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Note

A comparative study of certain aniline salts used in the determination of carbohydrates by spot elution chromatography

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Solutions of aniline hydrogen phthalate (AHP) in water-saturated butanol have commonly been used for the quantitative determination of carbohydrates on paper chromatograms[1–3]. The papers are dipped, dried, heated and the colored spots cut out, leached with solvents, and the color density of the solutions read in a spectrophotometer. Good color development is obtained with this reagent, but the spots are often streaked, and the background paper yields high blanks. Wilson[4] prepared the AHP in a mixture of butanol, diethyl ether, and water. This reagent produced compact spots without streaking. However, the objectional odor of butanol remained. Zentner[5] used a mixture of aniline and malonic acid in methanol for a dipping reagent which, he claimed, had a very light background color. Date[6] used a mixture of aniline and citric acid in aqueous alcohol. To the authors' knowledge no comparative study under identical conditions has been made to determine how the aniline salts compared with each other in ease of use and other qualities.

Accordingly, the above aniline salts and in addition aniline salts of borate, oxalate, succinate, and phosphate were prepared by us and checked as to their solubility in absolute ethanol, the least objectional solvent, the compactness of the carbohydrate spots after dipping, the amount of spot color produced, and the background color.

EXPERIMENTAL AND RESULTS

Crystalline aniline phthalate, malonate, citrate, oxalate, borate, succinate, and phosphate were prepared by adding aniline to solutions of the respective acids in ethanol. The white, crystalline precipitates that formed were recrystallized from ethanol, and their solubilities tested in order to prepare 0.1 *M* solutions in ethanol.

The phthalate, malonate, succinate, and citrate salts were soluble enough to prepare the 0.1 *M* alcoholic solutions while the remainder were unsuitable because of low solubility.

Paper chromatograms of solutions of mixtures of carbohydrate standards on Whatman No. 2 paper, developed according to the method of Colombo *et al.*[7], were dipped into the solutions of the soluble aniline salts, air dried for 1 h, and heated at 100° for 1 h. The resulting colored carbohydrate spots were cut out, together

with blanks from the paper adjacent to the colored spots, leached with 0.7 *M* HCl in ethanol, and the color density measured in a Beckman spectrophotometer.

Chromatograms developed with the phthalate solution often showed an uneven color reaction on drying, and the background color was darker than with the other salts. The other salts did not show the uneven color reaction, and the background colors were lighter, but the hexose spots were not as intense, except for the fructose and sucrose dipped in malonate solution. The carbohydrate spots on all the chromatograms were compact, without streaking. The malonate colored spots were more intense than the corresponding spots on the citrate chromatograms. Only one chromatogram was developed with the succinate solution, and it appeared similar to the malonate, but the spots took longer to develop. The colors of the spots produced by the different salts were different in appearance, but the absorption peaks were the same, indicating that the same Schiff base was produced with the same carbohydrates. The results are summarized in Table I.

The diphenylamine–aniline–phosphoric acid reagent of Kocourek *et al.*[8] was tested to see if it was suitable for use in the spot elution technique used above. It was found that the spot color was distinctly less intense relative to the background color than the aniline salts tested above, and in addition the paper deteriorated with this reagent. 3,5-Dinitrosalicylic acid left an intense yellow background color on the paper.

In our hands the alcoholic solution of aniline malonate was the most convenient and effective of the reagents tested in the above-described spot elution technique for determining carbohydrates on paper chromatograms. Aniline malonate was easily soluble in ethanol. 5% or 0.25 *M* solutions could be prepared. Aniline malonate gave the best color yield of the three salts tested with fructose, although, like with the other salts, the fructose spot appeared only after longer heating than was required with the aldose sugars, presumably because some transformation or decomposition must take place to free the reducing group. Erythrose, a tetrose, gave a yellow color. Mannoheptulose gave a brown color similar to that of the

TABLE I

COMPARISON OF MAXIMUM ABSORPTION PEAKS AND COLOR INTENSITIES OF CHROMATOGRAM SPOTS OF CARBOHYDRATE SOLUTIONS WITH CERTAIN ANALINE SALTS

Carbohydrate	*Maximum absorption (nm)*	*Aniline hydrogen phthalate*	*Aniline malonate*	*Aniline citrate*
Glucose	385	0.510*	0.440	0.370
Galactose	385	0.520	0.460	0.360
Xylose	345	0.670	0.650	0.540
Fructose	375	0.310	0.400	0.280
Rhamnose	375	0.510	0.540	0.390
Sucrose	385	0.110	0.120	0.060
Paper blanks (2 sq. in.)	385	0.115	0.075	0.105

* Each number represents an average of ten readings in a Beckman spectrophotometer of carbohydrate eluted from spots containing 100 μg per spot.

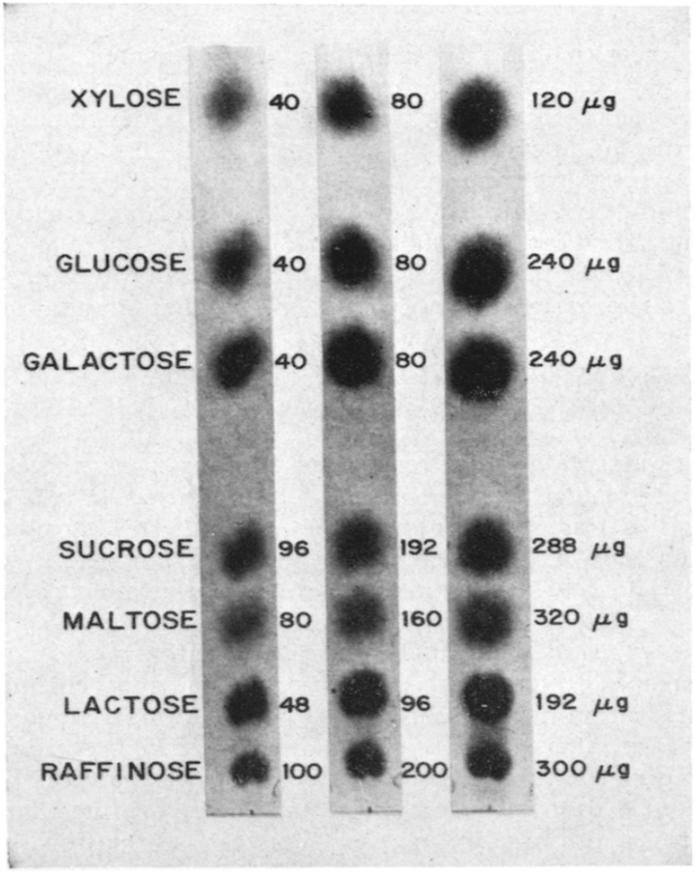

Fig. 1. Descending chromatography of a carbohydrate mixture for 72 h with the solvent system ethyl acetate–pyridine–water (180:50:57.5).

hexoses. Sucrose gave a spot only after prolonged heating. The color yield from sucrose from spots containing small amounts was proportionately greater than that from spots containing larger amounts. This was true also for the other sugars, but to a lesser degree. In the case of sucrose determinations the chromatographing should be repeated until the concentrations of unknown and standard carbohydrate on the same paper are within 20% or a series of standards of different concentrations should be run on the same paper. Maltose, lactose, and raffinose gave color readily (see Fig. 1), though not as much in proportion to weight of material present as did the monosaccharides. Trehalose, an α-glucopyranosyl-α-glucopyranoside, did not yield any color even with prolonged heating with any of the aniline salts tested. Aniline malonate reagent has been used by us to determine carbohydrates in numerous plant[9] and bacterial polysaccharides with good results.

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