

Structural Determination of Hypnosin, a Spore Germination Inhibitor of Phytopathogenic *Streptomyces* sp. Causing Root Tumor in Melon (*Cucumis* sp.)

YUU AOKI,[†] MASAHIRO YOSHIDA,[‡] HIROSHI KAWAIDE,[§] HIROSHI ABE,[§] AND MASAHIRO NATSUME*[§]

Science of Plant and Animal Production Course, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan, Department of Bioscience, School of Agriculture, Kyushu Tokai University, Minamiaso, Aso, Kumamoto 869-1404, Japan, and Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology, Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

The structure of a germination inhibitor, hypnosin, isolated from phytopathogenic *Streptomyces* sp. causing root tumor of melon was determined to be 3-acetylaminopyrazine-2-carboxylic acid (**1**) by mass spectrometry, computational chemical prediction of UV spectrum, and synthesis of candidates. The structure–activity relationship of hypnosin and anthranilic acid was examined, and it was concluded that pyrazinecarboxylic acid or pyridine-2-carboxylic acid was the fundamental structure with activity, that methylation of the carboxyl group or decarboxylation destroyed activity, and that the presence of an amino group was inhibitory to the activity, whereas acetylation or deletion of an amino group enhanced activity. Hypnosin inhibited spore germination of some *Streptomyces* spp. in addition to the species with which it was isolated.

KEYWORDS: Structural determination; computational chemistry; germination inhibitor; spore germination; *Streptomyces* sp.; root tumor of melon; *Cucumis* sp.

INTRODUCTION

The life cycle of microorganisms is often controlled by regulatory substances (1). The life cycle of *Streptomyces* has two important steps. One is the differentiation of aerial mycelia from substrate mycelia and subsequent sporulation. Aerial mycelia are clearly distinguishable from substrate mycelia in *Streptomyces* spp. Substrate mycelia are translucent and hydrophilic, while aerial mycelia show various colors and possess a characteristic hydrophobic surface structure called a sheath. Thus, the differentiation of aerial mycelia is a dramatic change and is easily distinguishable with the naked eye. Another reason why this step has received a lot of attention is that the differentiation of aerial from substrate mycelia and sporulation are closely related to secondary metabolite production. These characteristics have led many investigators to clarify the regulatory mechanism for differentiation; several regulatory substances control aerial mycelium formation, antibiotic production, or both in *Streptomyces* spp. Pamamycin induces the

formation of aerial mycelia and regulates the production of antibiotics or pigment in *S. alboniger* and other *Streptomyces* spp. (2–4). A butyrolactone, A-factor, restores streptomycin production in a nonproducing mutant of *S. griseus* (5). Many other butyrolactones were subsequently identified from various *Streptomyces* spp. and are recognized as quorum sensing signals (6).

Germination of spores is the other important event in the life cycle of *Streptomyces* spp. Ensign et al. reported that a gentle heat shock or treatment with some detergents eliminated the lag period preceding germination of *S. viridochromogenes* spores (7, 8) and found the germination inhibitor in the filtrate of germinating spore suspension and submerged cultured material (9). The inhibitor was isolated from the submerged cultured material by Petersen et al. and was determined to be 3-ethyl-4-hydroxy-6-(1-methylpropyl)-2H-pyran (germicidin) (10).

Yoshida et al. reported that about 10–20% of the spores of *Streptomyces* sp. B-9-1, the pathogen causing root tumor of melon, were ungerminated, that a heat-shock treatment increased the colony-forming rate to 110–115% compared to an untreated control, and that heat shock was more effective when the spores were placed in a sodium dodecyl sulfate solution (11). These observations led to the assumption that the spores contain some inhibitory factor to germination. We searched for the germina-

* To whom correspondence should be addressed. Fax: +81-42-367-5698. E-mail: natsume@cc.tuat.ac.jp.

[†] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology.

[‡] Kyushu Tokai University.

[§] Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology.

tion inhibitor in the liquid-cultured material of *Streptomyces* sp. B-9-1 and isolated anthranilic acid as a candidate inhibitor (12). However, its activity and content in the spores were not sufficient to explain the dormancy of the spores. We therefore re-examined the germination rate of spores and the activation conditions for spore germination with four strains of *Streptomyces* sp. that cause root tumor of melon and succeeded in isolating another germination inhibitor from strain CB-1-1 (13).

Here, we report on the structural elucidation and structure–activity relationship of this germination inhibitor, hypnosin, isolated from *Streptomyces* sp. CB-1-1 and its effect on other *Streptomyces* spp.

MATERIALS AND METHODS

General Procedures. High-performance liquid chromatography (HPLC) analysis was performed with a Shimadzu LC-10 system equipped with a SPD-M10A_{VP} photodiode array (PDA) detector. Liquid chromatography–electrospray ionization–time-of-flight mass spectrometry (LC–ESI–TOF MS) was performed with an Agilent 1100 LC system and a JEOL JMS-T100LC AccuTOF spectrometer. Chromatographic separation by HPLC and LC–MS was carried out on a reversed-phase column (Develosil ODS-UG, 5 μ m particle size, 150 \times 2.0 mm; Nomura Chemical, Seto, Japan) using isocratic elution with 10% aqueous MeOH at a flow rate of 0.1 mL min⁻¹. The ESI interface was operated in positive or negative mode [needle voltage of 2.0 kV, orifice 1 voltage of 40 V (100 V for collision-induced dissociation (CID) experiments), orifice 2 voltage of 5 V, ring lens voltage of 10 V, ion guide voltage of 500 V, desolvation temperature at 250 °C, orifice temperature at 100 °C, nebulizing gas flow rate of 1.0–1.5 L N₂ min⁻¹, and drying gas flow rate of 1.0–1.5 L N₂ min⁻¹].

Synthesis of Candidate Compound 1. 3-Aminopyrazine-2-carboxylic acid (**3**, 13.9 mg, Sigma-Aldrich Japan, Tokyo, Japan) was refluxed in a mixture of acetic acid (8 mL) and acetic anhydride (4 mL) for 3 h. The reaction mixture was mixed with toluene (ca. 10 mL) and concentrated in vacuo. The residue was suspended in water (5 mL), and the suspension was passed through a SepPak C-18 cartridge (360 mg). The cartridge was washed with H₂O, and **1** was eluted with 40% aqueous MeOH (5 mL). The eluate was concentrated in vacuo to obtain 3-acetylamino-pyrazine-2-carboxylic acid (**1**, 18.4 mg, quant.). Pale pink powder. UV (MeOH) λ_{\max} (ϵ): 258 (41 900) and 321 nm (33 300). LC–ESI–MS: m/z 182 [M + H]⁺; m/z 180 [M – H]⁻. ¹H NMR (600 MHz, CDCl₃) δ : 2.42 (s, 3, CH₃CO), 8.27 (d, 1, J = 2.2 Hz, H-6, =CH–), 8.69 (d, 1, J = 2.2 Hz, H-5, =CH–), 9.53 (s, 1, NH), 10.78 (s, 1, CO₂H). ¹³C NMR (CDCl₃) δ : 26.0 (CH₃CO), 136.5 (C-2), 140.1 (C-3), 141.7 (C-6), 148.0 (C-5), 168.6 (CO₂H), 169.2 (CH₃CO).

The other candidate **2** was also prepared from **3**.

Determination of Calculated UV Spectra. UV spectra were determined by the CAChe workgroup program version 7.5 (Fujitsu, Tokyo, Japan) in the following procedure; the most stable conformation of each compound was calculated by the CONFLEX program in a MM3 force field, and then its UV spectrum was determined by an improved ZINDO program based on INDO/1 parameters. The reliability of the calculated spectrum was confirmed by a comparison of the observed spectrum with the calculated one in tryptophan and anthranilic acid.

Microorganisms and Media. *Streptomyces* sp. A-1, B-9-1, CB-1-1, and KM-2-1 had been previously isolated from diseased melons by Yoshida et al. (14). Reference strains listed in **Table 1** were obtained from The National Institute of Technology and Evaluation, Biological Resource Center (NBRC, Kisarazu, Japan) or The Japan Collection of Microorganisms (JCM), RIKEN BioResource Center (Wako, Japan). *S. coelicolor* A3(2) was supplied by Dr. Keith Chater of the John Innes Centre, U.K.

Bioassay of Germination Inhibitory Activity. Germination inhibitory activity of samples was examined at eight concentrations from 1000 to 0.0128 μ g/mL by 5-fold serial dilution. Spores were incubated in the presence or absence of a sample in a 96-well microplate at 28

Table 1. Germination Inhibitory Activity of Hypnosin (**1**) on *Streptomyces* sp.

strain	spore germination inhibition rate by hypnosin (%)			germination rate (%)
	8 μ g/mL	40 μ g/mL	200 μ g/mL	
Strains Causing Root Tumor of Melon				
<i>Streptomyces</i> sp. A-1	12	37	80	84
<i>Streptomyces</i> sp. B-9-1	14	28	67	87
<i>Streptomyces</i> sp. CB-1-1	90	100	100	52
<i>Streptomyces</i> sp. KM-2-1	32	65	99	64
Other Species				
<i>S. acidiscabies</i> JCM 7913	30	70	93	69
<i>S. alboniger</i> NBRC 12738	37	54	100	72
<i>S. coelicolor</i> A3(2)	23	66	81	70
<i>S. griseus</i> NBRC 3237	14	51	77	86
<i>S. ipomoeae</i> NBRC 14508	12	43	52	85
<i>S. scabiei</i> NBRC 13767	25	79	100	72
<i>S. turgidiscabies</i> NBRC 16080	0	7	32	77
<i>S. viridochromogenes</i> JCM 4265	0	0	89	87

°C for 48 h. The absorbance at 595 nm was measured with a microplate reader, and the germination inhibition rate was calculated as previously reported (12).

Chemicals for Analysis of the Structure–Activity Relationship. Chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan), Sigma-Aldrich Japan (Tokyo, Japan), and Tokyo Chemical Industry (Tokyo, Japan).

RESULTS

Structural Elucidation of the Inhibitor. The germination inhibitor (0.25 mg) was isolated from agar cultured material of *Streptomyces* sp. CB-1-1 (3500 plates containing 10 mL of medium) by solvent extraction, silica gel column chromatography, and ODS–HPLC (13).

The molecular weight of the inhibitor was determined to be 181 from the molecular-related ion at m/z 180 and 182 in negative and positive mode, respectively, of LC–ESI–MS analysis. Two possible molecular formulas, C₇H₆N₃O₃ (calcd. 180.0409) or C₁₂H₆NO (calcd. 180.0449), were deduced from high-resolution LC–ESI–MS (negative mode, m/z 180.0407). A characteristic UV spectrum [λ_{\max} (10% aqueous MeOH) 263, 318, and 328 nm] was revealed by HPLC–PDA detector analysis.

The inhibitor was reacted with CH₂N₂, and the product was analyzed by LC–MS (positive mode). The untreated inhibitor showed a peak at a retention time of 3.7 min on the chromatogram monitored at m/z 182, which corresponded to the [M + H]⁺ ion, and no peak was observed on the chromatogram monitored at m/z 196 (parts **A** and **B** of **Figure 1**). Analysis of the reaction product showed a peak at a retention time of 4.4 min on the chromatogram monitored at m/z 196 but no peak at m/z 210 (parts **C** and **D** of **Figure 1**). The in-source CID spectrum of the [M – H]⁻ ion (m/z 180) showed peaks at m/z 136.0533 and 94.0431, which were deduced to be due to [M – CO₂]⁻ and [M – C₃H₂O₃ (CO₂ + C₂H₂O)]⁻, respectively (**Figure 3A**). These data suggested that the inhibitor has one carboxyl group with the molecular formula C₇H₇N₃O₃. We measured a ¹H nuclear magnetic resonance (NMR) spectrum; however, the spectrum obtained was uninformative.

We listed 516 structures that have the molecular formula C₇H₇N₃O₃ by searching the SciFinder database and our own design. Among these, structures that have a carboxyl group and an acetyl group or other functional group expected to produce

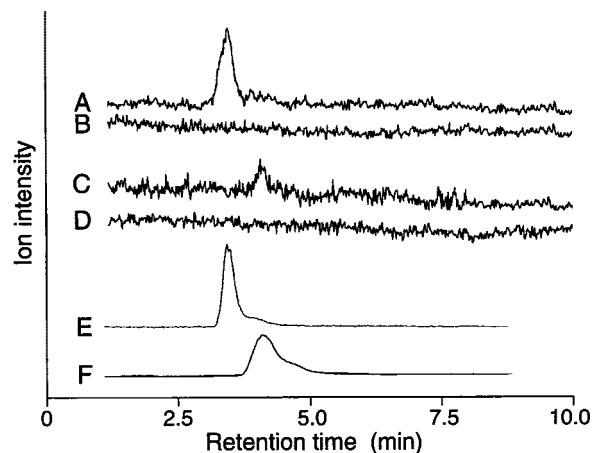


Figure 1. Mass chromatograms of the natural germination inhibitor and synthetic compound **1**. Analyses of the natural germination inhibitor monitored at m/z 182 (A) or m/z 196 (B). Analyses of the methylated derivative of the natural germination inhibitor monitored at m/z 196 (C) or m/z 210 (D). Analysis of synthetic **1** monitored at m/z 182 (E) and analysis of the methyl ester of **1** monitored at m/z 196 (F).

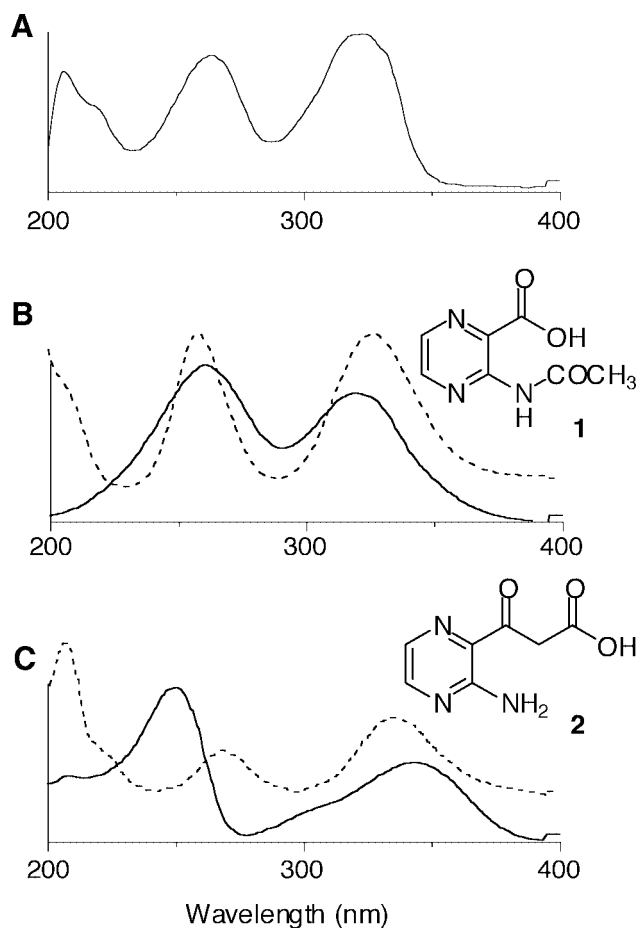


Figure 2. UV spectrum of the natural germination inhibitor and calculated and actual UV spectra of selected structural candidates **1** and **2**. The UV spectrum of the natural germination inhibitor (A) was recorded with a photodiode array detector during HPLC (elution solvent: 10% aqueous MeOH). Calculated UV spectra (dotted line in B and C) were determined by the CAChe program. Actual UV spectra (solid line in B and C) were recorded in a MeOH solution.

a $[M - C_3H_2O_3]^-$ fragment ion in an in-source CID spectrum were selected, narrowing the candidates down to 14 structures. UV spectra of these candidates were determined by the CAChe

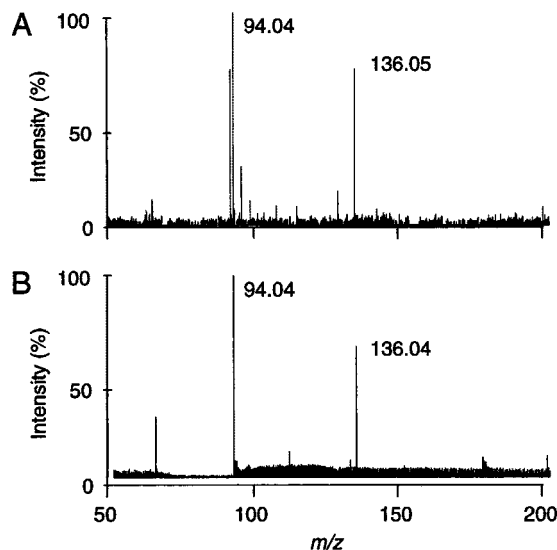


Figure 3. CID spectra of the natural germination inhibitor (A) and synthetic compound **1** (B). Product ions derived from the $[M - H]^-$ ion (m/z 180) were observed.

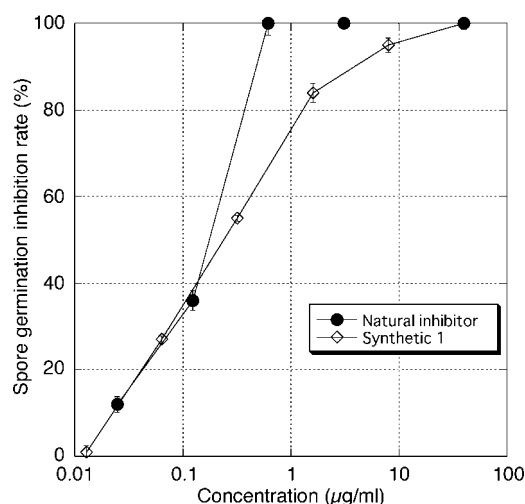


Figure 4. Germination inhibitory activities of the natural germination inhibitor and synthetic compound **1**.

program, and compounds that presented λ_{max} 250–280 and 300–340 nm with comparable intensity were selected. On the basis of these results, two candidates, 3-acetylaminopyrazine-2-carboxylic acid (**1**) and 3-(3-aminopyrazin-2-yl)-3-oxopropionic acid (**2**) were selected (Figure 2).

Comparison of the Germination Inhibitor and Synthetic Candidates. Candidate **1** was synthesized by refluxing of 3-aminopyrazine-2-carboxylic acid (**3**) in $Ac_2O/AcOH$. The UV spectrum of the synthetic **1** accorded well with that predicted by the calculation and with that of the natural inhibitor (Figure 2B). The retention time of compound **1** in LC–MS analysis was identical to that of the natural inhibitor (retention time of 3.7 min, Figure 1E). The methyl ester of synthetic **1** also showed the same retention time as that of the natural inhibitor (retention time of 4.4 min, Figure 1F). The in-source CID spectrum of synthetic **1** was identical with that of the natural inhibitor (Figure 3). These results suggested that the structure of the germination inhibitor is **1**.

The germination inhibitory activity of synthetic compound **1** was compared to that of the natural inhibitor (Figure 4). The dose–response curve of synthetic **1** was similar to that of the natural inhibitor, and the IC_{50} values of both compounds

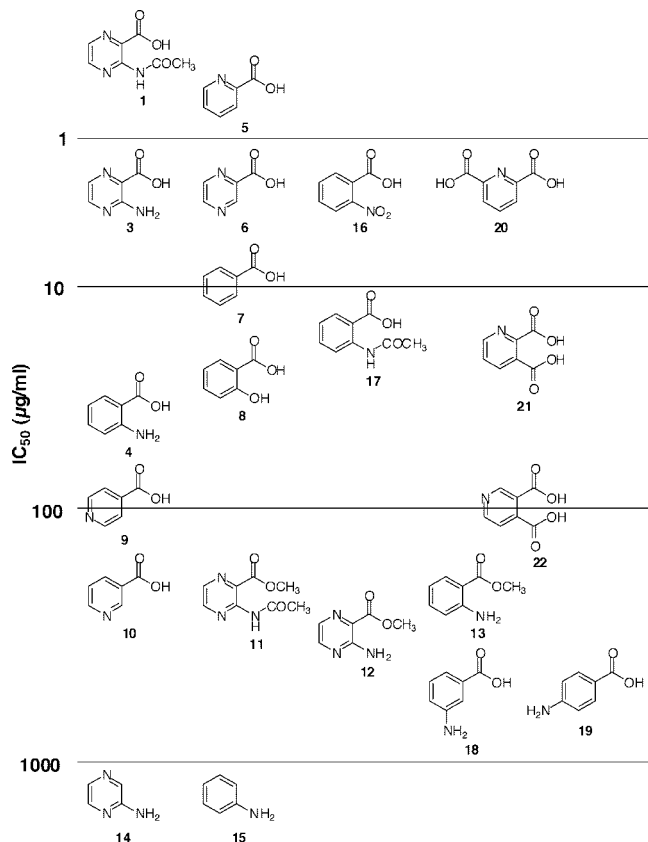


Figure 5. Structure–activity relationship of the natural germination inhibitor hypnosin (**1**) and related compounds. Structures are displayed according to their IC_{50} values.

were ca. 0.25 $\mu\text{g}/\text{mL}$. We, thus, concluded that the structure of the germination inhibitor isolated from *Streptomyces* sp. CB-1-1 is 3-acetylaminopyrazine-2-carboxylic acid (**1**). This is the first finding of compound **1** as a natural product; thus, we named it hypnosin.

Compound **1** was reported by Thurston et al. as a synthetic intermediate of an antitumor agent (**15**), which is the sole record of **1** in the SciFinder database. The ^1H NMR data of **1** that they reported were in discord with those of ours, but their data were coincident with those of **3**. They prepared **1** by partial hydrolysis of methyl 3-acetylamino-2-pyrazine carboxylate with LiOH; thus, there is a possibility that they obtained **3**, because they did not show mass spectral data.

The other candidate **2** was also prepared from **3**. Synthetic **2** showed a different UV spectrum from that of the natural inhibitor and calculated spectrum in wavelength and intensity of maxima (**Figure 2C**). Compound **2** eluted later than the natural inhibitor (retention time of 8.5 min) in LC–MS analysis, and it showed no activity.

Structure–Activity Relationship. The structural similarity of hypnosin (**1**) and a previously identified germination inhibitor, anthranilic acid (**4**) (**12**), led us to examine the germination inhibitory activity of related compounds (**Figure 5**).

Picolinic acid (**5**) showed comparable activity to hypnosin (**1**). The fundamental structures to show activity were pyrazinecarboxylic acids (such as **2**, **3**, and **6**) and pyridine-2-carboxylic acid (**5**); benzoic acid derivatives, such as anthranilic acid (**4**), benzoic acid (**7**), and salicylic acid (**8**) and regioisomers of pyridinecarboxylic acids (**9** and **10**) showed less than $1/10$ the activity of the heterocyclic acids. Methylation (from **2** to **11**, from **3** to **12**, and from **4** to **13**) or decarboxylation (from **3** to **14** and from **4** to **15**) eliminated activity, which showed

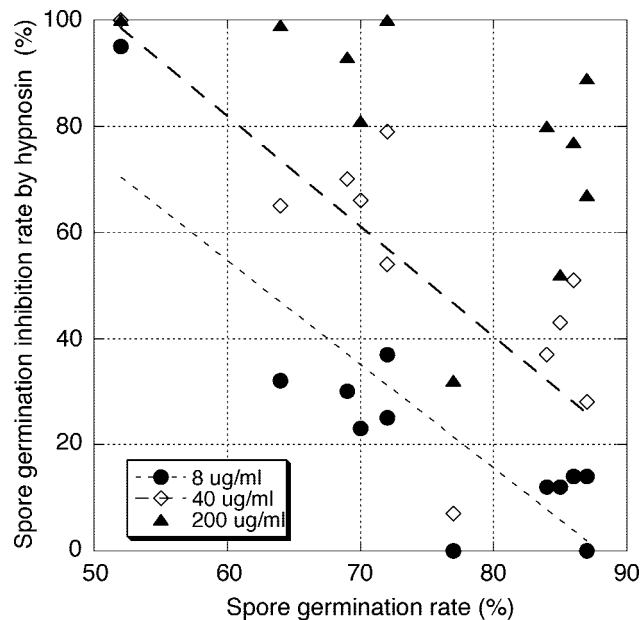


Figure 6. Relationship between the spore germination rate of *Streptomyces* sp. and the spore germination inhibition rate by hypnosin (**1**).

that the free carboxyl group is indispensable for germination inhibitory activity. The correlation between the pK_a value and activity, however, depended upon the situation; a good correlation was observed among carboxylic acids with various aromatic rings [**7** ($pK_a = 4.2$), **6** ($pK_a = 2.9$), and **5** ($pK_a = 0.9$)] but not among substituted benzoic acids [**4** ($pK_a = 5.0$), **7** ($pK_a = 4.2$), **8** ($pK_a = 3.0$), and **16** ($pK_a = 2.1$)]. Nicotinic acid (**10**, $pK_a = 2.2$) had $1/100$ the activity of hypnosin (**1**, $pK_a = 2.3$). Acetylation of the amino group (from **3** to **1** and from **4** to **17**), conversion of the amino group to a hydroxyl group (from **4** to **8**), or deamination (from **4** to **7**) increased activity, which suggested that a decrease in electron density in the aromatic ring increases inhibitory activity. This idea was supported by the elevation of activity in the derivative with an electron-withdrawing nitro group (**16**) but was contradicted by the result that regioisomers of anthranilic acid (**18** and **19**) were inactive. A ring nitrogen or nitrogenous group adjacent to the carboxyl group may have some contribution to activity. Dipicolinic acid (**20**) showed relatively strong activity, but its regioisomers, quinolinic acid (**21**) and cinchomeronic acid (**22**), had $1/10$ and $1/25$ the activity of **20**.

Effect of the Germination Inhibitor on Other *Streptomyces* spp. Spore germination inhibitory activity of hypnosin (**1**) on other strains of *Streptomyces* sp. causing root tumor of melon and on other *Streptomyces* species was examined. *Streptomyces* sp. CB-1-1, which was used for the isolation and bioassay of hypnosin, was most sensitive to hypnosin; *Streptomyces* sp. B-9-1, which was used in a previous study (**12**) was less sensitive (**Table 1**). Hypnosin also showed activity against other phytopathogenic species, such as *S. acidiscabies* and *S. scabiei*, and to a lesser extent against *S. ipomoeae* and *S. turgidiscabies*; these are causative agents of potato scab and sweet potato soil rot. Spores of typical actinomycetes *S. coelicolor* and *S. griseus* were also affected.

The germination rate of the strain used in the experiment was 52–87% (**Table 1**), which showed that the formation of spores that do not germinate easily is common in *Streptomyces* spp. Spore germination inhibition rates in **Table 1** were plotted against germination rates (**Figure 6**). A close correlation was observed between the spore germination inhibition rate by

hypnosin and the spore germination rate (correlation coefficient, R , at 40 $\mu\text{g/mL}$, -0.79 ; R , at 8 $\mu\text{g/mL}$, -0.86).

DISCUSSION

Spore formation-inducing substances and germination-inhibitory substances are well-known in fungi (1, 16–19), although their mode of action needs to be clarified. In *Streptomyces* spp., inducers of differentiation of aerial mycelia from substrate mycelia and subsequent sporulation and their mode of action are well-studied (2, 6, 20). Inhibitors of aerial mycelial differentiation have also been reported (21, 22). Hypnosin is the second germination inhibitor observed in *Streptomyces* spp.

The structure–activity relationship study revealed that picolinic acid (5) shows comparable activity to hypnosin. Picolinic acid has been reported as a germination inhibitor of *Pyricularia oryzae*, one of the most important phytopathogenic fungi in rice (23); picolinic acid reversibly inhibits spore germination of *P. oryzae* at 400 $\mu\text{g/mL}$. Spores of *P. oryzae* contain ca. 0.1% of picolinic acid as spore dry weight, which is entirely discharged from spores into water after 3 h of incubation, when the germ tube is hardly visible. Tamari and Kaji showed that it acts as a chelating agent (23, 24). This mode of action is relevant to our finding that active substances have a ring nitrogen or a nitrogenous group adjacent to a carboxyl group. However, in considering the result that picolinic acid showed activity at less than 1 $\mu\text{g/mL}$ in *Streptomyces* sp. CB-1-1, another mechanism should be developed.

Dipicolinic acid (DPA, 20) showed relatively strong activity. DAP was originally thought to be the germination inhibitor in *Bacillus* spp., but now, it is understood as a large depot for *Bacillus* spores (ca. 10% of the dry weight), and release of Ca^{2+} –DPA from and alternate penetration of water into spores is a crucial step in spore germination in *Bacillus* (25). Contrary to the above understanding concerning DPA, 6-carbamoylpyridine-2-carboxylic acid was isolated from *Bacillus subtilis* culture broth as a germination inhibitor of *Bacillus* spp. (26). However, it required a high concentration for inhibition (1 mg/mL), and there was no mention of whether it was contained in the spores, whether its activity was reversible, or whether it was inactive on mycelia. Taking these findings with our results, the action of hypnosin and related compounds on Gram-positive bacteria, such as *Streptomyces*, *Bacillus*, etc., needs to be reinvestigated.

Hypnosin showed inhibitory activity in many *Streptomyces* species, and the spore germination rate closely correlated with the spore germination inhibition rate by hypnosin. These results indicate that germination of *Streptomyces* spores is regulated by hypnosin or that hypnosin inhibits a common step of spore germination in *Streptomyces* spp. We have preliminary results that spores of some species contain hypnosin. The germination inhibitor hypnosin, thus, will be a powerful tool for the study of regulatory mechanisms in the germination of *Streptomyces* spores and as a lead compound for agrochemicals that control the germination of spores. We are now investigating the presence of hypnosin in various *Streptomyces* spp. to confirm that hypnosin is a universal germination inhibitor in *Streptomyces* spp. and its mode of action.

ACKNOWLEDGMENT

We thank Drs. Tohru Teraoka and Tsutomu Sato, Tokyo University of Agriculture and Technology, for helpful discussions on *Pyricularia oryzae* and *Bacillus subtilis*, respectively.

Supporting Information Available: Synthesis of candidate compound 2, mass chromatograms of the natural germination inhibitor and synthetic compound 2, and germination inhibitory activities of the natural germination inhibitor and synthetic compound 2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- (1) Khokhlov, A. S. *Microbial Autoregulators*; Harwood Academic Publishers: Chur, Switzerland, 1991.
- (2) Natsume, M. Differentiation of aerial mycelia—Pamamycins and calcium ion in *Streptomyces alboniger*. *Actinomycetologica* **1999**, *13*, 11–19.
- (3) Hashimoto, M.; Kondo, T.; Kozone, I.; Kawaide, H.; Abe, H.; Natsume, M. Relationship between response to and production of the aerial mycelium-inducing substances pamamycin-607 and A-factor. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 803–808.
- (4) Hashimoto, M.; Kozone, I.; Kawaide, H.; Abe, H.; Natsume, M. Biosynthetic route of pamamycin-607, a nitrogen-containing polyketide. *J. Antibiot.* **2005**, *58*, 722–730.
- (5) Khokhlov, A. S.; Anisova, L. N.; Tovarova, I. I.; Kleiner, E. M.; Kovalenko, I. V.; Krasilnikova, O. I.; Kornitskaya, E. Y.; Pliner, S. A. Effect of A-factor on the growth of asporogenous mutants of *Streptomyces griseus*, not producing this factor. *Z. Allg. Mikrobiol.* **1973**, *13*, 647–655.
- (6) Takano, E. γ -Butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation. *Curr. Opin. Microbiol.* **2006**, *9*, 287–294.
- (7) Hirsch, C. F.; Ensign, J. C. Heat activation of *Streptomyces viridochromogenes* spores. *J. Bacteriol.* **1976**, *126*, 24–30.
- (8) Grund, A. D.; Ensign, J. C. Activation of *Streptomyces viridochromogenes* spores by detergents. *Curr. Microbiol.* **1982**, *7*, 223–228.
- (9) Grund, A. D.; Ensign, J. C. Properties of the germination inhibitor of *Streptomyces viridochromogenes* spores. *J. Gen. Microbiol.* **1985**, *131*, 833–847.
- (10) Petersen, F.; Zähler, H.; Metzger, J. W.; Freund, S.; Hummel, R.-P. Germicidin, an autoregulative germination inhibitor of *Streptomyces viridochromogenes* NRRL B-1551. *J. Antibiot.* **1993**, *46*, 1126–1138.
- (11) Yoshida, M.; Nishiyama, T.; Yamaguchi, T.; Kobayashi, K. Spore germination and its activation of the pathogenic *Streptomyces* sp. causing root tumor of melon. *Ann. Phytopathol. Soc. Jpn.* **1994**, *60*, 711–716 (in Japanese with an English summary).
- (12) Aoki, Y.; Yoshida, Y.; Yoshida, M.; Kawaide, H.; Abe, H.; Natsume, M. Anthranilic acid, a spore germination inhibitor of phytopathogenic *Streptomyces* sp. B-9-1 causing root tumor of melon. *Actinomycetologica* **2005**, *19*, 48–54.
- (13) Aoki, Y.; Yoshida, M.; Kawaide, H.; Abe, H.; Natsume, M. Isolation and characterization of a spore germination inhibitor from *Streptomyces* sp. CB-1-1, a phytopathogen causing root tumor of melon. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 986–992.
- (14) Yoshida, M.; Kobayashi, K. Taxonomic characterization of the actinomycete causing root tumor of melon. *Ann. Phytopathol. Soc. Jpn.* **1991**, *57*, 540–548 (in Japanese with an English summary).
- (15) Thurston, D. E.; Bose, D. S.; Howard, P. W.; Jenkins, T. C.; Leoni, A.; Baraldi, P. G.; Guiotto, A.; Cacciari, B.; Kelland, L. R.; Foloppe, M.-P.; Rault, S. Effect of A-ring modifications on the DNA-binding behavior and cytotoxicity of pyrrolo[2,1-c][1,4]benzodiazepines. *J. Med. Chem.* **1999**, *42*, 1951–1964.
- (16) Katayama, M.; Marumo, S.; Hattori, H. The revised structure of sclerosporin, a sporogenic substance of *Sclerotinia fructicola*. The total synthesis of (\pm)-sclerosporin. *Tetrahedron Lett.* **1983**, *24*, 1703–1706.
- (17) Tanaka, S.; Wada, K.; Marumo, S.; Hattori, H. Structure of sporogen-AO 1, a sporogenic substance of *Aspergillus oryzae*. *Tetrahedron Lett.* **1984**, *25*, 5907–5910.
- (18) Natsume, M.; Takahashi, Y.; Marumo, S. Chlamyospore-like cell-inducing substances of fungi: Close correlation between chemical

- reactivity with methylamine and biological activity. *Agric. Biol. Chem.* **1988**, *52*, 307–312.
- (19) Ueno, T.; Miyagawa, H.; Turushima, T.; Inoue, M. Spore germination self-inhibitors from plant pathogenic fungi. *J. Pestic. Sci.* **1997**, *22*, 342–350.
- (20) Ohnishi, Y.; Yamazaki, H.; Kato, J.; Tomono, A.; Horinouchi, S. AdpA, a central transcriptional regulator in the A-factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 431–439.
- (21) Kondo, S.; Katayama, M.; Marumo, S. Carbazomycinal and 6-methoxycarbazomycinal as aerial mycelium formation-inhibitory substances of *Streptoverticillium* species. *J. Antibiot.* **1986**, *39*, 727–730.
- (22) Natsume, M.; Ooshiro, A.; Takahashi, T.; Kokido, F.; Hashimoto, M.; Aoki, Y.; Kawaide, H.; Abe, H. Isolation and structural properties of aerial mycelium differentiation-inhibitory substances against *Streptomyces scabiei* causing potato common scab. *J. Pestic. Sci.* **2007**, *32*, 131–134.
- (23) Tamari, K.; Ogasawara, N.; Kaji, J. Biochemical products of the metabolism of *Piricularia oryzae*. In *The Rice Blast Disease*; Johns Hopkins Press: Baltimore, MD, 1965; pp 35–68.
- (24) Tamari, K.; Kaji, J. The mechanism of the growth inhibitory action of fusaric acid on plants. *J. Biochem.* **1954**, *41*, 143–165.
- (25) Setlow, P. Spore germination. *Curr. Opin. Microbiol.* **2003**, *6*, 550–556.
- (26) Kobayashi, K.; Yamanaka, S.; Kuwahara, T.; Miyano, H.; Ishikawa, K.; Fudou, R. Spore germination inhibitor and food preservative containing 6-carbamoyl-2-pyridine carboxylic acid. Eur. Pat. Appl. EP 1125498 A2, 2001.

Received for review September 12, 2007. Revised manuscript received October 27, 2007. Accepted October 31, 2007.

JF072719X