A Colorimetric Method for the Determination of Sugars

VOLUMETRIC procedures have been used for the quantitative determination of sugars after separation by partition chromatography[1,2]. It has been our experience that these methods not only require considerable skill, but also they are lengthy and sensitive to slight variation of the conditions. We have therefore attempted to develop a simple quantitative colorimetric procedure. Preliminary experiments showed that the anthrone[3] and the α-naphthol sulphonate[4] reagents give good results with pure sugar solutions, but the presence of only traces of solvents such as butanol, phenol and propionic acid used in the chromatographic separation of the sugars rendered them useless.

It was then discovered that phenol itself in the presence of sulphuric acid provides a simple rapid method for the quantitative colorimetric determination of ketoses and aldoses and their methyl derivatives on a sub-micro scale. The method, applicable to all carbohydrates with either a free or potential reducing group, is particularly useful for determining sugars which have been separated by partition chromatography using phenol–water as the solvent. The orange-yellow colour, produced by adding sulphuric acid (5 ml.) to the sugar solution (2 ml.) containing phenol, is permanent; its optical density (measured at 490 mμ for hexoses and hexuronic acids and their derivatives and at 475 mμ for pentoses and their derivatives) when referred to a standard curve gives the concentration of the sugar.

This phenol–sulphuric acid reagent, which has enabled us to determine the composition of polysaccharides and their methyl derivatives on as little as 1 mgm. of material, thus offers an additional method for end-group analysis of polysaccharides[5]. The reaction is not limited to phenol, for certain amines such as N(1-naphthyl) ethylene diamine can replace phenol in the above reaction.

In conjunction with the above colorimetric method, it has been found convenient to extract the sugars from the strips of paper cut from the chromatogram by simply immersing them in water. Since the extraction is carried out at room temperature, there is no danger of decomposing the sugars[6]. Substances in filter paper which interfere with the sugar analysis can be largely removed by three or four 'chromatographic' washings with water; washing with dilute sodium hydroxide does not seem to improve the paper.

Further details of this work and its application to the determination of the structure of various polysaccharides will appear elsewhere.

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A New Paper Chromatography Solvent for Amino-Acids

PHENOL and collidine have remained the most widely used pair of solvents for two-dimensional paper chromatography of amino-acids since their introduction by Consden, Gordon and Martin[1]. The use of the latter solvent, however, is attended by certain disadvantages such as double spots and haloes[2], an offensive smell and possible toxic effects[3,4] and, in some countries, difficulty of supply. Alternative solvents have been suggested, for example, butanol – acetic acid[5], acetone – water[2] and pyridine – amyl alcohol[6]. Some defects of the latter system have recently been reported[7].

A new solvent giving an extremely wide distribution of R_F values is mesityl oxide, which has already been mentioned as a possible solvent for the paper partition chromatography of organic acids[8]. The difficulty encountered by Lugg and Overell[8], namely, the condensation of the solvent in the presence of formic acid, has been obviated by the use of re-distilled and fractionated solvent[9]. Because of the tendency to form peroxides on prolonged storage in contact with air, each batch of solvent should be tested for their presence before distillation. The fractionated solvent (b.p. 129–130°) alone does not give any movement of amino-acids; but the acid spots move when formic acid is added to the solvent system. The method of preparation of the most desirable mobile phase is to shake one volume of the solvent with one volume of formic acid (85 per cent) and two volumes of water. R_F values for the amino-acids in such a solvent are listed in the accompanying table, from which it can be seen that the spread of values is considerably higher than with collidine, and indeed almost as high as with phenol. An additional advantage with this solvent is its rapidity of migration, the solvent ascending about 50 cm. in twenty hours on Whatman No. 1 paper.

R_F VALUES OF AMINO-ACIDS

Amino-acid	R_F	Amino-acid	R_F
Alanine	0·35	Methionine	0·61
Arginine*	0·19	*nor*Valine	0·58
Aspartic acid	0·17	Ornithine†	0·08
Cystine	0·06	Phenylalanine	0·72
Glutamic acid	0·20	Proline	0·39
Glycine	0·22	Serine	0·20
Histidine	0·07	Threonine	0·26
*iso*Leucine	0·70	Tryptophane	0·81
Leucine	0·71	Tyrosine	0·63
Lysine	0·23	Valine	0·58

* Applied as monohydrochloride. † Applied as hydrobromide.

The tendency for mesityl oxide gradually to oxidize and polymerize on standing, with the formation of coloured substances, has been especially noticed in the acidified mobile phase, which after several days may develop a slight orange coloration. This difficulty has been avoided by making up small volumes of the mobile phase just prior to use and discarding any residual solvent after about two days. By this means an exceedingly high degree of reproducibility in R_F values has been obtained. Our usual practice is to allow the paper to become equilibrated thoroughly in the vapours of the stationary phase before introducing the mobile phase. The paper may be quickly and satisfactorily air-dried. Heating of the ninhydrin-sprayed sheet then brings up the spots in their characteristic colours. As with carboxylic acids, spots