BENTLEY AND WHITEHEAD[10] who reported that the R_F values of amino acids rose (for their solvent–paper system) with an increase in the water content of the solvent. The three-fold increase in the R_F values with equilibration (Table II) indicated that the "water of hydration" for the solvent–paper system employed in this study was high, and that omitting equilibration with Whatman No. 20 paper lowered the water-rich phase. This resulted in a different solvent composition as the moving solvent was dehydrated.

The data obtained in this study indicate a functional difference between Whatman No. 20 filter paper and the commonly employed Whatman filter papers No. 1 and 4. It is recommended that specific conditions be established for Whatman No. 20 paper for each solute–solvent–chamber-temperature system. It is also recommended that Whatman No. 20 paper be equilibrated for 18 to 24 h in the presence of freshly prepared solvent prior to irrigation with a critical volume of solvent.

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Chromatographic spray for the identification of tyrosine, histidine and their amines

In the course of estimating histamine in plant tissue, need arose for the specific detection of histamine on paper chromatograms. STEFANOVIÉ, CIRKOVIÉ AND BRESJANAC[1] described a colorimetric test for the detection of histamine in injection solutions. The method involved the coupling of histamine with diazotised *p*-aminobenzoic acid in alkaline solution. It was found that this test could be modified for use as a spray reagent to detect histamine on paper chromatograms.

TABLE I

LOWEST CONCENTRATIONS OF TYROSINE, TYRAMINE, HISTIDINE AND HISTAMINE DETECTABLE

	One-dimensional paper chromatography (μg)	*Two-dimensional paper chromatography* (μg)
Tyrosine	0.1	1.0
Tyramine	0.1	1.0
Histidine	0.05	0.1
Histamine	0.05	0.1

Reagent

The reagent can be used either as a single spray or as two sprays. When the reagent is used as two sprays, better results are obtained.

Spray 1. 10 ml of 1 % *p*-aminobenzoic acid dissolved in 2 *N* HCl is cooled in an icebath and 10 ml of freshly-prepared 5 % sodium nitrite is added slowly. This mixture is held at 2–3° for 15 min.

Spray 2. 20 % sodium carbonate.

Procedure

After spraying with (1) the chromatogram is dried and subsequently sprayed with (2). The compounds appear as red spots on a yellow background and are stable for several weeks.

The sensitivity of the reagent can be favourably compared with ninhydrin. Table I illustrates the smallest amounts of tyrosine, tyramine, histidine and histamine that can be detected in different chromatographic processes.

The colourless solution of diazotised *p*-aminobenzoic acid couples instantly with phenols and aromatic amines to produce azodyestuffs. The coupling takes place in the *para* position but if the *para* position is occupied, as in tyrosine, the coupling occurs in the position *ortho* to the hydroxyl group. In the case of imidazole derivatives, such as histidine and histamine, the coupling takes place in the position nearest to the imide group of the imidazole ring.

This reagent cannot be used when phenol has been used in the solvent. After drying at 100° for 4 h and standing in an air current overnight, some phenol still adheres to the paper and is subsequently dyed by the reagent.

This spray reagent can then be used to identify histidine, tyrosine and their amines, histamine and tyramine. Derivatives of either amino acid can also be detected, *e.g.* 3,4-dihydroxy-phenylalanine, 2-thiolhistidine or even carnosine, which is the dipeptide alanyl-L-histidine. Phenylalanine, however, does not couple with the reagent to give a coloured dyestuff.

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