GAS CHROMATOGRAPHY OF PHENYLTHIOHYDANTOIN AND DINITROPHENYL DERIVATIVES OF AMINO ACIDS

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Amino acids may be identified and estimated by gas chromatographic methods through the use of a variety of derivatives. Among the derivatives which have been used are the N-trimethylsilyl trimethylsilyl esters (Ruhlmann and Giesecke, 1961), N-acetyl amyl esters (Johnson, Scott and Meister, 1961) and N-trifluoroacetyl methyl esters (Saroff and Karmen, 1960; Wagner and Winkler, 1961; Weygand, Kolb and Kirchner, 1961; Bayer, 1958). These derivatives are not in wide use in amino acid work, and their chief value at the present time lies in their potential usefulness in gas chromatographic separations. We have investigated the gas chromatographic behavior of two types of derivatives which are widely used for the study of amino acids, peptides and proteins. These are the phenylthiohydantoin (PTH) and dinitrophenyl (DNP) derivatives. The gas chromatographic techniques were those developed in this laboratory for the separation of steroids and other natural products (Horning, Haahti and VandenHeuvel, 1961). The column packings contained relatively thin-film coatings of highly thermostable liquid phases, and these were used with a Lovelock argon ionization detection system.

Three different stationary phases were used in this work. They were (a) methyl silicone polymer SE-30 (VandenHeuvel, Sweeley and Horning,

1960), (b) fluoroalkyl silicone polymer QF-1 (VandenHeuvel, Haahti and Horning, 1961) and (c) phenyl silicone polymer PhSi (Sjövall, Meloni and Turner, 1961; Luukkainen, VandenHeuvel, Haahti and Horning, 1961). The gas chromatographic results are in the Table and Figure.

Relative Retention Times of PTH and DNP Derivatives of Amino Acids[a, b]

	SE-30		QF-1		PhSi	
Amino Acid	PTH	DNP[c]	PTH	DNP[c]	PTH	DNP[c]
	175°	175°	175°	194°	200°	200°
Alanine	0.81	1.93	0.63	2.33	0.35	0.44
Glycine	1.03	2.24	0.79	3.05	0.54	0.67
Proline	1.18	3.76	1.14	4.15	0.59	1.16
Valine	1.46	2.63	0.80	2.67	0.40	0.49
Leucine	1.80	3.23	1.21	3.30	0.53	0.56
Isoleucine	1.80	3.61	1.10	3.37	0.55	0.62
Androstane	1.00 (9.30)[d]	1.00 (3.30)[d]				
Cholestane			1.00 (11.7)[d]	1.00 (4.70)[d]	1.00 (9.70)[d]	1.00 (9.70)[d]
	200°	202°				
Aspartic Acid	2.12[e]	5.14	1.89[e]	6.95		1.47
Glutamic Acid	3.19[e]	7.88	2.31[e]	10.8		2.01
Methionine	3.95	8.80	3.04	6.77	2.03	2.32
Phenylalanine	5.38	12.4	3.52	11.6	3.10	3.94
Androstane	1.00 (4.20)[d]	1.00 (3.30)[d]				
Cholestane			1.00 (2.70)[d]	1.00 (4.70)[d]	1.00 (9.70)[d]	1.00 (9.70)[d]
	250°		255°		220°	
Tyrosine	1.12		2.77		9.67	
Histidine	1.29		4.07			
Tryptophan	2.84		5.46		32.3	
Cholestane	1.00 (8.20)[d]				1.00 (3.70)[d]	
Cholesterol			1.00 (1.30)[d]			

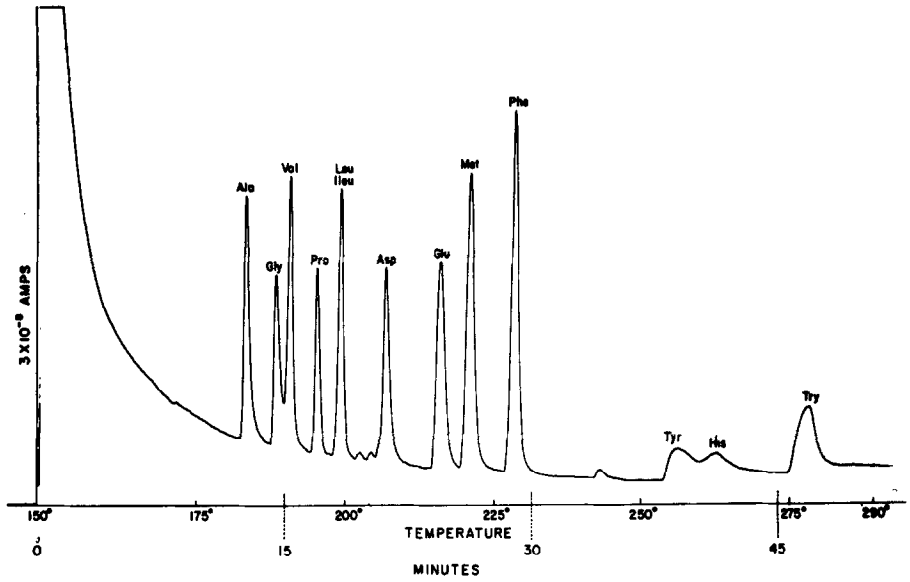
[a]PTH and DNP derivatives were obtained from Mann Research Laboratories, Inc., New York, or prepared according to established procedures.

[b]Column packing, 1% coating of the liquid phase on 100-140 mesh Gas-Chrom P; 6 ft. glass coils or U-tubes, 3.4-5.0 mm. I.D.; 12-25 psi argon; Lovelock argon ionization detection system.

[c]Chromatographed as methyl esters, prepared with diazomethane.

[d]Actual retention time, minutes.

[e]Chromatographed as methyl esters, prepared with BF_3-methanol.



Gas chromatographic separation of phenylthiohydantoin derivatives of amino acids. Conditions: column 6 ft x 3.4 mm I.D. glass coil, 0.75% SE-30 polymer on 100-140 mesh Gas-Chrom P; Argon pressure, 20 psi.

Most PTH derivatives chromatographed well. Derivatives of serine and threonine, of asparagine and glutamine, and of basic amino acids presented certain problems. The serine and threonine derivatives underwent dehydration, judging from the ultraviolet absorption spectra of the materials collected after chromatography. Asparagine and glutamine derivatives also yielded altered products, although a consistent pattern was obtained in each instance. Since the common amino acids have a wide range of molecular weight, it might be expected that SE-30 would be quite satisfactory for many separations. This was found to be true; SE-30 gave satisfactory separations except for leucine and isoleucine PTH derivatives. These were separated readily with a QF-1 phase.

DNP derivatives were separated as the methyl esters. Satisfactory results were obtained for derivatives of the simple neutral and acidic amino acids. Serine, threonine, tryptophan, tyrosine and histidine derivatives

gave unsatisfactory results (decomposition). Derivatives of basic amino acids were not chromatographed successfully.

Excellent procedures for the preparation of Edman (PTH) derivatives (Sjöquist, 1960) and DNP derivatives are now available. The speed, sensitivity and high resolving power of gas chromatographic methods suggest that these techniques will find use in end group analysis with PTH and DNP derivatives, and in other problems when only very small amounts of material are available. The present limitations (failure to yield satisfactory results with serine, threonine, and basic amino acids) indicate that gas chromatographic methods will not entirely supplant existing procedures for separating these derivatives. However, the fact that a considerable number of amino acids may be separated in this way provides a new method for amino acid work with PTH and DNP derivatives and further studies may resolve the problems associated with basic amino acids and with serine, threonine, glutamine and asparagine.

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