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Identification of 6-Prenylindole as an Antifungal Metabolite of *Streptomyces* sp. TP-A0595 and Synthesis and Bioactivity of 6-Substituted Indoles

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The biological control is an alternative measure to agrochemicals to control fungal pathogens in agricultural production. We recently reported the utilization of an actinomycete to the biological control in horticultural plant. Tissue-cultured seedlings of horticultural rhododendron suffer from significant damage caused by *Pestalotiopsis sydowiana*, a major pathogen of rhododendron, during the acclimatization. The inoculation of an endophytic *Streptomyces galbus* R-5 isolated from a field-grown rhododendron to the seedlings made them resistant to the pathogen[1~3]. Although the major antifungal metabolites of strain R-5, actinomycin X_2 and fungichromin, were speculated to be responsible for the resistance, their involvement in plant protection was not proved[2]. In spite of this observation, inoculation of such strains that produce secondary metabolites with antifungal activity is expected as a new strategy to control disease resistance in plants.

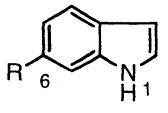
Alternaria brassicicola is a casual pathogen of *Brassica* species and causes damage and loss of quality to *Brassica* crops worldwide[4]. In our previous screening of the microbial secondary metabolites which suppress the disease symptom caused by *A. brassicicola* in the seedlings of Chinese cabbage, two positive strains producing metabolites antagonistic and non-antagonistic to the *in vitro* growth of *A. brassicicola* were obtained. The non-antagonistic compound, fistupyrone, was identified as a metabolite of *Streptomyces* sp. TP-A0569[5] with suppressive effect on the infection of *A. brassicicola* by inhibiting the formation of infection hypha. The antagonistic compound was isolated from *Streptomyces* sp. TP-A0595 and identified as 6-prenylindole (**1**, Fig. 1). In this paper, we describe the isolation of **1** and synthesis and biological activity of 6-substituted indoles.

The producing strain TP-A0595 was isolated from a leaf of leek *Allium tuberosum* collected in Toyama, Japan. Strain TP-A0595 grew well on both organic and synthetic media and formed spiral spore chains. Aerial mass color of the colony was grayish black to black. The whole-cell hydrolysate contained L,L-diaminopimeric acid, glycine, galactose, glucose and ribose. On the basis of these characteristics, strain TP-A0595 was considered to belong to the genus *Streptomyces*.

Strain TP-A0595 was cultured at 30°C for 4 days on a rotary shaker (200 rpm) in four 500-ml K-1 flasks each containing 100 ml of the seed medium consisting of soluble starch 1%, glucose 0.5%, NZ-case (Humco Scheffield Chemical Co.) 0.3%, yeast extract (Difco Laboratories) 0.2%, tryptone (Difco Laboratories) 0.5%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05% and $CaCO_3$ 0.3% (pH 7.0). Three-ml of the seed culture were transferred into one hundred 500-ml K-1 flasks each containing 100 ml of the production medium consisting of glucose 0.5%, glycerol 2%, soluble starch 2%, Pharmamedia (Difco Laboratories) 1.5%, yeast extract 0.3% and Diaion HP-20 (Mitsubishi Chemical Co.) 1%. The pH of the medium was adjusted to 7.0 before sterilization. The inoculated flasks were shaken on a rotary shaker (200 rpm) at 30°C for 6 days.

The fermented whole broth (10 liters) was stirred with acetone (10 liters) for 2 hours and centrifuged (8,000 rpm, 10 minutes) to separate the mycelium. The supernatant was concentrated and the pH was adjusted to 8.0. The aqueous solution was applied to a Diaion HP-20 column and eluted with 10% methanol and 80% acetone. After evapolation of the aqueous acetone eluent, the pH was adjusted to 2.0 and extracted with AcOEt (400 ml) twice. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to give a brown oil (4.94 g). The oily substance (4.0 g) was applied

Fig. 1. 6-Substituted indoles used in this study.



6-Prenylindole (**1**)	R= $(CH_3)_2C=CHCH_2$-
6-Allylindole (**8**)	R= $CH_2=CHCH_2$-
6-Phenylindole (**9**)	R= C_6H_5-
6-Isopropylindole (**12**)	R= $(CH_3)_2CH$-
6-Methylindole (**13**)	R= CH_3-

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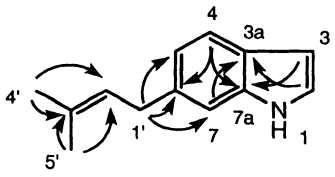
to a column of silica gel (Wakogel C-200, 120 g) and developed with $CHCl_3$ - MeOH (100 : 1~5 : 1). **1** (61.4 mg) was obtained as a pale brown oil which crystallized on standing in a refrigerator. Its structure was confirmed by the analysis of 2D-NMR spectra[6]. HMBC correlations observed with **1** are summarized in Fig. 2.

Isolation of **1** was first reported as a component of the liverwort (*Hepaticae*) without any description on the bioactivity[7]. It was reisolated from *Streptomyces hygroscopicus* in the screening for the growth inhibitor of SV-40 transformed cells[8]. The present study first describes the antifungal activity of **1** against plant fungal pathogens, *A. brassicicola* and *Fusarium oxysporum*. The inhibition diameters in paper disc assay are shown in Table 1. Antimicrobial activity of indole and its derivatives has been reported in 1974 by two groups[9,10]. Indole was shown to have a wide antibiotic spectrum against fungi such as *Candida*, *Pyricularia* and *Aspergillus* with the MIC of 100~200 μg/ml[9]. Among a number of indole derivatives having various substituents, halogenated indoles such as 6-chloro-2-methylindole were demonstrated to be most antifungal with the MIC of 10~100 μg/ml[10]. Since these studies did not include indoles having carbon-branch at 6-position as well as **1**, we synthesized 6-substituted indole derivatives for the comparison of antifungal activity.

Fig. 2. Significant HMBC correlations observed with **1**.



Synthetic scheme for 6-substituted indoles is shown in Fig. 3. 6-Bromoindole (**4**) was considered as an appropriate precursor for the palladium-catalyzed coupling toward the synthesis of 6-substituted derivatives. Batcho-Leimgruber reaction[11] was applied to the synthesis of **4**. 4-Bromo-2-nitrotoluene (**2**) was condensed with *N,N*-dimethylformamide dimethyl acetal (DMFDMA) in the presence of excess amount of pyrrolidine to provide the nitrostyrene (**3**). The reductive cyclization of **3** was conducted by the treatment with $FeSO_4/NH_4OH$ at reflux to give **4** in 30% yield from **2**. Then the NH group of **4** was protected by the treatment with NaH and benzenesulfonyl chloride to give a key intermediate **5**. 6-Allylindole (**8**) was synthesized by Stille coupling reaction[13] of **5** with allyltributyltin, followed by the hydrolysis of the protective group with KOH in MeOH-H_2O. 6-Phenylindole (**9**) was obtained using Suzuki coupling reaction[14] of phenylboronic acid with **5**, followed by the deprotection with Mg/MeOH. Synthesis of **1** was also investigated by applying Hikawa's condition[12]. Heck reaction of **5** with 1,1-dimethylallylalcohol afforded **6**, which was then treated with *p*-toluenesulfonic acid in benzene to give **7**. 1,4-Reduction and deprotection of **7** was simultaneously carried out by the treatment with Mg in MeOH to yield **1** in 20% yield from **4**. 6-Isopropylindole (**12**) was synthesized by Pd-C catalytic reduction of nitrostylene (**11**), which was

Table 1. Antifungal activity of 6-substituted indoles.

Compound	Substituent	Conc. (μg/disc)	Organisms: *A. brassicicola* TP-F0423	Organisms: *F. oxysporum* f. sp. *tulipae* TU-4-2
1	$(CH_3)_2C=CHCH_2$-	30	18	22
		15	12	17
8	$CH_2=CHCH_2$-	30	13	20
		15	+	-
9	C_6H_5-	30	-	-
12	$(CH_3)_2CH$-	30	-	-
13*	CH_3-	30	-	-

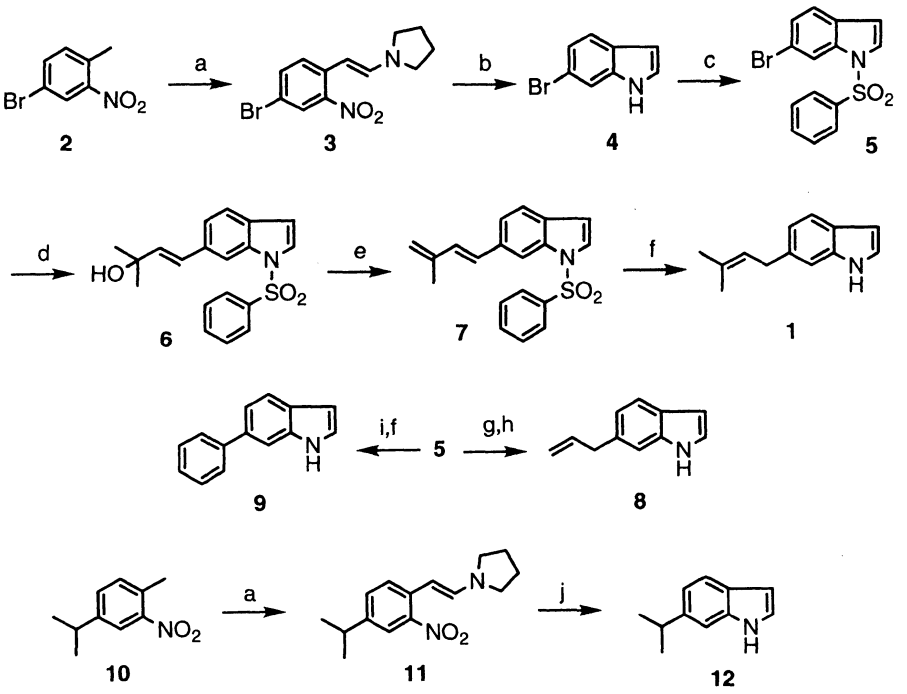
Samples were applied on 10 mm paper disks.

Values indicate diameters (mm) of inhibitory zones.

+: very weak inhibition, -: no inhibition

* purchased from Aldrich Chemical Co.

Fig. 3. Synthesis of 6-substituted indoles (**1**, **8**, **9**, **12**).



Reagents and conditions: a) DMFDMA, pyrrolidine, DMF, 100°C, 97% (**3**), 98% (**11**); b) $FeSO_4$, NH_4OH, reflux, 31%; c) benzenesulfonyl chloride, NaH, DMF, 0~rt., 92%; d) 1,1-dimethylallyl alcohol, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 100°C, 50%; e) $TsOH{\cdot}H_2O$, benzene, 44%; f) Mg, MeOH, 98% (**1**), 99% (**9**); g) allyltributyltin, $PdCl_2(PPh_3)_2$, Et_3N, DMF, 100°C, 87%; h) KOH, MeOH, H_2O, 80°C, 48%; i)phenylboronic acid, $PdCl_2(PPh_3)_2$, K_2CO_3, DMF, H_2O, 100°C, 59%; j) Pd-C, H_2, MeOH, r.t., 78%.

prepared in a similar manner as described for the synthesis of **3** starting from 4-isopropyl-2-nitrotoluene (**10**).

Antifungal activity of the 6-substituted indoles was tested in the paper disc assay against *A. brassicicola* TP-F0423 and *F. oxysporum* f. sp. *tulipae* TU-4-2 which is a cause of tulip bulb rot[15]. 6-Prenyl- and 6-allylindole inhibited the *in vitro* growth of these phytopathogenic fungi but 6-phenyl-, 6-isopropyl- and 6-methylindole did not show activity at the same concentrations (Table 1). These results indicate that the presence of allyl side chain is important to the bioactivity and the substituents on the side chain effect on the potency.

References

1) SHIMIZU, M.; Y. NAKAGAWA, Y. SATO, T. FURUMAI, Y. IGARASHI, H. ONAKA, R. YOSHIDA & H. KUNOH: Studies on endophytic actinomycetes (I) *Streptomyces* sp. isolated from rhododendron and its antifungal activity. J. Gen. Plant Pathol. 66: 360~366, 2000

2) SHIMIZU, M.; T. FURUMAI, Y. IGARASHI, H. ONAKA, T. NISHIMURA, R. YOSHIDA & H. KUNOH: Association of induced disease resistance of rhododendron seedlings with inoculation of *Streptomyces* sp. R-5 and treatment with actinomycin D and amphotericin B to the tissue-culture medium. J. Antibiotics 54: 501~505, 2001

3) SHIMIZU, M.; N. FUJITA, Y. NAKAGAWA, T. NISHIMURA, T. FURUMAI, Y. IGARASHI, H. ONAKA, R. YOSHIDA & H. KUNOH: Disease resistance of tissue-cultured seedlings of rhododendron after treatment with *Streptomyces* sp. R-5. J. Gen. Plant Pathol. 67: 325~332, 2001

4) TAKANO, K.: Alternaria leaf spot of Chinese cabbage (*Brassica campestris* L.) caused by *Alternaria brassicicola* (Sch.) Wiltshire. Proc. Assoc. Pl. Prot. Hokuriku 38: 36~41, 1990

5) IGARASHI, Y.; M. OGAWA, Y. SATO, N. SAITO, R. YOSHIDA, H. KUNOH, H. ONAKA & T. FURUMAI: Fistupyrone, a novel inhibitor of the infection of Chinese cabbage by *Alternaria brassicicola*, from *Streptomyces* sp. TP-A0569. J. Antibiotics 53: 1117~1122, 2000

6) 6-Prenylindole (**1**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.02 (br, 1H, NH), 7.54 (d, 1H, J=8.1 Hz, H4), 7.18 (s, 1H, H7), 7.12 (t, 1H, J=3.0 Hz, H2), 6.96 (dd, 1H, J=8.1, 1.2 Hz, H5), 6.49 (s, 1H, H3), 5.39 (m, 1H, H2′), 3.45 (d, 2H, J=7.3 Hz, H1′), 1.75 (s, 6H, H4′ and H5′); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 136.2 (C6), 135.9 (C7a),

132.0 (C3′), 126.0 (C3a), 124.0 (C2′), 123.6 (C2), 120.9 (C5), 120.5 (C4), 110.2 (C7), 102.4 (C3), 34.5 (C1′), 25.8 (C4′), 17.8 (C5′); EI-MS *m/z* 185 $(M)^+$.

7) BENEŠOVÁ, V.; Z. SAMEK, V. HEROUT & F. ORM: Plant Substrates. XXIX. Isolation and structure of two new indole alkaloids. Collect. Czech. Chem. Commun. 34: 1807~1809, 1969
8) YONEHARA, H.; N. OTAKE, K. ONODERA, T. SEGAWA, K. KIYO & T. SASAKI: Preparation of geldanamycin and other antibiotics. J. Patent S54-135300, October 20, 1979
9) OIMOI, M.; M. HAMADA & T. HARA: Antimicrobial activities of indole. J. Antibiotics 27: 987~988, 1974
10) WHITEHEAD, C. W. & C. A. WHITESITT: Effect of lipophilic substituents on some biological properties of indoles. J. Med. Chem. 17: 1298~1304, 1974
11) BATCHO, A. D. & W. LEIMGRUBER: Indoles from 2-methylnitrobenzenes by condensation with formamide acetals followed by reduction: 4-benzyloxyindole. Org. Synthe. 63: 214~221, 1984
12) HIKAWA, H.; Y. YOKOYAMA & Y. MURAKAMI: A short synthesis of optically active γ,γ-dimethylallyltryptophan (DMAT). Synthesis 214~216, 2000
13) YOKOYAMA, Y.; S. ITO, Y. TAKAHASHI & Y. MURAKAMI: Palladium-catalyzed cross-coupling reaction: Direct allylation of aryl bromide with allyl acetate. Tetrahedron Lett. 26: 6457~6460, 1985
14) MIYAURA, N. & A. SUZUKI: Palladium-catalyzed cross-coupling reactions of organoboron compounds. Chem. Rev. 95: 2457~2483, 1995
15) MUKOBATA, H.; K. NAHATA & T. KUSABA: Studies on a simple technique for evaluation of tulip cultivar resistance to bulb rot caused by *Fusarium oxysporum* f. sp. *tulipae*. (1) The bulb inoculation methods using a needle-prick or dipping in a conidial suspension. Proc. Assoc. Pl. Prot. Hokuriku 30: 91~96, 1982.