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THE STRUCTURE OF THE TOXIN FROM HELMINTHOSPORIUM CARBONUM

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ABSTRACT: Using Fast Atom Bombardment Mass Spectrometry and Mass Spectrometry/Mass Spectrometry, the structure of HC-toxin a metabolite of Helminthosporium carbonum, is postulated to be structure 3.

We report here the amino acid sequence of the host specific toxin from Helminthosporium carbonum which attacks corn (HC-toxin). The sequence, determined by mass spectrometry/mass spectrometry (MS/MS) (1) following ionization by fast atom bombardment (2), is different than that recently published by Leish, Sweeley, Stahfeld, Anderson, Weber, and Scheffer (3).

Research directed at elucidation of the HC-toxin dates back to the work of Pringle and Scheffer (4). They postulated that the toxin was a cyclic peptide ($C_{32}H_{50}N_6O_{11}$) containing proline, alanine, and an unknown amino acid. More recently, Leish, et al. have demonstrated that the toxin is a tetrapeptide of alanine (2 residues), proline, and the unusual amino acid, 2-amino-9,10-epoxy-8-oxodecanoic acid (AEO). Independently, and by different methods to be described elsewhere (5), these findings have been confirmed.

Conformation, dictated by amino acid sequence, obviously may be of paramount importance in a host-specific molecule. There are three possible sequences for the amino acids reported for HC-toxin (Figure 1). On the basis of fragmentation during EI-MS, Leish et al. proposed the

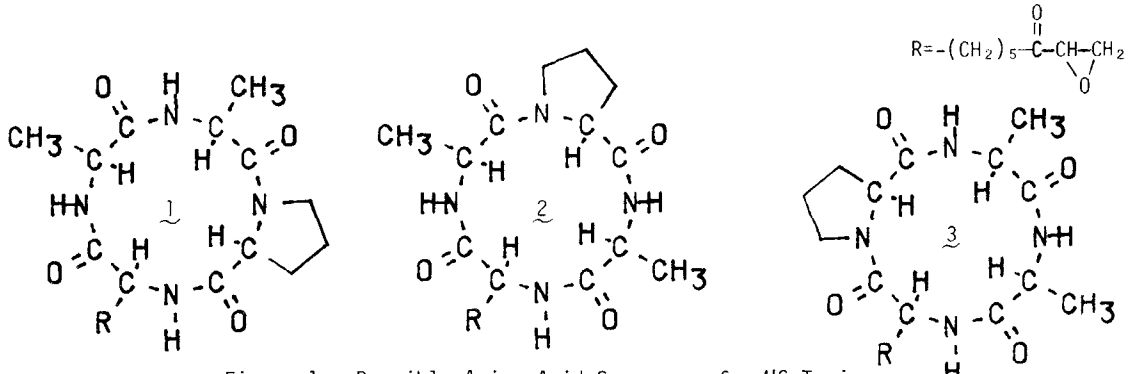


Figure 1. Possible Amino Acid Sequences for HC Toxin (MW = 436)

amino acid sequence labeled 1. However, sequence assignment by EI-MS is equivocable because of the possibility of rearrangements (6). This paper provides evidence that the sequence shown as 3 (Figure 1) is correct.

The elemental composition corresponding to the tetrapeptide has been confirmed by peak matching in both the CI (M+1 obs:437.23958, calc: 437.24001) and EI modes (obs:436.2333, calc:436.2323).

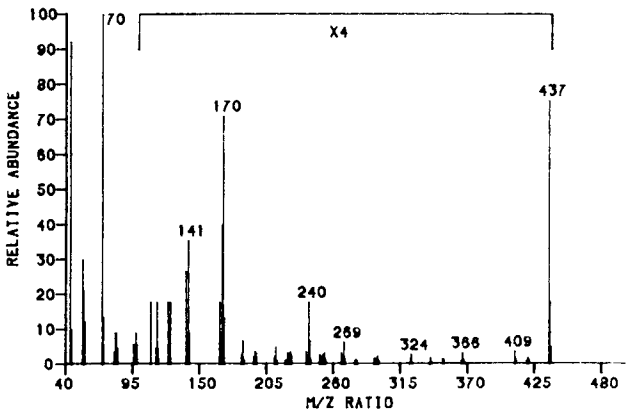


Figure 2. FAB Mass Spectrum of HC Toxin

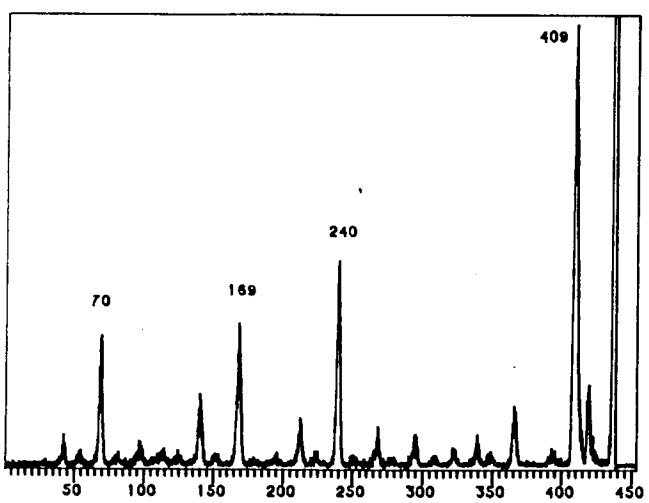


Figure 3. CID Spectrum of the $M + H^+$ of HC Toxin

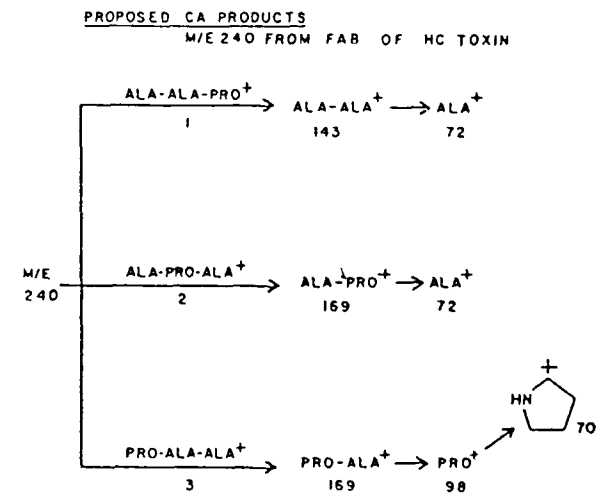
We obtained the FAB spectrum of the HC-toxin (see Figure 2) on a Kratos MS-50 triple analyzer mass spectrometer (7). The protonated molecule, $(M+H)^+$, was observed at m/z 437, and major fragment ions were found at m/z 240, 170, 169, 141.

The collision-induced decomposition spectrum of $(M+H)^+$ was obtained by selecting m/z 437 with MS-I (a double focusing MS), activating by collision with helium, and scanning the spectrum of products using MS-II (an electrostatic analyzer). The spectrum (see Figure 3) showed that the principal decomposition products were at masses 409, 240, 169, and 70. The ion of mass 240 is formed by loss of the epoxyketoamino acid fragment from the ring-opened MH ion and contains two alanine and one proline residues. The m/z 169 ion contains one alanine and one proline residue and loss of CO from this ion yields the m/z 141 ion. The ion at m/z 70 is proline less CO. The ion at mass 409 is due to loss of CO and is not diagnostic.

Since the m/z 240 ion contains the two alanine and one proline residues, its further fragmentation will define the sequence of the three residues. The possible structures of this ion arising from the three possible structures of the parent tetrapeptide along with their expected fragmentations are shown in Scheme 1. The ions of m/z 143 and 72 were not observed in the FAB spectrum while m/z 169 and 70 were found (see Figure 2), which indicates that structure 3 is the correct sequence (8).

Two further experiments were performed to corroborate this observation. The CID spectrum of m/z 240 was taken (Figure 4) and m/z 169, 141, and 70 were observed, in accord with the assignment of structure 3. To ensure that the ions observed in the CID spectrum arise from sequential losses, the CID spectrum of m/z 169 produced uniquely from m/z 240 was taken. This was accomplished by selecting m/z 169 produced by collisional activation of m/z 240 in a collision cell located after the source but before MS-I and taking its CID spectrum using MS-II (9). The result, an MS/MS/MS spectrum, compares favorably with the CID spectrum of m/z 169 formed in the FAB source (see Figures 5A & 5B). This confirms that the CID spectrum of m/z 169 is indeed representative of an ionic fragment of m/z 240 and not of another unanticipated precursor.

Thus, we propose that the normal spectrum produced by FAB ionization and, more importantly, the specific decomposition spectra produced by collisional activation are in accord with structure 3 and not structure 1.



Scheme 1.

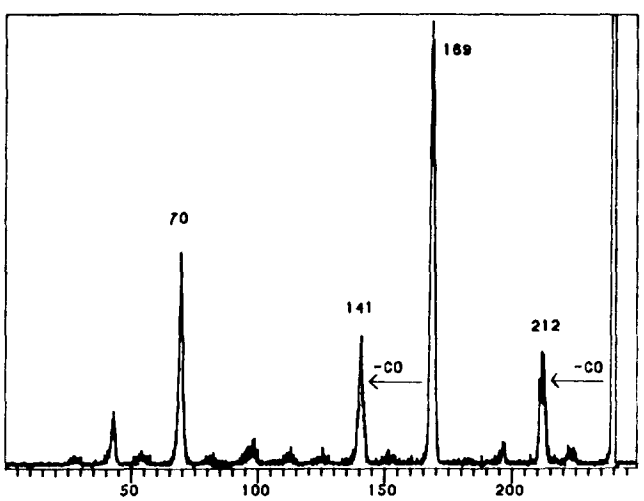


Figure 4. CID Spectrum of m/z 240.

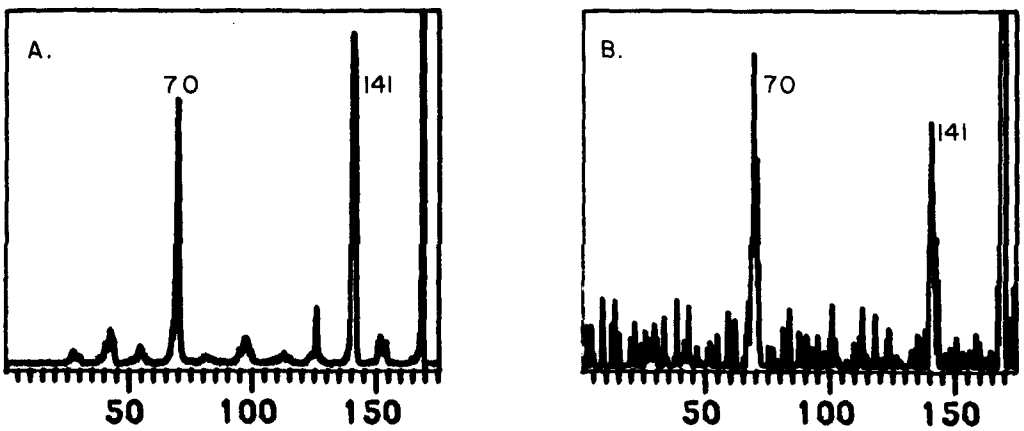


Figure 5A. CID spectrum of m/z 169 found in the FAB mass spectrum of HC Toxin.

Figure 5B. CID spectrum (collision cell 2) of m/z 169 produced uniquely from m/z 240 in collision cell 1.

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