Tetrahedron Letters,Vol.23,No.50,pp 5263-5266,1982 0040-4039/82/505263-04$03.00/0
Printed in Great Britain

STRUCTURE DETERMINATION OF A CYCLIC TETRAPEPTIDE : TENTOXINE BY MASS SPECTROMETRY USING FAST ATOM BOMBARDMENT FOR IONIZATION

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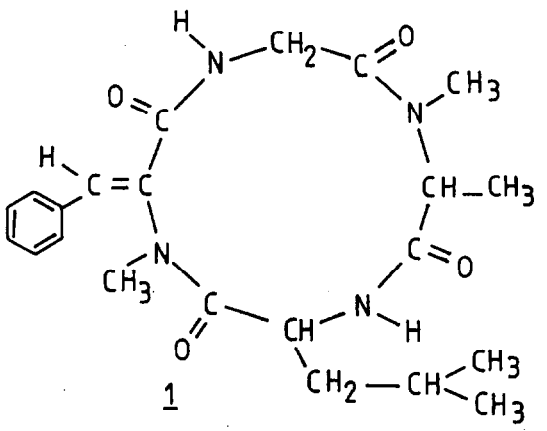
Summary : The molecular mass and the sequence of tentoxine, a cyclic tetrapeptide, are determined by the mass spectrum of this compound which was obtained by a mass spectrometer using the fast atom bombardment for ionization.

We study by use of physiological and biophysical tests mechanisms of phytotoxic action of a cyclic tetrapeptide : tentoxine 1 and some derivatives of this compound. We use mass spectrometry to identify compounds in complex mixtures and in this preliminary note we present possibilities of mass spectrometry for identification of tentoxine 1.



1

$C_{22}H_{30}O_4N_4$ M 414

Before study by mass spectrometry of peptides (compounds with low volatility), it is necessary to synthesisederivatives when electron impact is used for ionization (1). So, the published study on mass spectrum of underivatized tentoxine 1 obtained by electron impact (2) is not significant : there is no molecular ion in this spectrum. The formation of an ion characteristic of structure ($M + \overline{H}^{+}$; $M + \overline{Na}^{+}$; ...) is possible for peptides without derivatization by use of soft methods of ionization (3) and more specially by use of a very interesting me-

Schéma 1

GRAMICIDINE S M 1140

Val – Orn – Leu – Phe – Pro
Pro – Phe – Leu – Orn – Val

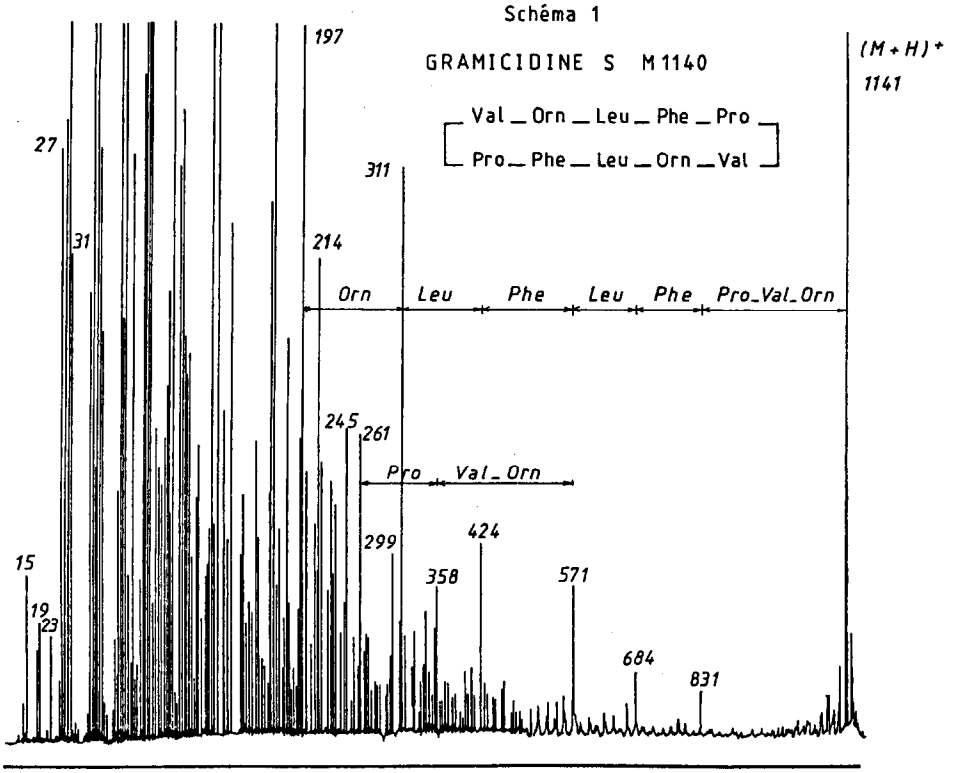
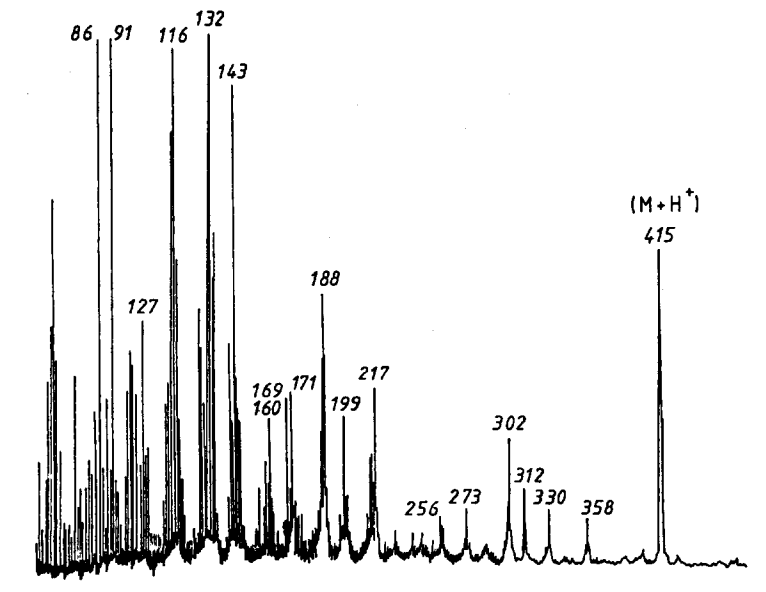


Schéma 2

TENTOXINE $C_{22}H_{30}O_4N_4$ M 414



thod : fast atom bombardment (4,5,6,7,8). This method shows two advantages : the observed signal is not transient as for mass spectra obtained by field desorption and there is neither charge-up of the target not the necessity to use high voltage tension as for mass spectra obtained by secondary ion mass spectrometry.

To prove possibilities of mass spectrometer that we built, we first obtained the FAB mass spectrum of decapeptide : gramicidine S (M 1140). It is possible with this spectrum (Schema 1) identical to already published results (9) to determine sequence. For tentoxine 1, the FAB mass spectrum (Schema 2) allows identification of structure.Masses of four residues are : Gly : 57-N-Methy-Ala : 85-Leu : 113 and Δ-Phe : 159. Ions $\frac{m}{2}$ 132 and $\frac{m}{2}$ 86 are characteristics of leucine : Leu + $\overline{H}^{+}$ and $N^{+}H_2 = CH - CH_2 - CH(CH_3)_2$. Structure of tentoxine is easily established form table. Note that formation of fragment ions in FAB spectra of these two peptides implicates simultaneous cleavage of two bonds.

For the study of complex mixtures we shall obtain in our study, we want to use a triple analyser mass spectrometer (electrostatic sector-magnetic sector-electrostatic sector) equiped with FAB source allowing MS/MS studies (10) for identification, detection and quantitative analysis of a compound in complex mixture without use in a first step of chromatographic method.

Table

OBSERVED IONS	REL. ABUNDANCE *	LOST NEUTRAL
415 : M + $\overline{H}^{+}$	100	-
358 : 415-57	15	Gly
330 : 415-85	17	N-Methyl-Ala
312 : 330-H_2O	24	-
302 : 415-113	40	Leu
273 : 415-85-57	17	N-Methyl-Ala-Gly
256 : 415-159	14	Δ-Phe
217 : 415-113-85	54	Leu-N-Methyl-Ala
199 : 415-159-57	48	Δ-Phe-Gly
143 : 415-159-113	150	Δ-Phe-Leu

* Abundance of ion $\frac{m}{2}$ 415 is choosen like reference.

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(Received in France 16 July 1982)