THIN-LAYER CHROMATOGRAPHY OF ALDONIC ACID LACTONES, ALDOSES AND ALDITOLS

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INTRODUCTION

Reductions of aldonic acid lactones to the corresponding aldoses are accompanied by the formation of alcoholic sugars as by-products. The course of these reductions may be advantageously followed, *inter alia*, by thin-layer chromatography. This method is rapid, simple and requires only minute amounts of material.

A series of papers[1–13] has been devoted to the separation of free sugars or their derivatives by chromatography on non-impregnated or impregnated thin layers in various solvent systems. The separation of the aldonic acid lactone–aldose–alcoholic sugar mixture which is described in the present paper has not yet been studied in detail in spite of the fact that separations of some other combinations of the compounds mentioned have been investigated[1–8].

In our experiments, silica gel G (Merck) has been employed on glass plates (100 × 200 mm and 26 × 76 mm). Various solvents (14 systems) and impregnating agents have been examined, *e.g.* with a pH 7 buffer (containing an aqueous solution of boric acid and borax) or with a pH 10 buffer (containing borax and sodium hydroxide). Thin layers have been also tested prepared using an 0.1 *M* aqueous boric acid solution instead of water. Notwithstanding, the chromatography was finally performed on non-impregnated layers of silica gel G in the methyl ethyl ketone–acetic acid–methanol (60:20:20) solvent system[20].

EXPERIMENTAL

Materials

Reagents. All reagents used were of analytical or practical grade and were obtained from the Laboratory Chemicals or Spolek pro chemickou a hutní výrobu (Czechoslovakia). The solvents employed for the development of chromatoplates were redistilled before use.

Sugars. Those used in this study are listed together with their sources, their R_F and R_G values, as well as the colors developed after the application of the spray reagents in Table I.

Apparatus

The preparation of the silica gel G layer has been described by STAHL[14]. Glass plates (100 × 200 mm, 2 mm thick) coated with a non-activated layer of 240–270 μ

TABLE I

R_F AND R_G VALUES, SPOT COLORS OF CARBOHYDRATES AND SOURCES OF THE SUGARS STUDIED

The developing solvent system was methyl ethyl ketone–acetic acid–methyl alcohol (60:20:20). Colors: B = black spot on white background; BG = blue-green to grey spot on white or yellow background; BR = brown-red spot on white or yellow background; GB = grey-brown spot on white or yellow background; P = pink spot on white or yellow background; PV = purple-violet spot on yellow background; W = white spot on blue background; YO = yellow-orange spot on white or yellow background.
Source: a = Laboratory Chemicals, Prague, Czechoslovakia; b = ref. 15; c = ref. 16; d = ref. 17; e = from the collection of carbohydrates of our laboratory; f = Farmakon, Olomouc, Czechoslovakia; g = Spolek pro chemickou a hutní výrobu, Prague, Czechoslovakia; h = Spofa, Prague, Czechoslovakia.

Sugar	R_F	R_G	*Color with reagent*					*Source*
			D_1	D_2	D_3	D_4	D_5	
D-Arabino-1,4-lactone	0.75 (0.08)	1.56 (0.17)	PV			W	B	a
D-Arabinitol	0.32	0.67				W	B	a
D-Arabinose	0.49	1.02		BR	BG	W	B	a
D-Ribonolactone	0.73 (0.12)	1.52 (0.25)	PV			W	B	e
Ribitol	0.37	0.77				W	B	a
D-Ribose	0.39	0.81		BR	BG	W	B	f
D-Xylonolactone	0.79 (0.10)	1.65 (0.21)	PV			W	B	a
Xylitol	0.26	0.54				W	B	e
D-Xylose	0.59	1.23		BR	BG	W	B	g
D-Glucono-1,4-lactone	0.75 (0.04)	1.56 (0.08)	PV			W	B	a
D-Glucitol	0.18	0.38				W	B	e
D-Glucose	0.48	1.00		YO	BG	W	B	h
L-Mannono-1,4-lactone	0.62 (0.06)	1.29 (0.13)	PV			W	B	g
D-Mannitol	0.28	0.58				W	B	e
D-Mannose	0.44	0.92		YO	BG	W	B	g
D-Galactono-1,4-lactone	0.78 (0.05)	1.63 (0.10)	PV			W	B	a
Galactitol	0.19	0.40				W	B	a
D-Galactose	0.40	0.83		YO	BG	W	B	a
D-Talono-1,4-lactone	0.68 (0.02)	1.42 (0.04)	PV			W	B	e
D-Talitol	0.31	0.65				W	B	e
D-Talose	0.36	0.75		YO	BG	W	B	g
L-Rhamnono-1,4-lactone	0.69 (0.11)	1.44 (0.23)	PV			W	B	e
D-Rhamnitol	0.38	0.79				W	B	e
L-Rhamnose	0.58	1.21		YO	BG	W	B	a
4,6-Dideoxy-L-*ribo*-hexono-1,5-lactone	0.57 (0.25)	1.19 (0.52)	PV			W	B	b
1,3-Dideoxy-D-*ribo*-hexitol	0.56	1.17				W	B	c
4,6-Dideoxy-L-*ribo*-hexose	0.49	1.02		P	GB	W	B	c
4,6-Dideoxy L-*xylo*-hexono-1,5-lactone	0.63 (0.29)	1.31 (0.60)	PV			W	B	d
1,3-Dideoxy-D-*xylo*-hexitol	0.51	1.06				W	B	d
4,6-Dideoxy-L-*xylo*-hexose	0.61	1.27		P	GB	W	B	c

thickness of silica gel G (E. Merck A.G., Darmstadt) were employed. The plates were allowed to dry at room temperature, 55–60 % relative humidity, for 1 to 3 days.

Procedure

The samples were dissolved in water 1 to 6 h before use. 2.5 μl of a solution containing 10 mg/ml of each compound were spotted with micropipettes along the starting line. The starting line was placed 20–25 mm from the lower edge of the plate. On the starting line of each plate there were seven to ten sample spots. The spots were allowed to dry at room temperature for 2–4 h (55–60 % relative humidity). The plates were run, utilizing a one-dimensional ascending technique in a glass tank (195 × 80 × 260

mm) saturated by lining three of the walls with a double layer of filter paper soaked with the solvent system employed. Prior to each chromatogram the chamber was saturated again by shaking with a new lot of the solvent system and equilibrated at 20° for about 5–10 min. The immersion line was 10–15 mm when 100–150 ml of the solvent system was used.

Development

The chromatoplates were developed to a height of 135–165 mm in a closed glass tank containing methyl ethyl ketone–acetic acid–methyl alchohol (60:20:20) as the solvent system. The solvent system was renewed in the chamber before each chromatogram. The average development time at 20° was 45–60 min. A warm stream of air from a hair dryer was used to dry the plates.

Detection

The dried plates were sprayed with the following reagents:

D_1: *Hydroxamate test*[18]. The chromatoplates were sprayed with a fresh 1 *N* methanolic hydroxylamine hydrochloride–1.1 *N* methanolic KOH (1:1) mixture, dried at room temperature for 5 min and heated for 5 to 10 min at 105°. The plates were then sprayed with a solution of ferric chloride (2 %) in aqueous HCl (1 %). Lactones gave purple spots.

D_2: *Aniline hydrogen phthalate*[18]. The chromatoplates were sprayed with a solution containing 0.93 g of aniline and 1.66 g of phthalic acid in 100 ml of water-saturated *n*-butyl alcohol. After heating the plates for 10 min at 105–110°, the aldoses appeared as red spots.

D_3: *Aniline–diphenylamine–phosphoric acid reagent*[18]. The chromatoplates were sprayed with a solution containing 4 g of aniline, 4 g of diphenylamine and 30 ml of concentrated phosphoric acid in 200 ml of methyl alcohol. Spots were developed by heating for 5 to 10 min at 110–120°. The aldoses appeared as brown or green spots.

D_4: *Potassium metaperiodate–benzidine reagent*[19]. The chromatoplates were sprayed with a saturated aqueous potassium metaperiodate solution and kept for 5 min at room temperature. The plates were then sprayed with a solution containing 2.57 g of benzidine hydrochloride in 20 ml of acetone and in 100 ml of 50 % aqueous ethyl alcohol. Compounds with a free α-diol configuration appeared as white spots on a blue background.

D_5: *Concentrated sulphuric acid reagent*. The chromatoplates were sprayed with concentrated sulphuric acid and then heated 3 to 10 min at 100–120°. The carbohydrates appeared as brown or black spots.

RESULTS AND DISCUSSION

The R_F and R_G (with D-glucose as reference compound) values of some pentoses, hexoses, 6-deoxyhexoses, and 4,6-dideoxyhexoses are summarized in Table I. All three corresponding monosaccharide derivatives (aldonic acid lactone–aldose–alcoholic sugar) are always of the same configurational series except L-mannonolactone and D-rhamnitol. Table I also contains color reactions of the compounds chromatographed as well as the source of the monosaccharides investigated.

The R_F and R_G values of aldonic acid lactones are supplemented with data in

brackets which are always lower and are probably due to the aldonic acid alone. Spots corresponding to the lower values can be detected only with the periodate–benzidine reagent (D_4) or with conc. sulfuric acid (D_5) but not by the hydroxamate test (D_1). On the other hand, the higher R_F or R_G value always corresponds to the lactone as expected. In addition to the reagents D_4 and D_5, the lactone spot may be detected also by the hydroxamate test (D_1). In comparison with the lactone, the spots corresponding to aldonic acids are more intense under the experimental conditions stated, especially in the case of D-arabino-1,4-lactone, D-xylonolactone and D-glucono-1,4-lactone. The difference in the intensity of the spots corresponding to the lactone and the parent acid is less pronounced in the case of D-ribonolactone, L-mannono-1,4-lactone, D-galactono-1,4-lactone, D-talono-1,4-lactone, 4,6-dideoxy-L-*ribo*-hexono-1,5-lactone and 4,6-dideoxy-L-*xylo*-hexono-1,5-lactone. On the other hand, the spot corresponding to L-rhamnonolactone in distinctly more intense than that of L-rhamnonic acid. When an aqueous solution of the lactone is chromatographed, the delactonisation equilibria have to be taken into consideration (the constitutional and configurational factors play an important part in this respect). Thus, *e.g.*, on the silica gel G chromatoplates prepared from fresh aqueous solutions of 4,6-dideoxy-L-*ribo*-hexono-1,5-lactone and 4,6-dideoxy-L-*xylo*-hexono-1,5-lactone in the methyl ethyl ketone–acetic acid–methanol (60:20:20) solvent system, the spots of the corresponding acids are less intense in comparison with those obtained from the same solutions which, however, had been stored for several months.

When the alcoholic sugars were detected with the reagent D_4, distinct white spots at the origin could be observed. Detection of aldoses with the reagent D_3 was also accompanied by the occurrence of spots at the origin.

No color reaction was truly specific for a particular compound or group of compounds under the conditions stated. In the case of dideoxyhexoses, the color of spots on the chromatoplates was somewhat different in comparison to the other aldoses, especially when the reagents D_2 and D_3 were used.

The development time of the chromatoplates under investigation was 45 to 60 min and only 10 min are required when microscope plates are used. In comparison with paper chromatography, thin layer chromatography on silica gel G is more rapid and more suitable to control the course of the reduction of aldonic acid lactones to the corresponding aldoses.

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SUMMARY

Thin layer chromatography on non-activated silica gel G in the solvent system methyl ethyl ketone–acetic acid–methanol (60:20:20) has been used to separate ten sugar triads (aldonic acid lactone–aldose–alcoholic sugar) from the group of pentoses,

hexoses, 6-deoxyhexoses and 4,6-dideoxyhexoses (R_F and R_G values given). This simple and rapid method may be used, *e.g.*, to control the course of the reduction of aldonic acid lactones to the corresponding aldoses.

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