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Note

p-Anisidine–phosphoric acid as a color reagent for sugar derivatives and halogen compounds

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p-Anisidine hydrochloride[1] and *p*-anisidine–phosphoric acid[2] are valuable reagents for the detection of sugars[1], polyols[2], uronic acids[1], sialic acids[3] and 5-(hydroxymethyl)-2-furaldehyde[4] on paper chromatograms and other technics. *p*-Anisidine gives different color reactions with different classes of carbohydrates.

In the present studies the derivatives of the hydrolysis product of lactose were oxidized with different reagents. All reaction steps were analyzed using thin-layer chromatography (TLC) and paper chromatography. Compounds detected using *p*-anisidine–phosphoric acid reagent[2], revealed unusual color spots which were found to be characteristic of uronic acids and some halogen-containing compounds.

MATERIALS AND METHODS

Chromatography paper, Whatman No. 1 (H. Reeve Angel, Clifton, N.J., U.S.A.) was used as well as silica gel G (Brinkmann, Westbury, N.Y., U.S.A.). *p*-Anisidine reagent was prepared by dissolving 0.5 g of *p*-anisidine in 3 ml of phosphoric acid (sirupy, 85.3%). The solution was diluted with 100 ml 80% aqueous methanol[2].

The developed TLC plates were sprayed with the *p*-anisidine reagent and heated for 10 min at 110–120°. The plates were examined frequently in the oven for color development. The color of some compounds changed very quickly during heating. It was noticed that uronic acids gave different colors on the TLC plates compared to those paper. The pH of the solvent had no influence on the reaction color. The 10% solution of reaction mixtures was spotted onto the TLC plates.

RESULTS AND DISCUSSION

Pertinent data are shown in Table I. Different color reactions of *p*-anisidine with different classes of carbohydrates on silica gel G plates were of exceptional value. They helped to reveal the presence of groups of sugar derivatives and the completeness of oxidation reaction. In addition to the colored sugar spots, the *p*-anisidine gave a colored spot with ClO_3^- ions. This color reaction was observed when sugar de-

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TABLE I

COLORS OF COMPOUNDS ON SILICA GEL G PLATES WITH *p*-ANISIDINE REAGENT[2]

Compound	*Color of spot*
D-Glucuronic acid	Orange
α-D-Galacturonic acid	Orange
D-Glucuronic acid lactone	Orange
Mucic acid	None
D-Gluconic acid	None
Common sugars	Yellow-brown, brown
Acetonation	
1,2,5,6-Di-O-isopropylidene-D-glucose	Yellow-brown
1,2,3,4-Di-O-isopropylidene-D-galactose	Yellow-brown
Mono-O-isopropylidene-D-glucoses	Yellow-brown
Mono-O-isopropylidene-D-galactoses	Yellow-brown
Pigments produced from acetone	Grey-brown
Tritylation	
Trityl–Cl	Several spots; yellow fades out; pink remains
Trityl–OH	Several spots; yellow fades out; pink remains
6-O-Trityl-D-galactose	Yellow, fades out at room temp. in 10 min
6-O-Trityl-D-glucose	Yellow, fades out at room temp. in 10 min
6-O-Trityl-tetra-O-acetyl-D-galactose	Yellow, fades out; pink remains
6-O-Trityl-tetra-O-acetyl-D-glucose	Yellow, fades out; pink remains
Tetra-O-acetyl-D-glucuronic acid	Yellow immediately; orange at 110°
Tetra-O-acetyl-D-galactose	Yellow, fades out
Tetra-O-acetyl-D-glucose	Yellow, fades out
Dialdose acetates	Pink–brown
Inorganic compounds	
Chlorine vapor	Violet-blue immediately at room temp.
Bromine vapor	Violet-blue immediately at room temp.
Iodine vapor	Yellow-brown immediately at room temp.
NaCl	No spot or very weak violet-blue
NaBr	No spot or very weak violet-blue
NaI	Yellow
$NaClO_3$	Violet-blue at 110°
$NaBrO_3$	Violet-blue immediately at room temp.; at 120° changed to yellow, finally white with violet-blue collar
$NaIO_4$	Violet-blue immediately at room temp.; stable at 120°
$HClO_4$	No colored spot
Cobalt acetate	Violet-blue at 110°, fades out

rivatives were oxidized with hypochlorite. Based on these observations the color reactions of *p*-anisidine were studied in detail.

All three common uronic acids (glucuronic acid, galacturonic acid and mannuronic acid) developed the same colored orange spots with *p*-anisidine on silica gel G plates. All other natural uronic acids, relatively rare in natural products, were not available for studies. The orange color spot was specific only for uronic acids. No other carbohydrate or organic acid tested, showed this color reaction. Sensitivity was 5 μg of uronic acids. Using TLC, the qualitative and approximately quantitative determination of the reaction mixture and the completeness of oxidation reaction was detected in 1 h.

Tritylated sugars and sugar acetates gave yellow spots, which faded completely at room temperature within 10 min. Acetonides of sugars gave yellow–brown spots, which were stable at room temperature. Common sugars like fructose, glucose, galactose, sucrose and lactose, gave yellow–brown spots. Hexodialdose acetates (1,2,3,4-tetra-O-acetyl-D-galactohexodialdose-1,5-pyranose and 1,2,3,4-tetra-O-acetyl-D-glucohexodialdose-1,5-pyranose) gave pink–brown spots.

Pigments produced from acetone in the acetonation reaction with H_2SO_4, gave a grey–brown color with *p*-anisidine reagent.

Violet–blue colored spots of *p*-anisidine were obtained with chlorine and bromine vapor and $NaClO_3$, $NaBrO_3$, and $NaIO_4$. For the testing of possible Br- and Cl-vapors in the air, the *p*-anisidine reagent was spotted on a chromatographic paper strip and the strip was exposed to air. In the presence of Cl- or Br-vapors the *p*-anisidine spot turned from colorless to violet–blue. Iodine vapor gave yellow–brown immediately. The paper strip, spotted with *p*-anisidine reagent, pulled over the reaction vessel or sodium hypochlorite solution, showed a violet–blue spot immediately at room temperature. A violet–blue color of *p*-anisidine has been reported previously with bromine vapor[5] and the bentonite (hydrated aluminium silicate)[6].

Using TLC for the investigation of commercial sodium hypochlorite solution and two commercial samples of bleach revealed that all three solutions contained ClO_3^- ions. A large amount of ClO_3^- was also detected in a reaction mixture, obtained by the hypochlorite oxidation of sugar derivatives. All tested solutions showed strong violet–blue spots corresponding to ClO_3^-, using $KClO_3$ as a standard. It was out of the scope of the present research to investigate why hypochlorite solutions showed a ClO_3^- spot only.

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