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Analysis of acetylated and trifluoroacetylated phenylthiohydantoin amino acids by gas chromatography

Reaction of phenylisothiocyanate with the N-terminal amino acid of a peptide or protein followed by cleavage in acid results in formation of a 3-phenyl-2-thiohydantoin (PTH) amino acid derivative[1,2]. Protein sequence studies have been facilitated by analysis of the isolated PTH using thin-layer or paper chromatography[3,4] and, more recently, gas chromatography (GC)[5,6]. Several PTH amino acids have been successfully separated and analyzed by GC as trimethylsilyl derivatives[7,8]. RODA AND ZAMORANI[9] separated six trifluoroacetylated PTH's by GC using a stainless-steel column containing 5 % SE-30 stationary phase. Prior to this investigation, glass columns alone had been used to analyze PTH's[5-8].

This report compares the GC behavior of trifluoroacetylated and acetylated PTH amino acids using a stainless-steel column.

Experimental

N-acetylation and N-trifluoroacetylation of PTH amino acids. Alanine, glycine, valine, proline, leucine, isoleucine, methionine and phenylalanine PTH's were purchased from Mann Research Laboratories. Acetates and trifluoroacetates were made by a procedure similar to that of RODA AND ZAMORANI[9]. To 2 mg of each PTH in 2 ml of chloroform was added 0.2 ml acetic anhydride or trifluoroacetic anhydride (TFAA). Reaction mixtures were allowed to stand at least 30 min at room temperature before GC.

Gas chromatography. A Varian Aerograph Model 204-1C gas chromatograph with dual-flame ionization detectors was used. Injector and detector temperatures were 220°. A 5 ft. × 1/8 in. O.D. stainless-steel column containing 1 % SE-30 on Chromosorb G (acid washed and silanized) was operated at 180° isothermally to obtain the data in Table I or programmed from 150–200°, 10°/min then held at 200° to obtain the chromatogram in Fig. 1. Helium carrier gas flow rate was 25 ml/min. Two-microliter samples were injected with a range of 10^{-11} and an attenuation of 16.

TABLE I

RELATIVE RETENTION TIMES OF AMINO ACID PHENYLTHIOHYDANTOIN ACETATES AND TRIFLUOROACETATES

Proline PTH, 5.75 min = 1.00.

	Acetates	*Trifluoroacetates*
Alanine	0.51	0.26
Glycine	0.60	0.33
Valine	0.71	0.37
Leucine, isoleucine	0.96	0.49
Methionine	2.36	—
Phenylalanine	2.88	1.51

Results

Table I gives retention times of acetates and trifluoroacetates relative to unreacted proline PTH. Since proline PTH was not N-acetylated, the retention time of

the derivative was not affected by acetic anhydride or TFAA treatment. Proline was the only compound which could be successfully chromatographed as the free PTH on the stainless-steel column and therefore it was chosen as a marker compound for derivatives. All PTH acetates separated except leucine and isoleucine. RODA AND ZAMORANI[9] also were unable to resolve these two as the PTH trifluoroacetates. Methionine PTH trifluoroacetate gave no peak and the solution turned dark brown upon standing for several hours at room temperature. Chromatography of methionine or phenylalanine PTH's was not attempted by RODA AND ZAMORANI[9]. The PTH acetates of these two amino acids were readily separated (Table I). The order of appearance of alanine and glycine derivatives (Table I) appears to conflict with that reported previously[9].

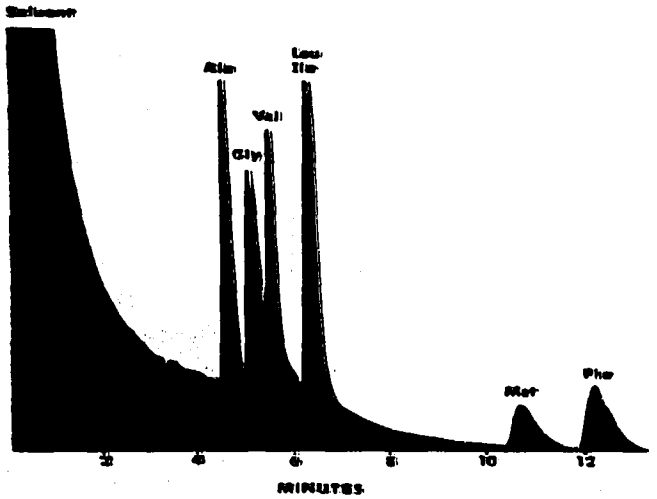

Fig. 1. An authentic gas chromatogram of acetylated phenylthiohydantoin amino acids (areas under the peaks are darkened).

Fig. 1 shows a chromatogram obtained with the PTH's as their acetates. Good separation was obtained with all but leucine and isoleucine. Thus, from a variety of considerations, acetic anhydride appears to be superior to TFAA for derivatization of simple amino acid PTH's.

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