MASS SPECTROMETRIC DETERMINATION OF THE TYPE OF AMIDE BOND IN α- AND γ-PEPTIDES OF GLUTAMIC ACID

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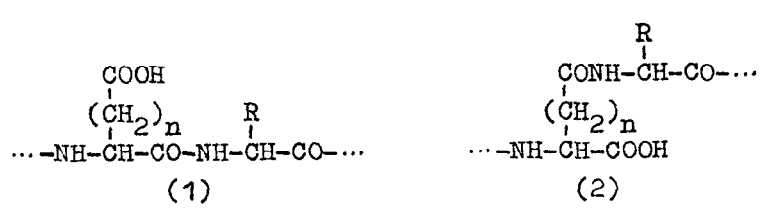
In a series of papers (Kiryushkin, et al., 1966; Shemyakin, et al., 1966; Wulfson, et al., 1965, 1966) we showed that mass spectrometry can be utilized to determine the amino acid sequence in polypeptides containing residues of the most varied amino acids, deciphering of the mass spectra being considerably facilitated by preliminary knowledge of the amino acid composition of the sample.

The analytical possibilities of the mass spectrometry in peptide chemistry are not confined, however, to only amino acid sequence determinations. Another type of analytical problems which could prove to be within the scope of the mass spectrometric method is elucidation of the type of amide bond in α- and ω-peptides of monoamino dicarboxylic acids (Formulae 1 and 2). The application of mass spectro-



(1) (2)

metry for solution of this problem could have several advantages over chemical and enzymatic methods.

In the present paper it has been shown that mass spectrometry can be used to determine the type of bond (α- or γ-) in peptides of glutamic acid. The dimethyl esters of the decanoyl peptides (3)-(8) were taken for the investigation, the peptides (3), (5) and (7) differing from (4), (6) and (8) in that the α-carboxyl group participates in the formation of the amide bond in the former and the γ-carboxyl group, in the latter.

Dec-Gly-Glu(OMe-γ)-Ala-Ala-Phe-OMe (3)

Dec-Gly-Glu(OMe-α)-Ala-Ala-Phe-OMe (4)

Dec-Gly-Leu-Val-Glu(OMe-γ)-Gly-Ala-Leu-OMe (5)

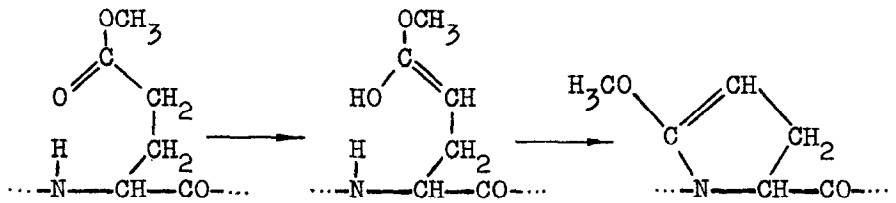
Dec-Gly-Leu-Val-Glu(OMe-α)-Gly-Ala-Leu-OMe (6)

Dec-Glu(OMe-γ)-Gly-Ala-Leu-OMe (7)

Dec-Glu(OMe-α)-Gly-Ala-Leu-OMe (8)

It turned out that the mass spectra of compounds (3)-(8) besides the earlier described (Shemyakin, et al., 1966; Wulfson, et al., 1966) types of fragmentation [amino acid type (A), ketiminic type (D), elimination of the elements of methanol (C), and elimination of the carbomethoxy group (D); see Figs. 1-2] that are common to all the compounds (3)-(8), also show a number of characteristic differences. For the α-peptides of glutamic acid possessing a γ-carbomethoxy group there is characteristic still another process involving the elimination of the elements of water from the molecular ion and from the all glutamic acid residue containing fragments (Route E). Elimination of the water, which occurs with participation of the γ-carbomethoxy group and one of

the neighboring amide groups and leads to the formation of a heteroring, most probably takes place in the following way:



It is clear that such a reaction could not be realized if the γ-carboxyl group of glutamic acid were the one involved in the formation of the amide bond. Indeed, in the mass spectra of peptides (3), (5) and (7) all peaks corresponding to fragments containing a glutamic acid residue with a γ-carbomethoxy group are accompanied by peaks displaced by 18 m.u. to the lower side of the spectrum (see Route E on Fig. 1). No such peaks are exhibited by the peptides (4), (6) and (8) whose γ-carboxyl group participates in the amide bond formation (see Fig. 2).

It should be mentioned that elimination of water occurs only at high temperatures (> 200°) so that the mass spectrometric method is especially convenient for analysis of low volatile compounds, such as those containing quite a large number of amino acid residues (tetra- and pentapeptides and higher).

The type of fragmentation (Type E) described here naturally does not reflect all the differences between the spectra of the α- and γ-peptides of glutamic acid.

The mass spectra were taken on the Hitachi RMU-6D mass spectrometer at 250-300°, the samples being introduced directly into the ion source.

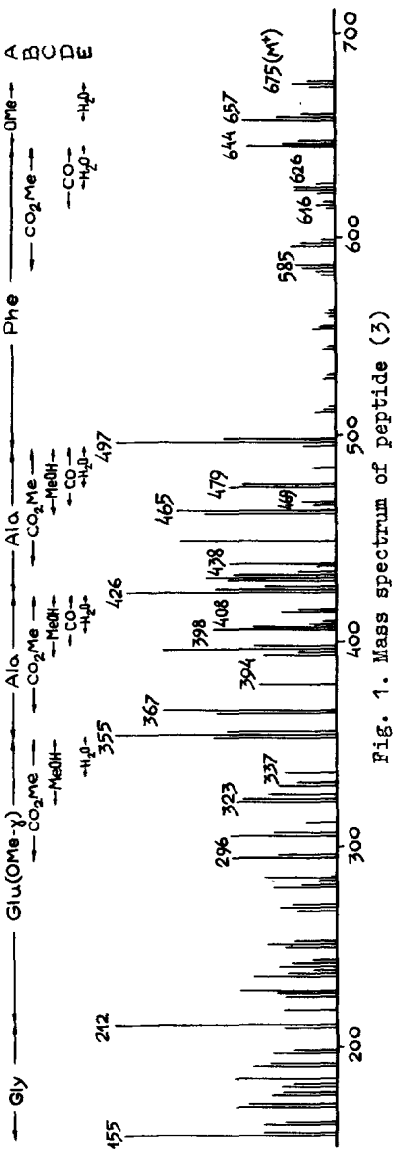


Fig. 1. Mass spectrum of peptide (3)

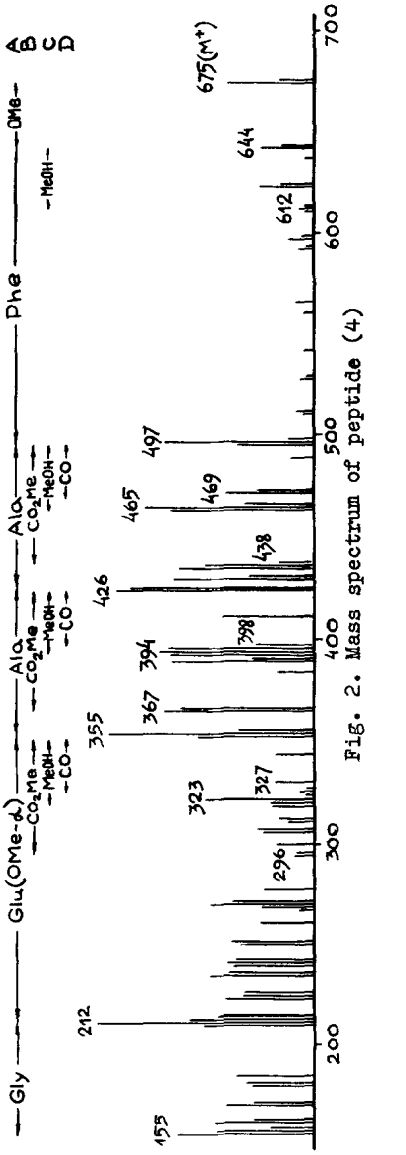


Fig. 2. Mass spectrum of peptide (4)

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