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Identification of Amino acids on Thin-layer Chromatography Plates with a New Reagent

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ABSTRACT

A new reagent has been introduced for the detection of amino acids. This reagent is capable for easy identification of amino acids on thin-layer chromatography plates by developing various distinguishable colors (detection limit: 0.08-5 µg). The colors thus obtained are more or less stable up to 6 -10 hrs.

Key words: Amino acids, TLC, 4- dimethylamino benzaldehyde, Isatin.

INTRODUCTION

Identification of amino acids has immense importance in the protein chemistry because they are the basic units of proteins and polypepeptides. Various spray reagents for the specific and non-specific detection of amino acids on thin-layer chromatograms have already been described[1-24]. Such identification is also useful when the amino acids occur in free state in numerous natural products and in the determination of the C-terminal amino acids of degraded proteins. Of the spray reagents used so far, ninhydrin is mostly used because of its remarkable high sensitivity[2] but it produces same purple color with all amino acids except in two cases; proline and hydroxylproline however, give a yellow color. The present communication deals with a reagent which can give various distinguishable colors with most of the amino acids with high sensitivities.

MATERIALS AND METHODS

Apparatus

Thin-layer Chromatographic plates (20 cm × 20 cm) were prepared from silica gel 'G' (Merck, India) having thickness of 0.1 mm by means of a Unoplan Coating Apparatus (Shandon, London, UK). Sample solutions were spotted on to the plates using a graduated micropipette (5 µL).

Reagents

Amino acid samples and Isatin were obtained from Sigma (USA), 4-dimethylamino benzaldehyde from Aldrich (USA) and n-propanol, acetone from Merck (India).

Detection on TLC plates

Standard solutions (5 mg/ml) of amino acids were prepared in 0.01M phosphate buffer (pH 8.0) and spotted on TLC plates with a graduated micropipette (5 µL). Spotting volume was always 1 µL; when necessary the solutions were diluted. The plates were subjected to TLC with n-propanol-water, 70+30 (v/v), as mobile phase. After development, plates were dried and sprayed with 1% 4-dimethylamino benzaldehyde in acetone (Reagent 1), kept at room temperature in air to evaporate acetone completely, heated in an oven at 110°C for 10 min, cooled and sprayed with 0.4% Isatin in ethanol (Reagent 2). After further drying in air the plates were heated at 110°C for 10 min; color and detection limits were recorded and placed in Table 1. Colors were always observed visually. Detection limits for the amino acids using ninhydrin reagent alone[2] are also depicted in Table 1.

RESULTS AND DISCUSSION

It has been observed that a lemon yellow color was observed in each of the amino acids after spraying with Reagent 1 with high detection limits ranging between 0.1-1.0 µg in cold condition. But after heating in an oven at 110°C for 10 min, the color intensities (lemon color) were markedly minimized and consequently not placed in Table 1. After spraying with Reagent 2 followed by heating can produce several distinguishable colors with high sensitivities. The mechanism leading to such color formation is uncertain but it is probable that the aldehyde group of 4-dimethylamino

Table 1: Colors formed by amino acids on TLC plates with 4-dimethyl aminobenzaldehyde-isatin as spray reagent with detection limits, detection limits for ninhydrin reagent and their R_f – values after development in n-propanol – water (70+30) system

Amino acid	Color observed after final heating	Detection limits µg	Detection limits for Ninhydrin reagent*(µg)	R_f-values in n-propanol –water (70:30)
Arginine	Brownish pink	5.0	0.01	0.02
Cysteine	Yellowish green	0.6	0.02	0.38
Cystine	Pista color	0.8	0.01	0.32
Histidine	Brownish pink	1.0	0.05	0.20
Isoleucine	Light pink	1.0	0.20	0.53
Glutamine	Reddish pink	0.2	0.10	0.15
Lysine	Ivory	0.6	0.005	0.03
Asparagine	Pale rose	1.0	0.10	0.14
Phenylalanine	Light ash	0.8	0.05	0.58
Serine	Light pink	1.0	0.008	0.35
Threonine	Talc color	1.0	0.05	0.37
Alanine	Pale rose	5.0	0.009	0.37
Glutamic acid	Light reddish pink	0.8	0.04	0.35
Valine	Rosy pink	0.9	0.01	0.45
Methionine	Talc color	1.0	0.01	0.51
Aspartic acid	Light brownish pink	5.0	0.10	0.33
Tyrosine	Pista color	0.4	0.03	0.57
Leucine	Pale rose	1.0	0.01	0.55
Glycine	Pinkish violet	1.0	0.001	0.32
Proline	Deep bluish violet	0.08	0.10	0.26
Hydroxyproline	Sky blue	0.1	0.05	0.34
Tryptophan	Light clay	1.0	0.05	0.62

* Reference 2.

benzaldehyde condenses with the amino group leading to the formation of imine-type intermediate which in turn forms a charge-transfer type complex with Isatin.

CONCLUSION

The detection limits obtained after final heating ranges between 0.08 µg to 5 µg. It is to be noted here that the identification of amino acids by color reaction with ninhydrin is in practice, difficult, despite of its remarkable high sensitivity[2] whereas the newly proposed reagent is more effective and convenient for identification of most of the amino acids, because it can produce a variety of distinguishable colors with the amino acids investigated. Moreover, the colors were more or less stable up to 6 to 10 hrs and not effected by mild acid vapour etc.

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