

Gas-Liquid Chromatographic Separation of Amino-acid Derivatives

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Summary A mixture of 2-trifluoromethyloxazolin-5-ones of ten α -amino acids was completely separated by gas-liquid chromatography.

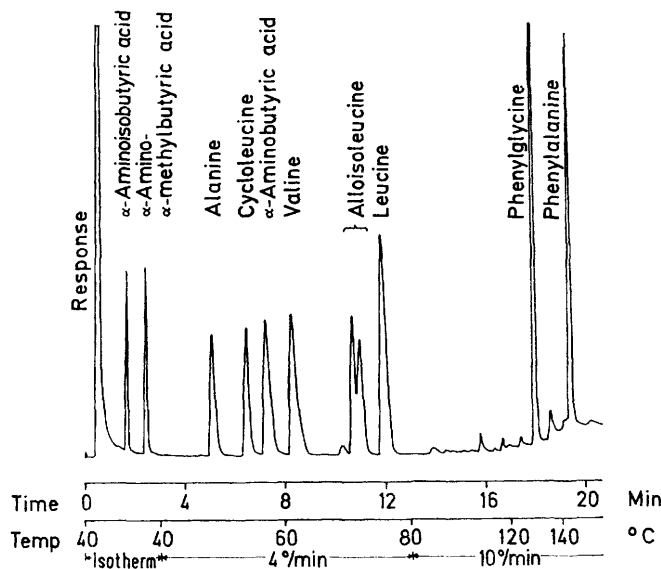
THE most widely used derivatives for gas chromatographic analysis of amino-acids are the *N*-trifluoroacetyl amino-acid *n*-butyl esters, which are synthesised by two separate

steps: esterification followed by acylation.¹ We report a simple, one-step method for the synthesis of the inner esters of *N*-trifluoroacetyl amino acids, the 2-trifluoromethyloxazolin-5-ones, which have excellent gas chromatographic properties.²

A mixture of 10 mg of the ten amino acids α -aminobutyric acid, α -aminoisobutyric acid, α -amino- α -methyl-

butyric acid, alanine, valine, leucine, cycloleucine, alloisoleucine, phenylglycine, and phenylalanine was treated with 2 ml of trifluoroacetic anhydride in a sealed tube which was heated at 150° for 10 min. The mixture was cooled to room temperature and evaporated under reduced pressure. A solution of the resulting oil in ethyl acetate was chromatographed (see Figure). Resolution of the ten oxazolin-5-

ones was complete. The oxazolin-5-ones of the α -methyl amino-acids and cycloleucine were more volatile than the derivatives of the isomeric amino-acids with hydrogen in the α -position. Elution of the latter followed the increase in molecular weight, as is also observed for the corresponding *N*-trifluoroacetyl amino-acid *n*-butyl esters. The 2-trifluoromethyloxazolin-5-ones are more volatile than the *N*-trifluoroacetyl amino acid *n*-butyl esters.

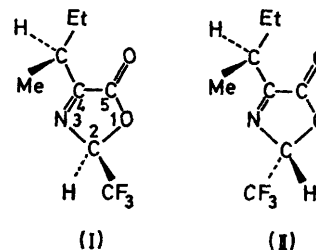


FIGURE

¹ 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, Montana, 1968.

² O. Grabi-Nielsen and E. Solheim, *J. Chromatog.*, 1972, **69**, 366.

³ F. Weygand, W. Steglich, D. Mayer, and W. von Philipsborn, *Chem. Ber.*, 1964, **97**, 2023.



The double bond in the 2-trifluoromethyloxazolin-5-ones is between the 3- and 4-positions.³ Accordingly, when these derivatives are synthesized from α -amino-acids, the chirality of the α -carbon atom is lost, and a new asymmetric centre, C-2 of the oxazolin-5-one ring, is created. Alloisoleucine has an asymmetric carbon atom in the side chain, and consequently the oxazolin-5-one will be a mixture of two diastereomers, (I) and (II), which give rise to the partly separated double-peak in the chromatogram.

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