## Note

# Polysaccharides of tropical grass species. II. Electrophoretic analysis and fractionation of *Setaria sphacelata* hemicellulose*

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Preliminary structural studies on *Setaria* hemicellulose[1] indicate a backbone of β-linked D-xylopyranose residues, with residues of L-arabinofuranose and D-glucuronic acid, or one of its methyl ethers, attached as side chains. Moving-boundary electrophoresis of solutions of the polysaccharide has revealed heterogeneity of the hemicellulose preparation, also detected by sedimentation velocity and by gel chromatography. The present communication reports these data, together with the preparation of the major electrophoretic fraction, by continuous electrophoresis on a filter-paper curtain.

Electrophoretic-mobility data obtained for *Setaria* hemicellulose in five buffer systems are summarized in Table I, about which the following points require comment. (*a*) Results for all five experiments were similar inasmuch as the ascending and descending patterns exhibited two migrating boundaries, the schlieren profiles for

TABLE I

ELECTROPHORETIC MOBILITIES OF *Setaria* HEMICELLULOSE IN VARIOUS BUFFERS

| *Buffer system* | *pH* | *Ionic strength* | *Mobility* × $10^5$ ($cm^2.sec^{-1}.volt^{-1}$) | |
|---|---|---|---|---|
| | | | *slow boundary*[a] | *fast boundary*[a] |
| acetate–acetic acid | 4.5 | 0.05 | −0.7 | −2.3 |
| acetate–acetic acid | 5.3 | 0.05 | −0.9 | −2.8 |
| Tris[b]–HCl | 7.0 | 0.05 | −1.1 | −2.4 |
| glycine–NaOH | 9.8 | 0.05 | −2.9 | −5.0 |
| carbonate–hydrogen carbonate | 10.5 | 0.05 | −3.0 | −7.3 |

[a]Values refer to migration of the descending-boundary system[2]. [b]Tris = tris(hydroxymethyl)aminomethane

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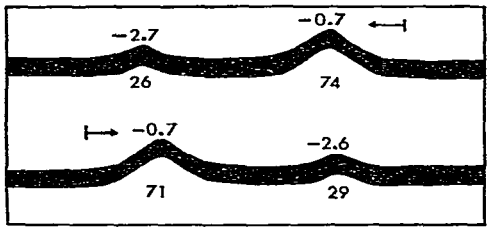


Fig. 1. Ascending (upper) and descending (lower) patterns obtained on electrophoresis of unfractionated *Setaria* hemicellulose in 0.05I acetate, pH 5.3, for 150 min with an applied potential gradient of 13.5 volts.cm$^{-1}$.

the hemicellulose in pH 5.3 acetate being shown in Fig. 1. (*b*) Various buffer salts were selected in order to eliminate migration resulting from specific complex-formation between ions of the supporting electrolyte and polysaccharide, rather than from movement of the hemicellulose itself: borate buffers[2] have therefore been excluded. It is noted that mobilities are relatively insensitive to pH and buffer systems in the range 4.5–7.0, suggesting that specific ion-effects are absent. (*c*) The relative distribution of polysaccharide between the two electrophoretic peaks was similar in all five experiments, the slower-moving material preponderating to the extent of about 3:1. (*d*) Acetate buffer, pH 5.3, was chosen for all subsequent physicochemical studies because enantiography of the ascending and descending patterns in terms of mobilities and relative areas of peaks was judged the most satisfactory in this environment: numbers above peaks in Fig. 1 refer to negative mobilities, whereas those below indicate the proportion of solute being ascribed the relevant mobility.

Analysis of the hemicellulose by velocity sedimentation in 0.05I acetate buffer, pH 5.3, indicated the presence of two molecular-size distributions, prolonged centri-

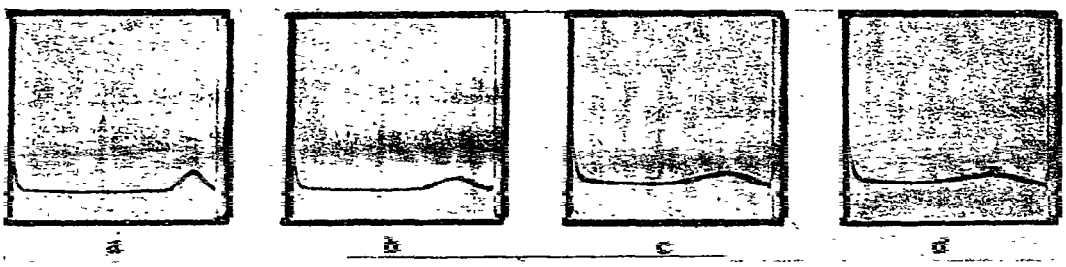


Fig. 2. Schlieren patterns obtained on centrifugation of *Setaria* hemicellulose at 59,780 r.p.m. for (*a*) 75 min; (*b*) 123 min; (*c*) 155 min; and (*d*) 187 min. Solvent: 0.05I acetate, pH 5.3. Sedimentation is from right to left.

fugation at 59,780 r.p.m. being required to obtain a schlieren pattern indicative of two partially resolved boundaries (Fig. 2). The sedimentation coefficient ($s_{20}$) of the slower-moving boundary is approximately 1.1 *S*, whereas that of the ill-defined, faster-moving boundary appears to be two or three times greater. Blake and Richards[3]

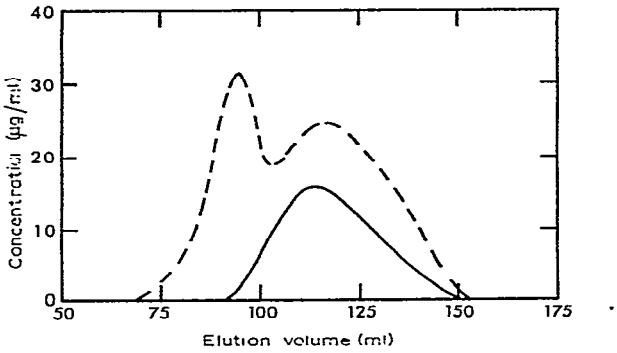


Fig. 3. Elution profiles resulting from application of 1-ml aliquots of unfractionated (---) and fractionated (——) hemicellulose to a 2.5 × 35 cm column of Sephadex G-200 equilibrated with 0.05I acetate, pH 5.3.

have also reported $s_{20}$ values in the vicinity of 1 *S* for the major fraction of hemicellulose preparations from other tropical grasses.

The elution profile obtained by gel chromatography of the polysaccharide, on Sephadex G-200 equilibrated with the pH 5.3 acetate buffer, is shown by the broken line in Fig. 3, from which it is evident that partial resolution into two molecular size-distributions has again been achieved. However, the appearance of a considerable proportion of applied carbohydrate in the eluate at the void volume of the column was unexpected on the basis of the sedimentation result, but parallels the work of Simonson[4] on a hemicellulose preparation containing lignin. Since delignification of the present sample is known to be incomplete (5.9% lignin), the faster-migrating carbohydrate zone in Fig. 3 may well reflect the chromatographic behaviour of a polysaccharide–lignin complex.

Before embarking on structural studies it seemed worthwhile to attempt purification of the slowly migrating fraction, which comprised the greater proportion (~70%) of the total polysaccharide. Initial attempts at fractionation by methods such as ion-exchange chromatography, ethanol and salt precipitation, and gel chromatography, were unproductive, but preparative curtain-electrophoresis of the hemicellulose in 0.01M ammonium acetate met with greater success.

The continuous electrophoresis apparatus was modified (see Experimental section) to allow a larger volume of sample to be applied to the filter-paper curtain, the rate of sample addition being a critical factor governing separation. In a typical experiment, 100 ml of 0.95% hemicellulose in 0.01M ammonium acetate was applied to the curtain over a period of 65 h, the current being maintained at 15 ±2 mA during this time and for a further 10 h to permit the sample to flow from the curtain into flasks situated beneath the 30 drip points at the bottom of the curtain. Since electro-endosmotic flow caused the slow-moving fraction to migrate cathodically despite its slight negative charge, the sample was not applied at the cathodic edge of the curtain (above tube 30) but at a position above tube 20 to allow for this drift. At the conclusion of electrophoresis, the contents of each collection flask (volume ~35 ml) were

assayed qualitatively for carbohydrate (Molisch test): those in which carbohydrate was detected, namely, fractions 8–25, inclusive, were then freeze-dried.

Electrophoresis in 0.05I acetate, pH 5.3, showed that fractions 8–17 contained polysaccharide corresponding to the faster-migrating peak in the unfractionated sample, whereas flasks 21–25 contained material that yielded only the more slowly migrating boundary: the contents of flasks 18 and 19 gave bimodal electrophoretic patterns. Electrophoretic analysis of fractions 21–25 individually yielded the same mobility ($-0.7 \times 10^{-5}$ cm$^2$.sec$^{-1}$.volt$^{-1}$), whereas a much wider spread of mobilities ($-2.6$ to $-4.2 \times 10^{-5}$ cm$^2$.sec$^{-1}$.volt$^{-1}$) was observed for polysaccharide cuts emanating from the faster-moving peak. In addition to being more heterogeneous electrophoretically than its slower-moving counterpart, this more-highly charged fraction was brown and absorbed at 280 nm, presumably because of the lignin contamination known to be present in the unfractionated hemicellulose preparation. These properties contrasted markedly with those of the slower-moving fraction, which was white and transparent in the 280 nm region.

Application of 0.95 g of the original hemicellulose to the curtain in an individual experiment yielded 0.41–0.42 g of purified, slow-moving fraction. The descending electrophoretic pattern for a sample pooled from several such preparative experiments

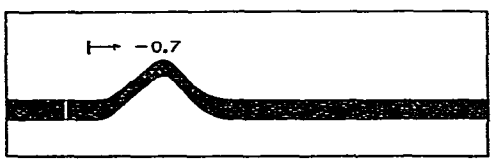


Fig. 4. Descending pattern obtained on electrophoresis of fractionated hemicellulose for 150 min under conditions identical with those applying to Fig. 1.

is shown in Fig. 4, and its gel-chromatographic behaviour is indicated by the solid line in Fig. 3. Although much of the larger molecular-weight material, including lignin, had been removed, examination of acid hydrolysates indicated that the chemical composition with regard to carbohydrate content was apparently unchanged from that of the crude polysaccharide (containing arabinose, xylose, and a trace of glucose). this successful fractionation of *Setaria* hemicellulose in relatively large amounts is significant in view of the difficulty[5,6] with which hemicellulose fractions may be purified by conventional methods.

## EXPERIMENTAL

*Isolation of the polysaccharide.* — Hemicellulose was isolated from *Setaria sphacelata* by essentially the procedure described by McIlroy[7]; see also ref. 1 for details of composition. Lignin was estimated by the method of Adams[8].

*Moving-boundary electrophoresis.* — Solutions of hemicellulose (~0.8%) were dialysed against the appropriate buffer (2 × 500 ml) for 24 h at 4° prior to electro-

phoresis at the same temperature in a Perkin–Elmer model 238 electrophoresis apparatus; a current of 8 mA through the standard 15-mm cell was used. Distances migrated and relative peak-areas were determined from enlarged tracings (×4) of schlieren patterns obtained with the diagonal-slit assembly. In calculations of mobilities, the resistances of the dialysed polysaccharide solutions were assumed to be those of the buffer against which they were dialysed. Quoted mobilities refer to migration at 0°, the temperature at which buffer resistances were measured in a Perkin–Elmer electrolytic conductivity-cell connected to a Leeds–Northrup conductivity bridge.

*Velocity sedimentation.* — Dialysed solutions (~0.8%) of polysaccharide in 0.05I acetate, pH 5.3, were centrifuged at 59.780 r.p.m. in a Spinco model E analytical ultracentrifuge, the R.T.I.C. unit being used to maintain the rotor temperature at 20°.

*Gel chromatography.* — Hemicellulose solution (1.0 ml, 0.3 mg/ml) was subjected to zonal gel-chromatography on a 2.5 × 35 cm column of Sephadex G-200 equilibrated at 20° with 0.05I acetate buffer, pH 5.3. The column effluent, maintained at a flow rate of 60 ml/h, was monitored continuously for carbohydrate by an automated orcinol procedure.

*Preparative electrophoresis.* — A Shandon continuous electrophoresis apparatus (model 2520) fitted with a 40 × 35 cm filter paper curtain (Whatman 31 E.T.) as supporting medium was used. Before application of sample the curtain was run for at least 24 h with the solvent to be used (10mM ammonium acetate): this and subsequent operations were carried out at 4°. In order to increase five-fold the volume of applied sample, a piece of Tygon tubing (0.015 in. i.d.) was passed through the supplied wick-support and connected to a 100-ml reservoir. This modification of sample volume necessitated replacement of the 15-ml collection tubes by 100-ml conical flasks. A current of 15 ±2 mA was maintained throughout the 65 h during which hemicellulose was being applied to the curtain and for a further 10 h thereafter. The contents of collection flasks yielding positive Molisch tests were freeze-dried and stored at 4°.

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