THE IDENTIFICATION OF SOME AMINO ACIDS IN THE PRESENCE OF 50% GLYCEROL, ON SILICA GEL THIN LAYERS

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INTRODUCTION

The effect of glycerol on the movement of amino acids on some thin layers has been reported previously[1]. The variation in the rate of movement and the shape of the spot made interpretation of the chromatograms very difficult and alternative procedures were investigated in order to identify the free amino acids present in grass pollen extracts preserved in 50% glycerol.

EXPERIMENTAL

The experimental details are as given previously[1], except that α-amino-*n*-butyric acid was also examined. The solvent systems were (I) 96% ethanol–water (70:30, v/v), (II) phenol–water (75:25, w/w; 20 mg NaCN added per 100 g mixture), (III) *n*-

TABLE I

EFFECT OF CHANGE IN pH OF SILICA GEL LAYER ON RATE OF MOVEMENT OF SOME AMINO ACIDS

Amino acid	*Average R_F × 100 values*					
	pH 3	*pH 4*	*pH 5*	*pH 6*	*pH 7*	*pH 8*
(i) Dissolved in distilled water						
L-Arginine	13	14	21	13	5	3
L-Arginine monohydrochloride	19	19	23	15	6	3
L-Histidine	21	20	18	11	11	15
L-Lysine monohydrochloride	13	19	13	9	3	1
DL-Asparagine	47	35	29	20	19	13
DL-Aspartic acid	47	36	33	20	17	21
L-Cysteic acid	49	47	41	21	21	27
L-Glutamic acid	51	47	49	32	27	31
L-Glutamine	53	46	39	33	31	24
α-Alanine	47	26	32	30	29	24
α-Amino-*n*-butyric acid	48	52	53	46	45	45
γ-Amino-*n*-butyric acid	47	48	41	28	33	24

(*continued on p. 83*)

TABLE I (*continued*)

Amino acid	*Average R_F × 100 values*					
	pH3	*pH4*	*pH5*	*pH6*	*pH7*	*pH8*
L-Cysteine hydrochloride	53	59	49	50	54	62
(two spots)	24	29	21	13	11	11
L-Cystine (pH 1.7)	32	30	20	14	9	10
Glycine	42	35	35	22	16	12
L-Hydroxyproline	49	47	42	35	33	25
DL-Leucine	41	31	51	61	56	53
DL-Methionine	64	62	43	63	55	52
DL-Norleucine	63	49	53	60	57	57
L-β-Phenylalanine	69	65	47	65	59	57
L-Proline	42	38	28	36	28	21
DL-Serine	49	36	31	23	14	13
L-Threonine	51	53	40	39	29	25
DL-Tryptophan	68	67	54	67	57	57
L-Tyrosine	49	24	50	51	61	55
DL-Valine	56	42	49	51	51	45
(ii) Dissolved in 50% glycerol						
L-Arginine	13	19	26	15	5	3
L-Arginine monohydrochloride	15	19	23	16	6	3
L-Histidine	21	20	18	12	11	15
L-Lysine monohydrochloride	13	19	17	12	3	1
DL-Asparagine	47	47	30	22	19	13
DL-Aspartic acid	41	32	33	20	18	21
L-Cysteic acid	48	47	41	25	21	27
L-Glutamic acid	46	37	47	33	27	31
L-Glutamine	45	42	39	31	30	23
α-Alanine	45	32	38	31	29	24
α-Amino-*n*-butyric acid	53	51	48	43	48	41
γ-Amino-*n*-butyric acid	36	46	40	28	30	24
L-Cysteine hydrochloride	43	53	49	46	54	61
(two spots)	25	30	23	13	11	12
L-Cystine (pH 1.7)	40	30	22	15	10	11
Glycine	41	33	35	24	15	12
L-Hydroxyproline	41	41	42	35	33	25
DL-Leucine	42	31	59	60	53	51
DL-Methionine	59	55	42	59	53	49
DL-Norleucine	63	49	43	60	56	53
L-β-Phenylalanine	59	55	42	57	52	48
L-Proline	39	35	28	36	28	23
DL-Serine	45	37	31	23	14	13
L-Threonine	51	47	40	38	29	25
DL-Tryptophan	56	48	51	62	57	57
L-Tyrosine	49	23	48	40	55	47
DL-Valine	56	49	51	51	50	42

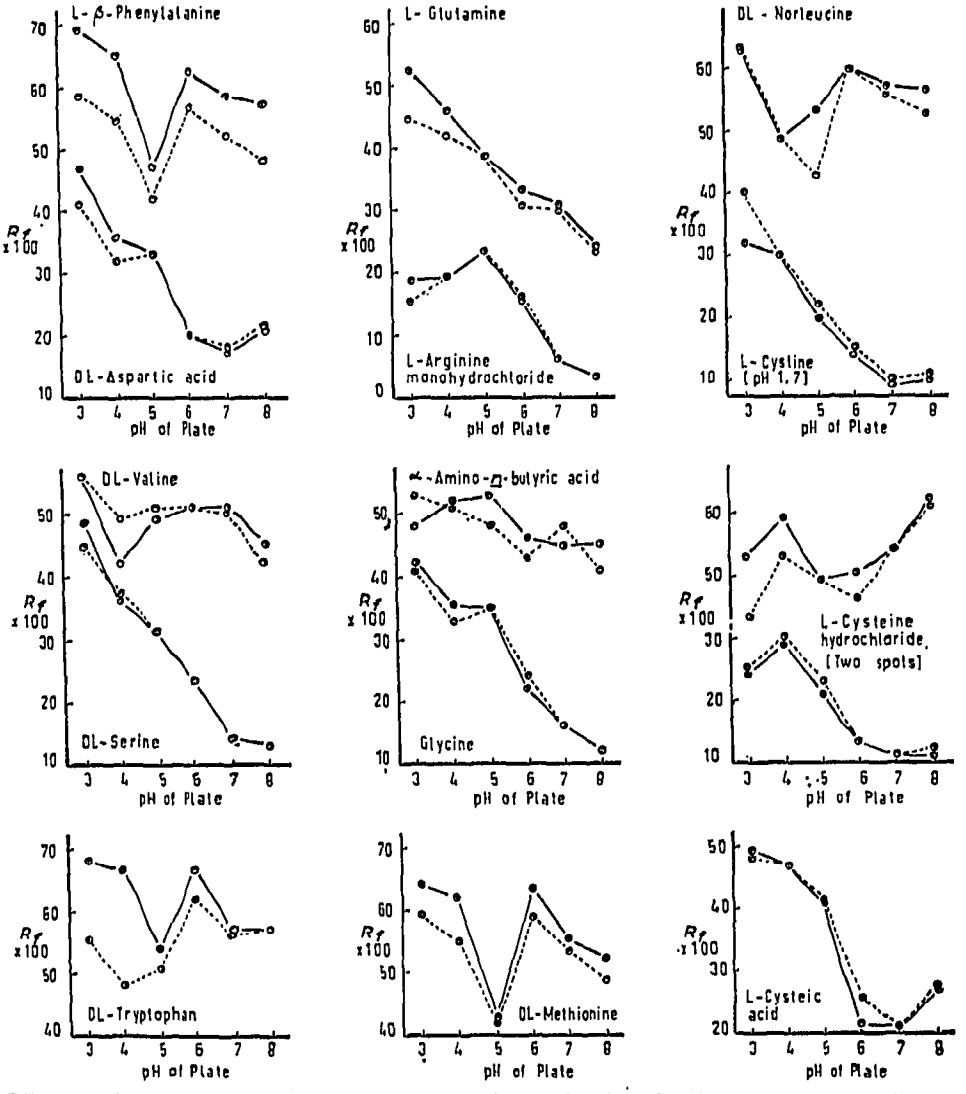

Fig. 1. $R_F \times 100$ values as a function of pH of silica gel layer. Solvent system I. (————) amino acid dissolved in distilled water; (– – – –) amino acid dissolved in 50% glycerol.

butanol–glacial acetic acid–water (80:20:20, v/v) and (IV) 96% ethanol–water–diethylamine (70:29:1, v/v). In addition McILVAINE's universal buffer[2] was used as the vehicle for preparation of the silica gel plates covering the pH range 3–8.

RESULTS AND DISCUSSION

Solvent system I; buffered silica gel layers pH 3–8

Since amino acids are amphoteric it was anticipated that variation in the pH of the layer would result in changes in the rate of movement of the amino acids on that layer. By virtue of differences in the patterns obtained by plotting R_F values against the pH of the layer it was hoped that specific amino acids could be identified.

R_F values obtained are given in Table I. The comparative graphs for each amino acid studied, dissolved in distilled water and in 50% glycerol, illustrated in Figs. 1 and 2, show R_F value plotted as a function of the pH of the layer. Reference to these figures shows that where the presence of glycerol alters the R_F values, the general

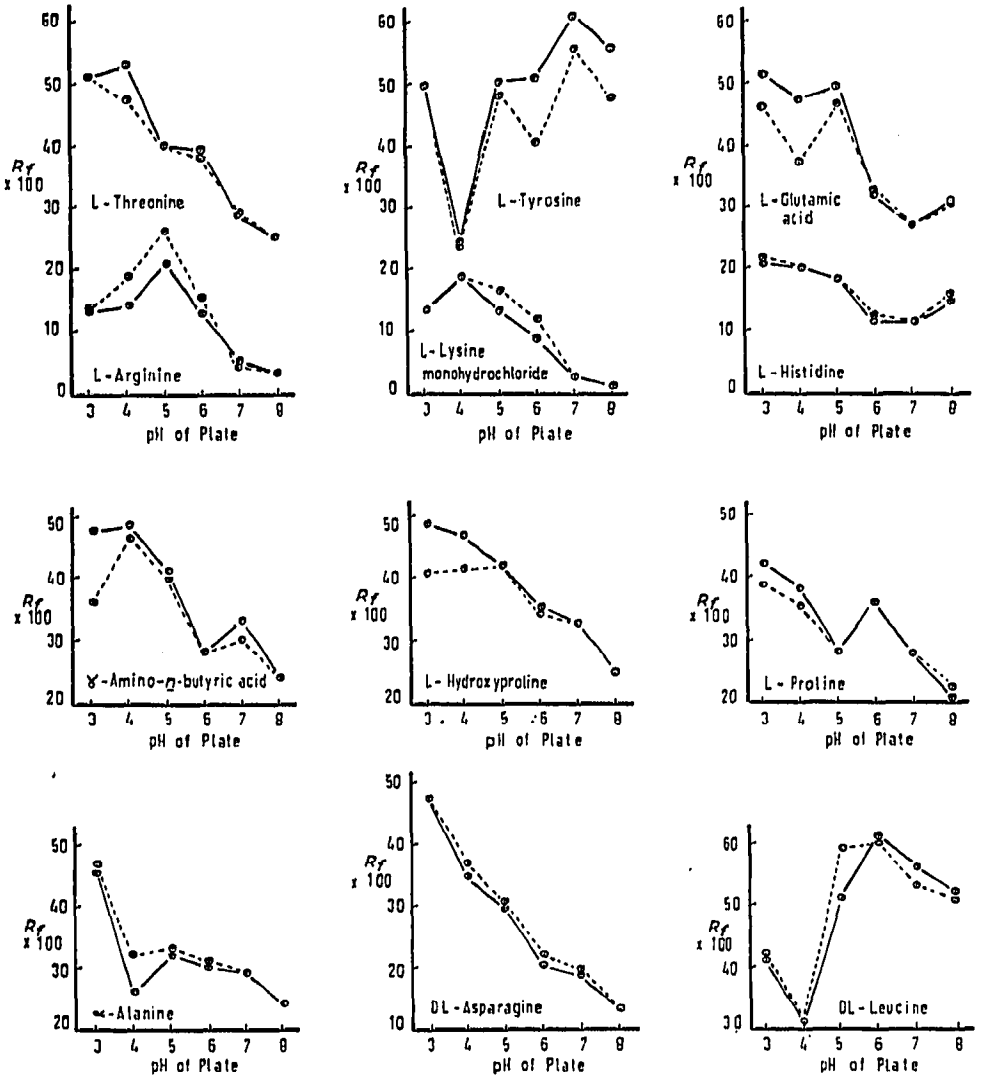

Fig. 2. $R_F \times 100$ values as a function of pH of silica gel layer. Solvent system I. (———) amino acid dissolved in distilled water; (– – – –) amino acid dissolved in 50% glycerol.

shape of the two curves remains the same. However, inspection of the curves for the following groups:

(a) L-glutamine, DL-serine (Fig. 1), L-hydroxyproline (Fig. 2)
(b) DL-asparagine (Fig. 2), L-cystine (pH 1.7) (Fig. 1)
(c) DL-methionine, L-β-phenylalanine (Fig. 1)

shows that the close similarity in shape makes identification within groups (a) and (c) impossible. Furthermore, the difference in colour on heating, after spraying with the ninhydrin reagent fails to distinguish L-glutamine from DL-serine (both pink) and DL-methionine from L-β-phenylalanine (both pink) although L-hydroxyproline (yellow) is differentiated from the other amino acids in group (a) and DL-asparagine (yellow) from L-cystine (grey) in group (b).

While identification of a number of amino acids is possible by considering the patterns obtained by plotting R_F as a function of pH of the silica gel layer, the overlap in pattern of those amino acids previously mentioned stimulated the investigation

TABLE II

MINIMUM AND MAXIMUM R_F VALUES IN SOLVENT SYSTEMS I–IV FOR SOME AMINO ACIDS

Amino acid dissolved in 50% glycerol	*R_F × 100 values in solvent system:*								*No. of determinations*
	I		*II*		*III*		*IV*		
	Min.	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	*Min.*	*Max.*	
L-Arginine	1	4	5	7	5	7	11	18	8
L-Arginine monohydrochloride	3	4	12	20	6	8	9	15	8
L-Histidine	15	26	18	22	6	8	33	43	11
L-Lysine monohydrochloride	1	3	5	10	3	5	5	9	11
DL-Asparagine	27	36	15	25	13	15	25	36	7
DL-Aspartic acid	25	47	1	10	15	20	45	56	12
L-Cysteic acid	52	65	1	9	9	12	63	67	8
L-Glutamic acid	29	52	3	10	17	24	37	70	11
L-Glutamine	31	35	20	32	13	15	36	48	6
α-Alanine	32	47	21	27	16	21	29	49	10
α-Amino-*n*-butyric acid	35	45	26	31	25	27	45	56	8
γ-Amino-*n*-butyric acid	25	31	21	27	20	26	23	24	10
L-Cysteine hydrochloride L-Cystine (pH 1.7 and 8.5)	Multiple spot formation								
Glycine	29	40	16	24	13	21	25	40	11
L-Hydroxyproline	35	40	25	35	15	30	38	41	8
DL-Leucine	45	60	43	56	30	43	50	72	12
DL-Methionine	41	53	34	40	34	66	47	54	10
DL-Norleucine	44	61	41	51	40	45	57	61	12
L-β-Phenylalanine	40	54	44	57	39	66	50	60	12
L-Proline	23	37	35	52	11	16	20	31	12
DL-Serine	35	51	13	20	13	23	29	47	12
L-Threonine	38	52	17	23	20	23	44	49	8
DL-Tryptophan	53	61	52	80	33	49	54	69	12
L-Tyrosine	50	63	29	47	24	43	34	63	12
DL-Valine	39	54	31	45	25	29	40	51	12

of the patterns produced when R_F is plotted as a function of the solvent system used for development of the layer.

Solvent systems I, II, III and IV; silica gel layers

Maximum and minimum R_F values are given in Table II rather than average values. This is to prevent misinterpretation of slight differences in the shape of the patterns obtained when the method is applied to the resolution of unknown amino acids in a mixture. BRENNER[3] reported the practical factors involved in determining the movement of solutes on thin layers but in spite of careful standardisation of technique variation in R_F values is well known in thin-layer chromatography. Although it is not the standard practice to quote the range of R_F values SHELLARD[4] strongly recommended the procedure because it gives a clear indication of the likely range within which R_F values for a particular substance may be expected.

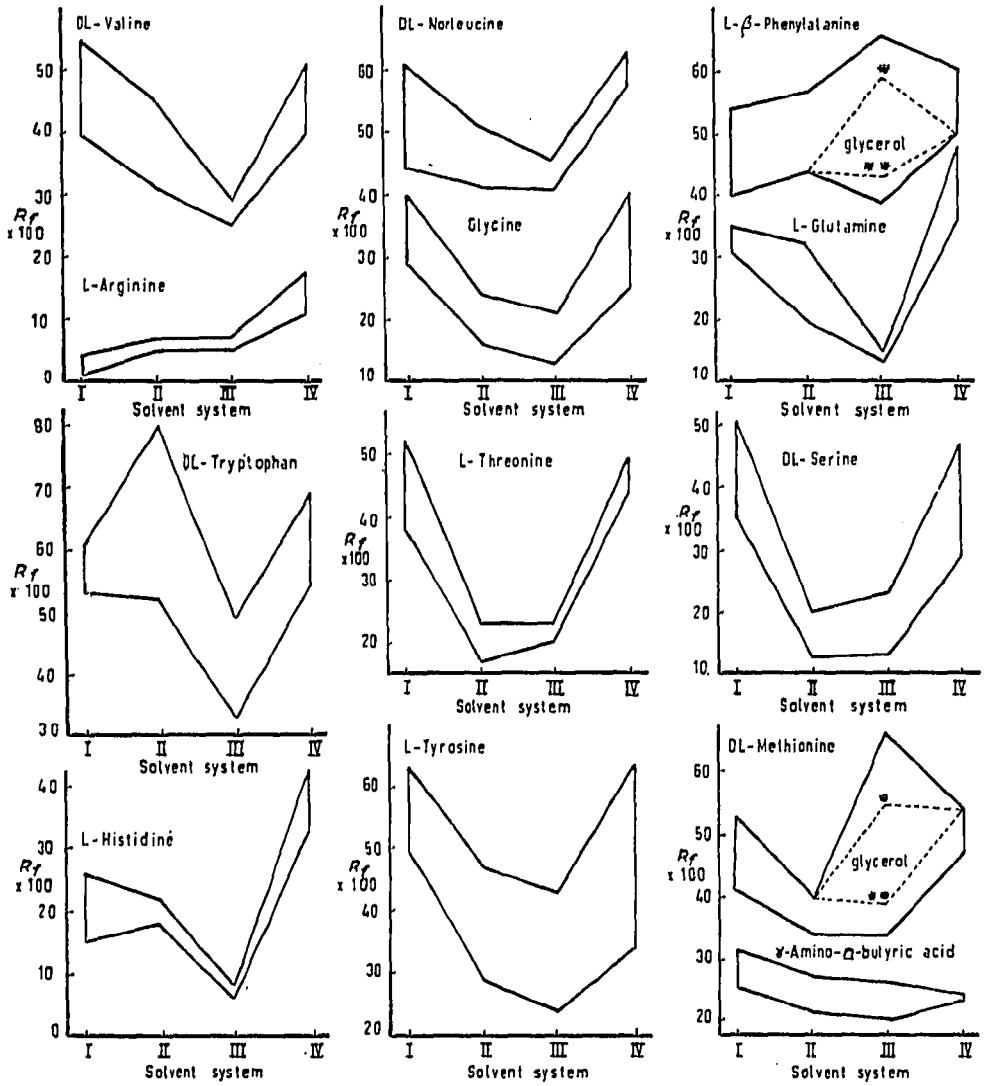

Fig. 3. $R_F \times 100$ patterns for some amino acids, dissolved in 50% glycerol and chromatographed on air-dried silica gel layers using solvent systems I–IV. L-β-Phenylalanine and DL-methionine: The dotted lines mark the limits of the pattern, * if the amino acid is carried ahead of the glycerol and ** if the amino acids lags behind the glycerol.

The patterns obtained by plotting the maximum and minimum R_F values as a function of the sequence of the solvent system used are shown in Figs. 3 and 4. From these it can be seen that the pattern is characteristic for each amino acid. However, the patterns for DL-aspartic acid and L-glutamic acid (Fig. 4) are almost identical but this would not prevent the method from being applied to the resolution of amino acid mixtures since these two amino acids give different colours on heating, after spraying with the ninhydrin reagent (DL-aspartic acid, blue; L-glutamic acid, pink).

This method has been successfully applied to the resolution of the free amino acid present in aqueous extracts of some grass pollens preserved in 50% glycerol. By plotting the patterns for the amino acids resolved, cutting them out and superimposing them on the patterns of the known amino acids also chromatographed in the presence of 50% glycerol, alanine, γ-amino-*n*-butyric acid, glycine, leucine, lysine, proline and serine were identified (details to be published later).

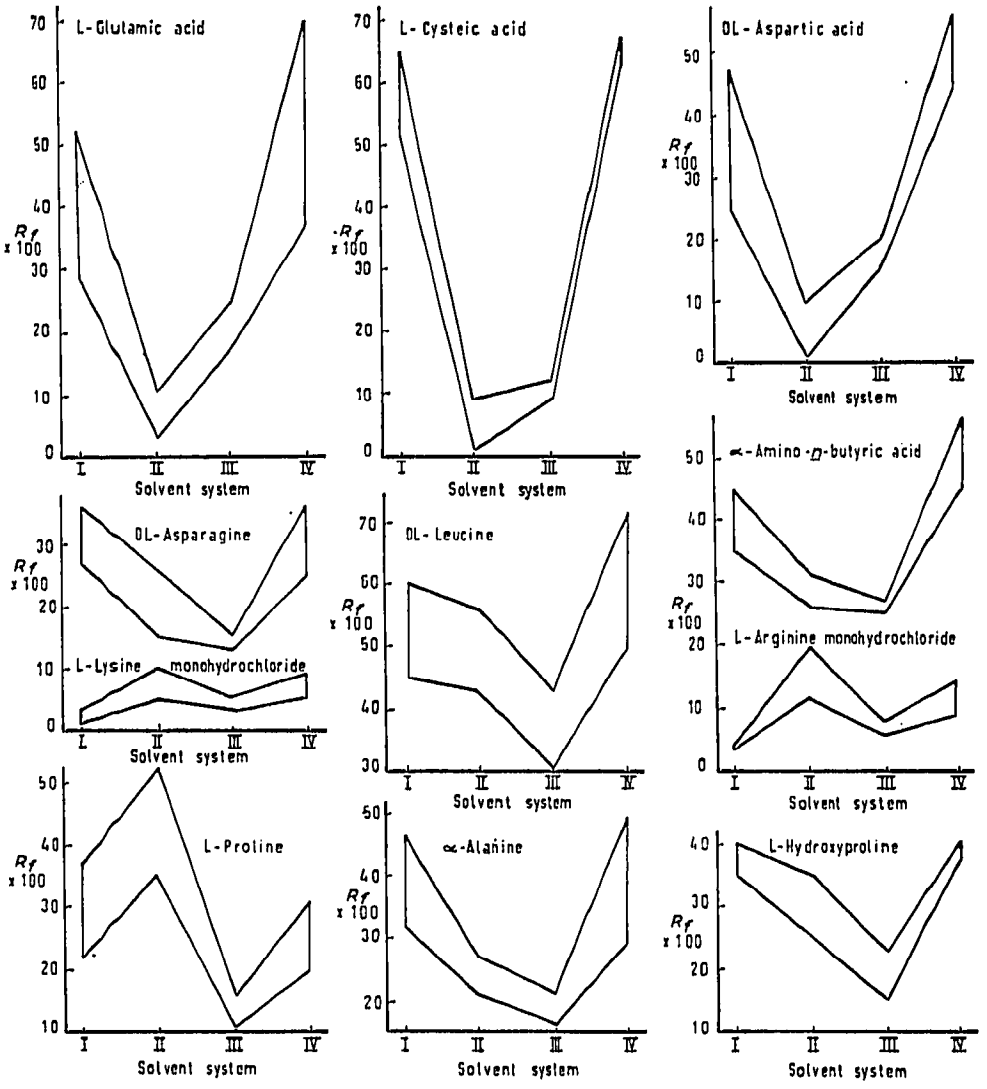

Fig. 4. $R_F \times 100$ patterns for some amino acids, dissolved in 50% glycerol and chromatographed on air-dried silica gel layers using solvent systems I–IV.

SUMMARY

In view of the difficulties previously reported on the effect of glycerol on the rate of movement of some amino acids on silica gel thin layers, two methods for the identification of amino acids in the presence of glycerol have been investigated. The patterns obtained by plotting R_F values as a function of the pH of the layer and as a function of the sequence of the solvent system used are presented. Consideration of these shows that the latter method is more specific and it has been successfully used in the resolution of the free amino acids present in grass pollen extracts preserved in 50% glycerol.

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