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Note

Thin-layer chromatography of neutral and acidic sugars from plant cell wall polysaccharides

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Thin-layer chromatography (TLC) has been used to separate carbohydrates[1–4]. However, these methods have been of limited use for the separation of mixtures of a large number of sugars, and so far no appropriate TLC technique exists which allows for a complete separation of all monomers obtained after hydrolysis of plant cell walls. The present report describes a two-dimensional thin-layer technique on unimpregnated cellulose for the separation of seven neutral and two acidic sugars such as those found in hydrolysates of plant cell wall polysaccharides.

EXPERIMENTAL

The thin-layer plates were prepared as follows: 15 g of cellulose MN 300 (Brinkman, Westbury, NY, U.S.A.) were homogenized with 100 ml of distilled water in a Wareing blender for 30 sec. The mixture was then spread onto glass plates (20 × 20 cm) at a thickness of 0.25 mm using an adjustable applicator (Desaga, Heidelberg, G.F.R.). After drying, the plates were heated for 15 min at 100°C. The sugars were applied to the lower corner of the plate at 2 cm from the bottom and 2 cm from the right edge. The following solvents were used: first direction, *n*-butanol–2-butenone–formic acid–water (8:6:3:3); second direction, phenol–water–formic acid (100:98:2, w/v/v organic phase only).

The sugar mixture was developed twice in the first direction. The plates were then chromatographed once in the second direction. Chromatography was carried out in glass tanks under saturated atmospheres. Visualization of the spots was achieved by spraying the plates with either anisidine phthalate[5] or *p*-aminobenzoic acid spray[6] and heating at 100°C for 10 min.

RESULTS AND DISCUSSION

Nitella axillaris cell walls were hydrolyzed in 2 *N* trifluoracetic acid for 1 h at 120°C. The hydrolysate was neutralized and chromatographed by the two-dimensional TLC method described above, and yielded the following sugars: glucose, galac-

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tose, mannose, xylose, arabinose, rhamnose, fucose, glucuronic acid and galacturonic acid (Fig. 1). The present method allowed for a clean separation of galactose and glucose, as well as arabinose and mannose which are difficult to separate in one-directional systems[1–3]. Quantitative evaluations of the separated sugars can be achieved by autoradiography[7] or fluorography[7]. Alternatively the sugars can be visualized directly with spray reagents. The colored spots are then decolorized with sodium borohydride to avoid quenching during the liquid scintillation radioassay[8].

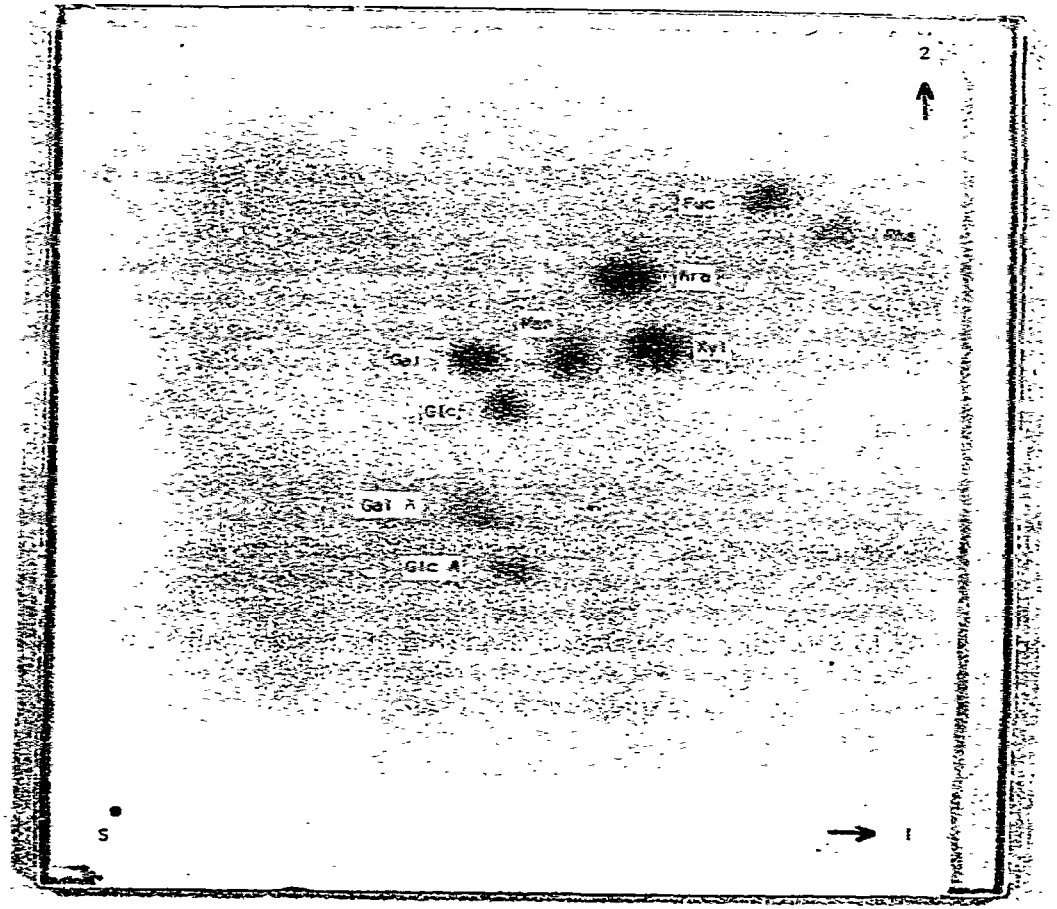

Fig. 1. Two-dimensional TLC on cellulose of a hydrolysate of *Nitella* cell wall polysaccharides. The solvents used are those described under Experimental. Spots: Glc = glucose; Gal = galactose; Man = mannose; Xyl = xylose; Ara = arabinose; Fuc = fucose; Rha = rhamnose; Glc A = glucuronic acid; Gal A = galacturonic acid. S = start; 1 = first direction; 2 = second direction. The plate was sprayed with anisidine phthalate. Development times: first solvent, 3 h; second solvent, 3.5 h.

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REFERENCES

1 A. Schweiger, *J. Chromatogr.*, 9 (1962) 374.
2 D. W. Vomhof and T. C. Tucker, *J. Chromatogr.*, 17 (1965) 300.
3 M. Ghebregzabher, S. Rufini, B. Monaldi and M. Lato, *J. Chromatogr.*, 127 (1976) 133.
4 M. Ghebregzabher, S. Rufini, G. M. Sapia and M. Lato, *J. Chromatogr.*, 180 (1979) 1.
5 J. B. Pridham, *Anal. Chem.*, 28 (1956) 1967.
6 J. K. Roy, *Analyst (London)*, 85 (1960) 294.
7 K. Randerath, *Anal. Biochem.*, 34 (1970) 188.
8 A. E. Gal, *J. Chromatogr.*, 34 (1968) 266.