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Note

Paper chromatography of plant sugars

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Liquid and gas chromatography are now commonly used for the separation of sugars from biological sources[1], but descending paper chromatography is still useful for applications in which flexibility and low cost are important. In the past the latter method has been used widely in botanical laboratories, but the poor separation of certain pairs of common monosaccharides, in most of the usual solvents, has sometimes caused confusion[2].

The range of solvents based on ethyl acetate and buffered with pyridine–acetic acid has been studied in this laboratory and a method capable of separating all the important monosaccharides occurring free or bound in plants can now be described.

EXPERIMENTAL

Chromatographically pure standard monosaccharides (0.5–2 mg/ml) were dissolved in water saturated with benzoic acid. Analytical-grade sucrose was prepared in aqueous solution (4 mg/ml) and used immediately. 0.5 μl of solution was usually applied, giving a spot 4 mm in diameter. Descending chromatography on Whatman No. 1 papers, all from the same batch, was carried out at 17° in a temperature-controlled room. Solvents were of analytical grade. $R_{Glucose}$ values were reproducible to $\pm$ 2% or better under these conditions, but would probably vary between laboratories. Sugars were detected with the alkaline silver reagents of Trevelyan *et al.*[3], the NaOH concentration being increased to 1 *M*.

RESULTS AND DISCUSSION

The resolution is increased by using a less polar solvent than usual and running for longer periods. This not only gives a wider spread of R_F values (see Fig. 1) but also reduces the size of the spots, since spread by diffusion depends on the mobility in the solvent chosen but only on the square root of the running time. Resolution is also improved by applying small quantities of sugar: The detection method described has an ultimate sensitivity of about 10–20 ng for glucose if the spot covers no more than 0.5 cm^2. Ultimately, the resolution is limited by the unevenness of flow through the paper.

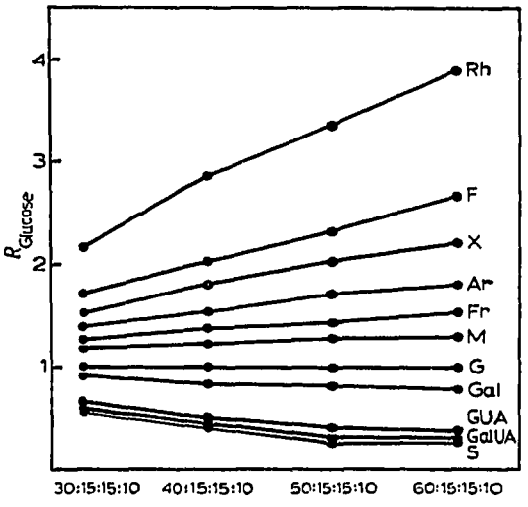

Fig. 1. Variation in $R_{Glucose}$ values with increasing proportions of ethyl acetate in the solvent system ethyl acetate–acetic acid–pyridine–water. Running time, 24 h. Rh = Rhamnose; F = fucose; X = xylose; Ar = arabinose; Fr = fructose; M = mannose; G = glucose; Gal = galactose; GUA = glucuronic acid; GalUA = galacturonic acid; S = sucrose.

The mobilities of most sugars are affected, to a lesser extent, by the acidity or otherwise of the solvent (Fig. 2). Thus glucose and galactose are poorly separated in most acidic solvents; arabinose and fructose, and sometimes also xylose and fucose, in basic solvents[4]. The solvent ethyl acetate–acetic acid–pyridine–water (50:12:18:10) is a suitable compromise, and can give a satisfactory separation of all the neutral sugars listed in 30 h, though if rhamnose is absent the resolution can be improved by

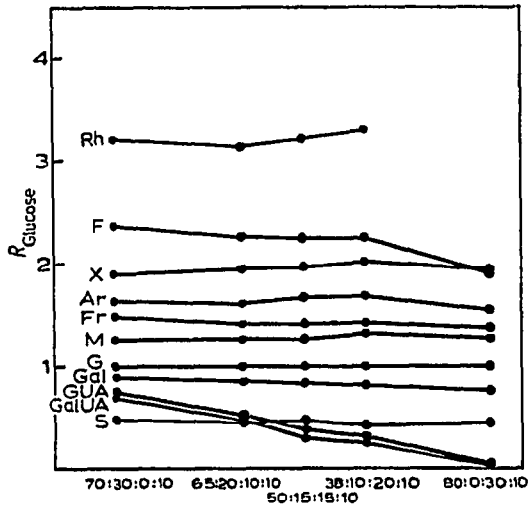

Fig. 2. Variation in $R_{Glucose}$ values with different proportions of acetic acid to pyridine. The proportion of ethyl acetate was adjusted to maintain the $R_{Glucose}$ value of sucrose close to 0.45. Abbreviations as in Fig. 1.

running for 40 h. If time is at a premium the proportion of ethyl acetate may be reduced with some loss of resolution. Longer running times, around three to four days, are required to separate uronic acids.

If maximum resolution is not required, 10- to 100-μg quantities may be analysed quantitatively by the aniline phthalate method of Wilson[5], or milligram quantities prepared by chromatography on Whatman No. 3 paper. It is useful to have similar separations in qualitative, quantitative and preparative work.

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