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Gas–liquid chromatographic separation of oxazolin-5-one derivatives of an amino acid

In recent years the gas–liquid chromatographic (GLC) separation of derivatives of amino acids has offered a valuable alternative to the classical amino acid analysis based on ion-exchange chromatography. Especially the trifluoroacetyl amino acid *n*-butyl esters, first used by ZOMZELY *et al.*[1], and later developed by the group of GEHRKE[2], have served as useful derivatives. A variety of other acyl and ester groups has also been successfully used[3].

The inner esters of *N*-acyl amino acids, oxazolin-5-ones, are in some cases distillable liquids[4,5]. This fact prompted an investigation of the GLC behavior of the oxazolin-5-ones obtained from the following acyl-leucines: formyl-, acetyl-, trifluoroacetyl-, and benzoyl-leucine.

Experimental

The 2-H-, 2-methyl-, and 2-phenyl-oxazolin-5-ones were synthesized by the action of dicyclohexylcarbodiimide on formyl-, acetyl- and benzoyl-leucine, respectively[6]. The 2-trifluoromethyl-oxazolin-5-one was synthesized directly from leucine by refluxing with trifluoroacetic anhydride[5]. The 2-H-, 2-methyl-, and 2-trifluoromethyl-4-isobutyl-oxazolin-5-ones were distilled under reduced pressure, and the 2-phenyl-4-isobutyl-oxazolin-5-one was recrystallized from petroleum ether.

Two microliters of an ethyl acetate solution containing about 1 mg/ml of each of the oxazolin-5-ones were chromatographed on an F & M Model 402 gas chromatograph equipped with a flame ionization detector. The glass column used was 1.2 m ×

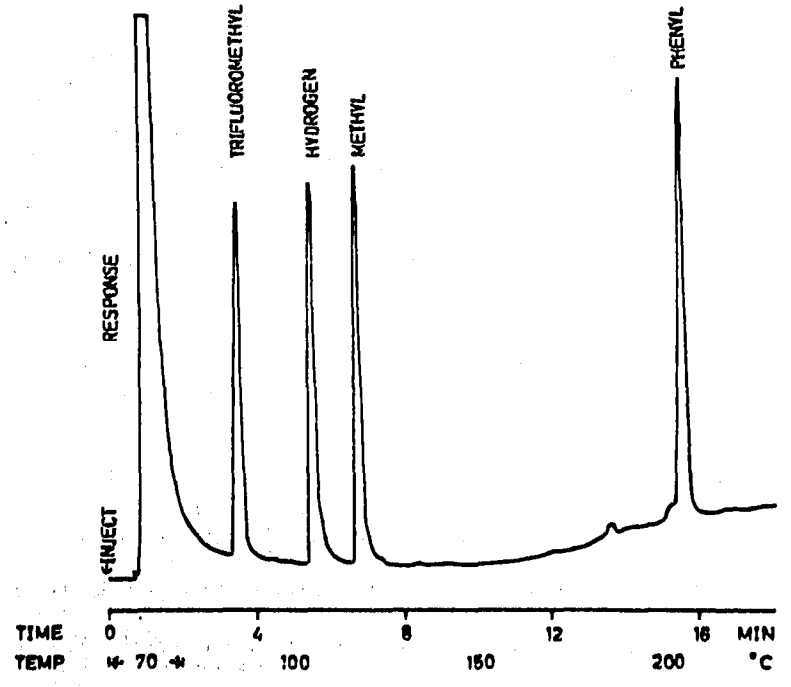

Fig. 1. Gas chromatogram of oxazolin-5-one derivatives of leucine.

3 mm I.D. packed with 5% w/w OV-17 coated on 80–100 mesh acid-washed (AW) Chromosorb W DMCS. Argon was used as carrier gas with a flow-rate of 35 ml per min. The column temperature was set at 70°, maintained at this temperature for 2 min after injection and thereafter increased 10° per min. The resulting chromatogram is presented in Fig. 1.

Discussion

The oxazolin-5-ones seem to be suitable derivatives of amino acids for GLC analysis. Although they are chemically very reactive, they are thermally stable as shown by the distinct peaks obtained. The volatility of 2-trifluoromethyl-4-isobutyl-oxazolin-5-one is greater than that of trifluoroacetyl-leucine *n*-butyl ester.

The synthesis of a 2-trifluoromethyl-oxazolin-5-one from the corresponding amino acid involves only a single step. Similarly, a 2-methyl-oxazolin-5-one may be synthesized in one step by the action of acetic anhydride on the amino acid[7].

Due to the excellent GLC behavior and the rapid and simple synthesis of the oxazolin-5-ones, these may be advantageous derivatives for the GLC analysis of amino acids.

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1 C. ZOMZELY, G. MARCO AND E. EMERY, *Anal. Chem.*, 34 (1962) 1414.
2 C. W. GEHRKE, D. ROACH, R. W. ZUMWALT, D. L. STALLING AND L. L. WALL, *Quantitative Gas–Liquid Chromatography of Amino Acids in Proteins and Biological Substances*, Anal. Biochem. Lab., Columbia, Mo., 1968.
3 B. W. MELVÅS, *Sv. Kem. Tidskr.*, 80 (1968) 430.
4 K. NOWAK AND I. Z. SIEMION, *Rocz. Chem.*, 35 (1961) 153.
5 F. WEYGAND, W. STEGLICH AND H. TANNER, *Justus Liebigs Ann. Chem.*, 658 (1962) 128.
6 I. Z. SIEMION AND K. NOWAK, *Rocz. Chem.*, 34(1960) 1479.
7 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Wiley, New York, 1961, p. 830.

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