Plumbagin, an Antifungal Factor in *Ceratostigma willmottianum* (Plumbaginaceae Plant)*

Michio MASUKO**, Hiroshi EGAWA**, Akinori UHEYAMA**,
Koichi KOSHIMIZU***, Kin-ichi KOBATA****
and Jiro FUMOTO****

益子道生**・江川 宏**・上山昭則**・小清水弘一****・木幡欣一****・麓 次郎*** :
コダチルリマツリの 1 抗かび成分, プルンバギンについて

Under natural conditions, it is hard to find the disease spots on the leaves of *Ceratostigma willmottianum*. The authors supposed that this is due to antifungal substance(s) contained in the plant. As a preliminary test, one gram of fresh leaves was dipped in ten ml of methanol for seven days and then the extract was used to examine the conidia germination of *Cochliobolus miyabeanus*, the leaf blight fungus of rice plants: no conidia germination was observed. This shows that inhibitory substance(s) for the conidia germination would be contained in the plant.

In order to characterize the active principle(s), the following experiment was planned.

After treatment as shown in Fig. 1, an antifungal factor was obtained as yellowish needle crystals. From the melting point (78-79°C), and the UV, IR and NMR spectra, it was identified as 2-methyl-5-hydroxy-1,4-naphthoquinone, Plumbagin which had been already isolated from *Plumbago europae*, *P. zeylanica* and *P. rosea*^{1,2,3}.

To clarify the physiological activity, conidia germination test was performed (Table 1): the result shows zero % in 100 γ /ml and 6.0% in 10 γ /ml. To evaluate the fungicidal activity, two ml containing 100 or 50 γ /ml of the compound was sprayed on each pot of rice seedlings

Fig. 1. Isolation of the antifungal substance from leaves of *C. willmottianum*.

Table 1. Effect of the conidia germination of *Cochliobolus miyabeanus* by the antifungal substance isolated from the leaves of *Ceratostigma willmottianum*

The antifungal substance (γ /ml)	Conidia germination	
	Percentage	Ratio
100	0.0	0
10	6.0	6
1	73.5	78
0.1	88.5	95
0.01	90.7	96
0	94.5	100

* Studies on the antimicrobial substances (1).

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** Pesticide Research Institute, Kyoto University, Kyoto, Japan. 京都大学農薬研究施設

*** Department of Food Science and Technology, Kyoto University, Kyoto, Japan. 京都大学食品工学教室

**** Kyoto Prefectural Botanical Garden, Kyoto, Japan. 京都府立植物園

Table 2. Inhibitory effect of the antifungal substance of *C. willmottianum* to the occurrence of helminthosporium blight disease of rice plant

Concentration (γ /ml)	Number of leaves	Disease spots			Disease outbreak index (Ratio) ^{a)}	Disease development index (Ratio) ^{b)}
		Large	Small	Total		
100	120	181	729	910	0.74 (71)	0.20 (69)
50	120	329	716	1045	0.88 (84)	0.32 (108)
0	120	374	930	1304	1.05 (100)	0.29 (100)

a) Number of spots per one cm leaf length.

b) Large spots/total spots.

Table 3. Antifungal substance found in several species of Plumbaginaceae plants

	Leaf	Stem	Root	Flower
<i>Armeria plantaginea</i>	—	—	—	—
<i>Armeria vulgaris</i>	+	+	+	—
<i>Ceratostigma willmottianum</i>	+	+	+	—
<i>Plumbago capensis</i>	+	+	+	—
<i>Plumbago zeylanica</i>	+	+	+	—

(variety "Kinmaze"). After 24 hours, conidia suspension of *C. miyabeanus* was inoculated by an atomizer, and then the pots were kept in a dark humid chamber at 28°C. The result is shown in Table 2.

The grade of the fungicidal activity was estimated as follows:

a) Rate of disease outbreak: (Total disease spots/leaf length (cm), 71 in 100 γ /ml and 84 in 50 γ /ml (non-treatment 100).

b) Rate of disease development: (Large spots/total spots), 69 in 100 γ /ml and 108 in 50 γ /ml (non-treatment 100).

The authors tried to compare the antifungal substance(s) in five species of Plumbaginaceae plants: one gram each of the roots, stems, leaves and flowers was dipped in ten ml of methanol, and the conidia germination test was performed by using the methanol extracts (Table 3).

As shown in Table 3, no inhibitory effect was recognized from all the parts of *Armeria plantaginea*, and from the flower part of *A. vulgaris*. In the other species, the antifungal substance(s) seemed to be distributed in all parts.

The methanol extract from the roots of five plant species was concentrated *in vacuo* up to the ratio of one gram fresh weight/one ml methanol, and then 0.1 ml of the concentrate was developed by paper-chromatography (*n*-hexane: acetone: methanol=1:1:1 (v/v)). After drying the chromatogram, conidia suspension of *C. miyabeanus* was sprayed on the chromatogram, and then kept in a dark humid chamber at 28°C for five days. The detection

Fig. 2. Paper chromatogram of the antifungal substance found in the roots of several species of Plumbaginaceae.

Paper chromatography: (Paper) Toyo-Roshi No. 51(Developer) *n*-Hexane: Methanol: Acetone=1:1:1 (V/V) Bioautography: (Test organism) *Cochliobolus miyabeanus* (Test method) Conidia germination test Samples: 1. *A. plantaginea*, 2. *A. vulgaris*, 3. *C. willmottianum*, 4. *P. capensis*, 5. *P. zeylanica*.

of the antifungal substance(s) was made under the microscope by searching non-germination zone on the paper chromatogram (Fig. 2).

As shown in Fig. 2, the antifungal bands were recognized from five plant species: two bands from *P. zeylanica*, *C. willmottianum* and *A. plantaginea*, and only one band from *A. vulgaris* and *P. capensis* respectively.

Antifungal band, which coincided with that of Plumbagin, was found in two plant species, *P. zeylanica* and *C. willmottianum*.

Characterization of the other antifungal substances are now in progress.

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和文摘要

コダチルリマツリの 1 抗かび成分, プルンバギンについて

益子道生・江川 宏・上山昭則・小清水弘一・木幡欣一・麓 次郎

コダチルリマツリ (イソマツ科植物) の葉には自然状態において病斑の形成がほとんど認められないことから抗かび成分が存在するのではないかと推定してその単離を試みた。

第 1 図の操作によって生葉 175 g から約 4 mg の結晶を得た。この物質は 2-methyl-5-hydroxy-1,4-naphthoquinone であって、すでに報告されているプルンバギンの化学構造と一致する。イネごま葉枯病菌分生胞子の発芽は 100 γ /ml で完全に阻止されるが, 10 γ /ml では 6% の発芽率をしめた。イネ幼苗にあらかじめ 50-100 γ /ml の液を散布したのち, イネごま葉枯病菌分生胞子を接種したところ, 100 γ /ml では標準区に比して 30% 程度の病斑減少効果が認められた。

つぎにイソマツ科植物 5 種について抗かび成分の有無を検索した。その結果, いずれの植物にも抗かび成分は認められたが, プルンバギンのほかにさらに別の抗かび成分を有する植物もあった。