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## Note

# Gas chromatographic separation of leucine and isoleucine phenylthiohydantoins

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In amino acid sequence determinations, the identification of the phenylthiohydantoin (PTH) derivatives of amino acids (from Edman degradation) may be accomplished by either gas–liquid chromatography (GLC) or thin-layer chromatography (TLC) or by a combination of these methods. In our GLC system the PTH derivatives have been analysed on a 5% SP-400 column[1] and found to be suitable for all the amino acid derivatives (some after silylation) except arginine. However, separation of leucine-PTH from isoleucine-PTH was not possible. The TLC system used[2] did not give a clear difference between these two derivatives either.

Trials with CFC blend[1], a mixture of equal volumes of 7.33% (w/v) SP-400, 5.33% OV-210 and 0.66% OV-225, as liquid phase for the GLC separation of Leu-PTH and Ile-PTH were not successful. We tried this method to avoid the need for

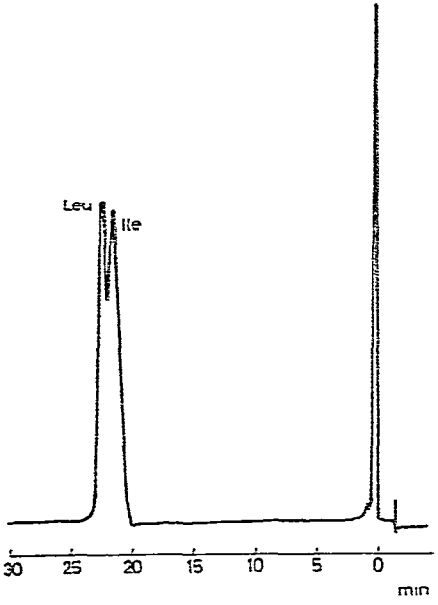


Fig. 1. Gas chromatographic separation of Leu-PTH from Ile-PTH on an OV-225 column.

silylation [with N,O-bis(trimethylsilyl)acetamide (BSA)] to distinguish between leucine and isoleucine. However, we have found now that by using only OV-225 as liquid phase at a comparatively low loading a reasonable resolution of the two compounds can be obtained.

Fig. 1 shows the separation of Leu-PTH and Ile-PTH on a 220 cm × 2 mm I.D. glass column filled with 2% OV-225 on Chromosorb W-HP, 80–100 mesh AW-DMCS. Further conditions were: GLC apparatus, Beckman GC-65; injector temperature, 290°; column temperature, 220°; detector temperature, 325°; carrier gas (nitrogen) flow-rate, 30 ml/min through the column and for detection an additional 50 ml/min; hydrogen flow-rate, 45 ml/min and air flow-rate, 300 ml/min. The retention times were 21.5 min for Ile-PTH (2230 plates/m) and 22.5 min for Leu-PTH (2330 plates/m). The resolution factor, which was 0.5, is not ideal for quantitative separation but sufficient for qualitative identification as in the present case. Using this in model experiments, the first two PTH residues, obtained by the automatic Edman degradation of the single chain of carboxymethylated β-lactoglobulin could be identified as being Leu-PTH and Ile-PTH, respectively, which is in agreement with the results published by Braunitzer *et al.*[3]. In our opinion, this proves the usefulness for sequence studies.

## REFERENCES

1 J. J. Pisano, T. J. Bronzert and H. B. Brewer, Jr., *Anal. Biochem.*, 45 (1972) 43.
2 D. A. Walz and J. Reuterby, *J. Chromatogr.*, 104 (1975) 180.
3 G. Braunitzer, R. Chen, B. Schrank and A. Stangl, *Hoppe-Seyler's Z. Physiol. Chem.*, 354 (1973) 867.