Chromatographic separation of 2,4-dinitro-5-aminophenyl derivatives of amino acids

Numerous compounds have been proposed for N-terminal determination and the chromatographic behaviour of the resulting derivatives has been described (for a review see MELOUN[1]). Nevertheless only a few derivatives are commonly used for the N-terminal estimation. Besides 2,4-dinitrofluorobenzene and 1-dimethylamino-naphthalene-sulphochloride, 2,4-dinitro-5-fluoroaniline offers some additional possibilities in N-terminal analysis of proteins and peptides. The sensitivity of this reagent is roughly the same as that of 2,4-dinitrofluorobenzene but the corresponding derivatives (2,4-dinitro-5-aminophenyl amino acids) are formed more readily and according to our experience the chromatographic separation of these derivatives is usually of a very good quality, does not suffer from tailing and high affinity to the carrier. However, apart from the original report[2] there are no data available dealing with any kind of chromatographic or electrophoretic separation of these compounds. The present paper is concerned with this problem.

Experimental

Preparation of the amino acid derivatives

1 μmole of the corresponding amino acid (or an equal amount of a peptide) was dissolved in 1 ml of double-distilled water and 20 mg of solid $NaHCO_3$ were added. To this mixture 2 ml of a solution of 2,4-dinitro-5-fluoroaniline (0.75 g of the reagent in 100 ml of absolute ethyl alcohol) were added. The reaction mixture was then vigorously stirred for 2 h. The dinitroaminophenyl peptides were hydrolysed in 6 *N* hydrochloric acid for 24 h.

Chromatographic separation

For separation, two kinds of chromatographic techniques were applied: thin-layer chromatography using Eastman Kodak Silica Gel sheets type K 301 R without any pretreatment and paper chromatography on Whatman No. 3 MM paper (other kinds of chromatographic paper are applicable equally well). The following solvent systems appeared suitable for the separation:

(*a*) *For thin-layer chromatography*

Chloroform–ethyl alcohol–acetic acid (8:4:1)
Chloroform–*n*-butyl alcohol–acetic acid (8:4:1)
Chloroform–*n*-amyl alcohol–acetic acid (8:4:1)
Chloroform–ethyl alcohol–acetic acid (10:2:1)
Benzene–*n*-amyl alcohol–acetic acid (8:5:1)
1.5 *M* phosphate buffer, pH 6.0.

(*b*) *For paper chromatography*

1.5 *M* phosphate buffer, pH 6.0
1 % solution of pyridine in water
5 % solution of acetic acid in water.

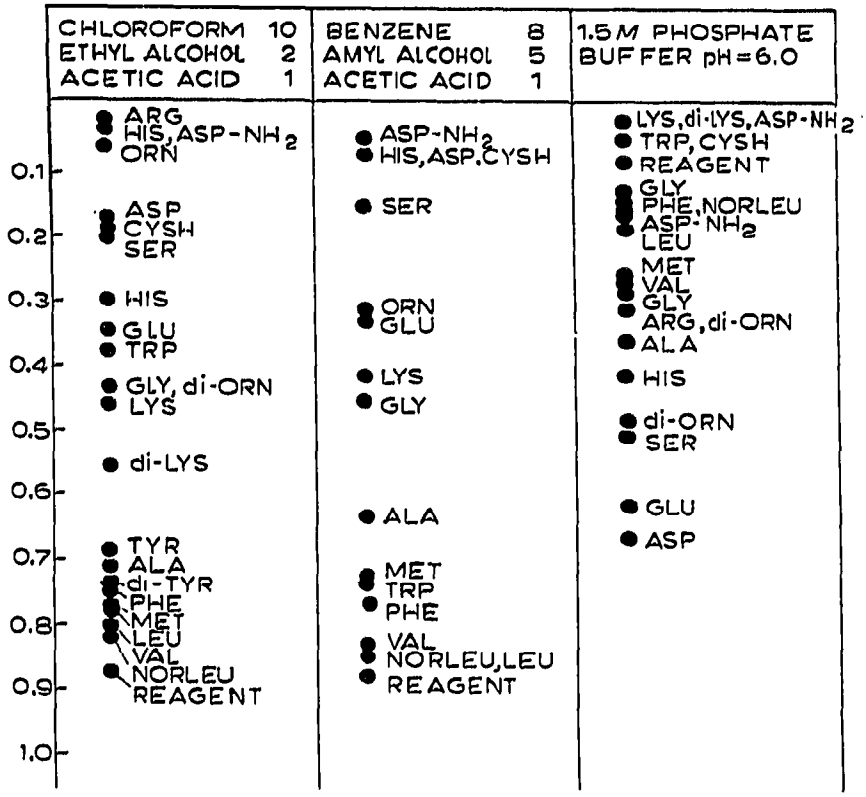

Fig. 1. Map of the 2,4-dinitro-5-aminophenyl derivatives of amino acids in different solvent systems. Separation on Eastman Kodak Silica Gel sheets.

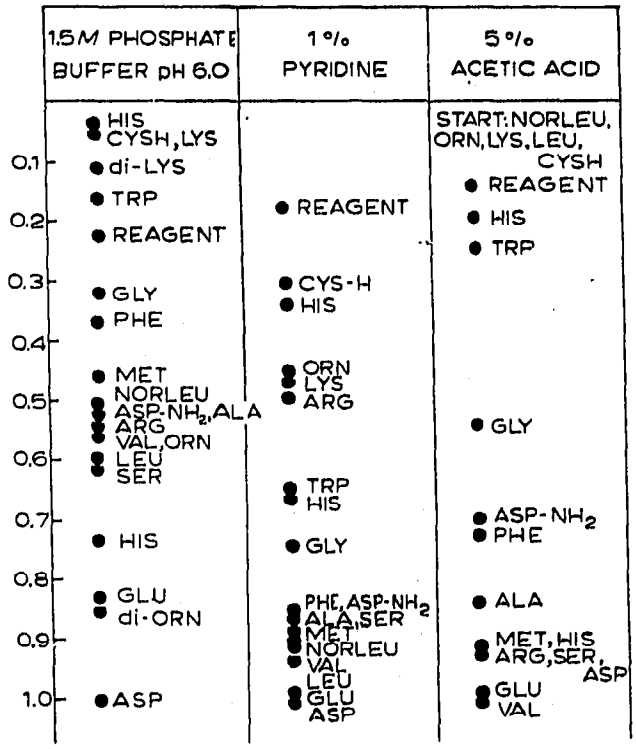

Fig. 2. Scheme of the chromatographic separation of 2,4-dinitro-5-aminophenyl derivatives of amino acids on paper Whatman No. 3 MM.

TABLE I

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

Carrier	*Solvent system*	*Unseparated combinations of amino acid derivatives*
Thin layer of silica gel	Chloroform–ethyl alcohol–acetic acid (8:4:1)	His–Arg; Val–Norleu; Ala–Trp; Met–Phe
	Chloroform–*n*-butyl alcohol–acetic acid (8:4:1)	Ser–His–Asp–CysH
	Chloroform–*n*-amyl alcohol–acetic acid (8:4:1)	Asp·NH_2–His; Lys–Glu; Met–Trp–Phe; Leu–Norleu
	Chloroform–ethyl alcohol–acetic acid (10:2:1)	Arg–His–Orn; Asp–CysH–Ser; Met–Leu–Val–Norleu
	Benzene–*n*-amyl alcohol–acetic acid (8:5:1)	Asp·NH_2–His–Asp–CysH; Norleu–Leu; Orn–Glu; Met–Trp–Phe
	1,5 *M* phosphate buffer, pH 6.0	Phe–Norleu; CysH–Trp–Lys–$AspNH_2$; Met–Val–Gly; Arg–Di–Orn
Whatman No. 3 MM paper	1.5 *M* phosphate buffer, pH 6.0	Norleu–Asp·NH_2–Ala–Arg–Val–di–Orn; His–CysH–Lys
	1 % pyridine	Phe–Asp·NH_2–Ala–Ser–Met–Norleu; Val–Leu; Glu–Asp
	5 % acetic acid	Norleu–Orn–Lys–Leu–CysH; Arg–Ser–Asp–Met–His

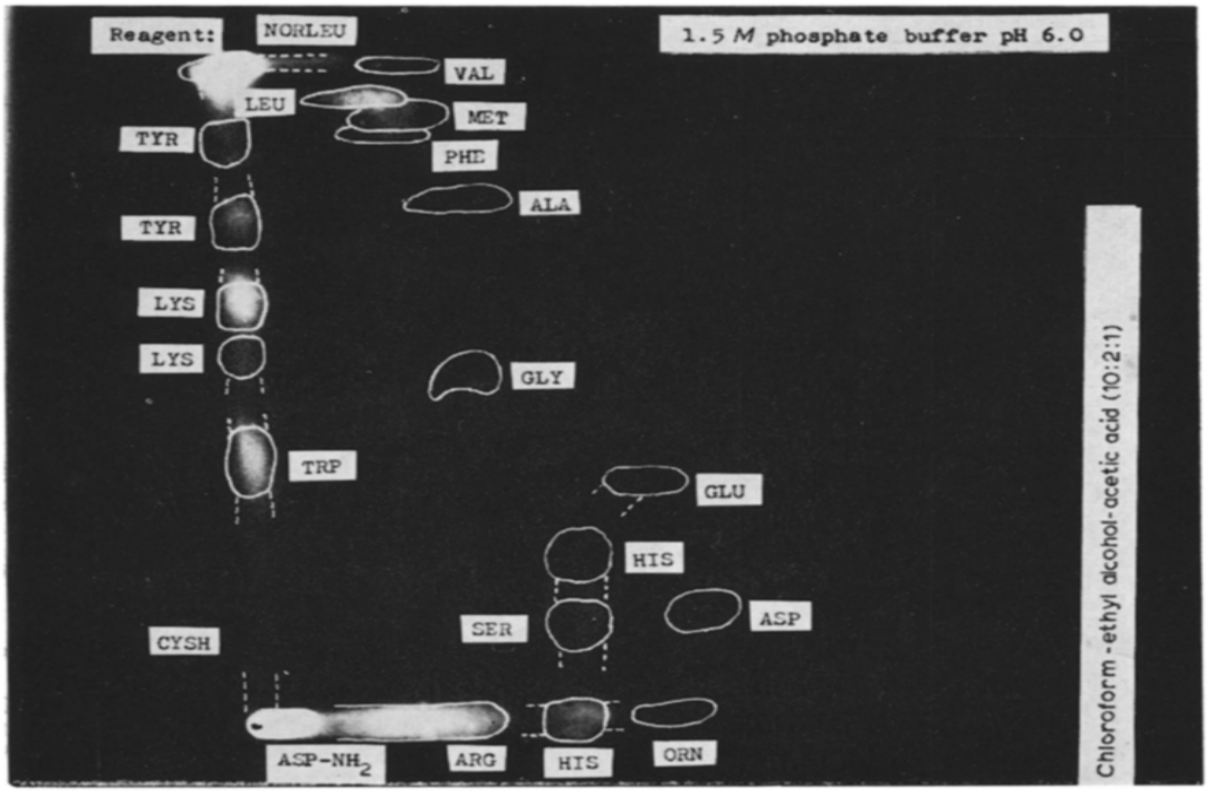

Fig. 3. An example of two-dimensional separation of 2,4-dinitro-5-aminophenyl derivatives of amino acids. Chromatography on Eastman Kodak Silica Gel sheets.

Results and discussion

The survey of results is presented in Figs. 1 and 2. Generally speaking the best results can be obtained on thin layers of silica using the system chloroform–*n*-amyl alcohol–acetic acid (8:4:1), while 1 % pyridine and 1.5 *M* phosphate buffer pH 6 are equally suitable for paper chromatography. Some of the series of the common amino acids remain unseparated in each solvent system. This is summarized in Table I. Two-dimensional chromatography, however, results in a complete separation of all the usual amino acids (see Figs. 3 and 4). For the two-dimensional separation on thin layers of silica the best way is to use any one of the alcoholic solvent systems in the first run; results of a separation in chloroform–ethyl alcohol–acetic acid (10:2:1) (first run) and phosphate buffer (second run) are presented in Fig. 3.

In the case of paper chromatography the first separation can be done in 1 % pyridine or in distilled water followed by the second run in pH 6.0 phosphate buffer (Fig. 4). Reversal of the solvents in both thin-layer and paper chromatography is not recommended as the presence of salts in the paper or in the silica layer always interferes with the second run.

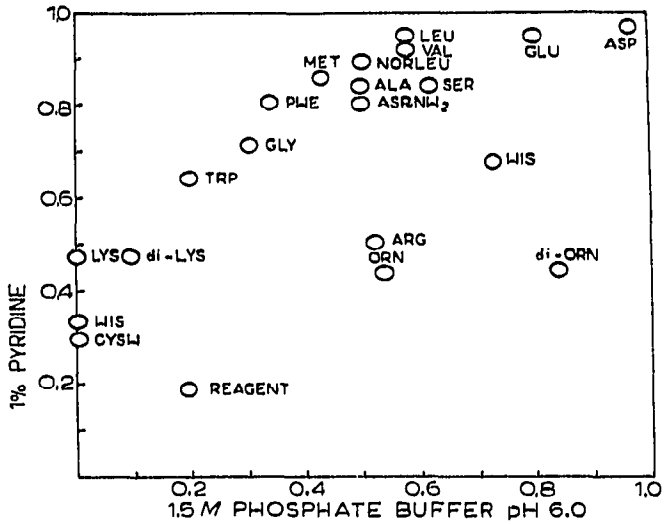

Fig. 4. Map of spots in two-dimensional paper chromatography (Whatman No. 3 MM) of 2,4-dinitro-5-aminophenyl derivatives.

Furthermore it is necessary to stress that amino acids with more than one amino group in the molecule result in multiple spots, *e.g.* lysine, ornithine, histidine and arginine. Some of these spots can be seen in the figures.

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2 E. D. BERGMANN AND M. BANTOV, *J. Org. Chem.*, 26 (1961) 1480.

Received March 23rd, 1967