Separation and Identification of Methylated Sugars on the Paper Chromatogram

THE identification of the products of hydrolysis of a methylated polysaccharide composed of many different sugar residues is a tedious and difficult task, especially when the polysaccharide is available only in small quantities. We have found, however, that many of the methylated sugars can be readily separated and identified on the paper chromatogram, using the same apparatus and solvents as described for the separation of the simple sugars[1,2]. The chromatogram is allowed to run until the solvent has advanced about 40 cm. from the starting line. The paper is then dried and sprayed with ammoniacal silver nitrate, and on warming, the positions of the sugars are indicated by brown spots (except in the case of tetramethylfructopyranose, which reduces ammoniacal silver nitrate only very slightly). As a standard, we use tetramethyl *D*-glucose, which moves rapidly on the chromatogram. The distance the sugar travels is measured from the starting line to the centre, not the edge, of the sugar spot, since the size of the spot and its leading edge vary with the concentration of sugar solution used. The figure R_G given in the accompanying table refers to the ratio between the distance the sugar travels and the distance through which the tetramethyl *D*-glucose has moved. (We use this in preference to the R_F value, which tends to vary with the distance the sugar has advanced from the starting line.)

Substance	R_G	Substance	R_G
Galactose	0·075	2 : 3-Dimethyl arabinose	0·63
Arabinose	0·135	2 : 3 : 4-Trimethyl galactose	0·64
Xylose	0·15	2 : 4-Dimethyl xylose	0·66
4-Methyl galactose	0·165	2 : 4 : 6-Trimethyl galactose	0·67
6-Methyl galactose	0·185	2 : 3-Dimethyl xylose	0·74
Talose, lyxose	0·19	2 : 4 : 6-Trimethyl glucose	0·755
2-Methyl galactose	0·205	3 : 4 : 6-Trimethyl mannose	0·795
Ribose, fucose	0·21	2 : 3 : 6-Trimethyl glucose	0·805
3-Methyl glucose	0·265	2 : 3 : 4 : 6-Tetramethyl galactose	0·88
Rhamnose	0·30	Tetramethyl fructopyranose	0·90
3 : 4-Dimethyl galactose	0·32	2 : 3 : 4-Trimethyl xylose	0·94
2-Methyl arabinose	0·36	2 : 3 : 5-Trimethyl arabinose	0·95
3 : 6-Anhydroglucose	0·37	2 : 3 : 4 : 6-Tetramethyl mannose	0·96
2-Methyl xylose	0·385	2 : 3 : 4 : 6-Tetramethyl glucose	1·0
2 : 4-Dimethyl galactose	0·405	2 : 3 : 4-Trimethyl rhamnose	1·01
2-Methyl fucose	0·51	Raffinose	0·0*
3 : 6-Dimethyl glucose	0·51	Sucrose	0·035
4-Methyl rhamnose	0·575	β-Methyl arabinoside	0·325
3 : 4-Dimethyl mannose	0·58	α-Methyl mannoside	0·30

* No movement.

The separation of the tetramethyl derivatives of glucose, galactose and mannose is readily achieved. Furthermore, closely related pairs of substances, for example, 2 : 3-dimethyl xylose and 2 : 4-dimethyl-xylose, and 2 : 3 : 4-trimethyl galactose and 2 : 4 : 6-trimethyl galactose, are separable. The method renders practicable a rapid examination of the hydrolysis products obtained from small quantities of methylated polysaccharides. It will facilitate also the examination of fractions of methylated sugars produced on distillation of the products of hydrolysis of methylated polysaccharides. The presence of methylated uronic acid derivatives causes tailing on the paper chromatogram; but we find that if these are first removed with 'Amberlite Resin', *IR4B*, the spots due to the various sugars may be readily differentiated in the majority of cases.

By this method we have confirmed the presence of the methylated sugar derivatives detected by the distillation procedure in methylated cherry gum[3], slippery elm mucilage[4] and sugar beet araban[5], and in some instances have found indications of the presence of other sugar derivatives. For example, 2-methyl galactose and 3 : 4 : 6-trimethylmannose were obtained from methylated cherry gum, and free rhamnose from methylated slippery elm mucilage.

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Electrophoretic Behaviour of Modified Ovalbumins

IT has been shown recently by Linderstrøm-Lang and Ottesen[1] that ovalbumin is transformed, by the action of a bacterial enzyme from *Bacillus subtilis*, into a protein that crystallizes as rectangular plates from ammonium sulphate. In an earlier paper[2], MacPherson, Moore and Longsworth report that an ovalbumin component, A_2 (characterized by its mobility in the electric field), increased at the expense of another one, A_1, during storage of a salt-free, isoelectric solution under toluene. It therefore seemed of interest to compare the electrophoretic behaviour of the enzyme-modified ovalbumin with the A_2-form of the original protein.

Ovalbumin recrystallized three times[3] was used as starting material. With the aid of a dry powder containing the bacterial enzyme from *Bacillus subtilis*, kindly furnished by Prof. Linderstrøm-Lang, the enzyme experiments were carried out as described by the Danish workers[1]. All the electrophoretic patterns were obtained after electrophoresis of a 1·2 per cent solution of the protein for four hours in a sodium phosphate buffer of *p*H 6·8 and 0·1 ionic strength at a potential gradient of 6·5 volts per cm. Under these conditions, superposition of the patterns permits direct comparison of the relative concentrations of the components and indicates roughly their mobilities. The patterns of the ascending boundaries are presented here because of better resolution. As a result of adjustments occurring at the δ boundary, peaks due to components having similar mobilities will not coincide precisely. The mobilities cited below were therefore computed from the descending patterns.

In Fig. 1 are superimposed the tracings of the patterns of ovalbumin before and after incubation with the bacterial enzyme, and also after recrystallization, as plates, of the enzyme-treated material. In the case of the ovalbumin before and after enzyme treatment, the qualitative appearance of the patterns is similar but the mobilities are different. Although this difference is small, it is sufficient to lead one to expect resolution of the two main components on mixing the enzyme-treated protein and ovalbumin.