Carbohydrate Research, 30 (1973) 218–219

Note

Separation of methyl ethers of sugars by gel filtration

EDWARD GRELLERT AND CLINTON E. BALLOU

Department of Biochemistry, University of California, Berkeley, California 94720 (U. S. A.)

(Received March 2nd, 1973; accepted for publication, April 2nd, 1973)

Partially methylated mono- and oligosaccharides have recently been prepared and characterized by gel filtration on Sephadex G-25 and Bio-Gel P-2 or P-4 resins[1,2]. It was found that a single methyl ether group increased the apparent volume of a sugar derivative by about 1.5 sugar units, so that 6-*O*-methyl-D-glucose was eluted between maltose and maltotriose, at a position equivalent to a degree of polymerization of 2.5 (Ref. 1). Moreover, the effect is additive. Thus, the fractionation of the partially methylated fragments obtained by Smith degradation of a methylated lipopolysaccharide allowed the separation of the products into peaks containing (a) glycerol and erythritol, (b) 1-*O*-methylerythritol and D-glucosylglycerol, (c) (6-*O*-methyl-D-glucosyl)glycerol and (6-*O*-methyl-D-glucosyl)erythritol, (d) 1-*O*-methyl-(6-*O*-methyl-D-glucosyl)erythritol and (3,6-di-*O*-methyl-D-glucosyl)erythritol, and (e) (3,6-di-*O*-methyl-D-glucosyl)-1-*O*-methylerythritol and (3,4,6-tri-*O*-methyl-D-glucosyl)erythritol[2]. Clearly, effective use can be made of the different molecular sizes resulting from different degrees of methylation.

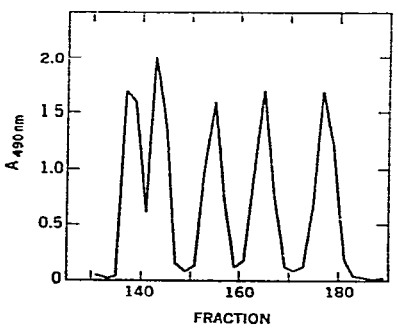

Fig. 1. Elution pattern of partially methylated D-glucose derivatives from a Bio-Gel P-4 resin column. From right to left, the peaks correspond to D-glucose, 6-*O*-methyl-D-glucose, 4,6-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose. Other details are given in the Experimental section.

In this note, we call attention to the usefulness of this procedure for the separation of typical fragments that might result from the methylation and hydrolysis

of a branched D-glycan. A synthetic mixture of D-glucose and its mono-, di-, tri-, and tetramethyl ethers was fractionated on a Bio-Gel P-4 column and the components were determined by the phenol–sulfuric acid reagent[3]. As shown in Fig. 1, the resolution was excellent. On the other hand, a mixture of isomeric mono-*O*-methyl-D-glucoses was not separated. Although comparable separations can be accomplished in other ways, gel filtration has the advantages of reasonable capacity, good recoveries, and minimal contamination from the column support if polyacrylamide gels are used.

EXPERIMENTAL

A column (2 × 200 cm) of Bio-Gel P-4 (−400 mesh) resin (Bio-Rad Laboratories Richmond, California 94804) was prepared according to the manufacturer's directions[4]. A mixture of the following sugars, about 3.5 mg each, was dissolved in 0.5 ml of water: D-glucose, 6-*O*-methyl-D-glucose, 4,6-di-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,3,4,6-tetra-*O*-methyl-D-glucose. The solution was applied to the column and the sample was eluted with water at a flow-rate of 7 ml per h. Samples of 3 ml were collected and analyzed by the phenol–sulfuric acid reagent[3]. The results are recorded in Fig. 1. The unequal separation of the different components suggests that there may be some small positional influence on the resolution. A mixture of 3-, 4-, and 6-*O*-methyl-D-glucose was not resolved.

ACKNOWLEDGMENT

This work was supported by Grant AM-884 of the Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, U. S. Public Health.

REFERENCES

1 E. A. GRELLERT AND C. E. BALLOU, *J. Biol. Chem.*, 247 (1972) 3236.
2 G. R. GRAY AND C. E. BALLOU, *J. Biol. Chem.*, 247 (1972) 8129.
3 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
4 *Gel Chromatography*, Bio-Rad Laboratories, Richmond, California, 1971, p. 32.