

With this apparatus* we have obtained good results for the separation of a great variety of substances, in particular amino acids and bile salts.

For special purposes, and in particular for stratifying mixtures of silica gel and silver nitrate^{5,6}, the apparatus has been constructed with the reservoir in plexiglas.

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A method for the detection of β -phenylethylamines and β -phenylethylamino acids

A convenient method for the detection of β -phenylethylamines on chromatographic paper has not been available. In the course of a study in which it was necessary to treat chromatograms with Ehrlich's reagent after they had been treated with ninhydrin, it was noted that each of a number of substituted β -phenylethylamines appeared as a pink spot. Since these compounds do not react with Ehrlich's reagent alone it was felt that this combination of color reagents might be useful in the detection of β -phenylethylamines. Accordingly, a systematic examination of a series of substituted β -phenylethylamines and related compounds was undertaken.

Procedure and results

The compounds studied were dissolved in methanol-water for application to paper chromatograms. 5 μ g of material were routinely chromatographed on Whatman No. 1 paper. The chromatograms were developed in butanol-acetic acid-water (4:1:1). Other solvent systems were also employed, and it was found that if the chromatograms were adequately air dried, the choice of solvent system did not significantly affect the results.

The dried chromatograms were then dipped in ninhydrin-pyridine reagent (0.2 % ninhydrin in acetone-pyridine 9:1), air dried, and heated for 1 min at 105°. After notation was made of the color, the chromatograms were dipped in a modified Ehrlich's reagent (2 % *p*-dimethylaminobenzaldehyde in acetone-conc. HCl 9:1). After the ninhydrin color had completely faded, certain compounds evidenced a pink color which slowly changed to orange. The pink color was maximum approximately 30 min after the strips were dipped in Ehrlich's reagent and the colors were therefore

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noted at that time. In the event that 5 μg of material failed to produce a color reaction, the procedure was repeated using 25 μg of the compound. Whether the compounds were applied as free bases, hydrochlorides, or other salts did not alter the results.

From Table I it can be seen that all compounds that produce a purple color with

TABLE I

RESPONSE OF AMINO COMPOUNDS TO THE SPECIFIC TEST FOR β -PHENYLETHYLAMINO COMPOUNDS

Compound (5 μg of chromatographed material)	Ninhydrin-pyridine reagent (color)*	Ehrlich's after ninhydrin-pyridine reagent (color)*	Response to test for β -phenylethyl- amino compounds
Ethylamine	p	neg.	—
Benzylamine	p	faint y	—
β -Phenylethylamine	p	pk-o	+
Tyramine	p	pk	+
Dopamine	p	pk	+
4-Hydroxy-3-methoxy- β -phenylethylamine	p	pk	+
3,4-Dimethoxy- β -phenylethylamine	p	pk	+
Mescaline	p	pk	+
Amphetamine	faint p	neg.**	weak
N-Methyl- β -phenylethylamine	faint p	faint pk	weak
Norepinephrine	p	faint y	—
Epinephrine	faint p	faint y	—
Ephedrine	neg.	neg.**	—
3-Phenyl-1-propylamine	p	neg.	—
Urea	neg.	y	—
<i>p</i> -Methylaminophenol	br	faint tan	—
Pyrrole	neg.	neg.**	—
<i>p</i> -Nitroaniline	y	o	—
N,N-Dimethylaniline	neg.	neg.	—
<i>p</i> -Anisidine	y-o	y	—
Naphthylamine	y-o (vis.)	y	—
Isatin	y (vis.)	faint y-o	—
Marsilid	neg.	neg.	—
Tryptamine	p	p	—
Serotonin	br-p	br-p	—
Bufotenine	faint y	p-b	—
N-Acetyl-5-methoxytryptamine	neg.	b	—
Glycine	p	faint green	—
Glutamic acid	p	faint y	—
Glutamine	p	faint y	—
Lysine	p	faint grey	—
Cysteine	p	faint y	—
Methionine	p	faint y	—
Proline	y	faint p-pk	—
Phenylserine	p	faint y	—
Phenylalanine	p	pk-o	+
3,5-Diiodotyrosine	p	pk	+
3,4-Dihydroxyphenylalanine	p	pk	+
Tryptophan	p	br-p	—
N-Carbobenzoxy-L-tryptophan	neg.	faint p	—

* Color code: p = purple; br = brown; y = yellow; o = orange; pk = pink; b = blue; (vis.) = spots were colored before ninhydrin-pyridine reagent.

** Chromatography of 50 μg of amphetamine revealed a spot faintly p with ninhydrin-pyridine and faintly pk with Ehrlich's after ninhydrin-pyridine. Chromatography of 25 μg of ephedrine revealed a spot p with ninhydrin-pyridine and faintly y with Ehrlich's after ninhydrin-pyridine. Chromatography of 25 μg of pyrrole revealed a spot faintly tan with ninhydrin-pyridine and faintly p-pk with Ehrlich's after ninhydrin-pyridine. All other compounds that failed to produce a color reaction with 5 μg of material did not produce any color with 25 μg .

ninhydrin and a pink color after subsequent treatment with Ehrlich's reagent were β -phenylethylamines and β -phenylethylamino acids. These compounds do not produce a pink color with Ehrlich's reagent alone. Therefore, a compound that:

1. forms a purple color with ninhydrin;
2. forms a pink color 30 min after subsequent treatment with Ehrlich's reagent; and
3. fails to form a pink color with Ehrlich's reagent alone, can be determined, presumptively, to be a β -phenylethylamino compound.

The satisfaction of these three conditions constitutes a positive response to this test. The test is positive for β -phenylethylamine, for β -phenyl-ethylamines substituted on the ring and for β -phenylethylamino acids. Mono substitution on the α -carbon of the amine or mono substitution on the amino group of the amine decreases the sensitivity of the test. The α -amino acids react strongly however. Tertiary amines do not react with ninhydrin¹ and these, therefore, do not give a positive response to this test. Further substitution on the β -carbon of the β -phenylethylamines, as in norepinephrine, produces a final yellow color and, therefore, the response of these compounds is also negative.

In order to determine whether these findings were dependent on the solvents used for the color reagents rather than the ninhydrin and the *p*-dimethylaminobenzaldehyde, ninhydrin reagent was dissolved in (1) acetone alone, (2) acetone-glacial acetic acid (9:1), (3) ethanol alone, (4) water alone. None of these variations affected the results of the test. When *p*-dimethylaminobenzaldehyde was dissolved in ethanol rather than acetone, this also did not alter the results.

Discussion

A study of the mechanism of this test has not been attempted. However, it has been observed that the intensity of the pink color formed after the application of the Ehrlich's reagent is proportional to the intensity of the purple formed after the application of ninhydrin. This suggests that the Ehrlich's reagent reacts with the Ruhemann's purple formed from the reaction of ninhydrin and the β -phenylethylamino compounds. This is also borne out by the fact that N mono substitution and α mono substitution weaken the reaction with ninhydrin and also produce a weaker response to this test. The sensitivity of this reaction has only been studied systematically to 5 μ g. However, sensitivity has been found to extend to the anticipated sensitivity with ninhydrin reagent alone.

The finding that many β -phenylethylamino compounds produce a pink color with this test provides a convenient method for the detection of these compounds on paper chromatograms.

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¹ I. SMITH, *Chromatographic and Electrophoretic Techniques*, Vol. 1, Heinemann, London, 1960.

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