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THIN-LAYER CHROMATOGRAPHIC SEPARATION OF THE DIPHENYL-INDENONESULPHONYL DERIVATIVES* OF AMINO ACIDS

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SUMMARY

Several chromatographic systems are proposed that give the possibility of determining the Disyl derivatives of all the amino acids commonly found in proteins in amounts up to 10^{-6} μmoles using one- and two-dimensional thin-layer chromatography.

INTRODUCTION

In two earlier papers[1,2], we reported the preparation of the derivatives of the amino acids usually found in proteins with 2-*p*-chlorosulphophenyl-3-phenyl-indenone[3]. In those papers we also described the characterization of these derivatives by means of melting-point determinations, elemental analyses and infrared spectroscopy.

We found that on treating Disyl derivatives with sodium ethylate on Silica Gel G chromatographic plates, the diphenylisobenzofuran derivatives, which show intensive yellow–green fluorescence in UV light (365 nm), are obtained. Based on those results, a new method for the determination of N-terminal groups was proposed. This method gives the possibility of determining 10^{-6} μmoles of the corresponding amino acids[4]. In the same paper, two solvent systems for the thin-layer chromatographic separation of the Disyl derivatives were proposed. The aim of the investigations reported in this paper was to find appropriate solvent systems for the thin-layer chromatography of the Disyl derivatives of the common amino acids.

EXPERIMENTAL

Preparation of Disyl derivatives

As described earlier[4], DIS-amino acids are prepared by adding DIS-chloride dissolved in acetone (1 mg/ml) to an equal volume of a solution of an amino acid mixture (the concentration of each amino acid is 5×10^{-4} μmoles per millilitre of 0.1 *M* sodium bicarbonate solution). If any sulphochloride is precipitated, acetone is added until a completely clear solution is obtained. After leaving the solution for

* In our earlier papers[1–4], these derivatives were termed (diphenyl)sulphoindonyl deriv-

3 h in a closed tube at room temperature, it is evaporated at low pressure. The residue is dissolved in 1 ml of acetone–methanol (1:1) and 20 μl (10^{-5} μmoles of each DIS-amino acid) are applied to the chromatographic plate. Standard solutions of the pure Disyl derivatives with concentrations of 10^{-5} and 10^{-6} μmoles in 20 μl were also prepared.

Chromatography

Thin-layer chromatography was carried out on Silica Gel G (Merck). The plates were of dimensions 200 × 200 mm with a layer thickness of 0.5 mm (8 g of silica gel), and 100 × 140 mm with a layer thickness of 0.25 mm.

Because of the similarity in the structures and chemical reactivity of DIS-chloride and DNS-chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride), different solvent systems recommended by many workers[5-12] for the separation of DNS-amino acids were first tested. Good results were achieved with only two of the systems examined; the other systems listed are proposed by us.

The best separations were achieved with the following systems:

(A) acetone–isobutanol–25 % ammonia (90:70:5)
(B) chloroform–ethyl acetate–methanol–propionic acid (70:30:8:1)
(C_a) toluene–ethylene chlorohydrin–25 % ammonia (60:100:40) (ref. 10)
(C_b) toluene–ethylene chlorohydrin–25 % ammonia (60:80:20)
(D) chloroform–ethyl acetate–methanol–acetic acid (70:30:8:5)
(E) chloroform–ethyl acetate–methanol–propionic acid (70:30:5:0.2)
(F) chloroform–ethyl acetate–acetic acid (50:66:2.5)
(G) *n*-butanol–toluene–25 % ammonia (80:10:10) (ref. 11)
(H) chloroform–ethyl acetate–methanol–propionic acid (70:30:8:0.5).

After chromatography, the plates were dried at 105° for 5 min, cooled to room temperature and a solution of sodium ethylate (5 g of Na per 100 ml of 96 % ethanol) was poured over the plates. The plates were immediately observed under a UV lamp (365 nm). The spots of the Disyl derivatives of the amino acids, as well as of the DIS-acid, the DIS-amide and the DIS-chloride, show yellow–green fluorescence.

RESULTS

The spots of the Disyl derivatives of the common amino acids obtained in different solvent systems are shown in Fig. 1.

In order to achieve better separation and to obtain circular spots in solvent systems A, B and E, the chromatograms were developed twice. The development of each chromatogram did not exceed half an hour. The solvent system was used once. In order to achieve reproducibility in the positions of the spots, it was necessary to saturate the chamber with the same solvent system for 1 h for plates with dimensions of 200 × 200 mm and for about 30 min for plates with dimensions of 100 × 140 mm.

Good separation was achieved by means of two-dimensional chromatograms (Figs. 2–5). For the first direction in the two-dimensional chromatograms, the

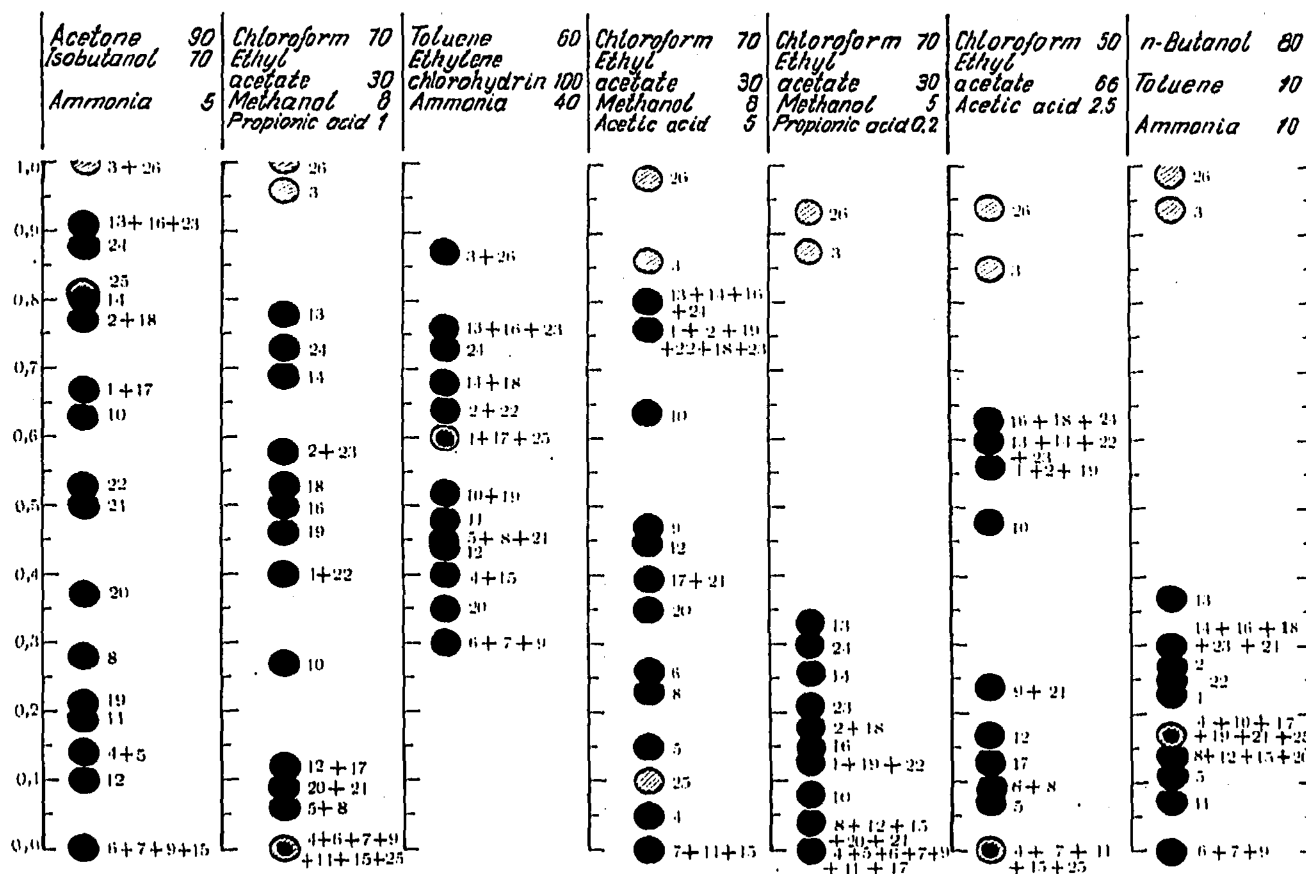


Fig. 1. R_F values of the Disyl derivatives of common amino acids in the solvent systems used 1 = DIS-α-alanine; 2 = DIS-α-aminobutyric acid; 3 = DIS-amide; 4 = DIS-arginine; 5 = DIS-asparagine; 6 = DIS-aspartic acid; 7 = DIS-cysteic acid; 8 = DIS-glutamine; 9 = DIS-glutamic acid; 10 = DIS-glycine; 11 = di-DIS-histidine; 12 = DIS-hydroxyproline; 13 = DIS-isoleucine; 14 = DIS-leucine; 15 = ε-DIS-lysine; 16 = di-DIS-lysine; 17 = DIS-methionine sulphone; 18 = DIS-phenylalanine; 19 = DIS-proline; 20 = DIS-serine; 21 = DIS-threonine; 22 = DIS-tryptophan; 23 = di-DIS-tyrosine; 24 = DIS-valine; 25 = DIS-acid; 26 = DIS-chloride.

ROSMUS[7] was used in all instances. Before developing the chromatogram in the second direction, the plates were dried and activated at 105° for 20 min.

It can be seen in Figs. 2 and 3 that the spots of the Disyl derivatives of isoleucine (13) and valine (24) are close together. The derivatives of α-aminobutyric acid(2) and phenylalanine (18) are also close together, as are di-DIS-lysine(16) and di-DIS-tyrosine (23). These three pairs are very well separated on small plates in system H, chloroform–ethyl acetate–methanol–propionic acid (70:30:8:0.5), by both single and double development of the chromatograms (Figs. 6a and 6b). The best separation of the pair di-DIS-lysine–di-DIS-tyrosine is achieved by using system C_b up to a distance 5 cm from the starting line and then system E in the same direction (Fig. 6c). Well formed circular spots were obtained in this case.

The Disyl derivatives of aspartic acid (6), glutamic acid (9), cysteic acid (7)

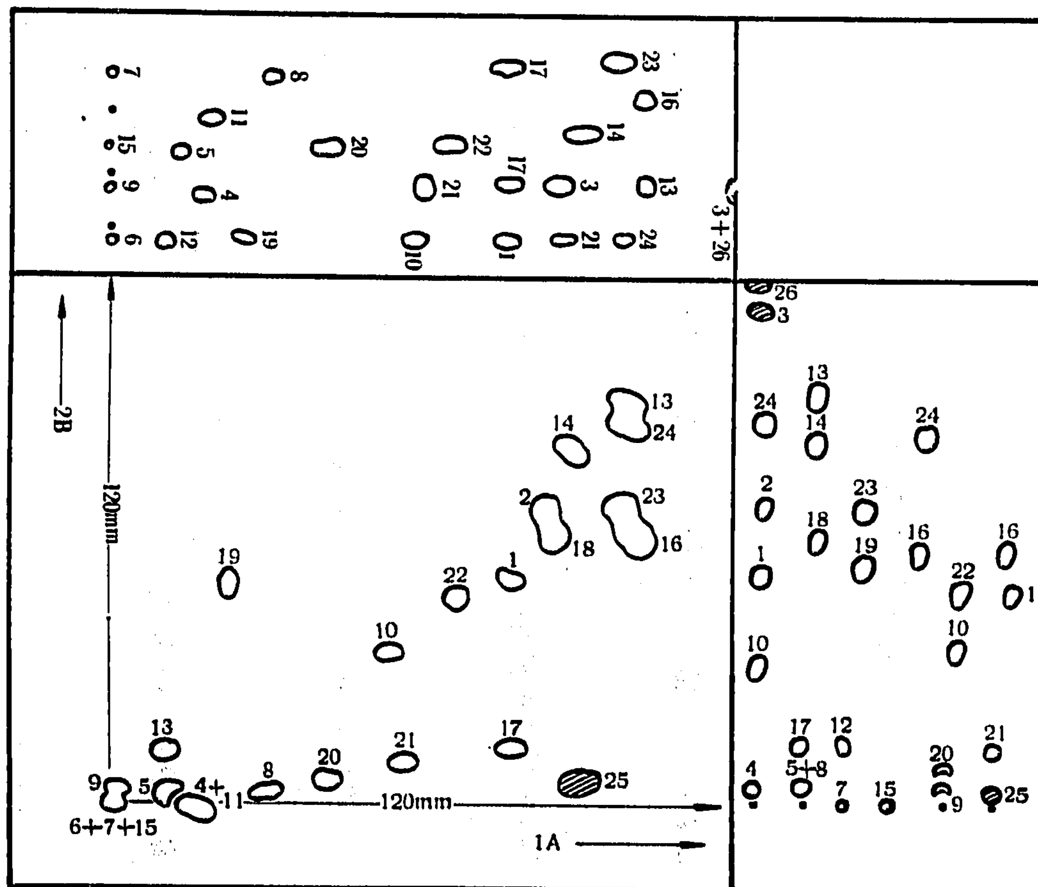
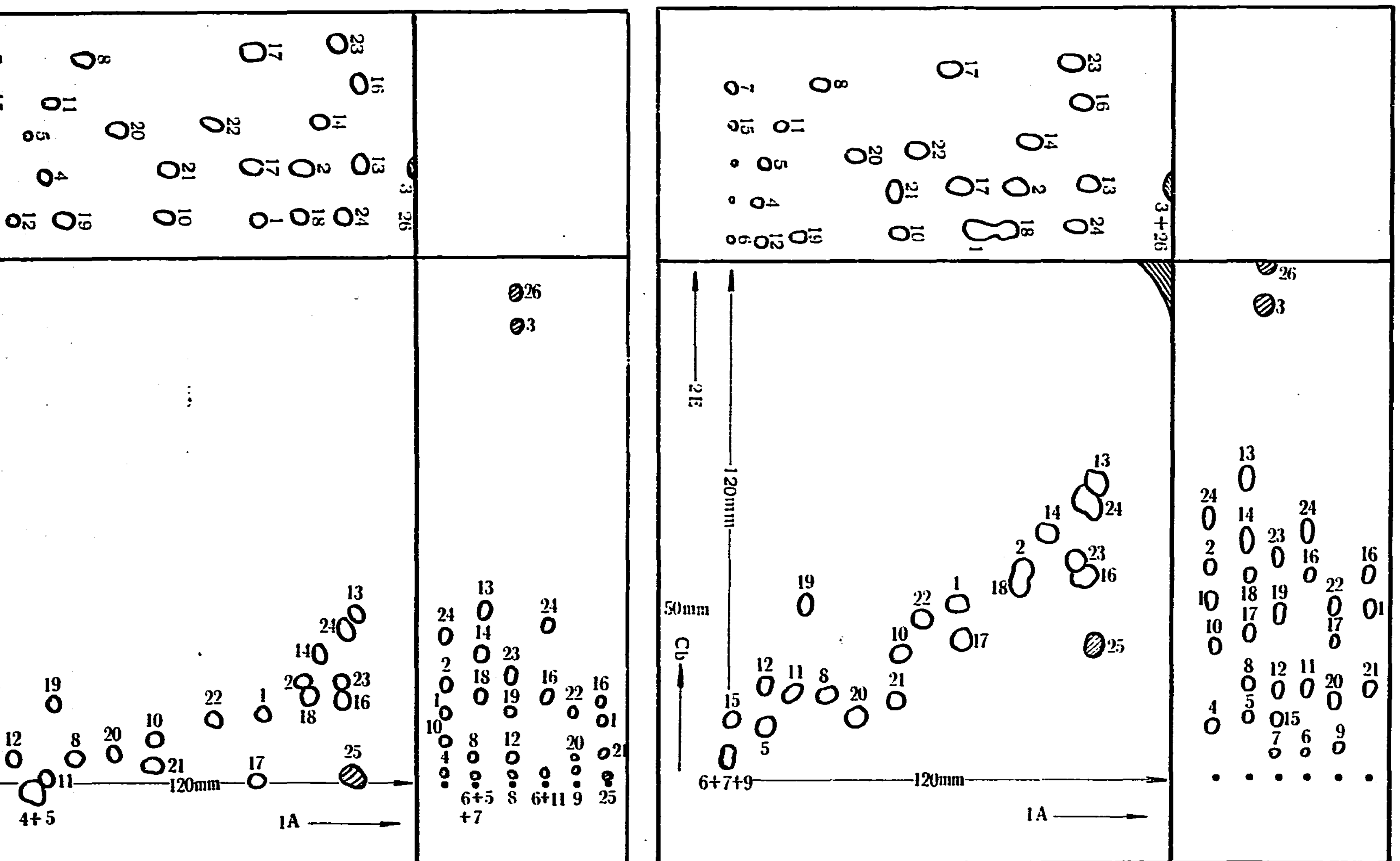


Fig. 2 Two-dimensional separation of common amino acid derivatives. Carrier: Silica Gel G (0.5 mm layer thickness). 1st run: system A, acetone–isobutanol–25% ammonia (90:70:5), developed twice. 2nd run: system B, chloroform–ethyl acetate–methanol–propionic acid (70:30:8:1), developed twice. Numbers of the spots as in Fig. 1.

from ε-DIS-lysine (which is usually present because of the lysine residues in the protein molecule) is achieved by the two-dimensional chromatogram shown on Fig. 4. It can be seen in this instance that in the second direction the chromatogram is developed to 5 cm in system C_b and after that it is developed twice in system E.

The separation of ε-DIS-lysine from the Disyl derivatives of aspartic, cysteic and glutamic acids can also be achieved by removing the silica gel layer of the spot from the starting line before pouring sodium ethylate over it, and after an elution with acetone a second development is carried out in system C_b. In this system, -DIS-lysine moves faster than the other derivatives mentioned.

A complete, reliable and comparatively rapid separation of the Disyl derivatives of aspartic, cysteic, and glutamic acids and of ε-DIS-lysine from the remaining, more slowly moving DIS-amino acids, such as arginine, asparagine, glutamine, histidine, hydroxyproline, serine, threonine and methionine sulphone, is accomplished by means of two-dimensional chromatography in the systems C_a–D (Fig. 5). This combination ensures very good separation with both large and small plates. The development in the two directions is a single development and can be carried out



Two-dimensional separation of common amino acid derivatives. Carrier: Silica Gel G (0.5 mm layer thickness). 1st run: system A, acetone–
.nol–25% ammonia (90:70:5), developed twice. 2nd run: system E, chloroform–ethyl acetate–methanol–propionic acid (70:30:5:0.2),
ıed twice. Numbers of the spots as in Fig. 1.

Two-dimensional separation of common amino acid derivatives. Carrier: Silica Gel G (0.5 mm layer thickness). 1st run: system A,
–isobutanol–25% ammonia (90:70:5) developed twice. 2nd run: system C_b, toluene–ethylene chlorohydrin–25% ammonia (60:80:20), up
ı from the starting line, and then system E, chloroform–ethyl acetate–methanol–propionic acid (70:30:5:0.2), developed twice. Numbers of the
s in Fig. 1.

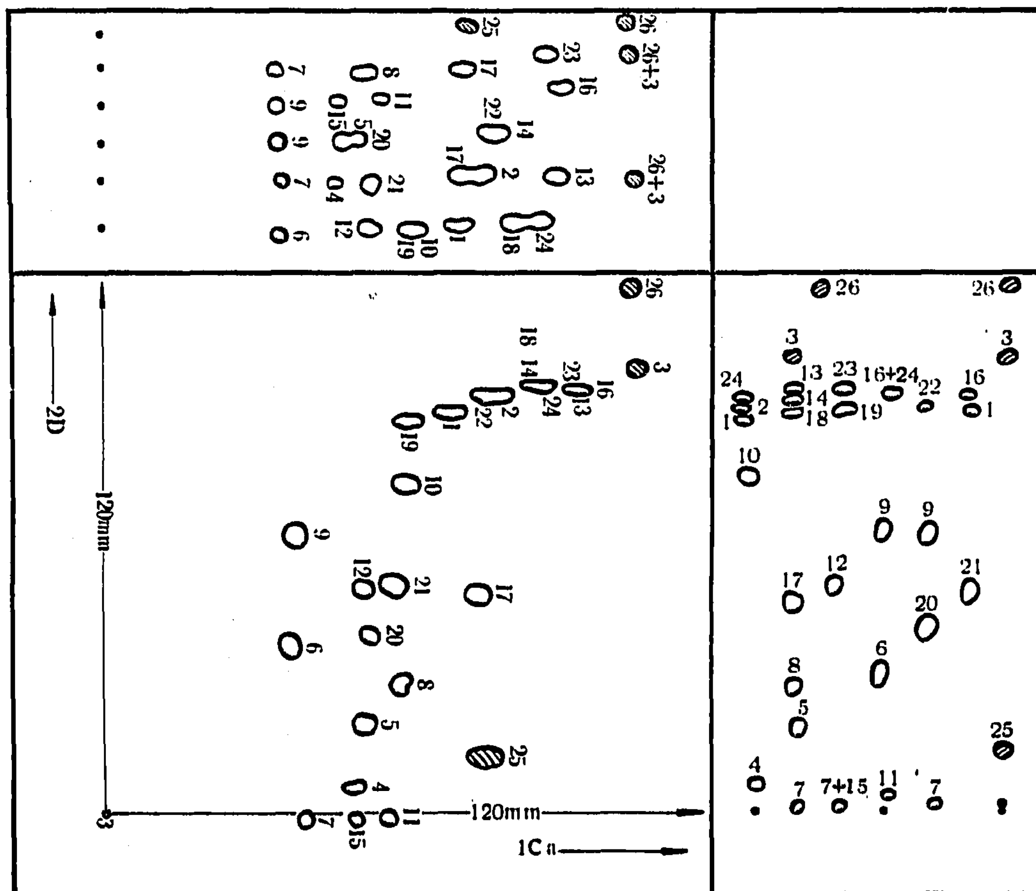


Fig. 5. Two-dimensional separation of common amino acid derivatives. Carrier: Silica Gel G (0.5 mm layer thickness). 1st run: system C_a, toluene–ethylene chlorohydrin–25% ammonia (60:100:40), developed once. 2nd run: system D, chloroform–ethyl acetate–methanol–acetic acid (70:30:8:5), developed once. Numbers of the spots as in Fig. 1.

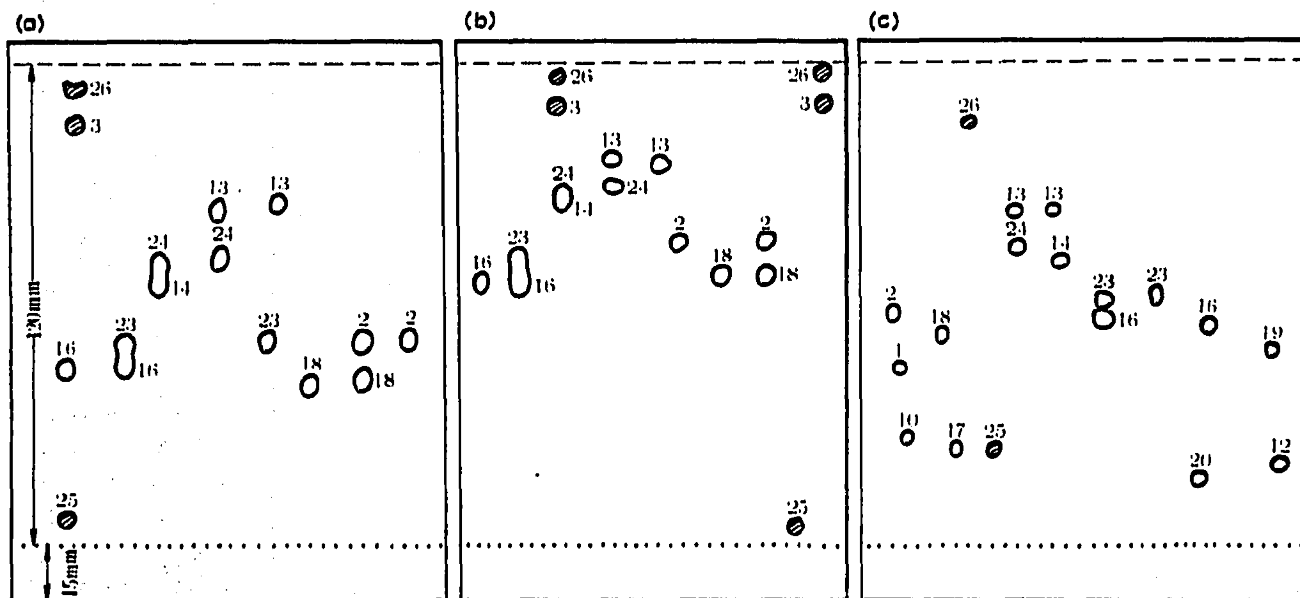


Fig. 6. (a) One-dimensional separation of DIS-isoleucine, DIS-valine, di-DIS-tyrosine, di-DIS-lysine, DIS-α-aminobutyric acid and DIS-phenylalanine in system H, chloroform–ethyl acetate–methanol–propionic acid (70:30:8:0.5), developed once. Carrier: Silica Gel G (0.25 mm layer thickness). (b) One-dimensional separation of DIS-isoleucine, DIS-valine, di-DIS-tyrosine, di-

It can be seen from all the two-dimensional chromatograms that the spots of the DIS-acid, the DIS-amide and the DIS-chloride do not hinder the separation because they are situated outside the region of the amino acids.

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