Title: Miticidal activity of α-pyrones and lactone homologs

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ABSTRACT

The miticidal activities against the house-dust mite, *Dermatophagoides pteronyssinus,* of the leaves, bark and twigs of fig trees (*Ficus carica* L) were studied. The leaves showed the strongest activity among the three parts, and the activity was dose dependent. The larger the weight of each part, the higher the miticidal activity was.

Miticidal activities of coumarin homologs were also studied. Coumarin homologs that have substituents on the coumarin skeleton showed lower miticidal activities than coumarin, suggesting they will not easily make contact with mites because of steric hindrance of bulky substituents. The α-Pyrone skeleton of coumarin homologs is important to have high miticidal activity and the benzene skeleton condensed to α-pyrone contributes to miticidal activity. Psoralen which has a furan ring condensed to coumarin skeleton, showed also high miticidal activity.

Saturated lactones such as γ-butyrolactone, γ-valerolactone and δ-valerolactone have weaker miticidal activities than cyclotene, maltol and crotonic acid which have the α-, β-unsaturated carbonyl system.

KEYWORDS: miticidal activity, α-pyrones, lactones, *Ficus carica*, *Dermatophagoides pteronyssinus*

1. INTRODUCTION

Leaves of the fig tree (*Ficus carica*) which is one of the Moraceae family, have been used as vermicide and for preventing of maggots in pit toilets. The leaves and root bark of fig trees contain high amounts of furocoumarine derivatives, psoralen and bergaptene[1] which have alleropathic activity[2,3] and help to control harmful insects on fig leaves.

It is known that coumarin, which is an α-pyrone derivative, exhibits one of the strongest miticidal activities of any natural product[4,5]. Several α-pyrone homologs have been found in plants and some of those plants are known to be poisonous and have been used in folk medicines, natural insecticides and so on. Psoralen and bergapten occur in the leaves and roots of fig trees as mentioned above. Scopoletin occurs in the root of *Scoplia japonica* Maxim and its roots are poisonous and used as anodyne and eyewash. Daphnetin is obtained by hydrolysis of daphnin which is 7,8-dihydroxycoumarin-7-β-D-glucoside and occurs in the bark and flowers of *Daphne* species.

In the course of our study on the biological activities of plant components against insects[4-8], especially acitivities against mites, we have studied the miticidal activities of α-pyrone homologs and fig leaves, which contain α-pyrone homologs, on the European house-dust mite (*Dermatophagoides pteronyssinus*) known as a causal organism which

causes asthma and atopic dermatitis.

2. MATERIALS AND METHODES

2.1. Plant materials

The leaves, bark and twigs of a fig tree (*Ficus carica* L.) were collected in July 2000 in Tsukuba, Ibaraki, Japan. Fresh plant materials were pulverized to the size of 1 mm or less in a house hold mixer.

2.2. Chemicals

All chemicals used for this experiment were special grade chemicals prepared by Wako Pure Chemical Industries, Ltd.

2.3. Bioassay for miticidal activities

The test mites were adult *Dermatophagoides pteronyssinus* that were cultured in a chamber maintained at room temperature and 70-80% relative humidity on a mixture of powdered mouse food and dry yeast (1:1 w/w). The miticidal activities were examined for each test by using the plate method described in the precedeing report[5]. Each determination was made with six replicates of 20-30 mites. The t-test was used to analyze the differences in the activities between the control and test samples.

3. RESULTS AND DISCUSSION

3.1 Miticidal activities of fig leaves, barks and twigs.

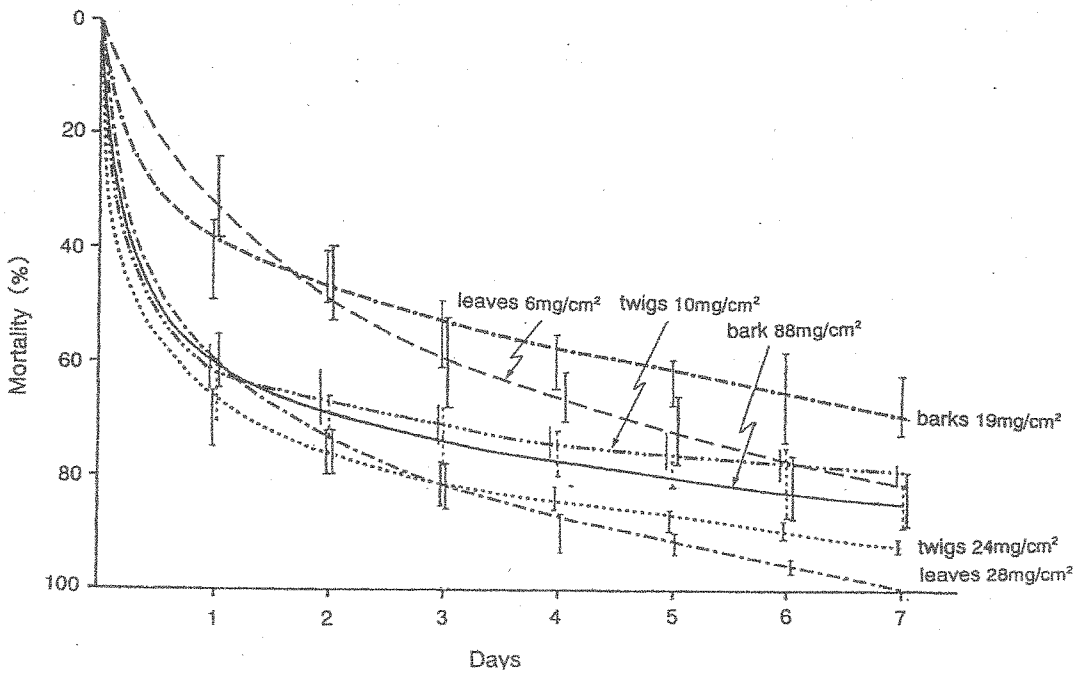

Fig. 1. Time-course of miticidal activities of fig leaves, bark and twigs. Graph shows the mortality based on the control (Mean±SD). Mite: *Dermatophagoides teronyssinus*

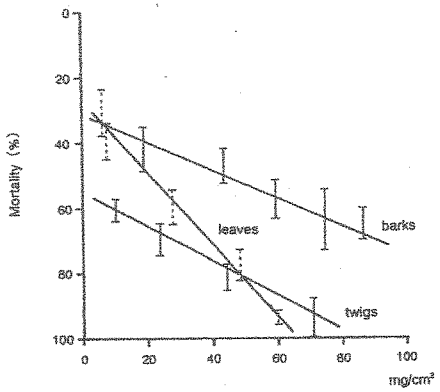

Fig. 2. Miticidal activities of fig leaves, bark and twigs. Graph shows the mortality based on the control (Mean ± SD) after one day.
Mite: *Dermatophagoides pteronyssinus*

Figure 1 shows the high miticidal activities of all three parts of fig trees one day after the tests were started. The miticidal activities remained even after 2 days, but the degree of activity decreased and was smaller than that at the beginning. As a result, the curves of miticidal acitivites showed a gentle slope after 2 days.

Figure 2 shows the dose response activity against mites of fig leaves, bark and twigs. The miticidal avtivites of the three materials were proportional to the weight of the materials, respectively. When the weight became large, the miticidal acitivity increased.

The degree of increase in miticidal activity was highest in the leaves. Water content of leaves, bark and twigs was 63, 22, and 20%, respectively. Therefore, the weight based on oven-dried material for the same weight of fresh materials was the smallest for the leaves, because the leaves had the highest water content. This suggests that the content of miticidal active compounds is larger in the leaves than in the other two parts.

3.2 Miticidal activities of α-pyrone and its homologs

Fig trees, which have strong miticidal activities as shown above have been used to control harmful insects like maggots and are known to be rich in α-pyrone compounds such as psoralen and bergapten. So, α-pyrone analogs were tested for miticidal activities. Figure 3 shows miticidal activities of α-pirones and its homologs. Coumarin shows the highest activity among the compounds in Fig. 3. Miticidal activity of p-coumaric acid, the α-pyrone skeleton of which is open, was lower than that of coumarin,

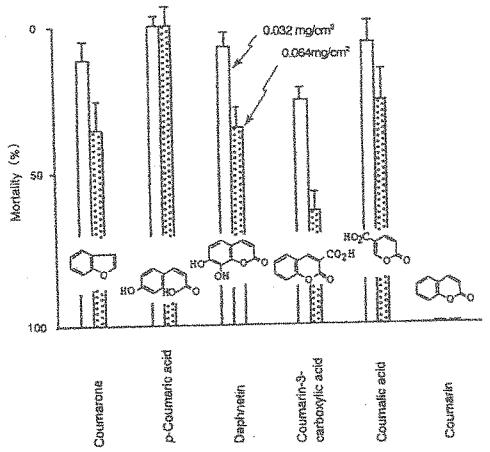

Fig. 3. Miticidal activities of coumarin homologs.
Graph shows the mortality based on the control (Mean ± SD) after one day.
Mite: *Dermatophagoides pteronyssinus*

daphnetin and coumarin-3-carboxylic acid, showing that the α-pyrone skeleton is important for high miticidal activity. Coumaric acid showed lower miticidal activity than coumarin, suggesting that the benzene skeleton condensed to α-pyrone contributes to miticidal activity. Daphentin and coumarin-3-caroxylic acid, which have α-pyrone and benzene skeletons, showed lower miticidal activities than coumarin. This fact suggests that substituents on coumarin make the compounds bulky and so difficult for them to come into contact with mites because of steric hindrance of bulky substituents. As a result, the miticidal activity of substituted coumarin decreases.

3.3 Psoralen and its homologs

Figure 4 shows the time-course of miticidal activity of coumarin homologs, all of which are natural products, on *D. pteronyssinus*. Figure 5 also shows the structures of naturally occurring coumarin homologs. Psoralen exhibited the highest activity at both dosages (100% of mortality after one day) among these three compounds. Scopoletin exhibited relatively weak activity compared with the other two compounds,

With about 60% of mites surviving at 0.032 mg/cm^2 on the third day. It is assumed that the weak activity of scopoletin was caused by the lack of furan ring on its structure, whereas psoralen and bergapten both have a furan ring.

Bergapten exhibited mild miticidal activity one day after the test was

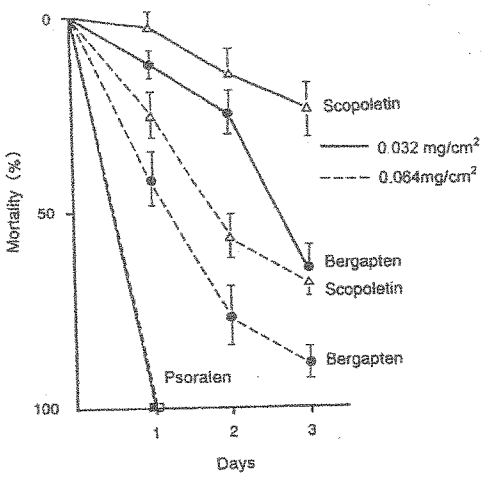

Fig. 4. Effect of coumarin homologs on the activity of *Dermatophagoides pteronyssinus*.

started, though mortality reached to 90% on the third day. The only difference in chemical structure between bergapten and psoralen which exhibited high miticidal activity is whether they have a methoxyl group or not. There is a possibility that the increased size of bergapten by substituting a methoxy group for an aromatic hydrogen of

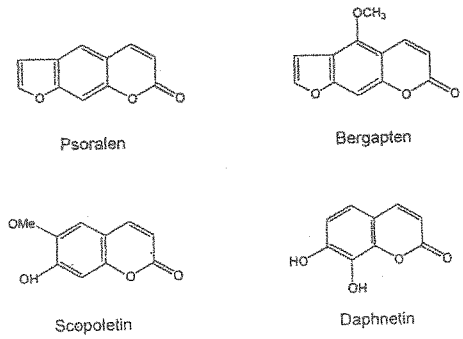

Fig. 5. Naturally occurring coumarin homologs used in this report.

psoralen is disadvantageous for the interaction at the receptor site of a mite. Another reason why a compound substituted with a methoxyl group has less activity than the original one may be the decreased volatility of bergapten relative to psoralen. In this report, bioassay for miticidal activity was done by contact of mites with samples on a piece of filter paper. Therefore, miticidal activity when only the headspace of compounds makes contact with mites will be studied in a future research.

Miticidal activity of psoralen, the lethal dose (LD_{100}) of which was $5x10^{-3}$ mg/cm^2, was slightly lower than that of coumarin (LD_{100} = $4x10^{-3}$ mg/cm^2) observed in this report. These two compounds showed higher miticidal activity than cedrol, which is a sesquiterpene alcohol, and was reported to have high miticidal activity[4].

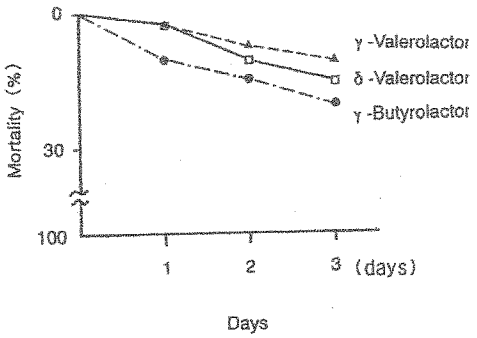

Fig. 6 Miticidal activities of saturated lactones. Graph shows the mortality based on the control (Mean ± SD).
Mite: *Dermatophagoides pteronyssinus*

Table 1 Miticidal activities of lactone analogs

	Amount of sample (mg/cm^2)	0.016	0.032	0.064	0.080
Tetrahydro Furfuryl alcohol		12± 2	22±6	28±5	32±6
γ- Butyrolactone		10±1	20±6	24±6	35±7
γ- Valerolactone		7±3	10±5	25±7	30±2
δ- Valerolactone		5±2	15±2	20±5	33±4
n- Valeric acid	$CH_3CH_2CH_2CH_2COOH$	20±5	25±5	30±3	57±6
Cyclotene		16±4	25±6	55±6	75±5
Maltol		10±2	15±5	46±8	61±7
Crotonic acid	CH_3CH=$CHCOOH$	5±1	35±7	60±7	72±6
Acetonyl acetone	$CH_3COCH_2CH_2COCH_3$	0	0	23±3	38±6

Notes: Figures show the avarage of two replicates of mortality of *Dermatophagoides pteronyssinus* after 3 days.

3.4 Miticidal activities of lactone homologs

As shown in Table 1, saturated lactones such γ-butyrolactone, γ-valerolactone and δ-valerolactone have weak miticidal activities. These three lactones also exhibited a small decrease of survival numbers every day (Fig. 6), while coumaric acid showed mild miticidal activity (mortality after one, two, three days: 6, 28, 47%, respectively). This fact suggests that the α, β-unsaturated carbonyl system in a lactone structure contributes to miticidal activity. Cyclotene, maltol and crotonic acid which have the α, β-unsaturated carbonyl system, exhibited mild activities (80 to 60% mortality at the dose of 0.08 mg/cm^2). Summarizing the results of Table 1, it is considered that the α, β-unsaturated carbonyl structure plays an important role in miticidal activity.

4. CONCLUSION

The leaves, bark and twigs of a fig tree showed high miticidal activities on the European house-dust mite (*Dermatophagoides pteronyssinus*) one day after the tests were started. The leaves showed the strongest miticidal activity among the above three parts, suggesting that the content of miticidal active compounds is larger in the leaves than in the other two parts.

Coumarin showed the strongest miticidal activity among coumarin homologs. The compounds with substituents on the coumarin skeleton showed lower miticidal activities, suggesting that they will not easily make contact with mites because of steric hindrance of bulky substituents. Psoralen showed slightly lower miticidal activity than coumarin (LD_{100} of psoralen: $5x10^{-3}$ mg/cm^2; coumarin $4x10^{-3}$ mg/cm^2).

The miticidal activities of saturated lactone compounds showed weak activities compared with α-pyrone homologs, showing that the α-, β-unsaturated carbonyl structure plays an important role in miticidal activity.

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