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Dual function of coronatine as a bacterial virulence factor against plant: possible COI1-JAZ-indipendent role

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Coronatine (COR, **1**) is a phytotoxin and structural mimic of the plant hormone (+)-7-*iso*-jasmonoyl-L-isoleucine (**2**). COR (**1**) functions as a ligand of the COI1-JAZ co-receptor, which is the exclusive receptor of **2**. Recently, a new role for **1** as a plant virulence factor for *Pseudomonas syringae* attracted the attention of plant scientists. Bacteria invade the plant apoplast through stomatal pores. The host plant then responds to the bacterial invasion by closing the stomatal pores (stomatal defense). COR (**1**) functions as a bacterial chemical weapon that secures the path of infection by reopening the closed stomata. The mechanism is thought to involve inhibition of abscisic acid (ABA)-signaling through the COI1-JAZ pathway. Thus, **1** plays an important role in plant-microbe interactions by abrogating the plant immune response. In this study, we synthesized seven analogues of **1** with naturally occurring α-amino acids and assessed their effect on stomata in a model plant, *Arabidopsis thaliana*. Structure-activity relationship studies of the analogues coupled with genetic studies and *in silico* docking analyses with COI1-JAZ strongly suggested that stomatal reopening induced by **1** may not rely on the COI1-JAZ signaling pathway. Our results suggest that stomatal reopening is triggered by **1** in conjunction with the conventional COI1-JAZ mode of action.

1. Introduction

Coronatine (COR, **1**, Figure 1) was first isolated as a phytotoxin produced by *Pseudomonas syringae*[1] and has played an indispensable role in the development of jasmonate bioscience.[2] COR (**1**) functions as a stable mimic of (+)-7-*iso*-jasmonoyl-L-isoleucine (JA-Ile, **2**), a genuine plant hormone involved in the biotic and abiotic stress responses of plants. JA-Ile (**2**) induces a protein-protein interaction (PPI) between the F-box protein COI1 and JAZ transcriptional repressor protein.[3, 4] This ligand-dependent PPI triggers the proteasome-mediated degradation of the JAZ repressor to release MYC transcription factors, which trigger the expression of JA-responsive genes.[2, 5-7] This process is known as the COI1-JAZ signaling pathway and is now considered the exclusive signaling pathway of **2**. Both **2** and **1** function as PPI inducers and facilitate the ligand-dependent assembly of the COI1-2/1-JAZ complex.[8]

A new role for 1 as a bacterial virulence factor has recently been proposed in the field of plant science. Pathogenic bacteria invade the plant apoplast via the stomatal pores. After bacterial entry, the host plant responds by closing the stomatal pores[9-11] in a process known as stomatal defense. However, plant pathogens are capable of forcing the stomata to reopen to facilitate entry and establish an infection. *Pseudomonas syringae* cells produce **1** in order to trigger stomatal reopening so that they can enter susceptible plants.[12] As **1** is known to be a mimic of **2**, it is believed that stomatal reopening[13] is under the control of the COI1-JAZ signaling pathway.[9, 14]

COR (**1**) is composed of two components, coronafacic acid (CFA, **3**), which acts as an acid, and coronamic acid (CMA, **4**), which is an α-amino acid. We previously reported the total synthesis of **1** and several derivatives, including (±)-**3**, and assessed their effect on stomatal reopening in *Ipomoea tricolor*.[15] Structure-activity relationship (SAR) analyses showed that the CFA moiety is more important than the CMA moiety for the stomatal reopening activity of **1**.

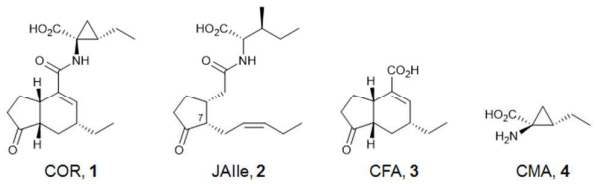

Figure 1. Structures of coronatine (COR, **1**) and related compounds.

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In this study, we synthesized seven COR analogues in which **4** was substituted with naturally occurring α-amino acids and assessed the stomatal reopening activity of the analogues using *Arabidopsis thaliana* as a model plant. SAR studies of the analogues coupled with genetic studies and *in silico* docking analyses with the COI1-JAZ co-receptor strongly suggest that stomatal reopening induced by **1** is not dependent on the COI1-JAZ signaling pathway.

2. Results and discussion

Coronafacyl amino acids: synthesis and biological evaluation

We carried out SAR studies of **1** for stomatal reopening activity using guard cells of *A. thaliana* as a model plant. We synthesized a racemic mixture of methyl coronafacate (**5**) using our previously reported procedure,[16] with minor modification (Scheme S1). The resulting (±)-**5** was then separated by chiral HPLC using a CHIRALPAK IA column to obtain naturally occurring (+)-**5** with an optical purity of 99.3%*ee* (Figure 2A). After hydrolysis, (+)-**3** ($[\alpha]_D^{23}$ = +120°; lit.[17] $[\alpha]_D^{22}$ = +122°) was coupled separately with seven naturally occurring L-amino acids (Figure 2B). These CFA-amino acids (CFA-AAs, **6**-**12**; CFA coupled with L-Ala, L-Val, L-Ile, L-Leu, L-Phe, L-Tyr, and L-Gln) were synthesized in moderate yields. JA counterparts of all of these CFA-AAs (JA-L-Ala, JA-L-Val, JA-L-Ile, JA-L-Leu, JA-L-Phe, JA-L-Tyr, and JA-L-Gln) are known to be produced by *A. thaliana*.[18, 19]

Biological evaluation of **6**-**12** was carried out by assessing their stomatal reopening activity using *A. thaliana* guard cells (Figure 3). In this assay, indole-3-acetic acid (IAA), which induces stomatal opening, was used as positive control.[9] CFA-AAs with a comparatively small size amino acyl moiety (i.e., **6**, **7**, **8**, and **9**) were as effective as **1** in inducing stomatal reopening of *A. thaliana* guard cells at a concentration on the order of 10^{-6} M. Surprisingly, CFA-AAs with comparatively bulky amino acids (i.e., **10**, **11**, and **12**) also effectively induced stomatal reopening of *A. thaliana* guard cells. Side-chain bulkiness thus has no effect on the stomatal reopening activity of the synthesized CFA-AAs. Considering the dense packing of **1** in the crystal structure of the COI1-**1**-JAZ1 complex,[8] it seems plausible that these bulky derivatives cannot function as ligands for the COI1-JAZ co-receptor. A previous study reported that the bulkiness of the amino acid side chains affects the affinity of (+)-jasmonoyl-L-amino acids (JA-AAs) for the COI1-JAZ co-receptor. In a pull-down assay, JA-AAs with small AAs, such as JA-L-Leu, JA-L-Val, and JA-L-Ala, triggered ligand-dependent assembly of the COI1-JA-AA-JAZ ternary complex as well as **2**, whereas JA-L-Phe and JA-L-Gln did not.[20] Based on the results of our SAR studies, we hypothesized that the stomatal reopening activity of **1** is independent of the COI1-JAZ signaling pathway. We then examined the effectiveness of the seven synthesized CFA-AAs as ligands for the COI1-JAZ co-receptor by modeling *in silico* docking and analyzing the expression of JA-responsive genes that function downstream of the COI1-JAZ co-receptor.

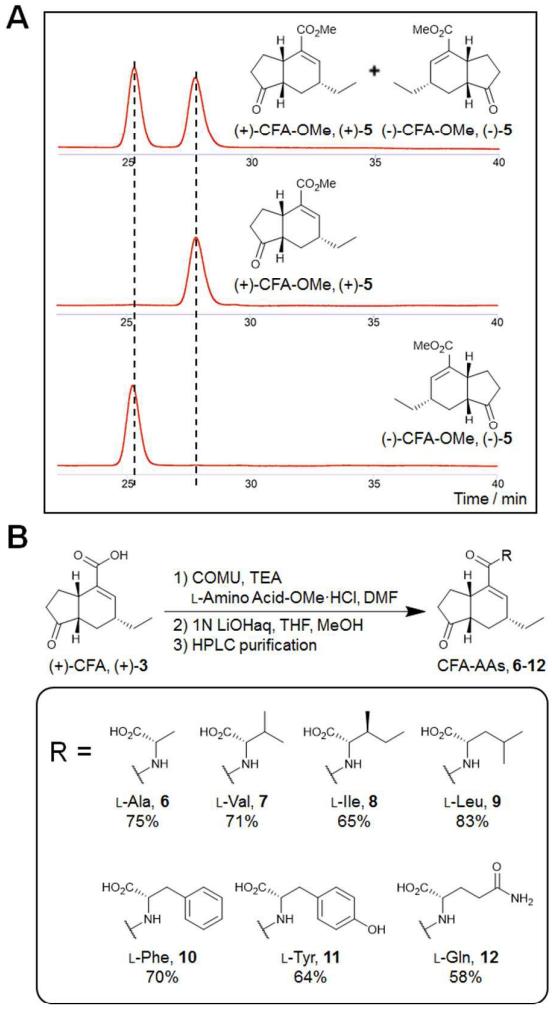

Figure 2. Optical purity of (+)-**5** and (−)-**5**. (**A**) and synthesis of CFA-AAs (**6**-**12**) (**B**). **A**: Optical purities were determined by chiral HPLC analyses on a Chiralpak IA ϕ 4.6 × 250 mm column (Daicel Co., Ltd., Japan) (mobile phase: 99% *n*-hexane containing 1% EtOH; flow rate: 0.5 mL/min). Good separation of each enantiomer was achieved: (−)-**5** at Rt = 25.4 min and (+)-**5** at Rt = 27.8 min. Enantiomeric excess was at calculated from the ratio of peak areas (mAUs) at 235 nm. Chiral HPLC analysis of 5 μg of synthetic (+)-**3** gave an optical purity of 99.3%*ee*. Chiral HPLC analysis of 5 μg of synthetic (−)-**3** gave an optical purity of 99.4%*ee*. **B**: CFA-AAs (**6**-**12**) were synthesized in moderate yields (58-82%).

In silico docking analysis of CFA-AAs and the COI1-JAZ1 coreceptor

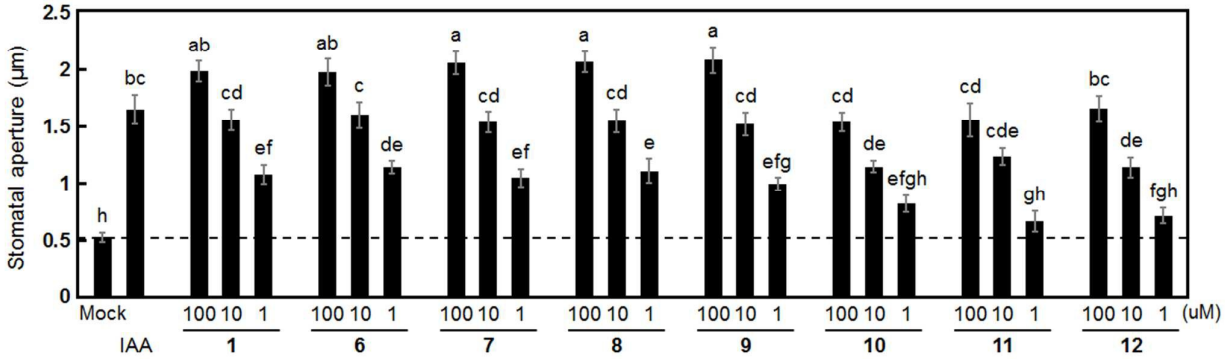

Figure 3. Effect of CFA-AAs (**6-12**) on stomatal reopening in *Arabidopsis thaliana*. Dashed line shows the control, which represents the mean stomatal aperture in buffer/2% EtOH and lacking test compound. Bars represent the mean stomatal aperture for test samples, and error bars indicate the standard error of the mean (n = 20). ANOVA: $P < 0.05$.

Based on the crystal structure of the COI1-**1**-JAZ1 complex (PDB ID: 3OGK),[8] *in silico* substitution of the CMA moiety of **1** into other amino acyl moieties was carried out (Table 1, Figure 4). The crystal structure of the COI1-**1**-JAZ1 complex indicated that the short 20–amino acid sequence within the Jas-motif of JAZ1 (Glu200-Val220) functions as a JAZ1 degron peptide, which is sufficient for full interaction of **1** with COI1-JAZ1.[8] Thus, we used the JAZ1 degron peptide for *in silico* analysis of docking between the COI1-JAZ1 co-receptor and CFA-AAs.[21] The following analyses were carried out using the most favored arrangement of the respective CFA-AAs in complex with the CO1-JAZ1 degron.

First, we evaluated the reliability of our approach. The structure of **1** was derived from the crystal structure of the COI1-**1**-JAZ1 complex *in silico*, and then we carried out an *in silico* docking simulation of **1** using AutoDock Vina 1.1.2[22] software. The obtained favored arrangement of **1** could be almost completely superimposed upon **1** in the crystal structure of the COI1-**1**-JAZ1 complex (Figure S1). Although the involvement of inositol-1,2,4,5,6-pentakisphosphate (IP_5) is also important for stabilization of the COI1-ligand-JAZ1 complex,[8] we could adequately evaluate the stability of COI1-ligand-JAZ1 complexes without additional consideration of IP_5.

We then carried out *in silico* docking simulations using CFA-AAs instead of **1** (Figures 4 and 5). Interestingly, the results contradicted those of the **1**-triggered stomatal reopening assay (Figure 3). As mentioned above, JA-AAs composed of small amino acids can trigger the ligand-dependent assembly of COI1-JA-AA-JAZ.[20] Their mimics (e.g., **6**, **7**, **8**, and **9**) would be expected to function as ligands of the COI1-JAZ1 receptor complex. The *in silico* docking study results suggested that COR-AAs **6**-**9** in complex with COI1-JAZ1 could be superimposed upon **1** in the crystal structure of COI1-**1**-JAZ1 (Figures S1 and S2). The mode of binding of COR-AAs **6**-**9** was very similar to that of **1** in the reported crystal structure (Figure S2, S3, S4 and Table S1).[8] In all of the docking models, **6**-**9** formed hydrogen bonds with R85, R348, Y386, R409, Y444, and R496 in COI1, as well as Y444 and R496 in JAZ1 (Figure 5 and Table S1). Also, we have conducted molecular dynamics (MD) simulations of the COI1-JAZ1 with some ligands (COR, CFA-Ile and CFA-Leu) in explicit water solvent condition for 10 nano second. The root mean square deviation (RMSD) of the ligands from the initial docked coordinates was evaluated to check the stability of the bound ligand structures. In all cases, the obtained ligand structures in equilibrated condition were shown to be almost close to those of the structures from *in silico* docking study (Figure S5). These results strongly suggested that **6**-**9** function as ligands of the COI1-JAZ1 receptor complex to stabilize the formation of the COI1-lignad-JAZ1 ternary complex in exactly the same manner as **1** and **2**. On the other hand, the docking study results also suggested that CFA-AAs with comparatively bulky amino acids (e.g., **10**, **11**, and **12**) cannot be accommodated in the ligand-binding cavity between the COI1 and JAZ1 proteins, even though **10**-**12** each exhibit stomatal reopening activity (Table S1, Figures 4

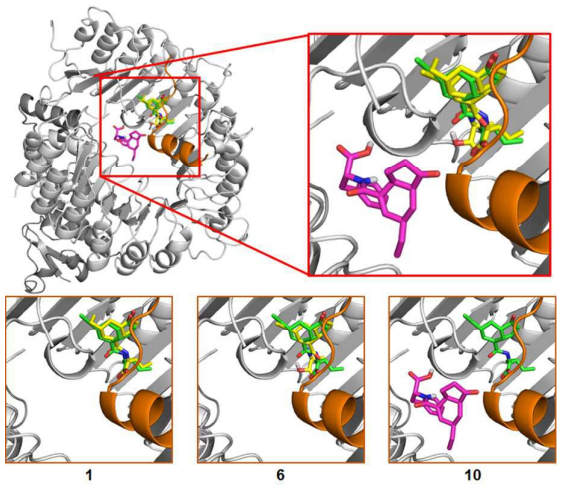

Figure 4. Model depicting the docking of COR (**1**), CFA-L-Ala (**6**), and CFA-L-Phe (**10**) in the COI1-JAZ1 co-receptor. Figure was prepared using Pymol 1.7.6.0 software. COR in the crystal structure (3OGM) is shown in green. Calculated COR (**1**) and CFA-L-Ala (**6**) are shown in yellow, and CFA-L-Phe (**10**) is shown in purple. COR (**1**) and CFA-L-Ala (**6**) are accommodated in the biding pocket of COI1- JAZ, whereas bulky **10** is not.

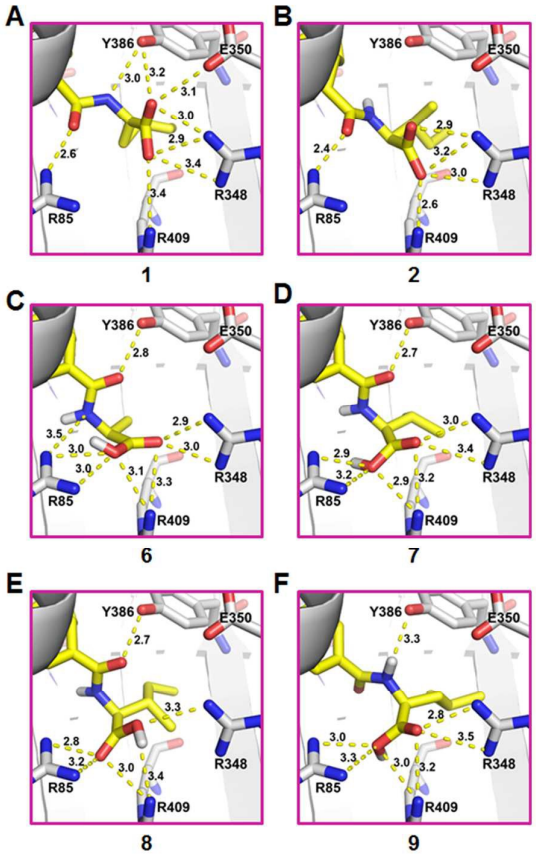

Figure 5. Models depicting the docking of **1**, **2**, and CFA-AAs (**6-9**) in the COI1-JAZ1 co-receptor. Figures were prepared using Pymol 1.7.6.0 software. Ligand and amino acid residues of COI1 located within 3.5 Å (R85, R348, E350, Y386 and R409) are connected by dotted yellow lines. **A**: COR (**1**). **B**: JA-Ile (**2**). **C**: CFA-L-Ala (**6**). **D**: CFA-L-Val (**7**). **E**: CFA-L-Ile (**8**). **F**: CFA-L-Leu (**9**).

and S1). These results of docking studies were strongly supported by *in vitro* pull-down experiment using recombinant COI1-GST and JAZ conjugated with maltose-binding protein (JAZ-MBP).[4] Figure 6 demonstrated that

ternary complex composed of COII1-CFAAA-JAZ can be pulled down only when we used **6**-**9** as ligand. Those pull-down results were also consistent with those of pull-down assays using JA-AAs with comparatively bulky amino acids, such as JA-L-Phe and JA-L-Gln.[20] These results of docking studies were strongly supported by *in vitro* pull-down experiment using recombinant COI1-GST and JAZ conjugated with maltose-binding protein (JAZ-MBP).[4] Figure 6 demonstrated that ternary complex composed of COII1-CFAAA-JAZ can be pulled down only when we used **6**-**9** as ligand. The bulkiness of the amino acid side chains in the CFA-AAs strongly affects their affinity for the COI1-JAZ1 co-receptor. Surprisingly, the affinities of CFA-AAs with COI1-JAZ coreceptor were inconsistent with results of stomatal reopening assays.

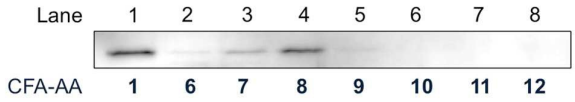

Figure 6. *In vitro* pull down assay of COI1-GST using MBP-fused JAZ3 with CFA-AAs. The conditions were as follows: input; COI1-GST (5 nM), JAZ3-MBP (10 nM), CFA-AAs (100 nM) in 50 mM Tris-HCl buffer (pH 7.8, 100 mM NaCl, 10% glycerol, 0.1% Tween20, 20 mM 2-mercaptoethanol, 1 μM IP5, protease inhibitor cocktail). These were pulled down with amylose resin and obtained COI1-GST was analyzed with SDS-PAGE and western blotting analyses detected with anti-GST antibody.

Genetic evaluation of coronafacyl amino acids: expression of *AOS* and *OPR3* in *A. thaliana*

In silico docking studies suggested the potential effectiveness

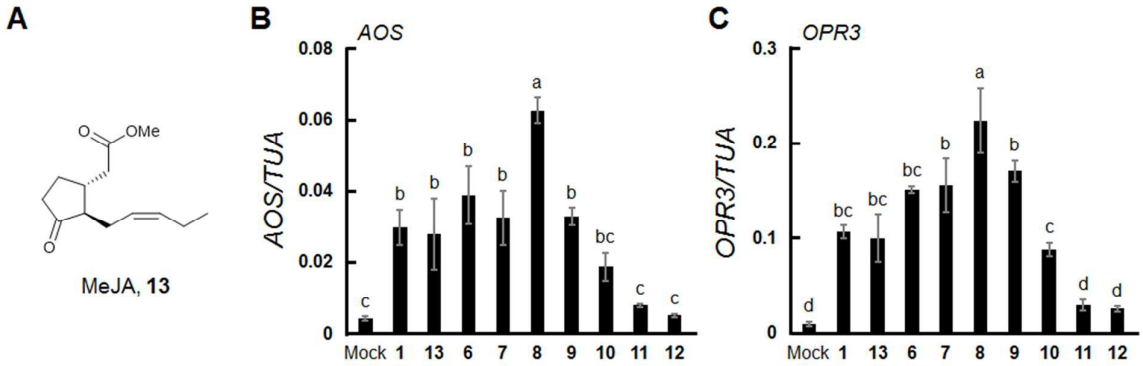

Figure 7. Structure of methyl jasmonate (MeJA, **13**) (**A**) and expression of the *AOS* (**B**) and *OPR3* (**C**) genes in *A. thaliana* treated with CFA-AAs. **B**, **C**: Quantitative RT-PCR analyses of relative *AOS* and OPR*3* transcript levels were performed after chemical treatment. 'Mock' indicates the control, representing the mean *AOS* or *OPR3* transcript level in buffer/2% EtOH without test compound. Bars represent the mean *AOS* or *OPR3* transcript levels for test samples, and error bars show the standard error of the mean (n = 3). ANOVA: $P < 0.05$.

of CFA-AAs as ligands of the COI1-JAZ co-receptor. However, the result of an *in silico* study should be validated using an independent method. Thus, we further examined the expression of various genes in *A. thaliana* treated with CFA-AAs (**6**-**12**). The expression of JA-responsive genes such as *AOS* and *OPR3*[23] is controlled by the COI1-JAZ signaling pathway and is upregulated following treatment with **1** or methyl jasmonate (**13**, MeJA). Analysis of the expression of *AOS* and *OPR3* following CFA-AA treatment provided validation of the observed affinity between the CFA-AAs and COI1-JAZ co-receptor in the *in silico* docking study. CFA-AAs such as **6**, **7**, and **9** were as effective as **1** and **13** in upregulating the expression of *AOS* and *OPR3* (Figure 7). Interestingly, **8** was found to upregulate the expression of these genes more than **1**. On the other hand, **11** and **12** did not induce the expression of *AOS* and *OPR3* in *A. thaliana* (Figure 7). CFA-AA **10** induced moderate upregulation of *AOS* and *OPR3* expression (Figure 7). The degree of upregulation in *AOS* and *OPR3* expression can be summarized as follows: **8** > **1** = **13** = **6** = **7** = **9** > **10** > **11** = **12** = control. With the exception of **10**, these results correlated well with those of the *in silico* docking studies.

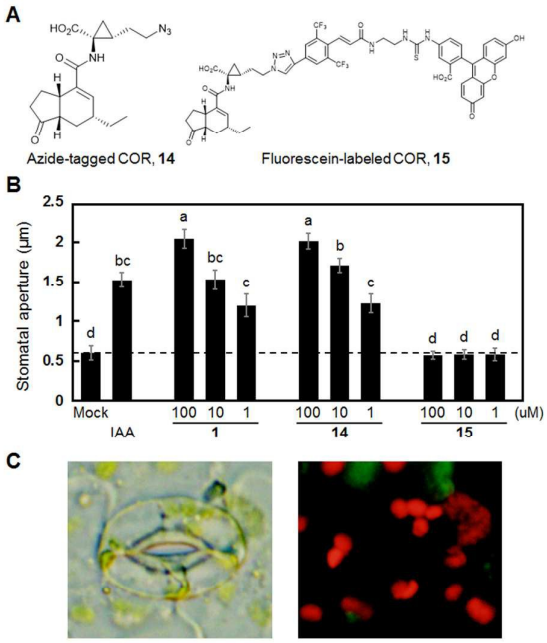

Figure 8. Structures of azido-COR (**14**) and fluorescein-labeled COR (**15**) for *in vivo* fluorescence imaging (**A**). Effect of **14** and **15** on the stomatal aperture size in samples of *A. thaliana* epidermis (**B**). A stoma in the epidermis of *A. thaliana* after treatment with **15** for 3 h (**C**). **B**: Dashed line indicates the mean stomatal aperture of the control incubated in buffer/2% EtOH lacking test compound. Bars represent the mean stomatal aperture, and error bars indicate the standard error of the mean (n = 20). ANOVA: $P < 0.05$. **C**: Optical (left) and fluorescent (right) images of an *Arabidopsis* stoma treated with **15** (left). The red spots in the right image are attributed to an autofluorescence from chloroplast.

The results of biological evaluations and *in silico* docking studies of the CFA-AAs demonstrated a distinct inconsistency between their effectiveness as COI1-JAZ agonists and their bioactivity in terms of inducing stomatal reopening (Figure 3). These results strongly suggest that **1** functions as a virulence factor, inducing stomatal reopening through a COI1-JAZ–independent mechanism.

Development of chemical tools for determining the subcellular localization of COR

The subcellular localization of bioactive substances often provides important clues regarding their mode-of-action (MOA).[24, 25] Determining the subcellular localization of **1** in *Arabidopsis* guard cells would aid in elucidating the COI1-JAZ dependence/independence of the compound's stomatal reopening activity, as Withers *et al.*[26] reported that COI1 and JAZ9 are localized in the nucleus in *A. thaliana* epidermal cells. Fluorescent-labeled COR probes would serve as important tools for further studies of the compound's MOA.

We previously reported the development of an azide-tagged COR (**14**) for use as a COR-based "Click"-mediated molecular probe.[27] Fluorescein-labeled COR (**15**) was easily prepared from **14** using "Click Chemistry" (Figure 8A).[28, 29] Unfortunately, **15** did not exhibit effective stomatal reopening (Figure 8B). Fluorescence imaging experiments using living *A. thaliana* guard cells showed a dispersed fluorescence pattern, indicating that **15** could not penetrate into the guard cells, even after incubation for 3 h (Figure 8C). The inability to penetrate the guard cells was attributed to the chemical characteristics of **15**, such as its high molecular weight and high polarity.[30] Thus, a molecular probe of smaller size which can be monitored in living guard cells is strongly desired.

As alternatives, we focused on alkyne-tagged COR (**16**). Alkyne **16** can be coupled with azide-tagged fluorescent dye to provide click-mediated fluorescent-COR. And 16 can be also used in alkyne-tag Raman imaging (ATRI) because Sodeoka *et*

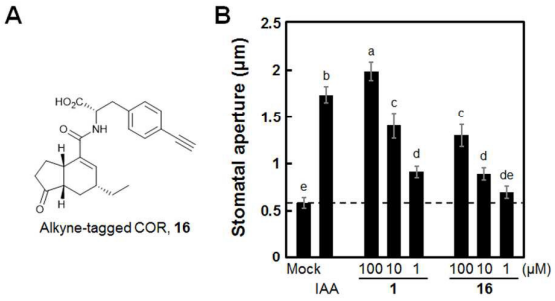

Figure 9. Structure of alkyne-tagged COR (**16**) (**A**). Effect of **1** and **14** on *A. thaliana* stomatal aperture size (**B**). **A**: Alkyne-tagged COR for ATRI. **B**: Dashed line indicates the mean stomatal aperture of the control incubated in buffer/2% EtOH without test compound. Bars indicate the mean stomatal aperture, and error bars indicate the standard error of the mean (n = 20). ANOVA: $P < 0.05$.

al.[31] used this technique to image small, alkyne-tagged EdU in living HeLa cells. Alkyne-tagged COR (**16**) was developed based on **10** (Figure 9A, Scheme S2) and was expected to retain stomatal reopening activity. As expected, **16** was one-tenth as effective as **1** and exhibited stomatal reopening activity at 100 μM (Figure 9B). The results of further subcellular localization studies using **16** will be reported elsewhere.

3. Conclusions

We synthesized seven CFA-AAs (**6**-**12**) and carried out SAR studies of their effect on stomatal reopening. The efficiency of each compound as an agonist of the COI1-JAZ co-receptor was evaluated using *in silico* docking studies, with validation through analyses of the induction of the expression of JA-responsive genes. The results of SAR and agonist efficiency analyses were inconsistent. For example, CFA-AAs with comparatively bulky side chains, such as **11** and **12**, induced stomatal reopening but did not function as agonists of the COI1-JAZ1 co-receptor. Thus, the MOA of **1** with respect to its stomatal reopening activity remains unknown.

We also developed a **1**-based molecular probe for further MOA studies. This alkyne-tagged probe (**16**) was shown to be a potentially useful tool for further ATRI studies of the subcellular localization of **1**.

COR (**1**) plays an important role in plant-microbe interactions because it is a chemical weapon used by bacteria to overcome the induced immune response of plants.[9] COR (**1**) was previously thought to reopen plant stomata by inhibiting stomatal closure by ABA through the COI1-JAZ signaling pathway.[14] However, our results suggests another possibility: that **1** has a dual MOA, triggering stomatal reopening through a COI1-JAZ–independent mechanism as well as COI1-JAZ–dependent inhibition of ABA.

4. Experimental

General methods

Both ^{1}H- and ^{13}C-NMR spectra were recorded on a JNM-ECS-400 (JEOL Inc., Japan) spectrometer in deuterium chloroform or deuterium methanol. High-resolution (HR) electrospray ionization (ESI)-mass spectrometry (MS) analyses were conducted using a micrOTOF II (Bruker Daltonics Inc., Germany) mass spectrometer with ESI techniques. Reagents and solvents were purchased from Kanto Chemical Co. Ltd., Japan; Wako Pure Chemical Industries Co. Ltd., Japan; and Nacalai Tesque Co., Ltd., Japan. All anhydrous solvents were dried using standard techniques and freshly distilled before use or purchased in anhydrous form from Fluka. HPLC solvents were purchased from Wako Pure Chemical Industries and Nacalai Tesque. All flash chromatography was carried out using dry-packed Chromatorex PSQ 100B silica gel (Fuji Silysia Chemical Co., Ltd., Japan). Reactions were monitored by thin layer chromatography, carried out on Kieselgel 60 PF254 (Merck, Germany) 0.2 mM plates. Unless stated otherwise, all reactions were carried out under air. FT/IR spectra were recorded on a JASCO FT/IR-4100 (JASCO Inc., Japan). Specific rotation was measured using a JASCO DIP-1000 polarimeter. HPLC chiral separations were performed using a JASCO PU-2086 plus HPLC system (JASCO UV-2075 and SSC-1310) equipped with a Chiralpak IA ϕ20 × 250 mm column (Daicel Co., Ltd., Japan). HPLC purifications were performed on a JASCO PU-2089 plus system (JASCO UV-2075 and Cosmosil SC_{18}-AR, ϕ20 × 250 mm column [Nacalai Tesque]). Samples were lyophilized using an EYELA FDU-830 freeze dryer system (Tokyo Rikakikai Co., Ltd., Japan) equipped with a Hitachi pump. Chiral COR was purchased from Sigma-Aldrich Japan Co. Ltd., Japan.

Assay of Stomatal Reopening

Arabidopsis thaliana ecotypes (Col-0) were grown on giffy-7 [SAKATA SEED CORPORATION, Japan] in a growth chamber at 22 °C under 12 h light (118 $\mu mol \cdot m^{-2} \cdot s^{-1}$; cool-white fluorescent light) / 12 h dark. The abaxial leaf epidermis of 6-8 weeks old plants were peeled and cut out of about 2 mm^2 square. The peels were submerged in MES buffer (50 mM KCl, 10 mM MES (Dojindo)-KOH, pH 6.2) at 22 °C for 3 hours under dark to close the stomata. After incubation for 3 hours with each test sample at 22 °C under dark, photographic images were taken with IX71 microscope equipped with a CCD camera (DP72; Olympus Corp., Japan). The length of stomatal apertures was analyzed using ImageJ 1.45S software (http://imagej.net/Welcome).

Quantitative RT-PCR

Col-0 were grown in 1/2 Murashige & Skoog (MS) liquid medium at 22 °C under 16 h light (118 $\mu mol \cdot m^{-2} \cdot s^{-1}$; cool-white fluorescent light) / 8 h dark at the sterile condition. 7-day-old plants were incubated with autoclaved 1/2 MS liquid medium containing 0.5% sucrose and 5 mM MES-KOH (pH 5.8) for 3 hours at 22 °C under dark. Plants were sampled after 1 more hour incubation with each filtered test sample at 22 °C under dark. Total RNA was isolated with an RNeasy Mini Kit [QIAGEN Co. Ltd., Germany]. First-strand cDNA was synthesized with ReverTra Ace [TOYOBO, Japan] using oligo dT primers. qPCR was performed by StepOnePlus Real-Time PCR System [Applied Biosystems, USA]. Sequences of all primers for qPCR are listed below. *Tubulin Alpha 5* was used as a reference gene.

Allene oxide synthase (*AOS*: AT5G42650)	5’ CTCCGTTAATTTCTCGTC 3’
	3’ GCAGCAACAGATTATACAAC 5’
Oxophytodienoate-reductase 3 (*OPR3*: AT2G06050)	5’ GACTGTTTATCGCAAACCCG 3’
	3’ ACTAGATAAACAACAAGATACATGACT 5’
Tubulin-alpha 5 (*TUA*: AT5G19780)	5’ GGTGAGTATGATGTTGAAGA 3’
	3’ AGAGATTTCCAAGAGTCGT 5’

In silico docking analyses

In silico docking analyses were carried out using AutoDock Vina 1.1.2 software.[22] Kollman united atom charges and polar hydrogens were added to the protein (COI1-JAZ1 co-receptor, PDB ID: 3OGK) and the ligand using AutoDock tools. All rotatable bonds in the ligands were kept free to allow for flexible docking (the torsions were as follows: **1**; 6, **2**; 11, **6**; 6, **7**; 7, **8**; 8. **9**; 8, **10**; 8, **11**; 9, **12**; 10, respectively). The grid size was set to 30 × 30 × 30 grid points (x, y, and z axes), with spacing between the grid points kept at 1 Å. The Lamarckian genetic algorithm was chosen to search for the best conformers. After the docking simulations, we further conducted MD simulations of the COI1-JAZ1 with several ligands in explicit water molecules under the constant temperature and pressure (T=300 K, P=1 atm) condition for 10 nano second. The COI1-JAZ1 with the several ligands were placed at the center of MD box that extended 12 Å from any solute atom. The box was then filled with water molecules to set the water density of the system at 1 g/cm^3. Berendsen thermostat and barostat were used to control the system

temperature and the pressure. Amber03 force field [Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T.; Caldwell, J.; Wang, J.; Kollman, P. *J. Comput. Chem.* **2003**, *24*, 1999.][32] and TIP3P water model [Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926..][33] were adopted for proteins and water molecule, respectively. Cutoff for the van der Waals (vdW) interaction was 12 Å. Particle mesh Ewald (PME) method [Darden, T.; York, D.; and Pederson, L. *J. Chem. Phys.* **1993**, *98*, 10089.][34] was adopted for the calculation of the coulomb electrostatic interactions. The time step for integration (Δt) was 2 fs. These MD calculations were carried out by the Amber11 program package. The root mean square deviation (RMSD) of the ligands from the initial docked coordinates was evaluated to check the stability of the bound ligand structures. After about 5 nano second, the structures were well equilibrated (Figure S5).

***In vitro* pull down assays**

The plasmid of GST-fused COI1 and ASK1 was obtained from Addgene (pFB-GTE-COI1 and pFB-HTB-ASK1). These proteins were co-expressed in insect cells and purified by glutathione affinity chromatography as previously reported by Sheard *et al*.[8] The plasmid of MBP-fused JAZ3 was kindly gifted from Drs. A. Chini and R. Solano, which was expressed in *E. coli* BL21 cells and purified in amylose resin as previously reported by A. Chini *et al*.[4] The pull down assay using COI1-GST, ASK1-GST, MBP-JAZ3 and each coronatine analogues were performed as previously reported by A. Chini et al. In the detail in each pull-down experiment, purified COI1-GST (5 nM) and coronatine analogues (CFA-AA, 100 nM) in 500 μL of incubation buffer (50 mM Tris-HCl, pH 7.8, containing 100 mM NaCl, 10% glycerol, 0.1% Tween 20, 20 mM 2-mercaptoethanol, 1 μM IP5, EDTA-free complete protease inhibitor cocktail according to the manufacturer's instructions (Roche)) was added to amylose resin-bound MBP-JAZ3 (25 μL suspension of amylose resin with 10 nM of MBP-JAZ3). After 4 hours incubation at 4 ºC under rotation, the samples were washed in triplicate with 500 μL of fresh incubation buffer. The washed amylose resin was resuspended in 50 μL of SDS-PAGE loading buffer containing maltose (20 mM). Following boiling for 10 min at 60 ºC, the samples were loaded on SDS-PAGE and analyzed with western blotting. The bound COI1-GST were detected using anti-GST HRP conjugate (RPN1236, GE Healthcare).

Synthesis of CFA-AAs (6-12)

COMU (1.05 eq.) and TEA (2.5 eq.) were added to a solution of (+)-CFA ([+]-**3**) in DMF (0.25 mL) at room temperature under an argon atmosphere. The reaction mixture was stirred for 10 min, and then L-alanine methyl ester hydrochloride (1.1 eq.) was added. After the reaction mixture was stirred for an additional 2.5 h, the reaction was quenched with saturated aqueous $NaHCO_3$. The mixture was extracted with EtOAc (3 × 5 mL). The organic layer was dried over Na_2SO_4 and then filtered. After evaporation, the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/1). Each fraction was concentrated by evaporation to give a mixture of compounds. The residue was dissolved in MeOH (0.2 mL), and then THF (0.1 mL) and 1 N aqueous LiOH (0.1 mL) were added. The mixture was stirred for 10 min, and then the reaction was quenched with 1 N aqueous $KHSO_4$. The mixture was extracted with EtOAc (3 × 5 mL). The organic layer was dried over Na_2SO_4 and filtered. After evaporation, the residue was purified by HPLC using a silica gel column (Cosmosil SC_{18}-AR, 20 × 250 mm, Nacalai Tesque) with 0.05% HCO_2H solution (MeOH/aqueous) as the mobile phase at a flow rate of 8.0 mL/min. Each collected fraction was concentrated by evaporation to give CFA-AAs **6-12** (58-75%) as colorless, crystalline solids (further details are provided in the supporting information).

*^{13}C signal of amido could not be detected, but in infrared absorption spectrometry, C=O stretching signal of amido could be detected around at 1620 cm^{-1}. And ^{13}C signal of α carbon of amino acids was lost in signals of $CDCl_3$

CFA-L-Ala (**6**)
^{1}H-NMR (400MHz, $CDCl_3$) δ_H; 6.40 (s, 1H), 6.36 (brs, 1H), 4.61 (1H, brs), 3.17 (m, 1H), 2.50-2.24 (m, 4H), 2.15 (m, 1H), 1.90 (dt. *J* = 12.8, 4.4 Hz, 1H), 1.68-1.32 (m, 6H), 1.07 (td. *J* = 12.8, 11.2 Hz, 1H), 0.99 (t. J = 7.6 Hz, 3H); ^{13}C-NMR (100MHz, $CDCl_3$) δ_c: 220.1, 168.6, 137.9, 135.1, 46.4, 38.1, 36.1, 28.0, 27.7, 25.9, 17.8, 11.3; IR (film) cm^{-1}: 3333, 2964, 2931, 2877, 1735, 1655, 1618, 1530, 1458, 1450, 1213, 1152, 753; HR MS (ESI, negative) *m/z* $[M-H]^-$ calcd. for $C_{15}H_{20}NO_4$: 278.1392, found: 278.1395; $[\alpha]_D^{23}$ +77° (*c* 0.10, $CHCl_3$).

CFA-L-Val (**7**)
^{1}H-NMR (400MHz, $CDCl_3$) δ_H; 6.41 (s, 1H), 6.27 (brs, 1H), 4.58 (brs, 1H), 3.18 (dt. *J* = 9.6, 7.2, 1H), 2.48-2.23 (m, 5H), 2.16 (m, 1H), 1.91 (dt. *J* = 12.8, 4.4 Hz, 1H), 1.63-1.33 (m, 3H), 1.07 (td. *J* = 12.8, 11.2 Hz, 1H), 1.03-0.96 (m, 9H); ^{13}C-NMR (100MHz, $CDCl_3$) δ_c: 220.1, 168.5, 137.6, 135.4, 46.4, 38.1, 37.4, 36.2, 30.1, 28.1, 27.8, 26.0, 19.2, 18.0, 11.3; IR (film) cm^{-1}: 3333, 2965, 2929, 2875, 1735, 1654, 1617, 1522, 1465, 1405, 1306, 1215, 1148, 755; HR MS (ESI, negative) *m/z* $[M-H]^-$ calcd. for $C_{17}H_{24}NO_4$: 306.1705, found: 306.1710. $[\alpha]_D^{23}$ +64° (*c* 0.11, $CHCl_3$).

CFA-L-Ile (**8**)
^{1}H-NMR (400MHz, $CDCl_3$) δ_H; 6.40 (s, 1H), 6.30 (brs, 1H), 4.64 (brs, 1H), 3.18 (m, 1H), 2.48-2.24 (m, 4H), 2.16 (m, 1H), 2.01 (m, 1H), 1.89 (dt. *J* = 12.8, 4.4 Hz, 1H), 1.69-1.33 (m, 4H), 1.30-1.21 (m, 1H), 1.07 (td. *J* = 12.8, 11.6 Hz, 1H), 1.00-0.92 (m, 9H); ^{13}C-NMR (100MHz, $CDCl_3$) δ_c: 220.2, 168.7, 137.7, 135.3, 46.4, 41.0, 38.1, 37.4, 36.2, 28.1, 27.7, 25.9, 25.1, 22.9, 21.9, 11.3; IR (film) cm^{-1}: 3345, 2965, 2935, 2877, 1734, 1654, 1622, 1521, 1463, 1405, 1385, 1329, 1217, 1145, 755; HRMS (ESI, negative) *m/z* $[M-H]^-$ calcd. for $C_{18}H_{26}NO_4$: 320.1862, found: 320.1867; $[\alpha]_D^{23}$ +33° (*c* 0.105, $CHCl_3$).

CFA-L-Leu (**9**)
^{1}H-NMR (400MHz, $CDCl_3$) δ_H; 6.38 (s, 1H), 6.16 (brs, 1H), 4.64 (brs, 1H), 3.16 (dt. *J* = 9.2, 6.8, 1H), 2.48-2.24 (m, 4H), 2.16 (m, 1H), 1.90 (dt. *J* = 12.8, 4.4 Hz, 1H), 1.82-1.32 (m, 6H), 1.07 (td. *J* = 12.8, 11.2 Hz, 1H), 1.00-0.94 (m, 9H); ^{13}C-NMR (100MHz, $CDCl_3$) δ_c: 220.2, 168.3, 137.6, 135.4, 46.4, 38.1, 37.6, 37.4, 36.2, 28.1, 27.8, 26.0, 25.3, 15.6, 11.6, 11.3; IR (film) cm^{-1}: 3308, 2958, 2936, 2872, 1734, 1653, 1617, 1532, 1465, 1444, 1333, 1270, 1231, 1152, 755; HRMS (ESI, negative) *m/z* $[M-H]^-$ calcd. for $C_{18}H_{26}NO_4$: 320.1862, found: 320.1862; $[\alpha]_D^{23}$ +36° (*c* 0.14, $CHCl_3$).

CFA-L-Phe (**10**)
^{1}H-NMR (400MHz, $CDCl_3$) δ_H; 7.36-7.27 (m, 3H), 7.21 (dd. *J* = 8.0, 1.6 Hz, 2H), 6.21 (s, 1H), 6.09 (brs, 1H), 4.86 (td. *J* = 6.0, 6.0 Hz, 1H), 3.34 (dd. *J* = 6.0, 1.6 Hz, 1H), 3.18 (dd. *J* = 6.0, 1.6 Hz, 1H), 3.07 (dt. *J* = 10.4, 6.8 Hz, 1H), 2.38-2.15 (m, 4H), 2.09 (m, 1H), 1.86 (dt. *J* = 12.8, 4.8 Hz, 1H), 1.54-1.28 (m, 3H), 1.00 (td. *J* = 12.8, 11.2 Hz, 1H), 0.94 (t. *J* = 7.6 Hz, 3H); ^{13}C-NMR (100MHz, $CDCl_3$) δ_c: 219.9, 168.7, 138.4, 135.8, 134.9, 129.4 (2C), 128.8 (2C), 127.4, 46.3, 38.1, 37.4, 36.9, 36.0, 27.9, 27.5, 26.0, 11.2; IR (film) cm^{-1}: 3333, 2959, 2932, 2872, 1739, 1653, 1617, 1521, 1497, 1456, 1269, 1218, 1152, 755; HRMS (ESI, negative) *m/z* $[M-H]^-$ calcd. for $C_{21}H_{24}NO_4$:354.1705, found: 354.1707; $[\alpha]_D^{22}$ +66° (*c* 0.10, $CHCl_3$).

CFA-L-Tyr (**11**)
^{1}H-NMR (400MHz, CD_3OD) δ_H: 7.05 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.4 Hz, 2H), 6.29 (s, 2H), 4.61 (brs, 1H), 3.19 (dd, *J* = 13.6, 4.4 Hz, 1H), 3.14-2.90 (m, 2H), 2.37-2.21 (m, 2H), 2.17-2.04 (m, 2H), 1.76 (dt, *J* = 13.2, 4.8 Hz, 1H), 1.53-1.34 (m, 3H), 1.07 (dt, *J* = 13.2, 10.8 Hz, 1H), 0.98 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100MHz, CD_3OD) δ_C: 222.9, 172.1, 157.3, 138.7, 138.3, 131.4 (2C), 129.6, 116.1 (2C), 84.0, 47.8, 38.7, 38.6, 37.6, 37.3, 29.1, 28.5, 27.2, 11.6; IR (film) cm^{-1}: 3333, 2964, 2931, 2854, 1733, 1653, 1616, 1516, 1457, 1261, 1226, 1146, 754; HRMS (ESI, negative) *m/z* [M-H]$^-$ calcd. for $C_{21}H_{24}NO_5$: 370.1654, found: 370.1658; $[\alpha]_D^{22}$ +45° (*c* 0.10, MeOH).

.

CFA-L-Gln (**12**)
^{1}H-NMR (400MHz, CD_3OD) δ_H: 6.52 (s, 1H), 4.59 (brs, 1H), 3.18 (dt. *J* = 10.4, 6.8 Hz, 1H), 2.44-2.28 (m, 6H), 2.26–2.12 (m, 2H), 2.05 (m, 1H) 1.80 (td. *J* = 12.8, 4.8 Hz, 1H), 1.68-1.35 (m, 3H), 1.14 (td, *J* = 12.8, 11.2 Hz, 1H), 1.01 (t, *J* = 7.6 Hz, 3H); ^{13}C-NMR (100MHz, CD_3OD) δ_c: 223.0, 170.5, 138.5, 136.4, 85.0, 47.9, 38.8, 38.7, 37.3, 33.0, 29.1, 29.0, 28.8, 27.1, 11.6; IR (film) cm-1; 3417, 2961, 2925, 2853, 1733, 1658, 1616, 1532, 1456, 1404, 1263, 1152, 757; HRMS (ESI, negative) *m/z* [M-H]$^-$ calcd. for $C_{17}H_{23}N_2O_5$: 335.1607, found: 335.1607; $[\alpha]_D^{22}$ +52° (*c* 0.17, MeOH).

Alkyne-tagged coronatine (**16**)
^{1}H-NMR (400MHz, CD_3OD) δ_H: 7.38 (d, *J* = 7.6 Hz, 2H), 7.24 (d, *J* = 7.6 Hz, 2H), 6.28 (s, 1H), 4.70 (dd, *J* = 10.0, 4.4 Hz, 1H), 3.42 (s, 1H), 3.22-3.02 (m, 3H), 2.36-2.21 (m, 3H), 2.16-2.05 (m, 2H), 1.76 (dt, *J* = 12.8, 5.2 Hz, 1H), 1.54-1.33 (m, 3H), 1.08 (dt, *J* = 12.8, 11.2 Hz, 1H), 0.98 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100MHz, CD_3OD) δ_c: 222.8, 174.6, 1671.1, 139.9, 138.5, 136.3, 133.1 (2C), 130.4 (2C), 122.3, 84.1, 78.6, 54.9, 47.7, 38.7, 38.5, 37.8, 37.2, 29.0, 28.5, 27.1, 11.6; IR (film) cm^{-1}: 3292, 2962, 2932, 2875, 2017, 1735, 1655, 1614, 1509, 1444, 1404, 1214, 1148, 1068, 827; HRMS (ESI, negative) *m/z* [M-H]$^-$ calcd. for $C_{23}H_{24}NO_4$: 378.1705, found: 378.1705; $[\alpha]_D^{23}$ +85° (*c* 0.10, $CHCl_3$).

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Notes and References

1 Ichihara, A.; Shiraishi, K.; Sato, H.; Sakamura, S.; Nishiyama, K.; Sakai, R.; Furusaki, A.; Matsumoto, T. *J. Am. Chem. Soc.* **1977,** *99*, 636.
2 Wasternack, C. *J. Plant Growth Regul.* **2015**, 1.
3 Thines, B.; Katsir, L.; Melotto, M.; Niu, Y.; Mandaokar, A.; Liu, G.; Nomura, K.; He, S. Y.; Howe, G. A.; Browse, J. *Nature* **2007,** *448*, 661.
4 Chini, A.; Fonseca, S.; Fernandez, G.; Adie, B.; Chico, J. M.; Lorenzo, O.; Garcia-Casado, G.; Lopez-Vidriero, I.; Lozano, F. M.; Ponce, M. R.; Micol, J. L.; Solano, R. *Nature* **2007,** *448*, 666.
5 Katsir, L.; Chung, H. S.; Koo, A. J.; Howe, G. A. *Curr. Opin. Plant Biol.* **2008,** *11*, 428.
6 Kazan, K.; Manners, J. M. *Plant Physiol.* **2008,** *146*, 1459.
7 Browse, J. *Annu. Rev. Plant Biol.* **2009,** *60*, 183.
8 Sheard, L. B.; Tan, X.; Mao, H.; Withers, J.; Ben-Nissan, G.; Hinds, T. R.; Kobayashi, Y.; Hsu, F. F.; Sharon, M.; Browse, J.; He, S. Y.; Rizo, J.; Howe, G. A.; Zheng, N. *Nature* **2010,** *468*, 400.
9 Melotto, M.; Underwood, W.; Koczan, J.; Nomura, K.; He, S. Y. *Cell* **2006,** *126*, 969.
10 Montillet, J. L.; Hirt, H. *Trends Plant Sci.* **2013,** *18*, 295.
11 McLachlan, D. H.; Kopischke, M.; Robatzek, S. *New Phytol.* **2014,** *203*, 1049.
12 Melotto, M.; Underwood, W.; He, S. Y. *Annu. Rev. Phytopathol.* **2008,** *46*, 101.
13 Mino, Y.; Matsushita, Y.; Sakai, R. *Ann. Phytopathol. Soc. Japan* **1987,** *53*, 53.
14 Zheng, X. Y.; Spivey, N. W.; Zeng, W.; Liu, P. P.; Fu, Z. Q.; Klessig, D. F.; He, S. Y.; Dong, X. *Cell Host Microbe* **2012,** *11*, 587.
15 Okada, M.; Ito, S.; Matsubara, A.; Iwakura, I.; Egoshi, S.; Ueda, M. *Organic & Biomolecular Chemistry* **2009,** *7*, 3065.
16 Okada, M.; Egoshi, S.; Ueda, M. *Biosci. Biotechnol. Biochem.* **2010,** *74*, 2092.
17 Nara, S.; Toshima, H.; Ichihara, A. *Tetrahedron* **1997,** *53*, 9509.
18 Staswick, P. E.; Tiryaki, I. *Plant Cell* **2004,** *16*, 2117.
19 Koo, A. J.; Gao, X.; Jones, A. D.; Howe, G. A. *Plant J.* **2009,** *59*, 974.
20 Katsir, L.; Schilmiller, A. L.; Staswick, P. E.; He, S. Y.; Howe, G. A. *Proc. Natl. Acad. Sci. U. S. A.* **2008,** *105*, 7100.
21 Nakamura, Y.; Paetz, C.; Brandt, W.; David, A.; Rendon-Anaya, M.; Herrera-Estrella, A.; Mithofer, A.; Boland, W. *J. Chem. Ecol.* **2014,** *40*, 687.
22 Trott, O.; Olson, A. J. *J. Comput. Chem.* **2010,** *31*, 455.
23 Kienow, L.; Schneider, K.; Bartsch, M.; Stuible, H. P.; Weng, H.; Miersch, O.; Wasternack, C.; Kombrink, E. *J. Exp. Bot.* **2008,** *59*, 403.
24 Nishimura, S.; Arita, Y.; Honda, M.; Iwamoto, K.; Matsuyama, A.; Shirai, A.; Kawasaki, H.; Kakeya, H.; Kobayashi, T.; Matsunaga, S.; Yoshida, M. *Nat. Chem. Biol.* **2010,** *6*, 519.
25 Kotake, Y.; Sagane, K.; Owa, T.; Mimori-Kiyosue, Y.; Shimizu, H.; Uesugi, M.; Ishihama, Y.; Iwata, M.; Mizui, Y. *Nat. Chem. Biol.* **2007,** *3*, 570.
26 Withers, J.; Yao, J.; Mecey, C.; Howe, G. A.; Melotto, M.; He, S. Y. *Proc. Natl. Acad. Sci. U. S. A.* **2012,** *109*, 20148.
27 Okada, M.; Egoshi, S.; Ueda, M. *Biosci. Biotechnol. Biochem.* **2010,** *74*, 2092.
28 Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angewandte Chemie-International Edition* **2001,** *40*, 2004.
29 Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002,** *67*, 3057.
30 Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Del. Rev.* **2001,** *46*, 3.
31 Yamakoshi, H.; Dodo, K.; Okada, M.; Ando, J.; Palonpon, A.; Fujita, K.; Kawata, S.; Sodeoka, M. *J. Am. Chem. Soc.* **2011,** *133*, 6102.
32 Duan, Y.; Wu, C.; Chowdhury, S.; Lee, M. C.; Xiong, G.; Zhang, W.; Yang, R.; Cieplak, P.; Luo, R.; Lee, T.; Caldwell, J.; Wang, J.; Kollman, P. *J. Comput. Chem.* **2003**, *24*, 1999.

33 Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.
34 Darden, T.; York, D.; and Pederson, L. *J. Chem. Phys.* **1993**, *98*, 10089.
35 Ueda, M. *Chem. Lett.* **2012,** *41*, 658.