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# Composition of the Water-Soluble Polysaccharide Isolated from the Leaves of *Cyclea burmanni*

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*ABSTRACT*

*The water soluble polysaccharide isolated from the leaves of* Cyclea burmanni *sets to a firm jelly at room temperature. It contained a high proportion of uronic acids and a small amount of neutral sugars. Galactose and arabinose are the major neutral sugars present. The high uronic acid content and the distribution of neutral sugars in the polysaccharide and partial hydrolysates suggest that the water soluble polysaccharide is probably a rhamnogalacturonan.*

## INTRODUCTION

*Cyclea burmanni* is a small woody twiner found in India and Sri Lanka (Jayaweera, 1982). It has been reported that the bruised leaf of this plant is used to stop bleeding from fresh wounds and also acts as a healing agent. In Sri Lanka the water extract of the leaves, which sets like a jelly at room temperature, is consumed as food in rural communities.

In this paper the distribution of uronic acid and neutral sugars present in the polysaccharide fractions isolated from the leaves of *Cyclea burmanni* is reported. The water soluble polysaccharide fraction showed characteristics of rhamnogalacturonan.

## MATERIALS AND METHODS

### Analysis of leaves

Leaves from *C. burmanni* were collected in Gampola (Sri Lanka), freeze dried and ground (Wiley mill) to pass a 1 mm screen, and the contents of



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dry matter, ash and crude protein were analysed by standard methods (AOAC Methods, 1980). The ground leaves were extracted with aqueous 80% ethanol and chloroform in a Soxhlet apparatus. The residue was analysed for starch Åman & Hesselman, 1984), Klason lignin (Åman & Nordkvist, 1983), and non-starch polysaccharide residues (Theander & Åman, 1979).

**Extraction and isolation of the polysaccharide fractions**

Fresh leaves (200 g, 72·2% DM (Dry Matter)) were chopped and extracted sequentially with each of the following reagents (2 × 1 litre) at room temperature for 2 × 8 h: 80% ethanol, distilled water, 2% aqueous EDTA and 10% NaOH.

*(i) Water soluble polysaccharides*
The viscous aqueous suspension was squeezed through a voile cloth, dialysed and concentrated under reduced pressure at < 40°C. The polymeric material was precipitated by the addition of acetone, dispersed in water, dialysed and freeze dried to obtain the crude water soluble polysaccharides (1·8 g).

*(ii) Aqueous EDTA soluble polysaccharides*
The EDTA extract was dialysed and freeze dried to give the crude EDTA soluble polysaccharides (0·7 g).

*(iii) Alkali soluble polysaccharides*
The NaOH extract was dialysed and freeze dried to give the crude alkali soluble polysaccharides (1·1 g).

The water, aqueous EDTA and alkali extracts were analysed for neutral sugar (Albersheim *et al.*, 1967) and uronic acid (Theander & Åman, 1979) content.

**Fractionation of the water soluble polysaccharides**

The crude polysaccharide (1·0 g) was dissolved in 0·1 M piperazine buffer (pH 6·5, 80 ml), introduced into a QAE-Sephadex (A-50) column (3·0 × 40 cm) and eluted with 0·1 M piperazine buffer. The absorbance (280 nm) of the effluent was monitored (UVICORD SII, LKB). Fractions (6 ml) were collected and their neutral sugar (Dubois *et al.*, 1956) and uronic acid (Blumenkrantz & Asboe-Hansen, 1973) contents were assayed. The column was then eluted successively with 0·3 M, 0·5 M and

1·0 M piperazine buffers (pH 6·5). Each fraction was monitored for carbohydrates (Albersheim *et al.*, 1967).

Fractions 1–4 obtained from the 0·1 M, 0·3 M, 0·5 M, and 1·0 M buffers respectively were dialysed, de-ionised (Dowex 50 $H^+$) and freeze dried. Each fraction was analysed for the neutral sugar composition by GLC of the derived alditol acetates.

**Partial acid hydrolysis**

The fraction eluted with 0·3 M piperazine buffer (40·5 mg) was treated with 0·05 M trifluoroacetic acid (2·5 ml) at 100°C for 1 h, neutralised ($BaCO_3$) and centrifuged.

**Fractionation of the partial hydrolysate**

The partially hydrolysed sample (25 ml) was fractionated on a Sepharose (CL-4B column (1·6 × 30 cm) by elution with water. The eluates (4 ml) were monitored for neutral sugars (Dubois *et al.*, 1956) and uronic acids (Blumenkrantz & Asboe-Hansen, 1973). The concentration in $\mu$g/ml was plotted against fraction number for neutral sugars and uronic acids respectively.

**Analysis of the column fractions by GLC**

Eluates obtained by gel-permeation chromatography (using the 0·3 M piperazine buffer) of the partial hydrolysate, which contained measurable amounts of neutral sugars and uronic acids, were separately air dried, hydrolysed with 2 M trifluoroacetic acid at 120°C for 1 h and each sample was analysed as the derived alditol acetate by GLC on an OV-225 capillary column at 190°C.

## RESULTS AND DISCUSSION

The chemical composition of the leaves of *C. burmanni* (Table 1) showed the presence of 34% non-starch polysaccharides, of which the major constituent was uronic acid (13·7%). Among the neutral sugars, high contents of glucose and xylose were detected. The crude protein content was quite significant.

The crude water-soluble polysaccharide isolated from the aqueous extract set to a firm jelly at room temperature. The neutral sugar compo-

**TABLE 1**
Chemical Composition of the Leaves, and the Water-Soluble Polysaccharides from *Cyclea burmanni*[a]

| *Component* | *Dried leaves* | *Water soluble crude polysaccharides* |
|---|---|---|
| Ethanol and chloroform soluble extractives | 30·1 | |
| Crude protein (N × 6·25) | 22·0 | 0·12 |
| Strach | 3·5 | |
| Non-starch polysaccharides | 34·4 | 1·20 |
| Rhamnose | 0·3 | 0·01 |
| Arabinose | 2·7 | 0·02 |
| Xylose | 5·9 | 0·00 |
| Mannose | 0·6 | 0·00 |
| Galactose | 1·3 | 0·04 |
| Glucose | 10·0 | 0·01 |
| Uronic acids | 13·7 | 1·12 |
| Klason lignin | 7·6 | |
| Ash | 5·3 | |

[a]% Dry matter of leaves.

**TABLE 2**
Chemical Composition of Polysaccharide Fractions Isolated from *C. burmanni*[a]

| *Component polysaccharides* | *Water-soluble extract* | *EDTA extract* | *NaOH extract* |
|---|---|---|---|
| Crude protein (N × 6·25) | 6·47 | 34·59 | 28·65 |
| Non-starch polysaccharides | 63·44 | 18·96 | 21·33 |
| Rhamnose | 0·32 | 2·41 | 1·24 |
| Arabinose | 1·24 | 2·41 | 2·11 |
| Xylose | 0·15 | 1·21 | 11·39 |
| Mannose | 0·21 | 1·05 | 1·34 |
| Galactose | 2·19 | 2·51 | 2·07 |
| Glucose | 0·44 | 1·69 | 3·18 |
| Uronic acids | 58·89 | 7·68 | 0·00 |

[a]% Dry matter of each extract.

sition and uronic acid analysis showed (Table 2) that it had a very high content of uronic acid (58·9%) and a small amount of neutral sugars (4·6%). Galactose and arabinose were the major neutral sugars present in the water-soluble polysaccharides. The high uronic acid content, ease of extraction with water and the neutral sugar composition indicate that the water-soluble polysaccharide is probably a rhamnogalacturonan. Table 1

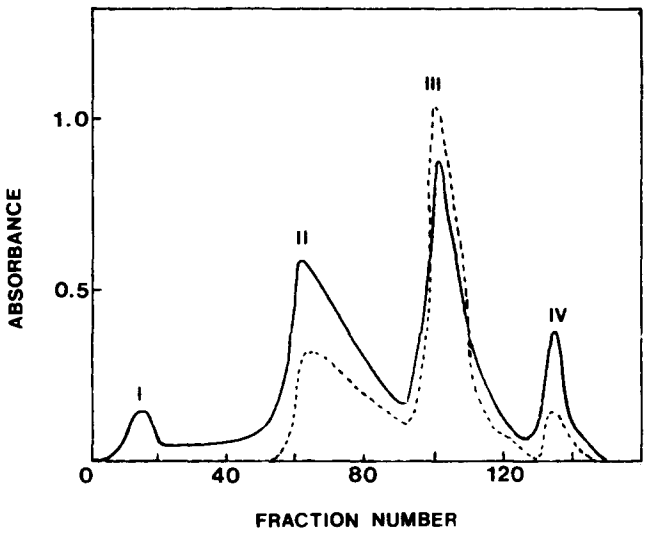


**Fig. 1.** Ion exchange chromatography of the water soluble polysaccharide on a QAE-Sephadex (A-50) column; (——) neutral sugars and (— — —) uronic acids.

shows that glucose and xylose were the more abundant neutral sugars present in the leaves. This indicates that the glucose- and xylose-rich polysaccharides, the xyloglucans, were not extracted with water. Xyloglucans are known to occur as a cell wall constituent associated with pectins (de Vries *et al.*, 1983).

Ion exchange chromatography of the crude water soluble polysaccharide on QAE-Sephadex gave four fractions which eluted with 0·1 M, 0·3 M, 0·5 M and 1·0 M piperazine buffers (pH 6·5) respectively (Fig. 1). The neutral sugar composition of the four fractions are given in Table 3. Glucose bleeding from the QAE-Sephadex column probably accounts for the unexpectedly high contents of glucose observed in each fraction. The relative composition of the neutral sugars without glucose is also given in Table 3. It can be seen that arabinose and galactose are the major neutral sugars in all four polymeric fractions. High amounts of rhamnose were observed in Fractions 2–4 which were eluted with relatively stronger base. This suggests that rhamnose probably forms an integral part of an inner chain which is constituted of uronic acids as in some pectins, which are easily extracted with water and have considerable gelling ability (Kennedy & White, 1979).

Fraction 2 was partially hydrolysed with 0·05 M trifluoroacetic acid for 1 h at 100°C. The partial hydrolysate was subjected to gel-permeation chromatography on a Sepharose CL-4B column and eluted with water.

**TABLE 3**
Relative composition of the neutral sugars in the water soluble polysaccharides and fractions 1–4[a]

| *Sample* | *Rhamnose* | *Arabinose* | *Xylose* | *Mannose* | *Galactose* | *Glucose* | *Galactose/Arabinose* |
|---|---|---|---|---|---|---|---|
| Water soluble polysaccharide | 7·02 | 27·19 | 3·29 | 4·61 | 48·03 | 9·87 | 1·8 |
| Fraction 1 | 5·12 | 22·78 | 0·88 | 4·24 | 41·25 | 25·73 | 1·8 |
| | (6·89) | (30·68) | (1·18) | (5·71) | (55·54) | — | (1·8) |
| Fraction 2 | 14·40 | 13·59 | 1·73 | 2·62 | 26·26 | 41·40 | 1·9 |
| | (24·57) | (23·20) | (2·94) | (4·47) | (44·82) | — | (1·9) |
| Fraction 3 | 13·66 | 19·51 | 8·78 | 7·80 | 26·83 | 23·41 | 1·4 |
| | (17·83) | (25·48) | (11·46) | (10·19) | (35·03) | — | (1·4) |
| Fraction 4 | 11·87 | 17·35 | 8·68 | 9·13 | 22·37 | 30·59 | 1·3 |
| | (17·11) | (25·00) | (12·50) | (13·16) | (32·24) | — | (1·3) |

[a]Figures in parentheses denote the relative composition without glucose.

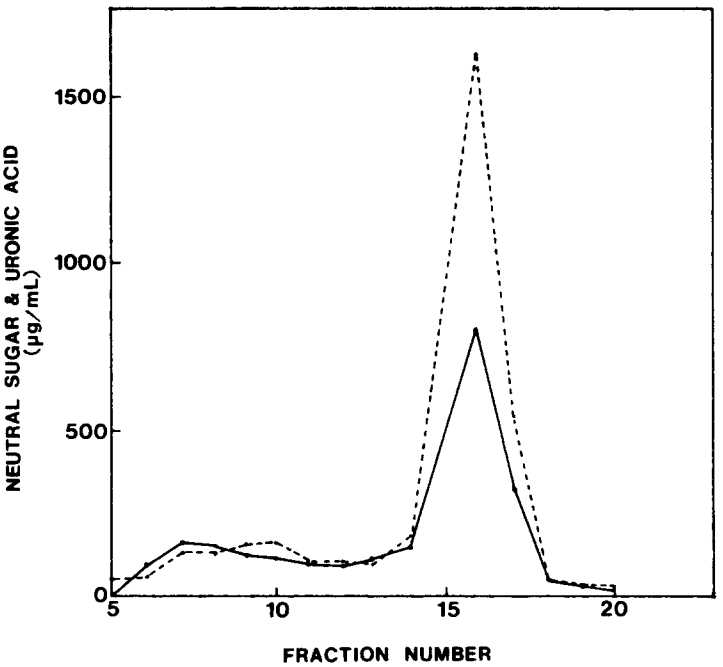


**Fig. 2.** Neutral sugar (——) and uronic acid (— — —) contents of the partial hydrolysate of Fraction 2 after gel permeation chromatography on Sepharose CL-4B.

The eluate when monitored for neutral sugars and uronic acid showed two peaks (Fig. 2). The neutral sugar composition of each fraction determined as the derived alditol acetates is shown in Figs 3A and 3B. The fractions which eluted close to the void volume ($V_o$) contained galactose as the major neutral sugar constituent. The contents of arabinose and glucose were significant, while small amounts of rhamnose, xylose, and mannose were detected. The fractions which eluted near the included volume ($V_i$) were richer in galactose, arabinose, rhamnose and glucose. The high contents of glucose eluted close to the void and included volumes is probably due to 'glucose bleeding' from the QAE-Sephadex column during ion exchange chromatography which was observed earlier (Table 3).

The distribution of rhamnose follows that of the uronic acids and may be considered as evidence for the presence of a rhamnouronan backbone. The pattern of distribution of arabinose and galactose and the way it is related to the distribution of rhamnose and uronic acids indicates the occurrence of neutral arabinogalactan side chains which are attached to the rhamnouronan backbone. The significant amounts of arabinose and

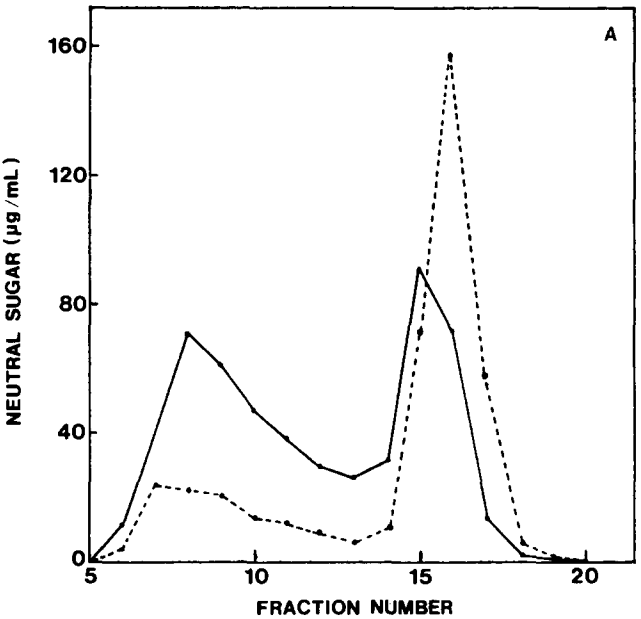


**Fig. 3A.** Galactose (——) and arabinose (— — —) contents of the partial hydrolysate of Fraction 2 after gel permeation chromatography on Sepharose CL-4B.

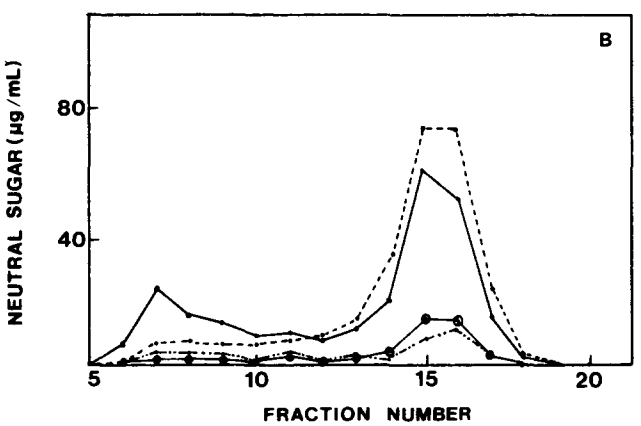


**Fig. 3B.** Glucose (——), rhamnose (— — —), mannose (o— — —o) and xylose (—··—··—) contents of the partial hydrolysate of Fraction 2 after gel permeation chromatography on Sepharose CL-4B.

galactose observed in the fractions which eluted close to the void volume suggest that the side chains are probably quite long and are stable under the conditions used for partial acid hydrolysis.

The EDTA extracted material showed (Table 2) the presence of a higher proportion of neutral sugars (11·3%) and a low content of uronic

acid (7·7%). Rhamnose, arabinose and galactose were the major neutral sugars present. A high content of protein too was observed.

The sodium hydroxide extracted material contained 21·3% of neutral sugars while uronic acids were completely absent (Table 2). Xylose and glucose constituted 53·4% and 14·9% respectively of the neutral sugars. The crude protein content of this extract too was high. This alkali extract probably constitutes the hemicellulosic material and consists mainly of a xyloglucan (Aspinall & Fanous,1984).

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