THIN LAYER CHROMATOGRAPHY OF DANSYL AMINO ACID DERIVATIVES

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INTRODUCTION

The stability of Dansyl (5-dimethylaminonaphthalenesulphonyl) derivatives of amino acids under acid hydrolysis conditions makes these compounds extremely useful for the determination of N-terminal amino acids in proteins and peptides[1]. Dansyl-amino acids can be detected by their fluorescence under ultra-violet light at levels of 10^{-3} μmol to 10^{-4} μmol, which is several times greater than the sensitivity achieved with fluordinitrobenzene derivatives. Several authors[2,3] have applied this reagent to N-terminal and sequence studies. The major difficulties occur in the separation of the Dansyl-amino acids. The paper chromatographic separation[3] is obscured by tailing and only those Dansyl derivatives that have markedly different R_F values can be separated in this way. Paper electrophoresis[1,2] is more successful, but this method uses thick paper and therefore needs more sample for analysis entailing a lowered sensitivity. Thin layer chromatography appeared to offer several possible advantages, namely the possibility of using the minimal quantity of sample and the prevention of tailing by appropriately adjusting the activity of the layer; various solvent systems were investigated. The findings are reported below.

EXPERIMENTAL

Preparation of Dansyl derivatives

According to GRAY AND HARTLEY[1], standard solutions of Dansyl amino acids were prepared by adding 1 ml of the Dansyl reagent (6 mg of Dansyl chloride in 1 ml of acetone) to an equal volume of amino acid (or peptide) solution. The concentration of the amino acids was 6.5 μmole/ml and the sample was dissolved in 1 ml 0.1 M $NaHCO_3$ to ensure the alkaline reaction of the mixture. The mixture was stood overnight; then 8 ml of acetone were added and the diluted sample was centrifuged. The supernatant was directly spotted on the chromatogram. Dansyl derivatives of peptides and proteins were prepared in a similar way. After the reaction was completed, peptides or proteins were hydrolyzed in 6 N hydrochloric acid within 12 h and the resulting hydrolysate spotted and chromatographed.

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Chromatography

Thin layer chromatography was performed on Silicagel G (RSCO) and aluminium oxide G (Merck) layers*.

For development, the following solvent systems were used:

(a) chloroform–ethanol–acetic acid (38:4:3),
(b) chloroform-benzyl alcohol-acetic acid (70:30:3),
(c) *n*-butanol–pyridine–acetic acid–water (30:20:6:24),
(d) benzene–pyridine–acetic acid (16:4:1),
(e) toluene–pyridine–ethylenechlorohydrin–0.8 *M* NH_4OH (100:30:60:60) upper layer,
(f) chloroform–*tert*.-butanol–acetic acid (6:3:1).

All these sytems were useful for chromatographic separation on Silicagel G layers. Systems b and c were used for chromatography on aluminium oxide layers.

Detection

The spots of amino acid derivatives have yellow fluorescence in U.V.- light, if the chromatography was performed on Silicagel G layer; in the case of aluminium oxide layers the resulting fluorescence is green. The spot of the hydrolyzed reagent has in both cases a blue fluorescence which does not disappear after spraying the chromatogram with 0.2 % solution of dichlorofluorescein in ethanol. After this spray, the spots of all the amino acids disappear and the spot of hydrolyzed reagent and the one due to the reaction product with ammonia, *i.e.* 5-dimethylaminonaphthalene-sulphonylamide, are visible in daylight as pink spots.

RESULTS

Maps of spots of the common amino acids are shown in Fig. 1. Each separation was performed in one run and did not usually last for longer than an hour. For a complete separation of most amino acids the two-dimensional arrangement was used. The order of use of the solvent systems in two-dimensional chromatography was found to be interchangeable, but the solvent from the first run must be completely removed before the second development. Solvent removal may be difficult especially when using Silicagel G as carrier and relatively non-volatile solvents like benzyl alcohol occur in the solvent system. It is therefore advantageous to perform the first run with a solvent system composed of volatile solvents and to use the less volatile solvents in the second solvent system. Even in two-dimensional separations (see maps on Figs. 2, 3, 4, etc.) some combinations of amino acids are scarcely separated. A survey of the usefulness of different solvent systems is given in Table I. Nevertheless, all the common amino acids could be identified in a complex mixture by two-dimensional chromatography. As in biological material one often also meets other amino-acid products and derivatives, we have summarized the chromatographic behavior on both Silicagel G and aluminium oxide layers of the more common amino compounds, including several simple peptides, in Table II.

* The Eastman Chromatogram sheets Type K301R were used as well as Silicagel layers. The R_F values are unchanged.

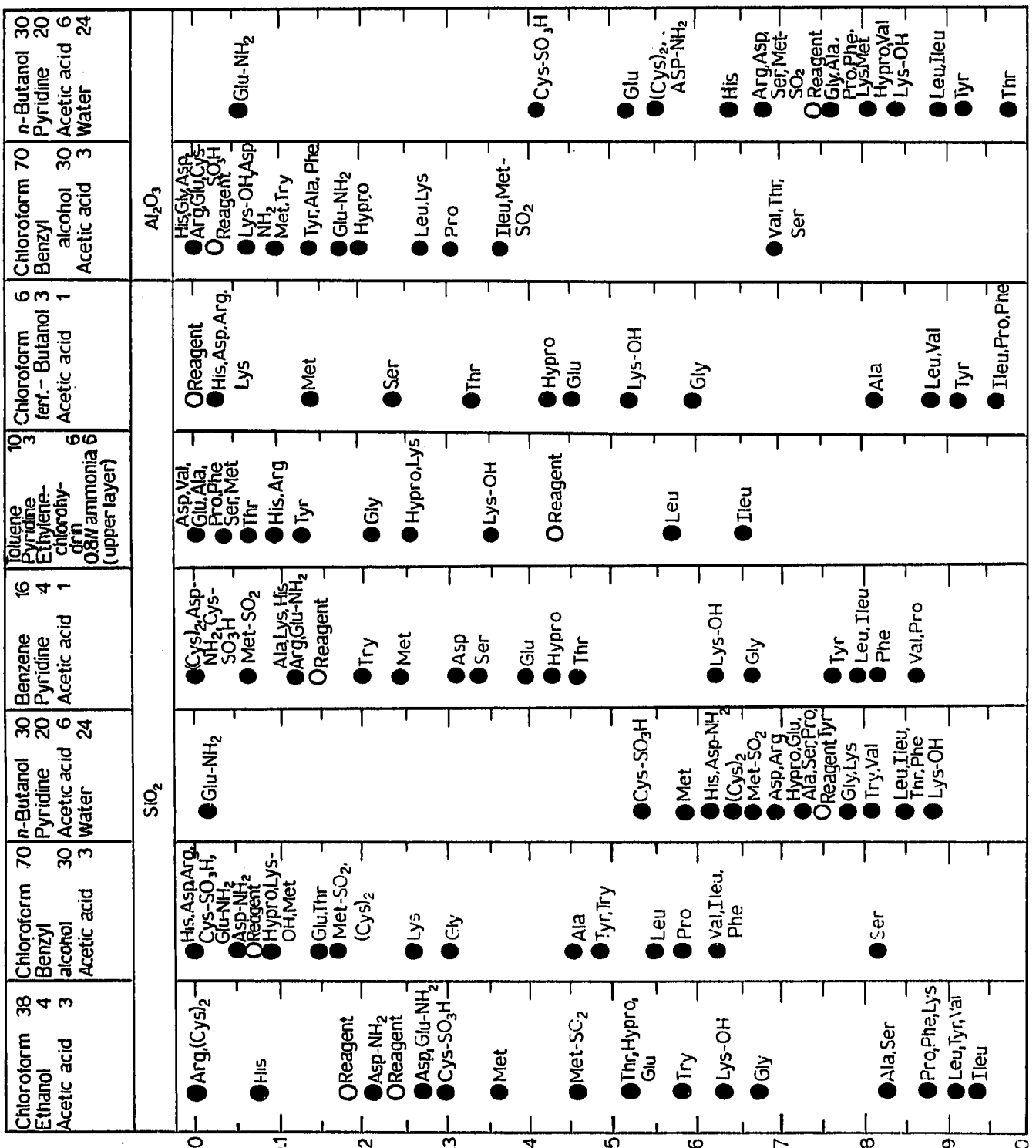

Fig. 1. R_F values of common amino acid derivatives in different solvent systems and on different carriers.

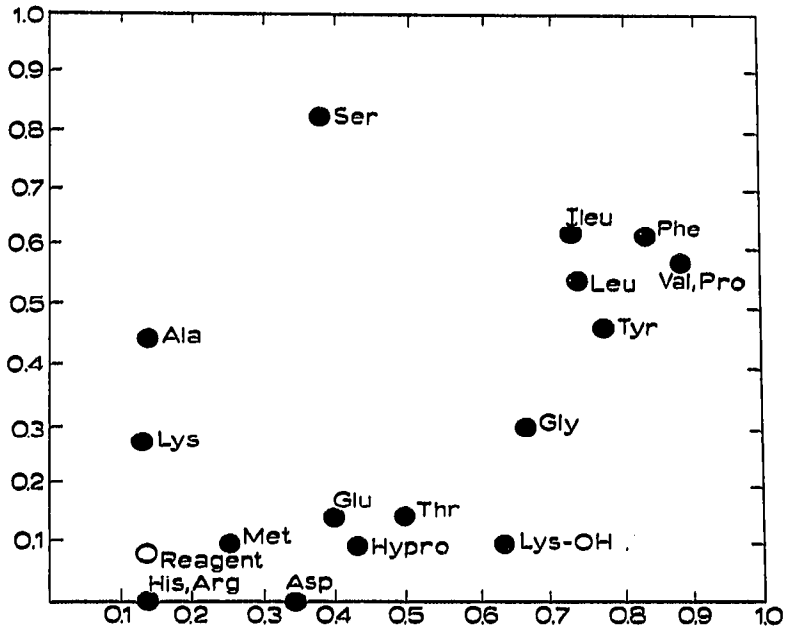

Fig. 2. Two-dimensional separation of common amino acid derivatives. Carrier: Silicagel G. 1st run: benzene–pyridine–acetic acid (16:4:1); 2nd run: chloroform–benzyl alcohol–acetic acid (70:30:3).

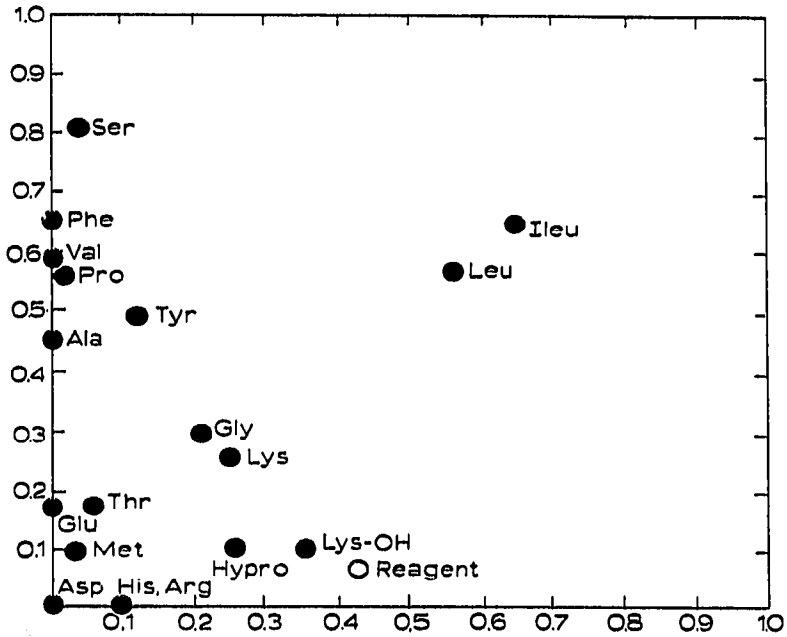

Fig. 3. Two-dimensional separation of common amino acid derivatives. Carrier: Silicagel G. 1st run: toluene–pyridine–ethylenechlorohydrin–0.8 *M* ammonia (100:30:60:60) (upper layer); 2nd run: chloroform–benzyl alcohol–acetic acid (70:30:3).

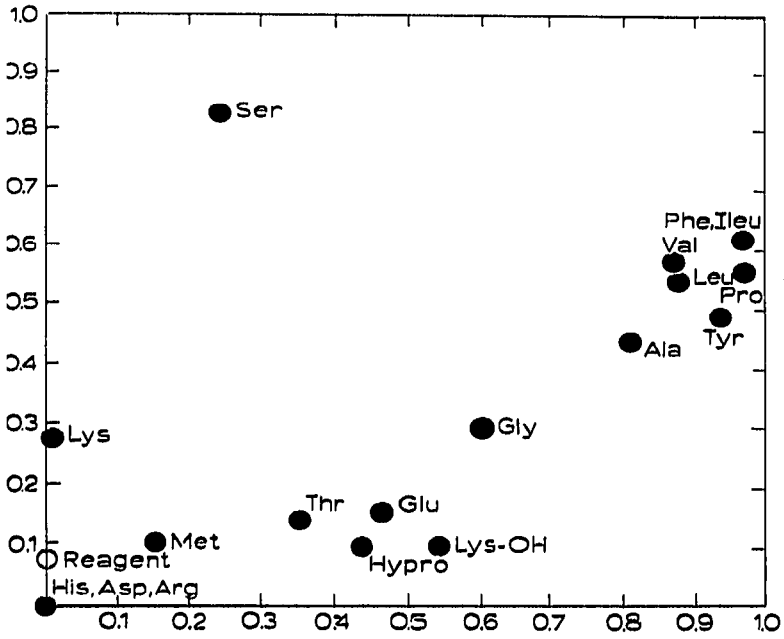

Fig. 4. Two-dimensional separation of common amino acid derivatives. Carrier: Silicagel G. 1st run: chloroform–*tert.*-butanol–acetic acid (6:3:1); 2nd run: chloroform–benzyl alcohol–acetic acid (70:30:3).

TABLE I

SUMMARY OF AMINO ACID DERIVATIVES NOT SEPARATED IN THE SOLVENT SYSTEMS DESCRIBED

Carrier	*Solvent system**		*Unseparated amino acid derivatives*
	1st run	*2nd run*	
Silicagel G	a	—	(Hypro-Glu) (Ala-Ser) (Pro-Phe-Lys-Leu-Tyr-Val-Ileu)
Silicagel G	b	—	(Ala-Tyr-Try) (Leu-Ileu-Val-Pro-Phe)
Silicagel G	c	—	Used only for two-dimensional chromatography in combination with (b) or (e)
Silicagel G	d	—	(Lys-His-Arg-Ala + reagent) (Asp-Ser) (Glu-Hypro-Thr) (Leu-Ileu)
Silicagel G	e	—	Used only for separation (Leu-Ileu)
Silicagel G	f	—	(His-Asp-Arg-Lys + reagent) (Leu-Val)
Aluminium oxide	b	—	Used only for separation (Val-Leu-Ileu)
Aluminium oxide	c	—	(Gly-Ala-Pro-Phe-Lys-Met-Reagent) (Arg-Asp-Ser) (Hypro-Val) (Leu-Ileu)
Silicagel G	d	b	(His-Arg) (Val-Pro)
Silicagel G	e	b	(Val-Pro-Phe) (Gly-Lys) (His-Arg)
Silicagel G	f	b	(His-Asp-Arg) (Val-Leu-Phe-Ileu-Pro-Tyr)
Silicagel G	c	b	(Asp-Arg) (Gly-Lys) (Ileu-Phe)
Silicagel G	a	b	(Val-Ileu-Phe-Leu)
Aluminium oxide	c	b	(Arg-Asp-His) (Pro-Lys) (Ala-Met-Phe)

* For key to solvent systems see text.

TABLE II

CHROMATOGRAPHIC BEHAVIOUR OF AMINO COMPOUNDS AND SIMPLE PEPTIDES

	R_F values							Colour of fluorescence	
	Solvent system a*		Solvent system b		Solvent system c		Solvent system d		
	SiO_2	Al_2O_3	SiO_2	Al_2O_3	SiO_2	Al_2O_3	SiO_2	SiO_2	Al_2O_3
ε-Amino-n-caproic acid	0.91	0.96	0.87	0.52	0.69	0.68	0.59	yellow	green
γ-Amino-n-butyric acid	0.87	0.93	0.65	0.48	0.69	0.68	0.48	orange	green
CBZ histidine	0.90	0.75	0.61	0.28	0.66	0.62	0.21	orange	orange
CBZ arginine	0.17	0.11	0	0.08	0.66	0.68	0.61	orange	green
Acetylhistidine	0.56	0.41	0.15	0.16	0.57	0.54	0.07	orange	orange
Carnosine	0.78	0.85	0.69	0.36	0.71	0.41	0.41	orange	orange
	0.65	0.66	0.23		0.54				
Glucosamine	0.22	0.57	0.07	0.16	0.74	0.78	0.09	yellow	green
Glycylphenylalanine	0.83	0.87	0.58	0.36	0.72	0.68	0.21	yellow	green
Alanyleucine	0.78	0.87	0.92	0.80	0.72	0.84	0.33	yellow	green
Glycylglycylalanine	0.30	0.21	0.07	0.08	0.63	0.62	0	yellow	green
Glycylglycine	0.44	0.16	0.15	0.04	0.57	0.59	0.07	yellow	green
Pentaglycine	0.0	0.04	0	0.80	0.53	0.46	0	yellow	green
Leucylglycylglycine	0.39	0.19	0.07	0.08	0.63	0.58	0.07	yellow	green
Citrulline	0.26	0.18	0.0	0.0	0.65	0.68	0	yellow	green
Ornithine	0.73	0.56	0.32	0.0	0.65	0.56	0.21	yellow	green
	0.07								
3-Iodotyrosine	0.84	0.35	0.41	0.08	0.74	0.68	0.21	yellow	green
Canavanine	0.08	0.18	0.0	0.11	0.65	0.68	0	orange	green
β-Aminobutyric acid	0.81	0.91	0.78	0.49	0.78	0.68	~ 0	yellow	orange
Mesolanthionine	0.34	0	0.07	0	0.65	0.36	0	orange	orange
2-Thiohistidine	0.46	0.57	0.09	0.15	0.71	0.55	0	orange	orange
Djenkolic acid	0.70	0	0.11	0.09	0.71	0.50	0	orange	green
1-Methylhistidine	0.87	0.52	0.0	0.08	0.55	0.46	0	yellow	green
β-Alanine	0.89	0.82	0.67	0.30	0.78	0.68	0.39	yellow	green
Norvaline	0.85	0.70	0.67	0.19	0.78	0.68	0.45	yellow	green
Sarcosine	0.00	0.57	0.52	0.17	0.74	0.68	0.30	yellow	green
meso-Homocystine	0.72	0.38	0.77	0.39	0.78	0.83	0.90	yellow	green
α-Amino-isobutyric acid	0.81	0.62	0.66	0.70	0.57	0.48	0.57	yellow	green
α-Amino-n-butyric acid	0.72	0.54	0.54	0.54	0.69	0.70	0.70	yellow	green
3,5-Diiodotyrosine	0.65	0.35	0.54	0.30	0.43	0.23	0.60	yellow	green
Reagent	0.06	0.04	0.04	0.05	0.73	0.68	start	blue-green	blue

* For key to solvent systems see text.

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SUMMARY

Dansyl derivatives of amino acids can be easily separated by thin layer chromatography. Dansyl derivatives of all naturally occurring amino acids can be identified in two-dimensional chromatographic separations. For their orientation in an unknown mixture the following combination of solvents was most useful on Silicagel G:

1st run: benzene–pyridine–acetic acid (16:4:1),
2nd run: chloroform–benzyl alcohol–acetic acid (70:30:3).

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