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Fluorescent visualization of trioses on silica gel thin-layer chromatograms

The present paper describes a method for the detection of glyceraldehyde and dihydroxyacetone on silica gel thin-layer chromatograms. When the trioses are heated with mineral acid, they are readily converted to pyruvaldehyde[1], and the latter reacts with chromotropic acid in sulfuric acid to form a soluble yellow condensation product which on irradiation at 380–510 mμ gives an intense green fluorescence[2]. So far, no report has appeared describing the application of the above reaction to the visualization of the trioses on chromatograms.

Experimental

Preparation of chromatoplates. 30 g of 'Kieselgel G nach Stahl' (E. Merck, A.G., Darmstadt, G.F.R.) was mixed with 60 ml of 0.1 *M* boric acid. The slurry obtained was applied to 20 × 20 cm glass plates in the usual manner (0.25 mm layer). These plates were allowed to stand overnight at room temperature and then heated for 1 h at 100–105°.

Solvent-development of chromatograms. 1 μl of a sample solution containing appropriate quantities of the trioses (preferably 1 μg per μl each) was applied to the chromatoplate with a microsyringe. The development was carried out by the one-dimensional ascending technique with a developing solvent of *n*-butanol–ethyl acetate–isopropanol–acetic acid–water (35:100:60:35:30) (ref. 3). Pre-equilibration of the plate in the developing tank (23 × 23 × 5 cm) was continued for more than 30 min. At the end of a 10-cm solvent run, the plate was dried by means of a fairly strong current of air, provided by a fan, for about 1 h at room temperature. Then, the odor of the solvent was very faint, if detectable at all.

Detection of the spots. After drying, the plate was sprayed with a slight excess of the following reagent solution: 0.5 g of chromotropic acid disodium salt dissolved in 100 ml of a 1:10 (v/v) mixture of sulfuric acid and water. The quantity of the reagent solution required to give satisfactory results was 0.04–0.05 ml per sq. cm. The plate was then heated in an oven at 100–102° for 10 min. To obtain good results it is advisable to raise the temperature of the oven up to 100° within 10 min after the plate has been placed in the oven; heating over 102° must be avoided. The finished chromatogram was submitted to UV irradiation in the dark to detect the trioses. In the present experiments, a 'Super-Light, Model LS-DI' (Osawa Shigaisen Kogyo Kenkyusho, Ltd., Tokyo, Japan) was used as a UV light source. With this instrument, it is possible to irradiate the chromatogram at 254 mμ or at 365 mμ. For reasons stated below, irradiation at 254 mμ was adopted. The irradiation revealed the trioses as spots showing a brilliant yellow fluorescence tinged with green on a violet background.

To obtain the most satisfactory results, it was required to complete the whole process described above, except for the preparation of the plates, within as short a time as possible because of the instability of the trioses and other sugars on the chromatoplates.

Results and discussion

When a mixture of an aqueous solution of a triose and a 1% solution of chromo-

tropic acid in 50 % sulfuric acid was heated in a test tube placed in boiling water[4], the resulting solution was brownish-yellow in transmitted light and exhibited a green fluorescence. By using a fluorescence spectrophotometer (Model MPF-2A, Hitachi, Ltd., Tokyo, Japan) it was found that the fluorescence was most intense when the solution was irradiated at 385–412 mμ. When the reaction was carried out on the chromatoplates as described above and the treated chromatograms were irradiated at 254 or 365 mμ with the above-mentioned instrument (Super-Light), however, it was observed that the fluorescence of the spots was yellow rather than green in color and that the irradiation at 254 mμ was more effective for the present purpose than at 365 mμ. The cause of disagreement between the results obtained in the above two cases has not been determined yet.

TABLE I

R_F VALUES (× 100) ON 0.1 *M* BORIC ACID-IMPREGNATED SILICA GEL

Solvent system: *n*-butanol–ethyl acetate–isopropanol–acetic acid– water (35:100:60:35:30). Detecting reagent[a]: naphthoresorcinol–sulfuric acid solution[3].

	R_F
Glycolaldehyde	44
DL-Glyceraldehyde	72
Dihydroxyacetone	57
D-Erythrose	45
L-Arabinose	42
D-Xylose	53
D-Lyxose	49
L-Rhamnose	60
D-Glucose	42
D-Mannose	44
D-Galactose	35
D-Fructose	36
L-Sorbose	34

[a] The fluorescence method, as described in the text, is fairly specific for trioses, and the detection of sugars other than the trioses is not very effective. For this reason, a universal detection reagent was used.

Experiments which were made using 13 monosaccharides listed in Table I as test samples (1 μl of each sample solution was chromatographed as described above), revealed that the limits of detection of the trioses were 0.10–0.12 μg per μl, respectively and that quantities of each less than 2.5 μg/μl of glucose, mannose, galactose, fructose and sorbose and 1.2 μg/μl of glycolaldehyde, erythrose, arabinose, xylose, lyxose and rhamnose gave no fluorescence. From these results, including the R_F values obtained (Table I), it seems likely that the present method can be used effectively for detecting the trioses in complicated monosaccharide mixtures.

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