# A Method of Peptide Sequencing

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*Summary* Sequencing of peptides has been carried out by degrading them to diketopiperazines and identification of the diketopiperazines by g.c./m.s. methods.

IN 1953, Albertson and McKay[1] proposed a method for sequencing peptides in which the peptide was first converted sequentially into a diketopiperazine mixture (I). The second step was to remove the *N*-terminal amino-acid to give another peptide which in turn was converted sequentially into a mixture of diketopiperazines (II). By examination of the mixtures (I), (II), the sequence of amino-acids in the original peptide can be deduced. Because in 1953 there was no suitable method for separating the mixture of diketopiperazines and identifying the components, the proposed scheme was left in abeyance. Recently, Mauger[2] has proposed a method of sequencing which is somewhat similar to that proposed here in that the peptide is thermally decomposed into diketopiperazines which are identified by g.c. Apart from the uncertainty concerning the strictly sequential nature of thermolytic methods of producing diketopiperazines, the use only of gas chromatography for identification leaves much to be desired and requires a knowledge of the retention times of all possible diketopiperazines. We have used the method proposed by Albertson and McKay and identified the diketopiperazines by combined g.c./m.s. The peptide, H.Gly.Leu.Leu.Gly.Gly.OH, was heated in refluxing glacial acetic acid for 12 h. The acetic acid was distilled off *in vacuo* and the residue dissolved in dimethylformamide and injected on to a column of 5% OV17 on celite at 240°. The only diketopiperazine detected was gly.leu (characteristic ion at *m/e* 170). The original pentapeptide was subjected to Edman degradation to give a tetrapeptide which, after treatment with glacial acetic acid, gave two diketopiperazines, leu.leu (characteristic ion at *m/e* 226) and gly.gly (*m/e* 114). From this information, the sequence of the original peptide can be assembled.

The method has a number of potentially attractive features in that the diketopiperazines give simple mass spectra and can be readily identified in amounts less than 1 $\mu$g. The above analysis could be carried out on about 10 nmol of pentapeptide. The diketopiperazines are very amenable to g.c.; no preparation of derivatives of the peptide is required whilst the conversion into diketopiperazines is simple. Further work is in progress with a range of amino-acids and in improving the yield of diketopiperazines which is about 25% at the moment.

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[1] N. F. Albertson and F. C. McKay, *J. Amer. Chem. Soc.*, 1953, **75**, 5323.
[2] A. B. Mauger, *Chem. Comm.*, 1971, 39.