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MASS SPECTROMETRIC DETERMINATION OF AMINO ACID SEQUENCE IN PEPTIDES
II. A CONVENIENT METHOD OF CONVERTING PEPTIDES TO ACYL-PEPTIDE ESTERS

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The amino acid type of fragmentation of acylpeptide esters we have described (1) makes it readily possible to determine mass spectrometrically the amino acid sequence in these compounds, in contrast to free peptides that are almost non-volatile and apt to undergo random fragmentation. In order to apply effectively mass spectrometry to structural-analytical problems of protein and peptide chemistry it is, therefore, essential to have at one's disposal a convenient and reliable method for converting free peptides to acylpeptide esters.

The direct esterification of peptides being rather involved, it seemed to be more convenient to convert the peptide first to acylpeptide and then to the corresponding ester.

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To this end, we have made use of N-hydroxysuccinimide esters (2) to be able to carry out the reaction in aqueous medium, the most suitable solvent for peptides. In addition, the use of activated esters allows one to do away with a number of side reactions unavoidable in the case of such methods of acylation as, for instance, the acid chloride method. The resulting acylpeptide can be then readily converted to the corresponding ester by diazomethane, isobutylene or any other suitable reagent. Thus, with N-hydroxysuccinimide ester of n-decanoic acid it proved possible to obtain a number of N-decanoylpeptides and then of their methyl esters (Comps. 1-5 in Table 1), the yield of reaction products after purification being above 60%.

It is of importance that mass spectrometric determination of amino acid sequence in peptides does not necessarily require any purification of acylpeptide ester, either by recrystallization or by chromatography. The difference in mass spectra of raw and analytically pure products is rather negligible and appears essentially in the low mass region (Figs. 1 and 2). This is especially important in determining the primary structure of proteins when one is dealing with microquantities of peptides formed on partial hydrolysis.

The method can be used to prepare a great variety of different N-acylderivatives of peptides. Thus, N-ethoxycarbonyloxysuccinimide is capable of converting free peptides to their N-ethoxycarbonyl derivatives, whose esters (such as Comp. 6 in Table 1) possess an advantage over the corresponding decanoyl derivatives in being more volatile and of lower molecular weight.

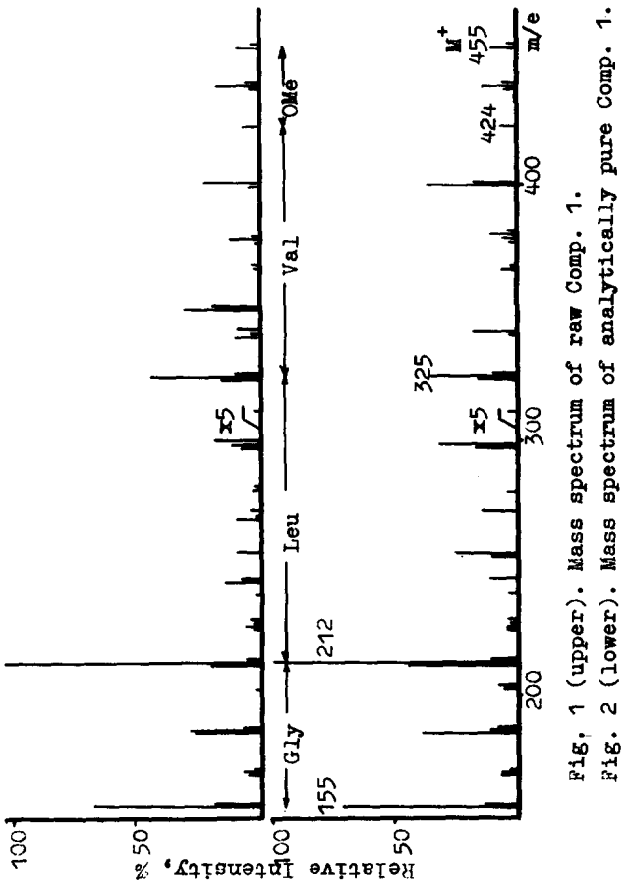

Fig. 1 (upper). Mass spectrum of raw Comp. 1.
Fig. 2 (lower). Mass spectrum of analytically pure Comp. 1.

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N	Compound	Yield (%)	M.p.	M^+	The sequence of residues eliminated (m/e of fragments observed)
1	Dec-Gly-DL-Leu-L-Val-OMe	75	151-152°	455	→ OMe (424) → Val (325) → Leu (212) → Gly (155)
2	Dec-Gly-L-Glu(OMe)-L-Tyr-OMe	61	150-151°	549	→ OMe (518) → Tyr (355) → Glu(OMe) (212) → Gly (155)
3	Dec-Gly-Gly-L-Pro-OMe	63	*	397	→ ProOMe (269) → Gly (212) → Gly (155)
4	Dec-Gly-Gly-L-Leu-Gly-OMe	60	135-137°	470	→ OMe (439) → Gly (382) → Leu (269) → Gly (212) → Gly (155)
5	Dec-L-Pro-DL-Ala-DL-Ala-L-Val-OMe	69	*	524	→ OMe (493) → Val (394) → Ala (323) → Ala (252) → Pro (155)
6	EtOCO-Gly-DL-Leu-L-Val-OMe	68	oil	373	→ OMe (342) → Val (243) → Leu (130) → Gly (73)

*Amorphous compound

The mass spectra of all acylpeptide esters prepared revealed the amino acid type of fragmentation as the dominating one (Table 1 and Figs. 2-4).

REFERENCES

(1) N.S.Wulfson, V.A.Puchkov, B.V.Rozinov, Yu.V.Denisov, V.N.Bochkarev, M.M.Shemyakin, Yu.A.Ovchinnikov, A.A.Kiryushkin, E.I.Vinogradova and M.Yu.Feigina, Tetrahedron Letters 1965, 2805.

(2) G.W.Anderson, J.E.Zimmerman and F.M.Callahan, J.Am. Chem. Soc. 86, 1839 (1964).

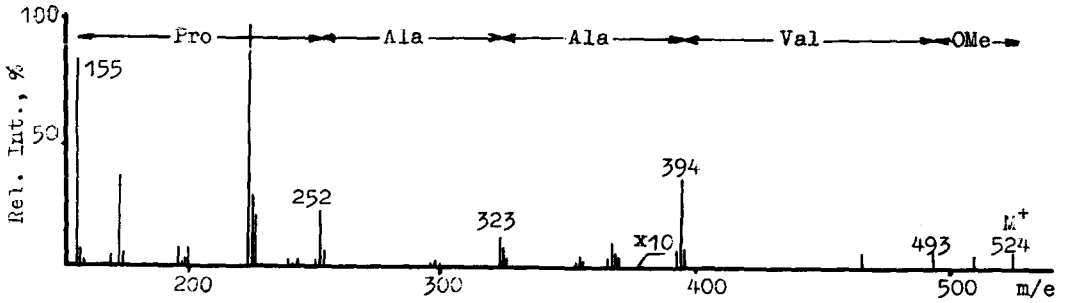

Fig. 3. Mass spectrum of Comp. 5.

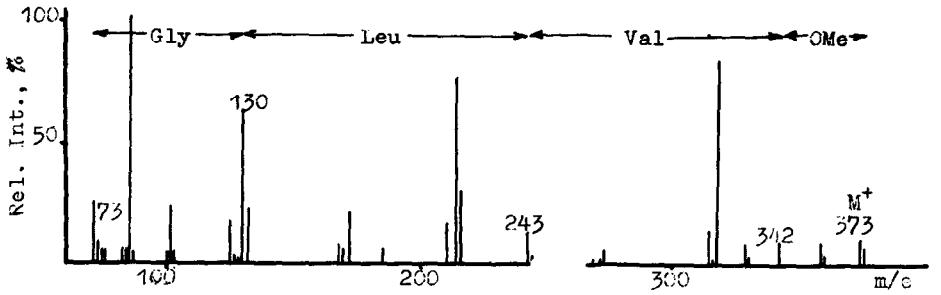

Fig. 4. Mass spectrum of Comp.