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MASS SPECTROMETRIC DETERMINATION OF AMINO ACID SEQUENCE IN PEPTIDES

III. PEPTIDES CONTAINING ASPARTIC AND GLUTAMIC ACID RESIDUES

N.S.Wulfson, V.N.Bochkarev, B.V.Rozinov*
M.M.Shemyakin, Yu.A.Ovchinnikov, A.A.Kiryushkin
and A.I.Miroshnikov**

Institute for Chemistry of Natural Products,
USSR Academy of Sciences, Moscow, USSR

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In the first communication of this series (1) it has been shown that acylpeptide esters undergo by electron impact the amino acid type of fragmentation characterised by consecutive elimination of amino acid residues, beginning from the C-terminus. A rapid method for determination of amino acid sequence in peptides can, therefore, be developed on the basis of mass spectrometric analysis of acylpeptide esters, readily obtained from free peptides (2).

To make the mass spectrometric method applicable for the structural investigation of any oligopeptides formed on partial hydrolysis of proteins, it was necessary to carry out a systematic study of the influence of the nature of the amino acid residue upon fragmentation of the peptide chain. We have

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*Laboratory of Mass Spectrometry of this Institute

**Laboratory of Antibiotic Chemistry of this Institute

previously exemplified by peptides built up of amino acids involving various aliphatic or aromatic radicals that the change in nature of the latter shows little or no influence on fragmentation of the peptide chain (1,2).

This communication concerns the results of mass spectrometric investigation of peptides containing a residue of monoaminodicarboxylic (aspartic or glutamic) acid, where the location of the positive charge at the ω-carboxylic group might result in different fragmentation routes which would hinder the interpretation of mass spectra.

We have taken mass spectra of a number of especially synthesised methyl and tert.-butyl esters of N-decanoylpeptides containing a residue of esterified dicarboxylic amino acid in different positions of the peptide chain. The decanoyl group was chosen because it had been found to protect reliably the N-terminus of the peptide and to impart the necessary volatility to the peptide (1,2), in good accord with the data reported by Lederer et al. (3-5) on natural lipopeptides.

The present study has shown that the presence of a residue of monoaminodicarboxylic acid in various positions of the peptide chain does not give rise to any deleterious effect. The amino acid type of fragmentation starting with the C-terminus of peptide always dominates whether this residue is at the C-terminus, in the middle of the chain or is N-terminal (Comps. 1-6 in Table 1, Figs. 1 and 2). It will thereby be noted that fragments containing a residue of aspartic acid are usually accompanied with fragments having m/e value smaller by 60m.u. This appears to be due to elimination of ω-carbomethoxyl group

TABLE 1

N	Compound	M^+	The sequence of residues eliminated (m/e of fragments observed)
1	Dec-Ala-Pro-Glu(OMe)-OMe	497	→ OMe (466) → Glu(OMe) (323) → Pro (226) → Ala (155)
2	Dec-$(Ala)_3$-Asp(OMe)-OMe	528	→ OMe (497) → Asp(OMe) (368) → Ala (297) → Ala (226) → Ala (155)
3	Dec-Ala-Asp(OMe)-Gly-OMe	443	→ OMe (412) → Gly (355) → Asp(OMe) (226) → Ala (155)
4	Dec-Asp(OMe)-Val-Ala-Leu-OBu^t	640	→ OBu^t (567) → Leu (454) → Ala (383) → Val (284) → Asp(OMe) (155)
5	Dec-Asp(OMe)-Leu-Val-Val-OMe	626	→ OMe (595) → Val (496) → Val (397) → Leu (284) → Asp(OMe) (155)
6	Dec-Glu(OMe)-Leu-Val-Val-OMe	640	→ OMe (609) → Val (510) → Val (411) → Leu (298) → Glu(OMe) (155)

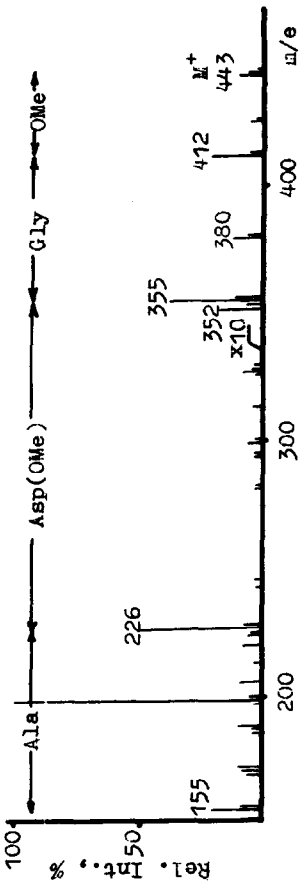

Fig. 1. Mass spectrum of Comp. 3.

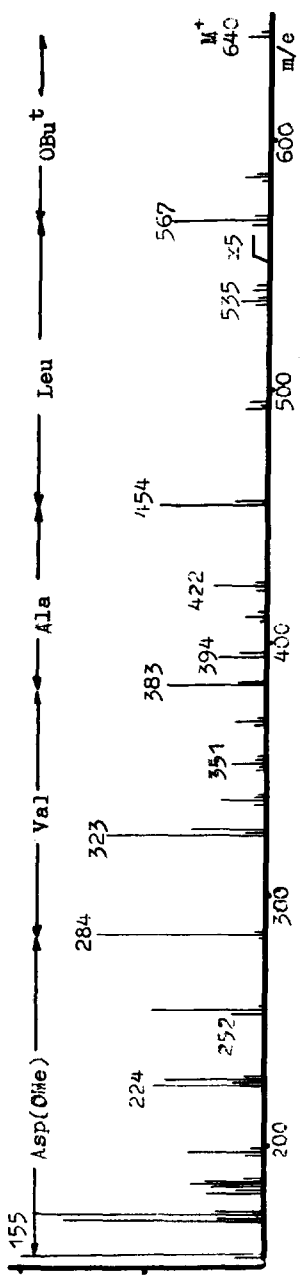

Fig. 2. Mass spectrum of Comp. 4.

and hydrogen atom from the residue of aspartic acid. It is also sometimes possible to observe elimination of methanol (32m.u.) from fragments containing a residue of this acid (Fig. 2). Other details of fragmentation of such peptides giving further information on the structure of the compounds under investigation will be reported in one of our future communications.

Mass spectra were taken at 170-200° and ionizing electron energy of 40eV, with the samples injected directly into the ion source.

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