Short Communications and Preliminary Notes

Phenyl thiohydantoins in amino acid analysis

In earlier papers[1,2,3] from this and other laboratories, partition chromatographic methods have been described for the identification of the various 3-phenyl-2-thiohydantoins obtained in the stepwise degradation of peptides[4]. The satisfactory chromatographic separation of the hydantoins and their strong absorption in the ultraviolet region in conjunction seemed to us to offer a simple way of determining quantitatively the composition of complex amino acid mixtures. The phenyl thiohydantoins of natural amino acids show closely similar light absorption curves, with a sharp peak at 269 mμ ($\varepsilon_M \sim$ 17,000). Automatic registration of the ultraviolet absorption at 269 mμ in the effluent from a chromatographic column would immediately give the composition of the mixture and this at the expense of extremely small amounts of material.

One necessary precondition was fulfilled in that a quantitative yield could be secured in the synthesis of the phenyl thiohydantoins (PTH) derivatives from extremely dilute solutions of the amino acids. The phenyl thiocarbamyl (PTC) amino acids were synthesized in aqueous acetone-sodium bicarbonate and the ring closure to the PTH-derivatives was then brought about in acetic acid-hydrogen chloride[5]. Our procedure makes it possible to obtain a quantitative yield from 10–20 μg of each amino acid.

For the partition chromatography, Hyflo Super-Cel was used as inert support[6] and heptane-amyl acetate or heptane-ethylene chloride served as moving phases and aqueous formic acid as stationary phase. The effluent from the column was led through a spectrophotometer absorption cell provided with quartz windows. A modulated light beam of 269 mμ from a Beckman Spectrophotometer Model DU was passed through the absorption cell to a photocell, the output of which was amplified and recorded against time on a Leeds and Northrup Speedomax recorder. Provisions were made to compensate for the drift of the hydrogen discharge lamp.

The record of a typical separation is presented in Fig. 1.

A more detailed report will appear later in this journal.

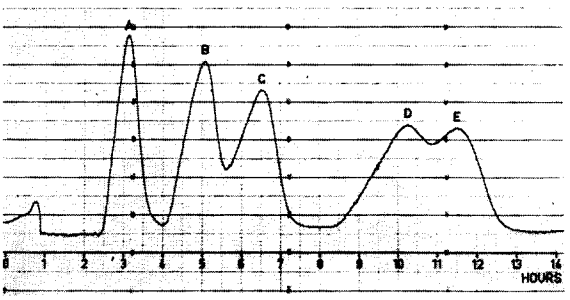

Fig. 1. Recorded transmission from a chromatography of PTH-leucine (A), PTH-valine (B), PTH-phenylalanine (C), PTH-proline (D), and PTH-methionine (E) in heptane-amyl acetate-formic acid (90%) (14:3:1). Mixture of 172.0 μg amino acids.

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