LETTERS TO THE EDITORS

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Application of Paper Partition Chromatography to the Separation of the Sugars and their Methylated Derivatives on a Column of Powdered Cellulose

THE analysis of mixtures of sugars resulting from the hydrolysis of a polysaccharide has been greatly facilitated by their separation[1] and quantitative estimation[2] on the paper chromatogram[3]. The application of this technique to the analysis of mixtures of sugars from methylated polysaccharides[4] has simplified a task previously difficult, which involved the fractional distillation of the methyl glycosides and examination of the fractions obtained. The latter method suffers from two disadvantages, in that it requires large quantities of methylated polysaccharides, and that it is very difficult to obtain a complete separation of the methyl glycosides.

The paper chromatographic technique now offers a rapid method of separating and estimating the sugars quantitatively as well as giving a strong indication as to their identities. The final proof of their constitution, however, still depends upon their separation and identification by determination of their physical constants, and the formation of characteristic derivatives. With this in mind we have attempted the separation of the sugars and their methylated derivatives on a column of powdered cellulose, a method similar to that tried by Synge[5], who used a column of potato starch, and similar to that employed by Bell[6], who used a column of silica gel. Using a column of cellulose (Whatman ashless filter tablets, rubbed through an 80-mesh sieve and tightly packed, as a powder, in a tube of 12 in. length and $1\frac{1}{4}$ in. diameter) and with *n*-butanol saturated with water containing 1 per cent of ammonia as the mobile phase, we have separated two- and four-component mixtures of sugars with individual recoveries of 95–100 per cent. The column is purified by washing it with the solvent until the eluate is non-reducing; this ensures that all the soluble impurities have been washed from the cellulose and that equilibrium is reached between the cellulose and the solvent. The maximum weight of any component sugar that can be placed on this column is proportional to its R_G value[4], and is of the order of 200 mgm. for galactose. The syrupy mixture of sugars to be separated is placed on the top of the cellulose and the solvent allowed to percolate down the column. At half-hourly intervals the receiver collecting the eluate is changed automatically. The time taken for any particular sugar to emerge is inversely proportional to its R_G value and is approximately 15 hr. for 2 : 3 : 4 : 6-tetramethyl glucose.

To determine which sugars are present in the receivers, a small spot from each is placed, in order of time, on the starting line of a paper chromatogram, and the sugars separated in the usual manner. On revealing the position of the sugars on the chromatogram by development with ammoniacal silver nitrate, a picture of the distribution of each sugar among the receivers is obtained. From such a chromatogram one may decide which portions of eluate to take in order to obtain a chromatographically pure sample of sugar. While such high recoveries as 95 per cent are quite practical where the components differ widely in R_G values, it is frequently expedient, where one has several components of closely similar R_G values with consequent overlapping of the sugars, to be content to divide the receivers in such a manner as to lead to a lower percentage recovery of a pure fraction. The ease of separation decreases as the R_G values of the particular sugars increase.

By use of the method described above, we have separated in the pure state and in yields of 95 per cent a two-component mixture consisting of *L*-rhamnose (300 mgm.) and *L*-arabinose (200 mgm.) and a four-component mixture consisting of *L*-rhamnose (50 mgm.), *D*-ribose (50 mgm.), *L*-arabinose (50 mgm.) and *D*-galactose (50 mgm.). The isolation of pure crystalline specimens of *L*-rhamnose hydrate and *D*-galactose and of *L*-rhamnose hydrate and *D*-xylose from *Sterculia Setigera* gum and from linseed (Var. Redwing) mucilage respectively has also been achieved.

Methylated sugar beet araban, on hydrolysis, yields in equimolecular amounts 2 : 3 : 5-trimethyl *L*-arabinose, 2 : 3-dimethyl *L*-arabinose and 2-monomethyl *L*-arabinose[7]; pure specimens of each of these sugars have been obtained by use of the above method.

The application of the above technique to the separation and identification of the hydrolysis products of various methylated polysaccharides will be the subject of a later publication.

L. HOUGH
J. K. N. JONES
W. H. WADMAN

University, Bristol.
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Oxidation of Ferrous Ions in Aqueous Solution by X- and γ-Radiation

THE oxidation of ferrous ions in dilute sulphuric acid solution by X- and γ-radiation has attracted attention since the work of Fricke[1,2] as a possible method of integral dosimetry for aqueous media[3]. A study has now been made which confirms that the rate of oxidation with dose is essentially independent of the concentration of ferrous ions in the region 10^{-3} to 10^{-4} *M*, and which also shows that this rate is maintained independently of the wave-length of the radiation over a much wider range of wave-length than has hitherto been used in studies of this type.

Four different types of system were employed.

(i) An ionization chamber was constructed of 'Perspex' with graphite electrodes and connected to a D.C. amplifier circuit, and a 'Perspex' irradiation cell was made of identical geometry to the chamber to contain the solution under investigation. The cell and chamber could be interchanged on a stand which was kept in a constant position relative to the tube of an industrial X-ray unit operating at 200 kV. peak and 10 m.amp. The thickness of the cylindrical