Phytogrowth-Inhibitory Activities of β-Dolabrin and γ-Thujapecin, 
Hinokitiol-Related Compounds and Constituents of Thujopsis dolabrata 
Sieb. et Zucc. var hondai MAKINO

Yoshikazu SAKAGAMI, Yoshiko INAMORI, Nami ISOYAMA, Hiroshi TSUJIBO, Toshihiro OKABE, 
Yasuhiro MORITA, and Nakao ISHIDA


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β-Dolabrin and γ-Thujapecin isolated from Thujopsis dolabrata Sieb. et Zucc. var hondai MAKINO, like hinokitiol, showed strong phytogrowth-inhibitory activities, and their growth-inhibitory activities were as high as that of sodium 2,4-dichlorophenoxyacetate used as a positive control. In particular, the phytogrowth-inhibitory activity of γ-Thujapecin was strong and it completely inhibited the germination of this seed of Brassica campestris L. subsp. rapa Hook f. et ANDERS at the concentration of 30 ppm. Both compounds exhibited inhibitory activities on B. campestris L. subsp. rapa Hook f. et ANDERS and Sesamum indicum LINNE, even at the low concentration of 10 ppm. At 7 d after treatment with β-dolabrin and γ-Thujapecin, the amount of chlorophyll in the cotyledons of B. campestris L. subsp. rapa Hook f. et ANDERS treated with both compounds was greatly decreased as compared with the control. The findings indicate that the phytogrowth-inhibitory action might be a common biological activity of hinokitiol-related compounds, suggesting that at least a part of their phytogrowth-inhibitory action seems to be related to a decrease in chlorophyll content.

Key words beta-dolabrin; gamma-Thujapecin; phytogrowth-inhibitory activity; chlorophyll content; hinokitiol-related compound

Hinokitiol (β-thujapecin, Chart 1), the main constituent of Thujopsis dolabrata Sieb. et Zucc. var hondai MAKINO, Thuja plicata and Chamaecyparis taiwensis, has already been reported to show phytogrowth-inhibitory activity. In addition to the phytogrowth-inhibitory activity, many papers have already dealt with the following biological activities of hinokitiol: antimicrobial activity, cytotoxic effect on the growth of mammalian cells and inhibitory activity of catechol-O-methyltransferase. Hinokitiol was also found to be effective as a preservative from the putrescence of vegetable, flower and mushroom, as well as a plant growth stimulator. We recently reported that hinokitiol, β-dolabrin and γ-Thujapecin (Chart 1), hinokitiol-related compounds isolated from Thujopsis dolabrata Sieb. et Zucc. var hondai MAKINO, showed inhibitory activities on carboxypeptidase A, collagenase and thermolysin. Among these enzymes inhibited, carboxypeptidase A has already been found to play an important role in the early stage of germination in the seeds of plants, suggesting that hinokitiol-related compounds seem to be closely related to phytogrowth-inhibitory activity. However, little is as yet known about the biological activity of β-dolabrin and γ-Thujapecin, because of their low yields in comparison with that of hinokitiol. Nor has there been any report about the phytogrowth-inhibitory activity of either compound.

In this work, to expand our knowledge of the phytogrowth-inhibitory activity of hinokitiol-related compounds, the phytogrowth-inhibitory activity of β-dolabrin and γ-Thujapecin was investigated and compared with that of hinokitiol. As a preliminary step to clarify the mechanisms of the phytogrowth-inhibitory activity of hinokitiol-related compounds, attention has been focused on the change in chlorophyll content in cotyledons treated with β-dolabrin and γ-Thujapecin.

MATERIALS AND METHODS

Chemicals Hinokitiol, β-dolabrin and γ-Thujapecin isolated from acid oil obtained by distillation of the wood of Thujopsis dolabrata Sieb. et Zucc. var hondai MAKINO, according to the method of Nozoe et al., were used for the phytogrowth-inhibitory activity test and quantitative analysis of chlorophyll content in the cotyledons of B. campestris L. subsp. rapa Hook f. et ANDERS. Hinokitiol-acetate was also used for the same biological tests. Sodium 2,4-dichlorophenoxyacetate (2,4-D, Tokyo Kasei Kogyo Co., Japan) was used as a positive control.

Plants As in previous papers, Brassica campestris L. subsp. rapa Hook f. et ANDERS, Sesamum indicum LINNE and Echinochloa utilis OHWI et YABUNO were chosen as test plants for the phytogrowth-inhibitory activity test.

Method The phytogrowth-inhibitory activity test was investigated according to the method reported previously. The chlorophyll content in the cotyledons of B. campestris L. subsp. rapa Hook f. et ANDERS treated with β-dolabrin and γ-

\[ \text{γ-Thujapecin} \quad \beta \text{-Dolabrin} \quad \text{Hinokitiol (β-Thujapecin)} \]

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* To whom correspondence should be addressed.
thujaplicin was measured by the A.O.A.C. method.  

**RESULTS AND DISCUSSION**

**Phytogrowth-Inhibitory Effects of β-Dolabrin and γ-Thujaplicin**

The growth-inhibitory effects of β-dolabrin and γ-thujaplicin were investigated using three kinds of plants. Hinokitiol and hinokitiol-acetate were examined as comparative agents for the phytogrowth-inhibitory activity test. As shown in Table 1, β-dolabrin and γ-thujaplicin completely inhibited the germination of seeds of three kinds of plants at the concentration of 50 ppm. The inhibitory activity of both compounds at the same concentration was as high as that of 2,4-D used as a positive control. In particular, γ-thujaplicin inhibited the germination of the seeds of *B. campestris* L. subsp. *rapa* Hook f. et Anders at the concentration of 30 ppm. Although γ-thujaplicin did not completely inhibit the germination of the seeds of *Sesamum indicum* Linne and *Echinocloa utilis* Ohwi et Yabuno at the concentration of 30 ppm, it showed strong growth-inhibitory activity on these plants at the same concentration.

γ-Thujaplicin showed inhibitory activity on three kinds of plants, even at the concentration of 10 ppm. β-Dolabrin also inhibited the germination of the seeds of three kinds of plants at the concentration of 50 ppm. Like γ-thujaplicin, the growth-inhibitory activity of β-dolabrin was as high as that of 2,4-D used as a positive control. Although β-dolabrin did not completely inhibit the germination of the seeds of three kinds of plants at the concentration of 30 ppm, it did show strong growth-inhibitory activity at the same concentration. The growth-inhibitory activity of both compounds was almost equal to that of hinokitiol used as a comparative agent. The growth-inhibitory activity of hinokitiol was as high as that of this compound in the previous paper. In this respect, the phytogrowth-inhibitory activity of both compounds is of considerable interest. The facts also suggest that phyto-growth-inhibitory action might be a common biological activity of hinokitiol-related compounds. Contrary to our expectation, the growth-inhibitory activity of hinokitiol-acetate on *B. campestris* L. subsp. *rapa* Hook f. et Anders was as high as those of other hinokitiol-related compounds, suggesting that the phytogrowth-inhibitory action is due to metal chelation between the carbonyl group at C-1 and the hydroxyl group at C-2 resulting from its hydrolysis. Judging from the findings that hinokitiol-acetate did not show metalloprotease inhibition or antimicrobial activity, we have recently concluded that the mechanism of the biological activity of hinokitiol seems to be due to metal chelation between the carbonyl group at C-1 and the hydroxyl group at C-2 in their tropolone skeletons. Outcomes have also been supported from the fact that in an assay of phytogrowth-inhibition, hinokitiol-acetate is in contact with water for long time, 7 d under irradiation, whereas its contact with water in the assay of enzyme inhibition is only for a few minutes in the dark. Hinokitiol-acetate is also in contact with water for 18 h in the dark in the assay of antimicrobial activity. Therefore, it is considered that in the phytogrowth-inhibitory activity, hinokitiol-acetate is hydrolyzed to hinokitiol, whereas hydrolysis does not occur in antimicrobial activity or inhibitory activity on metalloprotease. Although the phytogrowth-inhibitory activity of hinokitiol has previously been found, these activities of β-dolabrin and γ-thujaplicin are reported for the first time in this paper.

**Toxicity of β-Dolabrin and γ-Thujaplicin to Brassica campestris L. subsp. rapa Hook f. et Anders**

(A) Observation of the Growth Process: As the strong phytogrowth-inhibitory actions of β-dolabrin and γ-thujaplicin has been confirmed, we examined how the growth process of *B. campestris* L. subsp. *rapa* Hook f. et Anders was affected by treatment with β-dolabrin. As shown in Fig. 1, at 1 d after germination, growth differences were detected between the treatment groups and the control group. Thereafter, an growth-inhibitory effect, which increased with the passage of time, was recognized in every treated group. The growth-inhibitory process of *B. campestris* L. subsp. *rapa* Hook f. et Anders by γ-thujaplicin was similar to those of β-dolabrin and hinokitiol (data not shown). However, the inhibitory activity of γ-thujaplicin was higher than that of its isomer, hinokitiol.

(B) Changes in the Amounts of Chlorophyll in Cotyledons: Direct observation revealed that the surface of leaves turned white in every treated group. The leaves of groups treated with 10, 20 and 30 ppm of β-dolabrin and γ-thujaplicin were examined to determine the amount of chlorophyll at 7 d after germination. As shown in Table 2, the amounts decreased in the groups treated with both compounds as compared with the control group. In particular, the chlorophyll content was markedly decreased in groups treated with 30 ppm of β-dolabrin and γ-thujaplicin. The decrease in chlorophyll concentration in cotyledons treated with both compounds was lower than those treated with hinokitiol used as a comparative agent and 2,4-D, a positive
control. Like hinokitiol, 4) β-dolabrin, γ-thujaplicin and hinokitiol-acetate were also found to decrease the chlorophyll concentration in cotyledons of *Brassica campestris* L. subsp. *rapa* Hook f. et Anders (Table 2). The facts suggest that at least a part of the mechanism of the phytochemical-inhibitory action of hinokitiol-related compounds seems to be closely related to a decrease in chlorophyll concentration. However, it is not clear whether the cause of the decrease in chlorophyll concentration is due to (1) the inhibition of chlorophyll biosynthesis or (2) the acceleration of chlorophyll degradation. Studies on the relationship between the phytochemical-inhibitory activity and the decrease in chlorophyll concentration is in progress. Although coumarin, 18–20 γ-aminolevulinates, 21) kinetin, 22) fucoxacin 23) and hinokitiol 4) isolated from higher plants have already been found to decrease chlorophyll concentration, these effects of β-dolabrin and γ-thujaplicin are reported for the first time in this paper.

### REFERENCES