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# The mucilaginous polysaccharide material isolated from *ruredzo (Dicerocaryum zanguebarium)*

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The mucilage isolated from *ruredzo (Dicerocaryum zanguebarium)* by precipitation with ethanol and fractionation with copper II acetate contained galactose, xylose, arabinose and mannose in the ratio 21 : 19 : 12 : 1. Samples were analysed by TLC on Whatman K5 plates impregnated with $Na_2HPO_4$ and developed with ethyl acetate : pyridine : water (10 : 4 : 3 v/v). Plates were sprayed with *p*-anisidine in methanol or diphenylamine/aniline/phosphoric acid reagent to visualize spots. $R_f$ values for sample spots corresponding to galactose, xylose, and arabinose were 0·62, 0·81 and 0·76. Mannose was not detectable during TLC. For GC the retention times for the alditol acetates of samples corresponding to galactose, xylose, arabinose and mannose were 18·2, 9·1, 6·4, and 16·2 min, respectively. The polymer, whose molecular weight was 500 000, released arabinose residues after hydrolysis with a 50 mM $H_2SO_4$ solution but not a 5 mM $H_2SO_4$ solution. Gel chromatography of acid hydrolysates on Sepharose 6B showed fragments of decreasing molecular weight with increased periods of hydrolysis. The mucilage has a specific optical rotation of +4·5°, ash content of 7·6%, a protein content of 2·1% (Kjeldahl) and 1·8% (Lowry) and a uronic acid content of 8·1%.

## INTRODUCTION

Mucilages are polysaccharides that form viscous colloidal dispersions in water and occur in many plants. In Southern Africa the leaves of *ruredzo (Dicerocaryum Zanguebarium)* are occasionally used to prepare *derere*, a dish that is similar to others that are prepared from mucilaginous plant material (Woolfe *et al.*, 1977; El-Mahdy & El-Seibaiy, 1984). Distributed throughout Southern and Central Africa *ruredzo* is found as a prostrate plant with trailing annual stems that grow from a perennial rootstock (Wild, 1972; Plowes & Drummond, 1976). The sprawling stems, grow vigorously in summer and less so for the rest of the year. Covering an area of up to 10 m², the stems bear distinctive fruit with two sharp spines on the upper side which inspired its Latin name. When the plant is immersed and rubbed in water, copious amounts of a slimy mucilage are produced. *Ruredzo* has been used in the treatment of measles (Tredgold, 1986) with the affected person being washed in water containing liberal amounts of freshly picked leaves and stems. There is little evidence, however, that the mucilage has a bona fide therapeutic effect other than that resulting from thorough cleaning. *Ruredzo* leaves have also been used as a cleaning aid for hair. In this application, which was popular in rural communities until quite recently, the leaves are rubbed into moist hair alone or together with soap to form a creamy lather. There are claims that the mucilaginous material has also been used to facilitate difficult deliveries by humans and cows with the passage of the newly born being lubricated by the application of leaves macerated in water.

Since considerable amounts of *ruredzo* grow in some areas the mucilage from *ruredzo* could have potential use in industrial and other applications. An understanding of the structure and functional properties of the mucilage would be necessary in order to most effectively exploit the mucilage. No information is available on the nature, composition and structure of the mucilaginous material that occurs in *ruredzo*. A number of factors would be expected to complicate attempts to elucidate the structure of the polysaccharide material from any source. Variability in the structure of plant polysaccharides arises not only from the variety of monomers comprising the backbone but also from the degree of the incorporation of a variety of individual sugar units in highly branched molecules (Stephen, 1987). This together with heterogeneity in molecular

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weight makes the elucidation of polysaccharide structures more difficult than that of other biological polymers. A simplifying feature, however, is that polysaccharides have been found to be far less complex than would be predicted from the random combination of all the monomers that are theoretically possible. Also, of the many stereoisomeric monosaccharides, only a few are found in natural polysaccharides. This report describes studies on the mucilaginous material prepared from *ruredzo*.

## MATERIALS AND METHODS

### Extraction of mucilage

*Ruredzo* plants were harvested mainly during the growing season, and as soon as possible spread thinly on galvanized iron sheets and left to dry in the sun. Most of the material was collected from the edges of the cultivated land but some was obtained from the cultivated land itself and from untilled grazing land. Where necessary the dried material was stored in a dry place until required. Otherwise the thoroughly dried material was crushed and shaken vigorously by hand to separate the leaves from the stems. The stems were discarded and the dried leaves were ground in a laboratory mill.

Ground leaves (200 g) were suspended in distilled water (3 litres) and the mixture homogenized in a Waring blender. The mixture was heated in a stainless steel pot to near boiling, cooled and transferred to one-litre centrifuge tubes. The tubes were spun in an ICE centrifuge at 2000 rpm for 1 h at 4°C. After removal of supernatant the pellet was loosened with the addition of water and the mixture thoroughly agitated before further centrifugation. The supernatants were combined and two volumes of 96% ethanol were added with stirring to precipitate the mucilage. After centrifugation, the mucilage was washed twice in fresh 96% ethanol and dried in a preheated oven set at 90°C.

The dry mucilage was ground to a fine powder and stored in a dry place until required. To prepare solutions of the mucilage, powdered material was suspended in a suitable volume of water and the mixture allowed to hydrate overnight in a refrigerator at 4°C. After thorough stirring and centrifugation to remove lumpy material, any visible solids were removed by further centrifugation.

### Fractionation of mucilage with copper II acetate

To a 1% solution of the crude mucilage one volume of copper II acetate (7%) was added (Jones & Stoodley, 1965). In order to induce precipitation of the copper complex of the mucilage, one volume of 96% ethanol was added with stirring. After allowing the precipitate formed to stand for one hour the mixture was centrifuged and pellet resuspended in 0·5 mol litre$^{-1}$ EDTA. The mucilage was recovered from the solution by addition of two volumes of ethanol. After being washed three times in 96% ethanol the precipitate was dried as described earlier.

The resulting preparation was used for the determination of molecular weight by gel chromatography and the experiments in which samples were hydrolysed in acid; in order to determine the composition of the mucilage these were sometimes obtained by lyophilization.

### Determination of moisture and ash content

The ash content of the mucilage was determined by first weighing mucilage samples into oven-dried porcelain crucibles. Moisture was determined following the standard method 925.10 of the Association of Official Analytical Chemists (AOAC, 1990). The crucibles were then placed in a furnace at 500°C for at least 6 h. After cooling, the crucibles were weighed to determine the amount of ash.

### Determination of protein

The protein content was then determined by the Kjeldhal method using a factor of 6·25 to convert %N to % protein. Alternatively protein was determined by the folin–phenol method (Lowry *et al.*, 1951).

### Measurement of optical rotation

Optical rotation was determined by measuring the rotation of a 1% mucilage sample in a Belling and Stanley Model A type 3 polarimeter in which the light source was a sodium lamp.

### Acid hydrolysis

Mucilage prepared by fractionation using copper II acetate (1·9 g) was added to 5 mM $H_2SO_4$ solution (100 ml) left overnight to dissolve. The mixture was refluxed for a specified time after which 5 ml aliquots were removed, cooled and neutralized with barium carbonate (Churms & Stephen, 1970). Alternatively hydrolysis was performed using a 50 mM $H_2SO_4$ solution. After centrifugation, the clear supernatants were transferred to teflon stoppered bottles and stored at 4°C until required for analysis. The supernatants were analysed for reducing power by the 3,5-dinitrosalicylic acid method and the absorbance read at 570 nm.

For the identification of sugars, samples were applied onto a Whatman K5 plate impregnated with 0·2 mol litre$^{-1}$ $Na_2HPO_4$. After developing the plates in ethyl acetate : pyridine : water (10 : 4 : 3 v/v) the plates were sprayed with *p*-anisidine in methanol or diphenylamine/aniline/phosphoric acid reagent (Chaplin, 1986) and heated at 110°C to develop the spots.

Mucilage prepared by fractionation with copper II acetate (1 g) was dispersed in distilled water (100 ml) and the solution clarified by centrifugation. After dialysis of the supernatant against distilled water for about

18 h, samples (0·5 ml) were applied onto a Sepharose 6B column and the column eluted with 1 M aqueous sodium chloride at room temperature. The column had been calibrated with T dextrans, from Pharmacia, as standards. The concentration of the polysaccharide in the collected fractions was determined by the phenol—sulphuric acid method (Dubois *et al.*, 1956; Chaplin, 1986). Alternatively the concentration of carbohydrate in collected fractions was determined by the orcinol method (White & Kennedy, 1986).

The above procedure was repeated on hydrolysates in order to determine the distribution in molecular size of the polysaccharides remaining after each hydrolysis and, therefore, the extent of hydrolysis.

Samples of the mucilage were sent to Professor Milas of Centre de Recherches sur les Macromolecules Vegétales, Domaine Université, BP 53 X-38041, (Grenoble, France, where the polymer was characterized by size exclusion chromatography in conjunction with viscosity and light scattering measurements.

### Measurement of uronic acid

The uronic acid content of the mucilage was determined by the *m*-hydroxydiphenyl method (Blumekrantz & Asboe-Hansen, 1973).

### Gas chromatography

To standards (10 mg/10 ml) and neutralized samples, freshly prepared 0·5 M sodium borohydride solution (10 ml) was added (Supelco Inc. 1985). The mixtures were stirred slowly for 90 min at 25°C. Glacial acetic acid (8 ml) was added dropwise with constant stirring until bubbling had stopped. Methanolic-HCl (6 ml, 0·5 ml HCl in 100 ml methanol) was added and the liquid evaporated off in a rotary evaporator. Additions of methanolic-HCl and evaporation were repeated twice. The samples were dried over phosphorus pentoxide for 18 h. Dry samples were transferred to reaction vials and pyridine (0·2 ml) and acetic anhydride (0·4 ml) added. After sealing the vials with teflon lined caps and heating at 100°C for 1 h, the samples were centrifuged in a microcentrifuge and the supernatants stored in a refrigerator at 4°C.

A stainless steel column (ID 3 mm × 2 m) was packed with GP 3% SP-2330 on 100/120 Supelcoport material from Supelco. The column was placed in a Shimadzu GC-4CM PF Gas Chromatograph and analyses were done on 1-$\mu$l samples. The relative quantities of the sugars in the polymer were determined by cutting out the corresponding peak areas and weighing.

### Measurement of viscosity

Viscosity measurements were done using a Viscometers UK viscometer in which viscosity was directly read off in centipoise. Viscosity measurements were made at 25°C except when the effect of temperature was being investigated.

**Table 1. Summary of some of the properties of the mucilage isolate from *ruredzo* (*Dicerocaryum zanguebarium*). The uncertainties shown are standard deviations for at least three measurements**

| | |
|---|---|
| Moisture | 9·7±2·5% |
| Protein | |
| Kjeldhal | 2·1±0·4% |
| Lowry | 1·8±0·1% |
| Ash | 7·6±0·3% |
| Uronic acid | 8·1±1·1% |
| Specific rotation | +4·5±0·1° |
| Galactose : xylose : arabinose : mannose | 21 : 19 : 12 : 1 |
| Molecular weight | |
| Gel chromatography | 500 000 |
| Viscosity | 784 000 |
| Light scattering | 461 000 |

## RESULTS AND DISCUSSION

Crude *ruredzo* mucilage was a dull grey friable material with a fibrous appearance and the mucilage prepared using copper II acetate had a sparkling white colour. Mucilage dried in an inadequately heated oven was hard, horny and difficult to work with. The properties of the mucilage, summarized in Table 1, are similar to those of mucilages from other sources (Woolfe *et al.*, 1977; El-Mahdy & El-Seibaiy, 1984). TLC analyses of hydrolysates showed that the mucilage was composed of galactose, xylose and arabinose. The $R_f$ values for standards and samples are shown in Table 2. It can be seen that mannose could not be distinguished from arabinose partly because their $R_f$ values were nearly the same and partly because the relative amount of mannose was small. Examination of the spots developed on the TLC plates showed that hydrolysis of mucilage with 5 mM $H_2SO_4$ released only galactose and xylose and that arabinose was released only after further treatment with 50 mM acid. GC analyses showed that mannose was also a component and that the relative concentrations of galactose, xylose, arabinose and mannose were 21 : 19 : 12 : 1. The retention values for the alditol acetates of standards and samples are shown in Table 2.

It is not clear why arabinose units resisted release from the polysaccharide during hydrolysis with 5 mM acid but we expect that this resistance bears some rela-

**Table 2. $R_f$ values for samples and standards and retention times for the alditol acetates of standards and samples. The uncertainties shown for the $R_f$ values and retention times are standard deviations for at least 15 and 6 measurements respectively**

| Sample | $R_f$ | Retention time (min) |
|---|---|---|
| Arabinose | 0·76±0·02 | 6·4±0·4 |
| Galactose | 0·62±0·01 | 18·0±0·4 |
| Mannose | 0·76±0·01 | 16·2±0·5 |
| Xylose | 0·81±0·03 | 9·1±0·3 |
| Unknown 1 | 0·61±0·01 | 18·2±0·3 |
| Unknown 2 | 0·77±0·00 | 6·2±0·2 |
| Unknown 3 | 0·81±0·01 | 9·2±0·2 |
| Unknown 4 | not determined | 16±0·2 |

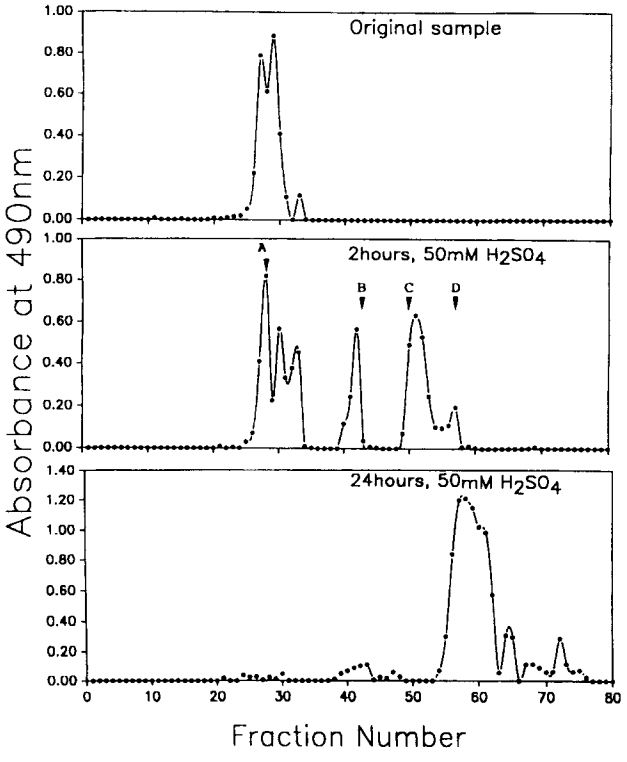


**Fig. 1.** Gel chromatography of samples of ruredzo mucilage hydrolysed in 50 mM $H_2SO_4$ acid for various periods. Samples of native and hydrolysed mucilage were applied onto a Sepharose 6B column and 3 ml fractions collected. A, B, C and D indicate positions corresponding to Pharmacia dextran standards T500, T70, T40 and T10 whose molecular weights are 500, 70, 40 and 10 kilodaltons respectively.

tionship to the structural position and chemical state of arabinose units in the polymer. It is known, for example, that glycosidic linkages that involve the reducing groups of glucuronic acids or 2-amino sugars are resistant to acid hydrolysis (Pazur, 1986). Ring size and the type of linkage also affect the rate of hydrolysis. Bonds that involve furanose forms are hydrolysed faster than those involving pyranoses and $\alpha$ linkages are more susceptible to hydrolysis than $\beta$ linkages. Further work is required to enable an understanding of why arabinose units unexpectedly resisted release by mild acid hydrolysis. The reducing power, expressed in equivalent amounts of glucose, increased from 20 to 420 $\mu$g/ml after hydrolysis for 60 h.

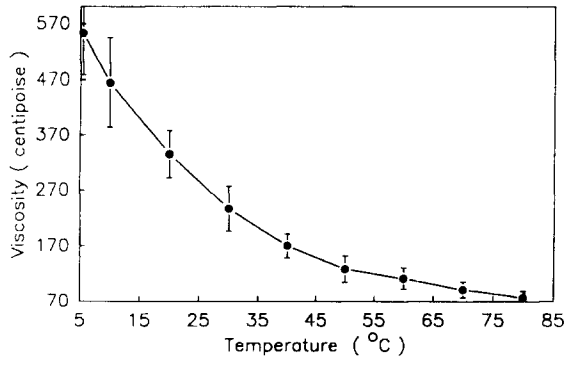


**Fig. 2.** Effect of temperature on the viscosity of *ruredzo* mucilage.

The progress of hydrolysis, as indicated by gel chromatography analysis of samples hydrolysed in acid for various lengths of time, is shown in Fig. 1. Unhydrolysed mucilage had a molecular weight of 500 000 based on the dextran standards used. It required hydrolysis periods of over 44 h to degrade the last traces of the fragments with a molecular weight of just under 10 kilodaltons. Although the cleavage of glycosidic linkages in polysaccharides by acids is believed to be more or less random, it is possible that regions of a polymer chain could be more susceptible to hydrolysis than others.

The viscosity of solutions of *ruredzo* mucilage increased with concentration and decreased with temperature as expected. At concentrations greater than about 2·5% the mucilage formed a viscous mass that cannot be properly regarded as a solution. The effect of temperature on the viscosity of ruredzo mucilage is shown in Fig. 2. At concentrations of 1% solutions of the mucilage behaved like non-Newtonian liquids.

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## REFERENCES

AOAC (1990). *Official Methods of Analysis* 5th edn, Association of Official Analytical Chemists, Washington DC, p. 777.

Blumenkrantz, N. & Asboe-Hansen, G. (1973). New methods for quantitative determination of uronic acids. *Anal. Biochem.*, **54**, 484–7.

Chaplin, M. F. (1986). In *Carbohydrate Analysis—A Practical Approach*, ed. M. F. Chaplin & J. F. Kennedy. IRL Press, Oxford, p. 15.

Churms, S. C. & Stephen, A. (1970). *Acacia podyalyriaefolia* gum. *Carbohydr. Res.*, **15**, 11–9.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–6.

El-Mahdy, A. R. & El-Seibaiy, L. A. (1984). Mucilages extracted from Okra, Taro, Jew's mellow and Fenugreek. *Food Chemistry*, **14**(11), 237–49.

Jones, J. K. N. & Stoodley, R. J. (1965). Fractionation using copper complexes. *Methods in Carbohydr. Chem.*, **5**, 37–8. Academic Press, New York, pp. 37–38.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–371.

Pazur, J. H. (1986). In *Carbohydrate Analysis—A Practical Approach*, ed. M. F. Chaplin & J. F. Kennedy. IRL Press, Oxford, pp. 79–81.

Plowes, D. C. H. & Drummond, R. B. (1976). Wild Flowers of Rhodesia, Longman, Salisbury, p. 121.

Stephen, A. M. (1987). Plant polysaccharides—how regular are they? *South Afr. J. Chem.*, **40**(2), 89–99.

Supelco, Inc. (1985). GC/HPLC Bulletin 774B, Bellefonte, PA, pp. 1–4.

Tredgold, M. H. (1986). *Food Plants of Zimbabwe*, Mambo Press Gweru, Zimbabwe, p. 7.

White, C. A. & Kennedy, J. F. (1986). In *Carbohydrate Analysis — A Practical Approach*, ed. M. F. Chaplin & J. F. Kennedy. IRL Press, Oxford, p. 37.

Wild, H. A. (1972). *A Rhodesian Dictionary of African and English Plant Names*, Government Printer, Salisbury, pp. 147.

Woolfe, M. L., Chaplin, M. F. & Otchere, G. (1977). Studies on the mucilages extracted from okra fruits (*Hibiscus esculentus* L.) and baobab leaves (*Adansonia digitata*). *J. Sci. Food Agric.*, **28**, 519–29.