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NOTE

Detection of amino acids on TLC plates with modified ninhydrin reagent

Sarmistha Basu and Amalendu Sinhababu*

Natural Product Laboratory, Department of Chemistry, The University of Burdwan, Burdwan-713 104, West Bengal, India

E-mail : sinhababu04@yahoo.co.in *Fax* : 91-342-2530452/2634200

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Abstract : Thin layer chromatography is an important tool for detecting amino acids by several spray reagents. Ninhydrin is the most well known spray reagent for identification of amino acids due to its high sensitivity. But, it produces same purple/violet color with all amino acids, except proline and hydroxy proline. A new modified ninhydrin reagent, *para*-chlorobenzaldehyde has been introduced here which produces distinguishable colors with the amino acids with moderately high sensitivity (0.03–1.0 μg). A probable mechanism for such color formation and the color pictures of the chromatograms by digital camera are also included here.

Keywords : Thin-layer chromatography, amino acids, *para*-chlorobenzaldehyde, ninhydrin.

Introduction

Identification of amino acids is a key necessity in the evaluation of protein structure, as these compounds are the structural units of protein and also for determination of the C-terminal units of degraded proteins. It is also essential for the determination of the presence of amino acids in natural products. Thin layer chromatography is an important tool for the detection of amino acids by variety of specific and non-specific spray reagents[1–22]. Among the reagents used, ninhydrin as a non-specific reagent is the most well known and widely used for its remarkable high sensitivity[23] but, it produces same purple/violet color with all amino acids, except proline and hydroxy proline (both produces yellow color). An auxiliary spray reagent, *p*-chlorobenzaldehyde reported here produces various distinguishable colors with ninhydrin and enables convenient and easy detection of them on silica gel G TLC plates with moderately high sensitivity (0.03–1.0) μg. The sensitivities are comparable to other reagents so far introduced/cited[1–22] and ninhydrin alone[23] and more sensitive in many occasions than some other reagents referred here[11–15,17–21]. A probable mechanism for such color formation has also been proposed.

Results and discussion

It is observed from Table 1 that ninhydrin gives various distinguishable colors with amino acids in the presence of reagent I before and after final heating. The detection limits are also substantially low before (0.1–1.0 μg) and after second heating (0.03–1.0 μg). The color pictures of the chromatograms are reflected in the Figs. 1 and 2. The detection limits (μg spot^{-1}) are substantially low (0.03–1.0 μg) which is quite comparable to ninhydrin alone. The detection limits before second heating are higher than those obtained after second heating except in case of glutamic acid and hydroxy proline. Color development is somewhat different almost in all the cases after second heating. Such color formations with high sensitivities of this modified spray reagent make it somewhat more useful than ninhydrin spray in the identification of amino acids on TLC plates.

The mechanism leading to such color formation is uncertain but a possibility may be ascertained as follows. An 'imine' intermediate is first formed by the reaction of amino acids with the reagent I and this then forms coloring complexes (C–T)[24] with ninhydrin (Scheme 1), colors of which are variable depending on the nature of amino acids. Another possibility is the reaction of amino acids (unreacted or derived from 'imine' intermediate) with ninhydrin in the usual way to produce Ruhemann

Table 1. Color formation of amino acids on TLC plates with *para*-chlorobenzaldehyde and ninhydrin

	Reagent I + Ninhydrin					
	Cold condition (before second heating)		Hot condition (after second heating)			
Amino acids	Color observed	Detection limit (μg)	Color observed	Detection limit (μg)	Detection limit of nihydrin[a] (μg)	R_f values[b]
Glycine (1)	Dirty yellow with pink ring	0.50	Yellow with pink ring	0.10	0.001	0.32
Alanine (2)	Brownish violet	1.00	Brown with pink ring	0.08	0.009	0.37
Valine (3)	Pinkish violet	1.00	Brown with pink ring	0.05	0.010	0.45
Leucine (4)	Brownish yellow with pink ring	1.00	Pinkish brown	0.05	0.010	0.55
Isoleucine (5)	Brownish yellow with pink ring	0.50	Brownish violet	0.30	0.200	0.53
Serine (6)	Pinkish brown	1.00	Deep pinkish brown	0.50	0.008	0.35
Threonine (7)	Pinkish yellow	1.00	Pinkish brown	0.50	0.050	0.37
Aspartic acid (8)	Light brownish violet	0.80	Deep violet	0.03	0.100	0.33
Asparagine (9)	Brownish yellow	1.00	Dirty yellow	0.10	0.100	0.14
Glutamic acid (10)	Brownish violet	1.00	Deep pink	1.00	0.040	0.35
Glutamine (11)	Yellowish purple	1.00	Pinkish yellow	0.80	0.100	0.15
Lysine (12)	Pinkish violet	0.10	Brown with pink ring	0.03	0.005	0.03
Histidine (13)	Brownish violet	0.80	Dark brown	0.50	0.050	0.20
Arginine (14)	Deep violet	0.30	Deep violet	0.30	0.010	0.02
Phenyl alanine (15)	Yellowish brown	1.00	Brownish pink	0.80	0.050	0.58
Tyrosine (16)	Brownish purple	0.10	Pinkish brown	0.01	0.030	0.57
Tryptophan (17)	Yellowish deep brown	0.30	Yellowish brown	0.08	0.050	0.62
Cysteine (18)	Pinkish pale cream	1.00	Pinkish pale cream	0.03	0.020	0.38
Cystine (19)	Light brownish violet	0.80	Pinkish brown	0.08	0.010	0.32
Methionine (20)	Brownish violet	0.80	Pinkish brown	0.30	0.010	0.51
Proline (21)	Light yellow	0.50	Lemon yellow with pink ring	0.05	0.100	0.26
Hydroxy proline (22)	Reddish pink	0.10	Mid cream	0.10	0.050	0.34

[a]Ref. : Stahl (1969).

[b]*n*-Propanol : water = 70 : 30 (v/v).

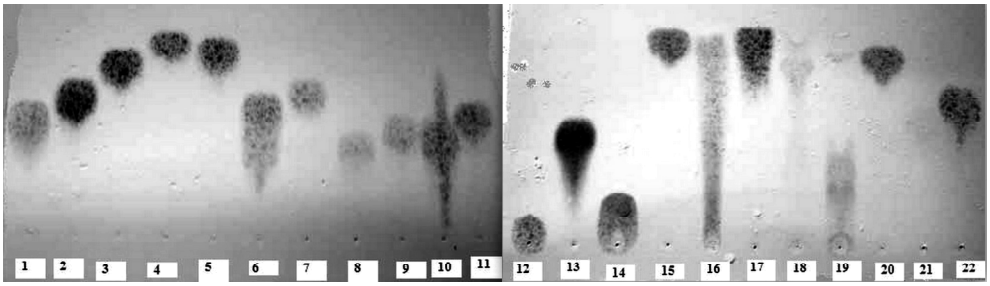


Fig. 1. Color of amino acids with ninhydrin in presence of reagent I in cold condition.

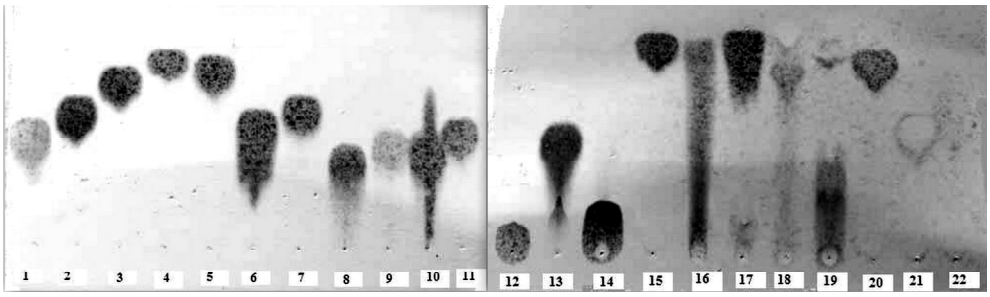
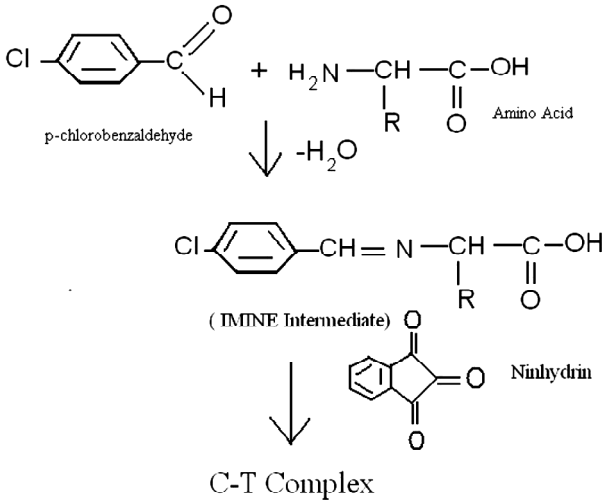
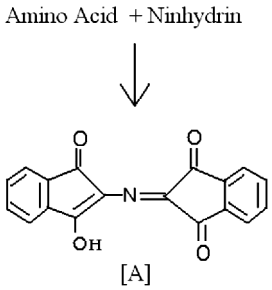


Fig. 2. Color of amino acids with ninhydrin in presence of reagent I in hot condition.

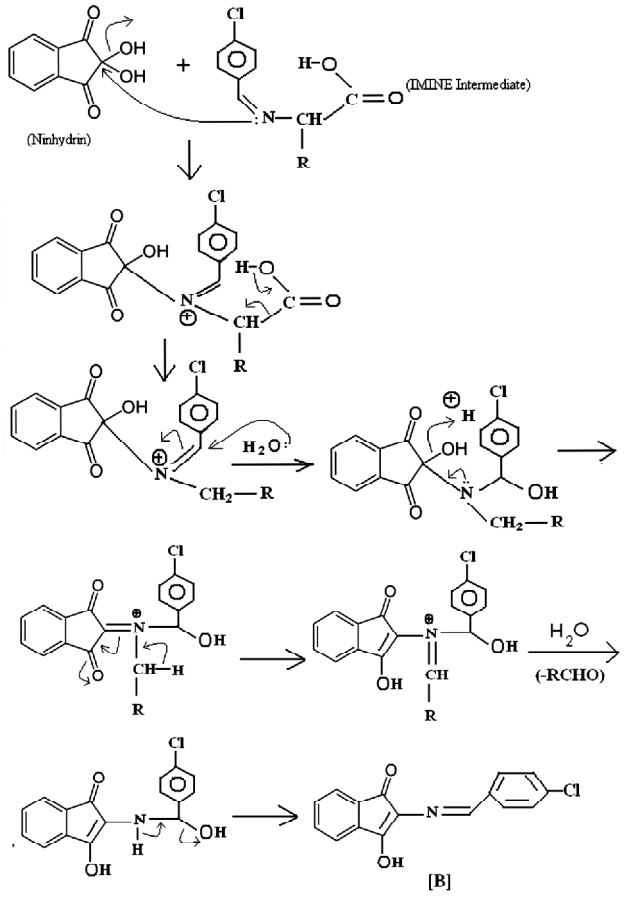


Scheme 1

Complex A (except for proline and hydroxyproline, Scheme 2a). On the other hand, the 'imine' intermediate may also undergo the reaction with ninhydrin in the following way (Scheme 2b) to produce the complex B. Since both complexes (A and B) are formed in unequal amounts depending on the nature of amino acids, we observed the variation of color contrast of the different amino acids.



Scheme 2a



Scheme 2b

Experimental

Apparatus :

Chromatography plates (20 × 20 cm; thickness 0.1 mm) were prepared with silica gel G (Merck, India) using Unoplan Coating apparatus (Shandon, London, UK). Photographs of the colored chromatograms were performed by Digital Camera (Nikon Coolpix L-25, China).

Reagents :

Standard amino acids were obtained from Sigma (USA) and *n*-propanol from Merck (India).

Reagent I : 1% *p*-chlorobenzaldehyde (Spectrochem Pvt. Ltd., Mumbai, India) in acetone.

Reagent II : 0.25% ninhydrin (Sigma, St. Louis, MO, USA) in acetone.

Detection on TLC plates :

Standard solutions (1 mg mL^{-1}) of amino acids were prepared in 0.01 mol L^{-1} phosphate buffer (pH 8.0) and spotted on the TLC plates by means of a graduated micropipette (5 μL). The solutions were diluted according to the required spot concentration. Plates were air-dried and subjected to TLC with *n*-propanol-water, 70 : 30 (v/v) as mobile phase. After development plates were dried and sprayed with the reagent I and then heated at 110 °C for 10 min in an oven. Plates were cooled and then sprayed with reagent II and colors were noted (Table 1). Colors were further observed after heating the plates at 110 °C for 10 min (Table 1). Colors were observed visually and the color pictures of chromatograms by digital camera are also recorded (Figs. 1 and 2). Detection limits for the amino acids after use of ninhydrin reagent alone is also given in Table 1.

Limit of detection :

The limit of detection of amino acids were determined by spotting a standard solution (1 mg mL^{-1}) of the concerned amino acid on to the TLC plate, which was developed with mobile phase and spot was visualized using the reagent and ninhydrin as described in the above section. This process was repeated with successive dilution of the standard amino acids solutions until no detection was possible. The amount of amino acid just detectable was taken as detection limit.

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