ONE-DIMENSIONAL CHROMATOGRAPHY OF DNS-AMINO ACIDS

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Edman's method in combination with dansylation is frequently employed for determining the amino-acid sequence of peptides, using two-dimensional chromatography to identify the dansyl[DNS]-amino acids [1].

However, not infrequently the one-dimensional method of separation in several solvent systems proves to be more reliable.

Here we propose a scheme for the successive use of five systems of organic solvents for the separation of 17 DNS-amino acids: 1) chloroform–tert-amyl alcohol–acetic acid (70 : 30 : 0.5) [2]; 2) chloroform–ethanol–acetic acid (45 : 4 : 0.75); 3) acetone–isopropanol–concentrated ammonia (44 : 36 : 5.6) [3]; 4) chloroform–ethanol–acetic acid (38 : 4 : 3) [4]; and 5) chloroform–isoamyl alcohol–acetic acid (45 : 5 : 2.5). Systems 2 and 5 have not been described before. Chromatography was performed on plates with dimensions of 10 × 10 cm with KSK-3 silica gel in a fixed layer (10 g of silica gel, 1 g of gypsum, 70 ml of water, 22 plates, Stahl apparatus for depositing the sorbent). Of the organic solvents, only the chloroform was additionally purified, and then 1.5% of ethanol was added to it. Chromatography was performed with the freshly prepared solvent systems (this applies particularly to systems 1 and 4). The plates were dried in a current of hot air for 10-15 min. After chromatography in systems 1 and 2, the hydrophilic amino acids, the dicarboxylic acids, and the dansyl-sulfonic acids remained at the start. All the hydrophobic amino acids were separated with the exception of alanine and tyrosine. If there was a spot corresponding to alanine or tyrosine, it was chromatographed separately in system 5. After systems 1 and 2, chromatography was performed in system 3, in which all the hydrophobic amino acids migrated upwards leaving a position free for the separation of the hydrophilic amino acids – serine and threonine – and the dicarboxylic acids remained at the start. Subsequent chromatography in system 4 led to the separation of aspartic and glutamic acids.

TABLE 1

DNS-amino acids	R_f in solvent systems			
	1, then 2	3	4	5
Dansylamide	0,91	—	—	—
Isoleucine	0,72	—	—	—
Valine	0,66	—	—	—
Leucine	0,59	—	—	—
Proline	0,47	0,54	—	—
Phenylalanine	0,43	—	—	0,40
Alanine	0,36	—	—	0,26
Tyrosine	0,36	—	—	0,34
Tryptophan	0,29	—	—	—
Glycine	0,16	—	—	0,23
Dansylsulfonic acid	0	0,54	—	—
Threonine	0	0,44	—	—
Serine	0	0,38	—	—
Lysine	0,43	—	—	0,22
Histidine	0,16	—	—	0,16
Arginine	0	—	—	—
Methionine	0,36	—	—	0,12

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So far as concerns the basic amino acids and methionine, on chromatography in systems 1 and 2 the arginine remained at the start, the R_f value of histidine coincided with that of glycine, that of lysine with phenylalanine, and that of methionine with alanine. These pairs were separated on additional chromatography in system 5. The results of the chromatographic separation of the DNS-amino acids in the five solvent systems are given in Table 1.

LITERATURE CITED

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