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Differentiation of dimethylaminonaphthalenesulphonic derivatives (DNS) of valine, leucine and isoleucine by chromatography on a thin layer of silica gel

Lately, dimethylaminonaphthalenesulphonic derivatives (DNS) of amino acids according to GRAY AND HARTLEY[1] have been used more and more for the determination of N-terminal amino acids in studies on protein structure. In this method, the identification of the DNS-amino acids by means of thin-layer chromatography proved to be very advantageous, the strong fluorescence of the DNS-amino acids in U.V. light (at 365 mμ)[2] being used for identification. The sensitivity of this method is greater by 2–3 orders than that of the more usual dinitrophenylation method[3].

The three solvent systems for thin-layer chromatography[2] hitherto described differentiate almost all of the DNS-amino acids. Only the differentiation of DNS-

leucine, -isoleucine, -valine and -phenylalanine is difficult, since their spots are situated very near to one another and they often fuse together.

In this paper solvent systems are presented allowing for the unambiguous separation of DNS-leucine, DNS-isoleucine, and DNS-valine.

Experimental

Apparatus. The standard technique of thin-layer chromatography by the ascending method as described by STAHL[4] was used.

Material and reagents. Kieselgel G (Merck) as well as Silicagel CH (Spolana-Velvary), prepared and classified by flotation as described by PITRA AND ŠTĚRBA[5,6], were used as carriers for chromatography. This silica gel had a particle size of 5–15 μ and it was mixed with plaster in a ratio of 25:5 prior to use.

Standard samples of DNS-amino acids were prepared from chromatographically pure amino acid preparations according to BOULTON AND BUSH[7]. The solvent systems were prepared from distilled solvents.

The following solvent systems were used:

(a) Petroleum ether–*tert.*-butanol–glacial acetic acid (75:15:15, v/v) (system Pe–t-B).

(b) Petroleum ether–2,4,6-collidine–methyl ethyl ketone–glacial acetic acid (75:5:24:3, v/v) (system Pe–Coll).

Results

The system Pe–t-B separates DNS-valine from a mixture of DNS-isoleucine and DNS-leucine in one-dimensional chromatography in 60–70 min, the solvent front migrating to a distance of 16 cm from the starting point. $DNSO_3H$ stays on the starting point. R_F values of DNS-amino acids are presented in Table I.

TABLE I

R_F VALUES OF DNS-AMINO ACIDS DURING CHROMATOGRAPHY ON A THIN LAYER OF SILICAGEL CH OR KIESELGEL G

DNS-amino acid	*Solvent system**	
	Pe–t-B	*Pe–Coll*
-Val	0.19	0.24
-Leu	0.26	0.24
-Ile	0.26	0.27
-Phe	0.16	0.13
Di-DNS-Lys	0.36	0.58
-Others	<0.10	<0.13
$-NH_2$	0.09	0.39
-OH	0.00	—

* See text for composition.

The system Pe–Coll separates DNS-isoleucine from a mixture of DNS-leucine and DNS-valine in one-dimensional chromatography in 75–85 min, the solvent front migrating to a distance of 16 cm from the starting point. $DNSO_3H$ was not detected in this system and the spot of $DNS\text{-}NH_2$ is very weak.

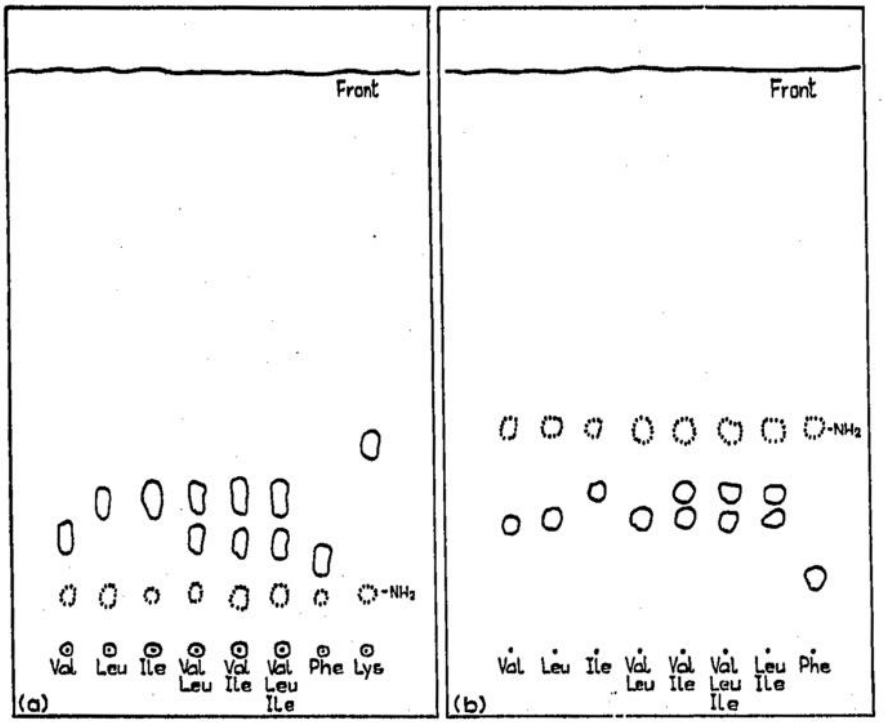

Fig. 1. Chromatographic separation of DNS-amino acids on a thin layer of Silicagel CH or Kieselgel G. Systems: (a) Petroleum ether–*tert.*-butanol–glacial acetic acid (75:15:15, v/v); (b) petroleum ether–collidine–methyl ethyl ketone–glacial acetic acid (75:5:24:3, v/v).

The relatively low R_F values of the separated components permit the utilization of a flow procedure for both systems described above, with flow periods of 120–140 min, giving a better separation of the spots, but the ascending arrangement is quite sufficient for the differentiation. A two-dimensional arrangement of the two systems did not result in a better separation than the combination of both systems in subsequent one-dimensional runs.

The N-terminal amino acids of the peptides 15 E_4 (ref. 8): Val. (Glu_3,Gly,Asn) and F 51 G (ref. 9): Leu·(Ile,Lys,Leu) isolated from bovine DIP-trypsin were determined in this way. The full details are to be published later[8,9].

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