Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1843–1850
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Structure of a highly substituted β-xylan of the gum exudate of the palm *Livistona chinensis* (Chinese fan)

Juliana Maurer-Menestrina, Guilherme L. Sassaki, Fernanda F. Simas, Philip A.J. Gorin,* Marcello Iacomini

Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Caixa Postal 19046, 81531-990 Curitiba, PR, Brazil

Received 28 April 2003; accepted 11 June 2003

Abstract

Structural features of the acidic, highly substituted glycanoxylan (LCP; 87% yield) from the gum exudate of the palm, *Livistona chinensis*, family Arecaceae, were determined. It had $[\alpha]_D$ −30°, M_w 1.9×10^5 and a polydispersity ratio M_w/M_n of ~1.0. Acid hydrolysis gave rise to Rha, Fuc, Ara, Xyl, and Gal, in a 1:6:46:44:3 molar ratio, and 12% of uronic acid was present. LCP had a highly branched structure with side-chains containing nonreducing end-units (% values are approximate) of Ara*f* (15%), Fuc*p* (4%), Xyl*p* (7%), Glc*p*A, and 4-Me-Glc*p*A, and internal 2-*O*- (5%) and 3-*O*-substituted Ara*f* (8%), and 2-*O*-substituted Xyl*p* (14%) units. The (1→4)-linked β-Xyl*p* main-chain units of LCP were substituted at O-3 (4%), O-2 (17%), and O-2,3 (16%). Partial acid hydrolysis gave 4-Me-α-Glc*p*A-(1→2)-[β-Xyl*p*-(1→4)]$_{0-2}$-Xyl, identified by showing that the uronic acids were single-unit side-chain substituents on O-2. Milder hydrolysis conditions removed from O-3 other side-chains containing Fuc*p* and Ara*f* nonreducing end-units and internal Ara*p*, and 2-*O*- and 3-*O*-substituted Ara*f* units. Carboxyl-reduced LCP contained 4-*O*-methylglucose and glucose in a 3.2:1 molar ratio, arising from Glc*p*A and 4-OMe-Glc*p*A nonreducing end-units, respectively. The gum contained small amounts of free α-Fuc*p*-(1→2)-Ara, which corresponds to structures in the polysaccharide. Free *myo*- and D- or L-*chiro*-inositol were present in a 9:1 ratio.

Keywords: *Livistona chinensis*; Arecaceae; Heteroxylan; Gum exudates

1. Introduction

A. M. Stephen, in his review,[1] classified gum xylans having (1→4)-linked β-Xyl*p* main-chains, as 'heavily substituted glycanoxylans'. These are found in exudates from angiosperms, some dicotyledons, and several monocotyledonous families. Although more complex in terms of side-chains, they are structurally related to the hemicellulose xylans occurring universally in cell walls of higher plants. However, few representatives of heavily substituted glycanoxylans have been isolated and characterized using a combination of classical and modern techniques.[2–8] Much more numerous are plant gum polysaccharides whose main chains contain uronic acid or (1→3)-linked galactopyranosyl units. The structures of xylan-based polysaccharides of gum exudates of palms are now being examined as part of a chemotaxonomic study, since preliminary hydrolysis studies on those from *Livistona chinensis* (Chinese fan),[9] *Syragus rommanzofiana* (Queen palm),[10] and *Scheelea phalerata* (Brazilian common name: uricuri)[11] showed that each were highly substituted xylans containing fucosyl units. Such a component is apparently unknown in plant gums, although they have been found in a glucuronoarabinoxylan from cultivated rose cells.[12] The structure of the polysaccharide from *L. chinensis* is now determined in detail.

* Corresponding author. Tel.: +55-41-3663372; fax: +55-41-2662042.
E-mail address: cesarat@ufpr.br (P.A.J. Gorin).

0008-6215/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00276-3

2. Results and discussion

2.1. Polysaccharide isolation and properties

L. chinensis polysaccharide (LCP) was isolated from the gum exudate in 87% yield and was soluble in water giving low-viscosity solutions. It contained 12% uronic acid, gave on acid hydrolysis Rha, Fuc, Ara, Xyl, and Gal in a 1:6:46:44:3 molar ratio, and had $[\alpha]_D^{25}$ −30° (Table 1). Other components were protein (2.3%) and moisture (12%). HSPEC–MALLS analysis of LCP showed it to have M_w 1.9×10^5 and to be monodisperse with a M_w/M_n ratio of ~1.0. Carboxyl-reduction of LCP gave a product containing 4-*O*-methylglucose and glucose (3.2:1 ratio), arising from 4-Me-Glc*p*A and Glc*p*A residues, respectively.

2.2. Methylation and NMR analysis of LCP polysaccharide

LCP was submitted to methylation–GC–MS analysis, using a DB-225 capillary column, but many resulting *O*-methylalditol peaks overlapped. One group consisted of 2,3,4-Me_3-Ara, 2,3,4-Me_3-Fuc, and 2,3,4-Me_3-Xyl and another was 2- and 3-Me-Xyl. The former group components could be resolved using a DB-23 column and the latter with DB-210, the relative content of each component being used to adjust the DB-225 percentages. The highly branched structure of LCP, in terms of neutral units, is of nonreducing end-units of Ara*f* (15%), Fuc*p* (4%), and Xyl*p* (7%), 2-*O*- (5%) and 3-*O*-substituted Ara*f* (8%), and 2-*O*- (~14%) and 4-*O*-substituted Xyl*p* units (~6%) from the side-chains (Table 2). Considering that the main chain of LCP is (1→4)-linked β-Xyl*p*, shown by a controlled Smith degradation (see below), this is substituted at O-2 (17%), O-3 (4%), and O-2,3 (16%). The Glc*p*A and 4-Me-Glc*p*A units, which together comprise 12% of the total, were found to be nonreducing end-units by a reduction procedure incorporating NaB^2H_4, which gave the acetate of 2,3,4-Me_3-Glc-^{2}H-1,$^2H_2$-6, which gave a typical ion at *m/z* 191.

Table 2
Neutral, partially *O*-methylalditol acetates formed on methylation analysis of *L. chinensis* polysaccharide (LCP), the product (LCP-SM) obtained on controlled Smith degradation, and that formed on partial acid hydrolysis (LCP-PH-0.5)

Alditol acetate	t_R [a]	% Fragment area		
		LCP	LCP-SM	LCP-PH-0.5
2,3,4-Me_3-Rha	6.73	1		
2,3,5-Me_3-Ara	7.12	15	7	
2,3,4-Me_3-Ara	7.42			4
2,3,4-Me_3-Fuc [b]	7.61	4	4	
2,3,4-Me_3-Xyl [b]	7.61	7	2	
3,5-Me_2-Ara	8.34	5		5
2,5-Me_2-Ara	8.56	8	10	4
2,3,4,6-Me_4-Gal	8.84	2		
2,3-Me_2-Ara	9.21		4	
2,3-Me_2-Xyl [c]	9.47	~6	44	~16
3,4-Me_2-Xyl [c]	9.47	~14		~13
2-Me-Xyl [d]	11.86	4	13	7
3-Me-Xyl [d]	11.86	17	16	51
Xyl	15.09	16		

[a] Retention times in minutes obtained with DB-225 column.
[b] These were resolved and quantified using a DB-23 column.
[c] These are approximate values obtained by comparison of the heights of ions with *m/z* 118 and 129 (2,3-isomer) with those with *m/z* 117 and 130 (3,4-isomer).
[d] These were resolved and quantified using a DB-210 column.

The ^{13}C NMR spectrum of LCP (Fig. 1(A)) contained signals of α-Ara*f* at δ 106.8–108.6, two CH_3 signals centered at δ 17.8 and 16.5. In terms of α-Glc*p*A and 4-Me-Glc*p*A units, they both gave rise signals of C-1 at δ 98.4 and of C-6 signal at δ 177.3. In agreement, Swamy and Salimath[13] showed that side-chains of α-Glc*p*A linked to O-2 of a (1→4)-linked β-Xyl*p* main-chain gave a signal at δ 98.5, and Cavagna and coworkers[14] found that 4-Me-Glc*p*A-(1→2)-β-Xyl*p*-(1→4)-Xyl also

Table 1
Monosaccharide composition and specific rotation of polysaccharide of *L. chinensis* (LCP), those obtained after 30 min (LCP-PH-0.5), 2 h (LCP-PH-2), and 4 h partial hydrolysis (LCP-PH-4), and that obtained on controlled Smith degradation (LCP-SM)

Polysaccharide	Uronic acid [a]	Rha [b]	Fuc [b]	Ara [b]	Xyl [b]	Gal [b]	Glc [b]	$[\alpha]_D$ (°)
LCP	12	1	6	46	44	3		−30
LCP-PH-0.5	9		5	28	60	4	3	−27
LCP-PH-2	18			20	80			−26
LCP-PH-4	17			8	92			+1
LCP-SM	4		3	22	71			nd

[a] Percentage content determined colorimetrically.[22]
[b] Values are percentages of neutral monosaccharides liberated on hydrolysis.

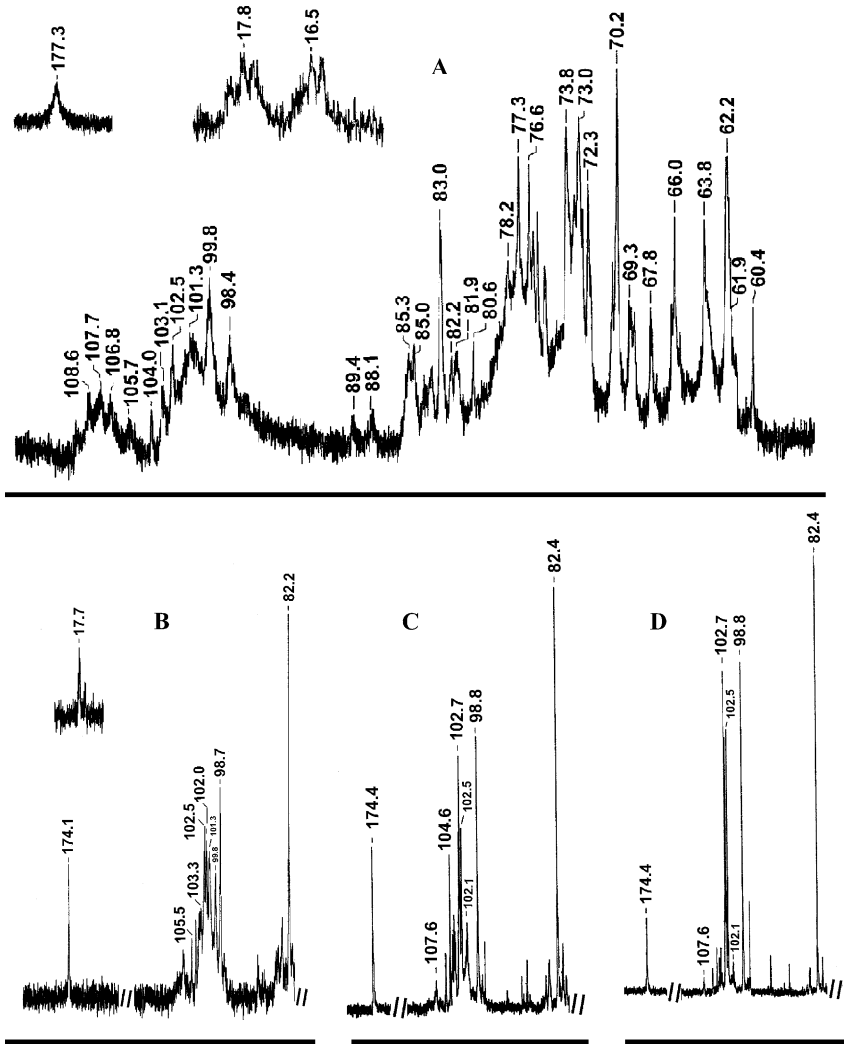

Fig. 1. ^{13}C NMR spectra of polysaccharide LCP from *L. chinensis* (A) and polysaccharides LCP-PH-0.5. LCP-PH-2, and LCP-PH-4, obtained after 30 min (B), 2 h (C), and 4 h (D) partial acid hydrolysis, respectively. Solvent D_2O at 30 °C with numerical values in δ (ppm). Inserts: CO_2H and CH_3 regions in spectrum A and CH_3 region in spectrum B.

showed a signal at δ 98.5. LCP also gave rise to a signal at δ 60.4 and 83.0 from $-CHOCH_3$-4 and $-CHOCH_3$-4 of 4-Me-α-Glc*p*A units, respectively.

2.3. Analysis of polysaccharides formed on partial hydrolysis of LCP

Partial hydrolyses were carried out on LCP with 0.1 M trifluoroacetic acid at 100 °C for 30 min and 0.1 M sulfuric acid at 100 °C for 2, and 4 h, giving polysaccharides with progressively better-defined ^{13}C NMR spectra (Fig. 1(B–D), respectively). A polymer (LCP-PH-0.5) was obtained after 30 min of partial hydrolysis. Hydrolysis gave rise to Fuc, Ara, Xyl, Gal, and Glc in a 5:28:60:4:3 molar ratio (GC–MS). The Glc arose from reduction of 9% of glucuronic acid residues (Table 1). Methylation analysis gave rise to partially *O*-methylated alditol acetates (Table 2), which showed that all of the nonreducing end-units of Ara*f* and some of those of Fuc*p* of LCP had been removed. This resulted in the exposure of nonreducing end-units of Ara*p* (4%) and retention of 2-*O*- (5%) and 3-*O*-substituted Ara*f* (4%) units. The C-1 and C-6 regions of its ^{13}C NMR spectrum (Fig. 1(B)) contained signal attributable to uronic acid at δ 98.8 and 174.1, respectively. Others were present at δ 82.2 ($CHOCH_3$-4), 60.9 ($CHOCH_3$-4; not shown) and 17.7 (CH_3 of fucose). Low-field α-Ara*f* signals were almost completely removed. After a 2-h partial hydrolysis, LCP gave a polysaccharide (LCP-PH-2; 19% yield) that contained 18% uronic acid, which upon hydrolysis gave Ara and Xyl in a 20:80 molar ratio, and had $[\alpha]_D$ −26° (Table 1). Its ^{13}C NMR spectrum (Fig. 1(C)) was similar to that of LCP-PH-0.5, except that the CH_3 signal was not present. A further 2 h of partial hydrolysis formed LCP-PH-4 (9% yield) that contained 17% uronic acid, gave on hydrolysis Ara and Xyl in a molar ratio of 8:92, and had $[\alpha]_D$ +1° (Table 1). Its ^{13}C NMR spectrum (Fig. 1(D)) retained the C-6, C-

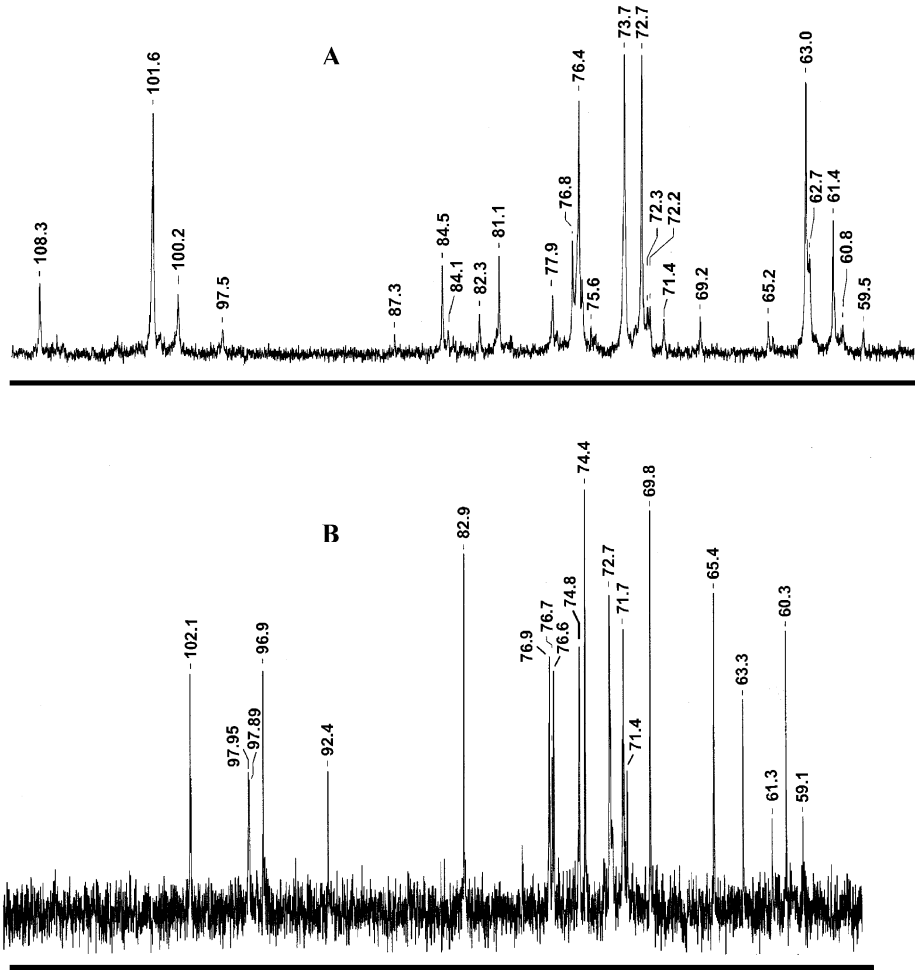

Fig. 2. Partial ^{13}C NMR spectra of LCP-SM, obtained by controlled Smith degradation of LCP (A), solvent 5% NaOD in D_2O at 30 °C, and aldotriouronic acid formed on partial hydrolysis (B), solvent D_2O at 30 °C. Numerical values in δ (ppm).

1, and C-4 signals of uronic acids at δ 174.4, 98.8, and 82.4 (–*C*HOCH$_3$-4), respectively, and those of C-1 of 4-*O*- and 2,4-di-*O*-substituted β-Xylp units at δ 102.7 and 102.5, respectively. This represented the more acid-resistant nucleus of LCP.

2.4. Analysis of polysaccharide formed on controlled Smith degradation of LCP

The presence of a (1→4)-linked β-Xyl*p* main-chain in LCP was confirmed by a controlled Smith degradation. The product (LCP-SM; 6% yield) contained 4% uronic acid and gave on acid hydrolysis Fuc, Ara, Xyl in a 3:22:71 molar ratio (Table 1). Methylation data (Table 2) showed nonreducing end-units of Fuc*p* (4%), Ara*f* (7%) and Xyl*p* (2%), 3-*O*-substituted Ara*f* (10%), 4-*O*-substituted Ara*p* and/or 5-*O*-susbstituted Ara*f* (4%) units, and 4-*O*- (44%), and 2,4- (16%), 3,4-di-*O*-substituted units of Xyl*p* (13%). The ^{13}C NMR spectrum of LCP-SM contained five main signals of (1→4)-linked β-Xyl*p* units (Fig. 2(A)).[15] The degradation thus resulted in the removal of uronic acid and 2-*O*-substituted Xyl*p* units in the side-chain (a reductive process carried out on permethylated LCP-SM did not show uronic acid residues). A signal at δ 108.3 showed that some α-Ara*f* side-chain units of LCP remained intact, agreeing with the data of mild acid hydrolysis. It can be seen that, based on the present data, the presence of a small proportion of (1→2)-links in the Xyl*p* main-chain cannot be eliminated.

2.5. Partial hydrolysis of LCP to form uronic acid-containing oligosaccharides

Partial acid hydrolysis of LCP with M TFA at 100 °C for 2 h gave oligosaccharides on PC in 1:1:1 *n*-BuOH–

Py–H_2O with R_{Lact} 0.87, 0.27, 0.23, and 0.19. That with R_{Lact} 0.87 was isolated by PC, although the other components could not be obtained pure, due to their similar R_{Lact} values. The R_{Lact} 0.87 component was 4-Me-α-Glc*p*A-(1→2)-Xyl, since methylation analysis involving a reducing step gave rise to acetates of 3,4-Me_2-xylitol-1-^2H and 2,3,4-Me_2-glucitol-1-^2H,6-$^2H_2$ and since its ^{13}C NMR spectrum contained –*C*HOCH_3-4 and –CHO*C*H_3-4 signals at δ 82.6 and 60.4, respectively. The R_{Lact} 0.27 fraction had a ^{13}C NMR spectrum (Fig. 2(B)) identical to that of 4-Me-α-Glc*p*A-(1→2)-Xyl*p*-(1→4)-αβ-Xyl,[14] although ESIMS in the negative-ion mode gave rise to molecular ions with *m/z* 471 and 457 in a ratio of ~7:1, corresponding to 4Me-Glc*p*A as well as Glc*p*A units, respectively. The R_{Lact} 0.23 fraction gave small molecular ions at *m/z* 603 and 589 (~4:1 ratio) arising from 4-Me-α-Glc*p*A-(1→2)-[β-Xyl*p*-(1→4)]$_n$-Xyl and α-Glc*p*A-(1→2)-[β-Xyl*p*-(1→4)]$_n$-Xyl ($n=2$), respectively. The R_{Lact} 0.19 fraction gave a molecular ions with *m/z* 735, showing an α-Glc*p*A-(1→2)-[β-Xyl*p*-(1→4)]$_3$-Xyl structure. Methylation analyses, carried out on each fraction, were consistent with the presence of 2-*O*- and 4-*O*-substituted Xyl*p* units and nonreducing ends of Glc*p*A and/or its 4-*O*-methyl derivative. These oligosaccharides indicate the presence of nonreducing end-units of α-Glc*p*A less than its 4-*O*-methyl derivative, linked to O-2 of the (1→4)-linked β-Xyl*p* main-chain.

2.6. Mild hydrolysis of LCP to form neutral oligosaccharides

In order to obtain other oligosaccharides containing Ara and Fuc from the side-chains of LCP, partial hydrolysis with 0.1 M trifluoroacetic acid at 100 °C for 30 min was carried out (these conditions were used above for degradation of LCP to LCP-PH-0.5). They were isolated via charcoal column chromatography, eluting with water, followed by 40% aqueous EtOH, to give fractions that contained fucose, arabinose, and xylose in a 1:2.8:1.3 ratio. ESIMS (positive-ion mode) showed the presence of molecular ions (Na^+ forms) with *m/z* 319 (Fuc-Pent) and 451 (Fuc-$Pent_2$), as well as 305 ($Pent_2$) and 437 ($Pent_3$), but methylation results on the mixture and borohydride-reduced material were too complex for accurate interpretation.

2.7. Free, reducing oligosaccharides present in the gum

Such oligosaccharides present in plant gums have been used to define acid-labile side-chain components of polysaccharides (for summary see Ref. 16). They were isolated from the gum as an ethanol supernatant and were fractionated on a charcoal column. The eluate with water, on PC examination gave rise to two spots with $R_{lact}0.86 > 1.00$, which proved to be *myo*- and D- or L-*chiro*-inositol in a ratio of 9:1 (GC–MS of acetates). Only *myo*-inositol has so far been characterized in gums, examples being its presence in gums arabic,[17] tragacanth,[18] and ghatti.[16]

The column was eluted with 40% aqueous ethanol, and the resulting material was fractionated to give a mixture having components with $R_{Gal} > 1.0$. ESIMS examination gave principally ion peaks with *m/z* 319 (Na^+ form) and 335 (K^+ form), corresponding to a disaccharide containing methylpentose and pentose. Small amounts of higher molecular weight material were present, however, since methylation–GC–MS analysis of sodium borohydride-reduced material gave rise to alditol acetates corresponding to nonreducing end-units of Fuc*p* and internal unit(s) of 2-*O*-substituted Ara*p*. Methylation analysis of the original eluate containing reducing sugars gave rise to alditol acetates of 2,3,4-Me_3-Fuc, 3,4- and 3,5-Me_2-Ara. The 3,4-isomer arose from the above-mentioned minor internal units of 2-*O*-substituted Ara*p*, but mainly from the reducing end-units of Fuc*p*-(1→2)-Ara, along with the 3,5-isomer, which is known to arise simultaneously on methylation of 2-*O*-substituted Ara reducing ends.[16] Another fragment was the alditol acetate of 2,3-Me_2-Ara from 4-*O*-substituted Ara*p* and/or 5-*O*-substituted Ara*f* units. ^{13}C NMR examination of the eluted mixture showed a signal at δ 97.4, which can be assigned to α-Fuc*p* nonreducing end-units, while a resonance at δ 82.0 is from C-2 of the 2-*O*-substituted reducing end and internal units of Ara*p*. The above data thus indicate a predominant α-Fuc*p*-(1→2)-Ara structure. As free reducing gum oligosaccharides have structures similar to those of the accompanying polysaccharide,[16] the presence of 2-*O*-substituted Ara*f* units in the polysaccharide (Table 1) shows that some of its terminal groups are α-Fuc*p*-(1→2)-Ara*f*.

2.8. Chemotaxonomic significance of LCP

The gum polysaccharide of the palm *L. chinensis* is a member of the group of highly substituted glycanoxylans[1] with a main chain of (1→4)-linked β-Xyl*p* units, mono- and di-*O*-substituted at both O-2 and O-3 with side-chains of up to at least three units. The main chain survived a controlled Smith degradation, a property that can be used to define such glycanoxylans. The complexity of the polysaccharide is evidenced by the presence of five different nonreducing end-units, including the unusual feature of that of fucopyranose, which may prove to be of chemotaxonomic interest, since it has not been detected in other polysaccharides of its group, except those of palm species.[9–11]

Side-chain structures have been described for other highly substituted xylans of members of the order Leguminosae, family Mimosaceae, such as *Cercidium praecox*, which are α-Glc*p*A-(1→2)-Xyl, 4-Me-α-

Glc*p*A-(1→2)-, and Ara*f*[2] and *Cercidium australe* with α-Glc*p*A-(1→2)-Xyl, α-Glc*p*A-(1→4)-Xyl, and 4-Me-α-Glc*p*A-(1→2)-Xyl groups.[3] α-Glc*p*A-(1→2)-β-Xyl*p*-(1→4)-Xyl and its 4-OMe analogue have been isolated from the polysaccharide of sapote gum (*Sapota achras*; Sapotaceae; Ebenales).[4] The polysaccharides from the corm-sacs of *Watsonia pyramidata*[5] and seed boxes of *Watsonia versveldii*[6] contain structures, in which substitution occurs at both O-2 and O-3 of the Xyl*p* units, the side-chains of the former contain Ara*f*, α-Gal*p*-(1→3)-Ara, and α-Gal*p*-(1→3)-α-Ara*f*-(1→2)-Ara groups, that of the trisaccharide being absent from the latter. *Puya chilensis* (order Bromeliales, family Bromeliaceae) contains Ara, Xyl, Gal, and uronic acid units linked as Glc*p*A-(1→2)-Xyl groups.[7] A distinct, highly substituted arabino(galactosyluronic acid)-rhamnosylxylan was isolated from *Plantago ovata*, order Plantaginaceae, family Scophulariales, which contained Gal*p*A-(1→2)-Xyl and Gal*p*A-(1→2)-Rha groups.[8]

The heteropolysaccharide from *L. chinensis* has now been shown to contain a (1→4)-linked β-xylan highly substituted with side-chains at O-2 including α-Glc*p*A and 4-Me-α-Glc*p*A units. The substituents at O-3 are less defined, but part of them should be terminated by α-Fuc*p*-(1→2)-Ara*f*- groups. The presence of nonreducing end units of fucose, also shown in preliminary studies on the polysaccharides of *Syragus rommanzofiana* and *Scheelea phalerata* gums[9–11] may be typical for palms, so that further investigations are being carried out on these and other palm species.

3. Experimental

3.1. Isolation of polysaccharide (LCP) and low-molecular weight material from gum

The gum exudate was collected in Caxambu, State of Minas Gerais, Brazil. It (20 g) was stirred vigorously in H_2O (1 L) to form a solution, and insoluble debris was removed by filtration. Addition of the filtrate to excess EtOH (300 mL) resulted in a precipitate, which was redissolved in H_2O, dialyzed successively against tap water (20 h) and distilled water (20 h), and then freeze-dried to give LCP (yield 87%).

The exudate (100 g) was dissolved in H_2O (700 mL), and the resulting solution was added to EtOH (3.0 L). The precipitate was filtered off, and the filtrate was evaporated to dryness (yield 0.3%). The residue was applied to a charcoal–Celite column, which was eluted with H_2O and then with 40% aq EtOH. A portion was fractionated on Whatman No. 3 filter paper (solvent: 5:3:3 *n*-BuOH–Py–H_2O) and mixed components with $R_{Gal} > 1.0$ were isolated.

3.2. General methods

Specific rotations of polysaccharide fractions were determined at 25 °C in 0.5% (w/v) aqueous solutions with a Rudolph polarimeter (model 589).

Polysaccharide samples were hydrolyzed with M TFA for 8 h at 100 °C to give monosaccharide mixtures. These were examined using Whatman No. 1 filter paper (solvent: 5:3:3 *n*-BuOH–Py–H_2O), and the products were detected by the acetone–$AgNO_3$ dip reagent.[19] The resulting monosaccharide mixtures were also analyzed by GC–MS of their derived alditol acetates, prepared by $NaBH_4$ or NaB^2H_4 reduction, followed by acetylation with Ac_2O–Py. GC–MS was performed using 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 to 220 °C (constant). He was the carrier gas.

The moisture content of LCP was determined by the method described by R.J. Smith,[20] and its protein content was determined according to Bradford[21] using bovine albumin serum as standard. Uronic acid contents of polysaccharide fractions were determined by the *m*-hydroxybiphenyl method.[22] The 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide–$NaBH_4$ method was used for carboxyl-reduction of LCP.[23]

3.3. Determination of molecular weight (M_w) and polydispersity (M_w/M_n) of LCP by HPSEC–MALLS

LCP was examined using a CMX 100 (Chromatix) light-scattering apparatus coupled to GPC columns of OH pak B-804 in series with one of OH pak B-805 Shodex, Showa Denko, Japan. A 0.1% aqueous solution of the polysaccharide was eluted at 1 L/min with a mixture of 0.1 M $NaNO_3$ and NaN_3 (0.2 g/L).

3.4. Methylation analysis of polysaccharides

Polysaccharides (~ 50 mg) were partially methylated by the method of Haworth[24] in order to render the products soluble in the Me_2SO-based reaction medium of Ciucanu and Kerek.[25] The per-*O*-methylated products were refluxed with 3% MeOH–HCl for 3 h, neutralized with Ag_2CO_3 and then hydrolyzed with 0.5 M H_2SO_4 at 100 °C for 15 h. After NaB^2H_4 reduction and acetylation with Ac_2O–Py, the resulting mixture of partially *O*-methyated alditol acetates was examined by GC–MS. But for this, a battery of capillary columns were necessary. A column of DB-225 was used for overall identification and quantification (Table 1). However, it neither resolved acetates of 2,3,4-Me_3-Ara, 2,3,4-Me_3-Fuc, and 2,3,4-Me_3-Xyl, nor those of 2- and 3-Me-Xyl. It was necessary to employ a column of DB-23 with the same dimensions of 30 m × 0.25 mm i.d., the

former group being resolved with retention times of 7.05, 7.11, and 7.16 min, respectively. DB-210 distinguished the latter group with retention times of 12.37 and 12.22 min, respectively.

In order to detect *O*-methyl fragments arising from glucuronic acid and/or 4-O-methylglucuronic acid residues in the above methanolysis step, the product was reduced with NaB^2H_4 in 0.1% NaOMe in MeOH at 70 °C for 2 h. The reduced material was then converted to a mixture of partially *O*-methylated alditol acetates as indicated above.

3.5. Partial acid hydrolyses of LCP

In order to obtain oligosaccharides containing uronic acid, LCP (2.4 g) was partially hydrolyzed in M TFA (40 mL) for 2 h at 100 °C. The resulting solution was evaporated to dryness, and the residue was applied to a charcoal–Celite column. This was eluted with H_2O and then with 40% aq EtOH, which gave rise to a mixture (0.61 g) with zones of oligosaccharides on PC at R_{Lact} 0.87, 0.27, 0.23, and 0.19 (solvent: 1:1:1 *n*-BuOH–Py–H_2O). A portion of the mixture was fractionated on Whatman No. 3 filter paper with the solvent 5:3:3 *n*-BuOH–Py–H_2O to give each component.

LCP (4.5 g) was dissolved in 150 mL of H_2O, and the solution was adjusted to pH 1.0 with dilute aq H_2SO_4. This was the divided into two parts, one of them being kept at 100 °C for 2 h with the other for 4 h. Each solution was neutralized with $BaCO_3$ and filtered, and the filtrate was treated with cation-exchange resin (Dowex 50W × 8, H^+, Sigma, St Louis, USA). The solution was then filtered and evaporated to a small volume. The partially hydrolyzed products obtained after EtOH precipitation were named LCP-PH-2 (19% yield; 2 h) and LCP-PH-4 (9.4% yield; 4 h).

LCP (0.52 g) was also partially hydrolyzed for 30 min in 0.1 M TFA at 100 °C. The resulting polysaccharide LCP-PH-0.5 was precipitated with excess EtOH. The supernatant was subjected to charcoal column chromatography. An aqueous eluate was discarded, and elution with 40% aq EtOH provided a mixture of oligosaccharides, fucose, and rhamnose (0.25 g).

3.6. Controlled Smith degradation of LCP

LCP (2.0 g) was dissolved in H_2O (500 mL) and oxidized with 0.05 M $NaIO_4$ (500 mL) for 2 days in the dark. Ethylene glycol (30 mL) was then added, and the solution was dialyzed and reduced with $NaBH_4$ (0.5 g). The solution was acidified with HOAc and redialyzed, successively, against tap (20 h) and distilled H_2O (20 h). The resulting solution was evaporated to 100 mL, adjusted to pH 2.0 with aq H_2SO_4, kept at 100 °C for 1 h, neutralized with $BaCO_3$, and filtered. The filtrate was evaporated to 20 mL and added to 200 mL EtOH, giving a precipitate (LCP-SM; 6% yield).

3.7. ^{13}C NMR spectroscopy

^{13}C NMR spectra were obtained in D_2O at 30 °C, except for the controlled Smith degraded polysaccharides, when 5% (w/v) NaOD in D_2O was used. Chemical shifts are expressed as in δ (ppm) based on Me_4Si ($\delta = 0$) as external standard.

3.8. ESIMS examination of oligosaccharides

Analyses were carried out using Micromass Quattro Ultima equipment. Each sample (~ 1 ηg/μL) was previously dissolved in H_2O and CH_3COCN added to give a 1:1 mixture. Samples were applied using a manual loop injector (10 μL volume) on to a flow rate of 20 μL/min of the 1:1 solvent. The positive-ion mode was used for neutral oligosaccharides and the negative-ion mode one for those containing uronic acid, which resulted in considerable formation of daughter ions, but was still preferred over the positive-ion mode, which did not produce any recognizable ions. The system was washed ($\times 6$) with the solvent after each run.

Acknowledgements

The authors wish to thank Dr Marguerite Rinaudo (Centre de Recherche sur les Macromolécules Végétales, Université Scientifique et Médicale de Grenoble, France) for determination of M_w and M_w/M_n values of the *L. chinensis* polysaccharide. We are also indebted to CNPq-Brazil for financial support (PRONEX-Carboidratos).

References

1. Stephen, A. M. In *The Polysaccharides*; Aspinall, G. O., Ed. Other plant polysaccharides; Vol. 2; Academic Press: New York, NY, 1983; pp 97–180.
2. León de Pinto, G.; Martinez, M.; Rivas, C. *Carbohydr. Res.* **1994**, *260*, 17–25.
3. Cerezo, A. S.; Stacey, M.; Webber, J. M. *Carbohydr. Res.* **1969**, *9*, 505–517.
4. Lambert, R. D.; Dickey, E. E.; Thompson, N. S. *Carbohydr. Res.* **1968**, *6*, 43–62.
5. Shaw, D. H.; Stephen, A. M. *Carbohydr. Res.* **1966**, *1*, 400–413.
6. Shaw, D. H.; Stephen, A. M. *Carbohydr. Res.* **1966**, *1*, 414–416.
7. Hamilton, D. R.; Spriesterbach, D. R.; Smith, F. J. *Am. Chem. Soc.* **1957**, *79*, 443–446.
8. Sandhu, J. S.; Hudson, G. J.; Kennedy, J. F. *Carbohydr. Res.* **1981**, *93*, 247–259.

9. Maurer-Menestrina, J.; Sassaki, G. L.; Simas, F. F.; Gorin, P. A. J.; Iacomini, M. *32nd Annual Meeting of the Brazilian Society of Biochemistry and Molecular Biology*, May 17–20, **2003**, M–56, p. 160.
10. Simas, F. F. Personal communication.
11. Simas, F. F.; Delgobo, C.; Leite, A.; Gorin, P. A. J.; Iacomini, M. *32nd Annual Meeting of the Brazilian Society of Biochemistry and Molecular Biology*, May 17–20, **2003**, M-91, p. 166.
12. Mollard, A.; Barnoud, F. *Carbohydr. Res.* **1975**, *39*, C13–C15.
13. Swamy, N. R.; Salimath, P. V. *Carbohydr. Res.* **1990**, *197*, 327–337.
14. Cavagna, F.; Deger, H.; Puls, J. *Carbohydr. Res.* **1984**, *129*, 1–8.
15. Ebringerová, A.; Hromádková, Z.; Petráková, E.; Hricovíni, M. *Carbohydr. Res.* **1990**, *198*, 57–66.
16. Tischer, C. A.; Iacomini, M.; Wagner, R.; Gorin, P. A. J. *Carbohydr. Res.* **2002**, *337*, 2205–2210.
17. Tischer, C. A.; Gorin, P. A. J.; Iacomini, M. *Carbohydr. Polym.* **2002**, *47*, 151–158.
18. Tischer, C. A.; Iacomini, M.; Gorin, P. A. J. *Carbohydr. Res.* **2002**, *337*, 1647–1655.
19. Trevelyan, W. E.; Procter, D. P.; Harrison, J. S. *Nature* **1950**, *166*, 444.
20. Smith, R. J. *Methods Carbohydr. Chem.* **1964**, *4*, 36–42.
21. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.
22. Blumenkrantz, N.; Asboe-Hansen, G. *Anal. Biochem.* **1973**, *54*, 484–489.
23. Stone, B. A.; Anderson, M. A. *Carbohydr. Polym.* **1985**, *5*, 115–120.
24. Haworth, W. N. *J. Chem. Soc.* **1915**, *107*, 8–16.
25. Ciucanu, I.; Kerek, F. A. *Carbohydr. Res.* **1984**, *131*, 209–217.