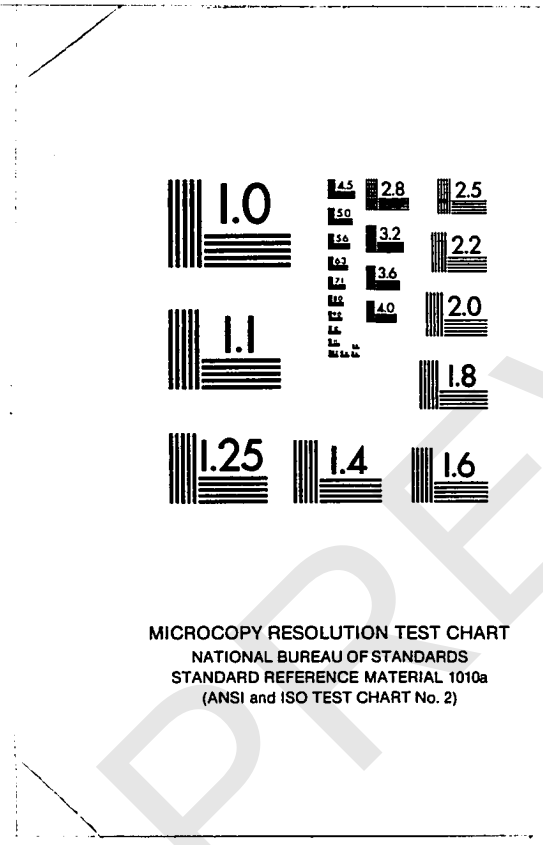

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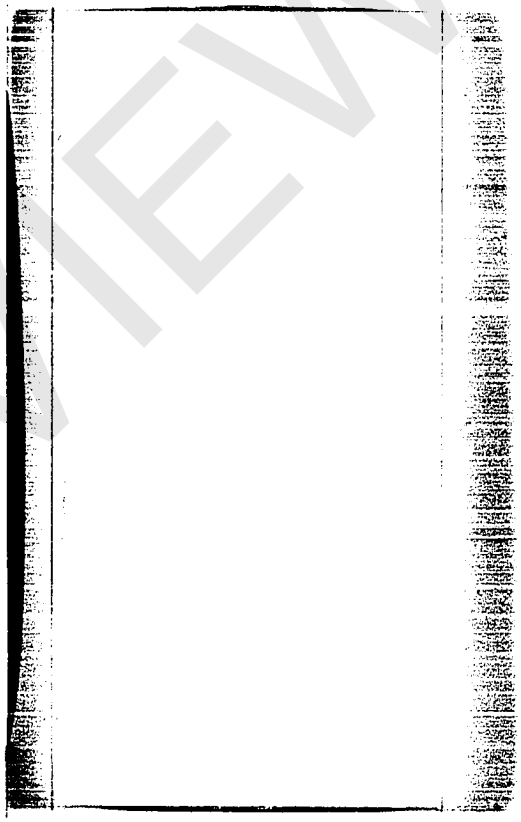

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CHEMISTRY AND BIOLOGICAL ACTIVITIES OF HC-TOXIN AND RELATED CYCLIC TETRAPEPTIDES

The University of Nebraska - Lincoln PH.D. 1986

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CHEMISTRY AND BIOLOGICAL ACTIVITIES OF HC-TOXIN AND RELATED CYCLIC TETRAPEPTIDES

by

Shin-Duk KIM

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Biological Sciences

Under the Supervision of Professor Herman W. Knoche

Lincoln, Nebraska

August, 1986

TITLE

Chemistry and Biological Activities of HC-Toxin and Related Cyclic Tetrapeptides

BY

Shin-Duk Kim

APPROVED	DATE
Herman W. Knoche	July 18, 1986
Robert V. Klucas	July 18, 1986
Robert B. Johnston	July 18, 1986
John P. Markwell	July 18, 1986
Leslie C. Lane	July 18, 1986

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CHEMISTRY AND BIOLOGICAL ACTIVITIES OF HC-TOXIN AND RELATED CYCLIC TETRAPEPTIDES

SHIN-DUK KIM, Ph.D.
UNIVERSITY OF NEBRASKA, 1986

ADVISER: Professor Herman W. Knoche

HC-toxin, a host-specific toxin produced by Helminthosporium carbonum, race 1, is a cyclic tetrapeptide, cyclo (L-Ala-D-Ala-L-Aoe-D-Pro). Aoe stands for an unusual amino acid, 2-amino-8-oxo-9,10-epoxydecanoic acid. Except for the epoxide group, structural features for the toxicity and host-specificity of HC-toxin were uncertain. Therefore, the contributions of various structural features to the biological activities of HC-toxin were investigated by chemical modification of HC-toxin and by comparing the action of HC-toxin to other naturally occurring cyclic tetrapeptides containing Aoe.

Two stereoisomers, produced by reduction of the ketone function in Aoe had no biological activity, suggesting that HC-toxin requires the ketone group for toxicity as well as the epoxide group.

A minor toxin in which glycine replaces the D-alanine residue was isolated. The structure of this minor toxin, called Gly-HC-toxin, was determined to be cyclo (L-Ala-Gly-L-Aoe-D-Pro). Bioassays indicated that the Gly-HC-toxin was only one-thirty-fifth as toxic as HC-toxin, but the toxicity was

specific for HC-toxin susceptible maize cultivars. Effectively, the substitution of a hydrogen atom for a methyl group caused a significant decrease in toxicity. NMR analysis indicated that the ring conformations of HC-toxin and Gly-HC-toxin were nearly identical.

The related cyclic tetrapeptides, CYL-2 and chlamydocin were isolated and their biological activities determined. Chlamydocin and CYL-2 were nearly as toxic as HC-toxin against maize. However, their activities were non-specific. Thus, molecular features that determine toxicity and cultivar specificity appear to be different. Specificity may be due to the overall conformation of the peptide ring.

A chlamydocin analog, containing an alanine residue instead of α-amino isobutyric acid, residue was isolated and characterized.

The binding of HC-toxin to a guanosine, GMP and isolated maize DNA was determined by fluorescence spectroscopy. Changes in fluorescence indicated that HC-toxin alkylated nucleotides and DNA non-specifically.

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Lastly, I wish to extend my deep appreciation to my family for their patience, moral support and sacrifice during my graduate studies in Lincoln.

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ABBREVIATIONS

Abo	2-amino-8-benzyloxyoctanoic acid
Aib	α-aminoisobutyric acid
Aoe	2-amino-8-oxo-9,10-epoxydecanoic acid
CAD	collision activated decomposition
CTAB	hexadecyltrimethylammonium bromide
DMSO	dimethylsulfoxide
EDTA	ethylenediaminetetraacetic acid
EI-MS	electron ionization mass spectrometry
FAB	fast atom bombardment
GMP	Guanosine 5'-monophosphate
HPLC	high performance liquid chromatography
me-Tyr	O-methyltyrosine
NBP	4-(p-nitrobenzyl)-pyridine
NMR	nuclear magnetic resonance
Pip	pipecolic acid
TLC	Thin layer chromatography

INTRODUCTION

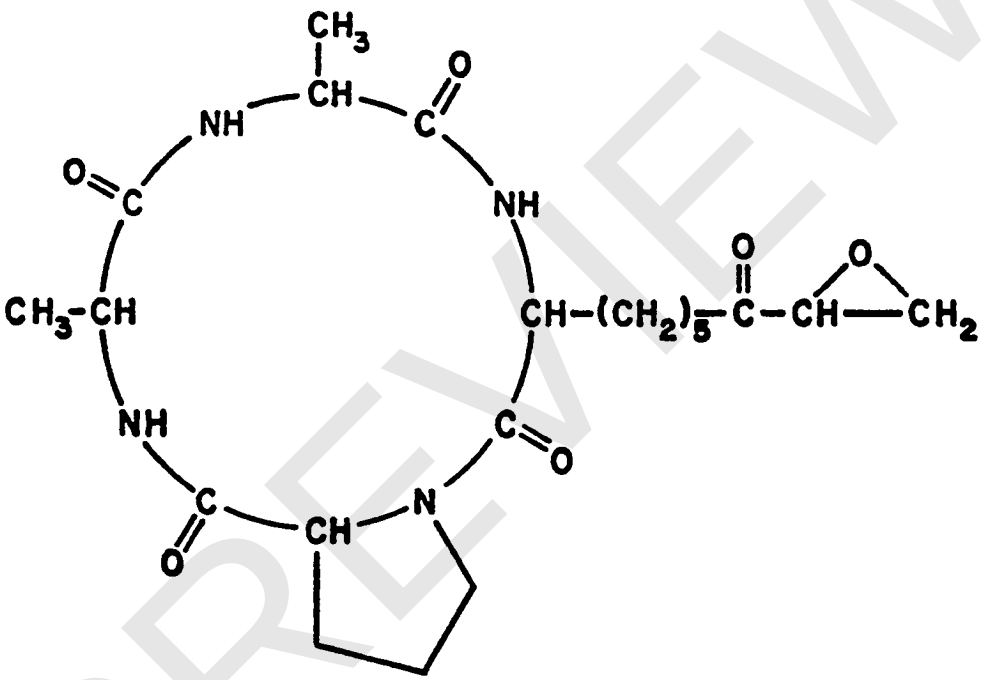
A major concern of plant pathology is to understand the molecular basis of disease development and disease resistance in plants. Obviously,this is a very difficult and complex problem. Progress will depend on division of the problem into simpler components.

Fungal host-specific toxins are valuable tools in the quest for these answers, because they cause all the visible and known biochemical symptoms that are induced in plants by the infecting fungi, without the complicating interactions between pathogen and host. Studies on the mode of action of toxins, therefore, have been accorded great promise for yielding details of the chemical mechanisms of pathogen-induced stress in plants.

The importance of knowing the structure of a toxin and availability of highly purified compounds can hardly be overemphasized since they are necessary for many areas of toxin research. In addition to knowing the primary structure and conformation of toxin molecules, precise chemical modification, the synthesis of structurally similar compounds, and isolation of natural analogs may provide clues concerning the interactions between the toxin and its primary effector site.

The structure of HC-toxin (figure 1), a host-specific toxin produced by Helminthosporium carbonum, race 1, has been determined to be a cyclic tetrapeptide, cyclo (L-Ala-D-Ala-L-Aoe-D-Pro), where Aoe is 2-amino-8-oxo-9,10-epoxydecanoic acid (Liesh et al. 1982, Walton et al. 1982, and Pope et al. 1983). However, cyclic tetrapeptides containing Aoe are not unique to H. carbonum. Cyclic tetrapeptides containing the unusual amino acid, Aoe and an imino acid, either proline or pipecolic acid (Pip), have been found in three other fungi. All of tetrapeptides

Figure 1. The structure of HC-toxin.



HC - TOXIN