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New structural features of the polysaccharide from gum ghatti (*Anogeissus latifola*)

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

Methylation and ^{13}C NMR analyses were carried out on the high-arabinose, acidic heteropolysaccharide of gum ghatti and the products obtained on three successive, controlled Smith degradations. The side chains contained mainly 2-*O*- and 3-*O*-substituted Ara*f* units. Of these the second degradation eliminated remaining α-Ara*f* units, and their β anomers became evident. The proportion of Gal*p* units gradually increased in the form of nonreducing end- and Gal*p* units, although 3,6-di-*O*- and 3,4,6-tri-*O*-substituted Gal*p* units diminished. After three degradations groups with consecutive 3-*O*-substituted β-Gal*p* units were formed. The proportion of periodate-resistant 3-*O*- and 2,3-di-*O*-substituted Man*p* units was maintained. As a guide to side-chain structures in the polysaccharide, seven of the 10 free reducing oligosaccharide fractions (PC) present in the gum were isolated and examined (NMR, ESIMS, and sometimes methylation analysis). Characterized are α-Ara*f*-(1→2)-Ara and three Ara-containing oligosaccharide fractions containing 2-*O*- and 3-*O*-substituted units. These gave respectively, ESIMS molecular ions arising from Ara_2, β-Ara*f* oligosaccharides with four units, β-Ara*f* oligosaccharides with seven units, and Hex_2-Ara_4. α-Rha*p*-(1→4)-GlcA, α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-Gal, and α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-β-Gal*p*-(1→6)-Gal represented other side chains.

Keywords: Gum ghatti; Polysaccharide; Free, reducing oligosaccharides; Structure

1. Introduction

The polysaccharide of gum ghatti has been extensively studied by Aspinall and co-workers from 1955 to 1965 and found to have an extremely complex structure,[1–5] with Ara, Gal, Man, Xyl, and GlcA in a 48:29:10:5:10 molar ratio and $< 1\%$ of rhamnose,[1] later found to occur as nonreducing end-groups.[2] Many structures were characterized, including **1**–**4**, but still unresolved was the nature of the substituent(s) at O-4 of the -Glc*p*A- units.

We now find that 6% of rhamnose in the polysaccharide that might contribute substantially to its structure as an α-Rha*p*-(1→4)-β-Glc*p*A- group, which is common in plant gum polysaccharides.[6–9] The structures

β-Gal*p*-(1→6)-[β-Gal*p*-(1→6)]$_{0\text{-}3}$-Ara*p*-

1

β-Gal*p*-[(1→6)-β-Gal*p*]$_{0\text{-}4}$-(1→6)-Gal*p*-

2

R-(1→3)-Gal*p*-(1→6)-Gal*p*-(1→6)-Gal*p*-(1→3)-Ara*p*-
(3 ↓ ; 4 ↑)

3

→4)-Glc*p*A-(1→6)-Gal*p*-(1→
(3 ↑)

4

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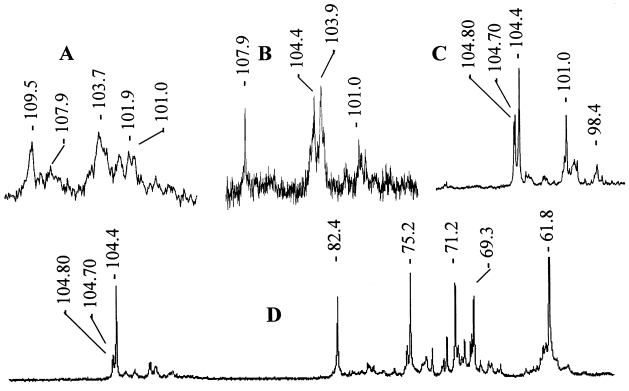
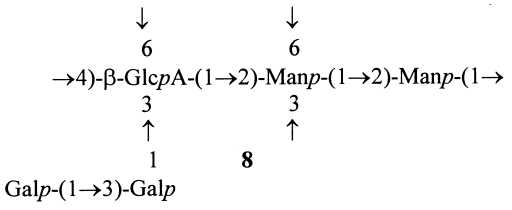


Fig. 1. C-1 portions of ^{13}C NMR spectra of the polysaccharide from gum ghatti as a gel in Me_2SO-d_6 (A) and products (D_2O solutions) obtained after one (B), two (C), and three controlled Smith degradations (D, entire spectrum).

present in the gum polysaccharide and its accompanying free, reducing oligosaccharides were therefore investigated. The latter should indicate other sequential polysaccharide structures which contain acid-labile Ara*f* units.

2. Results

The polysaccharide of gum ghatti (GC–MS of derived alditol acetates: *m*-hydroxydiphenyl method) contained Ara, Gal, Man, GlcA, and Rha in a molar ratio of 61:39:6:10:6, although previously found Xyl units,[1] were not detected. The presence of Rha units agreed with the presence of a substantial CH_3-6 signal at δ 16.9 in its ^{13}C NMR spectrum, obtained from a gel in Me_2SO-d_6. Four α-Ara*f* signals were present, the largest two being at δ 109.6 > 107.9 (Fig. 1(A)). Methylation analysis data (GC–MS of acetylated, partially *O*-methylated alditols-1-^2H), as indicated in Table 1, show nonreducing end-units of Rhap (4%), nonreducing end- (27%), 2-*O*- (12%), and 3-*O*-substituted Ara*f* units (10%), and 3-*O*- (10%), and 3,6-di-*O*- (12%), and 3,4,6-tri-*O*-substituted units of Gal*p* (7%). Man*p* was present as 3-*O*- (3%), and 2,3-di-*O*-substituted units (3%), in agreement with structures shown by Aspinall and coworkers, although 2,3,6-tri-*O*-substituted Man*p* units, as in structure **8** and attributed to the main chain,[3] could not be detected, which indicates a lack of 6-*O*-substitution.



Successive, controlled Smith degradations were carried out on the polysaccharide, and as expected, the first one eliminated the Rha*p* units forming a product (21% yield) containing Ara, Gal, Man, and GlcA in a 19:55:8:18 molar ratio. Polysaccharides obtained after two (18% yield) and three degradations (43% yield) contained Man, Gal, Ara, and GlcA in molar ratios of 9:68:20:3 and 7:68:21:2, respectively, the GlcA units being almost eliminated on the second degradation. Most of the Ara units are thus located in the periphery of the polymer, being gradually removed in contrast with internal Man and Gal units. Methylation analysis of the three Smith degradation products (Table 1) showed nonreducing end-, 2-*O*-, and 3-*O*-substituted Ara*f* units to be maintained and that Man*p* and 3-*O*-substituted Gal*p* units also showed resistance to oxidation. Significant features were the appearance of significant proportions of 6-*O*-substituted Gal*p* units (15%) after the first degradation and their nonreducing end-units (17%) after the third. The ^{13}C NMR spectrum (solvent: D_2O) of the product after one controlled Smith degradation contained one α-Ara*f* signal at δ 107.9 (Fig. 1(B)), whereas after two (Fig. 1(C)) and three degradations (Fig. 1(D)), no α-Ara*f* signals were detected. The presence of nonreducing end- and 2-*O*- and 3-*O*-substituted Ara*f* units after two and three degradations (Table 1) and the absence of low-field C-1 signals (Fig. 1(A) and (B)), show that they are β-linked, a feature also found in the heteropolysaccharide of the arabinogalactan of gum tragacanth.[10]

The free, reducing oligosaccharides, also present in plant gums, have been recently utilized to characterize sequences of acid-labile, side-chains containing Rha*p* and Ara*f* units in the accompanying polysaccharide.[6–8,10] These structural similarities would be expected independent of whether the oligosaccharides are produced via enzymatic degradation or autohydrolysis of the polysaccharide in situ, or are byproducts of its biosynthesis. This approach is now applied to gum ghatti.

Accordingly, an aqueous solution of the gum was added to excess ethanol, and the resulting precipitate was removed. The free, reducing carbohydrates in the filtrate, using allitol as internal standard, were reduced with sodium borohydride, and the product was acetylated to give a mixture of alditol acetates. GC–MS examination showed monosaccharides corresponding to Glc (0.020%), Gal (0.025%), Man (0.010%), Ara (0.203%), Rib (0.002%), and Rha (0.001%), which probably also correspond to side-chain components. In a larger scale experiment, the ethanol supernatant was applied to a charcoal column, which was eluted with water to remove *myo*-inositol and the monosaccharides, some of which overlapped with Ara*f*-containing oligosaccharides. This was followed with 40% aqueous

Table 1
Methylation analyses of: (1) polysaccharide (P), (2) polysaccharide formed by one controlled Smith degradation of P (P-SM × 1), (3) two degradations (P-SM × 2), (4) three degradations (P-SM × 3), (5) oligosaccharide fractions centered at R_{Lact} 0.99, (6) R_{Lact} 2.40, and (7) R_{Lact} 2.70, and (8) oligosaccharide with R_{Lact} 3.00

Acetylated alditol acetate (R_t) [a]	P (%)	P-SM × 1 (%)	P-SM × 2 (%)	P-SM × 3 (%)	R_{Lact} 0.99 (%)	R_{Lact} 2.40 (%)	R_{Lact} 2.70 (%)	R_{Lact} 3.00 (%)
2,3,4-Me_3-Rha (0.78) [b]	4	–	–	–	–	–	–	–
2,3,5-Me_3-Ara (0.78) [b]	27	12	21	16	19	20	36	50
3,5-Me_2-Ara (0.93)	12	9	12	8	23	62	24	26
2,5-Me_2-Ara (0.96)	10	12	8	7	13	9	11	–
2,3,4,6-Me_4-Man (0.98)	1	–	–	–	9	–	–	–
2,3-Me_2-Ara (1.03)	5	–	–	–	–	–	–	–
2,4-Me_2-Ara (1.045) [c]	–	–	–	–	–	4	13	–
3,4-Me_2-Ara (1.045) [c]	–	–	–	–	–	5	16	24
2,3,4,6-Me_4-Gal (1.05)	2	8	7	10	23	–	–	–
2,4,6-Me_3-Man (1.27)	3	5	8	5	3	–	–	–
2,4,6-Me_3-Gal (1.33)	10	18	26	32	–	–	–	–
2,3,6-Me_3-Gal (1.37)	–	–	–	–	5	–	–	–
2,3,4-Me_3-Gal (1.55)	3	15	3	2	5	–	–	–
4,6-Me_2-Man (1.59)	3	6	8	7	–	–	–	–
2,6-Me_2-Gal (1.63)	1	–	–		–	–	–	–
2,3-Me_2-Gal (2.00)	–	4	1	8	–	–	–	–
2,4-Me_2-Gal (2.13)	12	10	5	3	–	–	–	–
2-Me-Gal (2.45)	7	1	1	2	–	–	–	–

[a] Retention time compared with that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol using DB-225.
[b,c] These overlapped using a DB-225 column and were resolved using DB-210.

ethanol, which eluted rhamnose and oligosaccharides (0.5% yield). This fraction was shown by PC (1:1:1 *n*-BuOH–pyridine–H_2O) to be extremely complex, containing spots with R_{Lact} 0.20, 0.34, 0.45, 0.60, 0.67, 0.82, and 3.00 (R_{Gal} 1.61), of which those with R_{Lact} 0.20, 0.34, 0.45, and 3.00 were isolated pure by preparative PC. Other regions centered at R_{Lact} 0.99, 2.40 (R_{Gal} 1.28), and 2.70 (R_{Gal} 1.45) each contained many spots, and their components could not be resolved by preparative PC or HPLC. Each of the seven products were examined by methylation analysis (Table 1), ^{13}C NMR spectroscopy, ESIMS (Table 2), and their monosaccharide compositions.

Acidic disaccharide **9** *with* R_{Lact} *0.60.*—On acid hydrolysis, the oligosaccharide gave acetates of rhamnitol and a trace of glucitol (GC–MS), arising from glucuronolactone. Its ^{13}C NMR spectrum (Table 2) was identical to that of structure **9**[6] and its ESIMS (negative-ion mode) spectrum contained a molecular ion with m/z 339.

α-Rha*p*-(1→4)-GlcA
9

α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-Gal
10

α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-β-Gal*p*-(1→6)-Gal
11

α-Ara*f*-(1→2)-Ara
12

Acidic trisaccharide **10** *with* R_{Lact} *0.34.*—The oligosaccharide gave, on acid hydrolysis, acetates of rhamnitol, galactitol, and a trace of glucitol (GC–MS). Its ESIMS spectrum contained molecular ions with m/z 503 (H^+), 525 (Na^+) and 541 (K^+) and its ^{13}C NMR spectrum (Table 2) was identical to that of structure **10**.[7]

Acidic tetrasaccharide **11** *with* R_{Lact} *0.20.*—The oligosaccharide gave acetates of galactitol > rhamnitol > glucitol (GC–MS) following acid hydrolysis. Its ESIMS (negative-ion mode) spectrum contained a molecular ion with m/z 663. Its ^{13}C NMR spectrum (Table 2) corresponded to that of structure **11**.[6]

Disaccharide with R_{Lact} *3.00.*—ESIMS gave exclusively a molecular ion (Na^+ form) with m/z 305, consistent with an Ara_2 structure. The fraction, on methylation analysis, gave the acetate of 2,3,5-Me_3-Ara and those of 3,5- and 3,4-Me_2-Ara, each arising from 2-*O*-substituted reducing ends, depending on whether they were methylated in the furanosyl and pyranosyl form, respectively (Table 1), which is consistent with an α-Ara*f*-(1→2)-Ara structure (**12**). Its ^1H,^{13}C HMQC NMR spectrum (Table 2) contained a low-field C-1′ signal at δ 107.4 (α-Ara*f*) and C-1 signals at δ 91.1 and

93.3 from α and β-pyranosyl forms, respectively, and at δ 98.4 from traces of the α-furanosyl form.

Mixed fraction centered on R_{Lact} *2.70.*—Only arabinosyl units were present (GC–MS) and methylation-analysis indicated nonreducing end (36%), 2-*O*- (24%) and 3-*O*-substituted units of Ara*f* (11%) and reducing ends that were derivatized in the 2-*O*- (16%) and 3-*O*-substituted Ara*p* forms (13%). The nonreducing end percentage showed an average degree of polymerization of ~2.8. ESIMS under conditions optimized for formation of molecular ions, provided them (Na^+ form) with m/z 305 (Ara_2), 437 (Ara_3), and 569 (Ara_4), showing Ara-containing chains of up to four units. A ^{13}C NMR spectrum of the fraction (Table 2) contained C-1 signals at δ 101.9, 99.3, 97.1, 96.9, and 92.9 and the absence of low-field signals showed the Ara*f* units to have the β configuration.

Mixed fraction centered on R_{Lact} *2.40.*—It contained exclusively arabinose (GC–MS) and methylation analysis showed a mixture of nonreducing (20%), 2-*O*- (62%) and 3-*O*-substituted units of Ara*f*, (9%), and 2-*O*- (5%) and 3-*O*-substituted reducing end-units (4%) that were methylated as the Ara*p* form. The percentage of the nonreducing ends indicated an average degree of polymerization of ~5.0. ESIMS gave ions (Na^+ form) that were principally molecular with m/z 305 (Ara_2), 437 (Ara_3), 569 (Ara_4), 701 (Ara_5), 833 (Ara_6), and 965 (Ara_7), arising from Ara-containing chains of up to seven units. C-1 signals at δ 99.9 and 100.5 (Table 2) indicated Ara*f* units exclusively with the β configuration.

Mixed fraction centered on R_{Lact} *0.99.*—This was a complex mixture, as it contained Gal > Man = Ara units and which gave rise on methylation analysis to many partially *O*-methylated alditol acetates, but which showed linear structures (Table 1). ESIMS gave a predominant hexobiose molecular ion (Na^+ form) with m/z 365, and other ions, principally molecular, at m/z 497 (Hex_2Ara), 629 (Hex_2Ara_2), 761 (Hex_2Ara_3), and 893 (Hex_2Ara_4). C-1 signals of the fraction (Table 2) were present at δ 108.1 (α-Ara*f*), 103.5 (β-Gal*p*), 100.1, 96.8, 92.7, and 92.0.

Table 2
Key NMR spectral data for free, reducing oligosaccharides **9**–**12**, and fractions with R_{Lact} 0.99, 2.40, 2.70, and 3.00

Material	Key ^{13}C NMR signals[a]
Oligosaccharide **9** (R_{Lact} 0.60)	α-Rha*p*: 101.2 (C-1′) 16.9 (C-6′); αβ-Glc*p*A: 96.4 ($C\text{-}1_\beta$) > 92.5 ($C\text{-}1_\alpha$), 79.7 ($C\text{-}4_\beta$) > 80.8 ($C\text{-}4_\alpha$)
Oligosaccharide **10** (R_{Lact} 0.34)	α-Rha*p*: 101.2 (C-1″), 16.9 (C-6″); β-Glc*p*A′: 103.0 (C-1′), 79.6 (C-1′); αβ-Gal: 96.9 ($C\text{-}1_\beta$) > 92.8 ($C\text{-}1_\alpha$), 69.9 (C-6′, DEPT inverted)
Oligosaccharide **11** (R_{Lact} 0.20) 4″)	α-Rha*p*: 101.2 (C-1‴), 16.9 (C-6‴); β-Glc*p*A″: 103.0 (C-1′), 79.6 (C-4″); β-Gal*p*′: 103.6 (C-1′), 70.0 (C-6′, DEPT inverted); αβ-Gal: 69.9 and 70.0 (C-6, DEPT inverted); 96.9 ($C\text{-}1_\beta$) > 92.8 ($C\text{-}1_\alpha$)
Fraction, R_{Lact} 0.99	α-Ara*f*: 108.1 (C-1). Hex: 103.5 (C-1). β-Ara*f*: 100.1 (C-1), 96.8, 92.7, 92.6, 92.0
Fraction, R_{Lact} 2.40	β-Ara*f*: 97.2 > 97.6 (C-1), 82.6 (C-2). Small signals at 92.9, 92.8, 95.3, 98.1, 99.9, 100.5
Fraction, R_{Lact} 2.70	β-Ara*f*: 101.9, 99.3, 97.1, 96.9, 92.9 (C-1s)
Fraction, R_{Lact} 3.00 (**12**)	α-Ara*f*: 107.4 (C-1′), 83.9 (C-4′). Ara: 98.4, 93.3, 91.1 (C-1s)

[a] Shifts in δ (ppm).

3. Discussion

The structures present in the polysaccharide of gum ghatti are similar to those of the free, reducing oligosaccharides, as it contains side chains of Ara*f* units, which are in the α-form when close to the nonreducing ends, but which expose chains of nonreducing end-, 2-*O*- and 3-*O*-substituted β-Ara*f* units after two and three controlled Smith degradations (Table 1, Fig. 1(C) and (D)). The oligosaccharides have side chains containing nonreducing end, 2-*O*-, and 3-*O*-substituted Ara*f* units that are even longer than shown by the three above Smith degradations. The maximum length of consecutive β-Ara*f* units in Fraction R_{Lact} 2.70 is four units, whereas in Fraction R_{Lact} 2.40 it is seven. The Ara*f* units were present in α and β forms in Fraction R_{Lact} 0.99, and the oligosaccharide with the highest molecular weight was Hex_2Ara_3. In another oligosaccharide series, those with structures **9**, **10**, and **11** were characterized, each one being based on an α-Rha*p*-(1→4)-β-Glc*p*A group, which shows that the substituent at O-4 of Glc*p*A, not previously determined,[2,3] is α-Rha*p*.

Unexpectedly, the three controlled Smith degradations of the polysaccharide gave a product whose ^{13}C NMR spectrum (Fig. 1(D)) contained six prominent signals, arising from consecutive (1→3)-linked β-Gal*p* units. Such a structure has been characterized by ^{13}C NMR spectroscopy as a component of polysaccharides of gum arabic,[10] gums of the cashew nut tree[6] and angico branco,[7] and by other methodologies from those of gum arabic[11] and many other sources.[9]

4. Experimental

Source of gum ghatti.—The gum was obtained from Sigma-Aldrich Co.

Assay of reducing monosaccharides in the gum.—The gum (1.0 g) was dissolved in H_2O (75 mL), the solution

added to EtOH (500 mL), the resulting precipitate was filtered off, and the filtrate was evaporated to a residue (20 mg). Of this, 6.3 mg was dissolved in H_2O (0.5 mL), and allitol standard (1.72 mg) was added, followed by $NaBH_4$ (5 mg). The product was acetylated with Ac_2O–pyridine, and the resulting alditol acetates were examined using a capillary column of DB-225 (30 m × 0.25 mm i.d.), programmed from 50 °C (1 min) at 40 °C/min to 220 °C (constant temp.)

Controlled Smith degradations of polysaccharide.—Polysaccharide (15.0 g) was allowed to swell overnight in H_2O (600 mL), then dispersed using a blender, and on addition of $NaIO_4$ (~ 2.0 mol/mol; 43 g), an immediate loss of viscosity occurred. After 3 days, ethylene glycol was added, and the solution was dialyzed. The retained polyaldehyde was reduced with $NaBH_4$ (3.0 g). After 1 h HOAc was added, and the solution was dialyzed and evaporated to 100 mL. The solution was adjusted to pH 2.0 with dil aq H_2SO_4 and maintained at 100 °C for 30 min, neutralized with pyridine, and dialyzed. Yield on freeze drying: 3.10 g. A second degradation on this material (2.77 g) gave a product (yield, 510 mg). A third on 344 mg gave a polysaccharide (yield, 148 mg).

Isolation of oligosaccharides.—Gum ghatti (46.0 g), after being left to swell in H_2O (3 h; 900 mL), was dispersed by homogenization in a blender. The solution was added to EtOH (3.0 L) with gentle stirring, and the fibrous precipitate was isolated by decantation, resuspended in EtOH and filtered off. The solution was evaporated to a small volume, which was added to 6 vols of EtOH to precipitate the remaining polysaccharide, and the resulting solution evaporated to a residue (932 mg). Fractionation of the mixture (140 mg) by PC on Whatman No. 3 (solvent: 1:1:1 *n*-BuOH–pyridine–H_2O) gave fractions with R_{Lact} 0.20 (8 mg), 0.34 (19 mg), 0.60 (20 mg), and 3.00 (10 mg), along with those centered at R_{Lact} 0.99 (15 mg), 2.40 (22 mg), 2.70 (19 mg).

Determination of monosaccharide composition of polysaccharides.—Each sample was hydrolyzed with 2 M TFA for 8 h at 100 °C, and the product was reduced with $NaBH_4$ and acetylated with Ac_2O–pyridine. The resulting alditol acetates were analyzed by GC–MS as shown above. The original polysaccharide and products obtained by controlled Smith degradations were analyzed for uronic acid content using an *m*-hydroxydiphenyl colorimetric method in which neutral sugars do not interfere.[12]

Methylation analysis of polysaccharides.—The original polysaccharide and those obtained on one and two controlled Smith degradations were methylated by the method of Ciacunu and Kerek.[13] That obtained on three degradations proved to be insoluble in Me_2SO and a prior Haworth methylation[14] was necessary. Per-*O*-methylated products were treated with 3% MeOH–HCl, followed by hydrolysis with 1 M H_2SO_4 for 18 h at 100 °C, and the resulting partially *O*-methylated aldoses were converted to their corresponding monodeuterated alditol acetates by successive treatments with NaB^2H_4 and Ac_2O–pyridine. These were identified, using authentic samples, by their retention times and typical EI breakdown patterns.[15]

GC–MS analyses were carried out using a capillary column (30 m × 0.25 mm i.d.) of DB-225 (Table 1). This column did not resolve acetates of 2,3,5-Me_3-Ara from 2,3,4-Me_3-Rha and 3,4-Me_2-Ara from 2,4-Me_2-Ara, and these were resolved, respectively, using DB-210. The retention times were 2,3,5-Me_3-Ara (6.20 min), 2,3,4-Me_3-Rha (6.80 min),[6] 2,4-Me_2-Ara (12.8 min), and 3,4-Me_2-Ara (13.6 min). Each column was programmed from 50 °C (1 min) at 40 °C/min to 200 °C with He as carrier gas.

NMR data.—NMR spectra were obtained with a Bruker 400 MHz DRX Avance spectrometer from solutions in 99.9% D_2O at 30 °C (shifts expressed as δ in PPM, relative to external Me_4Si, $\delta = 0$). That of the polysaccharide was from a gel in Me_2SO-d_6. DEPT spectra were obtained according to the Bruker manual. ^{13}C shifts and assignments for oligosaccharides **9**–**12** and fractions with R_{Lact} 0.99, 2.40, and 2.70 are shown in Table 2.

ESIMS molecular weight determinations on oligosaccharides.—Analyses were carried out on individual samples using a Micromass double quadrupole Quattro LC equipment in the positive- and negative-ion modes on samples (~ 1 ηg/μL) dissolved in H_2O and CH_3COCN added to give a 1:1 solvent ratio. Samples were applied using a manual loop injector (10 μL volume) onto a flow rate of 20 μL/min of the 1:1 solvent. The system was washed (× 6) with the solvent after each run. The m/z values obtained from isolated oligosaccharides and fractions were as follows:

Oligosaccharide **9**: α-Rha*p*-(1→4)-αβ-Glc*p*A: 339 (Na^- ion).

Oligosaccharide **10**: α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-Gal: 503 (H^+ ion), 525 (Na^+ ion), 541 (K^+ ion).

Oligosaccharide **11**: α-Rha*p*-(1→4)-β-Glc*p*A-(1→6)-β-Gal*p*-(1→6)-αβ-Gal: 663 (negative ion).

Oligosaccharide **12** (fraction centered on R_{Lact} 3.00): 305 (Ara_2); Na^+ ion.

Fraction centered on R_{Lact} *2.70*: 569 (Ara_4), 437 (Ara_3), 305 (Ara_2); Na^+ ions.

Fraction centered on R_{Lact} *2.40*: 965 (Ara_7), 833 (Ara_6), 701 (Ara_5), 569 (Ara_4), 437 (Ara_3), 305 (Ara_2); Na^+ ions.

Fraction centered on R_{Lact} *0.99*: 893 (Hex_2Ara_4), 761 (Hex_2Ara_3), 629 (Hex_2Ara_2), 497 (Hex_2Ara), 365 (Hex_2); Na^+ ions.

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