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Structure of the arabinogalactan from gum tragacanth (*Astralagus gummifer*)

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Abstract

The polysaccharide obtained by ethanol precipitation from an aqueous solution of gum tragacanth contained arabinogalactan and tragacanthic acid, as well as starch (~0.6%). GC–MS, NMR, and ESI–MS analyses showed the structure of the arabinogalactan to be even more complex than previously determined, with core structures containing Ara*p*, β-Ara*f*, and α-Gal*p* units, as well as known terminal, and 2-*O*- and 3-*O*-substituted α-Ara*f* units. Analysis was aided by examination of free, reducing oligosaccharides present in the gum. In addition to maltose, maltotriose, maltotetraose, and maltopentaose, the following were characterized: mixed α-Ara*f* (1→2)-α-Ara*f*-(1→4)-Ara and α-Ara*f*-(1→2)-α-Ara*f*-(1→5)-Ara, which correspond to the side chains of the arabinogalactan, β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-Gal; and a mixture of β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-Gal and β-Glc*p*-(1→4)-β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-β-Gal*p*-(1→4)-Gal, which did not resemble side-chain structures of the arabinogalactan. The latter are suggested to be related to tragacanthic acid, which has been previously found to contain β-Gal*p* nonreducing end-units.

Keywords: Gum tragacanth; Arabinogalactan; Structure; Free, reducing oligosaccharides; Tragacanthic acid

1. Introduction

The structures of polysaccharides of gum tragacanth were investigated in detail by James and Smith,[1,2] followed by Aspinalll and Baillie in 1963,[3,4] who separated and analyzed the arabinogalactan and tragacanthic acid components. The complex structure of the arabinogalactan is now further elucidated using GC–MS, NMR, and ESI–MS techniques. Also examined are structurally similar free, reducing oligosaccharides present in gum tragacanth, which should arise via partial hydrolysis, partial enzymolysis, or as byproducts of biosynthesis. Their structures correspond to those of side chains and this approach has been successful in defining complex structures in the gum polysaccharides of the cashew-nut tree,[5] angico branco,[6] and gum Arabic,[7] since the oligosaccharides lend themselves more readily to NMR and ESI–MS analyses than the polysaccharides themselves.

Aspinall and Baillie[3] separated arabinogalactan from tragacanthic acid by several methods, including precipitation of the latter with Cetavlon. The remaining soluble arabinogalactan had a complex structure with many sequences containing Ara*f* units, mostly with the α configuration by virtue of a strongly negative specific rotation. These included nonreducing ends, 2-*O*- and 5-*O*-substituted units, but the formation of the acetate of 2,3-Me_2Ara on methylation analysis (GC of derived *O*-methyl methyl glycosides) was unclear as to whether it arose from 5-*O*-substituted Ara*f* and/or 4-*O*-substituted Ara*p* residues. In terms of galactose-containing structures, 3,6-di-*O*-substituted Gal*p* units were present, and on partial-acid hydrolysis a polysaccharide was formed containing mainly 6-*O*- and some 4-*O*-substituted units.[4] Partial hydrolysis gave β-Gal*p*-(1→6)-Gal, but neither the glycosidic configuration of the 4-*O*-substituted Gal*p* units, nor an existing sequence was determined. In the absence of this information, we have re-examined the structure of the arabinogalactan.

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2. Results and discussion

The polysaccharides of gum tragacanth were precipitated by the addition of an aqueous dispersion to excess ethanol. The mixture gave upon acid hydrolysis, the neutral monosaccharides Fuc, Ara, Xyl, Man, Gal, and Glc in a 3:52:29:6:5:5 molar ratio (GC–MS of derived alditol acetates). The Fuc and Xyl residues arose from tragacanthic acid.[3] A gel of the gum in Me_2SO-d_6 gave rise to a ^{13}C NMR spectrum with a C-1 region (Fig. 1(A)) containing signals at δ 107.7 = 108.4 > 107.1 = 109.5 (tr.) from α-Ara*f* units, δ 103.6, and 104.0, and δ 100.4; the last from a main chain of (1→4)-linked α-Gal*p*A units.[3]

Partial hydrolysis of the mixed gum polysaccharides under conditions which removed Ara*f* units[4] gave a polysaccharide in which its neutral monosaccharides were Rha, Fuc, Ara, Xyl, Man, Gal, and Glc, in a 12:9:24:29:1:14:10 molar ratio. The ^{13}C NMR spectrum (Fig. 1(B)) did not contain C-1 signals of α-Ara*f* units, indicating that its Ara units were in the pyranosyl form.

The arabinogalactan was prepared by removal of tragacanthic acid as a Cetavlon precipitate at pH 8.0.[3] The arabinogalactan contained Rha, Fuc, Ara, Xyl, Man, Gal, and Glc in a 1:1:68:2:5:22:1 molar ratio, the presence of only 2% of Xyl indicating that most of the tragacanthic acid had been removed, as did its uronic acid content of 4.7%. The arabinogalactan gave a main C-1 α-Ara*f* signal at δ 108.3, with smaller ones at δ 107.2, 107.7, 108.1, 108.6, and 109.8 (Fig. 1(C)), confirming its complex structure.[4]

^{13}C NMR examination and methylation analysis (GC–MS of derived *O*-methylalditol acetates) were carried out on the arabinogalactan and polysaccharides obtained following three successive, controlled Smith degradations of the mixed polysaccharide preparation. Methylation analysis of the original arabinogalactan (Table 1) provided partially *O*-methylated arabinose-containing fragments from nonreducing end- (29%), 2-*O*- (13%), and 3-*O*-substituted (3%) Ara*f* units. The formation of 2,3-Me_2Ara (7%) and 3-MeAra (13%) derivatives did not distinguish between furanosyl and pyranosyl units. Gal*p* units were mainly present as 3,6-di-*O*-substituted residues (11%). The only fragments detected corresponding to the tragacanthic acid were from nonreducing ends of Gal*p* (3%) and 2-*O*-substituted units of Xyl*p* (2%).

One controlled Smith degradation resulted in considerable periodate oxidation of the arabinogalactan, but a small amount of tragacanthic acid, since the product was formed in only 19% yield and had a uronic acid content of 69.5%. Its neutral monosaccharides were arabinose, galactose, and rhamnose in a 47:43:10 molar ratio. The ^{13}C NMR spectrum (Fig. 1(D)) contained C-1 signals of α-Ara*f* units at δ 106.8, 108.7, and 109.8 and the high proportion of uronic acid was reflected by a large, broad α-Gal*p*A signal at δ 100.9. Methylation analysis showed an increased proportion of 2-*O*-substituted (21%), when compared with nonreducing α-Ara*f* units (17%). The proportion of 2,3-Me_2Ara (6%) remained virtually unchanged. A lesser amount of 3-MeAra (4%) appeared, indicating oxidation of one of the branches attached to 2,5-di-*O*-Ara*f* and/or 2,4-di-*O*-substituted Ara*p* units.

A second controlled Smith degradation provided a polymer (23% yield: uronic acid 36.6%) containing arabinose, galactose, and rhamnose in a 51:39:10 molar ratio. The C-1 portion of the ^{13}C NMR spectrum contained a single α-Ara*f* signal at δ 109.9 (Fig. 1(E)), with α-Gal*p*A signals at δ 99.9 and 100.9. The presence of signals at δ 104.0 and 104.6 is consistent with β-Gal*p* and/or α-Ara*p* units. Methylation analysis (Table 1) showed an increase in 2-*O*-substituted Ara*f* to 28% over nonreducing end-units (14%). Each should arise from the β-form, since the δ 109.9 signal was small. 6-*O*-Substituted Gal*p* units increased to 17%.

A third degradation gave a product (23% yield) in which the uronic acid content was 2.7%, indicating that the tragacanthic acid products were virtually removed. The degradation product contained arabinose, galactose, rhamnose, and glucose in a 67:22:7:4 molar ratio. No α-Ara*f* signals were present in the C-1 region (Fig. 1(F)), and since methylation analysis showed 44% of 2-*O*-substituted Ara*f* units, a β configuration was indicated (nonreducing end-units of Ara*f* virtually disappeared). Formed in appreciable amounts were 4-*O*-substituted Gal*p* (15%) and 2,4-di-*O*-substituted Rha*p* units (10%) (Table 1).

Further information on the structure of the polysaccharide(s) was obtained by examination of the free, reducing oligosaccharides present in the gum. It was allowed to swell in water, and then treated in a blender to give a viscous dispersion. Addition of excess ethanol precipitated the polysaccharides, leaving free, reducing mono- and oligosaccharides in the supernatant (PC). These were converted to alditol acetates and examined by GC–MS which showed, using allitol as an internal standard, the monosaccharides Glc (0.34%), Gal (0.025%), Man (0.019%), Xyl (0.007%), Ara (0.084%), Rib (0.002%), and Rha (0.014%).

In a similar experiment on a larger scale, the trace oligosaccharide components were enriched by charcoal-column chromatography. Hexoses, pentoses, and *myo*-inositol were eluted with water (1.5% yield) and a fraction containing oligosaccharides plus rhamnose with 40% ethanol (0.7% yield). The latter was chromatographed on a cellulose column to give fractions that were homogeneous on PC in 1:1:1 *n*-BuOH–pyridine–water. The fastest R_{Lact} 1.29 was maltose by its monosaccharide composition, ^{13}C NMR spectrum, methylation data, and a molecular ion (Na^+ form) of *m*/*z* 365 (ESI–MS). Also isolated were the higher

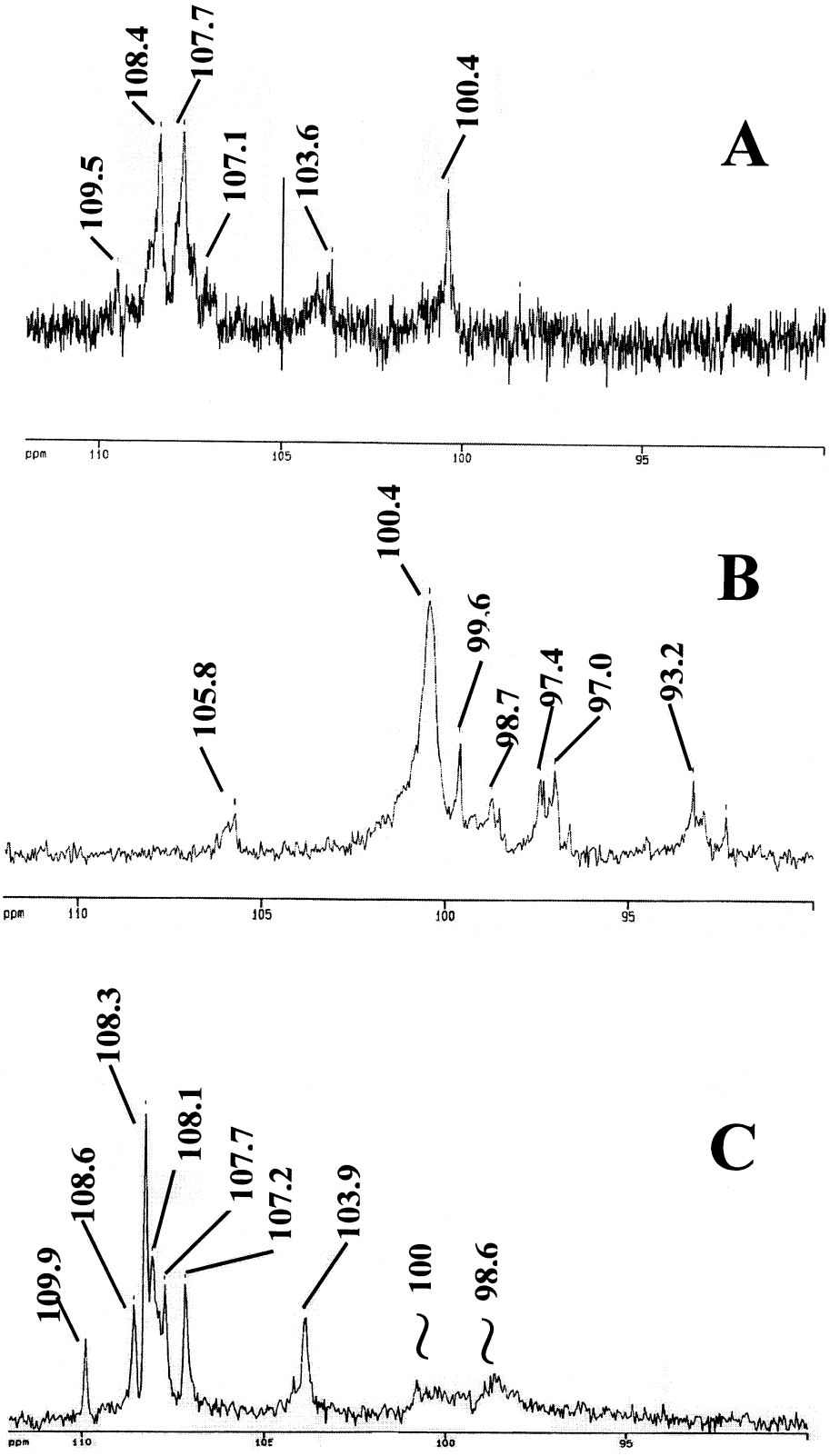

Fig. 1. C-1 region of ^{13}C NMR spectra of: gum tragacanth in Me_2SO-d_6 (A), polysaccharide formed on partial hydrolysis of gum polysaccharide (B), arabinogalactan (C), polysaccharide formed after one controlled Smith degradation of arabinogalactan (D), two degradations (E), and three degradations (F), each in D_2O.

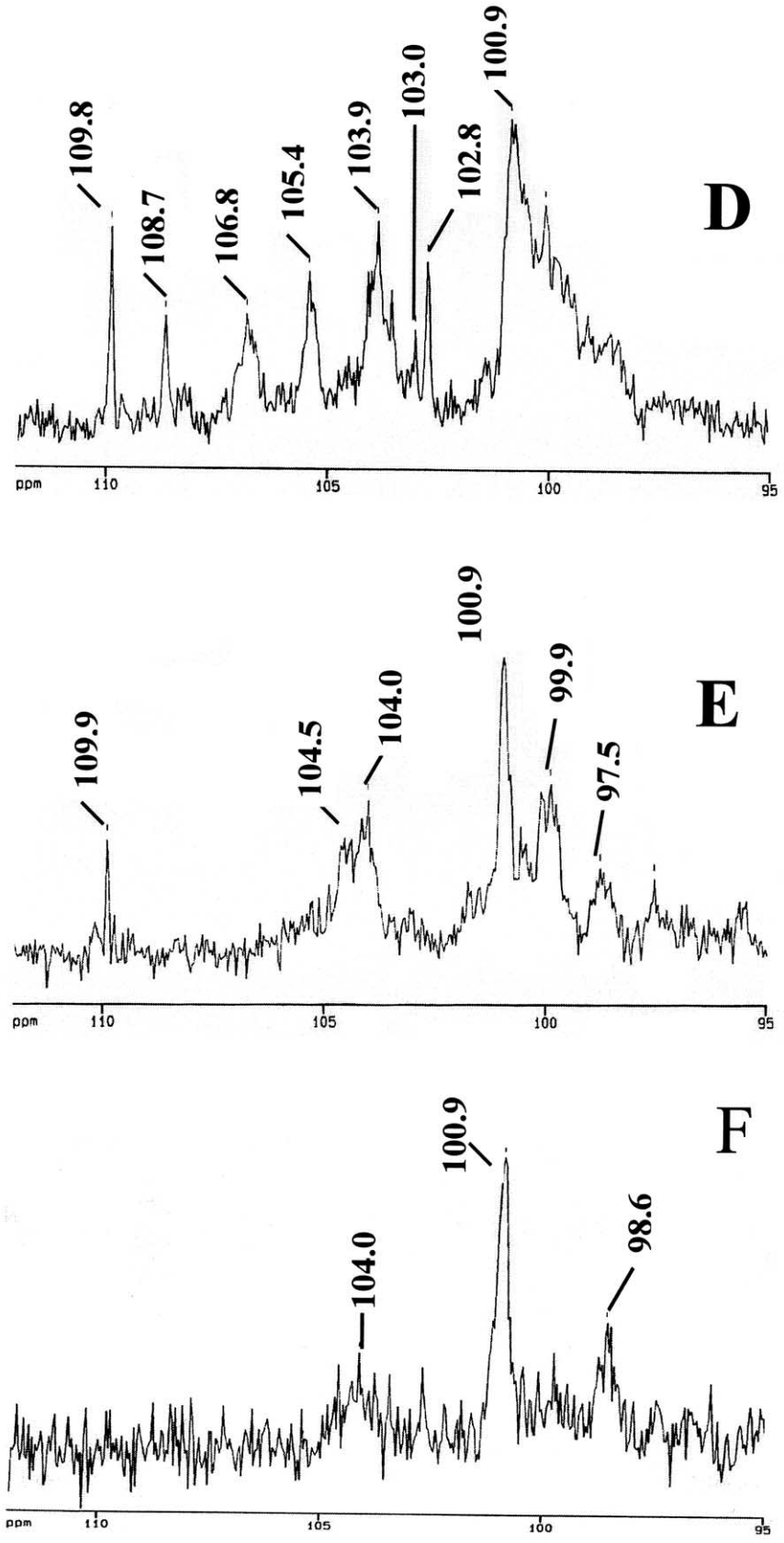

Fig. 1. (*Continued*)

Table 1
Methylation analyses of: (1) arabinogalactan (AR); (2) polysaccharide formed by one controlled Smith degradation of AR (AR-SMx1); (3) two degradations (AR-SMx2); (4) three degradations; (5) arabinotriose mixture **1** and **2**; (6) fraction with **3**, and (7) fraction with mixture of **4** and **5**

Acetylated Ald. Ac. (rt) [a]	AR (%)	AR-SMx1 (%)	AR-SMx2 (%)	AR-SMx3 (%)	Oligosaccharides **1**+**2** (%)	Oligosaccharides **3** (%)	Oligosaccharides **4**+**5** (%)
2,3,5-Me_3-Ara (0.80)	29	17	14	tr.	33		8
3,5-Me_2-Ara (0.94)	13	21	28	44			
3,4-Me_2-Fuc (0.96)	tr.	4					
2,5-Me_2-Ara (0.97)	3	3	3		3	34	
2,3,4,6-Me_4-Glc (1.00)				2			17
2,3-Me_2-Ara (1.03)	7	6	1		33	17	33
2,3,4,6-Me_4-Gal (1.05)	3	3	7	6		23	14
3,4-Me_2Xyl (1.09)	2						
2,3-Me_2Xyl (1.09)		2					
3-Me-Rha (1.21)	1	7	11	10			
3-Me-Ara (1.30)	13	4	1				
2,3,6-Me_3-Gal (1.33)	1	3	3	15		60	28
2,3,6-Me_3-Glc (1.36)	1	6	3				
Ara (1.48)	5						
2,3,4-Me_3-Gal (1.50)	4	5	17	6			
2,6-Me_2-Gal (1.58)		4	4				
2,4-Me_2-Gal (2.01)	11	14	9	6			
2-Me-Gal (2.29)	1	2					
Gal (2.65)	6						

[a] Retention time compared with that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

maltodextrins, maltotriose (R_{Lact} 0.95), -tetraose (R_{Lact} 0.76), and -pentaose (R_{Lact} 0.44), characterized by their ESI–MS molecular ions (Na^+ forms) and typical ^{13}C NMR spectra.[8] These are related to starch, since the ethanol precipitate of gum tragacanth gave a blue color with iodine. This starch component is consistent with 5% of glucosyl units present in the polysaccharide, although it was not observed by Anderson and Bridgeman.[9] The starch might be a true component or a contaminant, but although starch can be used as a lubricant in milling gums to powders,[10] the presence of free maltodextrins and glucose in the gum (see below) points to a natural component.

The charcoal-column fraction eluted with 40% ethanol was treated with amyloglucosidase to form glucose, leaving resistant components with PC R_{Lact} values of 0.34, 0.43, 1.45, and 1.03 (tr.). The glucose was removed by charcoal-column chromatography and the three principal oligosaccharides were isolated by preparative PC.

The R_{Lact} 1.45 fraction contained arabinose, xylose, mannose, and galactose in an 86:7:3:4 molar ratio (GC–MS of derived alditol acetates) and ESI–MS (+ve mode) gave rise exclusively to molecular ions with m/z 416 > 453 > 437 from H^+, K^+, and Na^+ adducts of arabinotriose, respectively. Methylation analysis (GC–MS of derived partly *O*-methylated alditol acetates on DB-225) provided fragments from 2,3,5-Me_3Ara, 3,5-Me_2-Ara, and 2,3-Me_2-Ara in a 1:1:1 ratio (Table 1). The ^1H,^{13}C HMQC NMR spectrum of the fraction contained C-1 signals at δ 108.4 and 109.8 (α-Ara*f*), δ 96.8 (α-Ara*p*) > 92.7 (β-Ara*p*) > 101.7 (α-Ara*f*) > 95.2 (β-Ara*f*). The shifts of the furanosyl reducing ends corresponded to those of arabinose, which gave rise to small α- and β-furanosyl signals at δ 101.5 and 95.6. The formation of 2,3-Me_2Ara shows that the reducing ends were 4-*O*- and 5-*O*-substituted units and that the trisaccharide fraction consisted principally of structure **1** with a smaller proportion of **2**. These sequential structures agree with those of individual monosaccharide units of side chains in the arabinogalactan.[4]

α-L-Ara*f*-(1→2)-α-L-Ara*f*-(1→4)-L-Ara*p* **1**

α-L-Ara*f*-(1→2)-α-L-Ara*f*-(1→5)-L-Ara*f* **2**

The fraction with R_{Lact} 0.34 contained galactose and arabinose in a 6:1 molar ratio. ESI–MS examination in the +ve mode gave molecular ions with m/z 689 (Na^+) > 705 (K^+), arising from a hexose tetrasaccharide. A small peak appeared at m/z 659 (Na^+) from a pent-hex_3 structure. Methylation analysis of the fraction showed the presence of a predominant nonreducing end (23%) and 4-*O*-substituted Gal*p* units (60%). As evidenced by a 2,3-Me_2Ara derivative, there were 5-*O*-substituted units of Ara*f* and/or 4-*O*-substi-

Table 2
^{13}C NMR chemical shifts of signals obtained from: (1) mixed gum tragacanth polysaccharide (MP); (2) derived arabinogalactan (AR); (3) polysaccharide formed by controlled Smith degradation of AR-SMx1; (4) two degradations (AR-SMx2); (5) three degradations (AR-SMx3); (6) polysaccharide formed by partial hydrolysis of MP ($MP-H^+$); and (7) oligosaccharide fractions **1**–**5**, isolated from gum tragacanth

Material	Chemical shifts of most significant signals, δ in PPM in D_2O
MP*	109.5, 108.4, 107.7, 103.6, 100.4, 87.8, 85.8
AR	173.7, 109.9, 108.6, 108.3, 108.1, 107.9, 107.7, 107.2, 103.9, ~100, ~98.6
AR-SMx1	109.9, 108.7, 106.8, 105.4, 103.9, 103.0, 102.8, 100.9, 98–100 (broad), 86.9, 84.7
AR-SMx2	109.9, 104.5, 104.0, 100.9, 99.9, 97.5
AR-SMx3	104.0 (broad), 100.9, 98,6, 82.9, 82.8, 81.1, 77.1, 75.5
MP-H^+	105.8, 100.4, 99.6, 98.7, 97.4, 97.0, 93.2, 92.4 78.8
1+**2**	109.8, 108.4, 101.7, 96.8, 95.2, 92.7.
3	108.0, 104.8, 96.9, 92.8, 81.3
4+**5**	108.0, 104.8, 96.9, 92.6, 84.4, 82.6, 81.3, 79.2, 77.9, 77.4

Spectrum obtained from gel in Me_2SO-d_6.

tuted units of Ara*p* (17%). α-Ara*f* units were indicated by a typical C-1 signal δ 108.0 (Table 2). Other signals were present at δ 104.8 (β-Gal*p*), and 96.9 > 92.8 (αβ-Gal*p* reducing ends), and δ 81.3 (C-4 of 4-*O*-substituted β-Gal*p*), consistent with main structure **3**.

β-D-Gal*p*-(1→4)-β-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Gal*p* **3**

The fraction with R_{Lact} 0.43 contained galactose, arabinose, and glucose in a 15:10:3 molar ratio. Its +ve mode ESI–MS spectrum contained molecular ions (Na^+ forms) with m/z 689 of a hexose tetrasaccharide and a smaller one with m/z 659 from a pent-hex_3 structure, accompanied by ions of equal magnitude with m/z 527 and 497, arising from a hexose trisaccharide and a pent-hex_2 moiety. Methylation analysis gave partly *O*-methylated alditol acetates (Table 1) which corresponded to reducing ends (8%) and 5-*O*-substituted Ara*f* units (33%), nonreducing end-units of Glc*p* (17%) and Gal*p* (14%), and 4-*O*-substituted units of Gal*p* (28%). The ^{13}C NMR spectrum was similar to that of the R_{Lact} 0.34 fraction with C-1 signals at δ 108.0 (α-Ara*f*), 104.8 (β-Hex*p*), 96.9 and 92.8 (αβ-Gal*p*), and 81.3 (C-4 of *O*-substituted β-Gal*p*). These data show a mixture, whose main components are a glucosyl unit in the tetrasaccharide (**4**) and a galactotriose (**5**), which would explain their greater PC mobility than that of galactotetraose **3**. The location of Ara units in the minor structures was not determined.

β-D-Glc*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Gal*p* **4**

β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Gal*p* **5**

Attempts were made to correlate structures **3**, **4**, and **5** with those of the polysaccharide side-chains. Although 4-*O*-substituted units were present in the arabinogalactan and derived polysaccharides (Table 1), there were β-Gal*p* units, which occur as nonreducing ends in tragacanthic acid.[3] Accordingly, partial-acid hydrolysis[4] was carried out which gave oligosaccharides with R_{Lact} 1.29, 0.95, and 0.76 (PC). These were isolated and the two faster components were found to be maltose and maltotriose, respectively, as shown by ^{13}C NMR spectra and ESI–MS molecular ions in the +ve mode. The only fraction (R_{Lact} 0.76) containing galactobioses (see Ref. 4), gave a ^{13}C spectrum consistent with a mixture of maltotetraose and 6-*O*-β-Gal*p*-Gal. The latter gave C-1 signals at δ 103.77 > 103.81 and 96.8 > 92.9 and inverted DEPT signals of C-6 at δ 69.8 > 70.0. Methylation analysis of the mixture provided partially *O*-methylated alditol acetates (GC–MS) of 2,3,4,6-Me_4-Gal (22%), 2,3,4,6-Me_4-Glc (21%), 2,3,6-Me_3-Glc (37%), 2,3,4-Me_3-Gal (17%), and 2,3,6-Me_3-Gal (3%) from a small proportion of 4-*O*-substituted Gal*p* units.

Partial acetolysis of the mixed polysaccharide preparation, followed by deacetylation gave a mixture which was fractionated on a charcoal column giving oligosaccharides with R_{Lact} values of 0.95 and 0.58. These were separated by PC and the faster fraction gave an ESI–MS molecular ion (Na^+ form) from a hexobiose structure and a ^1H,^{13}C HMQC spectrum with C-1 signals at δ 99.4, 97.1 > 93.3, showed an α-glycosidic configuration. The linkage was (1→6), since a C-6,H-6 doublet was present at δ 67.6 and that there were no other signals in the lower field, *O*-substituted region. The glycosidic linkage of the α-Gal*p*-(1→6)-Gal structure was confirmed by methylation analysis. The slower fraction was a hexotriose according to its ESI–MS molecular ion (Na^+ form). The HMQC spectrum contained C-1 signals at δ 100.6, 97.5 > 93.1, showing an α-glycosidic configuration and others from *O*-substituted nuclei at δ 79.4, 78.1, and 67.7. Methylation analysis showed a mixture of nonreducing end- and 4-*O*- and 6-*O*-substituted units, but an exact structural determination was not possible.

The structure of the arabinogalactan is thus confirmed as being extremely complex, with a core containing principally Gal*p*, Ara*p* and some 2-*O*-substituted β-Ara*f* units, and side-chain sequences now defined as **1** and **2**. However, a detailed polysaccharide analysis aimed at revealing consecutive 4-*O*-linked β-Hex*p* units, similar to those of oligosaccharides **3**, **4**, and **5** was not successful. It appears that such structures could be present as side chains in tragacanthic acid, which is

known to contain β-Gal*p* nonreducing-end and 4-*O*-substituted Gal*p* units.[3] A further investigation on the structure of tragacanthic acid will be carried out.

3. Experimental

Source of gum tragacanth.—Powdered gum was supplied by Sigma–Aldrich Co.

Preparation of arabinogalactan.—Gum tragacanth (110 g) was allowed to swell overnight in water (3.0 L), the gel was homogenized with a blender, and the resulting dispersion was added to EtOH (10.0 L). The polysaccharide precipitate was isolated and a portion fractionated by the method of Aspinall and Baillie[4] via precipitation of the tragacanthic acid component with Cetavlon at pH 8.0, the arabinogalactan being recovered from the supernatant.

Controlled Smith degradations of arabinogalactan.—The polysaccharide (10.1 g) in water (450 mL) was treated with $NaIO_4$ (30 g), and after 3 days the solution was dialyzed against tap and then distilled water. The retained solution was evaporated to 100 mL, $NaBH_4$ was added (1 h), and after 3 h, acidified with HOAc, and then the solution dialyzed. The pH was adjusted to 2.0 with dilute aq H_2SO_4, the solution was maintained at 100 °C for 30 min, neutralized with pyridine and dialyzed.

Determination of uronic acid contents. These were shown by an *m*-hydroxyldiphenyl colorimetric method, in which neutral sugars do not interfere.[11]

Partial hydrolyses of arabinogalactan.—The mixture of arabinogalactan and tragacanthic acid (10.7 g) was maintained in 0.05 M H_2SO_4 (450 mL) at 100 °C for 22 h.[4] The acid was then neutralized with pyridine and dialyzed (yield 1.20 g). On dissolving a portion in water, 0.61 g remained soluble (12:9:25:30:14:10 Rha–Fuc–Ara–Xyl–Gal–Glc ratio) and 0.30 g was insoluble.

Under more vigorous partial hydrolysis conditions, the polysaccharide mixture (2.4 g) was partly hydrolyzed in 0.5 M TFA (100 mL) at 100 °C for 1 h[4] to form oligosaccharides. The solution was evaporated and an aqueous solution of the residue was applied to a charcoal–Celite column.[12] This was eluted with water and then 40% aq EtOH, which liberated an oligosaccharide mixture (yield 0.22 g). The products had PC R_{Lact} values of 0.76, 0.95, and 1.29 in 1:1:1 *n*-BuOH–pyridine–water and were fractionated using Whatman 3 paper using the above solvent in a 5:3:3 ratio.

Trisaccharide with R_{Lact} *0.95 (10 mg).*—In 99 98%, D_2O at 70 °C: δ 5.37 (d, 1 H $J_{1,2}$ 3.5 Hz, H-1) > 4.67 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1) > 5.26 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1). HMQC at 30 °C: δ 100.2 (5.42), 96.4 (4.70), 92.4 (5.27), *O*-subst C-4 at δ 77.8 (3.57).

Di- and tetrasaccharide mixture with R_{Lact} *0.76 (5.0 mg).*—^{13}C in 99.98% D_2O at 30 °C: δ 100.30 > 100.11 > 100.08, 96.5 > 92.4, 77.75, 77.84, 77.92 (*O*-subst C-4′s); another set at δ 103.77 > 103.81, 96.8 > 92.9, with inverted 6-*O*-subst DEPT signals at δ 69.8 > 70.0.

Disaccharide with R_{Lact} *1.29 (4 mg).*—^{13}C NMR gave δ 100.20 (C-$1'_\beta$) > 100.28 (C-$1'_\alpha$), 96.4 (C-1_β) > 92.5 (C-1_α), 75.59 < 77.48 = 77.23 (*O*-subst C-4's).

Partial acetolysis of mixed polysaccharides.—The polysaccharide (1.10 g) was dissolved in a stirred mixture of 10:10:1 Ac_2O–HOAc–H_2SO_4 (20.1 mL). After 20 h, it was added to excess ice-water, and the mixture was extracted with $CHCl_3$, which was washed (× 3) with water. After evaporation, the residue was dissolved in $CHCl_3$ (20 mL) to which was added 0.1 M NaOMe in MeOH (10 mL). The deacetylated product was applied to a charcoal column, which was eluted with water and then 40% aq EtOH. This provided a mixture (0.24 g), which contained rhamnose and oligosaccharides with R_{Lact} 0.95 and 0.58 on PC in 1:1:1 *n*-BuOH–pyridine–water. This was fractionated on Whatman 3 filter paper (5:3:3 mixture) to give the oligosaccharide fractions in respective yields of 12 and 5 mg.

Assay of reducing monosaccharides in the gum.—The gum (1.0 g) was allowed to swell in water (75 mL), the gel was homogenized to give a dispersion, which was then added to EtOH (400 mL). The resulting precipitate was removed by centrifugation and the pellet was suspended in EtOH using a blender and the suspension centrifuged. The combined supernatants were evaporated to 10 mL, which was added to EtOH (50 mL), and after 18 h, the precipitate was filtered off. The filtrate was evaporated to a residue (74.0 mg), of which 26.2 mg was dissolved in water (2.0 mL), to which $NaBH_4$ (20 mg) was added, followed by an internal standard of allitol (1.0 mg). The product was acetylated with Ac_2O–pyridine and the resulting alditol acetates examined on a capillary column of DB-225 (30 m × 0.25 cm i.d.), programmed from 50 °C (1 min) at 40 °C/min to 230 °C (constant temperature).

Isolation of free, reducing oligosaccharides from the gum.—In the isolation of gum tragacanth polysaccharides (see above), a dispersion of the gum tragacanth (110 g) in water was added to EtOH to give a precipitate, which was centrifuged off, washed with EtOH, and filtered off. The combined supernatant and filtrate was evaporated to a residue (3.99 g), which was applied as an aqueous solution to a charcoal–Celite column. This was eluted with water to give a mixture (1.91 g) of reducing monosaccharides (mainly glucose) and *myo*-inositol (R_{Lact} 0.80 on PC with Whatman 1 paper in 1:1:1 *n*-BuOH–pyridine–water). Elution with 40% aq EtOH gave material (0.77 g), which in the same solvent gave PC spots with R_{Lact} 1.45 (22 mg), 1.29 (19 mg),

1.03 (5 mg), 0.95 (11 mg), 0.76 (6 mg), 0.67 (2 mg), 0.43 (4 mg), and 0.34 (4 mg) (non-maltose oligosaccharides, underlined).

In order to isolate greater amounts of the predominant maltodextrins, the oligosaccharide mixture (0.77 g) was fractionated on a cellulose column, which was eluted with mixtures of acetone–water. Fractions were obtained with monosaccharides (9:1 and 7:1; 2 L of each), and underlined maltose homologues with R_{Lact} 1.29 (7:1; 2 L; 92 mg), 0.95 (24 mg), 0.76 (9 mg), 0.44 (9 mg), and 0.34 (4 mg) with (2:1, 6 L).

Enzymolysis of oligosaccharide mixture.—The mixture (0.80 g), obtained on another charcoal–column fractionation, in water (10 mL) had pH ~5 and this was added to a solution in water (1 mL) of an amyloglucosidase (EC 3.2.1.3; 10 mg) from *Aspergillus niger* (Sigma), with an activity of 40 units/mg solid; 42 units/mg protein. After 18 h, homologous maltodextrins were absent (PC), being degraded to glucose, and the solution was applied to a charcoal column. This was washed with water and then 40% aq EtOH. The latter was evaporated to a residue (176 mg).

Methylation analysis of oligo- and polysaccharides.—In the case of reducing oligosaccharides, methylation was carried out by the method of Tischer et al.[7] The products obtained on 2 and 3 controlled Smith degradations of the arabinogalactan were submitted to a prior Haworth methylation,[13] to give products soluble in Me_2SO. These were completely methylated by the procedure of Ciacunu and Kerek.[14] Methylated polysaccharides were converted to monodeuterated *O*-methyl alditol acetates,[7] which were examined by GC–MS on columns of DB-23 and/or DB-225 (30 m × 0.25 cm i.d.) programmed from 50 °C (1 min) at 40 °C/min to 210 °C (const. temp.). A Varian model Saturn 2000 was used for obtaining GC–MS spectra and a less-sensitive system containing a Varian 3300 gas chromatograph linked to a Finnigan ion-trap model 800 mass spectrometer served for accurate quantification of peak areas. *O*-Methyl alditol acetates were identified by their retention times (Table 1) and typical EI breakdown patterns.[15]

Oligosaccharide fraction with R_{Lact} *0.43* (**4** + **5**).—On DB-225 were formed alditol acetates of: 2,3,5-Me_3Ara (8%), 2,3,4,6-Me_4-Glc (17%), 2,3-Me_2Ara (33%), 2,3,4,6-Me_4Gal (14%), and 2,3,6-Me_3Gal (28%).

Oligosaccharide fraction with R_{Lact} *0.34* (**3**).—2,3-Me_2Ara (17%), 2,3,4,6-Me_4Glc (23%), 2,3,6-Me_3Gal (60%).

Oligosaccharide fraction with R_{Lact} *1.45* (**1** + **2**).—2,3,5-Me_3Ara (34%), 3,5-Me_2Ara (32%), 2,3-Me_2Ara (34%).

NMR spectroscopic data of oligosaccharides.—NMR spectra were obtained with a Bruker 400 MHz DRX Avance spectrometer. Preliminary examinations were carried out by ^{13}C and ^{1}H NMR spectroscopy (^{1}H obs,^{13}C HMQC when only small quantities were available) spectroscopy in 99.98% D_2O at 30 °C (shifts expressed as δ in PPM, relative to external Me_4Si, $\delta = 0$). With analyses involving proton nuclei, presaturation was incorporated. DEPT spectra were obtained according to the Bruker manual.

ESI–MS molecular-weight determinations of oligosaccharides.—Analyses were carried out using Micromass double quadrupole Quattro LC and Quattro Ultima equipment in the positive-ion mode on samples (~ 1 ηg/μL) previously dissolved in water and CH_3COCN added to give a 1:1 solvent ratio. 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 system was washed (× 6) with the solvent after each run.

NMR and ESI–MS data of free, reducing oligosaccharides.—Arabinotrioses (**1** + **2**; R_{Lact} 1.45). ^{1}H,^{13}C HMQC NMR: main C-1,H-1 signals δ 109.8 (5.57), 108.4 (5.19), 101.7 (5.06) > 95.2 (5.33), 96.8 (4.76) > 92.7 (5.35). ESI–MS molecular ions: m/z 415 ($Ara_3.H^+$) > 453 ($Ara_3.K^+$) > 437 ($Ara_3.Na^+$).

Arabinose standard. ^{13}C and ^{1}H,^{13}C HMQC NMR: δ 92.9, large (5.108), 95.6 (5.15), 97.1, large (4.40), 101.5 (5.127).

Maltose (R_{Lact} 1.29). ^{13}C NMR: δ 100.18 (C-$1'_\beta$) > 100.26 (C-$1'_\alpha$), 96.4 (C-1_β) > 92.5 (C-1_α), 75.57 < 77.46 = 77.20 (*O*-subst C-4′s).

Maltotriose (R_{Lact} 0.95). ^{13}C NMR: δ 100.30 > 100.01 > 100.09, δ 96.5 > 92.4, 4 signals in C-4 subst region at δ 77.36 > shoulder at 77.40, δ 77.6 > 77.8, 60.46 > 60.53 (C-6′s); inverted DEPT, corresponding to authentic sample. ESI–MS + ve molecular ions: m/z 527 > 365 (Na^+ forms).

Maltotetraose (R_{Lact} 0.76). ^{13}C NMR: δ 100.4 > 100.26 > 100.37 = 100.15, 96.4 > 92.5, (C-1′s), 77.42, 77.63 > 77.48 (*O*-subst C-4′s). ESI–MS + ve ions: m/z 689 < 527 = 365 (Na^+ forms).

Maltopentaose (R_{Lact} 0.44). ^{13}C NMR: δ 100.19 > 100.07 = 100.27 = 100.39, 96.4 > 92.5, (C-1′s), 77.48, 77.67 (*O*-subst C-4′s). ESI–MS + ve ions: m/z 851 < 689, with 527, and 356 (Na^+ forms).

Oligo R_{Lact} 1.03. ^{1}H,^{13}C HMQC NMR: Main C-1′s, δ 107.9 (α-Ara*f*), 104.7, and 92.6 > 96.6, small C-1′s, δ 105.3, 102.9, 98.15, 91.9. Also, δ 84.4, 82.8, 81.3, 78.6, 77.4, 76.8, and 84.4.

Oligos R_{Lact} 0.43 (**4** + **5**), ^{1}H,^{13}C HMQC NMR: C-1′s, δ 108.0 (α-Ara*f*), 104.8 (β-Hexp), 96.9, and 92.8, O-subst C-4, δ 81.3. ESI–MS + ve molecular ions (Na^+ forms): m/z 689, 659, 527, 497.

Oligo R_{Lact} 0.34 (**3**), ^{1}H,^{13}C HMQC NMR: C-1′s, δ 108.0 (α-Ara*f*), 104.8 (β-Gal*p*), 96.9 > 92.8; *O*-subst C-4: δ 81.3. ESI–MS + ve molecular ions: m/z 689 (Na^+) > 705 (K^+) = 659 (Na^+).

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