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Note

Sulphosalicylic acid as spray reagent for the detection of sugars on thin-layer chromatograms

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A number of spray reagents are used for the detection of sugars in thin-layer and paper chromatography. The furfural-yielding reagents like aniline–diphenylamine–H_3PO_4[1], α-naphthol–H_3PO_4[1], orcinol–GCl[1], *p*-anisidine phthalate[2], aniline hydrogenphthalate[3], naphthoresorcinol–H_2SO_4[4], demidone–H_3PO_4[5], phenol–H_2SO_4[6], carbazole–H_2SO_4[6], anthrone–H_2SO_4[7], etc., under specific conditions permit the recognition of various classes of sugars. Alternatively, fluorogenic reagents such as ethylenediamine sulphate[8], malonamide[9], Dns hydrazine[10], fluoroglycine[11], *m*-phenylenediamine[12], aniline citrate[13], *o*-phenylphenol[14], aniline–H_3PO_4[15] and chromotropic acid–H_2SO_4[16] are used extensively for their high sensitivity.

In this paper we present another sensitive reagent, sulphosalicylic acid, which is capable of detecting sugars in amounts as low as 0.2 μg on silica gel G thin-layer plates.

EXPERIMENTAL

Chromatoplates (20 × 20 cm) were covered with a 0.1 mm thick coating of silica gel G [E. Merck (India)] using a Unoplan apparatus (Shandon, London, U.K.). A Shandon micropipette as used to spot the sample and a warm stream of air from a hair dryer was used to dry the spots. A 2% (w/v) solution of sulphosalicylic acid (SD's, India) in 0.5 *M* sulphuric acid was used as the spray reagent; it can be preserved in the dark for quite a long period. After development the thin-layer plate was thoroughly dried under a stream of air at room temperature, sprayed uniformly with the reagent and heated at 110°C for 15 min in an oven.

RESULTS AND DISCUSSION

Experiments made using the 23 sugars listed in Table I as samples revealed that the sensitivity of the spray reagent is quite high for all the aldoses, ketoses, uronic cids, 6-deoxy sugars, glucose-6-phosphate and disaccharides, but the reduced monosaccharides and inositol were either insensitive or very poorly sensitive. D-Glucosamine hydrochloride and D-galactosamine hydrochloride showed intermediate sensitivity. Fucose and rhamnose give a yellow colouration, whereas the spots of all

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TABLE I

SPOT COLOURS AND LIMITS OF DETECTION OF SUGARS ON SILICA GEL G PLATES WITH SULPHOSALICYLIC ACID REAGENT

Sugar	*Limit of detection (μg)*	*Colour of spot*
D-Ribose	1.0	Greyish brown
D-Xylose	2.0	Grey
L-Arabinose	1.5	Grey
L-Rhamnose	0.3	Yellow
L-Fucose	0.5	Yellow
D-Glucose	0.2	Steel grey
D-Galactose	0.5	Grey
D-Mannose	0.2	Greyish brown
L-Sorbose	0.7	Greyish brown
D-Fructose	0.3	Grey
D-Glucuronic acid	0.25	Grey
D-Galacturonic acid	1.0	Grey
D-Glucosamine hydrochloride	7.0	Greyish brown
D-Galactosamine hydrochloride	3.5	Greyish brown
D-Glucose-6-phosphate	0.3	Grey
Sorbitol	20.0	Grey
Mannitol	22.0	Grey
Dulcitol	–*	–
Inositol	–	–
Sucrose	0.8	Steel grey
Maltose	0.4	Grey
Lactose	0.3	Grey
Raffinose	0.7	Grey

* No spot was observed at 50 μg.

other sugars are usually grey, greyish brown or steel grey. The characteristic colouration produced by the above two 6-deoxy sugars can be utilized for their determination in a mixture of monosaccharides. It was also observed that a 20% solution of the reagent in water was also capable of producing similar colourations but with a slight background. The chemistry of the colour-forming reaction is assumed to be due to the formation of furfural which reacts with the excess of reagent producing the colouration. Thus, sulphosalicylic acid can be used for the detection of sugars on thin-layer plates, in addition to its accepted use for the detection of steroids and triterpenoids[1,7].

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