THE EFFECT OF GLYCEROL ON THE RATE OF MOVEMENT OF SIMPLE SUGARS ON SILICA GEL AND CELLULOSE THIN LAYERS

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INTRODUCTION

Thin-layer chromatography has been successfully applied to the separation of the sugars, the adsorbents used including aluminium oxide[1], calcium silicate[2], cellulose[3–6], kieselguhr[7–10], magnesium silicate[11] and silica gel[7,12–20]. Many different solvent systems have been used.

Preliminary work using buffered silica gel and cellulose layers with the solvent systems methyl ethyl ketone–glacial acetic acid–methanol (3:1:1)[7,8,17] and ethyl acetate–pyridine–water (60:25:20)[21] respectively yielded satisfactory results with solutions of some sugars in distilled water or isopropanol–water mixtures. Difficulty was encountered, however, when the same methods were applied to pollen extracts preserved in 50 % glycerol solution. Since glycerol is commonly used for the preservation of saccharides in biological fluids, the influence of the presence of glycerol on the rate of movement of some sugars was investigated on buffered silica gel and cellulose layers.

EXPERIMENTAL

Essential details are given in Table I.

Sugars

The following sugars were studied:

Monosaccharides:

aldohexoses	D-galactose, D-glucose, D-mannose
ketohexoses	D-fructose, L-sorbose
aldopentoses	L-arabinose, D-xylose
aldomethylpentoses	L-fucose, D-rhamnose

Disaccharides: cellobiose, lactose, maltose, sucrose
Trisaccharide: raffinose.

Spray reagents

(a) anisaldehyde–sulphuric acid[8]
(b) naphthoresorcinol–phosphoric acid[7,10]
(c) *p*-anisidine–phthalic acid[4].

TABLE I

SUMMARY OF EXPERIMENTAL PROCEDURE

Adsorbent	Silica gel G (Merck) buffered with boric acid	Cellulose MN300 (Macherey, Nagel & Co.)
Thickness	250 μ	250 μ
Activation	Air drying (1 h) 110° for 60 min	Air drying (1h) 105° for 10 min
Solvent system	Methyl ethyl ketone–glacial acetic acid–methanol (3:1:1)	Ethyl acetate–pyridine–water (60:25:20)
Method	Ascending in saturated chamber	Ascending in saturated chamber
Temperature	20–22°	20–22°
Distance	10 cm	10 cm
Load	20 μg (2 μl of 1 % solution)	20 μg (2 μl of 1 % solution)
% Glycerol in water	0, 10, 20, 30, 40 and 50	0, 10, 20, 30, 40 and 50

After spraying, the sugar was located by heating the plate at 100–105° for 20–30 min.

Glycerol can quite easily be distinguished from the sugars by all three spray reagents used, the colour of the glycerol spot being white or pale pink.

RESULTS AND DISCUSSION

Tracings of the results obtained are shown in Figs. 1–4.

Tracings of the movement of glycerol alone are not shown since the presence of the sugar does not affect the position or shape of the glycerol spot. However, the following observations on the behaviour of glycerol alone are pertinent:

(i) The rate of movement of glycerol depends on the concentration, the rate decreasing with increase in concentration.

(ii) The shape of the spot becomes distorted as the glycerol concentration increases. This effect is accentuated on cellulose layers.

The presence of glycerol in the sugar solutions affects both the *rate of movement* and the *shape* of the sugar spots thus making identification of specific sugars extremely difficult. No attempt, therefore, has been made to calculate the R_F values of the sugars.

Rate of movement

The sugars may be considered in two groups: (i) those whose movement is slow compared with that of glycerol and (ii) those whose movement is similar to that of glycerol.

(i) In general, the movement of the more slowly migrating di- and trisaccharides is not greatly affected by the presence of glycerol when chromatographed on buffered silica gel layers although the movement of cellobiose and sucrose (Fig. 3) is retarded in the presence of 40 and 50 % glycerol. The rate of movement of sorbose (Fig. 1) is unaffected by glycerol; other slow moving monosaccharides are retarded only when the glycerol concentration reaches a certain percentage, *e.g.* mannose and arabinose (Fig. 1) in 30 % glycerol and galactose, glucose and fructose (Fig. 1) in 40 % glycerol.

The behaviour of these sugars, in the presence of glycerol, is not identical when chromatographed on cellulose layers. Although there is little effect on the rate of

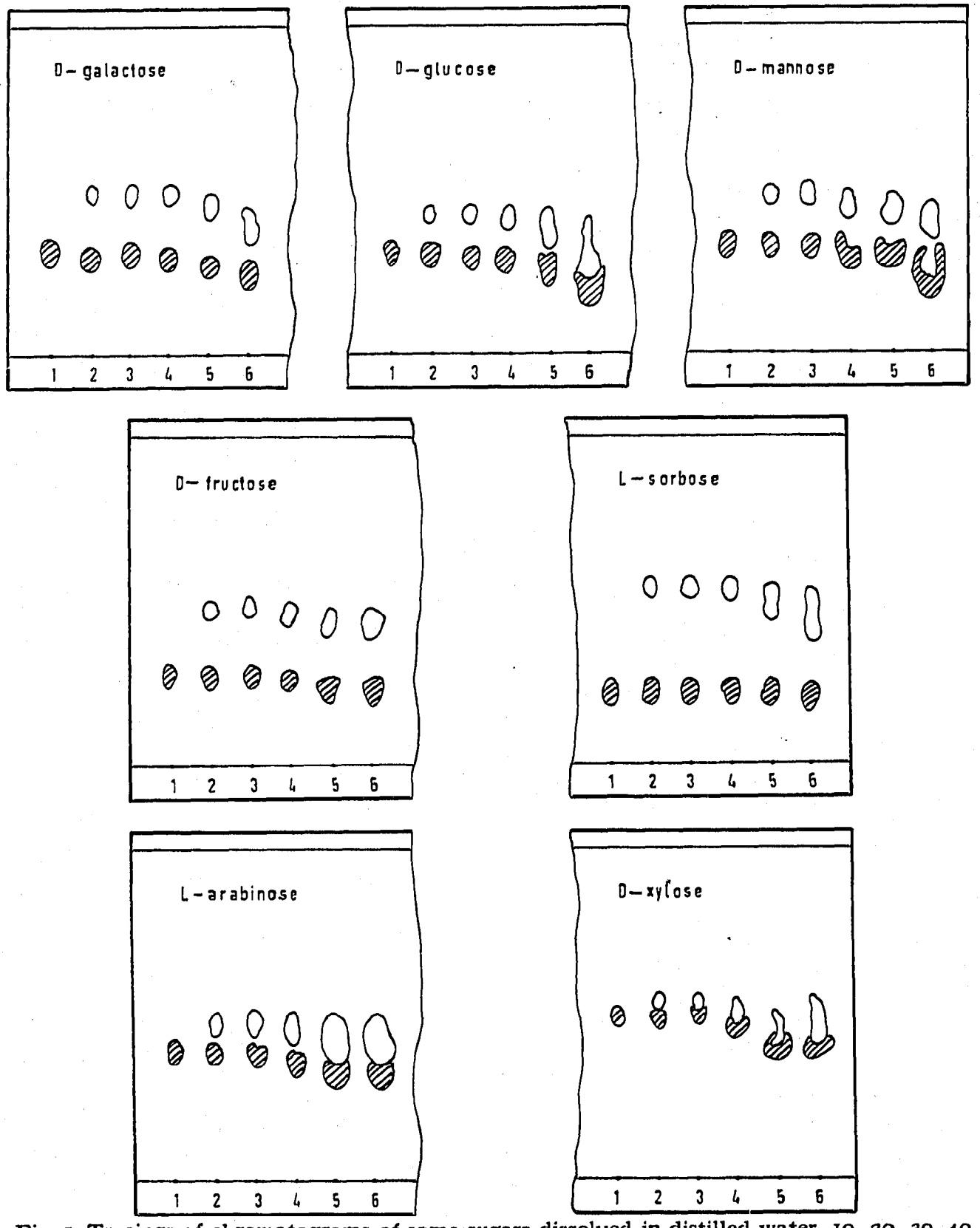

Fig. 1. Tracings of chromatograms of some sugars dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated nos. 1–6 respectively on the starting line. Layer: silica gel buffered with boric acid. Hatched areas: sugar; unhatched areas: glycerol.

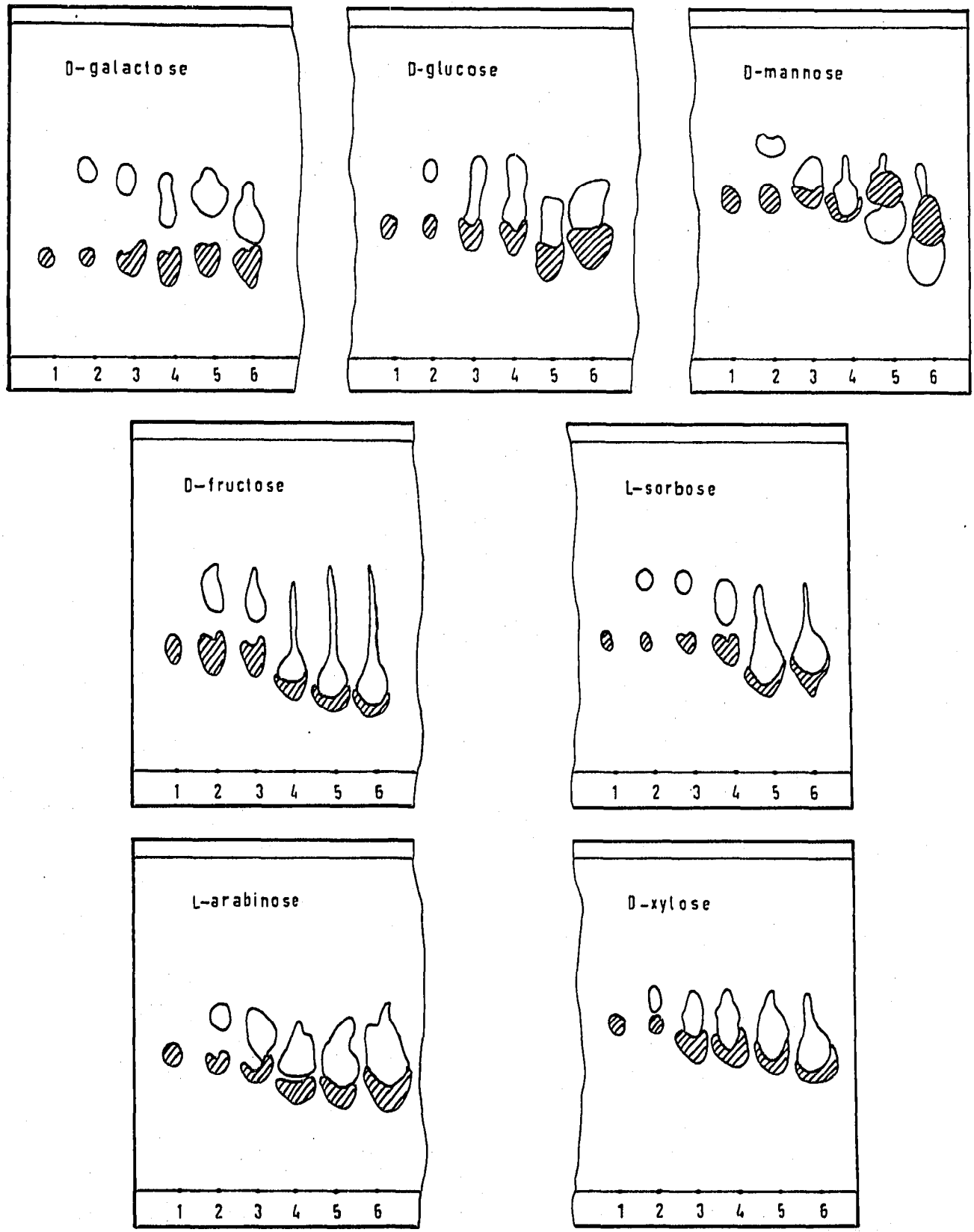

Fig. 2. Tracings of chromatograms of some sugars dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated nos. 1–6 respectively on the starting line. Layer: cellulose. Hatched areas: sugar; unhatched areas: glycerol.

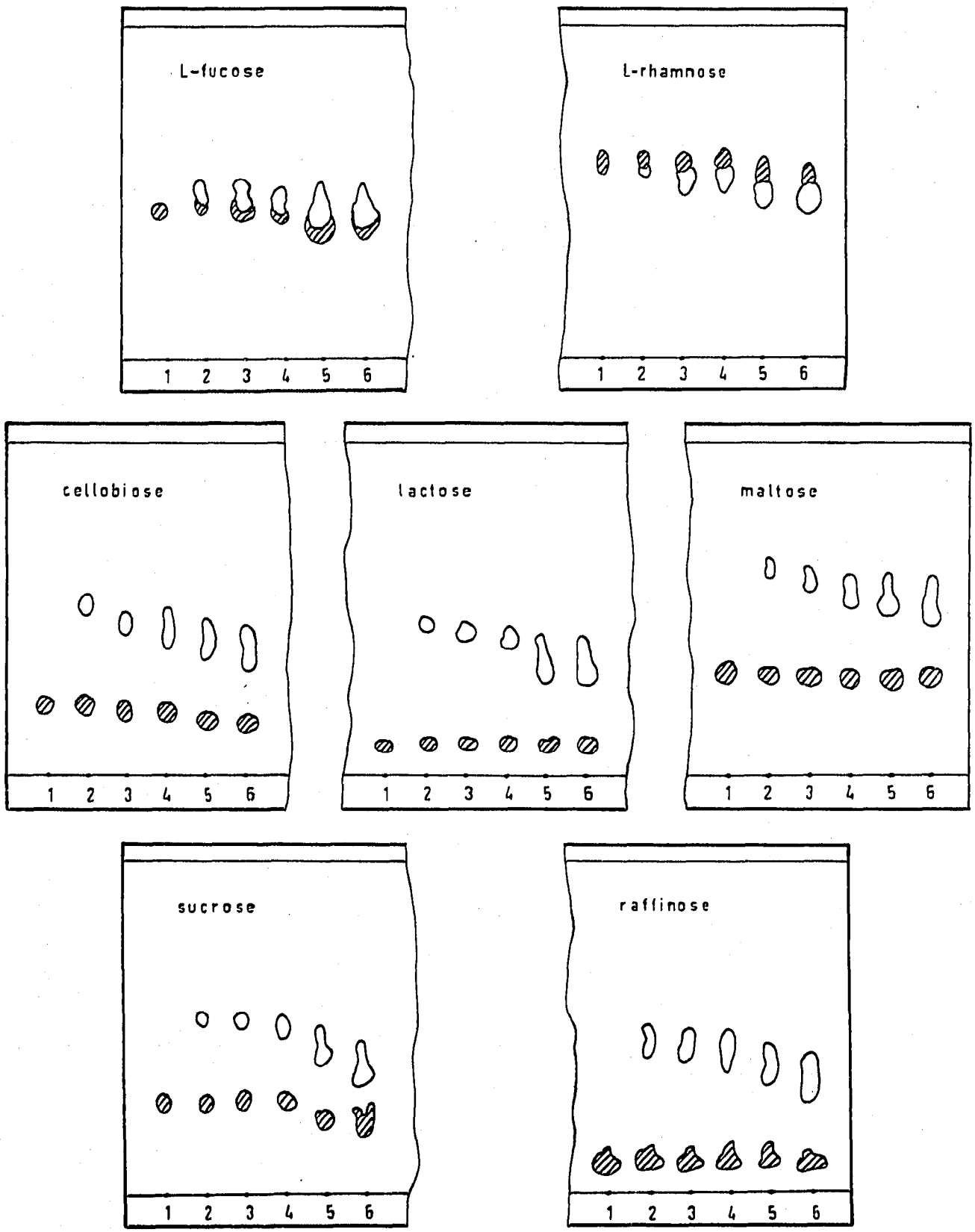

Fig. 3. Tracings of chromatograms of some sugars dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated nos. 1–6 respectively on the starting line. Layer: silica gel buffered with boric acid. Hatched areas: sugar; unhatched areas: glycerol.

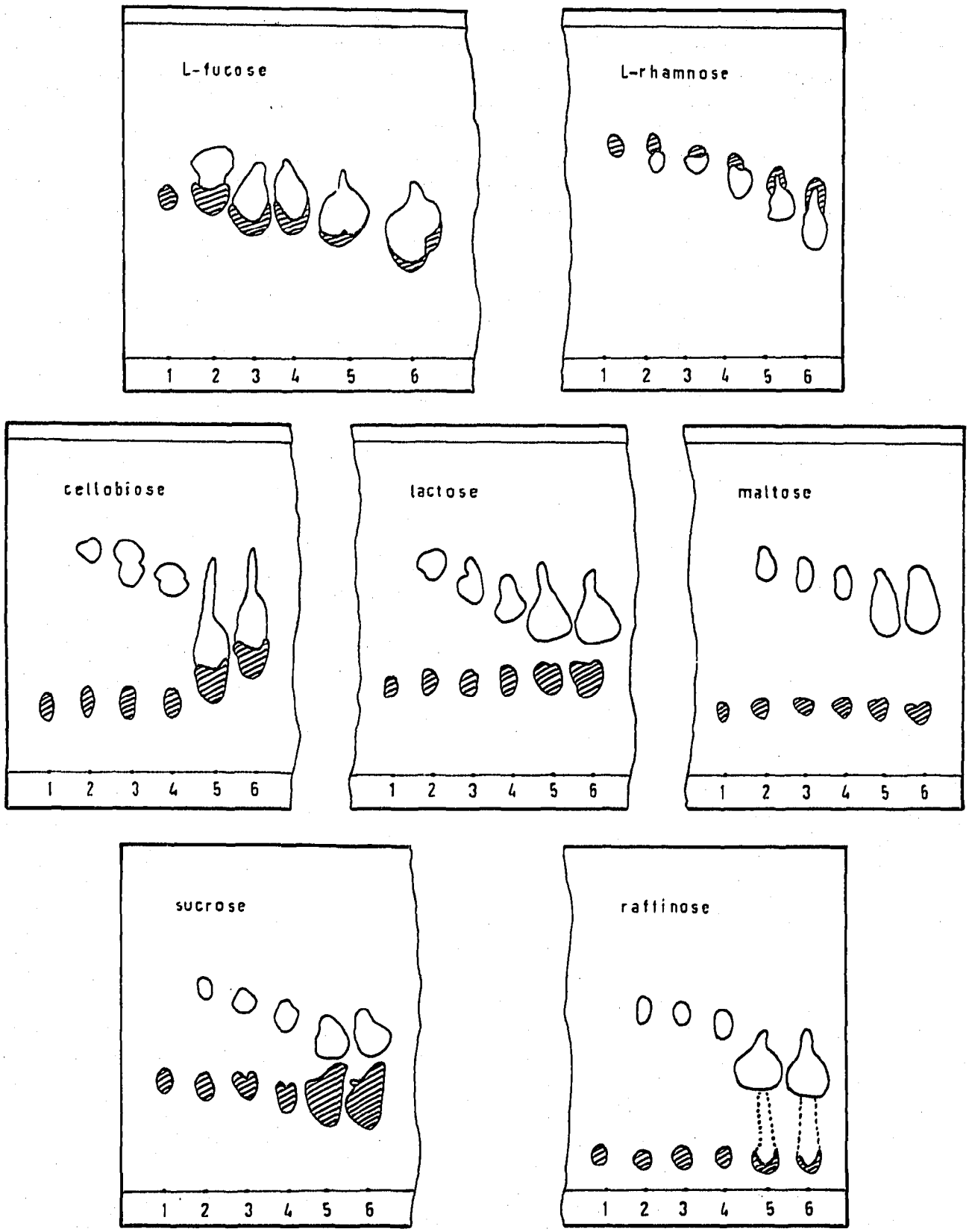

Fig. 4. Tracings of chromatograms of some sugars dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated nos. 1–6 respectively on the starting line. Layer: cellulose. Hatched areas: sugar; unhatched areas: glycerol.

movement of raffinose, lactose and maltose the movement of sucrose is retarded when the glycerol concentration reaches 30 % while that of cellobiose is markedly increased in 40 and 50 % glycerol (Fig. 4). It is difficult to compare the *rate of movement* of the monosaccharides chromatographed on cellulose layers (Fig. 2) with that on buffered silica gel (Fig. 1) because of interference in the movement of the sugars resulting from the considerable distortion in the shape of the glycerol spot particularly with the higher concentrations of glycerol. Nevertheless, there is a general tendency for increase in glycerol concentration to retard the movement of the sugar.

(ii) These sugars (xylose, fucose, rhamnose), whose movement is similar to that of glycerol, show a decrease in movement on both buffered silica gel (Figs. 1 and 3) and cellulose (Figs. 2 and 4) irrespective of whether the sugar moves just behind or in front of the glycerol. The effect is more marked on the cellulose layers, where for each sugar a glycerol concentration of 20 % retards the movement, whereas on buffered silica gel retardation is not observed until the glycerol concentration reaches 30 % (xylose, Fig. 1) or 40 % (fucose, rhamnose, Fig. 3).

The cause of this variation in the rate of movement is possibly a direct effect of the increase in the viscosity of the solutions which results in increased drag as the glycerol ascends the layers.

Shape

When chromatographed on buffered silica gel layers those sugars whose rate of movement is slow compared with that of glycerol appear as small, discrete, round to ovoid spots (*e.g.* galactose, fructose, sorbose, Fig. 1; cellobiose, lactose, maltose, Fig. 3). Only at the higher glycerol concentrations in the case of glucose, mannose and arabinose (Fig. 1) and sucrose (Fig. 3) is there a tendency for slight distortion in shape. Raffinose (Fig. 3) in water and all glycerol concentrations exhibits a small peak at the front edge of the spot. Where the movement of the sugar is similar to that of glycerol the shape of the spot is affected more if the sugar lies just behind the glycerol. A marked crescent-shaped spot results (*e.g.* xylose, Fig. 1; fucose, Fig. 3) especially as the concentration of glycerol increases. If the sugar moves just in front of the glycerol (*e.g.* rhamnose, Fig. 3) the shape of the spot is not influenced appreciably.

When chromatographed on cellulose layers the shape of the sugar spot is grossly distorted at glycerol concentrations as low as 10 % (*e.g.* fructose, arabinose, Fig. 2; fucose, Fig. 4). Distortion occurs even when the sugar and glycerol spots are well separated (*e.g.* sucrose and raffinose, Fig. 4).

VOMHOF[5] reported silica gel layers had not been a satisfactory medium for the naturally occurring sugars owing to the poor separations and low capacity of the plates. However, comparison of the spots obtained in the two systems used shows that in the presence of glycerol there is greater distortion in the shape of the spot on the cellulose layers than on buffered silica gel.

To overcome the difficulties encountered in separation of sugars in extracts preserved in 50 % glycerol solution it is desirable to dilute the glycerol concentration to as low a value as possible commensurate with the ability to detect the sugar. On buffered silica gel layers a glycerol concentration of 20 % produces little or no distortion in shape of change in rate of movement compared with the standard sugar in distilled water.

SUMMARY

The movement of some saccharides in the presence of varying concentrations of glycerol has been investigated to account for the marked variation in R_F values obtained for some sugars in pollen extracts preserved in 50% glycerol. Buffered silica gel and cellulose layers have been compared and, in the presence of glycerol, the former are preferred. It is recommended, commensurate with the ability to detect the quantity of sugar present, that before chromatographing the solution is diluted to contain 10 or 20% glycerol.

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