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Structure of the exudate gum from *Meryta sinclairii*

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Abstract

A gum that exudes from the wounded trunk of the New Zealand native tree *Meryta sinclairii* has been isolated. The gum was completely precipitated by the β-glucosyl Yariv reagent and was thus determined to be an arabinogalactan-protein (AGP). It contained >95% w/w carbohydrate and only 2% w/w protein with a high level of hydroxyproline. SEC-MALLS showed that the gum had a weight-average molecular weight of 4.45×10^6 Da compared with 6.02×10^5 Da for gum arabic. Constituent sugar and linkage analyses were consistent with polymers comprised of a highly branched backbone of 1,3-linked galactopyranosyl (Gal*p*) residues, with side-chains made up of arabinofuranose- (Ara*f*) containing oligosaccharides, terminated variously by rhamnopyranosyl (Rha*p*), arabinopyranosyl (Ara*p*), Gal*p* and glucuronopyranosyl (Glc*p*A) residues. Analysis by one-dimensional and two-dimensional ^{1}H and ^{13}C NMR experiments confirmed the linkage analyses. The structure of the gum is discussed in comparison with the structure of gum arabic and other AGPs.

Keywords: *Meryta sinclairii*; Araliaceae; Plant gum; Arabinogalactan-protein; Structural analysis

1. Introduction

Many plants exude polysaccharide gums in response to external stresses such as wounding or disease. Typically, the sticky gums ooze from the trunks and branches of the plants and often dry to a hard glass as a physical barrier. The gums are usually complex acidic heteroglycans and some have associated protein or terpenoid material (Stephen, Churms, & Vogt, 1990). A number of these have been exploited commercially, including gum arabic (from *Acacia senegal* and *A. seyal*), gum ghatti (*Anogeissus latifolia*), gum tragacanth (*Astragalus gummifer*) and gum karaya (*Sterculia urens*), of which gum arabic is the most widely used.

The plant gums can be classified into various polysaccharide types; type II arabinogalactans (AGs, e.g. gum arabic), substituted glucuronomannans (e.g. gum ghatti), or substituted rhamnogalacturonans (e.g. gum karaya). Gum tragacanth has a mixture of type II AG and galacturonan type regions (Stephen & Churms, 1995). Gum arabic and related exudate gums, are proteoglycans (arabinogalactan-proteins, AGPs) which contain 2–10% (w/w) covalently attached protein rich in hydroxyproline and serine (Fincher, Stone, & Clarke, 1983). The polysaccharide moieties are composed of mostly arabinose and galactose, together with varying proportions of rhamnose, glucuronic acid and 4-*O*-methyl-glucuronic acid. Other sugars may be present in minor amounts. Structural models for AGPs suggest that they are compact molecules consisting of a protein core to which highly branched polysaccharide chains are attached. The predominant carbohydrate to protein linkage is thought to be an *O*-linkage to hydroxyproline.

Meryta sinclairii (puka, family Araliaceae) is a tree growing up to 8 m tall. It occurs naturally on islands off the coast of northern New Zealand, but is also extensively cultivated in gardens throughout the north island of New Zealand. When wounded, the trunk of the tree exudes a clear gum which dries to a hard crust. Brooker, Cambie, and Cooper (1987) report that this gum ‘was chewed as a remedy for long-standing stomach trouble’. The only other report of gums from members of the Araliaceae is that from *Cussonia spicata* (Churms & Stephen, 1971). This gum contained L-arabinose (L-Ara*f*; 58 mol%), D-galactose (D-Gal*p*; 23%), D-glucuronic acid (D-Glc*p*A; 12%) and L-rhamnose (L-Rha*p*; 7%). It appeared to be a type II AG since it contained 3,6- and 3,4,6-linked D-Gal*p*, 5-linked L-Ara*f* and 4-linked D-Glc*p*A residues, with terminal L-Ara*f*, L-Ara*p*, D-Glc*p*A and L-Rha*p* residues.

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In this present study we have investigated the composition and structure of the gum collected from the trunks of *M. sinclairii* trees. Our findings are discussed in relation to gum arabic and other type II AG gum exudates.

2. Experimental

2.1. Isolation of polysaccharides

The bark of *M. sinclairii* trees growing locally was cut and gum allowed to exude to form tears. The gum tears (25.0 g) were collected and dispersed in hot water (250 ml, 80 °C) for 1 h to dissolve completely. The hot solution was filtered under pressure (Whatman GF-B) and freeze-dried (MS gum). This material was redissolved in distilled water (250 ml), dialysed (mwco 12–14,000) against distilled water for 48 h and freeze-dried (MSd gum).

Gum arabic (*Acacia senegal*; Hopkin and Williams Ltd, UK) was dissolved in distilled water (10 mg ml^{-1}), dialysed (mwco 12–14,000) against distilled water for 48 h and freeze-dried.

2.2. General analysis of gum

Total water-soluble carbohydrate was determined by the phenol-sulphuric acid method using galactose as a standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). AGPs in MSd gum were precipitated with β-glucosyl Yariv reagent as described by Gane et al. (1995). MSd gum (50 mg) was dissolved in 1% w/v NaCl at a concentration of 2 mg ml^{-1} and an equal volume of β-glucosyl Yariv reagent (2 mg ml^{-1} in 1% w/v NaCl; Biosupplies Australia Ltd) added. The mixture was incubated overnight at 4 °C and the insoluble β-glucosyl Yariv–AGP complex collected by centrifugation (10,000*g*, 20 min, 4 °C), washed with 1% w/v NaCl (3 × 25 ml) then MeOH (3 × 25 ml). The pellet was dried, dissolved in 10 ml sodium dithionite (30% w/v) and stirred for 2 h at 20 °C. The resultant solution was dialysed (mwco 12–14,000) exhaustively against distilled H_2O and freeze-dried.

2.3. Amino acid analysis

Amino acid composition was determined by narrow-bore reverse-phase HPLC, after conversion to phenylisothiocyanate (PITC) derivatives as described by Hubbard (1995, 1996) and Hubbard and McHugh (1996). The analysis was conducted at the Protein Microchemistry Facility, Department of Biochemistry, University of Otago, NZ.

2.4. High performance size-exclusion chromatography coupled with multi-angle laser light scattering (SEC-MALLS)

The SEC-MALLS system consisted of a Waters 2690 Alliance separations module, a Waters 450 variable wavelength detector set at 280 nm, a DAWN-EOS multi-angle laser light scattering detector with a laser at 690 nm (Wyatt Technology Corp., Santa Barbara, CA) and a Waters 2410 refractive index monitor. Samples (2 mg ml^{-1}) were filtered (0.45 μm) before injection (20 μl) and eluted with 0.1 M $LiNO_3$ containing 0.02% NaN_3 (0.7 ml min^{-1}) from two columns (TSK-Gel G5000PWXL and G4000PWXL, 300 × 7.8 mm, Tosoh Corp., Tokyo, Japan) connected in series. Data for molecular weight determination and conformation were analysed using ASTRA software (Version 4.73.04, Wyatt Technology Corp.) using a *dn*/*dc* of 0.145 ml g^{-1} (determined experimentally).

2.5. Reduction of uronic acid residues

For constituent sugar and glycosyl linkage analyses, uronic acid residues were reduced as described by Sims and Bacic (1995). Thus, MSd gum or gum arabic (5 mg) was dissolved in 500 mM imidazole-HCl (5 ml, pH 8.0) and cooled to 4 °C. Three aliquots of $NaBD_4$ (each 1 ml, 100 mg ml^{-1}) were added at 30 min intervals and the samples maintained for 1 h at 4 °C. Excess $NaBD_4$ was destroyed by addition of excess acetic acid and the samples were dialysed (mwco 12–14,000) for 24 h against distilled water and freeze-dried. The samples were redissolved in 50 mM MES-KOH (2 ml, pH 4.75). 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide-*metho*-*p*-toluenesulphonate (400 μl, 500 mg ml^{-1}) was added to each sample and the mixtures were maintained for 3 h at 30 °C. Tris-HCl buffer (1 ml, 2 M, pH 8.0) was then added to each sample and the samples were reduced (18 h, 4 °C) with $NaBD_4$ (1 ml, 70 mg ml^{-1} in 50 mM NaOH). Excess reductant was destroyed by addition of excess acetic acid and the samples were dialysed for 24 h against distilled water and freeze-dried to provide carboxyl-reduced gums.

2.6. Constituent sugar analysis

Native or carboxyl-reduced gums (100–200 μg) were hydrolysed with 2.5 M TFA (200 μl, 4 h, 100 °C), concentrated to dryness under an air stream at 40 °C, then reduced with 1.0 M $NaBH_4$ in 2.0 M NH_4OH (200 μl) overnight at 35 °C. The reaction was stopped by the addition of glacial acetic acid (50 μl). Borate was removed as volatile trimethylborate by addition of 5% v/v acetic acid in MeOH (3 × 0.5 ml), and concentrating under an air stream at 40 °C, followed by addition of MeOH (3 × 0.5 ml) and concentrating to dryness under an air stream at 40 °C. The resulting alditols were acetylated in acetic anhydride (100 μl) and TFA (100 μl) for 10 min at 50 °C and extracted into CH_2Cl_2 for analysis. The alditol acetates were separated by GC on a SGE BPX70 capillary column (25 m × 0.25 mm i.d., 0.22 μm film thickness) with the GC oven programmed from 70 °C (held for 1 min) to 140 °C at a rate of 25 °C min^{-1}, and then to 230 °C at a rate of 3 °C min^{-1} and analysed by MS using a Hewlett Packard 5970 MSD.

Identifications were based on peak retention times and by comparison of electron impact mass spectra with published spectra.

2.7. Glycosyl linkage analysis

Carboxyl-reduced gum (100–200 μg) was methylated using NaOH and CH_3I in DMSO (Ciucanu & Kerek, 1984) as described by McConville, Homans, Thomas-Oates, Dell, and Bacic (1990). The methylated polysaccharides were hydrolysed, reduced and acetylated, and analysed by GC-MS as described above. Identifications were based on peak retention times and by comparison of electron impact mass spectra with published spectra.

2.8. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-500 spectrometer with an inverse probe at 70 °C. Samples were dissolved in D_2O (99.6 atom%; 100 mg in 1 ml), and transferred to 5 mm o.d. NMR tubes. ^{1}H and ^{13}C chemical shifts were measured relative to an internal standard of Me_2SO (^{1}H, 2.70 ppm; ^{13}C, 39.5 ppm). Assignments were made from double quantum filtered (DQF) COSY, heteronuclear multiple quantum coherence (HMQC) COSY and HMQC TOCSY experiments and by comparing the spectra with published data.

For ^{1}H NMR spectra at 499.8 MHz, 64 scans were collected with 4.096 s data acquisition time for free induction decay (fid) after a 6 s relaxation delay and 90° pulse with a free 8000 Hz sweep width resulting in 32 K data points.

For proton decoupled ^{13}C NMR spectra at 125.7 MHz, 42 K scans were collected with 1.3 s data acquisition time, a relaxation delay of 9 s and a 90° pulse with a free 25 kHz sweep width resulting in 32 K data points.

3. Results and discussion

3.1. Composition

The yield of filtered and freeze-dried *M. sinclairii* gum was 17.75 g, representing 71% w/w of the original exudate. This material was almost completely recovered after dialysis and was totally precipitated with β-glucosyl Yariv reagent, and thus the gum was deduced to be a high molecular weight arabinogalactan-protein (AGP; Fincher et al., 1983). The dialysed gum (MSd) contained a high proportion of carbohydrate (>95% w/w) and only 2% w/w protein (calculated from the amino-acid composition).

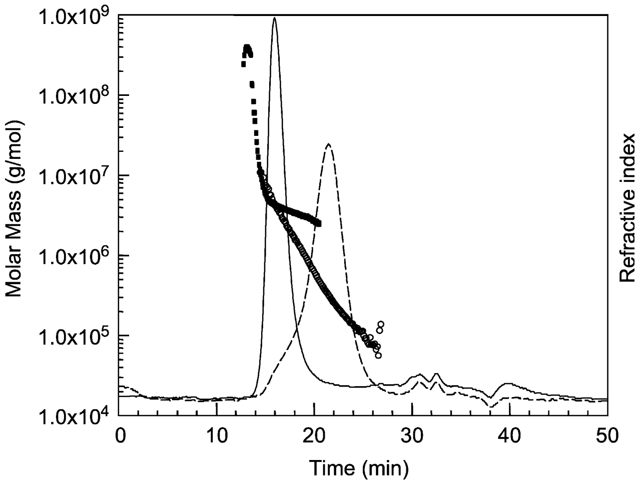

Fig. 1. Molecular weight analysis by size-exclusion/multi-angle laser light scattering (SEC-MALLS). I. MSd gum refractive index (—) and molar mass (■), II. Gum arabic refractive index (- -) and molar mass (○).

MSd gum eluted as a single peak on SEC-MALLS close to the V_0 of the columns (Fig. 1). The gum had a weight-average molecular weight (M_w) of 4.45×10^6 Da and a relatively low polydispersity index (M_w/M_n) of 1.03. Gum arabic had a M_w 6.02×10^5 Da and a higher M_w/M_n than MSd gum of 1.65.

Constituent sugar analysis of native MSd gum and gum arabic gave Ara, Gal and Rha (Table 1). The ratio of Ara/Gal was 2.3 and 2.6 for MSd gum and gum arabic, respectively. Following reduction of uronic acid residues to their respective 6,6′-dideuterio neutral sugars, constituent sugar analysis revealed that in addition to the neutral sugars above, MSd gum also contained GlcA and 4-*O*-Me-GlcA, and gum arabic contained GlcA (Table 1). The ratio of Ara/Gal obtained following carboxyl-reduction was lower than for the native gums, 1.3 and 1.0 for MSd gum and gum arabic, respectively. The relative amount of Rha in native, unreduced gum arabic was similar to the Rha content of *Acacia seyal*, but following carboxyl-reduction was more characteristic of the Rha content of *Acacia senegal* (Williams & Phillips, 2000). These observations indicated that hydrolysis of the native gums was incomplete and suggested that the uronic acid residues present in the native gums resulted in the polysaccharides being more resistant to

Table 1
Comparison of the constituent sugar analyses of native and carboxyl-reduced MSd gum and gum arabic

Sugar	Relative amount (mol%)[a]			
	Meryta sinclairii gum		Gum arabic	
	Native	Reduced	Native	Reduced
Rhamnose	10	7	2	14
Arabinose	63	49	71	40
Galactose	27	38	27	40
Glucose	–[b]	tr	–[b]	tr
4-*O*-methyl-GlcA	n.d.[c]	3	n.d.[c]	tr
Glucuronic acid	n.d.[c]	3	n.d.[c]	5

[a] Average of duplicate determinations.

[b] –, Not detected.

[c] n.d., Not determined.

acid hydrolysis. The amino-acid composition of MSd gum showed high proportions of Hyp, Ser and Glx (Table 2). Published amino-acid compositions of gum arabic, from both *Acacia senegal* and *A. seyal*, show similarly high proportions of Hyp and Ser, but lower levels of Glx (Table 2; Williams & Phillips, 2000).

3.2. Linkage composition analyses

MSd gum was subject to linkage analysis following reduction of carboxyl groups to their respective $6,6'$-dideuterio neutral sugars, and the results were compared with the linkage analysis of carboxyl-reduced gum arabic (Table 3). The analyses of the two gums are consistent with polymers comprised of a highly branched backbone of 1,3-linked Gal*p* residues, with side-chains made up of Ara*f*-containing oligosaccharides, terminated variously by Rha*p*, Ara*p*, Gal*p* and Glc*p*A residues.

Studies of the side-chain structures of gum arabic and other type II arabinogalactan polysaccharides have suggested that Glc*p*A are usually capped with terminally linked Rha*p* residues (Delgobo, Gorin, Tischer, & Iacomini, 1999; Tishcer, Gorin, & Iacomini, 2002). In addition to 4-linked Glc*p*A which may be terminated with Rha*p* residues, linkage analysis data revealed MSd gum contained a similar amount of terminally linked Glc*p*A. Constituent sugar analysis showed that MSd gum contained equal amounts of GlcA and 4-*O*-Me-GlcA (Table 1) and thus the terminally linked GlcA*p* was deduced to be naturally 4-*O*-methylated. MSd gum and gum arabic contained similar amounts of terminal Ara*f*, but MSd gum also contained terminal Ara*p*, which was almost absent in gum arabic. MSd gum did not contain terminal Gal*p*, but this residue was present gum arabic. MSd gum and gum arabic contained similar amounts of intra-chain Ara*f* residues, but whereas MSd gum contained almost equal amounts of 3-Ara*f* and 5-Ara*f*, gum arabic contained only 3-Ara*f*.

Table 2
Amino acid composition of MSd gum, compared to published data for gum arabic from *Acacia senegal* and *A. seyal* (Williams & Phillips, 2000)

Amino acid	Relative amount (mol%)		
	M. sinclairii	*A. senegal*	*A. seyal*
Protein (%)	1.7	2.3	1.0
Hyp	33.4	25.6	24.0
Asx	3.4	9.1	6.5
Thr	2.3	7.2	6.2
Ser	13.5	14.4	17.0
Glx	12.9	3.6	3.8
Pro	3.9	6.4	7.3
Gly	8.0	5.3	5.1
Ala	2.4	2.8	3.8
Cys	–	0.3	–
Val	2.1	3.5	4.2
Met	0.2	0.2	–
Ile	3.5	1.1	1.6
Leu	4.4	7.0	8.5
Tyr	–	1.3	1.3
Phe	2.8	3.0	2.4
His	3.3	5.2	5.1
Lys	2.5	2.7	1.8
Arg	1.5	1.5	1.1

Table 3
Comparison of the linkage compositions of carboxyl-reduced MSd gum and gum arabic, with the published data for the exudate gum from *Cussonia spicata* (Churms & Stephen, 1971)

Sugar	Deduced glycosyl linkage[a]	Relative amount (mol%)[b]		
		MSd	Gum arabic	*C. spicata*
Rha*p*	Terminal	9	10	3
Ara*p*	Terminal	8	1	10
Ara*f*	Terminal	23	22	30
	2-	tr	tr	tr
	3-	7	16	tr
	5-	9	–[c]	14
	2,5-	tr	–	tr
	2,3,5-	tr	–	–
Gal*p*	Terminal	tr	12	–
	3-	–	1	5
	4-	–	–	
	6-	tr	1	
	3,4-	–	1	–
	3,6-	19	18	24
	3,4,6-	9	12	–
Glc*p*	4-	1	1	–
GlcA*p*	Terminal[d]	8	1	7
	4-	6	8	6

[a] Terminal Rha*p* deduced from 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tetra-*O*-methylrhamnitolitol, etc.

[b] Average of duplicate determinations.

[c] –, Not detected.

[d] Presumed to be all 4-*O*-methylated.

3.3. NMR spectroscopy

The 1D ^{13}C and ^{1}H NMR spectra of MSd gum are compared with those of gum arabic in Figs. 2 and 3 and 2D HMQC COSY and DQF COSY spectra for MSd gum are shown in Figs. 4 and 5, respectively. The 2D HMQC TOCSY spectrum for MSd and the 2D spectra for gum arabic were recorded, but are not shown. The assignments for MSd gum and gum arabic are reported in Tables 4 and 5, respectively. The ^{13}C NMR spectra of both gums showed one group of C-1 signals from 108 to 110 ppm and a second group of C-1 signals from 103 to 104 ppm. In addition, MSd gum had two further C-1 signals at 102.0 and 101.1 ppm, while gum arabic had three additional C-1 signals at 101.1, 100.4 and 99.6 ppm. The ^{1}H NMR spectra for the two gums were similar and showed several H-1 signals in the 5.02–5.43, 4.69–4.81 and 4.38–4.48 ppm ranges. There were no carbonyl signals present in the ^{13}C NMR spectrum of MSd gum recorded at 70 °C, but when a spectrum was recorded at 90 °C two carbonyl signals were observed at 174.4 and 173.9 ppm, respectively. The ^{13}C NMR spectrum of gum

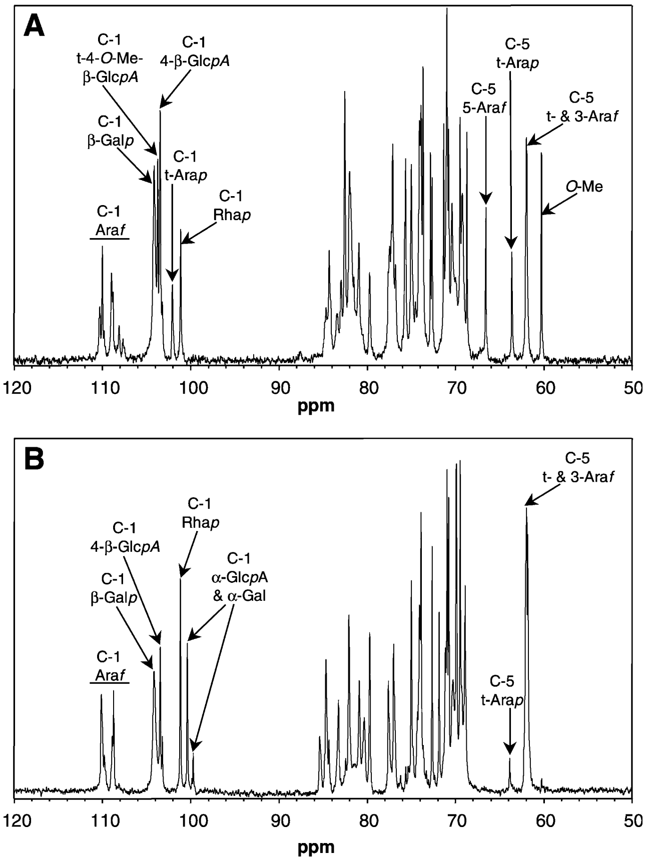

Fig. 2. ^{13}C-NMR of (A) MSd gum and (B) gum arabic.

arabic recorded at 70 °C contained one carbonyl signal at 175.3 ppm. Spectra of MSd gum contained one *O*-methyl signal (^{13}C 60.2 ppm, ^{1}H 3.48 ppm) and one *C*-methyl signal (^{13}C 17.2 ppm, ^{1}H 1.25 ppm), while only the *C*-methyl signal was present in gum arabic.

HMQC COSY spectra of both MSd gum (Fig. 4) and gum arabic contained C-1 resonances between 108.7 and 110.1 ppm with corresponding H-1 cross-peaks between 5.22 and 5.43 ppm that due to their downfield position were deduced to represent α-L-Ara*f* residues. MSd gum contained an additional C-1 resonance at 108.1 ppm with a corresponding H-1 cross-peak at 5.08 ppm that was also deduced to represent an α-L-Ara*f* residue. Linkage analysis data showed that in addition to terminal Ara*f* and 3-Ara*f* that was present in both MSd gum and gum arabic, MSd gum contained 5-Ara*f*, and thus these latter resonances were deduced to represent this residue (Table 3). This was also consistent with NMR assignments for 5-α-L-Ara*f* of arabinogalactan from the leaves of *Nerium indicum* (Dong & Fang, 2001) and arabinomannan from *Mycobacterium bovis* (Nigou, Gilleron, Brando, Vercellone, & Puzo, 1999). In addition to the deduced 5-α-L-Ara*f* H-1 resonance at 5.08 ppm, the HMQC COSY spectrum of MSd gum contained a second resonance with C-1, H-1 102.0/5.08 ppm. The linkage analysis of carboxyl-reduced MSd gum identified 8 mol% terminal Ara*p* (Table 2), also identified as a component of AGs from *Larix dahurica* (Odonmazig, Ebringerova, Machova, & Alfoldi, 1994), *Larix occidentalis* (Ponder & Richards 1997) and *Anadenthera colubrina* (Delgobo et al., 1999). In the two *Larix* species the terminal Ara*p* was identified as β-Ara*p* and, specifically, as β-L-Ara*p* by Ponder and Richards (1997). The C-1 resonance for the β-L-Ara*p* residue was at 101.2 ppm (Ponder & Richards, 1997). The C-1 resonance for methyl β-L-Ara*p* (102.1–102.5 ppm) is several ppm upfield of that for methyl α-L-Ara*p* (105.8–106.5 ppm) and

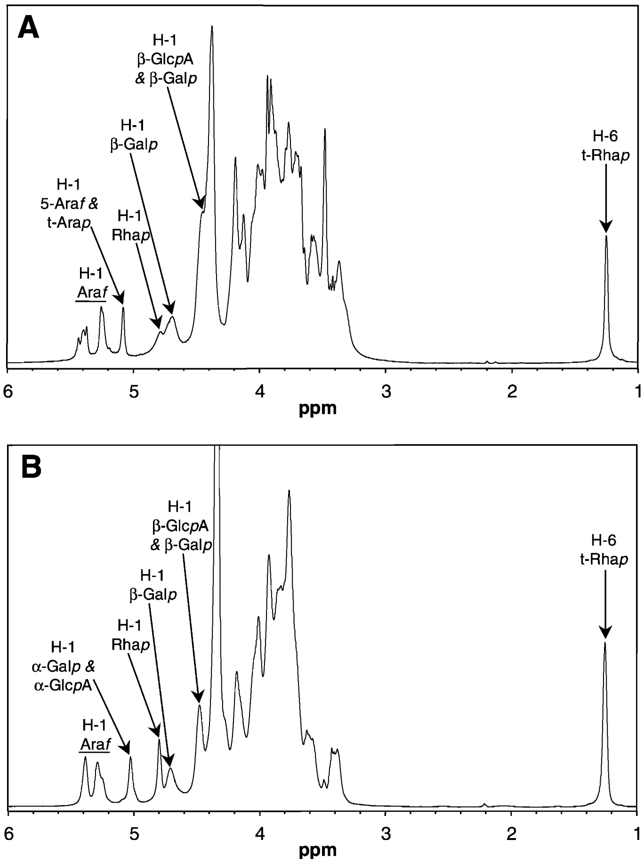

Fig. 3. ^{1}H-NMR of (A) MSd gum and (B) gum arabic.

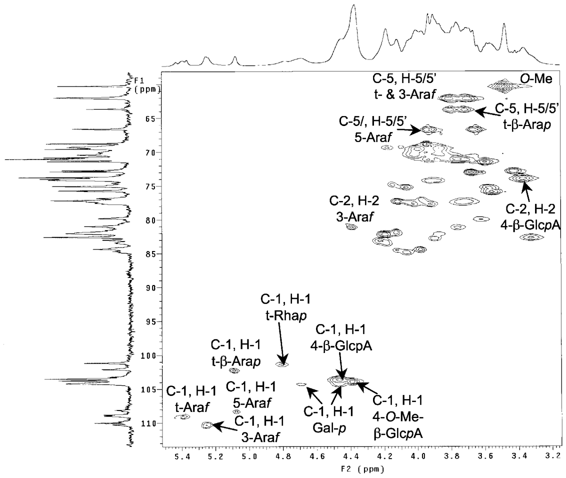

Fig. 4. Double quantum filtered COSY of MSd gum.

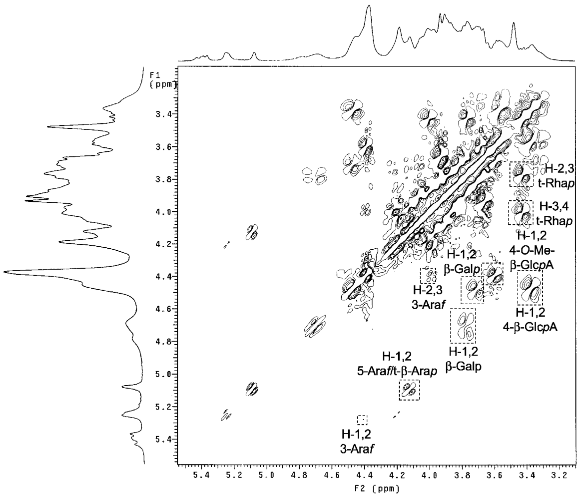

Fig. 5. Heteronuclear multiple quantum coherence COSY of MSd gum.

the H-1 resonance for methyl β-L-Ara*p* (5.00 ppm) is downfield of that for methyl α-L-Ara*p* (4.58 ppm) (Joseleau, Chambat, Vignon, & Barnoud, 1977; Mitzutani, Kasai, & Tanaka, 1980). Thus, the second C-1, H-1 resonance at 102.0/5.08 ppm was deduced to be terminal β-L-Ara*p*.

The remaining resonances deduced to represent α-L-Ara*f* residues were due to terminal α-L-Ara*f* and 3-α-L-Ara*f*. Some authors have assigned the C-1 resonance for terminal α-L-Ara*f* 0.9–1.0 ppm downfield from that of 3-α-L-Ara*f* (Odonmazig et al., 1994; de Pinto, Martinez, Ocando, & Rivas, 2001; Ponder & Richards, 1997), while others have assigned the C-1 resonance for terminal α-L-Ara*f* upfield from that of 3-α-L-Ara*f* (Delgobo et al., 1999; Tischer et al., 2002). The C-3 resonance of 3-α-L-Ara*f* has been assigned at 84.5–85.3 ppm, while that for terminal α-L-Ara*f* has been assigned at 77.6–77.8 ppm. Thus, a resonance in the HMQC TOCSY spectrum of gum arabic at 85.4 ppm that correlated with a C-1, H-1 cross-peak at 110.1/5.29 ppm was deduced to be C-3 of an α-L-Ara*f* residue, and thus this system was assigned as 3-α-L-Ara*f*. Similarly, a resonance at C-1, H-1 108.9/5.39 ppm was correlated with a deduced C-3 at 77.6 and this system was assigned as terminal α-L-Ara*f*. The corresponding anomeric resonances in the spectra for MSd gum were assigned to terminal α-L-Ara*f* and 3-α-L-Ara*f*. Resonances in the spectra for the two gums at C-1, H-1 110.0/5.25 ppm could not be assigned further. Thus, we conclude that the C-1 resonance for a terminal α-L-Ara*f* residue is in fact ca. 1 ppm upfield of that for 3-α-L-Ara*f* residue in our gums.

The ^{13}C spectrum of MSd gum had three distinctive resonances at 61.9, 63.6 and 66.5 ppm arising from C-5 of Ara residues with corresponding ^{1}H cross-peaks at 3.71/3.81, 3.82/3.81 and 3.66/3.93 ppm, respectively. The ^{13}C spectrum of gum arabic had two major resonances at 61.8 and 62.9 ppm with corresponding ^{1}H cross-peaks at about 3.68/3.84 ppm and a minor resonance at 63.8 ppm with corresponding ^{1}H cross-peaks at 3.71/3.97 ppm. The C-5 resonances for terminal Ara*f* and 3-Ara*f* have been assigned in the region 61.6–62.4 ppm (Delgobo et al., 1999; Odonmazig et al., 1994; Ponder & Richards, 1997), and thus similar resonances in the spectra of MSd gum and gum arabic were assigned to terminal α-L-Ara*f* and 3-α-L-Ara*f*.

Table 4
Summary of ^{13}C and ^{1}H NMR chemical shifts (δ, ppm) for MSd gum

Residue	Deduced linkage	Chemical shifts (ppm)					
		C-1 H-1	C-2 H-2	C-3 H-3	C-4 H-4	C-5 H-5/5′	C-6 H-6/6′
α-L-Ara*f*	3-	110.2	80.9	84.2	–	61.7	
		5.27	4.40	3.99	–	3.81/3.71	
	?	110.0	81.9	–	–	61.7^{a}	
		5.25	4.20	–	–	3.81/3.71^{a}	
	Terminal	108.8	–	–	61.7		
		5.39	–	–	–	3.81/3.71	
	5-	108.1	81.7^{a}	–	–	66.5	
		5.08	4.13^{a}	–	–	3.93/3.66	
β-L-Ara*p*	Terminal	102.0	81.7^{a}	–	–	63.4	
		5.08	4.13^{a}	–	–	3.81/3.72	
β-D-Gal*p*	3,6-^{b}	104.1	–	–	–	–	68.3^{a}
		4.47	3.72	–	–	–	4.00/3.87^{a}
	3,4,6-^{b}	104.1	–	–	–	–	70.3^{a}
		4.70	3.78	–	–	–	4.02/3.87^{a}
β-D-Glc*p*A	Terminal	103.7	71.3	–	–	–	–
	4-*O*-Me	4.38	3.60	–	–	–	–
	4-	103.5	73.7	75.0	–	–	–
		4.45	3.37	3.57	–	–	–
α-L-Rha*p*	Terminal	101.1	–	–	72.6	69.3	17.2
		4.80	–	3.78	3.44	4.01	1.25

[a] Assignments are only tentative.
[b] Assignments are interchangeable

Table 5
Summary of ^{13}C and ^{1}H NMR chemical shifts (δ, ppm) for gum arabic

Residue	Deduced linkage	Chemical shifts (ppm)					
		C-1 H-1	C-2 H-2	C-3 H-3	C-4 H-4	C-5 H-5/5′	C-6 H-6/6′
α-L-Ara*f*	3-	110.1	80.9	85.4	83.2	61.9	
		5.29	4.38	3.95	4.28	3.68/3.84	
	?	109.8	–	–	–	–	
		5.25	–	–	–	–	
	Terminal	108.7	82.1	77.6	77.0	61.8	
		5.39	4.18	3.92	3.97	3.68/3.84	
	Terminal	108.1	–	–	–	–	
		5.39	–	–	–	–	
β-D-Gal*p*	3,6-[a]	104.1	–	–	–	–	–
		4.46	3.72	–	–	–	–
	3,4,6-[a]	104.1	–	–	–	–	–
		4.70	3.78	–	–	–	–
α-D-Gal*p*	?	99.7	68.9[b]	–	–	–	–
		5.03	3.83[b]	–	–	–	–
α-D-Glc*p*A	?	100.3	68.9[b]	–	–	–	–
		5.02	3.83[b]	–	–	–	–
β-D-Glc*p*A	4-	103.4	74.1	75.0	79.7	70.9/77.1	175.3
		4.48	3.38	3.58	3.62	3.76	–
α-L-Rha*p*	Terminal	101.1	–	70.9	72.6	69.5	17.2
		4.80	–	3.76	3.43	4.00	1.25

[a] Assignments are interchangeable
[b] Assignments are only tentative.

These authors assigned C-5 of β-Ara*p* in the region 63.7–64.5 ppm and thus the resonance at 63.6 ppm in the ^{13}C spectrum of MSd gum and the weak resonance at 63.8 ppm in the ^{13}C spectrum of gum arabic was deduced to be C-5 of terminal β-L-Ara*p*. The C-5 resonance of 5-α-L-Ara*f* was shown to be 5–6 ppm downfield of those for α-L-Ara*f* not substituted at C-5 (Joeseleau et al., 1977). Taken together with the absence of 5-Ara*f* in gum arabic from methylation analysis and no resonance at 66–67 ppm in the ^{13}C spectrum, the resonance at 66.5 ppm in the ^{13}C spectrum of MSd gum was deduced to be C-5 of 5-α-L-Ara*f*.

^{13}C resonances between 103.4 and 104.1 ppm which were correlated with ^{1}H resonances between 4.37 and 4.70 ppm in spectra of both gums were initially deduced to be β-Gal*p* residues. Linkage analysis showed that both MSd gum and gum arabic contain similar amounts of 3,6-Gal*p* and 3,4,6-Gal*p*, with gum arabic also containing terminal Gal*p*. The DQF–COSY spectrum for gum arabic showed three C-1/H-1 cross-peaks (104.1/4.46, 104.1/4.70 and 103.4/4.48 ppm) and the HMQC-COSY showed H-1/H-2 cross-peaks (4.70/3.78, 4.48/3.38, 4.46/3.72 ppm). In addition to these resonances, MSd gum had additional cross-peaks at 103.7/4.38 ppm in the DQF–COSY spectrum (Fig. 4) and 4.38/3.60 ppm in the HMQC–COSY spectrum (Fig. 5). In previous studies of gum arabic and other arabinogalactans the H-2 resonances in 3,6-β-Gal*p* and 3,4,6-β-Gal*p* residues have been assigned at 3.65–3.76 ppm (Delgobo et al., 1999; Gane et al., 1995; Ponder & Richards, 1997; Tischer et al., 2002). The H-1 resonances at 4.70 and 4.46/4.47 ppm in both gums were thus deduced to represent β-D-Gal*p* residues, although it was not possible to distinguish the different linkages. Delgobo et al. (1999) and Tischer et al. (2002) assigned a H-1 resonance at 4.50 ppm with H-2 at 3.38 ppm to 4-β-GlcA*p*. Thus, the H-1 resonances for MSd gum and gum arabic at 4.45–4.48 ppm that were correlated with H-2 signals at 3.37–3.38 ppm were deduced to be 4-β-GlcA*p*. In addition to 4-β-GlcA*p*, linkage analysis showed that MSd gum also contained terminal 4-*O*-Me-GlcA*p*. This residue was almost absent from gum arabic. The C-1, H-1 resonances for 4-*O*-Me-α-D-GlcA*p* of xylans from luffa fruit fibres, jute bast fibres and quince tree seed mucilage have been assigned at 98.8–98.9/5.09–5.12 ppm (Vignon & Gey, 1998). A C-1 resonance at 99.8 ppm for the AG gum exudate from *Spondias purpurea* has also been assigned to 4-*O*-Me-α-D-GlcA*p* (de Pinto et al., 1996). Such resonances were not present in the NMR spectra of MSd gum. As the β-D-Gal*p* residues and 4-β-GlcA*p* residue were assigned in both MSd gum and gum arabic, the C-1/H-1 resonance at 103.7/4.38 ppm in the DQF–COSY spectrum (Fig. 4) and the H-1/H-2 4.38/3.60 ppm in the HMQC–COSY spectrum of MSd gum, that was not present in the spectrum gum arabic, was tentatively assigned to terminal 4-*O*-Me-β-GlcA*p*. Further experiments will be necessary to confirm this assignment.

^{13}C and ^{1}H resonances at 17.2 ppm and 1.25 ppm, respectively, in the spectra of MSd gum and gum arabic were deduced to be from the *C*-methyl group of α-Rha*p*. Strong correlations in DQF–COSY spectra enabled H-5 to

H-3 of this residue to be assigned at 4.01, 3.44 and 3.78 ppm, respectively. From these assignments, the HMQC COSY spectrum enabled C-3 to C-5 to be assigned (Tables 4 and 5). These assignments were consistent with previous assignments for α-Rha*p* (Delgobo et al., 1999; Oxley & Wilkinson, 1992). Unfortunately, H-2 and C-2 of this residue could not be assigned and thus unambiguous identification of H-1 and C-1 was not possible. Anomeric resonances for terminal α-L-Rha*p* have been assigned at H-1 4.74–4.78 ppm and C-1 101.2–101.5 (Delgobo et al., 1999; McIntyre, Ceri, & Vogel, 1996; Tischer et al., 2002). Resonances at C-1, H-1 101.1/4.80 ppm in the HMQC COSY of MSd gum (Fig. 4) and gum arabic were thus assigned to terminal α-L-Rha*p*.

There were two resonances in the HMQC–COSY spectrum of gum arabic (C-1, H-1 100.3/5.02 and 99.7/5.03) that were not present in the spectrum of MSd gum. McIntyre et al. (1996) assigned a resonance at C-1, H-1 100.8/5.05 to α-D-Glc*p*A and Tischer et al. (2002) assigned a resonance at C-1,H-1 100.5/5.02 to α-D-Gal*p*. Based on these data, the two resonances in the HMQC–COSY spectrum of gum arabic were assigned to α-D-Glc*p*A and α-D-Gal*p*, but it was not possible to distinguish them unequivocally. Thus, gum arabic contained Glc*p*A residues in both the α- and β-configurations. MSd gum contained both 4-Glc*p*A and terminal 4-*O*-Me-Glc*p*A (Tables 1 and 2), but NMR assignments indicate that both of these residues were in the β-configuration.

4. Conclusions

MSd gum exuded from the bark of *Meryta sinclairii* (Araliaceae) is an extremely high-molecular-weight AGP. SEC-MALLS showed that the gum had an weight average molecular weight (M_w) of 4.45×10^6 Da, compared with M_w 6.02×10^5 Da for gum arabic. This is in distinct contrast to the estimated molecular weight of the gum isolated from *C. spicata* (M_w ~125,000 Da), another member of the family Araliaceae (Churms & Stephen, 1971). Exuded AG type gums from *Acacia* species show a wide range of molecular weights ranging from M_w 12,000 for *A. cyclops* and *A. pycnantha* to M_w 2.3×10^6 for *A. arabica* (Stephen & Churms, 1995).

Constituent sugar, linkage and NMR analysis showed that although MSd gum and gum arabic are both type II arabinogalactans there are distinct structural features that distinguish them. Both gums probably consist of a protein core with *O*-linked, highly branched 1,3-galactan substituents, but with different side-chain structures. While both gums have side-chains containing 3-Ara*f* and 4-GlcA*p*, terminated with Ara*f* and Rha*p*, MSd gum also has side-chains containing 5-Ara*f* and terminal GlcA*p*. Terminal Gal*p* residues present in gum arabic are absent from MSd gum. It appears that MSd gum shares some structural features with the gum from *C. spicata*, namely the presence of similar amounts of terminal Ara*f* and terminal GlcA*p* (Churms & Stephen, 1971). However, while both 3-Ara*f* and 5-Ara*f* were present in MSd gum, the gum from *C. spicata* contained only 5-Ara*f*, and the 3,4,6-Gal*p* present in MSd gum, was absent from the gum from *C. spicata*.

The high molecular weight and complex composition of MSd gum make detailed analysis of its structure difficult. Many structural features of AGs from other species have been deduced by using controlled degradation techniques, such as enzymic hydrolysis, partial acid hydrolysis, Smith degradation and alkali degradation (Dong & Fang, 2001; Odonmazig et al., 1994; de Pinto et al., 1996; 2001; 2000; Saulnier, Brillouet, Moutounet, de Penhout, & Michon, 1992). Recently, structural features of the gum from *Anadenanthera colubrina* (Delgobo et al., 1999) and gum arabic (Tischer et al., 2002) have been characterised by isolating and analysing the structure of oligosaccharides which are naturally present in the gum. The use of similar degradative and analytical techniques could enable detailed structural features of MSd gum to be determined.

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