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Some properties of a polysaccharide preparation that is isolated from the fruit of *Cordia abyssinica*

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Abstract

An acidic polysaccharide gum was isolated from the fruit of *Cordia abyssinica* by extraction with alkali and precipitation with acid. The polysaccharide preparation had a specific optical rotation of −50°, moisture content of 9.1%, ash content of 0.7% and a uronic acid content of 8.7%. In the acid hydrolysates of *C. abyssinica* polysaccharide, galactose was identified by HPLC to be the main monosaccharide released (27%), followed by rhamnose (21%) and mannose (17%). Xylose (11%), glucose (10%), arabinose (9.5%) and uronic acids (5%) were also components of the polymer. At present, it is not clear whether the small amount of protein present (2.6%) was covalently bound or not. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Plant hydrocolloids, long chain high molecular weight polymers that dissolve or disperse in water to give a thickening, stabilising or gelling effect, are generally polyuronides composed of more than one type of monosaccharide unit (Sharma, 1981; Zapsalls & Berck, 1985). Hydrocolloids share similar characteristics that are responsible for their ability to form viscous solutions in water and dispersions that possess suspending and stabilizing properties. The polymers are used to improve or manipulate the texture of food products because of their ability to retard flow, modify gelling characteristics, and preserve emulsions and suspensions (Balmaceda, Rha, & Huang, 1973). Except for gelatin, most hydrocolloids that are used in industry are polysaccharides of plant origin.

The effectiveness of hydrocolloids, in modifying the rheological properties of solutions, depends on the distribution of molecular weight in a given polymer, the degree of hydration of the molecules, the extent of interaction with different molecules or molecules of the same type, concentration of the polymer and environmental conditions, such as temperature and the presence of other types of molecules (Krumel & Sarkar, 1975).

Cordia abyssinica is an evergreen shrub or small tree that grows at low altitude, in warm, moist riverine bushveld of southern Africa (Van Wyk & Van Wyk, 1998). The fruit of *C. abyssinica* is a drupe about 30 mm in diameter which is green when unripe and turns yellow on ripening. The fruit pulp, a sweet mucilaginous flesh that is highly viscous, has been used as glue by rural school children because of its excellent adhesive properties. Although not widely consumed by humans, the fruit is used as food by monkeys and other wild animals.

In preliminary studies we have shown that, at low temperature and concentrations of at least 1.5%, the polysaccharide that is isolated from the fruit of *C. abyssinica* can form gels in water (Benhura & Katayi, 2000). The viscosity and solubility properties of the polysaccharide in aqueous solutions have been studied (Benhura & Chidewe, 2000). No information is available on the chemical structure of *C. abyssinica* polysaccharide.

An understanding of the structure and functional properties of the gum would be necessary in order to most effectively exploit the gum. An understanding of the properties of the polysaccharide of *C. abyssinica* should contribute towards our understanding of the origin of adhesiveness in polysaccharides. Methods that are available for the analysis of complex polysaccharides are based mainly on determination of their constituent sugar residues, obtained after chemical hydrolysis of the native polymers (DeRuiter, Schol,

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Voragen, & Rombouts, 1992). Analysis of the composition of polysaccharides has typically been based on hydrolysis procedures using hydrochloric, sulphuric or trifluoroacetic acid, at elevated temperatures (Yu lp, Manam, Hepler, & Hennessey, 1992). Trifluoroacetic acid has become the preferred acid for most carbohydrate analysis due to its effectiveness in hydrolysing glycosidic bonds, without causing extensive destruction of the resulting monosaccharide components and due to its volatility, which minimises its interference with subsequent procedures such as acetylation of the monosaccharide components.

Uronic acids are often involved in very acid-resistant glycosidic linkages, such that the solubilisation and hydrolysis of uronic acid-containing polymers is incomplete. The incomplete hydrolysis of acidic polysaccharides is reported to be due to the unusual stability of the glycosyl uronic acid linkage, which hinders quantitative depolymerisation under normal acid hydrolysis conditions (Selvendran et al., 1979). The factors affecting hydrolysis of polyuronides have been reviewed by Dutton (1973).

Procedures that are commonly applied for quantification of the liberated sugars have disadvantages, mainly originating from the necessity for derivatisation of the monosaccharide to alditol acetates and trimethyl silyl ethers or inadequate separation by HPLC of the monosaccharides. In addition, the effectiveness of the hydrolysis method applied cannot be assessed after derivatisation, as oligomers resulting from incomplete hydrolysis often escape detection. A considerable improvement in the sensitivity of analysis of monosaccharides was obtained by the development of high performance anion-exchange chromatography, using pellicular resins and pulsed amperometric detection (PAD). With this method carbohydrates can be analysed accurately without any derivatisation, with a sensitivity of 0.1 nmol per injected amount (DeRuiter et al., 1992).

We set out to study the chemical composition and some of the physical properties of the polysaccharide that is isolated from the fruit of *C. abyssinica*. Such information would facilitate the use of the polysaccharide in food, pharmaceutical and other technical applications. Use of the polysaccharide in industrial and technical applications would contribute towards the sustainable use of plant resources.

2. Materials and methods

2.1. Collection of material and extraction of the gum

Unripe but otherwise mature fruits of *C. abyssinica* were collected between December and April, in south eastern Zimbabwe, transported to the laboratory, stored at 4 °C and processed within 7 days of collection.

The polysaccharide was extracted by first manually squashing the fruit to release the stones, which were covered in pulp. The stones were placed in a strong plastic or stainless steel container and agitated with a robust wooden stirrer, during which process the pulp cleanly separated from the stones as a thick sticky mass.

2.2. Precipitation of the polysaccharide with acid

The extracted pulp was suspended in Na_2CO_3 (1 M) with stirring. The final concentration of Na_2CO_3 was adjusted to 0.1 M using water and the mixture centrifuged in a BHG Hemle ZK 401 centrifuge at 6000 rpm for 30 min to remove debris. To precipitate the polysaccharide, 0.2 M HCl was added to the supernatant with stirring until no further precipitation could be observed. The precipitated polysaccharide was washed five times with water and freeze-dried in a Christ Alpha 2-4 freeze-drier.

2.3. Chemical analysis

Protein content of the polysaccharide preparations was analysed using the Kjeldahl method with the nitrogen content being multiplied by a factor of 6.25 to give crude protein (James, 1995).

Moisture and ash contents were determined using the method described by Rodriguez-Otero, Paseiro, Simal, Cepeda (1994) and James (1995). Uronic acids were analysed using the m-hydroxybiphenyl method with a galacturonic acid standard (Chaplin & Kennedy, 1986).

The optical rotation of solutions at concentrations up to 0.25% was measured on an Otago polax-D polarimeter using the D-line of polarised sodium light and a 100 mm cell (Ibrahim & Bangudu, 1992).

2.4. Determination of component sugars

The component monosaccharides were determined following the method used by Adams, with minor modifications (Adams, 1965). The polysaccharide (1.5 g) was suspended in 120 ml of 2.5% solution of sulphuric acid. The mixture was refluxed for 24 h with 40-ml samples being withdrawn after 7, 15, and 24 h. The 40-ml

Table 1
Summary of some properties of the polysaccharide isolated from *Cordia abyssinica* polysaccharide[a]

Parameter	Quantity
Moisture	9.1±0.9%
Ash	0.7±0.1%
Specific optical rotation	−50°±0.0
Protein	2.6±0.2%
Uronic acids	8.7±0.4%

[a] The uncertainties shown are standard deviations for at least three measurements.

aliquots were neutralised with barium carbonate, filtered and the filtrate concentrated to 10 ml in a rotary evaporator at 50 °C.

Hydrolysis with sulphuric acid was done using the standard Saeman method (De Ruiter et al, 1992). The sugars were released by treatment of 2 mg of polysaccharide with 225 μl of 12 M H_2SO_4 (72% w/w) for 1 h at 30 °C. Distilled water was added to give 1 M H_2SO_4 acid and the mixture was further heated for 3 h at 100 °C.

2.5. Hydrolysis of polysaccharide using 2 M trifluoroacetic acid (Stephen, Churms, & Vogt, 1990)

The polysaccharide (10 mg) was suspended in 1 ml of 2 M trifluoroacetic acid (TFA) in a screw-cap vial. The vial was tightly sealed, heated for up to 3 h at 120 °C allowed to cool and the contents centrifuged for 5 min at 2000 ***g***. The TFA in the supernatant was allowed to evaporate off in the fume hood and the remaining mixture was freeze-dried, after which the dried material was dissolved in 50 μl of distilled water. Samples of the hydrolysed mixtures were applied onto a Whatman linear K silica gel TLC plate, size 20×20. After developing the plates in ethylacetate: pyridine: water (20:7:5 v/v) the dry plates were sprayed with diphenylamine: aniline: phosphoric acid (5:5:1 v/v), dried and heated at 110 °C.

The hydrolysates were also analysed by HPLC using a Shimadzu HPLC system, a refractive index detector and a Zorbax NH_2 column. Acetonitrile–water (85:15 v/v) was used as the solvent at a flow rate of 2 ml/min and 20 μL of samples or standards were injected (Nikolov, Meager & Reilly, 1985; Supelco, 1985). The concentration of uronic acids in the hydrolysates was determined by the m-hydroxydiphenyl sulphuric acid method (Chaplin & Kennedy, 1986).

3. Results and discussion

3.1. Chemical analysis

Some of the chemical and physical properties of the gum that was isolated from *C. abyssinica*, are summarised in Table 1. In contrast to the polysaccharide, earlier isolated from *Azanza garkeana*, which was sticky, hygroscopic and difficult to store dry (Benhura, Mbuya, & Machirori, 1999), the polysaccharide preparation from *C. abyssinica* was easy to store in a dry state. Thus stickiness of the polysaccharide in aqueous solution was not necessarily associated with hygroscopic properties. It is noteworthy that, of the polysaccharides prepared in our laboratory, *Dicerocaryum zanguebarium* polysaccharide is neither sticky nor hygroscopic, that of *C. abyssinica* is sticky but not hygroscopic, and that from *A. garkeana* is both sticky and hygroscopic. Further study of the polysaccharide from *C. abyssinica* should contribute to an understanding of the adhesive properties of the polysaccharide in solution. The ash content of less than 1% is lower than that obtained for the polysaccharide from *D. zanguebarium* at 7.3% and *A. garkeana* at 7.6% (Benhura & Marume, 1993; Benhura et al., 1999). The protein content of 2.6% was slightly higher than that of *D. zanguebarium* at 2.1% and slightly lower than that of the arabinogalactan-protein that is a major component of the mucilage filling *Gladiolus* style canal, at 3% (Aspinall, 1983; Benhura & Marume, 1993). The uronic acid content of 8.7% was consistent with the acidic nature of the polysaccharide.

Table 2
Thin-layer chromatography on Whatman linear K silica gel plates, of sugars released on hydrolysis of *Cordia abyssinica* polysaccharide with sulphuric acid following the method of Adams, (1965)[a]

Sample	R_f value
Xylose	0.53±0.03
Galactose	0.19±0.02
Arabinose	0.37±0.01
Rhamnose	0.72±0.04
Sample at 7 h	0.53±0.04
Sample at 15 h	Smear from origin to rhamnose position
Sample at 24 h	Smear from xylose to rhamnose position

[a] Uncertainties shown are standard deviations for at least three measurements.

3.2. Hydrolysis using sulphuric acid

On hydrolysis, a substantial proportion of the polysaccharide material was very resistant to hydrolysis, with solid material remaining at the end of the hydrolysis procedure. Attempts to change the conditions of hydrolysis, in order to achieve complete hydrolysis of the polymer, were not successful. The degradation of the polysaccharide from *C. abyssinica* is probably complicated by its acidic nature. The hydrolytic degradation of polysaccharides depends on the solubility of the polymer, which in turn is influenced by pH, ionic strength, and the presence of any complexing ions. The incomplete hydrolysis of acidic polysaccharides arises from the unusual stability of the glycosyl uronic acid linkage, which hinders quantitative depolymerisation under normal acid hydrolysis conditions (Selvendran et al., 1979).

After the polysaccharide was hydrolysed with sulphuric acid for 7 h using the method of Adams, a single spot, corresponding to xylose, was observed as shown in Table 2. On increasing duration of hydrolysis to 15 h, a smear, that started from the origin to the position corresponding to rhamnose standard, was observed. The smear that was observed after 15 h of hydrolysis most likely arose from the production of oligometric fragments comprising a spectrum of different molecular

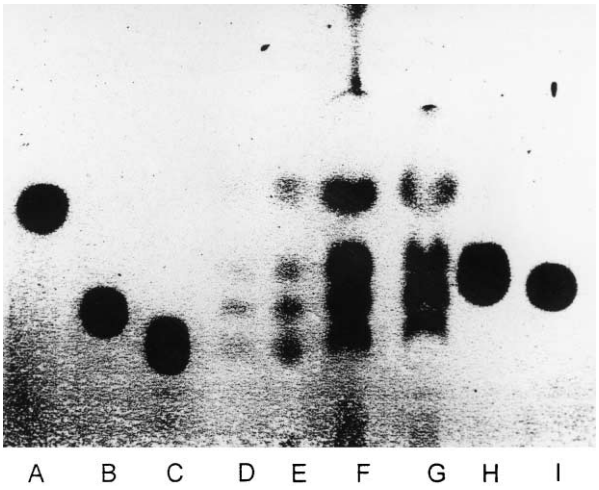

Fig. 1. Thin-layer chromatography of sugars released during the time dependent hydrolysis of *Cordia abyssinica* polysaccharide with 2 M TFA. TLC sheet: Whatman LK 5 silica gel plate 20×20. Solvent: Ethyl acetate–Pyridine–Water (20:7:5). Spray reagent: Diphenylamine–Aniline–Phosphoric acid (5:5:1). Lanes A, B, C, H, and I are standards xylose, glucose, galactose, arabinose and mannose, respectively. Lanes D, E, F and G are samples hydrolysed for 0.5, 1, 2 and 3 h respectively.

sizes. When hydrolysis was carried out for 24 h, a smear, that started from the position of xylose to the position of the rhamnose standard, was observed as the oligomers were further degraded.

When the polysaccharide was hydrolysed following the Saeman procedure, followed by TLC, only two spots were observed, one corresponding to rhamnose and a faint spot corresponding to galactose. The galactose was not detectable when the samples were analysed by HPLC, reflecting extreme resistance of the polysaccharide to acid hydrolysis.

3.3. Hydrolysis using 2 M TFA

It can be seen in Fig. 1 that hydrolysis with TFA was most effective in degrading the polysaccharide into component monosaccharides. The sugars galactose, glucose, arabinose, xylose and rhamnose were released, together with oligosaccharides and uronic acids occurring near and at the origin, respectively. An unidentified spot, that moved with the solvent front, was also observed. As the duration of hydrolysis was increased from 30 min to 2 h, there was a corresponding increase in the intensity of spots on the TLC chromatograms with maximum intensity being observed after 2 h of hydrolysis, as shown in Fig. 1. Increasing the duration of hydrolysis beyond 2 h resulted in decreased intensity of spots, reflecting the degradation of the sugars that had been released.

TLC and HPLC analyses of the samples that had been treated with trifluoroacetic acid for 2 h showed that the gum was composed of the monosaccharides, galactose, glucose, mannose, arabinose, xylose, rhamnose and uronic acids, as shown in Table 3. Analysis of hydrolysates by HPLC showed presence of galactose, rhamnose, mannose, xylose, glucose, arabinose and uronic acids in the ratio 27:21:17:11:10:9:5, respectively. In the TLC analysis, mannose and glucose were not distinguishable.

Table 3
High performance liquid chromatography on a Zorbax-NH2 column, of sugars released on hydrolysis of *Cordia abyssinica* polysaccharide with 2 M TFA[a]

Sample	Retention time	Identity
Glucose	7.00±0.13	
Galactose	7.70±0.01	
Arabinose	4.86±0.09	
Xylose	4.48±0.05	
Fucose	4.29±0.06	
Rhamnose	3.54±0.16	
Mannose	6.36±0.09	
Samples	3.54±0.16	Rhamnose
	6.38±0.07	Mannose
	4.52±0.05	Xylose
	4.78±0.2	Arabinose
	7.01±0.13	Glucose
	7.72±0.18	Galactose

[a] Uncertainties shown are standard deviations for at least five measurements.

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