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# Coronatine elicits phytoalexin production in rice leaves (*Oryza sativa* L.) in the same manner as jasmonic acid

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**Abstract**

The phytotoxin coronatine induced the accumulation of the flavonoid phytoalexins sakuranetin and momilactone A in rice leaves. Coronatine-inducible sakuranetin production was under the control of kinetin and ascorbic acid (AsA), as observed with jasmonic acid (JA). The effects of kinetin and AsA on the activity of coronatine indicated that coronatine might elicit sakuranetin production in a manner similar to JA. The similarity of both their structures and the manner of elicitation of coronatine and JA suggest that they might interact at the same active site(s) to lead to phytoalexin production. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* Coronatine; Jasmonic acid; Phytoalexin; Rice plants

## 1. Introduction

The phytotoxin coronatine (**1**, Fig. 1) was isolated from a *Pseudomonas syringae pv. atropurpurea* culture broth as a chlorosis-inducing compound against Italian ryegrass leaves (Ichihara et al., 1977). Coronatine is an amide of coronafacic acid and coronamic acid (Ichihara et al., 1977). The structure of coronatine is somewhat related to that of jasmonic acid (JA, **2**), which is believed to act as a signaling compound in plants (Farmer and Ryan, 1992). JA activates self-defense systems in plants, such as by inducing formation of protease inhibitors and various secondary metabolites (Farmer and Ryan, 1992; Gundlach et al., 1992). Coronatine has a biological activity similar to that of JA, including senescense promotion and induction of formation of various secondary metabolites in plants (Boland et al., 1995; Greulich et al., 1995; Krumm et al., 1995), and thus coronatine is believed to mimic JA (Weiler et al., 1994).

JA-inducible secondary metabolites include antifungal compounds called phytoalexins. Phytoalexin production contributes to the self-defense systems in plants, since they show high antifungal activity and accumulate around infection sites soon after pathogen attack (Mansfield, 1982). Many phytoalexins have been isolated from various kinds of plants and their importance in the self-defense systems in plants has been discussed. In rice plants, momilactone A and B, oryzalexin A–F, and the flavonoid phytoalexin sakuranetin were isolated as phytoalexins (Kodama et al., 1992). Exogenously applied JA elicits the production of phytoalexins, such as momilactone A and sakuranetin (Fig. 2) in suspension-cultured rice cells or whole rice leaves (Nojiri et al., 1996; Rakwal et al., 1996). A rapid rise and fall in the levels of endogenous JA was observed in stressed rice plants, indicating that JA might work as a cellular signal transducer, which activates phytoalexin induction (Tamogami and Kodama, 1998).

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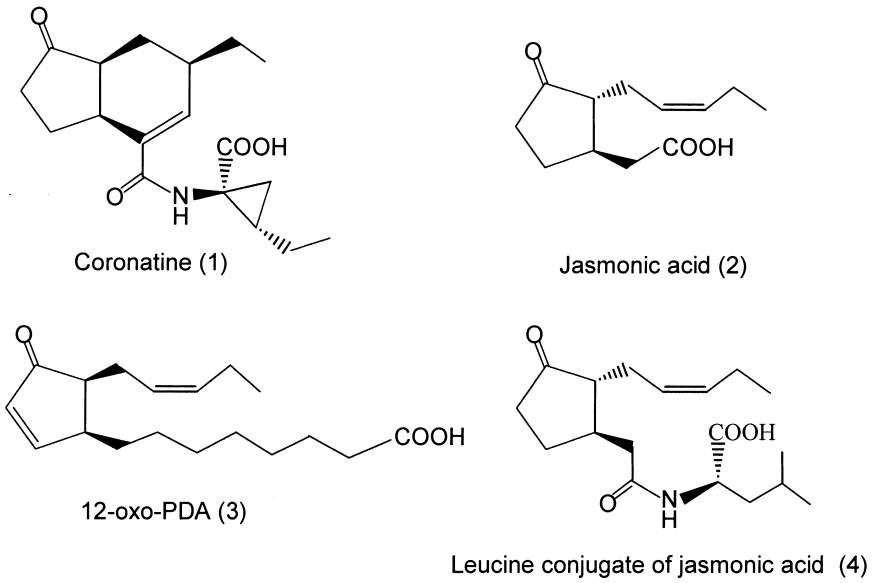


Fig. 1. Structures of coronatine and other related compounds.

If the mode of action of coronatine does indeed mimic that of JA, as described previously (Weiler et al., 1994), then coronatine may also elicit rice phytoalexin production, like JA. It was reported that 12-oxophytodienoic acid (12-oxo-PDA, **3**), an intermediate in JA biogenesis, mimicked JA-inducible phytoalexin production in parsley cell cultures (Dittrich et al., 1992). Thus, we were interested in the elicitor activity of these JA-related compounds in rice phytoalexin production, and investigated how these compounds might elicit sakuranetin production in relation to JA.

## 2. Results and discussion

Exogenously applied coronatine induced the production of sakuranetin in rice leaves (Fig. 3[I]). Coronatine showed a strong activity for sakuranetin production in rice leaves, like JA. The application of 0.05 mM coronatine effectively induced a significant amount of sakuranetin in rice leaves, and this production was dose-dependent. Although linolenic acid, a starting material in JA biosynthesis, was inactive in phytoalexin production (Li et al., 1991), 12-oxo-PDA, a cyclic intermediate in JA biosynthesis, was almost as active as JA in sakuranetin production (Fig. 3[II]). Our previous work showed that amino acid conjugates of JA, such as the leucine conjugate of JA (**4** in Fig. 1) were also active in sakuranetin production, like JA (Tamogami et al., 1997a). The leucine conjugate of JA increased in response to stress in rice leaves (Tamogami and Kodama, 1998). Thus, both a precursor and metabolites of JA were active in sakuranetin production, like JA itself. These JA-related compounds might, therefore, act as a cluster of active functional compounds in rice leaves.

Previous results have shown that coronatine was more active than JA with regard to tuber formation,

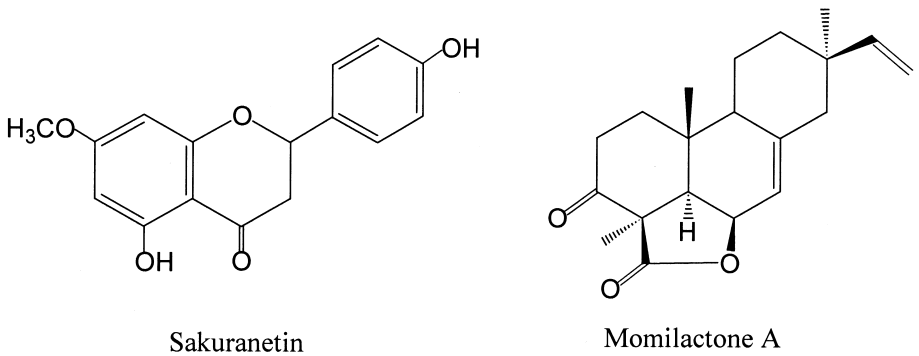


Fig. 2. Structures of sakuranetin and momilactone A.

tendril coiling and the production of other secondary metabolites (Weiler et al., 1994). The much higher activity of coronatine compared to that of JA has been explained by its rigid *cis*-orientation in its bi-cyclic skeleton (Koda et al., 1992; Ward and Beale, 1993; Tappken et al., 1994; Koda et al., 1995). Unexpectedly, coronatine was almost as active as JA for the production of sakuranetin, suggesting that the *cis*-orientation in coronatine did not affect this activity.

In our previous study, JA-inducible sakuranetin production was shown to be affected by substances, such as plant hormones and reductants (Tamogami et al., 1997b). Kinetin and zeatin significantly counteracted JA-inducible sakuranetin production in rice leaves at very low concentrations (Tamogami et al., 1997b). On the other hand, ascorbic acid (AsA), glutathione and tocopherol dramatically accelerated JA-inducible sakuranetin production (Tamogami et al., 1997b). Interestingly, the present results show that coronatine-inducible sakuranetin production is also under the control of kinetin and AsA, as with JA (Fig. 4). Coronatine-inducible sakuranetin production, at a concentration of 0.1 mM, was effectively counteracted by a low concentration of kinetin (25 μM). On the other hand, AsA (5 mM) significantly accelerated coronatine-inducible sakuranetin production (Fig. 4). The effects of kinetin and AsA on the activity of coronatine indicated that coronatine might elicit sakuranetin production in a manner similar to JA. The similarity of both their structures and the manner of elicitation of coronatine and JA suggest that they might act at the same active site(s) to lead to sakuranetin production.

The coronatine-induced production of momilactone A in rice leaves was higher than that induced by JA (Fig. 5). Interestingly, kinetin did not affect the production of momilactone A induced by either coronatine or JA (Fig. 5). These results suggested that the processes for eliciting momilactone A might be controlled differently from those for sakuranetin.

In our previous study regarding sakuranetin production by JA, we proposed that an active oxygen species (AOS) might be important in the elicitation processes, and speculated that hydroxy radical generated by a Fenton reaction might act as an important signaling substance downstream from JA elicitation (Tamogami et al., 1997b). Metal ions are known to catalyze AOS generation by the Fenton reaction, and also to initiate lipid peroxidation (Minotti and Aust, 1987). The effect of a metal-chelating agent, ethylene diamine tetraacetate (EDTA), on sakuranetin production by coronatine and JA was investigated. As a result, 10 mM of EDTA significantly counteracted both coronatine-inducible and JA-inducible sakuranetin production (Fig. 4). These results suggested that coronatine and JA might require metal ions to elicit sakuranetin production, and it was possible that AOS generation or the resulting lipid peroxidation might be involved in the elicitation processes for both coronatine and JA. If AOS is involved in the elicitation by JA, chloroplasts might be associated with such elicitation, since chloroplasts generate AOS by photosynthesis reactions. Chloroplasts are known to be sensitive to cytokinins, coronatine and JA. Cytokinins are known to affect chloroplasts and keep them in long-term good health, while coronatine and JA are known to promote senescence (Ueda and Kato, 1980). This observation implied that the physiological events in

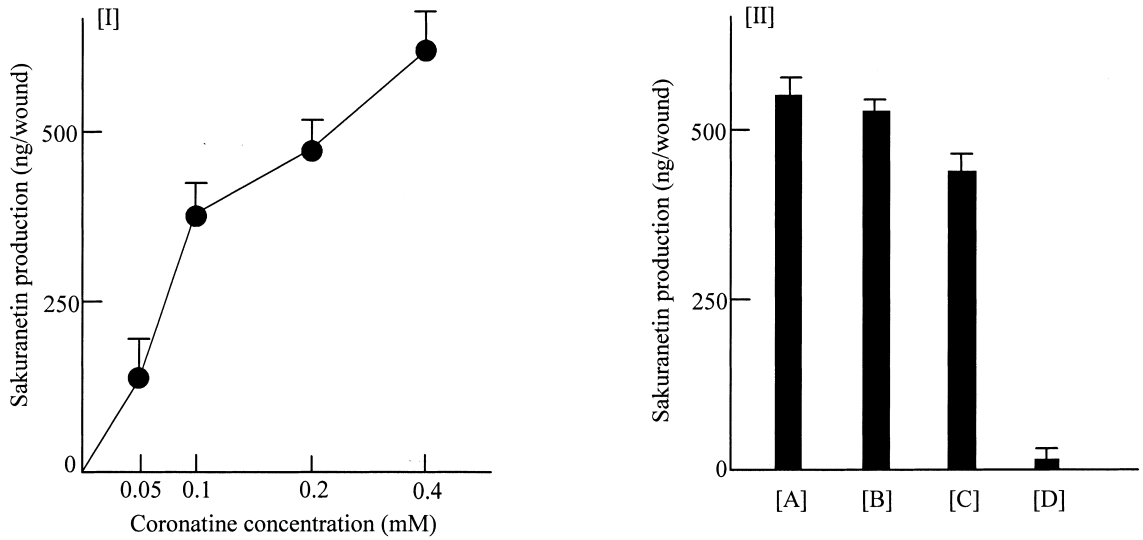


Fig. 3. [I] Dose-dependent sakuranetin production in rice leaves by coronatine. [II] Sakuranetin production by coronatine and other related compounds. [A] Coronatine (0.5 mM), [B] JA (0.5 mM), [C] 12-oxo-PDA (0.5 mM), [D] control (water). Sakuranetin was quantified 48 h after treatments.

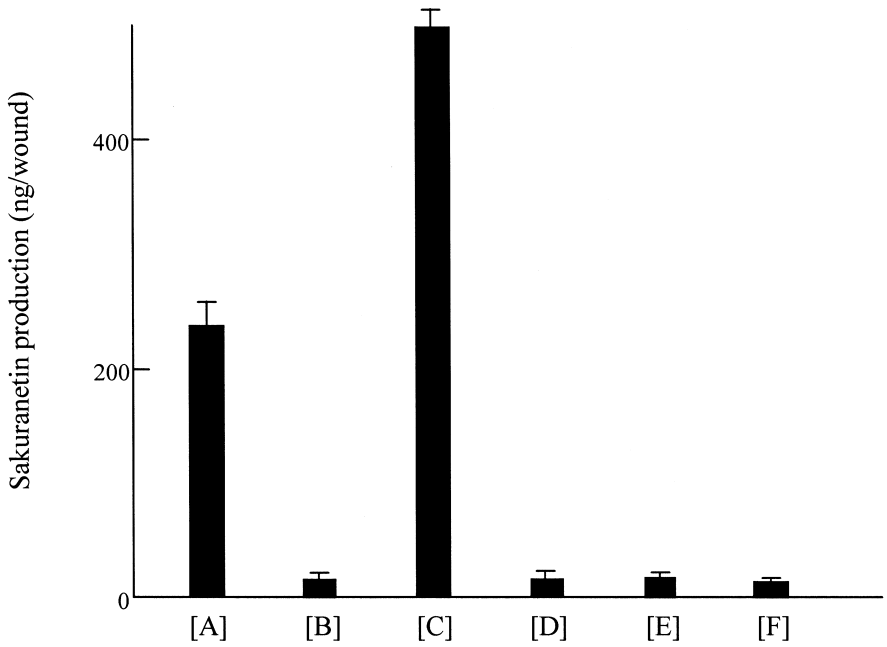


Fig. 4. Effects of kinetin, AsA and EDTA on coronatine-inducible sakuranetin production. [A] Coronatine (0.1 mM), [B] coronatine (0.1 mM) + kinetin (25 μM), [C] coronatine (0.1 mM) + AsA (5 mM), [D] coronatine (0.1 m) + EDTA (10 mM), [E] JA (0.1 mM) + EDTA (10 mM), [F] control (water). Sakuranetin was quantified 48 h after treatments.

chloroplasts might be associated with sakuranetin production.

The present results indicate that coronatine-inducible sakuranetin production is affected by cytokinins and reductants, such as AsA, and provide important information on the action mechanism of the phytotoxin coronatine.

## 3. Experimental

### 3.1. Chemicals

(+)-Coronatine was a kind gift from Dr H. Toshima of Hokkaido University. Racemic JA was prepared according to the method reported by Büchi

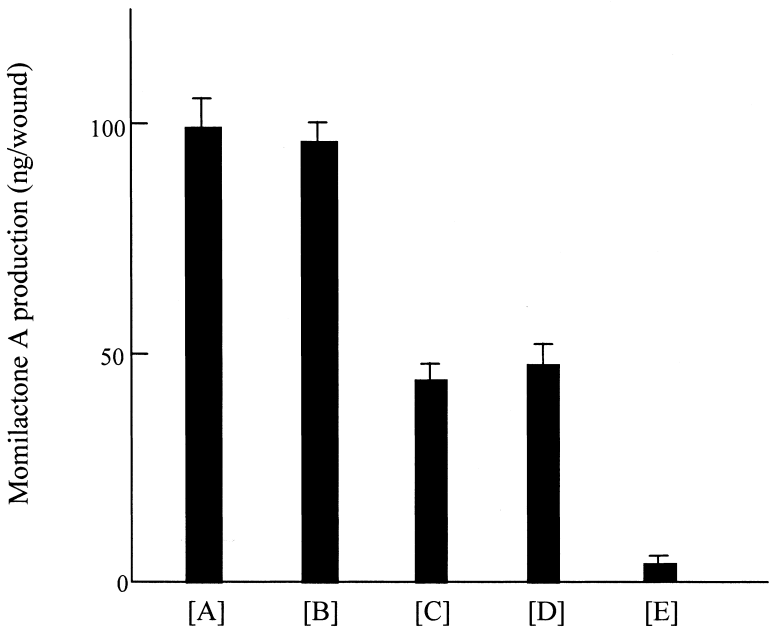


Fig. 5. Effects of kinetin on momilactone A production by coronatine and JA. [A] Coronatine (0.1 m), [B] coronatine (0.1 mM) + kinetin (25 μM), [C] JA (0.1 mM), [D] JA (0.1 mM) + kinetin (25 μM), [E] control (water). Momilactone A was quantified 48 h after treatments.

and Egger (1971), and successive enantiomeric separation of JA was performed as described previously (Kramell et al., 1990). 12-oxo-PDA was purchased from Cayman (Ann Arbor, MI, USA). Sakuranetin was obtained by the selective methylation of naringenin (Aldrich, Milwaukee, WI, USA), as described elsewhere (Aida et al., 1996). Kinetin, zeatin and adenine were purchased from Sigma (St. Louis, MO, USA). Ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid, disodium salt, EDTA and AsA were purchased from Wako (Tokyo, Japan).

### 3.2. Plant material

Rice plants (*Oryza sativa* L. Nipponbare) were cultivated and used as described previously (Rakwal et al., 1996).

### 3.3. Elicitation and quantification of sakuranetin and momilactone A

Droplets (20 μl) of a test solution were applied to press-injured spots on rice leaves. After an appropriate incubation period, sakuranetin was extracted and quantified by HPLC as described by Rakwal et al. (1996). Momilactone A was extracted and quantified by an LC-MS/MS technique. An HP 1100 HPLC (HEWLETT PACKARD, Waldbronn, Germany) equipped with an Inertsil ODS-2 column (4.6 mm × 150 mm, i.d. 5 mm, GL Sciences, Japan) was used. Elution with 80% acetonitrile (containing 0.1% formic acid) was carried out at a flow rate of 0.5 ml/min. A Sciex API-300 (Perkin-Elmer SCIEX Instruments, Foster City, CA) was used for the HPLC MS/MS system equipped with an APCI inlet system in a positive-ion mode. Nitrogen was used as the collision gas. Momilactone A was detected at a combination of *m*/*z* 315/271 in MRM mode.

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