CHEMISTRY LETTERS, pp. 679-680, 1989.

Structure of Halo-toxin Produced by Phytopathogenic Bacterium, Pseudomonas syringae pv. mori

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Halo-toxin was isolated from the incubated medium of pseudomonas syringae pv. mori which causes halo bright disease against the leaves of mulberry trees. The structure of this compound was revealed to be Pro-Phe-Pro-Gly-Pro-Ile by spectroscopic means and amino acid sequence analysis.

Recent developments and improvements of agricultural techniques have caused new plant diseases. The halo bright disease of mulberry trees is one of this kind of diseases, being recorded in 1971 by one of the authors. The causal bacterium was found to be pseudomonas syringae pv.mori from bacteriological and serological view points.[1] As for phytopathogenic Ps. syringae, it's known that there are several kinds of pathover and each pathover produce each phytotoxin.[2] Thus, we started out to search the phytopathogenic substances produced by the title bacterium, as a part of our chemical studies on phytotoxins.

Pseudomonas syringae pv. mori isolated from infected mulberry leaves was incubated in the King's B medium at 28 °C for 3 days. And incubated medium was extracted with n-butanol and the organic layer was subjected to a combination of chromatographies over Bondapak C-18(40-50% MeOH) and silica gel($CHCl_3$:MeOH:H_2O= 8:2:0.1) to afford a homogeneous compound, designated as Halo-toxin.

Halo-toxin came as a pale yellow powder of $[\alpha]_D^{22}$ -114.0°(c 0.4, MeOH) and exhibited positive ninhydrin test. The ^{13}C-NMR spectrum[3] showed that this compound was composed from phenylalanine, glycine, isoleucine and proline, and furthermore amino acid analysis[4] revealed the composed ratio of these amino acids as 1:1:1:3. The molecular ion peak $[M+H]^+$ at m/z 627 in the positive FAB-MS showed that neither N- nor C-terminals of this peptide attach to other molecule. The result of amino acid sequence analysis[5] confirmed the primary structure of this hexapeptide to be Pro-Phe-Pro-Gly-Pro-Ile.

Although this hexapeptide had already been synthesized by Okai in the course of their studies on bitterness of casein,[6] we synthesized the peptide having the same sequence as shown in Fig.1 for the purpose of the identification. The behavior on TLC, the mass spectrum, the value of $[\alpha]_D$ of Halo-toxin were well

coincided with those of the synthesized peptide.

Lastly, both the natural and synthesized peptides showed toxic activities against mulberry leaves at the concentration of 1γ.

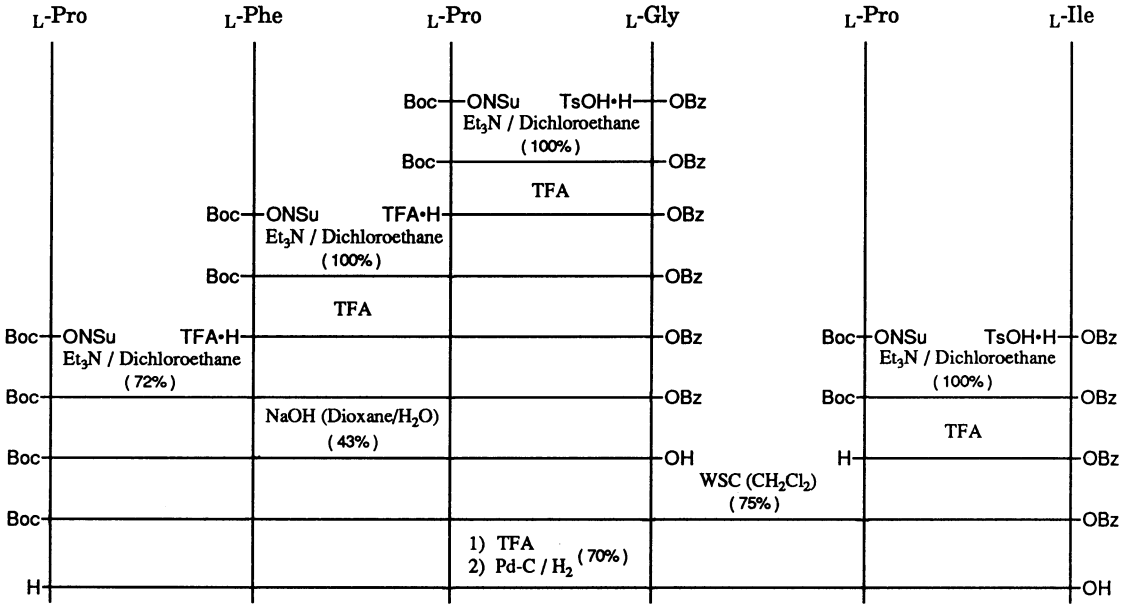

Fig.1. Synthesis of Halo-toxin.

We gratefully thank for the support from Ministry of Education, Sciences, and Culture, Japan (Grant No.633030013) and measurement of amino acid sequence analysis by Dr. Yoji Sengoku(Applied Biosystems Co.).

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3) The ^{13}C-NMR(100 MHz) spectrum was measured in DMSO-d_6 at 90 °C and all signals were assigned as follows, by referring to the report of C.Grathwohl and K.Wüthrich[J. Magn. Reson., 13, 217 (1974)]. δ : 51.7(Phe,α-CH), 36.6(Phe,β-CH_2), 137.2(Phe,Ar-1), 128.1(Phe,Ar-2,6), 129.3(Phe,Ar-3,5), 126.4(Phe,Ar-4), 171.2(Phe,C=O), 41.4(Gly,α-CH_2), 167.1(Gly,C=O), 56.7(Ile,α-CH), 37.6(Ile,β-CH), 24.9(Ile,γ-CH_2), 15.6(Ile,γ-CH_3), 11.1(Ile,δ-CH_3), 169.9(Ile,C=O), 59.9,59.7,59.4(Pro,α-CH), 28.8,28.6,29.9(Pro,β-CH_2), 24.3,24.2,21.8(Pro,γ-CH_2), 46.7,46.3(Pro,δ-CH_2), 172.6,171.2(Pro,C=O).

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(Received January 23, 1989)