p* units, although this remains to be clarified.

Such a structure, however, contrasts with those of another acidic arabinogalactan group (Stephen, 1983) comprising gums of *Acacia* spp. One example is that of *Acacia senegal*, which although containing (1 → 3)- and (1 → 6)-linked β-Gal*p* units, survived seven successive degradations with its 3-*O*- and 3,6-di-*O*-substituted β-Gal*p* units to form polymers with predominant (1 → 3)-linkages (Anderson, Hirst, & Stoddart, 1966).

The structure of the fruit gum polysaccharide (PPNF) was investigated in less detail, but could be compared with that of with PPNA. A reason for possible differences could be seasonal variation (Stephen, 1983), not the case here, although we obtained our gums from different varieties of *P. persica* growing in proximity. PPNF was homogeneous with some differences from PPNA in its monosaccharide ratios (Table 1), containing more Xyl and uronic acid, and less Gal. Methylation analysis showed PPNF to be less branched, with more 3,6-di-*O*- and less 3,4,6-tri-*O*-substituted Gal*p* units, although component structures were qualitatively similar (Table 2). This was also reflected in the close similarity of their ^{13}C NMR (Fig. 2a, b), HMQC (Fig. 3a, b), and TOCSY correlation spectra, starting from H-1 (Fig. 4a, b).

References

Anderson, D. M. W., Hirst, E. L., & Stoddart, J. F. (1966). Studies of uronic acid materials. Part XVII. Some structural features of *Acacia senegal* (gum Arabic). *Journal of the Chemical Society*, 1959–1966.

Andrews, P., Ball, D. H., & Jones, J. K. N. (1953). The isolation of oligosaccharides from gums and mucilages. Part I. *Journal of the Chemical Society*, 4090–4095.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research, 131*, 209–217.

Delgobo, C. L., Gorin, P. A. J., Jones, C., & Iacomini, M. (1998). Gum heteropolysaccharide and free reducing mono- and oligosaccharides of *Anadenanthera colubrina*. *Phytochemistry, 47*, 1207–1214.

Delgobo, C. L., Gorin, P. A. J., Tischer, C. A., & Iacomini, M. (1999). The free reducing oligosaccharides of angico branco (*Anadenanthera*

colubrina) gum exudate: An aid for structural assignments in the heteropolysaccharide. *Carbohydrate Research, 320*, 167–175.

Filisetti-Cozzi, T. M. C. C., & Carpita, N. C. (1991). Measurement of uronic acids without interference from neutral sugars. *Analytical Biochemistry, 197*, 157–162.

Gorin, P. A. J., & Mazurek, M. (1975). Further studies on assignment of signals in ^{13}C magnetic resonance spectra of aldoses and derived methyl glycosides. *Canadian Journal of Chemistry, 53*, 1212–1224.

Hartree, E. F. (1972). Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analytical Biochemistry, 48*, 422–427.

Jones, J. K. N. (1950). The structure of peach gum. Part I. The sugars produced on partial hydrolysis of the gum. *Journal of the Chemical Society*, 534–537.

Joseleau, J. P., Chambat, G., Vignon, M., & Barnoud, F. (1977). Chemical and ^{13}C NMR studies on two arabinans from the inner bark of *Rosa glauca*. *Carbohydrate Research, 58*, 165–175.

Kardošová, A., Rosík, J., & Kubala, J. (1978). Neutral oligosaccharides from the enzyme hydrolysate of the polysaccharide of peach-tree gum (*Prunus persica* (L.) Batsch). *Collection of Czechoslovak Chemical Communications, 43*, 3428–3432.

Kardošová, A., Rosík, J., Kubala, J., & Kovácik, V. (1979). Structure of neutral oligosaccharides from the enzyme hydrolysate of polysaccharide of peach gum (*Prunus persica* (L.) Batsch). *Collection of Czechoslovak Chemical Communications, 44*, 2250–2254.

Kubala, J., & Rosík, J. (1977). The structures of three neutral oligosaccharides obtained by partial acid hydrolysis. *Collection of Czechoslovak Chemical Communications, 42*, 2809–2814.

Perlin, A. S., & Casu, B. (1969). Carbon-13 and proton magnetic resonance spectra of D-glucose-1-^{13}C. *Tetrahedron Letters, 34*, 2919–2924.

Rosík, J., Bruteničová-Sósková, M., Zitko, V., & Kubala, L. (1966). Polysaccharide from the resin of the peach tree *Prunus persica* (L.) Batsch. *Chemicke Zvesti, 20*, 577–585.

Rosík, J., Kardošová, A., & Kubala, J. (1967). Aldobiuronic acids of the polysaccharides of gums from the trees of genus *Prunus* (I): Methylation analysis. *Chemicke Zvesti, 21*, 739–744.

Rosík, J., Kubala, J., Kardošová, A., & Kovácik, V. (1973). Isolation and identification of 4-*O*-β-D-xylopyranosyl-L-arabinopyranose from the peach gum polysaccharide. I. *Chemicke Zvesti, 27*, 688–691.

Saeman, J. F., Moore, W. E., Mitchell, R. L., & Millet, M. A. (1954). Techniques for the determination of pulp constituents by quantitative paper chromatography. *Technical Association of the Pulp and Paper Industry, 37*, 336–343.

Sassaki, G. L., Gorin, P. A. J., Souza, L. M., Czelusniak, P. A., & Iacomini, M. (2005). Rapid synthesis of partially *O*-methylated alditol acetate standards for GC–MS: Some relative activities of hydroxyl groups of methyl glycopyranosides on Purdie methylation. *Carbohydrate Research, 340*, 731–739.

Sims, I. M., & Furneaux, R. H. (2003). Structure of the exudate gum from *Meryta sinclairii*. *Carbohydrate Polymers, 52*, 423–431.

Stephen, A. M. (1983). Other plant polysaccharides. In G. O. Aspinall (Ed.). *The polysaccharides* (Vol. 2, pp. 97–180). New York, NY: Academic Press.

Taylor, R. L., & Conrad, H. E. (1972). Stoichiometric depolymerization of polyuronides and glycosaminoglycuronans to monosaccharides following reduction of their carbodiimide-activated carboxyl groups. *Biochemistry, 11*, 1383–1388.

Tischer, C. A., Gorin, P. A. J., & Iacomini, M. (2002). The free reducing oligosaccharides of gum arabic: Aids for structural assignments in the polysaccharide. *Carbohydrate Polymers, 47*, 151–158.

Trevelyan, W. E., Procter, D. P., & Harrison, J. S. (1950). Detection of sugars on paper chromatograms. *Nature, 166*, 444.

Wagner, R., Woranovicz-Barreira, S. M., Iacomini, M., Delgobo, C. L., Pimentel, N. M., & Gorin, P. A. J. (2004). Structure of the glycoglucuronomannan from *Vochysia lehmannii* (Quaruba). *Carbohydrate Polymers, 57*, 269–275.