A visualizing reagent for α-hydroxy acids, α-keto acids and mercaptans in paper chromatography*

Carboxylic acids are generally identified on paper chromatograms with the aid of an indicator spray[1] or by running them as hydroxamate derivatives and visualization as brown spots with a ferric chloride spray[2]. Since the R_F alone is normally an insufficient criterion, the spots being too close together, the absolute identification of some of the above acids by paper chromatography cannot be made[3].

BUCH, MONTGOMERY AND PORTER[3] overcame this difficulty by using four separate visualizing agents. A comparison of the reaction of the acids investigated with each of the four reagents enabled their absolute identification.)

The existing methods however do not differentiate between α-hydroxy acids and α-keto acids or between these and other carboxylic acids.

A critical analysis of the properties of the ceric ammonium nitrate reagent of BUCH *et al.*[3] revealed that this reagent may be used to differentiate between α-hydroxy and α-keto acids on the one hand, and other carboxylic acids on the other hand. This reagent was further modified to enable the differentiation of α-hydroxy and α-keto acids and mercaptans. In the course of the work, the reagent was found to react with tyrosine, tryptophan, and methionine, and was specific for the first two amino acids.

Experimental

Reagents

Reagent A. A stock solution is prepared by dissolving 20 g ceric ammonium nitrate in 50 ml of 0.5 *M* nitric acid. This solution is diluted with three volumes of water before use. (This solution was found to be more convenient than that used by BUCH *et al.*[3] because it was stored at a high concentration.)

Reagent B. Ceric ammonium nitrate, 8.2 g, mercuric nitrate 5 g, and potassium permanganate, 100 mg, are dissolved in 100 ml water. The mercuric nitrate is necessary for the detection of sulfhydryl groups. It may be omitted if none are present.

Procedure

The paper chromatogram is run in the normal manner, either ascending or descending with any solvent normally used for carboxylic acids; the air dried chromatogram is then dipped, at room temperature, in a shallow dish containing reagent A or B. Excess liquid is removed by placing the chromatogram on clean filter paper. The identifying colors develop without any additional treatment. These are listed in the results.

Results

Reagent A

A yellow background was formed with the cerium salt. This color was stable for 2 h.

The following compounds gave a white spot with the above reagent (range 0.5 to 5 μmole):

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α-Hydroxy acids. α-Hydroxyglutaric acid; lactic acid; ascorbic acid; glycolic acid; malic acid; methylmalic acid; citramalic acid; glucuronic acid; glyceric acid and tartaric acid.

α-Keto acids. Glyoxylic acid, α-ketoglutaric acid and pyruvic acid.

Mercaptans. Cysteine; cysteamine and thioglycolic acid.

Other acids. Malonic acid, oxalic acid and methionine gave white spots. Tryptophan gave a brown spot, and tyrosine gave a yellow spot.

The following gave no reaction, or reacted very slowly, if at all: unsaturated acids—acrylic acid, maleic acid and oleic acid, all other common amino acids, β-hydroxy acids, aliphatic mono- and dicarboxylic acids and their esters, alcohols, mono- and disaccharides, sulfonic acids, amides and imides.

Reagent B

An orange background, due to the permanganate, appeared at once. It was stable for 30 min.

The following compounds gave a yellow spot immediately turning to white within 2 min (range 0.5 to 5 μmole):

α-Hydroxy acids. Ascorbic acid; glycolic acid; α-hydroxyglutaric acid; lactic acid; methylmalic acid; glucuronic acid and glyceric acid.

The following compounds gave a white spot immediately:

α-Keto acids. Glyoxylic acid; α-ketoglutaric acid; pyruvic acid.

The following compounds gave a yellow color immediately changing to brown within 3 min: cysteine; cysteamine; thioglycolic acid (mercaptans) and tryptophan.

The following gave a yellow color blending with the background after 3 min: acrylic acid; maleic acid; oleic acid (unsaturated acids), as well as tyrosine. Malonic acid gave a weak yellow color after 2 min. Oxalic acid gave a yellow color at once.

Compounds which did not react or reacted very slowly, if at all, include all the other common amino acids, β-hydroxy acids, aliphatic mono- and dicarboxylic acids and their esters, alcohols and polyalcohols, mono- and disaccharides, sulfonic acids, amides and imides.

Discussion

The ceric ammonium nitrate reagent of BUCH *et al.*[3], was a 2 % solution in 1 *N* nitric acid. They report that pyruvic and glycolic acids did not react with the above reagent. We found, however, that 10 μl of solution of the above acids at the concentration used by them (15 mg/ml) did react with the above reagent.

The ceric ammonium nitrate reagent (reagent A) does not differentiate between the α-hydroxy, α-keto acids and mercaptans. The white color which develops on the yellow background is almost indistinguishable for these three types of compounds. The reagent becomes much more specific with the addition of potassium permanganate. Here an orange background is obtained and the rate of bleaching of the color is different for the different types. The α-hydroxy acids reduce the permanganate color at once and the ceric ammonium nitrate more slowly. The yellow spot, therefore, remains for about 2 min before turning white. The α-keto acids on the other hand, reduce the ceric ammonium nitrate more rapidly so that the white color is obtained at once.

Unsaturated acids react at once with permanganate but not with ceric ammo-

nium nitrate. A yellow spot finally blending into the background is, therefore, obtained.

Mercaptans rapidly reduce both permanganate and ceric ammonium nitrate. Mercuric nitrate is therefore necessary to differentiate this class of compound. The mercuric nitrate is reduced by these mercaptans, and not by the α-hydroxy or α-ketoacids. A brown color is therefore obtained with these mercaptans and not with the other acids.

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