MASS-SPECTROMETRIC ANALYSIS OF SOME DERIVATIVES OF α,β-DIDEHYDROTRYPTOPHAN AND PEPTIDES INCLUDING IT

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UDC 547.962

An α,β-didehydrotryptophan (ΔTrp) residue is present in a number of polypeptide antibiotics [1-2] and metabolites of microbial origin [4, 5]. The difficulties in the determination of the amino acid sequences of the antibiotics that are connected with the ready decomposition or modification of the ΔTrp residue in strongly acidic or alkaline media, and also under the action of various nucleophiles and oxidizing agents, are well known. It is obvious that the special properties of the ΔTrp residue mentioned above have been the reason for errors in the determination of its positions in the peptide chains of the antibiotics telomycin [2, 6] and A-128-OP [6, 7]. Consequently, we consider that one of the rational approaches to the investigation of the structures of ΔTrp peptides must be not the chemical but the mass-spectrometric method of analysis. In actual fact, on analyzing the mass spectra (LKB 9000 instrument, 70 eV, 290°C) of the simplest derivatives (I, II) of α,β-didehydrotryptophan and a number of protected peptides containing it (III-VII) (Figs. 1-7), which we had obtained previously [8, 9], it was found that in addition to the peak of the ion (VIII) with m/e 130 that is characteristic for tryptophan [10], there were new peaks of ions with m/e 156 and 157 corresponding to the fragments (IXa, b) (Fig. 8). The spectra of some peptides containing ΔTrp and their derivatives each have a peak at m/e 183 corresponding to the ion (X), which is likewise not observed in the mass spectra of tryptophan peptides. Thus, the strong peaks of ions with m/e 130, 156 (157), and 183 are characteristic for the mass spectra of peptides of α,β-didehydrotryptophan and their derivatives.

The direction of fragmentaiton of peptides (III-V) with a ΔTrp residue at the N-terminus corresponds to the usual amino acid type of fragmentation [11] and begins with the splitting out of the C-terminal amino acid or its fragments with the formation of an ion with m/e 288 probably having the structure of the azlactone of N-benzoyl-α,β-didehydrotryptophan (I).

In the peptides (VI) and (VII) with a C-terminal ΔTrp residue the splitting out of the N-and the C-terminal amino acid residues is observed. However, in these cases, as well, the stable ion of the azlactone of N-acetyl-α,β-didehydrotryptophan (XI) is formed, which is represented by an intense peak with m/e 225 and which subsequently decomposes into fragments

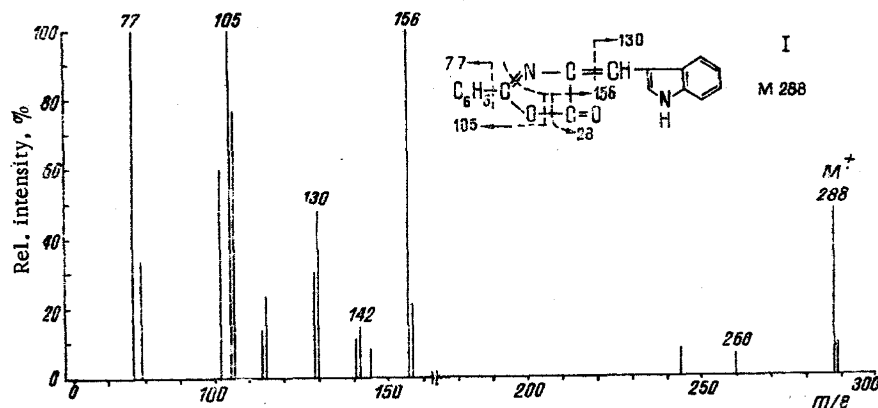

Fig. 1

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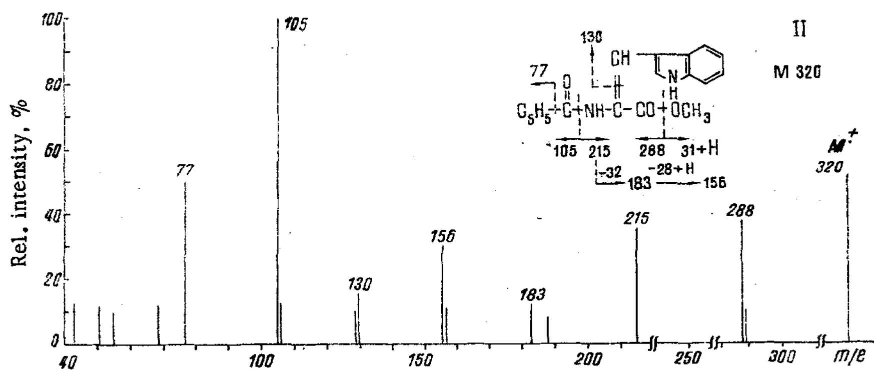

Fig. 2

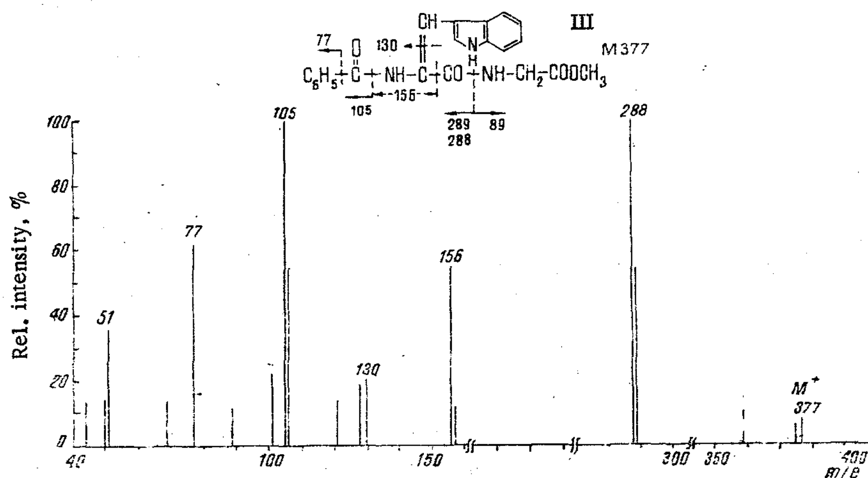

Fig. 3

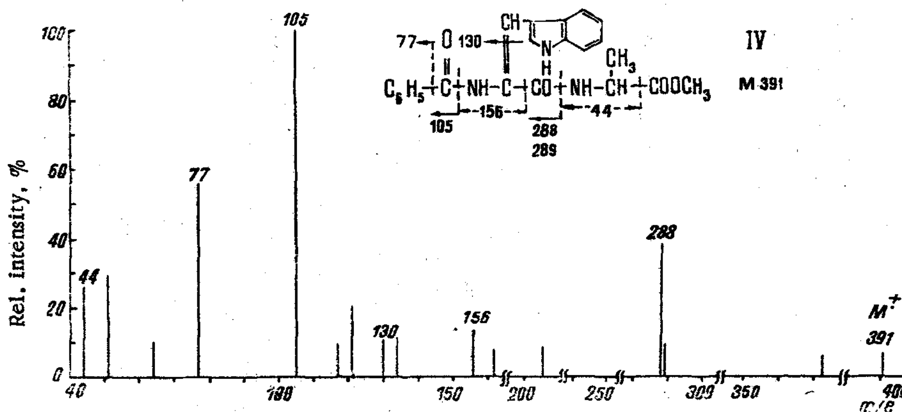

Fig. 4

with m/e 183, 156, and 130 (the characteristic peaks of the ΔTrp residue) and ketene with m/e 42, showing the presence of the -Gly-ΔTrp- sequence in the peptide. The formation in the course of the mass spectrometric fragmentation of ΔTrp peptides of an intense peak of the ion of a N-acyl-ΔTrp azlactone readily permits the determination of the amino acid acylating the NH_2 group of the α,β-didehydrotryptophan, i.e., the sequence -X-ΔTrp in the peptide.

The results obtained in the present work show the promising nature of the use of the mass-spectrometric method both for the identification of the ΔTrp residue in peptides and for

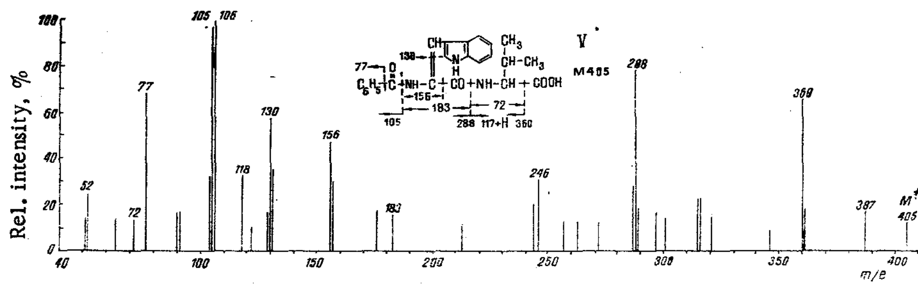

Fig. 5

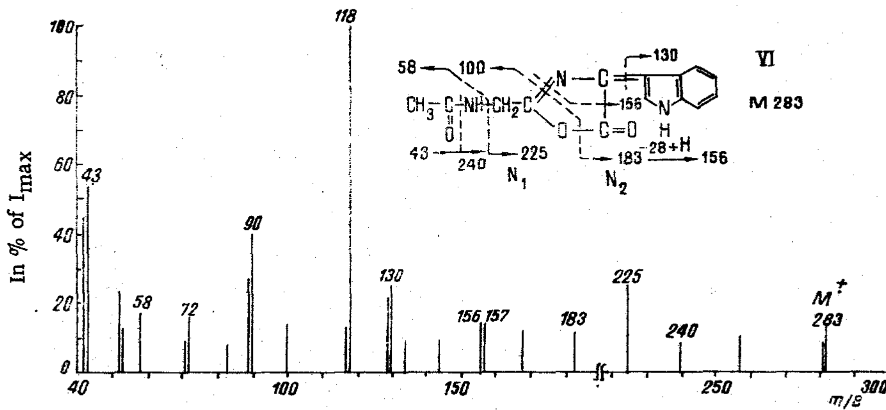

Fig. 6

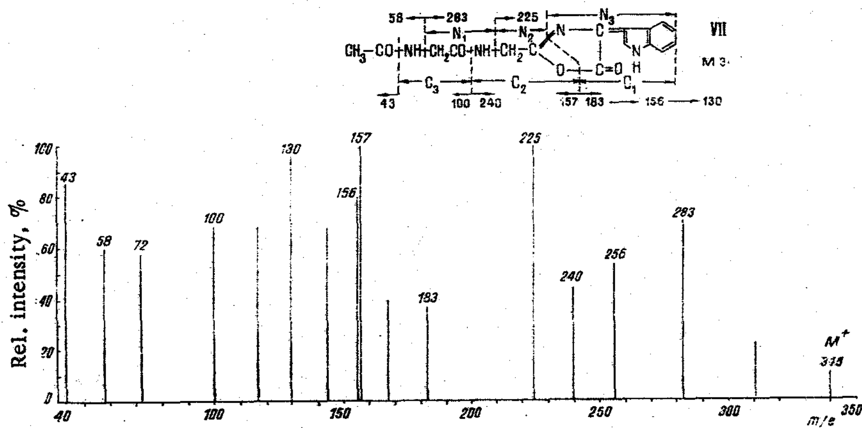

Fig. 7

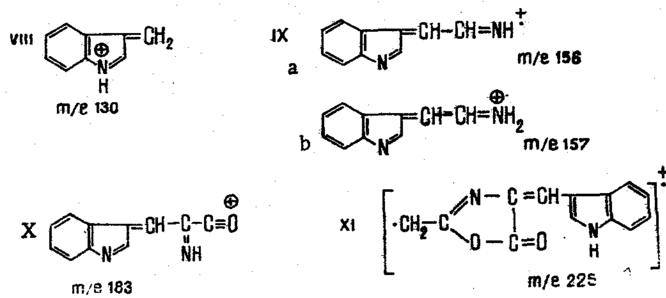

Fig. 8

determining the amino acid sequence of peptides containing a ΔTrp residue. The most important advantage of this method of analyzing the ΔTrp peptides in comparison with chemical methods of analysis is the absence of the side reaction characteristic for ΔTrp, the rapidity and ease of obtaining the results, and the possibility of working with micro amounts of difficulty accessible peptides.

SUMMARY

It has been established that the mass spectra of N-Bz, and N-Ac derivatives of peptides containing α,β-didehydrotryptophan residues have characteristic peaks of ions with m/e 130, 156 (157), and 183; these permit the identification in peptides of the α,β-didehydrotryptophan residue, which is unstable on hydrolysis. In the course of the fragmentation of dehydropeptides by the amino acid route, the formation of the intense peak of the ion of an azlactone of a N-acyl-α,β-didehydrotryptophan is characteristic. The mass-spectrometric method can be used for determining the amino acid sequences of peptides containing α,β-didehydrotryptophan residues.

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SEPHAROSE 4B—DNP-HEXAMETHYLENEDIAMINE AS A SORBENT FOR THE CHROMATOGRAPHY OF CARBOXYLIC PROTEINASES

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We have previously reported the production of the sorbent Sepharose 4B—N-(2,4-dinitrophenyl)-hexamethylenediamine and its use for chromatography of porcine pepsin and pepsinogen and of the carboxylic proteinase of *Aspergillus awamori* (aspergillopepsin A) at pH 5.6 [1]. However, as has been shown for sorbents with peptide ligands, under these conditions a considerable contribution to sorption is made by ionic interactions [2]. At pH 5.6, pepsin and proteinases related to it are charged negatively. The addition of amines to Sepharose activated with cyanogen bromide leads to the formation of isourea derivatives which have pK_a values of about 10 [3] and are charged positively under the conditions of chromatography. This creates the prerequisites for ionic interaction between ligand and enzyme. We set ourselves the task of evaluating the contribution of ionic and hydrophobic forces in the interaction of sorbents containing a DNP group with a number of carboxylic proteinases.

Chromatography of a purified preparation of porcine pepsin with a specific activity of 42-46 activity units/optical unit was carried out on the sorbent Sepharose 4B—DNP-hexamethylenediamine having a ligand concentration of 7.1 μmole/ml. At pH 4.5 and 5.6 in 0.1 M acetate

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