THIN-LAYER CHROMATOGRAPHY OF SUBSTITUTED GLYCOSIDES

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INTRODUCTION

Even though the principle of thin-layer chromatography dates back thirty years[1], it began to be applied widely only about ten years ago with the introduction of the procedures of STAHL[2-7]. Use of thin-layer chromatography for separation of carbohydrates was first reported in 1961[8,9]. Since then it has been applied to a wide variety of derivatized sugars—esters[10-21], ethers[14,22-29], cyclic acetals[14,19,22,29,30], deoxy sugars[20,31-33], and others[34-38].

Thin-layer chromatography has also been a useful tool in monitoring carbohydrate reactions[19,29,30,40] by determining the reactant–product ratios at various times.

This paper describes the use of thin-layer silica gel chromatography for (a) the separation of partially or completely derivatized carbohydrates, (b) the monitoring of carbohydrate alkylations, and (c) the separation of α- and β-anomers of glycosides of benzylated mono- and oligo-saccharides.

EXPERIMENTAL

The compounds used in this study were prepared by the authors. Most were synthesized using published methods. The benzylated maltooligosaccharides were synthesized by WING[41], and all alkylations were effected by the same procedure described for methylation by WING[41], substituting ethyl bromide, 2-chloropropane, 1-bromobutane or 2-bromobutane for methyl iodide[42]. Alkylations using the larger alkyl halides required longer reaction times for two reasons: (a) substitution of a larger group, and (b) use of a less reactive halide.

Several variables effect the reproducibility of the R_F values obtained by thin-layer chromatography. These variables have been reviewed by RANDERATH[43] and have been investigated further by the authors to give reproducible results.

Preparation of silica gel plates

A slurry of Silica Gel H (Brinkmann Instruments, Inc., Cantiague Road, Westbury, Long Island, N.Y.) (30 g) in distilled water (80 ml) was applied to five clean glass plates (20 cm × 20 cm × 0.3 cm) at a thickness of 0.25 mm, using a Shandon Spreader outfit (Colab Laboratories, Inc., Chicago Heights, Ill.). This apparatus uses an inflatable bag which presses the plates up against the underside of two guide rails so that plates which differ in thickness will still have a uniform layer thickness.

Since Silica Gel H contains no calcium sulfate or organic binder, the length of time between the addition of water and the spreading is not as critical as with other adsorbents with binders. Therefore, the slurry was stirred with a glass stirring rod for ten minutes to assure complete mixing.

After preparation, the plates were exposed to the air for two hours before activation at 130° for 2 h. Edges of the plates were then scraped with a spatula to make them straight, and the plates were stored at room temperature. Since room humidity has an influence on the activity of the gel for separation, the plates were placed in a 130° oven for 30 min and cooled just before use.

Irrigants

Redistilled A.C.S. certified petroleum ether (30–60°) and acetone were used in this study in the ratios given in Table I. Freshly prepared irrigants were used for each development.

TABLE I

SOLVENT RATIOS IN IRRIGANTS

Irrigant	*Petroleum ether (30–60°) (ml)*	*Acetone (ml)*
A	100	15
B	100	20
C	100	25
D	100	30
E	100	40

Sample application

The samples (1 % solutions in chloroform) were applied with capillary tubes 1.5 cm from the bottom edge, 3.0 cm from each side and 1.5 cm apart.

Development of the plates

The appropriate irrigant was added to the tank (Shandon TLC Chromatank) and a piece of Whatman No. 1 filter paper was dipped through the solvent and attached to the sides of the tank. The tank was equilibrated for 30 min before insertion of the plate to decrease the "edge phenomenon" as much as possible[5,43]. The tank was placed in the same position for each ascent; the temperature was 31°. The depth of the solvent in the tank was approximately 1 cm.

Only one plate at a time was placed in the tank, and the time was recorded. Approximately 35 min were required to reach a height of 16 cm from the sample origin. When this height was reached, the top was removed from the tank; and the solvent front was marked before the plate was removed from the tank.

Detection of components

After removal, the plate was dried for 2 min before spraying lightly with a 50 % sulfuric acid–ethanol solution. The plate was then placed directly into a 130° oven for 15 min. When the plates were removed from the oven, the center of each spot was

marked so that the R_F values could be calculated when the plates were cooled. Because the spots sometimes faded, this preliminary marking was helpful.

Plates for monitoring carbohydrate alkylations

Plates used for monitoring carbohydrate alkylations were prepared as previously described except, after the first spot (zero time) was made, the plate was left exposed to the ambient air.

RESULTS AND DISCUSSION

This procedure for thin-layer chromatography has been applied to a large number of derivatized carbohydrates. The derivatives made were ethers [methyl, ethyl, isopropyl, *n*-butyl, *sec.*-butyl (2-butyl), trityl (triphenylmethyl), and benzyl], esters [acetyl, benzoyl and tosyl (*p*-tolysulfonyl)], cyclic acetal (benzylidene), deoxy, and deoxy thiocyano. These derivatives were made of the methyl glycosides of D-glucose, D-galactose, D-mannose and maltooligosaccharides.

In Table II are recorded R_F values for some derivatized monosaccharides using irrigants A, B, D and E.

In Table III are recorded R_F values for some α- and β-anomers of perbenzylated

TABLE II

R_F VALUES OF DERIVATIZED MONOSACCHARIDE GLYCOSIDES

Compound	*R_F in irrigant*			
	A	*B*	*D*	*E*
Methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-glucopyranoside	0.34	0.41	0.55	—
Methyl 2,3-di-O-acetyl-4,6-O-benzylidene-α-D-glucopyranoside	0.27	0.35	0.50	—
Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside	0.35	0.53	0.74	—
Methyl 2,3-di-O-benzyl-α-D-glucopyranoside	0.03	0.07	0.15	0.25
Methyl 2,3-di-O-benzyl-6-O-trityl-α-D-glucopyranoside	0.28	0.41	0.60	—
Methyl 6-O-trityl-α-D-glucopyranoside	0.00	0.00	0.03	0.06
Methyl 6-O-trityl-α-D-mannopyranoside	0.00	0.03	0.06	0.10
Methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside	0.10	0.20	0.39	0.54
Methyl 2,3,6-tri-O-benzoyl-4-O-methyl-α-D-glucopyranoside	0.29	0.38	0.56	—
Methyl 2,3,6-tri-O-benzoyl-4-O-tosyl-α-D-glucopyranoside	0.08	0.21	0.42	0.53
Methyl 2,3,6-tri-O-benzoyl-4-deoxy-4-thiocyano-α-D-galactopyranoside	0.19	0.32	0.56	—
Methyl 2,3,6-tri-O-benzoyl-4-deoxy-α-D-glucopyranoside	0.30	0.45	0.66	—
Methyl 2,3-di-O-benzoyl-4-O-tosyl-6-O-trityl-α-D-glucopyranoside	0.28	0.40	0.62	—
Methyl 2,3,4-tri-O-benzoyl-6-O-trityl-α-D-glucopyranoside	0.24	0.38	0.61	—
Methyl 2,3-di-O-benzoyl-4-O-tosyl-α-D-glucopyranoside	0.04	0.08	0.19	0.33
Methyl 2,3-di-O-benzoyl-6-O-trityl-α-D-glucopyranoside	0.21	0.29	0.47	0.64
Methyl 2,3,6-tri-O-methyl-6-O-trityl-α-D-mannopyranoside	0.42	0.50	0.67	—
Methyl 2,3,6-tri-O-methyl-6-O-trityl-α-D-galactopyranoside	0.34	0.44	0.61	—
Methyl 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranoside	0.19	0.32	0.55	—
Triphenylcarbinol	0.49	0.55	0.70	—
Chlorotriphenylmethane	0.49	0.55	0.71	—

TABLE III

R_F VALUES OF THE α- AND β-ANOMERS OF PERBENZYLATED METHYL 4-O-SUBSTITUTED-D-GLUCOSIDES

Compound	*R_F in irrigant*							
	A		*B*		*C*		*D*	
	α	β	α	β	α	β	α	β
Methyl 2,3,6-tri-O-benzyl-D-glucopyranoside	0.20	0.24	0.31	0.36	0.41	0.45	0.52	0.56
Methyl 2,3,6-tri-O-benzyl-4-O-methyl-D-glucopyranoside	0.36	0.40	0.47	0.52	0.57	0.61	0.67	0.70
Methyl 2,3,6-tri-O-benzyl-4-O-ethyl D-glucopyranoside	0.38	0.43	0.48	0.52	0.61	0.64	0.70	0.73
Methyl 2,3,6-tri-O-benzyl-4-O- isopropyl-D-glucopyranoside	0.40	0.45	0.50	0.54	0.62	0.65	0.71	0.74
Methyl 2,3,6-tri-O-benzyl-4-O-*n*-butyl-D-glucopyranoside	0.42	0.46	0.52	0.56	0.64	0.66	0.72	0.76
Methyl 2,3,6-tri-O-benzyl-4-O-*sec.*-butyl-D-glucopyranoside	0.41	0.46	0.52	0.55	0.63	0.67	0.72	0.76
Methyl 2,3,4,6-tetra-O-benzyl-D-glucopyranoside	0.35	0.39	0.47	0.50	0.57	0.60	0.66	0.69

methyl 4-O-substituted-D-glucosides using irrigants A, B, C and D. These reactions were also monitored by TLC; the plates are shown in Figs. 1–5.

In Table IV are recorded R_F values of perbenzylated methyl terminal-4-hydroxymaltooligosaccharides and in Table V R_F values of perbenzylated methyl terminal-4-O-methylmaltooligosaccharides using irrigants A, B and D.

All reported R_F values are averages of three values; the precision in each case is ± 0.01. The migration of the compound in a particular irrigant is dependent upon the degree of substitution. As the number of unsubstitituted hydroxy groups or polar substituents increases, a corresponding decrease in R_F is noted. By increasing the

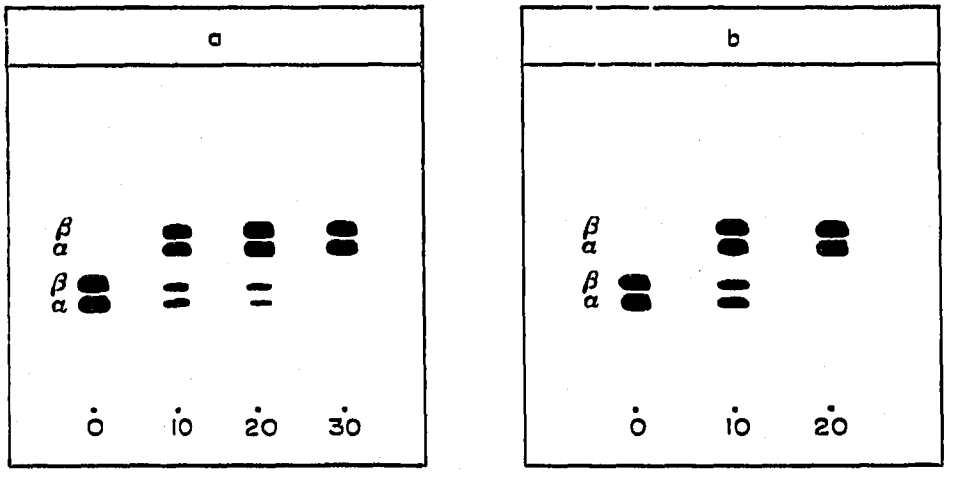

Fig. 1. Monitoring reaction of methyl 2,3,6-tri-O-benzyl-α,β-D-glucopyranoside with methyl iodide on Silica Gel H using an irrigant of petroleum ether (30–60°)–acetone (100:20, v/v). Numbers refer to time in minutes after the addition of methyl iodide. (a) Reaction mixture cooled to —40° until the addition of methyl iodide. (b) Reaction mixture kept at room temperature throughout the entire reaction.

proportion of acetone in the irrigant, the R_F values will increase so a useful value can be obtained.

During methanolysis (24 h) of tri-O-benzylamylose, α- and β-methyl glucosides of a homologous series of oligosaccharides are obtained (Table IV). Upon methylation[41], perbenzylated methyl terminal-4-O-methyl-α,β-maltooligosaccharides result (Table V). If the methanolysis is extended to 125 h, the major products are the methyl α- and

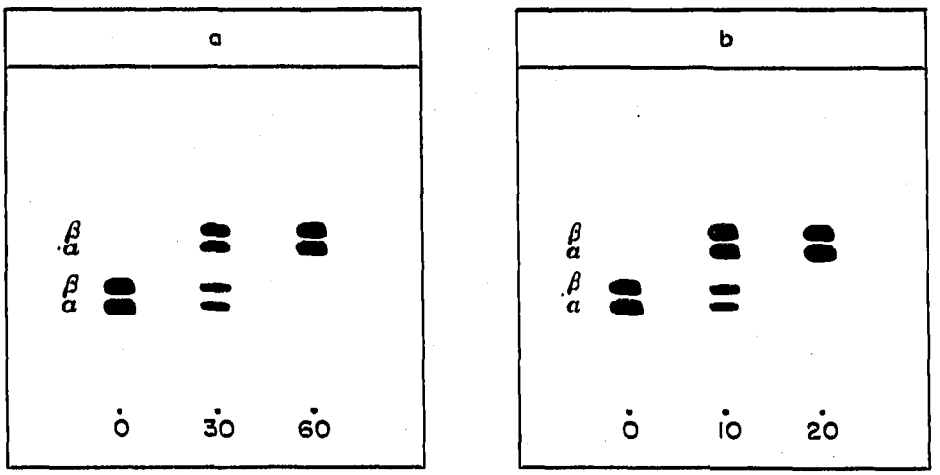

Fig. 2. Monitoring reaction of methyl 2,3,6-tri-O-benzyl-α,β-D-glucopyranoside with ethyl bromide on Silica Gel H using an irrigant of petroleum ether (30–60°)–acetone (100:20, v/v). Numbers refer to time in minutes after the addition of ethyl bromide. (a) Reaction mixture cooled to —40° until the addition of ethyl bromide. (b) Reaction mixture kept at room temperature throughout the entire reaction.

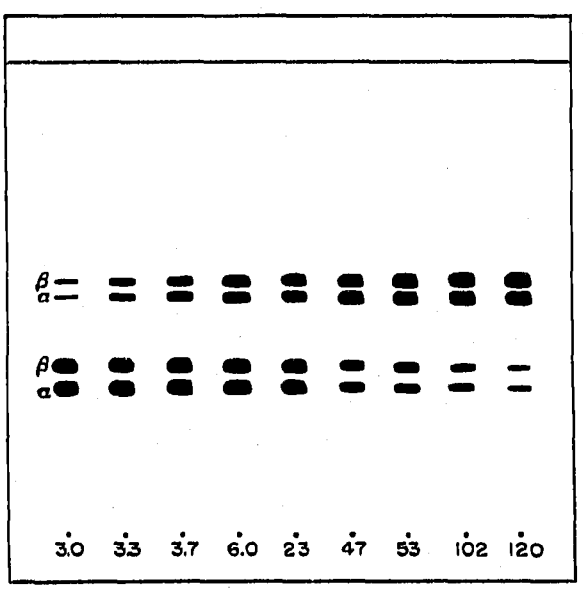

Fig. 3. Monitoring reaction of methyl 2,3,6-tri-O-benzyl-α,β-D-glucopyranoside with 2-chloropropane on Silica Gel H using an irrigant of petroleum ether (30–60°)–acetone (100:20, v/v). Numbers refer to time in hours after the addition of 2-chloropropane.

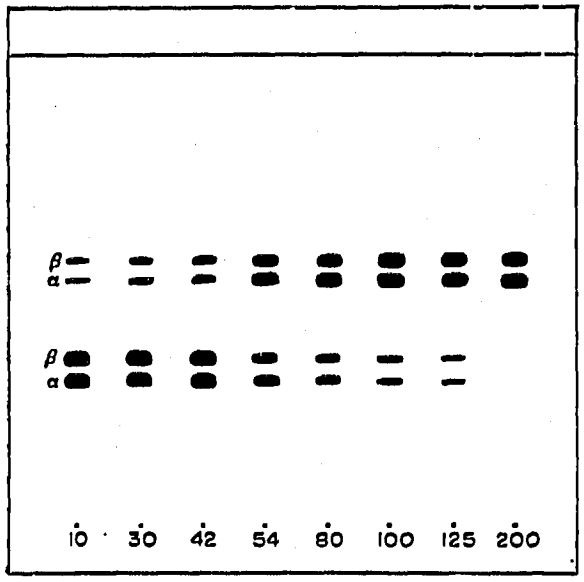

Fig. 4. Monitoring reaction of methyl 2,3,6-tri-O-benzyl-α,β-D-glucopyranoside with 1-bromobutane on Silica Gel H using an irrigant of petroleum ether (30–60°)–acetone (100:20,v/v). Numbers refer to time in minutes after the addition of 1-bromobutane.

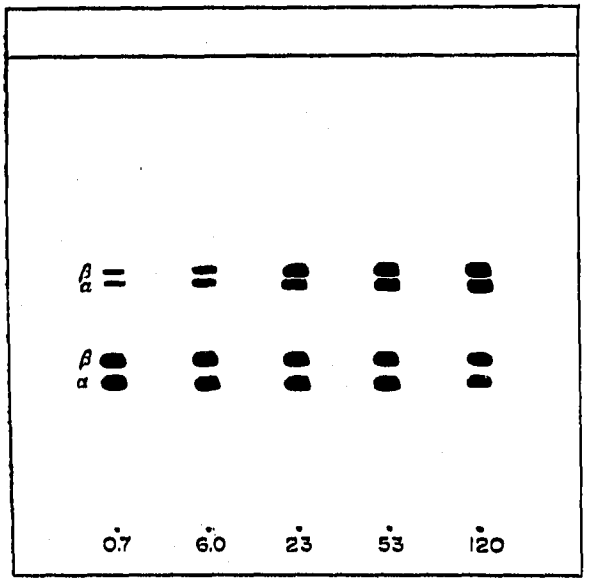

Fig. 5. Monitoring reaction of methyl 2,3,6-tri-O-benzyl-α,β-D-glucopyranoside with 2-bromo butane on Silica Gel H using an irrigant of petroleum ether (30–60°)–acetone (100:20, v/v). Numbers refer to time in hours after the addition of 2-bromobutane.

β-glycosides of 2,3,6-tri-O-benzyl-D-glucose. Alkyl derivatives were made from this mixture of anomers using the conditions of WING[41]. If the temperature is increased from —40° to 31°, a faster reaction occurs as shown in Figs. 1b and 2b (as compared to Figs. 1a and 2a). When monitoring these reactions, no effect in R_F was noticed by the sodium halide formed in the reaction. Therefore, thin-layer chromatography is a rapid, advantageous method for the investigation of reaction rate and reaction completeness for these glucosides.

TABLE IV

R_F VALUES OF THE α- AND β-ANOMERS OF PERBENZYLATED METHYL TERMINAL-4-HYDROXY-MALTOOLIGOSACCHARIDES

Compound	R_F in irrigant					
	A		B		D	
	α	β	α	β	α	β
Methyl tetra-O-benzyl-D-glucopyranoside	0.35	0.39	0.47	0.52	0.66	0.69
Methyl tri-O-benzyl-D-glucopyranoside	0.20	0.24	0.31	0.36	0.52	0.56
Methyl hexa-O-benzyl-maltoside	0.15	0.17	0.22	0.25	0.38	0.42
Methyl nona-O-benzyl-maltotrioside	0.07	0.11	0.16	0.18	0.28	0.33
Methyl dodeca-O-benzyl-maltotetraoside	—	—	0.10	0.12	0.20	0.24
Methyl pentadeca-O-benzyl-maltopentaoside	—	—	0.06	0.09	0.16	0.19

TABLE V

R_F VALUES OF THE α- AND β-ANOMERS OF PERBENZYLATED METHYL TERMINAL-4-O-METHYL-MALTOOLIGOSACCHARIDES

Degree of polymerization	R_F in irrigant					
	A		B		D	
	α	β	α	β	α	β
1	0.36	0.40	0.47	0.52	0.67	0.70
2	0.27	0.32	0.41	0.44	0.56	0.62
3	0.21	0.24	0.33	0.37	0.45	0.51
4	0.15	0.18	0.25	0.29	0.34	0.39
5	0.08	0.11	0.18	0.22	0.25	0.29

It can be noted from the R_F values that only a small change in separation of the α- and β-anomers of the oligosaccharides results when changing the irrigant from A to D. By increasing the quantity of acetone and using several ascents, it should, therefore, be possible to observe the components with a degree of polymerization greater than 5.

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SUMMARY

A thin-layer chromatographic procedure useful for the separation of various derivatized carbohydrates is described. The method is also useful for the separation of the anomeric α- and β-D-glycosides of partially or completely derivatized carbo-

hydrates. The method has been used to monitor the alkylations of benzylated carbohydrates; it shows no decrease in resolution even in the presence of salts. The method involves irrigation of Silica Gel H with petroleum ether (30–60°)–acetone mixtures in volume to volume ratios of 100:15 to 100:40.

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