CHROM. 3496

The separation of glucosamine and galactosamine and their glycitols on an amino acid analyser

The usual method of determining the reducing sugar unit in an oligosaccharide is by analysis before and after reduction with sodium or potassium borohydride[1,2], the alditol thus obtained being derived from the terminal reducing sugar. Where the amino sugars glucosamine and galactosamine are components of the oligosaccharides, the difference in composition after reduction is difficult to determine, for although glucosamine and galactosamine can be analysed simultaneously on an amino acid analyser,

separation obtained is shown in Fig. 1. Where glucosamine and glucosaminitol together are the only amino sugars present, the same routine analysis is effective (Fig. 2). However when glucosamine and galactosamine and their alcohols are to be analysed, the separation is performed on 150 × 0.9 cm columns of Aminex MS Q 150, using pH 5.28 buffer to which boric acid is added to 0.2 *M*, to give a final pH of 5.09. The chromatogram obtained is shown in Fig. 3, and the retention times relative to glucosamine

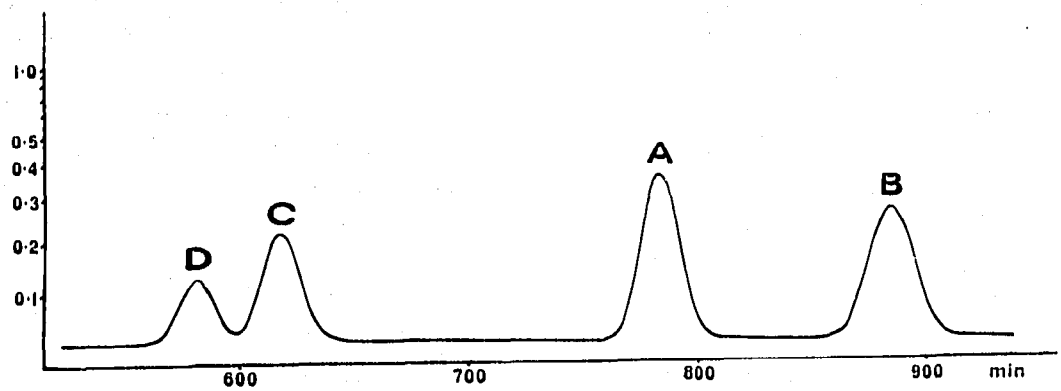

Fig. 3. The resolution of galactosaminitol (D), glucosaminitol (C), glucosamine (A) and galactosamine (B) on a 150 cm column of Aminex MS Q 150 resin with pH 5.09 buffer containing 0.2 M borate.

are galactosaminitol 0.74, glucosaminitol 0.80 and galactosamine 1.13. For normal use a load of 50–300 μg of each of the sugar bases was required, and the method gave results of the degree of accuracy to be expected with this type of amino acid analyser (*i.e.* ±2–3%) for the determination of standard mixtures. With a high sensitivity cuvette 5–30 μg was the usual loading, but the accuracy was less (±5%). The retention times of glucosamine and galactosamine were practically the same as in buffers of similar pH containing no borate, these sugars being unable to form strong complexes of the C_1–C_2 type. The more strongly complexing alditols emerge from the column with decreased retention times consistent with the decrease in basicity which the complex formation might be expected to confer on the molecules.

Acknowledgements

This work was supported by a grant from the Medical Research Council. The author would like to thank Prof. W. T. J. MORGAN for his encouragement.

The Lister Institute of Preventive Medicine,
Chelsea Bridge Road,
London, S.W.1 (Great Britain)

A. S. R. DONALD

1 M. ABDEL-AKHER, J. K. HAMILTON AND F. SMITH, *J. Am. Chem. Soc.*, 73 (1951) 4691.
2 S. PEAT, W. J. WHELAN AND J. G. ROBERTS, *J. Chem. Soc.*, (1956) 2258.
3 R. MONTGOMERY, Y. C. LEE AND Y. WU, *Biochemistry*, 4 (1965) 566.
4 P. D. BRAGG AND L. HOUGH, *J. Chem. Soc.*, (1957) 4347.
5 W. R. C. CRIMMIN, *J. Chem. Soc.*, (1957) 2838.
6 D. H. SPACKMAN, W. H. STEIN AND S. MOORE, *Anal. Chem.*, 30 (1958) 1190.

Received March 4th, 1968