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SIMILARITIES OF THE BIOLOGICAL ACTIVITIES OF CORONATINE AND CORONAFACIC ACID TO THOSE OF JASMONIC ACID

YASUNORI KODA, KIYOSHI TAKAHASHI, YOSIO KIKUTA, FRIEDEMANN GREULICH,* HIROAKI TOSHIMA† and AKITAMI ICHIHARA†

Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan; *Institute für Mikrobiologie, Friedrich-Schiller-Universität Jena, Philosophenweg 12, D-07743 Jena, Germany; †Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

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Key Word Index—*Solanum tuberosum*; Solanaceae; *Glycine max*; Leguminosae; *Avena sativa*; Gramineae; potato tuberization; expansion of potato cells; growth of soybean callus; leaf senescence; coronatine; coronafacic acid; jasmonic acid.

Abstract—Coronatine, a phytotoxin produced by *Pseudomonas syringae* pv. *atropurpurea* and an amide of coronafacic acid and coronamic acid, is known to induce the expansion of cells in potato tubers just as jasmonic acid (JA) does. Furthermore, the chemical structure of coronafacic acid resembles that of JA to some extent. These observations led us to postulate that coronatine and related compounds might have biological activities similar to those of JA. We compared the biological activities of coronatine and coronafacic acid to those of JA in four jasmonate-responsive assay systems, namely, in assays for tuber-inducing activity (with single-node segments of potato stems), for cell expansion-inducing activity (with cells of potato tubers), for cell division-inhibiting activity (with soybean callus) and for senescence-promoting activity (with oat leaves). Coronatine had a positive effect in all these assays and its activity was 100 to 10000 times higher than that of JA in terms of the threshold concentration for activity. Coronafacic acid also gave a positive result in all the assays, but its activity was slightly weaker than that of JA in two assay systems. These results suggest that the special configuration of side chains with respect to the plane of the cyclopentanone ring, namely, the 1*R*, 2*S* configuration in JA and the 3a*S*, 7a*R* configuration in coronatine and coronafacic acid, is necessary for these various biological activities.

INTRODUCTION

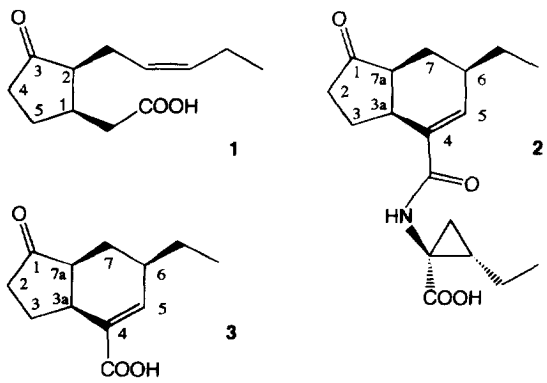
We demonstrated previously that jasmonic acid (JA) is capable of inducing both potato tuberization *in vitro* [1] and the expansion of cells in potato tubers [2], and we suggested that the expansion-inducing activity of JA might be associated with its tuber-inducing activity since tuberization of potato is initiated mainly by the expansion of cells at the sub-apical region of a stolon. The absolute configuration of naturally occurring JA is thought to be 1*R*,2*S*(+ epi-JA, **1**) [3]. Among the four stereoisomers of methyl jasmonate (JA-Me), the 1*R*,2*S* isomer has the strongest activity in each of four assay systems, among which is the assay for tuber-inducing activity [4].

Coronatine (**2**) was first isolated from cultures of *Pseudomonas syringae* pv. *atropurpurea* [5] as a compound that induces chlorosis on the leaves of Italian ryegrass and expansion of cells in potato tubers [6, 7]. This compound is an amide of coronafacic acid (**3**) and coronamic acid, and both moieties are indispensable for the expansion-inducing activity [8]. Since the chemical structure of **3** resembles that of **1** to some extent, it seems likely that **2** and **3** might have biological activities similar to those of JA, such as tuber-inducing activity [9]. Weiler *et al.* [10] reported that **2** induced coiling of tendrils of *Bryonia dioica* just as JA does and that **3** had no such activity.

In the present study, we compared various biological activities of **2** and **3** to those of JA.

RESULTS

Tuber-inducing activities of **2** and **3** were examined in cultures of single-node segments of potato stems *in vitro*. Compound **2** had tuber-inducing activity at concentrations above 10^{-10} M (Fig. 1). Single-node segments failed to survive at concentrations above 10^{-6} M. Furthermore, the tubers induced by **2** were smaller than those induced by JA, suggesting that **2** might be toxic to potato



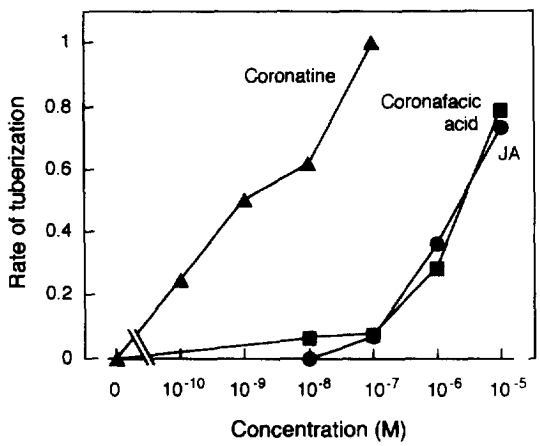


Fig. 1. Effects of coronatine (2), coronafacic acid (3) and jasmonic acid (JA) on potato tuberization *in vitro*. Single-node segments of etiolated potato shoots were cultured aseptically on test media for 3 weeks.

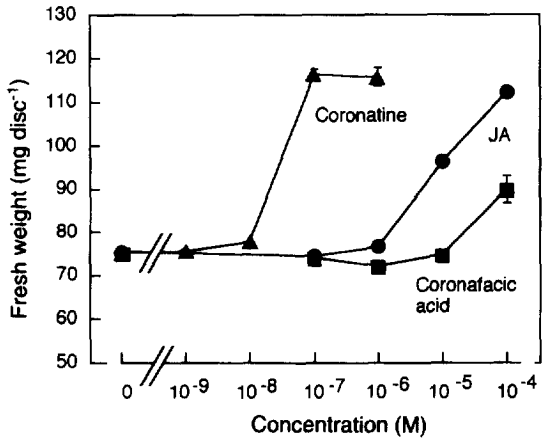


Fig. 2. Effects of **2**, **3** and JA on the expansion of cells in tissue discs of potato tubers. Tissue discs (6 mm $\phi \times 1$ mm) were cultured on test media for 5 days. Increases in fresh weight were due exclusively to the expansion of cells. Each value represents a mean $\pm$s.e. ($n = 20$). The initial fresh weight of tissue disc was 56.0 $\pm$0.5mg ($\pm$s.e., $n = 20$).

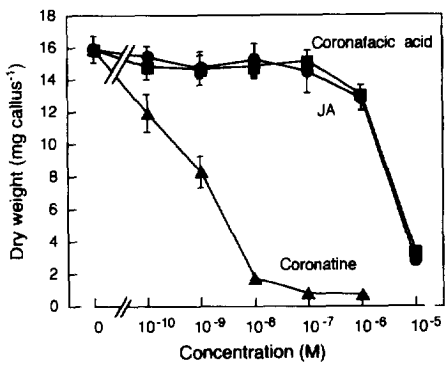


Fig. 3. Eeffects of **2**, **3** and JA on the growth of soybean callus induced by 3×10^{-8} M zeatin riboside. Dry weights of callus were measured after 4 weeks. Each value represents a mean $\pm$ s.e. ($n = 6$).

shoots even if it does have tuber-inducing activity. The activities of **3** and JA were somewhat similar.

To examine cell expansion-inducing activity, tissue discs of potato tubers were cultured *in vitro* for five days on medium that contained each compound. The increase in fresh weight of the discs observed during this culture period was due exclusively to expansion of cells, as reported previously [2]. Compound **2** induced expansion of cells at concentrations above 10^{-8} M (Fig. 2), the threshold concentration for the activity being one-hundredth of that of JA (10^{-6} M). Compound **3** also had similar activity, but its activity in terms of the threshold concentration was lower than that of JA.

JA and JA-Me are known to inhibit cytokinin-induced growth of soybean callus [11]. As shown in Fig. 3, compound **2** at concentrations above 10^{-10} M severely inhibited zeatin riboside-induced growth of soybean callus. The callus barely grew at all at concentrations above 10^{-8} M. The activity of **2** was about 10000 times stronger than that of JA in terms of the threshold concentration for activity. Compound **3** and JA were almost equally effective.

The effects of these compounds on leaf senescence were examined in the oat leaf assay. Compound **2** had senescence-promoting activity at concentrations above 10^{-9} M, but the activity was not very strong and the activity did not increase any further when the concentration was raised above 10^{-7} M (Fig. 4). The activity of **3** was weaker than that of JA.

DISCUSSION

The results presented herein indicate that **2** and its constituent, **3**, have similar biological activities to those of JA. The activities of **2** were always much stronger than those of **3** and JA. Although we cannot explain why the condensation between **3** and coronamic acid, to yield **2**, increases the activities so much, these results do suggest that the special configuration of the attachments at the C-1 and C-2 positions of the cyclopentanone ring,

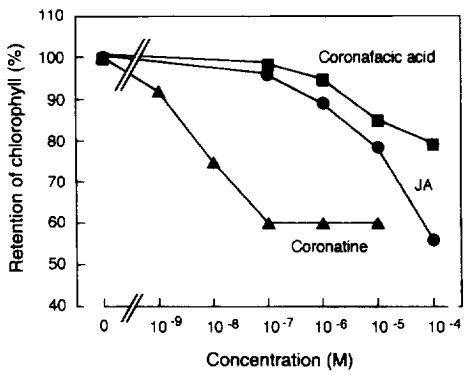


Fig. 4. Effects of **2**, **3** and JA on senescence of oat leaves in the presence of 10^{-6} M benzyladenine. Chlorophyll that remained in the leaves was extracted with hot 80% ethanol 3 days after treatment, and the amount was determined from the absorbance at 665 nm. The value of the control represents a mean ± s.e. ($n = 5$).

namely, the 1*R*,2*S* configuration in JA and the 3a*S*,7a*R* configuration in **2** and **3**, is very important for the various activities. The effectiveness of **3** in inducing potato tuberization and in inhibiting cell division was almost the same as that of JA (Figs 1 and 3). The JA that we used in the present study was a mixture of four isomers and only about 3.5% of the total amount was considered to be the 1*R*,2*S* isomer [12]. This low level suggests the greater effectiveness of 1*R*,2*S*-JA than of **3**.

All the compounds tested herein inhibited the growth of soybean callus (Fig. 3). The strength of the inhibition by **2** was particularly noteworthy. Since the growth of callus is mainly due to cell division, the results indicate that these compounds are all capable of inhibiting cell division. JA-Me disrupts cortical microtubules in potato and tobacco cells [13, 14]. It seems likely that the inhibition of cell division by these compounds is caused by disruption of spindles, which consist predominantly of tubulin.

Shiraishi *et al.* [8] reported that **3** was inactive in inducing the expansion of cells in potato tubers (the authors referred to this phenomenon as a hypertrophy response). In the present study, by contrast, the compound was able to induce the expansion of cells, even though the activity was low (Fig. 2). The difference between results might be due to differences in methodology. Shiraishi *et al.* incubated large discs of potato tubers (20 mm ϕ × 10 mm) with the compounds to be tested for five days under non-sterile conditions, while we cultured small discs (6 mm ϕ × 1 mm) on White's medium that contained the compound to be tested under sterile conditions for the same period of time.

Kenyon and Turner [15] reported that **2** enhanced the production of ethylene in tobacco leaves and discussed the possibility that the various biological effects of **2** might be due to its action as a promoter of ethylene synthesis. Since JA-Me is able to enhance the production of ethylene in tomato and apple fruits [16], it is possible that ethylene is also involved in the various biological activities of JA and JA-Me. However, ethylene has no tuber-inducing activity [17] and does not appear to be involved in the leaf senescence that is induced by JA-Me [18]. It seems unlikely that the various actions of **2** and JA can be attributed to some change in the level or rate of production of a single factor.

Weiler *et al.* [10] showed that **2** induced the coiling of tendrils of *B. dioica*, as did JA, and that **3**, which is structurally similar to JA, was inactive. In their assay system, 12-oxophytodienoic acid (12-oxo-PDA), a precursor to JA, was also active. They pointed out the structural similarity between **2** and 12-oxo-PDA and proposed that **2** might exert its biological activities by mimicking 12-oxo-PDA, rather than JA. However, in the present study, **3** had activity in each of the four JA-responsive assay systems tested. Furthermore, in preliminary tests, we found that 12-oxo-PDA had only very low tuber-inducing activity. These results indicate that the biological activity of **2** is attributable to its structural similarity to JA. These discrepancies suggest differences in the structural requirements for promotion of the individual biological activities. As suggested previously [4], there seem to be different receptors that trigger the reactions that lead to the various different responses.

EXPERIMENTAL

Chemicals. JA was prepd from JA-Me (Tokyo Kasei Co.) by hydrolysis with 1 M KOH. This compound was racemic, as well as a diastereomeric mixt. About 3.5% of the prepn seemed to be the 1*R*,2*S* isomer. Compound **3** was isolated from cultures of *P. syringae* pv. *atropurpurea*, and **2** was synthesized from isolated **3** and optically active coronamic acid, as described previously [19]. The authenticity of **2** and **3** was confirmed by ^{1}H NMR. Both compounds were purified by HPLC before their activities were examined: column, Novapak C_{18}, Radial Pak cartridge, 100 mm × 8ϕ; solvent, 50% MeOH that contained 0.1% HOAc; flow rate, 1 ml min^{-1}; detected at 210 mn. R_t (min), **2** (19.44), **3** (10.90).

Bioassays. Bioassay for tuber-inducing activity was carried out using cultures of single-node segments of potato stems (*Solanum tuberosum* L. cv. Irish Cobbler) *in vitro*, as reported previously [20]. Induction of the expansion of potato cells was examined with cultures of tissue discs of potato tubers *in vitro*, as reported previously [2]. Senescence-promoting activity was determined by the oat leaf assay (with leaves of *Avena sativa* L. cv. Victory No. 1) and cell division-inhibiting activity was tested by the soybean callus assay [with callus derived from cotyledons of *Glycine max* (L.) Merril. cv. Acme], respectively, as reported previously [4]. All assays were repeated at least twice, and results were fully reproducible in each case.

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