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The free reducing oligosaccharides of angico branco (*Anadenanthera colubrina*) gum exudate: an aid for structural assignments in the heteropolysaccharide

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Abstract

A novel method is described for the determination of sequential side-chain structures in the complex, high-arabinose polysaccharide of the gum exudate of angico branco (*Anadenanthera colubrina*), using as basis the structurally similar reducing oligosaccharides present in small quantities. Of the ten detected, eight were characterized as disaccharides (**2**, **3**, and **9**), linear trisaccharides (**1** and **4**), branched pentasaccharides (**5** and **6**), and a doubly branched heptasaccharide (**8**). The oligosaccharides are substituents of the polysaccharide, which has a (1→3)-linked β-D-galactopyranosyl main chain, and with two exceptions they had 6-O-substituted galactopyranosyl reducing ends, probably corresponding to its main-chain units. Characterization was effected through their 1D and 2D NMR correlation spectra, which were better resolved and more readily interpretable than those of the polysaccharide. These spectral data were supported by monosaccharide composition and rotation values. Controlled Smith degradations and methylation analyses were carried out when it was necessary. These data were confirmed by field-desorption MS.

Keywords: Angico branco; *Anadenanthera colubrina*; Gum; Polysaccharide; Free, reducing oligosaccharides; Structural similarity

1. Introduction

Structural investigations on the gum exudate that appears on trunks of angico branco (*Anadenanthera colubrina*) showed it to contain, in addition to a polysaccharide, small amounts of free reducing mono- and oligosaccharides [1,2]. A more detailed study [3] indicated a complex high-arabinose heteropolysaccharide with a (1→3)-linked β-D-Gal*p* main chain with many different side-chains, consisting of groups with α- and β-Ara*f*, α- and β-Ara*p*, β-Glc*p*A, Rha*p*, and structures containing -β-Gal*p*-(1→6)-. Its complexity was evidenced by its 500 MHz NMR spectra, which contained at least 26 H-1 and 20 C-1 signals. The gum also contained four monosaccharides and at least ten oligosaccharides, all reducing and many of the latter arabinose-containing. The only oligosaccharide identified was α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-αβ-Gal, although it was not completely certain whether the Rha*p* to Glc*p*A linkage was (1→3) or (1→4). When

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compared with the polysaccharide of angico branco, the NMR spectra of this and other oligosaccharides were less complex and had narrower NMR line widths; hence spectral interpretation was more straightforward. This approach is particularly useful for the present example of a high-arabinose polysaccharide, since oligosaccharides containing Ara*f* units are extremely difficult to obtain from the polysaccharide by conventional methods. They are now characterized and used as an aid in determination of the complex structure of the polysaccharide.

An indication that such free oligosaccharides could be used as models for structural analysis of the accompanying polysaccharide became evident in the case of the gum of the Brazilian cashew-nut tree, *Anacardium occidentale* [4,5]. Their less complex structures appeared to be similar to those of the side chains of the polysaccharide: 3-O-substituted β-Gal*p* structures, typical of the polysaccharide main chain, were absent. The only exception applied to reducing 6-O-substituted galactosyl units, often present, and which probably corresponded to those of the main chain.

2. Results

The oligosaccharides of angico branco gum were isolated by chromatography on a charcoal column, followed by preparative paper chromatography (PC), to give fractions, homogeneous according to 1D ^{13}C and ^{1}H NMR spectroscopy, PC, and HPLC, and when only quantities of ~ 1 mg were available, by HMQC. Their monosaccharide composition, specific rotation, molecular weight, and monosaccharide sequence (field-desorption MS) were then determined.

Examination of ^{13}C NMR spectra of the oligosaccharides showed many structural similarities between them. Of the ten oligosaccharides with typical PC R_{Lact} values, eight are now characterized.

(A) Trisaccharide **1** *with* R_{Lact} *0.38.*—The oligosaccharide ($[\alpha]_D - 23°$) gave Rha, Gal, and a trace of Glc on acid hydrolysis (GC–MS), the glucose arising from glucuronolactone formed by sodium borohydride reduction in the alditol acetate derivatization process. ESIMS and MALDI-TOF examination (Table 1) showed Rha, Glc*p*A, and Gal as components of a trisaccharide. The ^{13}C NMR spectrum contained anomeric reducing-end signals at $96.9 > 92.8$ ($>$ and $<$ refer to one signal being larger or smaller than the other, respectively), corresponding only to those of αβ-Gal*p* [6] and which were O-substituted at C-6 (δ $69.73 > 69.82$; DEPT inverted). An internal residue of β-Glc*p*A was evidenced by anomeric resonances at δ $102.88 > 102.85$, which was O-substituted (δ 79.6), and also gave rise to a C-6 signal of uronic acid residues (δ 175.6). The anomeric signal of α-Rha*p* was at δ 101.2: COSY–TOCSY, starting from H-1 and H-6, followed by HMQC examination of the trisaccharide, confirmed this as a non-reducing end of α-Rha*p* as its ^{13}C resonances corresponded to those of Me α-Rha*p* [6].

However, difficulties were encountered in the characterization of the Rha*p* to Glc*p*A linkage. Using the COSY–TOCSY sequence,

Table 1
NMR and MS data for free reducing oligosaccharides **1–4**

Oligosaccharide	Key NMR signals [a]; *m/z* values of molecular and daughter ions
1	α-Rha*p*″: 101.2 (C-1), 4.75 (H-1), 16.9 (C-6), 1.35 (H-6). β-Glc*p*A′: 102.88 > 102.85 (C-1′), 79.6 (C-4′), 175.6 (C-6′). αβ-Gal*p*: 92.8 < 96.9 ($C_{\alpha\beta}$-1), 69.82 < 69.73 ($C_{\alpha\beta}$-6; DEPT inverted) ESIMS negative ions: 501, 355 (-Rha), MALDI-TOF positive ion: 547 (Na^+ form of Na salt)
2	α-Rha*p*′: 101.2 (C-1), 4.75 (H-1), 17.0 (C-6), 1.35 (H-6). αβ-Glc*p*A: 92.5 < 96.4 ($C_{\alpha\beta}$-1′), 80.1 < 79.7 ($C_{\alpha\beta}$-4), 3.75 (H-5), 175.9 (C-6) ESIMS negative ions: 339, 163 (Rha)
3	β-Gal*p*′: 103.67 < 103.63 (C-1), 61.82 < 61.50 (C-6). αβ-Gal*p*: 92.8 < 96.9 ($C_{\alpha\beta}$-1), 69.83 < 69.70 ($C_{\alpha\beta}$-6) No MS data
4	α-Ara*f*″: 109.4 (C-1), 5.26 (H-1), 61.6 (C-5, DEPT inverted). β-Gal*p*′: 103.40 < 103.44 (C-1), 75.4 (C-4). αβ-Gal*p*: 92.7 < 96.8 ($C_{\alpha\beta}$-1), 61.6 (DEPT inverted), 69.65 < 69.44 ($C_{\alpha\beta}$-6; DEPT inverted) ESIMS positive Na^+ ions: 497, 365 (-Ara), negative ions: 473, 311 (-Gal), 179 [-(Gal + Ara)], 149 (-Gal_2)

[a] Shifts in δ (ppm).

an apparent H-3′ signal of β-Glc*p*A units appeared at δ 3.59, but HMQC showed that it correlated with two ^{13}C resonances at δ 74.8 and 79.6, due to the overlapping of H-3′ and H-4′ signals. Further spectral examination did not resolve this problem. The ROESY spectrum showed correlation of H-1″ of α-Rha*p* (δ 4.75) with the same signal at δ 3.59, and the HMBC spectrum indicated coupling with the ^{13}C signal at δ 79.6, along with internal coupling with resonances at δ 69.4 (C-5″) and 70.5 (C-2″ or C-3″). Thus, to distinguish between a (1→3)- and a (1→4)-linkage, a controlled Smith degradation was carried out on the trisaccharide. HMQC examination of the product showed the absence of anomeric signals, consistent with complete oxidation as would only occur with a (1→4)-linkage. HMBC coupling of H-1′ of β-Glc*p*A (δ 4.52) was found with the C-6 of a 6-O-substituted Gal*p* reducing end (δ 69.7), but the ROESY experiment could not be interpreted, due to multiple correlations.

(B) Disaccharide **2** *with* R_{Lact} *0.55.*—The oligosaccharide ($[\alpha]_D - 14°$) gave acetates of rhamnitol > glucitol (GC–MS) following acid hydrolysis. Its ESIMS spectrum showed that it was a disaccharide with Rha and GlcA (Table 1), which was confirmed by its ^{13}C NMR spectrum with anomeric carbon resonances, typical of an α-Rha*p* non-reducing (δ 101.2) and αβ-Glc*p*A reducing ends (δ 92.5 < 96.4). No inverted DEPT signals of CH_2 were detected. Typical O-substituted α- and β-Glc*p*A signals appeared at δ 80.1 and 79.7, respectively, and since the β isomer gave rise to signals similar to those of **1**, the disaccharide can be assigned as α-Rha*p*-(1→4)-αβ-GlcA (**2**).

α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→6)-αβ-D-Gal*p*
1

α-L-Rha*p*-(1→4)-αβ-D-Glc*p*A
2

β-D-Gal*p*-(1→6)-αβ-D-Gal*p*
3

α-L-Ara*f*-(1→4)-β-D-Gal*p*-(1→6)-αβ-D-Gal*p*
4

(C) Disaccharide **3** *with* R_{Lact} *0.80.*—Acid hydrolysis gave rise to galactose only (GC–MS), and its ^{13}C NMR spectrum (Table 1) corresponded to that of a (1→6)-linked disaccharide (inverted DEPT C-6 signals at δ 61.50 > 61.82) with a β-glycosidic configuration (anomeric resonances at δ 103.63 > 103.67), data which demonstrate a β-Gal*p*-(1→6)-αβ-Gal structure (**3**).

(D) Trisaccharide **4** *with* R_{Lact} *0.85.*—The oligosaccharide ($[\alpha]_D - 17°$) gave, on hydrolysis, galactose and arabinose in a molar ratio of 2:1 (GC–MS), corresponding to a trisaccharide, which was confirmed by its ESIMS spectrum (Table 1). The anomeric region of its ^{13}C NMR spectrum contained a low-field signal at δ 109.4, typical of α-Ara*f* units, with others of internal β-Gal*p* and 6-O-substituted αβ-Gal*p* reducing units (Table 1). A (TOCSY + COSY)–HMQC examination showed α-Ara*f*, but not β-Gal*p* non-reducing end-units. The DEPT spectrum did not resolve whether the trisaccharide contained α-Ara*f*-(1→4)- or α-Ara*f*-(1→6)- units, due to possible overlap of C-4 and an inverted C-6′ signal. However, the 2D approach showed an H-4′ signal (δ 4.01), which correlated with that of C-4′ at δ 75.4, consistent with an O-substitution α-shift of + 6.4 ppm, when compared with δ 69.0 of C-4 of an unsubstituted β-Gal*p* residue [6]. Confirmation of the α-Ara*f*-(1→4)-β-Gal*p*-(1→6)-αβ-Gal structure (**4**) was obtained by methylation analyses of the reducing sugar and the alditol derived by $NaBH_4$ reduction, which each furnished 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol (GC–MS). The linkage between the two Gal*p* units was confirmed as (1→6), since the HMBC spectrum showed coupling between H-1′ (δ 4.48) and the O-substituted C-6 of the β-Gal*p* reducing end (δ 69.44).

(E) Acidic pentasaccharide **5** *with* R_{Lact} *0.23.*—On acid hydrolysis, the oligosaccharide ($[\alpha]_D - 18°$) gave rise mainly to alditol acetates of Rha, Ara, Gal, with a trace of Glc (GC–MS), which arose from GlcA. ESIMS showed a negative molecular ion peak at m/z 795, corresponding to a pentasaccharide with two Gal, and one each of Rha, GlcA, and Ara units. Its ^{13}C NMR spectrum also indicated that it was a pentasaccharide, and since it contained signals common to those of α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-αβ-Gal and α-

Table 2
NMR and MS data for free reducing oligosaccharides **5**, **6**, **8**, and **9** and derived oligosaccharide polyol **7**

Oligo-saccharide	Key NMR signals [a]; m/z values for molecular and daughter ions
5	α-Rha*p*: 101.2 (C-1), 4.78 (H-1), 16.9 (C-6), 1.26 (H-6). β-Glc*p*A: 103.38, (C-1); 4.53 (H-1), 79.6 (C-4), 176.4 (C-6). β-Gal*p*′: 103.60 < 103.57, 4.50 (C-1s and H-1), 70.53 < 70.62 (C- 6; DEPT inverted), 75.45 (C-4′). α-Ara*f*: 109.4 (C-1), 5.26 (H-1), 61.6 (C-5; DEPT inverted). αβ-Gal*p*: 92.9 < 96.8 ($C_{\alpha\beta}$-1), 69.65 < 69.44 ($C_{\alpha\beta}$-6; DEPT inverted) ESIMS negative ions: 795, 663 (-Ara), 649 (-Rha), 633 (-Gal), 517 [-(Ara + Rha)], 501 [-(Gal + Ara)], 487 [-(Gal + Rha)], 340 (Rha + GlcA), 311 [-(Gal + Rha + GlcA)], 132 (Ara)
6	β-Ara*p*: 100.5 (C-1), 5.00 (H-1), 63.7 (C-5; DEPT inverted). -(1 → 3)-α-Ara*f*-(1 → 3: 110.1 (C-1), 5.27 (H-1), 84.5 (C-3). α-Ara*f*-(1 → 4: 108.7 (C-1), 5.40 (H-1). β-Gal*p*′: 103.42 < 103.47 (C-1), 75.3 (C-4), 80.3 (C-3), 70.05 < 69.92 (C-6; DEPT inverted). αβ-Gal*p*: 92.8 < 96.9 ($C_{\alpha\beta}$-1), 69.62 < 69.60 ($C_{\alpha\beta}$-6; DEPT inverted). Two α-Ara*f*; (61.70, 61.80; DEPT inverted) MALDI-TOF positive ions: 761, 629 (-Ara), 497 ($-Ara_2$), each Na^+ form, 365 ($-Ara_3$, H^+ form)
7	α-Ara*f*″: 109.8 (C-1), 5.24 (H-1). β-Gal*p*′: 103.3 (C-1), 4.52, $J = 7.6$ Hz (H-1), 80.8 (C-3) ESIMS Na^+ ions: 379 ≫ 407
8	α-Rha*p*: 101.2 (C-1), 4.74 (H-1), 16.9 (C-6), 1.23 (H-6). β-Glc*p*A: 103.37 (C-1), 79.6 (C-4). β-Ara*p*: 100.6 (C-1), 5.00 (H-1). α-Ara*f*-(1 → 3-: 110.0 (C-1), 5.27 (H-1), 84.6 (C-3). α-Ara*f*-(1 → 4: 108.6 (C-1), 5.40 (H-1). β-Gal*p*′: 103.54 < 103.45 (C-1), 80.1 (C-3), 74.2 (C-4), 70.3 (C-6; DEPT inverted). αβ-Gal*p*: 92.85 < 96.9 (C_{ab}-1), 70.10 < 69.96 ($C_{\alpha\beta}$-6; DEPT inverted) MALDI-TOF positive ions: 1083 (Na^+) > 1105 (Na salt, Na^+), Na^+ ions with 937 (-Rha), 762.5 [-(Rha + GlcA)], 629.5 [-(Rha + GlcA + Ara)]
9	β-Ara*f*′: 99.5 < 99.3 (C-1s); 5.19 $J = 2.4$ Hz (H-1s). α-Ara*p* (greater than β-anomer): 97.1 (C-1); 4.57, $J = 6.9$ Hz (H-1), 66.6 (C-6; DEPT inverted), β-Ara*p*: 92.9 (C-1); 5.27, $J = 3.3$ Hz (H-1) ESIMS positive ions: 305.5 (Na^+), 283 (H^+), negative ions: 281, 149 (-Ara)

[a] Shifts in δ (ppm).

Ara*f*-(1 → 4)-β-Gal*p*-(1 → 6)-αβ-Gal (Table 2), a composite structure was suggested. An overall structure **5** was confirmed by the m/z values of the negative daughter ions, which were formed via cleavage starting both from the reducing and two non-reducing ends (Table 2). The COSY–TOCSY and HMQC spectra indicated non-reducing end-units of α-Ara*f* and α-Rha*p*.

The HMBC spectrum of the pentasaccharide showed that the H-1 signal of the α-Rha*p* units (δ 4.78) was coupled with that of an O-substituted C-4 residue of β-Glc*p*A at δ 79.6, while that of β-Gal*p* (δ 4.50) coupled with the 6-O-substituted resonance of β-Gal*p* reducing end-units at δ 69.4. A ROESY spectrum showed correlation of the α-Ara*f* H-1 signal at δ 5.26 with that at δ 4.03 (H-4), with HMQC coupling to a resonance at δ 75.45 (O-substituted C-4 of β-Gal*p*).

α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-αβ-D-Gal*p*
4
↑
1
α-L-Ara*f*
5

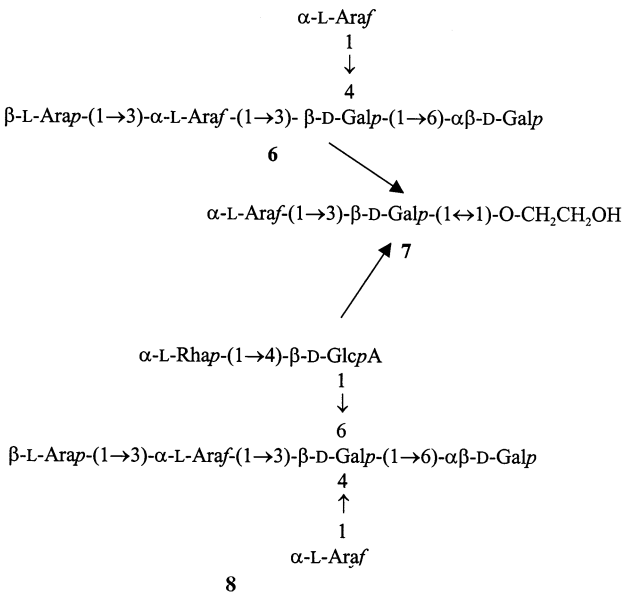
All monosaccharide units of **5** were oxidized during a controlled Smith oxidation, as shown by HMQC examination of the product, which did not give a signal in the H-1, C-1 anomeric region.

(F) Pentasaccharide **6** *with* R_{Lact} *0.70.*—The oligosaccharide ($[\alpha]_D + 4°$) gave on acid hydrolysis galactose and arabinose in a 2:3 ratio (GC–MS). A pentasaccharide structure containing these monosaccharides was confirmed by its MALDI-TOF spectrum, which had a molecular ion at m/z 761 (Na^+), PSD giving daughter ions that indicated removal of one, two, and three Ara units (Table 2). Its ^{13}C NMR spectrum also showed a pentasaccharide structure with two α-Ara*f*, a β-Ara*p*, and a β-Gal*p* linked (1 → 6) to an αβ-Gal*p* reducing end unit. (TOCSY + COSY)–HMQC examination showed non-reducing end-units of β-Ara*p* and α-Ara*f*. The other α-Ara*f* unit gave rise to a C-3 signal at δ 84.5, which showed 3-O-substitution with an O-substitution α-shift of + 7.2 ppm. The signal at δ 75.3 was from C-4 of internal β-Gal*p* O-substituted by α-Ara*f* units, assigned by comparison with

arabinosyl galactobiose (**4**), whose related structure gave rise to a signal at δ 75.45.

A controlled Smith degradation of **6** gave **7**, whose COSY and HMQC spectra showed a structure with α-Ara*f*-(1→3)-β-Gal*p*- linked to ethylene glycol or glycerol (Table 2). The (1→3)-linkage was confirmed by ROESY–HMQC analysis. Positive-ion mode ESIMS gave Na^+ ions at m/z 379 ≫ 409 and no daughter ions (no response occurred in the negative mode), showing much more α-Ara*f*-(1→3)-β-Gal*p*-(1↔1)-ethylene glycol than the -(1↔1)-glycerol derivative, formed via an undissociated 2-formate ester (Scheme 1).

ROESY examination of the pentasaccharide showed correlation of the H-1 signal of non-reducing end β-Ara*p* (δ 5.00) with H-3 of the adjacent α-Ara*f* unit (δ 3.95). HMQC, in turn, indicated correlation with C-3, whose low field of δ 84.6, when compared with unsubstituted C-3 (δ 77.3), indicated 3-O-substitution. The H-1 signal of this α-Ara*f* unit (δ 5.27) correlated by ROESY with the H-3 signal of β-Gal*p* (δ 3.85), corresponding to the δ 80.1 signal of (C-3 of 3-O-substituted β-Gal*p*). As confirmation, H-1 of the other non-reducing α-Ara*f* unit at δ 5.40 showed ROESY correlation with a resonance at δ 4.16 (HMQC: δ 75.3 of 4-O-substituted β-Gal*p*). The HMBC spectrum showed H-1 (δ 5.40) coupling with δ 75.3 (4-O-substituted β-Gal*p*).



Scheme 1.

(G) Acidic heptasaccharide **8** *with* R_{Lact} *0.20.*—The oligosaccharide ($[\alpha]_D - 10°$) gave mainly Rha, Ara, Gal and a trace of Glc (GC–MS) on acid hydrolysis, the latter arising via derivatization from glucuronic acid. A uronic acid determination accounted for a content of 11%. The anomeric region of its ^{13}C NMR spectrum indicated a heptasaccharide structure, as did MALDI-TOF in the positive mode, whose molecular ions at m/z 1083 (Na^+) > 1105 (Na^+ of Na salt), and which also showed that the monosaccharide components were three arabinoses, two galactoses, and one each of glucuronic acid and rhamnose. PSD daughter ions were only removed sequentially from the non-reducing end (Table 2). This observation, together with comparison of the NMR data with those of **1** and **6**, suggested the net structure **8**.

2D NMR confirmation of this structure was effected as follows: (TOCSY + COSY)–HMQC examination showed non-reducing ends of α-Rha*p*, β-Ara*p*, and α-Ara*f*. As with structure **6**, the other Ara*f* unit was 3-O-substituted, since its C-3 signal was at δ 84.6.

ROESY examination showed correlation between H-1 of one of the terminal α-Ara*f* units (δ 5.40) and H-4 (δ 4.16) of the internal β-Gal*p* units (HMQC: C-4, δ 74.2), H-1 of the 3-O-substituted α-Ara*f* units (δ 5.27) and H-3 (δ 3.86) of the same β-Gal*p* units (HMQC: C-3, δ 80.1), H-1 of α-Rha*p* units (δ 4.74) and H-3 (δ 3.58) of those of β-Glc*p*A (HMQC: δ 79.6), and H-1 of β-Ara*p* units (δ 5.00) with H-4 (δ 3.93) of the internal α-Ara*f* units (HMQC: C-3, δ 84.6). With the exception of the last result, confirmation was obtained by HMBC (H-1 → ^{13}C data), which showed coupling between δ 5.27 and 80.1, δ 5.40 and 74.2, and δ 4.74 and 79.6.

A controlled Smith degradation gave rise to α-Ara*f*-(1→3)-β-Gal*p*-(1↔1)-ethylene glycol (**7**) and a trace of its -(1↔1)-glycerol derivative, as shown by ESIMS peaks at m/z 379 ≫ 409 (Na^+ forms).

(H) Disaccharide **9** *with* R_{Lact} *2.50.*—The oligosaccharide gave arabinose on hydrolysis and a MALDI-TOF measurement showed an arabinobiose structure, by virtue of ion peaks at m/z 305.5 and 283, corresponding to Ara-

β-L-Ara*f*-(1→3)-L-Ara
9

Ara-Na^+ and Ara-Ara-H^+, respectively. This was confirmed by its ESIMS profile, which showed peaks with m/z 281 and 149 (-Ara) in the negative-ion mode. Its strongly positive $[\alpha]_D + 130°$ indicated predominant β-L-Ara*p* and/or β-L-Ara*f* components.

It gave ^{13}C and ^1H NMR reducing-end signals at δ 97.1 (C-1) and δ 4.57, $J_{1,2}$ 6.9 Hz (H-1) of the α isomer > δ 92.9 (C-1) and δ 5.27, $J_{1,2}$ 3.3 Hz (H-1) of the β isomer. Non-reducing end C-1′ signals were present at δ 99.3 > 99.5 (only one correlated HMQC H-1′ signal at δ 5.19, $J_{1,2}$ 2.4 Hz). COSY–TOCSY–HMQC showed a non-reducing end of β-Ara*f* units and a H-3 signal of the α-Ara*p* reducing end at δ 3.77, which correlated with an O-substituted C-3 signal at δ 79.1. Only intraunit correlations were found in ROESY and HMBC spectra. Consequently, the structure of the disaccharide was confirmed by methylation analysis, which gave rise to acetates of 2,4-Me_2- and 2,3,5-Me_3-arabinitol (GC–MS), arising from the β-Ara*f*-(1→3)-αβ-Ara*p* (**9**) structure.

This disaccharide was also formed by partial hydrolysis of the angico polysaccharide. In view of the presence of Ara*f* and not Ara*p* units O-substituted at C-3 in the original polysaccharide [3], the free reducing disaccharide should be associated with β-Ara*f*-(1→3)-α-Ara*f*- structures.

3. Discussion

In terms of NMR analysis of the oligosaccharides, their TOCSY, COSY, and HMQC spectra were examined, and the ^{13}C resonances of each unit were compared with those of the structurally analogous methyl glycoside [6]. This showed whether the monosaccharide unit was non-reducing terminal, O-substituted and sometimes, in which position. DEPT spectra were used to assign C-5 and C-6 signals of pentosyl and hexosyl units, respectively. The position of the glycosidic linkages was principally determined by ROESY and HMBC correlation, which was successful in most cases. HMBC spectroscopy also proved useful to detect C-6 of the glucuronic acid residue in **2**, when only a small quantity was available, by correlation with H-5. The overall NMR approach was applicable in most of the oligosaccharide characterizations, but occasionally, signal overlap occurred in the COSY and TOCSY spectra, which then became apparent on HMQC examination. This ambiguity was encountered with **1**, which required a controlled Smith degradation. Methylation analyses were used for final confirmation of structures of **4** and **9**. The field-desorption MS data invariably agreed with those of NMR spectroscopy.

The structures of lower-molecular-weight oligosaccharides provided additional information on the side-chain sequences in the angico branco polysaccharide. As with the acidic galactan of cashew nut tree gum [4,5], no oligosaccharide had a 3-O-substituted Gal*p* unit, present in the (1→3)-linked β-Gal*p* main-chain of the polysaccharides, but the reducing-end units of αβ-Gal*p* would correspond in most instances, to those present in the main chain. Also to be considered in structural comparisons with the polysaccharides are:

α-Ara*p*-(1→6)-αβ-Gal
10

β-Gal*p*-(1→6)-β-Gal*p*-(1→6)-αβ-Gal
11

β-Glc*p*A-(1→6)-β-Gal*p*-(1→6)-αβ-Gal
12

β-Glc*p*A-(1→6)-αβ-Gal
13

oligosaccharides **10**, **11**, **12**, and **13** (Table 3), previously obtained via partial acid hydrolysis of the polysaccharide [3]. Structure **10** accounts for the α-Ara*p* component, the last of the four arabinosyl isomers known to be a component of the polysaccharide by NMR examination [3], but which lacked representation in the free oligosaccharides.

Each oligosaccharide, isolated from the angico branco gum, showed spectral evidence that supports many structural similarities; therefore the structures of lower oligosaccharides could be used as an aid in characterization of those of higher molecular weight, and finally the polysaccharide. In support of these

Table 3
NMR data of oligosaccharides formed on partial acid hydrolysis of angico branco polysaccharide

Oligosaccharide	Key NMR signals [a]
10	α-Ara*p*′: 103.0 (C-1), 4.57 (H-1). αβ-Gal: 92.6 < 96.7 ($C_{\alpha\beta}$-1), 69.8 < 69.6 ($C_{\alpha\beta}$-6)
11	β-Gal*p*″: 103.7 (C-1). β-Gal*p*′: 103.6 < 103.4 (C-1), 69.5 (C-6). αβ-Gal*p*: 92.6 < 96.7 ($C_{\alpha\beta}$-1), 69.9 < 69.6 ($C_{\alpha\beta}$-6; DEPT inverted)
12	β-Glc*p*A″: 103.0 (C-1), 176.5 (C-6). β-Gal*p*′: 103.6 < 103.4 (C-1), 69.5 (C-6; DEPT inverted). αβ-Gal*p*: 92.3 < 96.4 ($C_{\alpha\beta}$-1), 69.9 < 69.6 ($C_{\alpha\beta}$-6; DEPT inverted)
13	β-Glc*p*A′: 102.69 and 102.64 (C-1s), 176.1 (C-6). αβ-Gal*p*: 92.6 < 96.7 ($C_{\alpha\beta}$-1), 69.64 < 69.54 ($C_{\alpha\beta}$-6; DEPT inverted)

[a] Shifts in δ (ppm).

similarities, the possible mechanisms of oligosaccharide formation are (i) via autohydrolysis of the polysaccharide in its natural state, although this was previously eliminated [3], since the acidic conditions were not sufficiently strong to promote glycosidic cleavage, even of arabinofuranosyl linkages; (ii) by the action of *endo*- and *exo*-hydrolases with emphasis on cleavage of the main chain; or (iii) formation as byproducts of biosynthesis of the polysaccharide. According to the definition of Kandler and Hopf [7], oligosaccharides formed via the first two mechanisms can be considered as secondary. Those formed as biosynthetic byproducts would be primary.

The oligosaccharides can be used to assign H-1 shifts of the original polysaccharide [3], namely those at δ 109.9 (β-Ara*p*-(1→3)-α-Ara*f*-(1→3)-β-Gal*p*-), 108.6 (α-Ara*f* linked (1→4) to β-Gal*p*, also 3-O-substituted with α-Ara*f* and 6-O-substituted with β-Glc*p*A), 108.2 (α-Ara*f* linked (1→4) to β-Gal*p*, also 3-O-substituted with α-Ara*f*), 103.2, 103.4, 103.0 (β-Gal*p*, α-Ara*p*, and β-Glc*p*A), 101.5 (α-Rha*p*-(1→4)-β-Glc*p*A-), and 100.8 (β-Ara*p*-(1→3)-α-Ara*f*-), 99.3, (β-Ara*f*-(1→3)-Ara-), with the exception of those at δ 109.5, 99.1, 98.1, and 97.0.

4. Experimental

Collection of gum exudate.—Samples were collected from trees growing in Curitiba, State of Paraná, Brazil, giving preference to those least contaminated with bark, leaves and other contaminants.

Isolation of oligosaccharides from the gum.—Following removal of debris, the gum, as previously described [3], was dissolved in water, the polysaccharide was precipitated with 10 vols of a mixture of 3:2 MeOH–EtOH (v/v), and the low-molecular-weight carbohydrates were recovered from the supernatant. The product was applied to a column of activated 1:1 charcoal powder–diatomaceous earth (w/w), which was eluted with water to give the monosaccharides. Passage of up to 40% aq EtOH gave a mixture of oligosaccharides (yield 0.9%), which on PC in 1:1:1 *n*-BuOH–EtOH–H_2O: v/v, gave spots with R_{Lact} 2.50, 1.10, 0.85, 0.80, 0.70, 0.56, 0.50, 0.38, 0.23, and 0.20 (R_f values compared with that of lactose), that with R_{Lact} 0.55 being the most abundant (it was found to be composed of an acidic and a later unidentified component). Those with R_{Lact} 2.50, 0.85, 0.80, 0.70, 0.56, 0.38, 0.23, and 0.20 were isolated using Whatman 3MM filter paper in the same solvent system and characterized.

Uronic acid content of oligosaccharide.—This was determined by the *m*-hydroxybiphenyl method [8].

Determination of monosaccharide ratios in oligosaccharides.—Each sample (ca. 1 mg) was hydrolyzed in 2 M TFA for 8 h at 100 °C, and the product was successively reduced with NaB^2H_4 and acetylated with Ac_2O–pyridine. The product was examined by GC–MS using a capillary column of DB-225 (30 m × 0.25 cm i.d.), programmed from 50 °C (1 min) at 40 °C/min to 220 °C (constant temperature).

Methylation analysis.—This was carried out using the method of Ciacunu and Kerek [9]. Per-O-methylated product was cleaved with refluxing 3% MeOH for 3 h, followed by 1 M H_2SO_4 at 100 °C for 18 h. The resulting mixture of partially O-methylated aldoses was reduced, and then acetylated, to give O-methyl alditol acetates, which were examined by GC–MS, as described above.

Controlled Smith degradation of oligosaccharides.—The neutral oligosaccharide sample (8 mg) in water (0.5 mL) containing $NaIO_4$ (30 mg) was left for 3 days. The solution was

successively deionized, reduced with $NaBH_4$, partly hydrolyzed with TFA at pH 2.0 for 30 min, and evaporated to give the product. Acidic oligosaccharide was treated with H_5IO_6, which was removed, along with resulting HIO_3, with $BaCO_3$, and the filtrate was processed as above.

Determination of oligosaccharide structure by NMR spectroscopy.—A preliminary examination was carried out by ^{13}C and ^{1}H NMR spectroscopy in D_2O at 30 °C using a Bruker 400 MHz DRX spectrometer (shifts expressed as δ ppm relative to external Me_4Si, $\delta = 0$). COSY, TOCSY, HMQC, HMBC, ROESY, and DEPT spectra were obtained, according to the Bruker manual, and the complete results presented below.

Trisaccharide **1** *(*R_{Lact} *0.38).*—α-Rha*p* non-reducing end (δ values of ^{1}H and ^{13}C, respectively; N = nucleus): N-1 (4.75, 101.2), N-2 (3.97, 70.7), N-3 (3.78, 70.5), N-4 (3.47, 72.3), N-5 (4.05, 69.4), N-6 (1.35, 16.9). Internal β-Glc*p*A: N-1 (4.52, 102.88/102.85), N-2 (3.38, 73.8), N-3 (3.59, 74.8), N-4 (3.59, 79.6), N-5 (3.75, 76.6), C-6 (175.6). α-Gal*p* reducing end: N-1 (5.27, 92.8), N-2 (3.82, 68.8), N-3 (3.88, 69.22), N-4 (4.05, 69.64), C-6 (69.82). β-Gal*p* reducing end: N-1 (4.55, 96.9), N-2 (3.52, 72.4), N-3 (3.67, 73.1), N-4 (3.98, 69.22), C-6 (69.73).

Disaccharide **2** *(*R_{Lact} *0.55).*—α-Rha*p* non-reducing end: N-1 (4.75, 101.2), N-2 (3.96, 70.8), N-3 (3.75, 70.6), N-4 (3.45, 72.45), N-5 (4.02, 69.4), N-6 (1.35, 17.0). β-Glc*p*A reducing end: N-1 (4.63, 96.4), N-2 (3.32, 74.88), N-3 (3.58, 74.81), N-4 (3.58, 79.7), N-5 (3.75, 76.95), C-6 (175.9). α-Glc*p*A reducing end: N-1 (5.25, 92.5), N-2 (3.62, 72.1), N-3 (3.75, 71.9), N-4 (3.58, 80.1), N-5 (4.13, 72.45), C-6 (175.9).

Disaccharide **3** *(*R_{Lact} *0.80): NMR data.*—β-Gal*p* non-reducing end: C-1 (103.63 > 103.67), C-6 (61.50 > 61.82. αβ-Gal*p* reducing end: C-1 (96.9 > 92.8), C-6 (69.70 > 69.83).

Trisaccharide **4** *(*R_{Lact} *0.85).*—α-Ara*f* non-reducing end: N-1 (5.26, 109.4), N-2 (4.20, 81.7), N-3 (3.93, 76.95), N-4 (4.07, 84.5), N-5 (3.82, 61.6). Internal β-Gal*p*: N-1 (4.48, 103.44 > 103.49), N-2 (3.58, 71.4), N-3 (3.78, 73.45), N-4 (4.01, 75.4), C-6 (61.7). β-Gal*p* reducing end: N-1 (4.59, 96.8), N-2 (3.49, 72.2), N-3 (3.65, 73.1), N-4 (3.96, 69.2), C-6 (69.44). α-Gal*p* reducing end: N-1 (5.27, 92.8), N-2 (3.80, 68.7), N-3 (4.02, 69.4), N-4 (3.86, 69.2), C-6 (69.65).

Pentasaccharide **5** *(*R_{Lact} *0.23).*—α-Ara*f* non-reducing end: N-1 (5.26, 109.4), N-2 (4.22, 81.85), N-3 (3.98, 77.0), N-4 (4.12, 84.5), C-5 (61.6). α-Rha*p* non-reducing end: N-1 (4.78, 101.2), N-2 (3.92, 70.78), N-3 (3.75, 70.53), N-4 (3.45, 72.4), N-5 (3.98, 69.40), N-6 (1.26, 16.9). 4-O-Substituted β-Glc*p*A: N-1 (4.53, 103.38), N-2 (3.38, 73.8), N-3 (3.60, 74.8), N-4, (3.60, 79.6), C-6 (176.4). 4,6-Di-O-substituted β-Gal*p*: N-1 (4.50, 103.57 > 103.60), N-2 (3.72, 71.3), N-3 (3.83, 73.4), N-4 (4.03, 75.45), C-6 (70.53 < 70.62). β-Gal*p* reducing end: N-1 (4.60, 96.8), N-2 (3.55, 72.3), N-3 (3.72, 73.1), N-4 (3.95, 69.40), C-6 (69.44). α-Gal*p* reducing end: N-1 (5.28, 92.9), N-2 (3.81, 69.06), N-3 (3.88, 69.29), N-4 (4.05, 69.81), C-6 (69.65).

Pentasaccharide **6** *(*R_{Lact} *0.70).*—α-Ara*f* non-reducing end: N-1 (5.40, 108.7), N-2 (4.16, 81.8), N-3 (3.93, 77.3), N-4 (4.08, 84.5), N-5 (3.88, 61.8). β-Ara*p* non-reducing end: N-1 (5.00, 100.5), N-2 (3.82, 68.8), N-3 (3.86, 69.04), N-4 (4.00, 69.4), N-5 (3.65, 63.7). 3-O-substituted Ara*f*: N-1 5.27 (110.1), N-2 (4.35, 80.7), N-3 (3.95, 84.5), N-4 (4.27, 83.3), N-5 (3.78, 61.8). 3,4-di-O-substituted β-Galp: N-1 (4.53, 103.5), N-2 (3.72, 71.1), N-3 (3.85, 80.3), N-4 (4.16, 75.3 or 81.8), C-6 (61.7). β-Gal*p* reducing end: N-1 (4.57, 96.9), N-2 (3.50, 72.3), N-3 (3.65, 73.1), N-4 (3.96, 69.27), C-6 (69.60). α-Gal*p* reducing end: N-1 (5.27, 92.8), N-2 (3.81, 68.76), N-3 (3.85 (69.04), N-4 4.02 (69.76), C-6 (69.62).

Controlled Smith degradation product of **6**.—The product **7** gave rise to signals of: α-Ara*f*: N-1 (δ 5.24, 109.8), N-2 (δ 4.21, 81.9), N-3 (δ 3.95, 77.1), C-4 (84.4). β-Gal*p*: N-1 (δ 4.52, *J* 7.6 Hz; 103.3), N-2 (δ 4.10, 69.1), N-3 (3.70, 80.8).

Heptasaccharide **8** *(*R_{Lact} *0.20).*—α-Ara*f* non-reducing end: N-1 (5.40, 108.6), N-2 (4.16, 81.9), N-3 (3.90, 77.3), C-5 (61.7). β-Ara*p* non-reducing end: N-1 (5.00, 100.6), N-2 (3.82, 68.78), N-3 (3.86, 69.03), N-4 (3.98, 69.28), C-5 (63.7). α-Rha*p* non-reducing end: N-1 (4.74, 101.2), N-2 (3.92, 70.8), N-3 (3.75, 70.5), N-4 (3.42, 72.4), N-5 (4.00, 69.41), N-5 (1.23, 16.9). 3-O-Substituted α-Ara*f*: N-1

(5.27, 110.0), N-2 (4.33, 80.7), N-3 (3.93, 84.6), C-5 (61.7). 4-O-Substituted β-Glc*p*A: N-1 (4.50, 103.37), N-2 (3.38, 73.75), N-3 93.58, 74.8), N-4 (3.58, 79.6), C-6 (175.6). 3,4,6-Tri-O-substituted β-Gal*p*: N-1 (4.50, 103.45 > 103.54), N-2 (3.72, 71.1), N-3 (3.86, 80.1), N-4 (4.16, 74.3 or 81.9), N-6 (4.02, 70.3). β-Gal*p* reducing end: N-1 4.60 (96.9), N-2 (3.48, 72.3), N-3 (3.65, 73.1), N-4 (3.95, 69.28), N-6 (4.06, 69.96). α-Gal*p* reducing end: N-1 (5.26, 92.85), N-2 (3.79, 68.78), N-3 (3.85, 69.03), N-4 (4.02, 69.80), C-6 (70.10).

A controlled Smith degradation gave rise to **7**: NMR data, was identical with that described above for the product obtained from **6**.

Disaccharide **9** *with* R_{Lact} *2.50.*—β-Ara*f* non-reducing end: N-1 (5.19, $J = 2.4$ Hz; 99.3 > 99.5), N-2 (4.19, 76.8), N-3 (4.22, 73.6 and 73.8), N-4 (3.92, 82.2). α-Ara*p* reducing end: N-1 (4.57, $J = 6.9$ Hz; 97.1), N-2 (3.60, 70.8), N-3 (3.77, 79.1), N-4 (4.11, 66.1), N-5 (3.96, 66.1). β-Ara*p* reducing end: N-1 (5.27, $J = 3.3$ Hz; 92.9), N-2 (3.92, 67.4), N-3 (3.99, 75.8).

Field-desorption molecular-weight determinations on oligosaccharides.—M/z values of molecular and daughter ions were measured using MALDI-TOF (with PSD) and ESIMS.

MALDI-TOF and subsequent PSD analyses were carried out in the positive ionization mode. Approximately 2 mg of sample was dissolved in water (1 mL), and a further tenfold dilution was carried out. Samples were run in matrices of 2,5-dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid (Alpha C), and the better spectra were selected. Spectra were compared with those of blanks. Fragments containing uronic acid could sometimes not be detected.

ESIMS examination was carried out in the negative-ion mode for examination of oligosaccharides containing uronic acid. For neutral oligosaccharides, the positive-ion mode gave rise principally to the molecular Na^+ ion, with small daughter ions. The negative-ion mode gave more complex spectra, with more emphasis on daughter ions. For examination of oligosaccharide polyols, the positive-ion mode was employed. These analyses were carried out using a Micromass Quattro LC, with or without prior fractionation by HPLC on a Shimadzu LC-10AD apparatus using a C_{18}, amino-form column with eluant, 3:1 AcCN–H_2O (v/v) incorporating a refractive index detector. The samples (ca. 100 μg) were applied in the solvent systems (1:1 AcCN–water; 2 mL, v/v for direct application, and 3:1 v/v for HPLC).

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