Polyamide layer chromatography of phenylthiohydantions (PTH) of amino acids

Since EDMAN's original work on PTH amino acids[1] for the determination of the N-terminal group of proteins and peptides, there have been many successful identification methods of PTH amino acids by paper partition chromatography[2]. Recently, BRENNER *et al.*[3] used silica gel G thin-layer chromatography for the identification of PTH amino acids. Later, PATAKI[4,5] reported the excellent reproducibility of his method and actually applied it to the stepwise degradation of peptides. The chromatographic behavior of polyamide has been studied in our laboratory[6] using polyamide layer prepared according to WANG[7]. Our most recent application of polyamide layer chromatography to phenylthiohydantoins of amino acids is described here.

Table I shows the R_F values of sixteen PTH amino acids; these were obtained by five solvent systems. The distribution of R_F values is very good except for leucine and isoleucine.

Figs. 1 and 2 are drawings of chromatograms after two-dimensional development. After drying (80°) the chromatograms, the PTH amino acids show up as dark spots or bright yellow spots (tryptophan, phenylalanine and tyrosine) on a slightly fluorescent background when irradiated with an ultraviolet lamp (2537 Å). These three yellow spots, tryptophan, phenylalanine and tyrosine, facilitate the identification of other

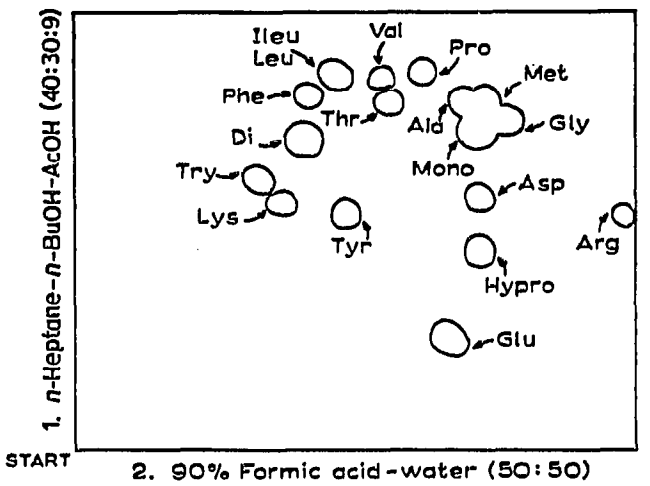
TABLE I

R_F VALUES OF SIXTEEN PTH AMINO ACIDS

Polyamide layer prepared according to WANG[7] using Amilan CM 1011 of Toyo Rayon Co., Tokyo, Japan.
Solvents: I = 90% formic acid–water (1:1); II = *n*-heptane–*n*-butanol–glacial acetic acid (40:30:9); III = carbon tetrachloride–glacial acetic acid (9:1); IV = benzene–glacial acetic acid (9:1); V = 2-butanone–*n*-hexane (1:3).
Detection: Visible under U.V.-lamp (2537Å: Mitamura Riken Kogyo Inc., Tokyo, Japan) after drying (80°). Distance: 10 cm.

Solvents	*I*	*II*	*III*	*IV*	*V*
Time (min)	*75*	*180*	*60*	*45*	*40*
PTH-L-Try	0.24	0.40	0.14	0.48	0.22
PTH-L-Lys	0.29	0.39	0.19	0.55	0.03
PTH-L-Phe	0.38	0.65	0.55	0.66	0.56
DPTH*	0.37	0.65	0.32	0.70	0.23
PTH-DL-Tyr	0.43	0.34	0.04	0.16	0.04
PTH-DL-Leu	0.44	0.74	0.63	0.80	0.72
PTH-L-Ileu	0.44	0.76	0.60	0.76	0.75
PTH-L-Thr	0.46	0.63	0.51	0.71	0.56
PTH-DL-Glu	0.51	0.12	0.00	0.31	0.00
PTH-DL-Met	0.51	0.63	0.48	0.72	0.50
PTH-L-Val	0.57	0.70	0.53	0.74	0.53
PTH-L-Pro	0.61	0.75	0.85	0.90	0.77
PTH-L-Asp	0.65	0.25	0.03	0.15	0.00
PTH-DL-Ala	0.68	0.60	0.43	0.67	0.47
MPTH**	0.70	0.65	0.67	0.52	0.04
PTH-Gly	0.71	0.65	0.30	0.63	0.29
PTH-L-Hypro	0.73	0.14	0.00	0.00	0.00
PTH-L-Arg	0.90	0.40	0.00	0.06	0.00

* DPTH = *sym*-diphenylthiourea. ** MPTH = monophenylthiourea.



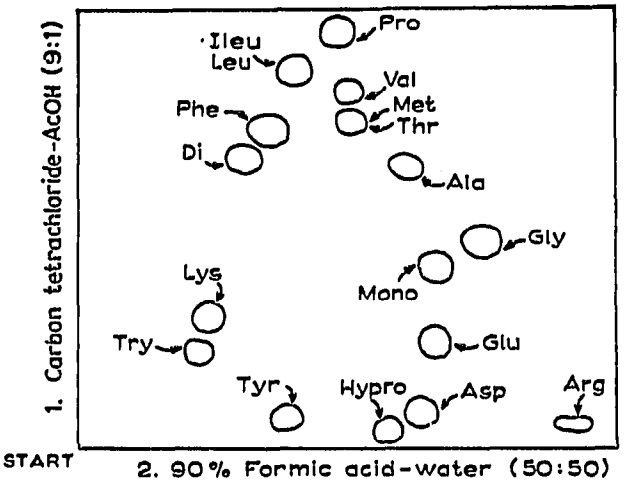


Fig. 1. Two-dimensional chromatogram. Solvent: 1st dimension: *n*-heptane–*n*-butanol–glacial acetic acid (40:30:9) (solvent II); 10 cm; 180 min; 2nd dimension: 90% formic acid–water (1:1) (solvent I); 10 cm; 75 min. Layer: ε-polycaprolactam resin CM 1007s (Toyo Rayon Co., Tokyo, Japan). Loading: 8 μg for each PTH amino acid. Detection: visible under U.V. light (2537 Å) after drying at 80°. Symbols: Mono = monophenylthiourea; Di = *sym*-diphenylthiourea.

Fig. 2. Two-dimensional chromatogram. Solvent: 1st dimension: carbon tetrachloride–glacial acetic acid (9:1) (solvent III); 10 cm; 60 min; 2nd dimension: 90% formic acid–water (1:1) (solvent I); 10 cm; 75 min. Layer: ε-polycaprolactam resin CM 1007s (Toyo Rayon Co., Tokyo, Japan). Loading: 8 μg for each PTH amino acid. Detection: visible under U.V. light (2537 Å) after drying at 80°. Symbols: Mono = monophenylthiourea; Di = *sym*-diphenylthiourea.

spots. Eight μg of phenylthiohydantoins are detectable by this method and the spraying of chromatograms with fluorescent reagent is not necessary.[2]

The phenylthiohydantoins are a new type of compound which can be separated by polyamide. The exact center of sorption is not yet known but this is the second example of heterocyclic compounds that can be separated by polyamide. The first example was the indole derivatives[8] whose acidic nitrogen seemed to be the cause of sorption by polyamide. We are investigating more PTH amino acids by different solvent systems and the stepwise degradation of peptides. A detailed report will be published in the near future.

This work was supported in part by Chemistry Research Center grant (Grant No. CRC-5508).

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Received June 14th, 1966