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Identification of Dansyl amino acids using a single solvent and a layer composed of three sorbents

Since the fundamental paper of GRAY AND HARTLEY[1] concerning chromatographic separations of Dansyl (dimethylaminonaphthalenesulphonyl) amino acids considerable advances have been made. Since the separations in a flat bed arrangement first described by DEYL AND ROSMUS[2], and SEILER AND WIECHMANN[3] a number of papers on this topic have appeared, and were recently reviewed[4]. Though a method for the identification of Dansyl amino acids has been published[5] with only a single chromatographic run, the chance of possible errors in identification of an unknown Dansyl derivative is still high. The recent suggestion of LEDERER[6] that layers with different sorbent paths should be used with a single solvent system seemed quite promising and resulted in the data presented here. The spreading device has been divided into three equal parts and silica gel (Silica Gel Woelm 210, neutral, 67 g per 100 ml of water), aluminium oxide (Aluminium Oxide G Woelm 113, 67 g per 100 g of water) and polyamide (Polyamide Woelm 410, 11 g per 100 ml of methanol) were used and spread in the individual parts of the device. The layers of silica gel and alumina were spread together in the first run of the spreader. The plates were then heated to 105° for 3 h and the second movement of the spreader was used to prepare the layer of polyamide. After being spread with polyamide, the layer was allowed to stand at room temperature for at least 15 min and finally dried again at 60° in a ventilated oven. The layers were approximately 250 μ thick and were stored in a desiccator over silica gel. Cellulose was not used for layer preparation since Dansyl derivatives tend to stick to the starting line and tail badly in all kinds of solvent systems.

The preparation of the Dansyl amino acids was done by the routine technique as described many times previously[1-6] (in sodium bicarbonate media at 37° for 2 h with an ethyl acetate extraction of the excess reagent) and the results were visualized in UV light. Out of the solvent systems tested, the following two proved suitable for the identification procedure (A) chloroform–benzyl alcohol–acetic acid (70:30:3) (Fig. 1a) and (B) *n*-butanol–pyridine–acetic acid–water (30:20:6:24) (Fig. 1b). As shown in the figures both systems allow a complete identification of eighteen common acids in the form of their Dansyl derivatives. The usefulness of this technique becomes clear from some examples: In the solvent system based on chloroform the application of the third sorbent (polyamide) permits the identification of Asp, Arg and His in the presence of each other, a combination which is otherwise very hard to separate. Val and Ile is another combination which, without using the third sorbent (aluminium oxide), cannot be distinguished. In the aqueous system (see Fig. 1b) similar combinations are Asp-Lys-Arg-Pro or Gly-Ala. In order to facilitate identification a simple system for describing the three spots belonging to a particular amino acid derivative has been used: Assuming that the accuracy of R_F estimation lies within the range of 0.05 R_F, each spot on a particular sorbent is characterized by the nearest R_F value ending in 0 or 5 (0.05, 0.10, 0.15 etc.). This is the origin of the

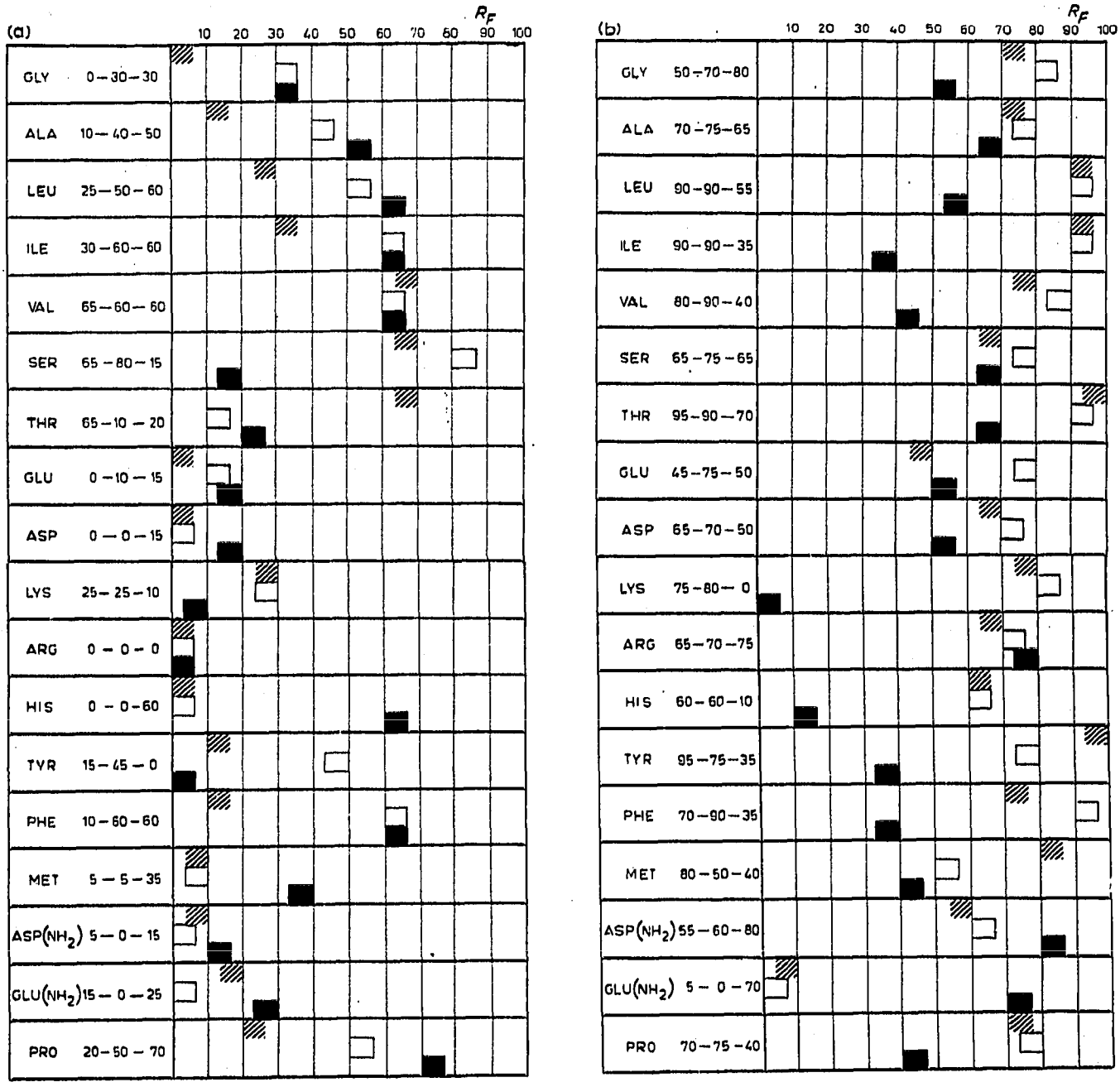

Fig. 1. Identification scheme for eighteen common amino acids in the form of their Dansyl derivatives. Solvents: (a) chloroform–benzyl alcohol–acetic acid (70:30:3) and (b) *n*-butanol–pyridine–acetic acid–water (30:20:6:24). Sorbents used: aluminium oxide (hatched squares), silica gel (open squares), and polyamide (filled squares). Numbers in the left column of the figure correspond to the R_F values of the Dansyl amino acid derivatives on the same series of sorbent in the same order.

numbers given in the left-hand column of both figures, and these help to make the system quite handy without using a lot of layers for record purposes.

It seems likely that similar systems consisting of several sorbents and one

rather universal solvent system may be very useful for the separation and identification of complex mixtures of compounds of similar chemical nature.

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ZDENĚK DEYL
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