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The core carbohydrate structure of *Acacia seyal var. seyal* (Gum arabic)

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ABSTRACT

The structure of gum arabic (*Acacia seyal*) has been studied using methylation analysis and 2D (COSY, TOCSY, HMQC and HMBC) NMR spectroscopy. Galacturonic acid (13.66%) is a major component not previously identified. The backbone is made up of 1,3-linked galactopyranosyl (Gal*p*) residues substituted at O-2, O-6 or O-4 positions, which are terminated with mainly arabinofuranosyl (Ara*f*), galacturonopyranosyl (Gal*p*A), rhamnopyranosyl (Rha*p*), but occasionally with arabinopyranosyl (Ara*p*), and glucuronopyranosyl (Glc*p*A) residues. There are long side chains of →3)-α-L-Ara*f*-(1→ and →2)-α-L-Ara*f*-(1→ linked to the backbone.

1. Introduction

The commercial Acacia gums found in Africa fall into two taxonomic series, Gummiferae Benth (=subgen Acacia vas.) and Vulgares Benth: (=subgen Aculeiferum vas.) as originally proposed by Bentham in 1850 and subsequently refined by others (Jurasek, Varga, & Phillips, 1995; Ross, 1979). *Acacia senegal* falls into the Vulgares series and *Acacia seyal* into the Gummiferae series but in regulatory terms both are considered to be "gum arabic". The Codex Alimentarius Commission at its 23rd Session in Rome, 28 June–3 July 1999 adopted the following substantive definition of gum arabic "Gum arabic is a dried exudation obtained from the stems and branches of *A. senegal* (L) or *A. seyal* (fam. Leguminosae)" (Dondain & Phillips, 1999; FAO/WHO, 1999). There are important differences between the two species of gum arabic which can be summarized as follows (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005):

***Acacia seyal* (Gummiferae)**

Positive optical rotation.
Low viscosity.
Arabinose/galactose ratio > 1.
Low rhamnose.

***Acacia senegal* (Vulgares)**

Negative optical rotation.
More acidic.
More viscous.
Higher proportion of rhamnose.
Arabinose/galactose ratio less than 1.
Higher proportion of rhamnose.

The structure of *A. senegal* has recently been significantly revised as a result of a new recent structural investigation (Nie et al., 2012). However, despite a significant number of investigations the current position is that there is still no detailed identified structure for *A. seyal*. (Anderson, Dea, & Hirst, 1968; Elmanan, Al-Assaf, Phillips, & Williams, 2008; Flindt, Al-Assaf, Phillips, & Williams, 2005; Hassan, Al-Assaf, Phillips, & Williams, 2005; Siddig et al., 2005).

The early work of Anderson's group (Anderson et al., 1968) could not distinguish any different fundamental linkages between *A. seyal* and *A. senegal*. However, they noted that long chains of periodate-resistant β 1-3-linked galactose residues are not such a dominant structural feature of *A. seyal* as for *A. senegal*. It was suggested that *A. seyal* has a galactan framework which is more highly branched than *A. senegal*. In these studies also, Anderson noted a considerable variation in composition between different nodules of well-

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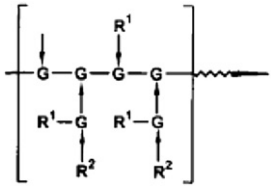
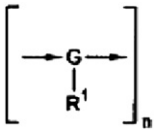
authenticated *A. seyal*. In a re-interpretation of previous work this group again suggested that *A. seyal* is more highly branched than *A. senegal* (Street & Anderson, 1983). They considered that the available data relating to the difference between the structure of *A. senegal* and *A. seyal* could be explained in terms of two basic core structures (Types 1 and 2) (Street et al., 1983).

Type 1 repeat unit: R^1 is an arabinose chain of varying length; R^2 may be H, U = Uronic acid or U − *R*; at the end of a branch.



Type 2 repeat unit: R^1 is an arabinose chain as in Type 1



A. senegal is suggested to contain only Type 1 repeat units whereas *A. seyal* consists of small blocks of two or three modified Type 1 repeat units separated by significant blocks of Type 2 repeat units. *A. seyal* is proposed, therefore, to be more highly branched than *A. senegal*.

The historical conclusion, therefore, is that *A. seyal* is highly branched polysaccharides with backbone composed of β-1,3,6-linked galactose residues with the branching points at 3-O or 6-O positions (Anderson & Herbich, 1963). Here the structure of the bulk carbohydrate component of *A. seyal* is re-visited. The techniques used are methylation analysis and 2D NMR spectroscopy, including homonuclear $^1H/^1H$ correlation spectroscopy (COSY, TOCSY), and heteronuclear $^{13}C/^1H$ multiple quantum coherence experiments (HMQC, HMBC). Significant structural and compositional differences have been found in relation to the historical proposals about *A. seyal* structure.

2. Materials and methods

2.1. Materials

A. seyal var seyal was used in this investigation. It will be referred to elsewhere in this paper also as *A. seyal*. Authenticated samples were provided by Phillips Hydrocolloids Research Centre, Wales, UK.

2.2. Chemical composition of A. seyal

Monosaccharide composition was determined as previously described (Nie et al., 2011): *A. seyal* samples were hydrolyzed with H_2SO_4 at 100 °C for 3 h, diluted, and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Nie et al., 2011). Uronic acid was measured using an enzymatic-HPLC method (Nie et al., 2011). The sample was dissolved in 0.5 mL 50 mM sodium acetate buffers at room temperature and 0.5 mL of driselase was added. After incubation for 48 h, the mixture was diluted, and filtered using 0.45 μm filters. The samples were quantitatively analyzed by injection to the HPAEC-PAD and compared with authentic, galacturonic acid and glucuronic acid standards (Nie et al., 2011).

2.3. Methylation and GC–MS study of A. seyal

Uronic acids were reduced to neutral polysaccharides according to the previously reported procedure (Kang, Cui, Chen, et al., 2011; Taylor & Conrad, 1972). Methylation analysis was carried out using the method of Ciucanu and Kerek (Ciucanu & Kerek, 1984) with a slight modification, as described (Nie et al., 2011). Aliquots of PMAA were injected into the GC–MS system (ThermoQuest Finnigan, San Diego, CA) fitted with an SP-2330 (Supelco, Bellefonte, Pa) column (30 m × 0.25 mm, 0.2 μm film thickness, and were first heating to 160–210 °C at 2 °C/min, and then 210–240 °C at 5 °C/min). The system was equipped with an ion trap MS detector (Nie et al., 2011).

2.4. NMR analysis

After drying in a vacuum oven (~80 °C) for 6 h, the sample was dissolved in 5 mL deuterium oxide (D_2O) (~4%, w/v) and freeze dried. This procedure was repeated three times to completely replace H with D, with the sample being finally dissolved and kept in D_2O at room temperature for 3 h before NMR analysis.

Both ^1H and ^{13}C spectra were recorded on a Bruker AMX 500FT spectrometer. The spectra of ^1H, ^{13}C, and homonuclear $^1H/^1H$ correlation experiments (COSY, TOCSY), and Heteronuclear Multiple–Quantum Correlation (HMQC) and Heteronuclear Multiple Bond Correlation (HMBC) experiments were carried out at 25 °C.

3. Results and discussion

3.1. Chemical composition of A. seyal

A. seyal is made up of arabinose, galactose, rhamnose, galacturonic acid and glucuronic acid and can thus be regarded as mainly an arabinogalactan with uronic acids attached (Table 1). The previously reported 4-O-methyl-D-glucuronic acid was not found and galacturonic acid (13.66%) was present in greater amount than previously reported. The amounts of arabinose, galactose, rhamnose and glucuronic acid were in accord with previous reports (Anderson et al., 1968; Flindt et al., 2005). There are some differences from the previously reported on molar ratio of monosaccharides, which could be due to the natural variability which has been previously associated with *A. seyal*. In particular, the ratio of galacturonic acid (13.66%) to glucuronic acid (0.64%) is different from previous results (Anderson et al., 1963; Anderson et al., 1968; Flindt et al., 2005). This difference could be due to the previously used colorimetric method for uronic acid determination, which does not differentiate galacturonic acid from glucuronic acid (Flindt et al., 2005).

3.2. Methylation analysis of A. seyal

The high uronic acids content found makes it difficult to carry out the methylation analysis, since uronic acids are liable to β-elimination in alkali conditions, and are generally resistant to acid hydrolysis. Therefore, the linkage between uronic acids and neutral sugars could be lost during methylation. Reducing the carboxyl groups can avoid this problem (Cui, 2005, chap. 3). Therefore, *A. seyal* was first reduced and then methylated and

Table 1
Chemical composition of *Acacia seyal*.

	Rhamnose (%)	Arabinose (%)	Galactose (%)	Galacturonic acid (%)	Glucuronic acid (%)
Acacia seyal	1.79%	35.48%	27.33%	13.66%	0.64%