

p-Anisidine-Phosphoric Acid as a Color Reagent for Sialic Acid Compounds on Paper Chromatograms

SIR: Many reagents have been used for the detection of sugars on paper chromatograms (1). A preferred reagent is one which gives different color reactions with different classes of sugars, and *p*-anisidine meets this desideratum (2).

In recent years the necessity has arisen to distinguish sialic acid and sialic acid-containing compounds from other sugars on paper chromatograms. Benzidine-trichloroacetic acid in aqueous ethyl alcohol has been recommended for that purpose (4). However, *p*-anisidine-phosphoric acid (2) has been found to be a more satisfactory reagent for differentiation of several classes of sugars and polyols, and, in the present research, for distinguishing sialic acid-containing compounds from other sugars.

The paper chromatogram is dipped in a solution of *p*-anisidine (0.5 gram) and phosphoric acid (3 ml. of 85 to 88%) in methanol (100 ml. of 80% aqueous) (2). The paper is blotted to

remove excess reagent and heated for approximately 10 minutes at 105° to 110° C. Sialic acid and sialic acid-containing compounds appear slowly, giving purplish-gray spots in about 10 minutes. The color of the spots distinguishes sialic compounds from all other sugars tested. The background with this reagent is colored pale yellow. Neutral sugars appear after 1 minute at 105° to 110° C., aldoses as brown spots and ketoses as yellow spots; polyols appear as white spots in 5 to 10 minutes (2). Amino hexoses give brown spots. In parallel tests the *p*-anisidine reagent has proved to be more sensitive than the benzidine reagent for sialic acid-containing compounds because the background is much lighter and the spots are seen more readily.

Sialidolactose and sialic acid prepared from bovine colostrum (4), and 6- α -D-sialyl-*N*-acetylgalactosamine, *N*-glycolylsialic acid, and sialic acid prepared from mucin (3) were among the tested substances chromatographed on filter paper. All these and some other

noncharacterized sialic acid-containing substances (sialic acid obtained on hydrolysis) gave purplish-gray spots, which distinguish these compounds from all other sugars tested.

LITERATURE CITED

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