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Note

Gas–liquid chromatographic separation of dipeptide derivatives

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The gas–liquid chromatographic (GLC) separation of peptide derivatives was first carried out by Weygand *et al.*[1]. Sufficient volatility of the peptides was obtained by acylation of the terminal amino group with the trifluoroacetyl group and esterification of the terminal carboxyl group with methyl ester. The GLC separation of peptide derivatives combined with mass spectrometric analysis has been developed as an important technique in the sequence analysis of peptides and proteins. Combinations of different acyl and ester groups have been employed for volatilization. More recently, permethylation of N-acyl peptides has given derivatives which were suitable for GLC analysis[2].

In two previous communications[3,4], it was demonstrated that the inner esters, azlactones or oxazolin-5-ones, were useful derivatives of amino acids for GLC analysis. This paper describes the GLC separation of dipeptides where the terminal carboxyl group is converted into an oxazolin-5-one. The terminal amino group is protected with a urethane group, the *tert.*-butyloxycarbonyl (BOC) group, which is used extensively in peptide synthesis.

EXPERIMENTAL AND RESULTS

The four protected peptides BOC–Gly–Ala–OMe, BOC–Gly–Val–OMe, BOC–Gly–Leu–OMe and BOC–Gly–Ile–OMe were synthesized by the dicyclohexylcarbodiimide (DCCI) method. After the normal work-up of the reaction mixtures, the products were saponified for 30 min with sodium hydroxide in methanol and worked up in the normal manner. BOC–Gly–Leu–OH was recrystallized from methanol (m.p. 131–132°) and the other BOC-peptides were used without further purification.

The conversion of the terminal carboxyl group of the BOC-dipeptides was achieved with DCCI. Approximately 40 μmole of each of the BOC-dipeptides BOC–Gly–Ala–OH, BOC–Gly–Val–OH and BOC–Gly–Leu–OH were dissolved in ethyl acetate, 1.2 ml of a 0.1 *M* solution of DCCI in ethyl acetate were added, and the total volume of the reaction mixture was made up to 10 ml, *i.e.*, the concentration of each of the dipeptides was *ca.* 4 μmole/ml and of DCCI *ca.* 12 μmole/ml. A 1-μl volume of this solution was chromatographed on an F & M Model 402 gas chromatograph equipped with a flame ionization detector, using a glass column (1.2 m × 3 mm I.D.) packed with 3% OV-17 coated on 80–100 mesh Chromosorb W AW DMCS. Argon was used as the carrier gas at a flow-rate of 35 ml/min.

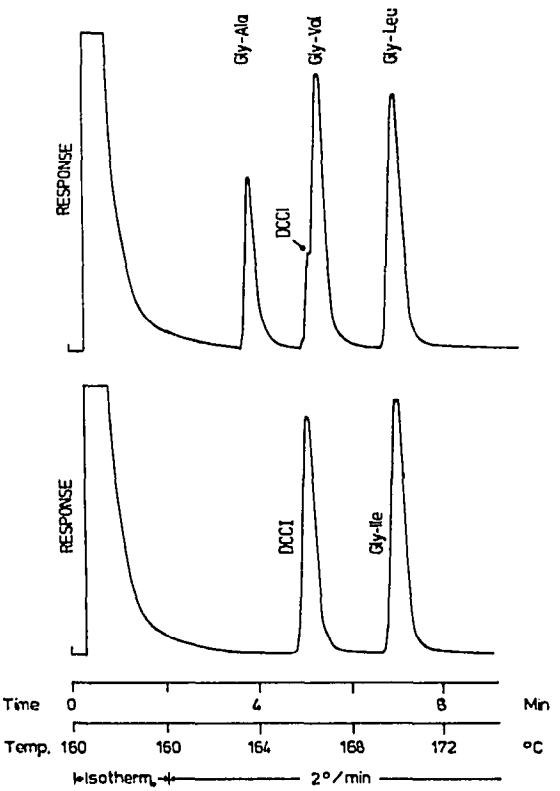


Fig. 1. Gas chromatogram of a mixture of the oxazolin-5-ones of the dipeptides BOC–Gly–Ala–OH, BOC–Gly–Val–OH and BOC–Gly–Leu–OH plus trace amounts of DCCI (above), and a mixture of DCCI and the oxazolin-5-one of BOC–Gly–Ile–OH (below).

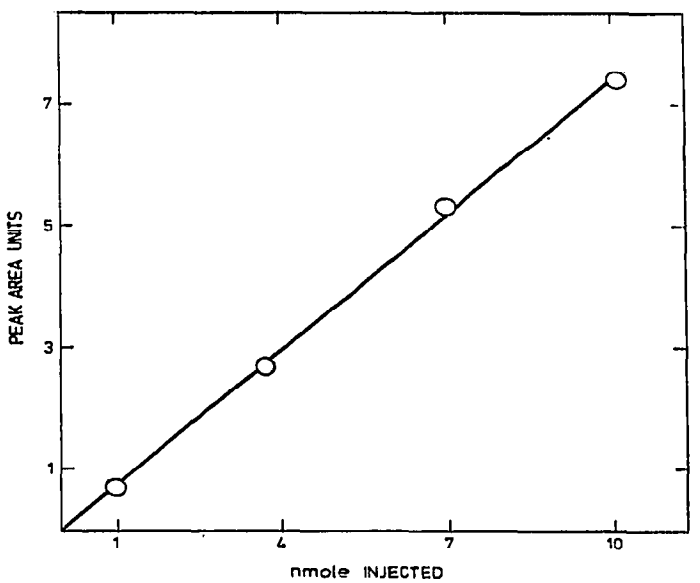
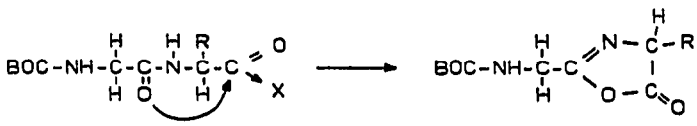


Fig. 2. Plot of peak response *versus* concentration of BOC–Gly–Leu–OH.

The resulting chromatogram is shown in Fig. 1, together with a chromatogram of a solution containing *ca.* 4 μmole/ml of BOC–Gly–Ile–OH and 6 μmole/ml of DCCI. A calibration curve based on four solutions with different concentrations of BOC–Gly–Leu–OH is shown in Fig. 2.

DISCUSSION

By nucleophilic attack of the amide oxygen of the peptide bond adjacent to the terminal carboxyl group in a peptide on the activated carboxyl carbon, an inner ester is formed[5]:



This reaction is well known in peptide synthesis, where it competes with the formation of the peptide bond and may lead to unwanted racemization.

Peptide oxazolin-5-ones have been synthesized by activation of the carboxyl group with DCCI[6,7].

Based on our experience with oxazolin-5-ones of acylamino acids[3,4], the synthesis and GLC behaviour of peptide oxazolin-5-ones were investigated. Because of the widespread use of the BOC group for the protection of amino groups in peptide synthesis, it was of interest to see if this group could be applied in GLC.

The chromatograms show that these peptide derivatives are well suited for GLC analysis. The symmetrical form of the peaks demonstrate that both the BOC group and the oxazolin-5-one ring are stable under the conditions used. It can be seen from the calibration curve in Fig. 2 that the method is reproducible and can be used for quantitative analysis. The three peptide derivatives in the mixture were completely separated. The oxazolin-5-one derivatives of BOC–Gly–Leu–OH and BOC–Gly–Ile–OH had identical retention indices and the separation of these two isomers consequently could not be achieved.

DCCI gave a distinct and symmetrical peak. It was completely separated from the alanine and leucine/isoleucine derivatives, but overlapped partly with the peak from the valine derivative. The peak response of DCCI was approximately twice as high as that of BOC–Gly–Ile–oxazolin-5-one on a molar basis. The GLC properties of DCCI make it possible to follow the disappearance of DCCI in reactions where this reagent is used. Surprisingly, the reaction product of DCCI, dicyclohexylurea, was also eluted from the column, although with a much higher retention time.

The procedure for derivatization of the terminal carboxyl group was very simple and rapid. DCCI was kept in a stock solution, and after adding the desired volume of this to the solution of the BOC-dipeptides, the reaction mixture could be injected as soon as proper mixing had been accomplished, *i.e.*, within 1–2 min, without any work-up. The ring formation seemed to be very rapid for all derivatives, and no difference between carboxyl terminal residues with different sized side-chains, *i.e.*, alanine and isoleucine, could be noted.

The reproducibility of the method was independent of the time lapse between mixing of the reactants and injection of the sample. This method competes favourably

with esterifications, which, depending on the reagent used, need up to several hours of reaction time followed by work-up of the reaction mixture. In the present case, where BOC is used for protection of amino groups, esterification under acidic conditions would have been impossible, as the BOC group is very labile towards acids.

In conclusion, the conversion of the carboxyl terminal residues of BOC-protected dipeptides by DCCI into oxazolin-5-one is a convenient, rapid and reproducible method of obtaining volatile derivatives that are suitable for GLC analysis.

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