Paper-chromatographic differentiation between α-monoamino acids and other ninhydrin-positive substances

In the course of studies in this laboratory on certain derivatives of α,β-diaminopropionic acid[1] a need arose for a paper-chromatographic method for distinguishing between α-monoamino acids and other ninhydrin-positive compounds including amino derivatives of the latter. Previously published methods[2–4] utilizing complex formation between all α-amino acids and Cu^{++} did not prove satisfactory for the present purpose. Hence, an alternative procedure has been developed, based on specific masking of the α-amino acid grouping with Cu^{++} to such an extent as to make it virtually insusceptible to subsequent ninhydrin treatment.

The dried chromatograms, run in standard solvent systems, are dipped through a methanolic solution containing 0.25 % $Cu(NO_3)_2 \cdot 3H_2O$ and 2 % anhydrous CH_3-COONa, and dried for 2 min at 70°. Subsequent dipping of the chromatograms

TABLE I

COLOUR REACTIONS AND INTENSITIES OF VARIOUS AMINO-SUBSTITUTED COMPOUNDS AFTER NINHYDRIN TREATMENT WITH AND WITHOUT PRETREATMENT WITH COPPER SALT

Compound	*Development with ninhydrin*				*Development with Cu++-reagent followed by ninhydrin*			
	Immediately after treatment		*20 h after treatment*		*Immediately after treatment*		*20 h after treatment*	
	*Colour**	*Intensity***	*Colour**	*Intensity***	*Colour**	*Intensity***	*Colour**	*Intensity***
1. L-Alanine	V	++	V	+++	V	((+))	V	(+)
2. L-α-Aminobutyric acid	V	++	V	+++	V	((+))	V	(+)
3. L-Valine	V	++	V	+++	V	((+))	V	(+)
4. L-Leucine	V	++	V	+++	V	((+))	V	(+)
5. L-Isoleucine	V	++	V	+++	V	((+))	V	(+)
6. L-Serine	V	++	V	+++	V	((+))	V	(+)
7. L-Threonine	V	++	V	+++	V	((+))	V	(+)
8. L-Methionine	V	++	V	+++	V	((+))	V	(+)
9. L-Aspartic acid	V	+	V	++			Y-Bn	(+)
10. L-Glutamic acid	V	++	V	++	V	((+))	V	(+)
11. DL-α-Phenylglycine	Y	+	V	++			Y-Bn	(+)
12. L-Phenylalanine	V	++	V	++			V	(+)
13. L-Tyrosine	V	((+))***	V	+			V	(+)
14. L-Histidine	V	+	V	++	Y-Gr	((+))	Y-Bn	(+)
15. L-Tryptophane	V	+	V	++	V	((+))	V	(+)
16. L-α-Amino-γ-guanidino-butyric acid	V	++	V	++	V	((+))	V	(+)
17. L-Arginine	V	++	V	++	V	((+))	V	(+)
18. L-α-Amino-ε-guanidino-caproic acid	V	++	V	++	V	((+))	V	(+)
19. L-α-Amino-β-ureidopropionic acid§ (Albizziine)	V	++	V	++	V	((+))	V	(+)
20. L-α-Amino-γ-ureidobutyric acid	V	++	V	++	V	((+))	V	(+)
21. L-Citrulline	V	++	V	++	V	((+))	V	(+)
22. L-α-Amino-ε-ureidocaproic acid	V	++	V	++	V	((+))	V	(+)

* The following abbreviations have been used to indicate the colours: V, violet; Y, yellow; R, red; O, orange; Bn, brown; Gr, grey.

** Intensities are specified as follows: +++, very strong; ++, strong; +, medium; (+), faint and ((+)), very faint.

*** The weakness is due to the limited solubility of tyrosine in water.

§ New compounds (*cf.* ref.[1]).

through an 0.2 % solution of ninhydrin in acetone is followed by air-drying for 1 min and heating at 70° for 40–50 sec.

Whereas α-monoamino acids under these conditions do not at all appear, or at most as very faint violet spots, other amino acids, such as α,β-diaminopropionic acid, β-alanine, γ-aminobutyric acid, ornithine and lysine, rapidly produce intense and characteristic, individual colours. Glycine represents the sole exception to the above rule because this amino acid as well as a number of glycyl derivatives yield persistent yellow or orange spots, which may possibly be of diagnostic value for the identification of glycine and glycyl peptides. On prolonged keeping at room temperature all α-amino acids produce visible violet spots, but of weak intensity compared to those on the chromatograms exclusively treated with ninhydrin.

In Table I are listed the results obtained with 10 μg of each of 50 individual, ninhydrin-positive compounds subsequent to paper chromatography on Whatman paper No. 1 in *n*-butanol–acetic acid–water (12 : 3 : 5). Chromatographic results obtained with other solvent systems have been equally consistent and the procedure

TABLE I, *continued*

Compound	*Development with ninhydrin*				*Development with Cu++-reagent followed by ninhydrin*			
	Immediately after treatment		*20 h after treatment*		*Immediately after treatment*		*20 h after treatment*	
	*Colour**	*Intensity***	*Colour**	*Intensity***	*Colour**	*Intensity***	*Colour**	*Intensity***
23. L-Cysteine·HCl	V	+	V	+			Y-Bn	(+)
24. L-Asparagine	V	+	R-Bn	+			Gr-Bn	(+)
25. L-Proline	Y	+	Y	+	Y	((+))	Gr-Bn	(+)
26. β-Alanine	V	((+))	V	+	Bn	++	Bn	++
27. Taurine	V	(+)	V	+	R-Bn	++	R-Bn	++
28. L-α,β-Diaminopropionic acid·HCl	V	++	V	++	Y-Bn	++	Y-Bn	++
29. L-α-Ureido-β-amino-propionic acid §	V	(+)	V	+	Y-Bn	++	Y-Bn	++
30. γ-Aminobutyric acid	V	+	V	++	R	++	R	++
31. L-α, γ-Diaminobutyric acid	V	++	V	++	Y-Bn	++	R-Bn	++
32. L-Ornithine·2HCl	V	++	V	++	R-V	++	R-V	++
33. L-Lysine·HCl	V	++	V	++	R-V	++	R-V	++
34. Glycine	V	++	V	++	O	++	O	++
35. Glycylglycine·HCl	Y-Bn	+	V	++	Y	(+)	Y	+
36. Glycylglycylglycine	Y-Bn	+	V	++	Y	(+)	Y	+
37. Glycyl-L-leucine	R-Bn	+	V	++	Y	(+)	Y	+
38. Glycyl-L-tryptophane			V	+	Y	(+)	Y	+
39. Glycinamide, $0.5H_2SO_4$	Y-Bn	+	V	++	Y	(+)	Y	+
40. Glycine methyl ester, HCl	Y	+	V	++	Y	(+)	Y	+
41. DL-Alanylglycine	V	+	V	++	Y	(+)	R-Bn	+
42. L-α-Glutamyl-L-methionine	V	+	V	++	Y	(+)	V	+
43. L-Tyrosinamide, $0.5H_2SO_4$	V	+	V	++	Y	(+)	Y-Bn	+
44. DL-Alanine methyl ester·HCl	V	+	V	++	Y	(+)	V	+
45. L-Leucine ethyl ester·HCl	V	+	V	+	Y	(+)	V	+
46. DL-Phenylalanine ethyl ester·HCl	V	+	V	++	Y	(+)	Y-Bn	+
47. Ethanolamine	V	+	V	++	V	+	R-V	++
48. 3-Amino-1-propanol	V	+	V	++	V	+	R-V	++
49. Benzylamine	Y	++	V	++	Y	+	R-Bn	++
50. Ethylendiamine	V	++	V	++	R-V	+	R-Bn	++

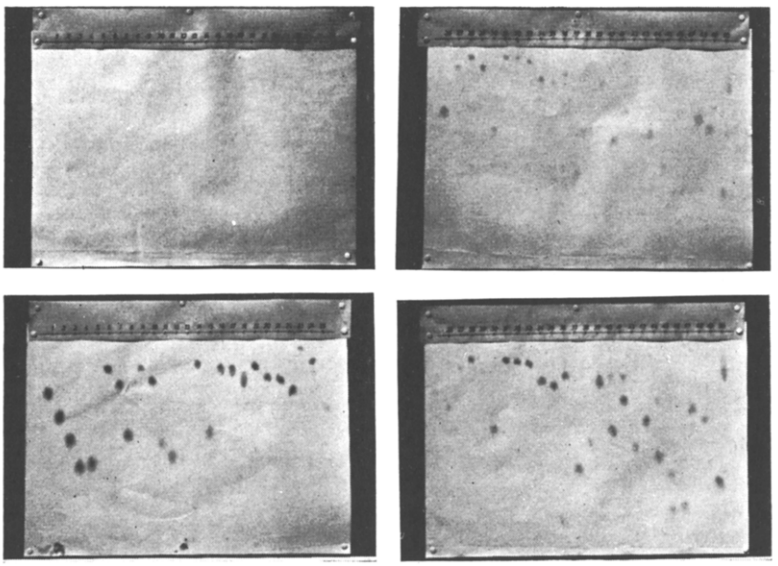

Fig. 1. Photographic reproduction of descending paper chromatograms of the compounds No. 1–50, listed in Table I, on Whatman paper No. 1 in *n*-butanol–acetic acid–water (12 : 3 : 5). Upper row: ninhydrin-treated papers after impregnation with copper salt. Lower row: Same compounds treated with ninhydrin without copper impregnation.

has proved useful also in spot-test technique on filter paper. Photographic reproductions of ninhydrin-treated paper chromatograms of all compounds listed in Table I on copper-impregnated as well as untreated paper are presented in Fig. 1.

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Chloramphenicol and chlortetracycline inhibition of amino acid incorporation into proteins in a cell-free system from *Tetrahymena pyriformis*

Growth of *Tetrahymena pyriformis* "W" in 2.5 % proteose peptone (Difco) was considerably delayed or completely arrested by chloramphenicol at the relatively high levels ranging from 25 to 150 μg/ml medium. Similar concentrations of the

Abbreviations: ATP, GTP, CTP, UTP, the triphosphates of adenosine, guanosine, cytidine and uridine respectively; PEP, phosphoenol pyruvate; RNA-ase, ribonuclease; Tris, tris(hydroxymethyl)aminomethane.