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Note

Specific colour reaction for the detection of 1,2-linked reducing disaccharides on paper and thin-layer chromatograms

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Diphenylamine–aniline–phosphoric acid (DAAP) reagent, which is used for the detection of mono- and oligo-saccharides on paper and thin-layer chromatograms, gives a wide range of colours with carbohydrates of different structures[1–4]; this reagent has also been used for distinguishing between 1,4-linked and 1,6-linked disaccharides[5,6]. Here, we describe a new application of this reagent in differentiation of 1,2-linked reducing disaccharides from other disaccharides on paper or thin-layer chromatograms or paper electropherograms.

EXPERIMENTAL

General methods

The kojibiose, sophorose, nigerose and laminaribiose used were gifts; lactulose, neolactose, 2-deoxy-D-glucose and 2,3,4,6-tetra-O-methyl-D-glucose were prepared in our laboratory, and all other carbohydrates used were commercially available.

The sugars were applied to papers and plates as 2% aqueous solutions. Sheets (40 × 40 cm, and 36 × 14.5 cm) of Toyo No. 514 filter paper were used for paper chromatography and paper electrophoresis, respectively. Paper chromatograms were subjected to three successive developments with *n*-butanol–pyridine–water (6:4:3), with the solvent ascending. Paper electrophoresis was carried out in 0.05 *M* borate buffer (pH 9.8) for 3 h at 400 V, and the M_G values were calculated[7]. Thin-layer chromatograms were developed in propanol–water (8.5:1.5) (see ref. 8).

Detection of the spots

We used a reagent freshly prepared by dissolving diphenylamine (4 g) and aniline (4 ml) in 200 ml of acetone and mixing the solution with 20 ml of orthophosphoric acid (sp.gr. 1.70 at 20°)[6]. The air-dried paper chromatograms and paper electropherograms were dipped in this reagent, dried again and then heated at 80° for 5 min until the background became faintly gray. The thin-layer chromatograms were sprayed with the reagent, allowed to stand until they were nearly dry, then heated at 80° for 15 min.

TABLE I

COLOUR REACTIONS ON PAPER CHROMATOGRAMS, PAPER ELECTROPHEROGRAMS AND THIN-LAYER CHROMATOGRAMS OF SUGARS WITH DAAP REAGENT

*Sugar**	*Paper chromatography*			*Paper electrophoresis*		*Thin-layer chromatography*		
	R_G**	*Colour*	*Sensitivity (μg)*	M_G	*Colour*	R_F	*Colour*	*Sensitivity (μg)*
2-Deoxyglucose	1.22	Pink	5	0.31	Pink	0.74	Red	1
Kojibiose (1,2)	0.77	Orange	10	0.32	Orange	0.26	Brown	1
Sophorose (1,2)	0.83	Orange	5	0.31	Orange	0.32	Brown	1
Nigerose (1,3)	0.88	Dark green	5	0.68	Yellowish green	0.34	Pale blue	1
Laminaribiose (1,3)	0.94	Dark green	5	0.66	Yellowish green	0.38	Pale blue	1
Maltose (1,4)	0.84	Bright blue	5	0.32	Blue	0.31	Bright blue	1
Cellobiose (1,4)	0.82	Blue	5	0.26	Dark blue	0.31	Blue	1
Lactose (1,4)	0.73	Blue	5	0.39	Dark blue	0.23	Blue	1
Gentiobiose (1,6)	0.75	Gray-green	5	0.71	Dark yellowish green	0.25	Blue	1
Melibiose (1,6)	0.67	Gray-green	5	0.77	Dark yellowish green	0.22	Blue	1
Sucrose	0.97	Dark green	5	0.18	Dark green	0.37	Green-black	1
Turanose (1,3)	0.95	Violet	10	0.64	Violet	0.35	Reddish chocolate brown	1
Neolactose (1,4)	0.83	Blue	5	0.66	Blue	0.27	Blue	1
Lactulose (1,4)	0.79	Gray-green	10	0.68	Dark yellowish green	0.27	Chocolate brown	1
Trehalose	0.77	White***	300		Nil	0.23	Blue	10
								1§

* Type of linkage shown in parentheses when appropriate.

** Mobility relative to D-glucose.

*** Yellowish green after 1 day.

§ After being heated at 80° for 45 min.

RESULTS AND DISCUSSION

The colours derived from the various disaccharides by the DAAP reagent are summarized in Table I. With this reagent, 1,2-linked reducing disaccharides gave a brown colour on thin-layer chromatograms, and orange on paper chromatograms or paper electropherograms, whereas 1,4-linked disaccharides gave various shades of blue (and 1,3- and 1,6-linked disaccharides of green) on paper. Thin-layer chromatograms of 1,3- and 1,6-linked saccharides gave various shades of blue (identical with the 1,4-linked saccharides). Thus, the reagent offered a useful means for tentative identification of 1,2-linked reducing disaccharides on paper and thin-layer chromatograms.

The coloured spots should be observed as soon as possible, after their formation; however, the characteristic colour of 1,2-linked reducing disaccharides changes little in 2 or 3 days.

The ratios of relative absorbance in the 610 to 620-nm and 450 to 470-nm regions (*cf.* Schwimmer and Bevenue[6]) in the spectra of acetone extracts from the spots were as follows. Kojibiose, 0.84; laminaribiose, 1.21; maltose, 1.69; and gentiobiose, 1.28.

The sensitivity of the DAAP reagent is also shown in Table I. Each sugar was tested with solvent development at levels of *ca.* 1, 5 and 10 μg; trehalose was tested at levels of 100, 300 and 500 μg. The limits of detection of the disaccharides on paper and thin-layer chromatograms were in the range of 5 to 10 μg and 1 μg, respectively, except for trehalose on paper.

This DAAP reagent was successfully applied to the characterization of transfer disaccharides from lactose by microbial β-galactosidases; the disaccharide tentatively identified as a 1,2-linked sugar by this reagent was proved to be 2-O-β-D-galactopyranosyl-D-glucose by methylation analysis[9].

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