CHROM. 5340

Application of thin-layer chromatography to the purification and characterization of some methyl glucosides

The recent elucidation of the structure of the monomycolylglucoses of some corynebacteria and mycobacteria[1] involved synthesis of a number of methyl glucosides. Several methods were investigated to prepare pure samples of these compounds. The most satisfactory and rapid procedure employed preparative thin-layer chromatographic (TLC) systems. This note describes the application of these systems to the purification of α- and β-methyl tetra-O-methylglucosides and the anomers of methyl 2,3,4-tri-O-methylglucoside. The characterization of these compounds by TLC and other methods is also described.

Experimental

Preparation of the methyl glucosides. Two precursors of methyl glucosides were used, *viz.* methyl α-D-glucoside and isomaltose (both obtained from Koch-Light). The methylation of methyl α-D-glucoside was based on the procedure of PEAT[2]. Methyl α-D-glucoside (350 mg) was dissolved in 3 ml of distilled anhydrous methanol. To this were added 1.26 g of freshly prepared Ag_2O and 1.55 g of CH_3I. The mixture was refluxed for short spells over a 4-h period and allowed to cool for the intermittent times. At the end of this period further portions of CH_3I and Ag_2O were added. The mixture was stirred at room temperature for 5 h and finally refluxed while stirring for 15 min. The solution was filtered and the Ag_2O washed with warm methanol. The filtrates were concentrated, extracted with distilled diethyl ether and the extract was dried over anhydrous sodium sulphate, filtered and concentrated to give a clear viscous syrup.

Methylation of isomaltose was carried out in two ways. The first employed the method of KUHN *et al.*[3] as follows: 20 mg of isomaltose, 2 ml of dimethylformamide, 2 ml of CH_3I and 2 g of Ag_2O were placed in a test tube and shaken vigorously for 24 h. Similar amounts of Ag_2O and CH_3I were then added and the solution shaken for a further 24 h. The mixture was filtered and the Ag_2O was washed with $CHCl_3$. The filtrates were concentrated and equal volumes of water and benzene added and the mixture shaken. The upper benzene layer was washed several times with water and finally concentrated to yield a yellow syrup which was dried *in vacuo* over P_2O_5.

The methylation procedure of BRIMACOMBE *et al.*[4] was also applied to isomaltose as follows: isomaltose (15 mg) was dissolved in 1 ml of dimethylformamide. Sodium hydride (50 mg) was then added, followed by CH_3I (1 ml). The solution was stirred for 15 min at room temperature and a further 1 ml of dimethylformamide was added. After 2-h stirring at room temperature the mixture was added to chloroform–water (1:1). The chloroform layer was repeatedly washed with water, finally dried over sodium sulphate and evaporated to dryness.

Methanolysis and hydrolysis. The permethylated isomaltose obtained by both procedures was methanolysed in a sealed tube with 3 ml of 4.6% anhydrous methanolic HCl at 105° for 18 h. The tube was opened, methanol was removed on a rotary evaporator and HCl in a desiccator over KOH. Reducing sugars were obtained from the corresponding methyl glucosides by incubating in sealed tubes at 100° with 2 *N* HCl for 2 h.

Chromatographic systems. Preparative TLC was carried out on wide plates (40 × 20 cm) of Silica Gel H (0.5 mm thick). Chloroform–methanol (47:3) (solvent A)[5] was used to obtain full resolution of the methyl glucosides with different numbers of methyl groups arising from methyl α-D-glucoside. This solvent was not satisfactory for resolving the α- and β-anomers of the methyl glucosides, in which case benzene–ethanol–water (170:47:15, upper layer)[6] (solvent B) was used. Reducing sugars were chromatographed on thin-layer plates of Silica Gel G (0.25 mm thick) in acetone–25% (w/v) aqueous ammonia–water (250:1.5:3)[7] (solvent C) or on sheets of Whatman No. 1 paper in butanol–ethanol–water (50:10:4, upper layer). Gas–liquid chromatography (GLC) was carried out on columns of 10% diethylene glycol succinate (DEGS) on Chromosorb W, 100/120 mesh, at 175° and with a nitrogen flow rate of 45 ml/min. A column of 2% neopentyl glycol succinate (NPGS) on Chromosorb W, temperature programmed at 3° per min from 120° to 250°, and with a nitrogen flow rate of 45 ml/min, was also used to indentify the α- and β-anomers of the methyl tri-O-methylglucoside.

Results and discussion

The methylated glucosides obtained from the methylation of methyl α-D-glucoside were dissolved in chloroform and applied to four preparative thin-layer plates and chromatographed in solvent A. The plates were divided into nine equal bands parallel to the line of origin and these were cut out except for two narrow strips (1.5 cm wide) on each margin of the plate. The strips were sprayed with 50% H_2SO_4 and heated at 110° for 20 min. Four compounds were present (Table I). These were eluted from the respective bands of silica gel with chloroform–methanol (2:1). The mono- and di-O-methylglucosides were not examined further. The tetra-O-methylglucoside in solvent B had an R_F value of 0.49 identical to methyl 2,3,4,6-tetra-

TABLE I

RESOLUTION OF SOME METHYL GLUCOSIDES BY TLC

Source	*R_F value*		*Relative amounts*	*Structure*
	Solvent A	*Solvent B*		
Methylated methyl α-D-glucoside	1.00		+	methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside
	0.63		+ + + +	methyl 2,3,4-tri-O-methyl-α-D-glucopyranoside
	0.32		+ +	a methyl di-O-methyl-α-D-glucopyranoside
	0.12		+	a methyl mono-O-methyl-α-D-glucopyranoside
Methylated, methanolysed isomaltose		0.62	+ + +	methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside
		0.49	+ +	methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside
		0.35	+ + +	methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside
		0.31	+ +	methyl 2,3,4-tri-O-methyl-α-D-glucopyranoside

O-methyl-α-D-glucopyranoside (a gift from Professor P. O'COLLA, University College, Galway). GLC of this material showed a retention time of 6.9 min identical to that of methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside. PC of the reducing sugar showed one spot (detected with aniline–phthalate) with an $R_{Glucose}$ value of 4.28, the same as that for 2,3,4,6-tetra-O-methylglucose. TLC of the reducing sugar in solvent C again showed one spot with an R_F value of 0.78 identical to 2,3,4,6-tetra-O-methylglucose.

In solvent B the tri-O-methyl-α-D-glucoside had an R_F value of 0.30 identical to that of methyl 2,3,4-tri-O-methyl-α-D-glucopyranoside (a gift from Dr. G. BATHGATE, Guinness Research Laboratories, Dublin). GLC of this material on the DEGS column showed a retention time of 27.6 min identical to the methyl 2,3,4-tri-O-methyl-α-D-glucopyranoside. PC of the reducing sugar showed an $R_{Glucose}$ value similar to that of 2,3,4-tri-O-methylglucose.

Permethylated isomaltose was methanolysed and the product applied to preparative thin-layer plates and chromatographed in solvent B. When strips from the plates were charred with 50% H_2SO_4, four bands were observed (Table I). The methyl glucosides were eluted and identified by TLC and PC as described above. GLC of the methyl 2,3,4,6-tetra-O-methyl-β-D-glucoside showed a retention time of 5.1 min compared with 7.5 min for the α-anomer. The methyl 2,3,4-tri-O-methyl-β-D-glucopyranoside had a retention time of 19.8 min compared to 27.6 min for the α-anomer.

Solvent A has proved very effective for the resolution of mono-, di-, tri- and tetra-O-methylglucosides and solvent B can separate the anomeric forms of the methyl tri-O-methylglucosides and the methyl tetra-O-methylglucosides. A combination of these solvents is a useful adduct to GLC[8] for locating the position of fatty acids on the glucopyranose of the acylglucoses[1,9].

This work was supported by a grant from the National Science Council.

Department of Biochemistry, Trinity College, Dublin 2 (Ireland) PATRICK J. BRENNAN

1 P. J. BRENNAN, D. P. LEHANE AND D. W. THOMAS, *Eur. J. Biochem.*, 13 (1970) 117.
2 S. PEAT, *J. Chem. Soc.*, 83 (1903) 1037.
3 R. KUHN, H. TRISCHMANN AND I. LOW, *Angew. Chem.*, 67 (1955) 32.
4 J. S. BRIMACOMBE, B. D. JONES, M. STACEY AND J. J. WILLARD, *Carbohyd. Res.*, 2 (1966) 167.
5 T. S. STEWART, P. B. MENDERSHAUSEN AND C. E. BALLOU, *Biochemistry*, 7 (1968) 1843.
6 C. PROTTEY AND C. E. BALLOU, *J. Biol. Chem.*, 243 (1968) 6196.
7 P. J. STOFFYN, *J. Amer. Oil Chem. Soc.*, 43 (1966) 69.
8 G. MARTIN AND J. ASSELINEAU, *J. Chromatogr.*, 39 (1969) 322.
9 P. J. BRENNAN, M. P. FLYNN AND P. F. S. GRIFFIN, *FEBS Letters*, 8 (1970) 322.

Received March 8th, 1971