THIN-LAYER CHROMATOGRAPHY OF ACYL DERIVATIVES OF SUGARS

J. O. DEFERRARI, R. MUCHNIK DE LEDERKREMER, B. MATSUHIRO AND J. F. SPROVIERO

Laboratorio de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (Argentina)

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In connection with our work[1] on acyl derivatives of aldoses we adapted thin-layer chromatography for the separation and characterisation of anomers.

On acylation of sugars a mixture of anomers is generally obtained. Thin-layer chromatography is a rapid, advantageous method for the investigation of the purity of the acyl derivative isolated. This is very important, because mixtures of anomers have been described as pure compounds in the literature. On the other hand, these acyl derivatives of sugars cannot be characterised by ordinary paper chromatography, unless the paper is specially treated. MICHEEL AND SCHWEPPE[2,3] reported the separation of sugar acetates by reversed-phase chromatography on acetylated paper, and WICKBERG[4] has described the separation of acetylated sugars by chromatography on papers impregnated with polar solvents. We consider thin-layer chromatography superior to paper chromatography in simplicity and speed, for the analytical separation of these sugar derivatives.

STAHL[5], who applied thin-layer chromatography to a wide range of compounds, has recently described the separation of free sugars on Kieselgur-G layers.

We separated acetyl and benzoyl derivatives of sugars by using glass plates coated with a mixture of silicic acid with 10% starch as binder. Development by the ascending method was employed with benzene, alone, or mixed with more polar solvents. A solvent system of 30% v/v ethyl acetate in benzene was one of the best for the resolution of fully acetylated sugars; the benzoyl derivatives were separated using benzene alone or a mixture of benzene with 0.5% methanol. Mixtures of chloroform and benzene were also used. It generally took 15–20 min to reach a height of about 13 cm.

In all cases we detected the spots with the silver nitrate–ammonia–sodium methylate reagent recommended by CADENAS AND DEFERRARI[6]. After spraying, the plates were heated for 10 min at 110°.

All acetates and benzoates showed up as light brown spots which were fluorescent when examined under ultraviolet light. Acetates reacted more quickly than benzoates, and acetylated disaccharides much more slowly than acetylated monosaccharides.

Acetates were resolved with more polar solvents than the corresponding benzoates.

EXPERIMENTAL

Smooth glass plates (15 × 18 cm) were used. They were coated with an even layer, 0.5 mm thick, of an adsorbent mixture of silicic acid Mallinckrodt, chromatographic

grade, with 10% starch as binder. The coating mixture for nine plates was prepared by a method similar to that reported by KIRCHNER, MILLER AND KELLER[7]. Thirty grams of silicic acid were thoroughly mixed with 3 g of starch; both had been sifted through a 200 mesh sieve. The mixture was stirred with 69 ml of distilled water, while heating on a water bath at 80–85° until it thickened. It was then cooled with stirring to room temperature and spread on the glass plates. These plates were activated by heating for 2 h at 110°. The chromatoplates were kept in a desiccator over potassium hydroxide. The starting points were marked on the plates at 15 mm from the base.

Ten milligrams of the sample were dissolved in 1 ml of chloroform and two drops of the solution were applied with a micropipette on the marked points. After evaporation of the solvent, the plates were placed in the chromatographic chamber, containing sufficient solvent to wet *ca.* 0.5 cm of the plates. The jar had to be saturated with the solvent vapours for 2 hours before development. The time of development for a height of about 13 cm was 15 min. After development, the plates were removed and the solvent was allowed to evaporate. The dried plates were sprayed with the silver nitrate–ammonia–sodium methylate reagent[6], and heated for 10 min at 110°.

All acetates and benzoates were detected as brown spots which were fluorescent when examined under ultraviolet light.

The acetyl and benzoyl derivatives investigated were of our own making.

RESULTS

The separation of fully benzoylated monosaccharides is shown in the chromatograms of Figs. 1, 2 and 3, and the R_F values are given in Tables I and II.

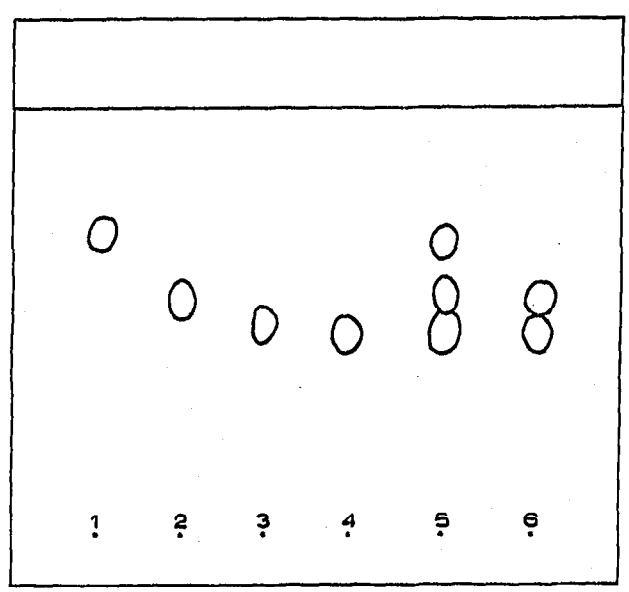

Fig. 1. Thin-layer chromatogram of benzoylated sugars. Solvent system: chloroform–benzene, 3:7 (v/v). (1) Tetra-O-benzoyl-α-D-lyxopyranose; (2) penta-O-benzoyl-α-D-glucopyranose; (3) penta-O-benzoyl-β-D-glucopyranose; (4) hexa-O-benzoyl-D-*glycero*-α-D-*galacto*-heptose; (5) mixture of 1, 2 and 3; (6) mixture of 2 and 3.

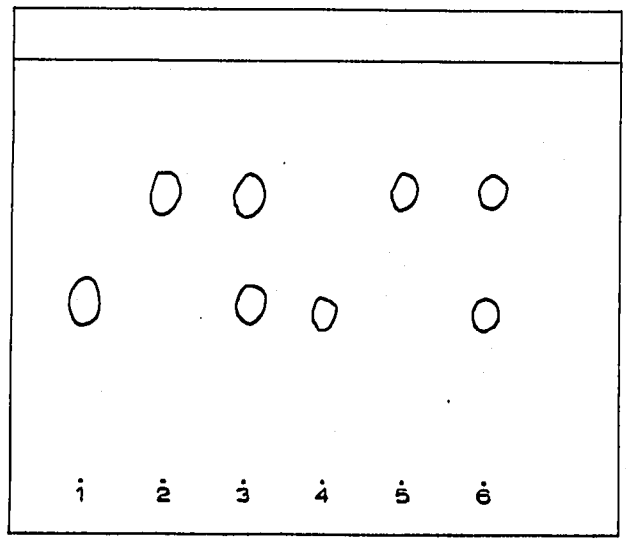

Fig. 2. Thin-layer chromatogram of anomeric benzoylated sugars. Solvent system: 0.5% methanol in benzene. (1) Hexa-O-benzoyl-D-*glycero*-α-L-*manno*-heptose; (2) hexa-O-benzoyl-D-*glycero*-β-L-*manno*-heptose; (3) mixture of 1 and 2; (4) hexa-O-benzoyl-D-*glycero*-α-D-*gulo*-heptose; (5) hexa-O-benzoyl-D-*glycero*-β-D-*gulo*-heptose; (6) mixture of 4 and 5.

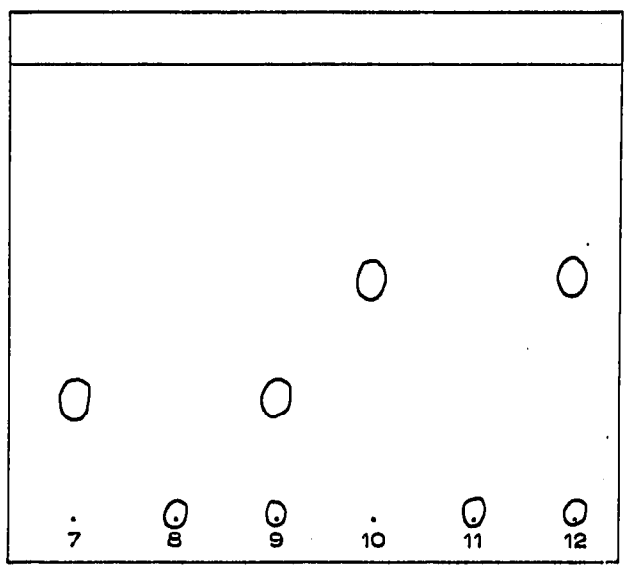

Fig. 3. Thin-layer chromatogram of anomeric benzoylated sugars. Solvent: benzene. (7) Hexa-O-benzoyl-D-*glycero*-α-D-*galacto*-heptose; (8) hexa-O-benzoyl-D-*glycero*-β-D-*galacto*-heptose; (9) mixture of 7 and 8; (10) penta-O-benzoyl-α-D-galactopyranose; (11) penta-O-benzoyl-β-D-galactopyranose; (12) mixture of 10 and 11.

TABLE I

R_F VALUES OF BENZOYLATED SUGARS

Fig. 1 No.	*Compound*	R_F [a]
1	Tetra-O-benzoyl-α-D-lyxopyranose	0.70
2	Penta-O-benzoyl-α-D-glucopyranose	0.56
3	Penta-O-benzoyl-β-D-glucopyranose	0.49
4	Hexa-O-benzoyl-D-*glycero*-α-D-*galacto*-heptose	0.39

[a] Solvent system: chloroform–benzene, 3:7 (v/v).

TABLE II

R_F VALUES OF ANOMERIC BENZOYLATED SUGARS

Figs. 2 and 3 No.	*Compound*	R_F
1	Hexa-O-benzoyl-D-*glycero*-α-L-*manno*-heptose	0.42[a]
2	Hexa-O-benzoyl-D-*glycero*-β-L-*manno*-heptose	0.68[a]
4	Hexa-O-benzoyl-D-*glycero*-α-D-*gulo*-heptose	0.40[a]
5	Hexa-O-benzoyl-D-*glycero*-β-D-*gulo*-heptose	0.69[a]
7	Hexa-O-benzoyl-D-*glycero*-α-D-*galacto*-heptose	0.26[b]
8	Hexa-O-benzoyl-D-*glycero*-β-D-*galacto*-heptose	0.0[b]
10	Penta-O-benzoyl-α-D-galactopyranose	0.52[b]
11	Penta-O-benzoyl-β-D-galactopyranose	0.0[b]

[a] Solvent system: 0.5% methanol in benzene.
[b] Solvent: benzene.

As is readily seen, the mobilities of the anomeric benzoates show considerable differences, while, for the anomeric acetates investigated, the R_F values of a pair of anomers differ only slightly (Fig. 4, Table III).

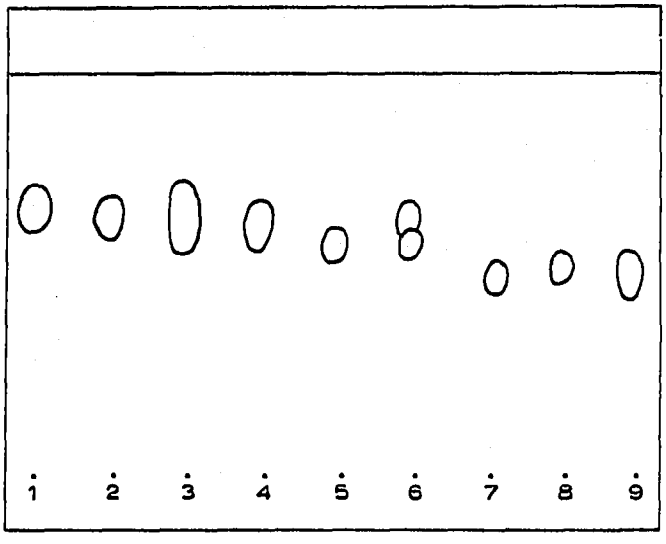

Fig. 4. Thin-layer chromatogram of anomeric acetylated sugars. Solvent system: ethyl acetate–benzene, 3:7 (v/v). (1) Penta-O-acetyl-α-D-glucopyranose; (2) penta-O-acetyl-β-D-glucopyranose; (3) mixture of 1 and 2; (4) penta-O-acetyl-α-D-galactopyranose; (5) penta-O-acetyl-β-D-galactopyranose; (6) mixture of 4 and 5; (7) hexa-O-acetyl-D-*glycero*-α-L-*manno*-heptose; (8) hexa-O-acetyl-D-*glycero*-β-L-*manno*-heptose; (9) mixture of 7 and 8.

TABLE III

R_F VALUES OF ANOMERIC ACETYLATED SUGARS

Fig. 4 No.	*Compound*	R_F [a]
1	Penta-O-acetyl-α-D-glucopyranose	0.66
2	Penta-O-acetyl-β-D-glucopyranose	0.65
4	Penta-O-acetyl-α-D-galactopyranose	0.64
5	Penta-O-acetyl-β-D-galactopyranose	0.57
7	Hexa-O-acetyl-D-*glycero*-α-L-*manno*-heptose	0.49
8	Hexa-O-acetyl-D-*glycero*-β-L-*manno*-heptose	0.52

[a] Solvent system: ethyl acetate–benzene, 3:7 (v/v).

MICHEEL AND SCHWEPPE could not separate the anomeric penta-acetyl-glucoses on acetylated paper but WICKBERG resolved anomeric acetates on papers impregnated with polar solvents.

It is interesting to note that in all cases the benzoyl or acetyl derivative with a 1,5-*trans* configuration had a higher R_F value than the respective anomer, with the exception of D-*glycero*-D-*gulo*-heptose. On the other hand, R_F values were found to be very dependent on the molecular weight for the fully acetylated and benzoylated sugars (Tables IV and I).

TABLE IV

R_F VALUES OF ACETYLATED SUGARS

Figs. 5 and 6 No.	*Compound*	R_F	
		a	*b*
1	Tetra-O-acetyl-α-D-lyxopyranose	0.78	0.74
2	Tetra-O-acetyl-β-D-xylopyranose	0.71	0.69
3	Penta-O-acetyl-β-D-glucopyranose	0.68	0.63
4	Penta-O-acetyl-β-D-mannopyranose	0.57	0.52
5	Octa-O-acetyl-6-β-D-glucopyranosyl-α-D-mannose	0.28	0.27
6	Octa-O-acetyl-gentiobiose	0.27	0.22

[a] Solvent system: ethyl acetate–benzene, 3:7 (v/v).
[b] Solvent system: methanol–benzene, 2:98 (v/v).

The acetylated sugars were resolved with two solvent systems, 30% v/v ethyl acetate in benzene (Fig. 5) or 2% v/v methanol in benzene (Fig. 6).

Table V lists the R_F values of benzoyl-pentoses. The mobilities of these isomeric compounds are rather similar (Fig. 7).

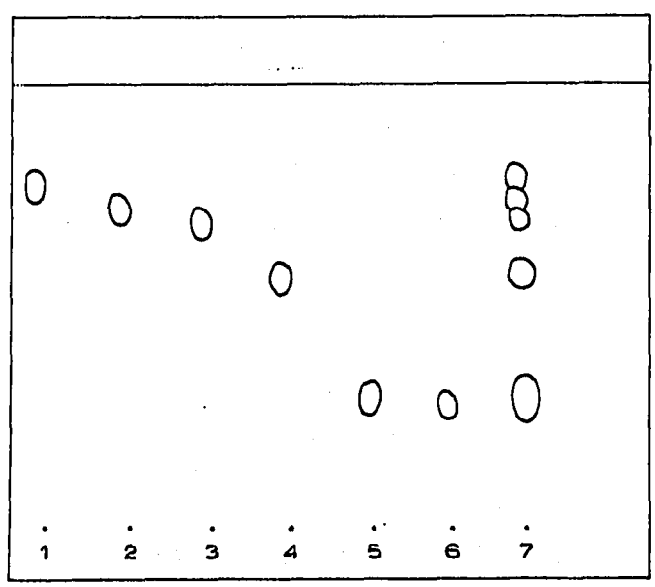

Fig. 5. Thin-layer chromatogram of acetylated sugars. Solvent system: ethyl acetate–benzene, 3:7 (v/v). (1) Tetra-O-acetyl-α-D-lyxopyranose; (2) tetra-O-acetyl-β-D-xylopyranose; (3) penta-O-acetyl-β-D-glucopyranose; (4) penta-O-acetyl-β-D-mannopyranose; (5) octa-O-acetyl-6-β-D-glucopyranosyl-α-D-mannose; (6) octa-O-acetyl-gentiobiose; (7) mixture of 1, 2, 3, 4, 5 and 6.

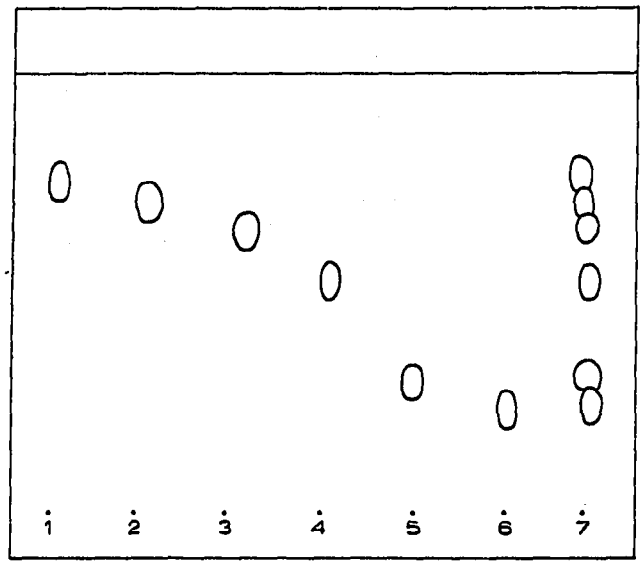

Fig. 6. Thin-layer chromatogram of acetylated sugars. Solvent system: methanol–benzene, 2:98 (v/v). (1) Tetra-O-acetyl-α-D-lyxopyranose; (2) tetra-O-acetyl-β-D-xylopyranose; (3) penta-O-acetyl-β-D-glucopyranose; (4) penta-O-acetyl-β-D-mannopyranose; (5) octa-O-acetyl-6-β-D-glucopyranosyl-α-D-mannose; (6) octa-O-acetyl-gentiobiose; (7) mixture of 1, 2, 3, 4, 5 and 6.

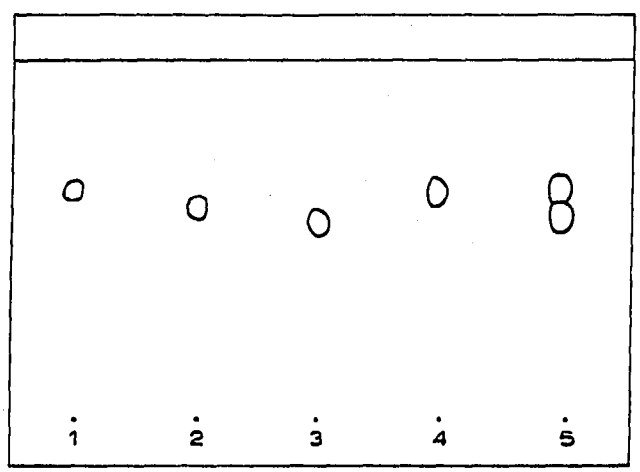

Fig. 7. Thin-layer chromatogram of tetra-O-benzoyl-pentoses. Solvent system: ethyl acetate–benzene, 3:97 (v/v). (1) Tetra-O-benzoyl-α-D-xylopyranose; (2) tetra-O-benzoyl-α-D-lyxopyranose; (3) tetra-O-benzoyl-α-D-ribopyranose; (4) tetra-O-benzoyl-β-L-arabinopyranose; (5) mixture of 1, 2, 3 and 4.

TABLE V

R_F VALUES OF TETRA-O-BENZOYL-PENTOSES

Fig. 7 No.	*Compound*	R_F[a]
1	Tetra-O-benzoyl-α-D-xylopyranose	0.64
2	Tetra-O-benzoyl-α-D-lyxopyranose	0.59
3	Tetra-O-benzoyl-α-D-ribopyranose	0.54
4	Tetra-O-benzoyl-β-L-arabinopyranose	0.62

[a] Solvent system: ethyl acetate–benzene, 3:97 (v/v).

We could distinguish the furanoid from the pyranoid form of penta-O-acetyl-galactoses. The penta-O-acetyl-α-D-galactopyranose travelled faster than the corresponding furanoid derivative, and the penta-O-acetyl-β-D-galactopyranose had a higher R_F value than the penta-O-acetyl-β-D-galactofuranose (Table VI, Fig. 8).

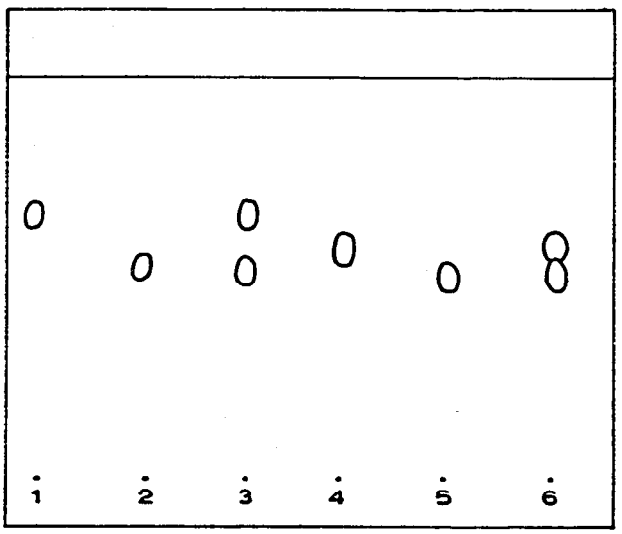

Fig. 8. Thin-layer chromatogram of penta-O-acetyl-galactoses. Solvent system: ethyl acetate–benzene, 3:7 (v/v). (1) Penta-O-acetyl-α-D-galactopyranose; (2) penta-O-acetyl-α-D-galactofuranose; (3) mixture of 1 and 2; (4) penta-O-acetyl-β-D-galactopyranose; (5) penta-O-acetyl-β-D-galactofuranose; (6) mixture of 4 and 5.

We also succeeded in separating a mixture of partially benzoylated glucoses (Fig. 9). As was to be expected, the R_F values increased with acylation (Table VII).

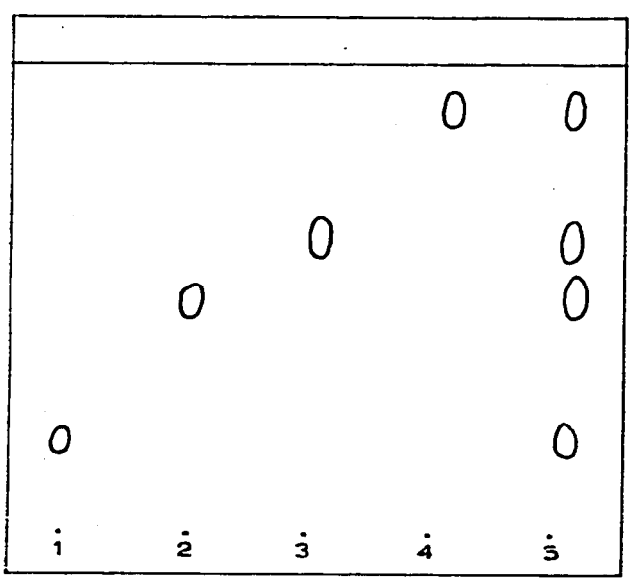

Fig. 9. Thin-layer chromatogram of partially benzoylated glucoses. Solvent system: ethyl acetate–benzene, 4:6 (v/v). (1) 5,6-Di-O-benzoyl-D-glucofuranose; (2) 1,2,3-tri-O-benzoyl-D-glucopyranose; (3) 3,5,6-tri-O-benzoyl-D-glucofuranose; (4) 3,4,5,6-tetra-O-benzoyl-aldehydo-D-glucose; (5) mixture of 1, 2, 3 and 4.

TABLE VI

R_F VALUES OF PENTA-O-ACETYL-GALACTOSES

Fig. 8 No.	*Compound*	R_F[a]
1	Penta-O-acetyl-α-D-galactopyranose	0.65
2	Penta-O-acetyl-α-D-galactofuranose	0.52
4	Penta-O-acetyl-β-D-galactopyranose	0.56
5	Penta-O-acetyl-β-D-galactofuranose	0.49

[a] Solvent system: ethyl acetate–benzene, 3:7 (v/v).

TABLE VII

R_F VALUES OF PARTIALLY BENZOYLATED GLUCOSES

Fig. 9 No.	*Compound*	R_F[a]
1	5,6-Di-O-benzoyl-D-glucofuranose	0.18
2	1,2,3-Tri-O-benzoyl-D-glucopyranose	0.47
3	3,5,6-Tri-O-benzoyl-D-glucofuranose	0.61
4	3,4,5,6-Tetra-O-benzoyl-aldehydo-D-glucose	0.88

[a] Solvent system: ethyl acetate–benzene, 4:6 (v/v).

SUMMARY

Acyl derivatives of sugars have been separated and characterised by thin-layer chromatography. We consider this method of great value for the resolution and identification of a mixture of anomers.

Separation was completed within 20 minutes, with benzene alone or mixed with more polar solvents.

In all cases the acyl derivative with a 1,5-*trans* configuration had a higher R_F value than the respective anomer, with the exception of D-*glycero*-D-*gulo*-heptose.

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