The effect of glycerol on the rate of movement of some amino acids on silica gel thin layers

Difficulties due to the presence of glycerol have been reported[1] in the movement of simple sugars on silica gel and cellulose thin layers. Similar difficulties have been experienced in attempting to identify the free amino acids in pollen extracts preserved in 50 % glycerol solution.

Amino acid mixtures have been resolved using thin-layer chromatography (TLC) on layers of acetylated cellulose[2], aluminium oxide [3–5], calcium hydroxide[6], calcium oxide[7], calcium phosphate[6], calcium sulphate[8], cellulose[9–11], iron oxide hydrate[12], kieselguhr[4], magnesia[7], magnesium trisilicate hydrate[13], polyacrylonitrile[14] and polyamide [15] powders, sephadex[16–17] and silica gel[4,18–27]. Since the latter has found greatest application in TLC of amino acids, silica gel was selected as the adsorbent in preliminary work on the effect of the presence of glycerol on the rate of movement of some amino acids, prior to applying the method to the resolution of the free amino acids in pollen extracts.

Air-dried silica gel layers are recommended although RANDERATH[28] suggests they may also be dried at an elevated temperature provided they are exposed to air for at least 30 min before use. Activation at 110° for 15 min with subsequent exposure to air for periods of 30–60 min resulted in greater variation in R_F values than when when air-dried plates were used.

Experimental

Essentials details are given in Table I.

TABLE I

SUMMARY OF EXPERIMENTAL PROCEDURE

Layer	Silica Gel G (Merck), 250 mμ, air dried overnight
Solvent systems	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*-Butanol–glacial acetic acid–water (80:20:20, v/v) IV 96 % Ethanol–water–diethylamine (70:29:1, v/v)
Method	Ascending, in saturated chamber; 20–22°; 15 cm
Load	20 μg (2 μl of 1 % solution in 0,10,20,30,40 and 50 % glycerol) (L-Tyrosine + trace of NH_4OH; DL-aspartic acid, 8 μg; L-cystine and L-glutamic acid, 10 μg).
Amino acids	
Basic	L-Arginine, L-arginine monohydrochloride, L-histidine, L-lysine monohydrochloride
Acidic	DL-Asparagine, DL-aspartic acid, L-cysteic acid, L-glutamic acid, L-glutamine
Neutral	α-Alanine, γ-amino-*n*-butyric acid, L-cysteine hydrochloride, L-cystine, glycine, L-hydroxyproline, DL-leucine, DL-methionine, DL-norleucine, L-β-phenylalanine, L-proline, D-serine, L-threonine, DL-tryptophan, L-tyrosine, DL-valine.

Detection of amino acids

The plates were dried at 110° for 10 min and sprayed with modified ninhydrin reagent[29]. After spraying the plates were further heated to give optimum colour development of the amino acid spots.

TABLE II

AVERAGE R_F × 100 VALUES OF SOME AMINO ACIDS IN THE PRESENCE OF VARYING CONCENTRATIONS OF GLYCEROL IN FOUR SOLVENT SYSTEMS
M = Multiple spots; S = streaked.

Compound	*Solvent I* % *Glycerol*						*Solvent II* % *Glycerol*						*Solvent III* % *Glycerol*						*Solvent IV* % *Glycerol*					
	0	*10*	*20*	*30*	*40*	*50*	*0*	*10*	*20*	*30*	*40*	*50*	*0*	*10*	*20*	*30*	*40*	*50*	*0*	*10*	*20*	*30*	*40*	*50*
L-Arginine	3	3	3	3	3	3	5	5	5	5	6	6	5	5	6	6	6	6	16	16	15	15	15	15
L-Arginine monohydrochloride	4	4	4	4	4	4	19	19	19	19	19	19	6	6	6	6	6	6	9	9	9	9	9	9
L-Histidine	24	24	24	24	23	23	21	21	21	21	21	20	5	5	5	6	7	7	58	57	51	48	44	43
L-Lysine monohydrochloride	2	2	2	2	2	2	7	7	7	6	6	6	5	5	5	6	5	5	7	7	7	7	7	7
DL-Asparagine	35	35	34	33	34	34	25	25	24	25	24	25	13	13	13	13	13	14	33	33	32	31	31	29
DL-Aspartic acid	49	50	48	42	43	33	3	3	3	4	4	8	14	14	15	15	15	16	59	62	61	61	58	55
L-Cysteic acid	65	63	61	59	56	55	1	1	2	3	7	8	10	10	10	10	10	10	64	64	63	62	64	63
L-Glutamic acid	35	38	39	39	37	35	4	4	5	5	7	8	17	17	19	20	18	17	52	59	59	59	57	49
L-Glutamine	37	38	39	37	37	35	28	28	28	28	28	28	13	14	14	14	15	15	40	39	39	37	37	37
α-Alanine	55	58	57	54	51	47	26	26	26	25	23	25	21	22	21	21	19	19	56	54	50	47	42	41
γ-Amino-n-butyric acid	29	29	29	29	28	29	27	27	27	27	27	27	25	25	24	24	23	23	24	24	23	24	23	24
L-Cysteine hydrochloride	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
L-Cystine (pH 1.7)	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
L-Cystine (pH 8.5)	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Glycine	41	41	40	39	38	35	19	19	19	19	19	19	20	20	20	21	21	21	41	41	40	39	39	41
L-Hydroxyproline	49	48	45	44	39	37	34	37	36	36	35	33	27	26	27	27	27	27	53	53	51	49	45	40
DL-Leucine	63	63	59	59	57	56	41	52	53	56	57	55	37	37	35	36	35	33	63	69	71	71	67	61
DL-Methionine	43	58	56	52	50	47	27	30	37	39	38	39	63	65	67	67	66	63	70	71	67	59	56	49
DL-Norleucine	50	56	53	52	48	44	40	47	46	51	48	48	48	47	47	45	44	41	12	10	9	7	7	7
L-β-Phenylalanine	56	55	51	49	47	44	51	49	47	47	45	44	52	51	52	65	65	66	66	67	67	64	63	60
L-Proline	42	41	40	38	35	33	55	55	54	53	49	45	13	14	15	17	17	15	22	22	22	23	23	21
D-Serine	45	43	41	39	36	35	14	15	16	18	20	20	13	14	14	14	15	13	40	41	39	35	33	30
L-Threonine	49	49	47	48	48	41	17	17	17	17	17	17	23	23	23	23	23	23	47	49	49	48	48	47
DL-Tryptophan	69	67	65	63	63	61	81	81	81	79	79	80	42	42	40	38	37	33	69	70	73	71	70	68
L-Tyrosine	S	S	S	S	S	S	S	S	S	S	S	S	42	42	41	39	37	34	52	52	48	47	39	34
DL-Valine	63	62	61	57	54	53	36	37	38	38	41	41	31	31	30	30	28	27	55	57	54	50	46	51

The position of the glycerol was readily distinguished as a whitish zone on a pinkish-buff background on prolonged heating of the sprayed plate at 110°.

Results and discussion

R_F values, in four solvent systems, for the amino acids investigated are given in Table II and tracings of some of the results obtained are shown in Figs. 1–2.

Tracings of the movement of glycerol alone are not included since the concentration of amino acid is small by comparison with that of glycerol. Variation in shape and position of the glycerol spots at higher concentrations is more likely to result from the shape of the adsorption isotherm for the substance than from the presence of the amino acid. SHELLARD[30] has indicated that since the amount of substance adsorbed per mg of adsorbent varies with the concentration of the substance, in TLC, substances with a concave isotherm will show a decrease in rate of movement with increase in concentration.

Consideration of the R_F values in Table II shows that the presence of glycerol in the solution does not adversely affect the rate of movement of all the amino acids studied. The spread of R_F values at the various glycerol concentrations for L-arginine, L-arginine monohydrochloride, L-lysine monohydrochloride, DL-asparagine and L-glutamine is within the limits of expected variation and no distortion in the shape of these spots was observed.

The presence of glycerol in some of the amino acid solutions, however, affects both the *rate of movement* and the *shape* of the amino acid spot to such an extent that identification of specific amino acids in a mixture would be extremely difficult.

Rate of movement

Basic amino acids. In general these are not adversely affected by the presence of glycerol in the four solvent systems studied, with the exception of L-histidine in solvent IV (Fig. 1).

Acidic amino acids. The effect in this group is more varied. DL-Aspartic acid and L-cysteic acid (Fig. 1) with solvent I show a decrease in rate of movement with increase in glycerol concentration whereas with solvent IV aspartic and glutamic acids (Fig. 1) show an apparent increase in rate of movement at the lower glycerol concentration followed by a subsequent decrease with increase in glycerol concentration. In this latter solvent system the movements of the amino acids concerned and glycerol are similar and the initial increase in R_F value at low glycerol concentrations may result from the amino acid being mechanically dragged upwards with the more viscous glycerol. This effect is reversed as the glycerol concentration is increased since the area of the glycerol spot is increased resulting in retardation in the movement of the amino acid.

Neutral amino acids. The variability in movement within this group is marked. In solvent I there is consistent retardation with increase in glycerol concentration; in II, III and IV increase, decrease or constancy in rate may occur while DL-leucine and DL-tryptophan (solvent IV) show an initial increase (10 and 20% glycerol) with subsequent decrease as the concentration of glycerol increases. Selected examples are illustrated in Figs. 1 and 2.

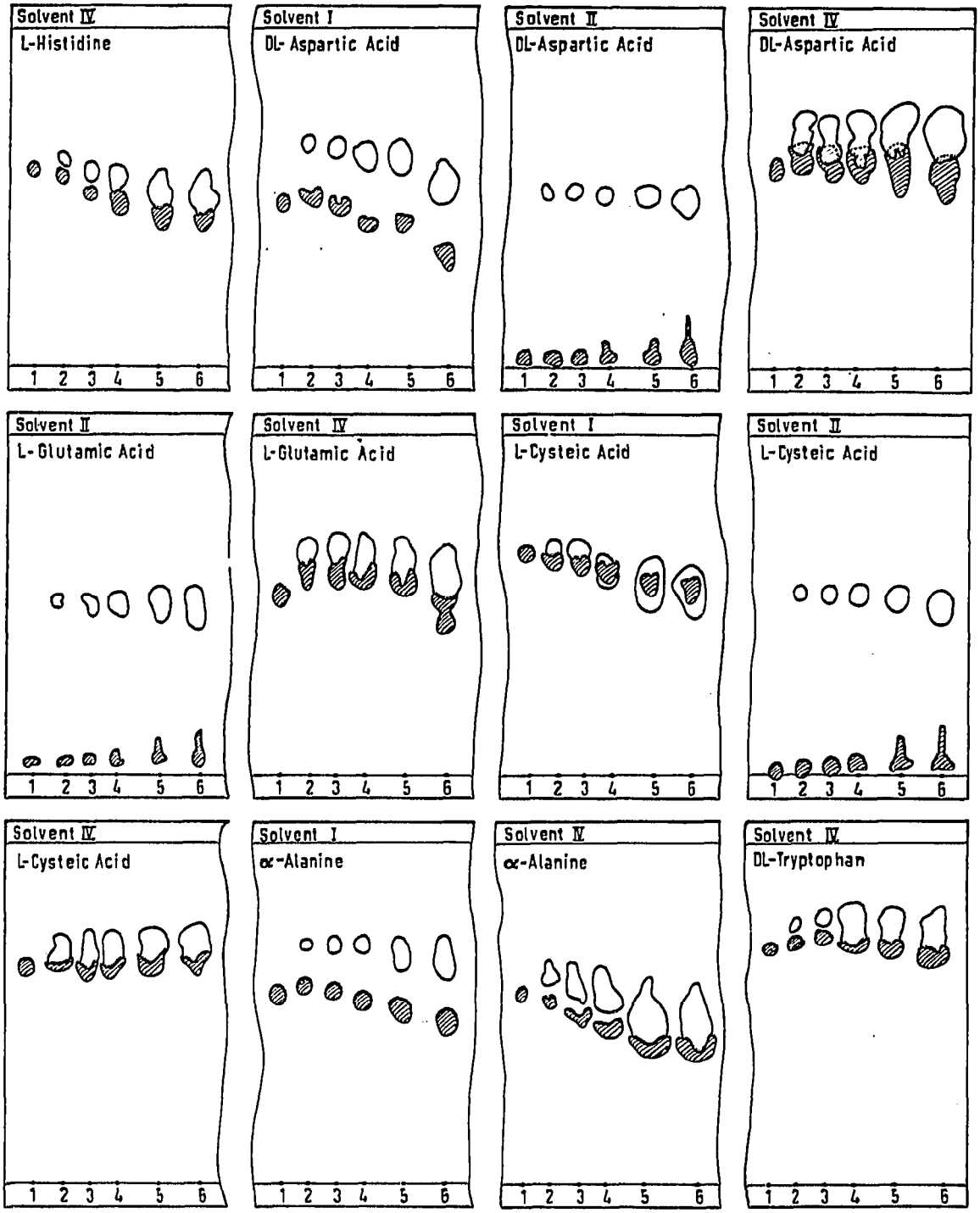

Fig. 1. Tracings of chromatograms of some amino acids dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated Nos. 1–6 respectively on the starting line. Amino acid (●); glycerol (○).

Shape

In TLC of amino acids it is known that while good separations are obtained with solvent systems rich in ethanol the spots may be diffuse, whereas solvent systems such as II and III have the effect of compacting the spots. Observation of Figs. 1 and

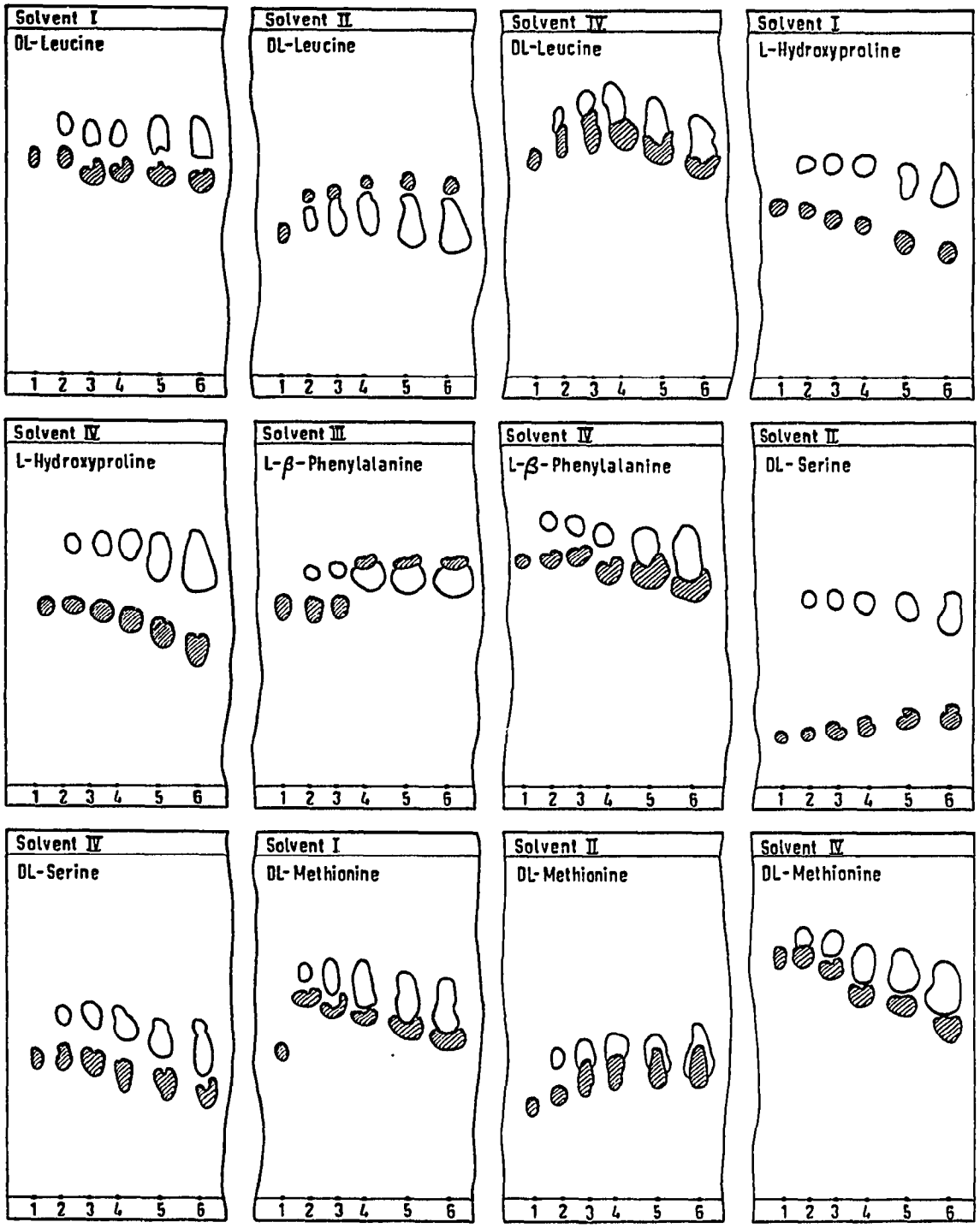

Fig. 2. Tracings of chromatograms of some amino acids dissolved in distilled water, 10, 20, 30, 40 and 50% glycerol solution and designated Nos. 1–6 respectively on the starting line. Amino acid (●); glycerol (○).

2 will clearly show that the diffusion of the amino acid spots in the presence of glycerol is not entirely a function of the solvent system used.

The diffusion of the amino acid spot in the presence of glycerol makes quantitative estimation based on the area of the spot impractical. FISHER *et al.*[31,32] described

a procedure for the quantitative estimation of substances on one-dimensional paper chromatograms and such a method has been appled in TLC[20,33], the area of the spots being proportional to the logarithm of the weight of the compound chromatographed. Observation of Figs. 1 and 2 shows this relationship does not hold since the amount of a given amino acid is constant in the varying concentrations of glycerol.

The difficulties encountered in the resolution of amino acids in pollen extracts preserved in 50% glycerol will not, therefore, be overcome by dilution of the glycerol concentration. The presence of the lowest concentration (10%) of glycerol examined adversely affects either the rate of movement or the shape of the spot of some of the amino acids investigated in one or more of the solvent systems used.

The results of work at present proceeding on the resolution of amino acid mixtures in the presence of 50% glycerol are encouraging and will be reported later.

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