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Structure of BZR-cotoxin I Produced by *Bipolaris zeicola* race 3, the Cause of Leaf Spot Disease in Corn

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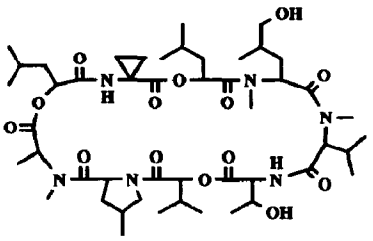
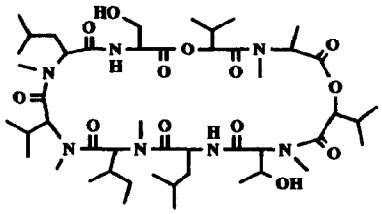
Key Words: *Bipolaris zeicola*; phytotoxicity; susceptibility-inducing factor; cyclic depsipeptide; non-protein amino acid

Abstract: *The structure of BZR-cotoxin I, a component of BZR-toxin produced by B. zeicola race 3, which causes leaf spot disease in corn, was determined to be a cyclic nonadepsipeptide (2).*

The pathogenic races of *B. zeicola*, which is the pathogen of leaf spot disease in corn, have been differentiated according to the symptoms caused on different corn genotypes.[1, 2] *B. zeicola* race 1 produces a host-specific HC-toxin.[3] In 1990 BZR-toxin was isolated as a susceptibility-inducing factor produced by race 3; artificial inoculation of this factor to rice plant induced high pathogenicity. The factor consists of four components, namely BZR-cotoxin I, II, III and IV. While the components separately showed little activity, in combination they exhibited potent phytotoxicity and susceptibility-inducing activity in rice plant.[4] We have undertaken the structural study of BZR-toxin and elucidated the structure of BZR-cotoxin II (**1**).[5] We report here the structure of BZR-cotoxin I (**2**), another component of BZR-toxin.



BZR-cotoxin II (1)



BZR-cotoxin I (2)

Compound **2** was isolated as a colorless amorphous powder, which was soluble in chloroform, acetone,

and alcohols and insoluble in hexane and water. It showed only end absorption in UV-spectrum in methanol. Its molecular weight 967 was indicated from its LSIMS spectrum. The molecular formula was established as $C_{48}H_{85}N_7O_{13}$ by HRLSIMS on the (M+H) ion at 968.6282. In the ^{1}H-NMR spectrum of **2** in $CDCl_3$, four singlet signals at 2.85 ppm (3H), 2.93 ppm (3H), 3.21 ppm (3H) and 3.27 ppm (6H) indicated the presence of five methyl groups connected with amide nitrogen. Two deuterium exchangeable signals at 7.28 ppm and 8.11 ppm were assigned to be amide NH. Some of the signals between 4 and 6 ppm were considered to be α-protons of amino acid residues. The signals around 1 ppm indicated that BZR-cotoxin I included some branched amino acid residues, and the IR spectrum indicated presence of ester bonds besides amide bonds. ^{1}H-NMR spectrum: δ ($CDCl_3$) (ppm) 0.73 (3H, d, J = 6.7Hz), 0.81 (3H, d, J = 6.4Hz), 0.86 - 1.20 (28H, m), 1.07 (3H, d, J = 6.7Hz), 1.20-2.45 (11H, m), 1.25 (3H, d, J = 6.4Hz), 1.39 (3H, d, J = 6.7Hz), 2.85 (3H, s), 2.93 (3H, s), 3.12 (3H, s), 3.27 (3H, s), 3.57 (1H, dd, J = 9.8Hz, 5.8Hz), 3.80 (1H, br.), 4.23 (1H, br.), 4.64 (1H, m), 4.71 (1H, m), 4.86 (2H, m), 5.00 (1H, d, J = 8.5Hz), 5.06 (1H, d, J = 10.4Hz), 5.14 (1H, d, J = 10.7Hz), 5.43 (1H, q, J = 6.7Hz), 5.52 (1H, d, J = 10.1Hz), 7.28 (1H, br.; NH), 8.11 (1H d J = 8.2 Hz; NH). IR spectra: ν max (cm^{-1}) (CH_2Cl_2 soln.) 3430 (br.), 3300 (sh.), 2950, 2890, 1755, 1670, 1645.

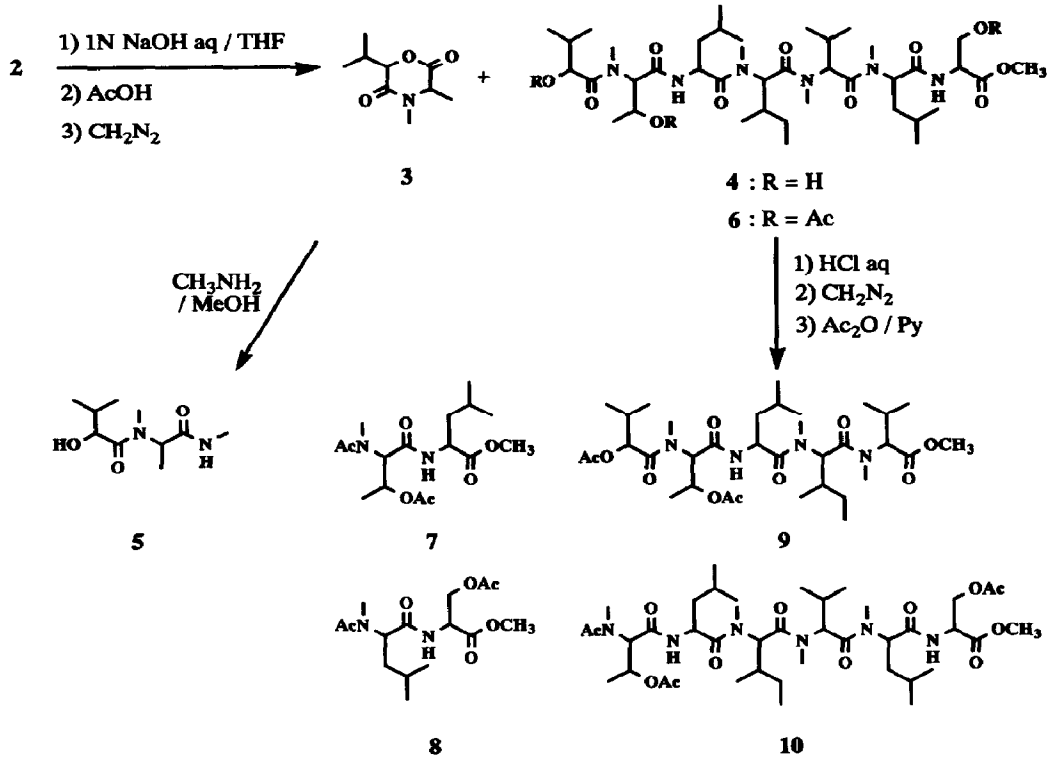
Compound **2** reacted with acetic anhydride under presence of pyridine to give a diacetyl derivative, as judged by two new $COCH_3$ signals, and the shift of the appropriate protons to lower field. Hydrolysis of **2** with 6N hydrochloric acid gave seven ninhydrin positive spots on silicagel TLC. They were identified as *N*-methylvaline, *N*-methylleucine, serine, *N*-methylthreonine, leucine, *N*-methylisoleucine and *N*-methylalanine by comparison of their Rf values with authentic samples. 2-Hydroxy-3-methylbutanoic acid was also isolated from the hydrolyzed mixture and the structure was determined by comparison of its retention time with authentic compounds by HPLC.

Partial hydrolysis of **2** in basic condition followed by neutralization with acetic acid and treatment with diazomethane gave two compounds **3**[6] and **4**[7]. It was determined from ^{1}H-NMR spectrum that compound **3** had no methoxycarbonyl group. Since treatment of **3** with metylamine-methanol solution gave a methylamide **5**[8], compound **3** was determined to be the cyclic didepsipeptide that consisted of *N*-methylalanine and 2-hydroxy-3-methylbutanoic acid residues.

Compound **4** reacted with acetic anhydride under presence of pyridine to give a triacetyl derivative **6**[9], as judged by three new $COCH_3$ groups around 2 ppm and the shift of the four protons to lower field. The ^{1}H-NMR and LSIMS spectra of **6** suggested the presence of residues of 2-acetoxy-3-butanoic acid, *O*-acetyl-*N*-methylthreonine, leucine, *N*-methylisoleucine, *N*-methyvaline, *N*-methylleucine, and *O*-acetylserine. But the peptide linkage could not be revealed because of two couples of amino acid, leucine / *N*-methylvaline and *N*-methylisoleucine /*N*-methylleucine which had the same residue weights 113 and 127

Partial hydrolysis of **4** with hydrochloric acid followed by treatment of diazomethane and acetic anhydride-pyridine solution gave **7**[10], **8**[11], **9**[12], **10**[13] and several products which could not be identified. Compound **7** was determined by its ^{1}H-NMR spectrum to be a dipeptide that consists of *N*, *O*-diacetyl-*N*-methylthreonine and leucine methyl ester. ^{1}H-NMR and LSIMS spectra of compound **8** determined it also to be a dipeptide that consists of *N*-acetyl-*N*-methylleucine and *O*-acetylserine methyl ester.

By the detailed analysis of LSIMS spectrum of **6** and the determination of structures of **7** and **8**, all peptide linkages of **5** and **6** were determined to be as shown in the figure. The structures of **9** and **10**, which were determined by their ^{1}H-NMR and LSIMS spectra also supported the structures of **5** and **6**.



Compound **2** had no carboxyl group since it did not react with diazomethane. Addition of the formulas for **4** ($C_{40}H_{74}N_6O_{11}$) and **5** ($C_{10}H_{20}N_2O_3$) gave $C_{50}H_{94}N_8O_{14}$. The formula for **2** was determined to be $C_{48}H_{85}N_7O_{13}$. Subtraction of the two formulas yielded C_2H_9NO, which was determined to be the sum of a methanol molecule (CH_4O) and a methylamine molecule (CH_5N). Therefore, the structure of BZR-cotoxin I (**2**) was concluded to be a cyclic depsipeptide formed by condensation of **4** and **5** between the carboxyl groups and hydroxyl groups of α-hydroxycarboxylic acid residues.

As stated above, the structure of BZR-cotoxin I was determined to be the cyclic nonadepsipeptide **2** that consists of two 2-hydroxy-3-methylbutanoic acid residues, five *N*-methylated α-amino acid residues and two protein amino acid residues.

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5. K. Ueda, J.-Z. Xiao, N. Doke and S. Nakatsuka, *Tetrahedron Lett.*, **33**, 5377 (1992).
6. ^{1}H-NMR ($CDCl_3$) δ (ppm) 1.00 (3H, d, J = 6.7Hz), 1.12 (3H, d, J = 7.0Hz), 1.64 (3H, d, J = 7.0Hz), 2.37 (1H, m), 2.99 (3H, s), 4.15 (1H, q, J = 7.0Hz), 4.63 (1H, d, J = 4.6Hz).
7. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.73 (3H, d, J = 6.7Hz), 0.77-1.03 (24H, m) 1.00 (1H, m), 1.07 (3H, d, J = 6.7Hz), 1.15-1.70 (6H, m), 1.19 (3H, d, J = 6.4 Hz), 1.78 (1H, m), 1.92 (1H, m), 2.12 (1H, m), 2.37 (1H, m), 3.02 (3H, s), 3.04 (3H, s), 3.07 (3H, s), 3.17 (3H, s), 3.78 (3H, s), 3.82-3.98 (2H, m), 4.30-4.40 (2H, m), 4.58 (1H, m), 4.90 (1H, m), 5.08-5.14 (2H, m), 5.24 (2H, d, J = 11.0Hz) 6.87 (1H, d, J = 7.3 Hz; NH), 7.11 (1H, d, J = 8.5 Hz; NH).
8. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.81 (3H, d, J = 6.7Hz), 1.07 (3H,d, J = 6.7Hz), 1.38 (3H, d, J = 7.0Hz), 1.91 (1H, m), 2.78 (3H, d, J = 4.9Hz), 2.93 (3H, s), 4.23 (1H, br.), 5.12 (1H, q J = 7.0Hz), 6.01 (1H, br; NH).
9. MS (LSIMS) M/Z 963 (MNa^+), 941 (MH^+), 780, 653, 540, 300, 272, 115. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.74 (3H, d, J = 6.7Hz), 0.77-0.94 (21H, m), 0.99 (3H, d, J = 7.0Hz), 1.03 (3H, d, J = 6.7Hz), 1.05 (1H, m), 1.20-1.65 (6H, m), 1.23 (3H, d, J = 6.4Hz), 1.76 (1H, m), 1.95-2.22 (2H, m), 2.03 (3H, s), 2.06 (3H, s), 2.11(3H, s), 2.38 (1H, m), 2.98 (3H, s), 3.01 (3H, s), 3.05 (3H, s), 3.10 (3H, s), 3.77 (3H, s), 4.30 (1H, dd, J = 11.6Hz, 4.0Hz), 4.41 (1H, dd, J = 11.6Hz, 4.0Hz), 4.77 (1H, dt, J = 8.0Hz, 4.0Hz), 4.86 (1H, m), 4.95 (1H, d, J = 6.7Hz), 5.16-5.27 (4H, m), 5.51 (1H, m), 6.52 (1H, d, J = 8.0Hz; NH), 6.77 (1H, d, J = 8.0Hz; NH).
10. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.90 (3H, d, J = 6.4Hz), 0.92 (3H, d, J = 6.4Hz), 1.29 (3H, d, J = 6.4Hz), 1.35-1.65 (3H, m), 2.03 (3H, s), 2.13 (3H, s), 2.97 (3H, s), 3.73 (3H, s), 4.52 (1H, m), 5.17 (1H, d, J = 9.2Hz), 5.50 (3H, dq, J = 9.2Hz, 6.4Hz), 6.45 (1H, br.; NH).
11. MS (LSIMS) M/Z 353 (MNa^+), 331 (MH^+), 170, 43. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.91 (3H, d, J = 6.4Hz), 0.95 (3H, d, J = 6.7Hz), 1.41-1.72 (3H, m), 2.06 (3H, s), 2.13 (3H, s), 2.90 (3H, s), 3.79 (3H, s), 4.29 (1H, dd, J = 11.6Hz, 3.7Hz), 4.53 (1H, dd, J = 11.6Hz, 3.7Hz), 4.77 (3H, dt, J = 3.7Hz, 7.9Hz), 5.16 (1H, dd, J = 8.9Hz, 6.7Hz), 6.88 (1H, d, J = 7.9Hz; NH).
12. MS (LSIMS) M/Z 685 (MH^+), 540, 300, 115. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.76 (3H, d, J = 6.7Hz), 0.82-1.08 (22H, m), 1.18-1.62 (6H, m), 1.26 (3H, d, J = 6.4Hz), 2.13 (3H, s), 2.16 (3H, s), 2.22 (1H, m), 2.93 (3H, s), 3.04 (3H, s), 3.08 (3H, s), 3.70 (1H, m), 4.60 (1H, d, J = 4.6Hz), 4.86 (1H, d, J = 10.4Hz), 4.88 (1H, m), 5.18-5.25 (2H, m), 5.55 (1H, dq, J = 10.4Hz, 6.4Hz), 6.65 (1H, d, J = 8.9Hz; NH).
13. MS (LSIMS) M/Z 863 (MNa^+), 841 (MH^+), 680, 553, 440, 200, 43. ^{1}H-NMR ($CDCl_3$) δ (ppm) 0.74 (3H, d, J = 6.7Hz), 0.80-1.09 (22H, m), 1.20-1.80 (8H, m), 1.24 (3H, d, J = 6.7Hz), 2.02 (1H, m), 2.03 (3H, s), 2.14 (3H, s), 2.38 (1H, m), 2.98 (6H, s), 3.01 (3H, s), 3.06 (3H, s), 3.77 (3H, s), 4.30 (1H, dd, J = 11.6Hz, 4.0Hz), 4.41 (1H, dd, J = 11.6Hz, 4.0Hz), 4.78 (1H, dt, J = 4.0Hz, 7.9Hz), 4.87 (1H, m), 5.18-5.26 (4H, m), 5.46 (1H, m), 6.62 (1H, d, J = 8.5Hz; NH), 6.78 (1H, d, J = 7.9Hz; NH).

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