

Control of the Bacterial Wilt of Tomato Plants by a Derivative of 3-Indolepropionic Acid Based on Selective Actions on *Ralstonia solanacearum*

Kazuhiko Matsuda,^{*,†} Hideyoshi Toyoda,[‡] Hitomi Nishio,[†] Takatsugu Nishida,[†] Mitsue Dohgo,[‡] Miki Bingo,[‡] Yoshinori Matsuda,[§] Satoshi Yoshida,^{||} Satoshi Harada,^{||} Hiroshi Tanaka,^{||} Koichiro Komai,[†] and Seiji Ouchi[‡]

Departments of Agricultural Chemistry and Agronomy, Faculty of Agriculture, and Institute for Comprehensive Agricultural Sciences, Kinki University, 3327-204 Nakamachi, Nara 631-8505, Japan; and Research Institute, Kagome Company, Ltd., 17 Nishitomiya, Nishinasuno-machi, Nasu-gun, Tochigi 329-2762, Japan

3-Indolepropionic acid (IPA)-related compounds having a benzo[*b*]thiophene or an indazole ring and derivatives having various substituents in the propionic acid moieties were tested for their antibacterial activity against *Ralstonia solanacearum*. Substitution of the indole ring for other aromatic rings resulted in lowered activity, whereas addition of a methyl or a trifluoromethyl group to the propionic acid moiety had little effect. Of the derivatives, 3-(3-indolyl)butanoic acid (3-IBA) was as active as IPA, exhibiting a 10-fold higher activity with the *S* configuration than with the *R* configuration. In contrast with the strong phytotoxicity of IPA, 3-IBA was able to suppress bacterial wilt without affecting the growth of tomato plants.

Keywords: 3-Indolepropionic acid; 3-(3-indolyl)butanoic acid; *Ralstonia solanacearum*; bacterial wilt; tomato plants

INTRODUCTION

Ralstonia solanacearum (previously classified as *Pseudomonas*) is a pathogen that causes wilt of many *Solanaceae* plants in warmer climates in most parts of Japan. To date, no measures other than sterilization of soils by fumigants such as chlorpicrin or by sunlight are used for controlling the pathogen. To circumvent this problem, we have screened indole derivatives and related compounds to inhibit the growth of *R. solanacearum* on the basis of our finding that indole secreted by *Klebsiella oxytoca* was capable of inhibiting the growth of the pathogen (Matsuda et al., 1990). This screening led to the discovery of 3-indolepropionic acid (IPA), which is much more active than indole with selective toxicity when tested against *R. solanacearum* (Toyoda et al., 1991).

We have tested the antibacterial activity of IPA derivatives with a variety of substituents on the indole ring as well as those having side-chain moieties that differ in length or functional groups to clarify the structural factors that contribute to their antibacterial action (Toyoda et al., 1991; Matsuda et al., 1993). It was found that the compounds having a substituent such as fluorine on position 4 of the indole nucleus were capable of inhibiting bacterial growth at low concentra-

tions. A change of the carboxylic acid group and the length of the propionic acid moiety abolished the activity.

To further extend structure–activity studies of the indolepropanoids, we synthesized IPA derivatives and related compounds in which the indole ring is replaced by either a benzo[*b*]thiophene or an indazole ring and in which the α - or β -proton of the propionic acid moiety is substituted by various groups and tested for their antibacterial actions. We here show that a methylated derivative of IPA is as potent as IPA and much less phytotoxic to tomato plants than IPA, thereby exhibiting the capability of controlling bacterial wilt.

MATERIALS AND METHODS

1. Instrumental Analyses. High-resolution mass spectra (HRMS) were recorded on a Shimadzu 9100-MK using electron impact at 70 eV. ¹H nuclear magnetic resonance (NMR) spectra were measured on a JEOL JNM 270 EX spectrometer in deuterated chloroform (CDCl₃) or methanol (CD₃OD) with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in parts per million downfield from the internal standard and coupling constants (*J*) in hertz (Hz). Elementary analyses were performed in the Department of Pharmaceutical Science of Kyoto University. Melting points are uncorrected.

2. Reagents. All reagents for chemical syntheses were purchased from Sigma-Aldrich Japan K.K., Tokyo, Japan, or Wako Pure Chemical Industries, Osaka, Japan, and used without further purification. Lipase AK was a gift from Amano Pharmaceutical Co., Aichi, Japan, and porcine liver esterase was purchased from Sigma-Aldrich Japan K.K. Silica gels (Wakogel C-200) for column chromatography and silica gel-coated TLC plates with fluorescence were purchased from Wako Pure Chemical Industries.

3. Synthesis. Structures of IPA and related compounds are shown in Figure 1. 3-Benzo[*b*]thiophenepropionic acid

* Author to whom correspondence should be addressed (telephone +81-742-43-1511, ext. 3306; fax +81-742-43-1445; e-mail kmatsuda@nara.kindai.ac.jp).

[†] Department of Agricultural Chemistry.

[‡] Department of Agronomy.

[§] Institute for Comprehensive Agricultural Sciences.

^{||} Research Institute.

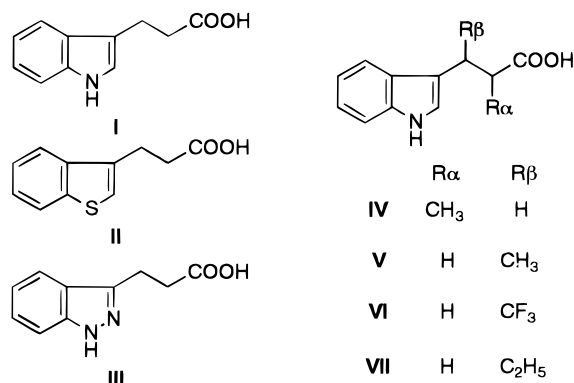


Figure 1. Indolepropanoids and related compounds tested for their antibacterial activity against *R. solanacearum*.

(Campaigne and Knapp, 1970), 3-indazolepropionic acid, and 2-methyl-(3-indolyl)propionic acid were synthesized from the formyl derivatives of benzo[*b*]thiophene, indazole, and indole, respectively. IPA derivatives having an alkyl branch on the β -carbon were synthesized according to the literature (Oikawa et al., 1978; Kato et al., 1993). Of these compounds, 3-indazolepropionic acid and 2-methyl-(3-indolyl)propionic acid have not yet been described; thus, their syntheses are shown below.

IPA derivatives having the substituent in the propionic acid moiety have an asymmetric carbon. We separated optical isomers of 3-IBA according to the method of Kato et al. (1993) using lipase AK for selectively hydrolyzing the ethyl ester of the *S* isomer, followed by porcine liver esterase. Optical purities of the isomers were confirmed to be >98% ee by HPLC using a Chiralcel OD column (4.6 \times 250 mm, Daicel Chemical Industries, Tokyo, Japan) with a mixture of hexane, 2-propanol, and trifluoroacetic acid (9:1:0.5) at a flow rate of 1 mL/min.

3.1. 3-Indazolepropionic Acid. 3-(3-Indazolyl)acrylic acid (100 mg, 531 nmol; lit. Dikopolova and Suvorov, 1979) in 5 mL of methanol was hydrogenized by stirring with 10 mg of 5% palladium carbon under 1 atm of hydrogen at room temperature for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. Recrystallization from hexane containing a small portion of ethyl acetate afforded colorless fine needles (67.7 mg, 67.0%); mp 170–171 °C; $^1\text{H NMR } \delta$ (CD_3OD) 2.79 (2H, t, $J = 7.5$), 3.25 (2H, t, $J = 7.5$), 7.08–7.77 (4H, m). Anal. Calcd for $\text{C}_{10}\text{H}_{10}\text{N}_2\text{O}_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 62.95; H, 5.30; N, 14.61.

3.2. 2-Methyl-3-(3-indolyl)propionic Acid (2-MIPA). **3.2.1. Ethyl 2-Methyl-3-(3-indolyl)acrylate.** To a solution of indole-3-carbaldehyde (300 mg, 2.07 mmol) in 20 mL of anhydrous benzene was added 1.34 g (3.70 mmol) of phosphorane prepared from triphenylphosphine and ethyl 2-bromopropionate, and the solution was heated under reflux for 4 h. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography with hexane/ethyl acetate (2:1); subsequent recrystallization from hexane containing a small amount of ethyl acetate afforded colorless plates: yield 450 mg (95%); mp 135–136 °C; HRMS 229.1095, calcd for $\text{C}_{14}\text{H}_{15}\text{NO}_2$ 229.1102; $^1\text{H NMR } \delta$ (CDCl_3) 1.38 (3H, t, $J = 7.0$), 2.19 (3H, s), 4.30 (2H, q, $J = 7.0$), 7.18–7.84 (5H, m), 8.06 (1H, s), 8.64 (1H, s).

3.2.2. Ethyl 2-Methyl-3-(3-indolyl)propionate. To a solution of the acrylate (300 mg, 1.31 mmol) in 10 mL of methanol was added 220 mg of 5% palladium-coated carbon, and the mixture was stirred under hydrogen (1 atm) at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by a silica gel column eluting with hexane/ethyl acetate (4:1) to yield 290 mg of colorless oil: yield 95.8%; HRMS 231.1257, calcd for $\text{C}_{14}\text{H}_{17}\text{NO}_2$ 231.1259; $^1\text{H NMR } \delta$ (CDCl_3) 1.19 (3H, t, $J = 7.0$), 1.21 (3H, s), 2.83 (2H, d, $J = 9.5$), 3.18 (1H, m), 4.09 (2H, q, $J = 7.0$), 6.99–7.63 (5H, m), 8.00 (1H, s).

3.2.3. 2-MIPA. Ethyl 2-methyl-3-(3-indolyl)propionate (300 mg, 1.30 mmol) was dissolved in a mixture of 5 mL of ethanol

and 5 mL of 5% aqueous NaOH solution, and the solution was heated under reflux for 2 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in water. After acidification with 2 N HCl solution, white precipitates were yielded. Recrystallization from hexane containing a small portion of ethyl acetate gave colorless fine needles (160 mg, 60.7%); mp 130–131 °C; $^1\text{H NMR } \delta$ (CD_3OD) 1.16 (3H, d, $J = 6.5$), 2.81 (2H, d, $J = 9.5$), 3.08–3.19 (1H, m), 6.95–7.55 (5H, m). Anal. Calcd for $\text{C}_{12}\text{H}_{13}\text{NO}_2$: C, 70.92; H, 6.45; N, 6.89. Found: C, 70.92; H, 6.47; N, 6.77.

4. Antibacterial Activity. Antibacterial activity of the compounds toward *R. solanacearum* was measured as described (Matsuda et al., 1993). Test solution was prepared by adding methanol stock of the compound in distilled water and mixing with an equal volume of 2 \times PCG medium (20 g of casamino acid and 20 g of glucose in 1 L of water). Three milliliters of the test solution was inoculated with 60 mL of the bacterial suspension (5×10^5 cells/mL) and cultured with shaking for 24 h at 30 °C. The minimum inhibitory concentration (MIC, $\mu\text{g/mL}$), required to suppress the bacterial numbers to <5% of control, was determined for each compound.

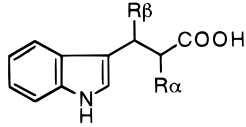
5. Effects on Growth of Tomato Seedlings. Seeds of tomato (*Lycopersicon esculentum* Mill cv. Poderosa) were placed on moistened paper in a Petri dish and incubated at 26 °C for 2–3 days in the dark for germination and root initiation. Germinated seeds with 1–2 mm roots were transferred to a filter paper supplied with different concentrations of test compounds and subsequently incubated for a further 3 days. The auxinic effect of test compounds was examined by measuring the length of radicles and hypocotyls. Alternatively, the auxinic effect of the compounds was examined using 1-month-old tomato seedlings. That is, germinated seeds were hydroponically cultured for 1 month in a growth chamber controlled at 26 °C and then transferred to an aqueous solution containing the test compounds. After 10 days of incubation, the appearance of stem winding was recorded and scored from 1 to 3 for slight, moderate, and great windings, respectively.

6. Control of the Bacterial Growth by 3-IBA in a Hydroponic Culture of Tomato Plants. The antibacterial action of 3-IBA on the pathogen was examined in a hydroponic culture of the tomato plants. The pathogen was first inoculated at a concentration of $\sim 1 \times 10^8$ cells/mL into a hydroponic culture medium containing 4.0 mM KNO_3 , 1.5 mM $\text{Ca}(\text{NO}_3)_2$, 1.0 mM MgSO_4 , 0.66 mM $\text{NH}_4\text{H}_2\text{PO}_4$, 0.057 mM FeEDTA , 0.048 mM H_3BO_3 , and 0.009 mM MnSO_4 on day 0, followed by the addition of 3-IBA on the next day (day 1). Certain numbers of the tomato plants grown for 1 month were transferred to the culture on day 2, and numbers of the wilted plants were counted every day for 6 days ($n = 3$).

RESULTS AND DISCUSSION

1. Role of Indole Ring in the Antibacterial Activity. Indazole has been shown to mimic indole for the compounds exhibiting 5-HT₃ antagonist activity (Bermudez et al., 1990), probably due to resemblance of the physicochemical properties with those of indole moiety (Palmer and Kennedy, 1994; Palmer et al., 1975). However, in the antibacterial activity, replacement of the indole moiety of IPA for indazole (compound III) abolished the antibacterial activity, as with benzo[*b*]thiophene (compound II) (data not shown). Similar results were obtained with aromatic moieties of naphthalene or quinoline, regardless of whether the propionic acid moiety extends from position 3 or 4 of these aromatic rings (data not shown), suggesting an important role of the five-membered ring in the antibacterial action.

2. Substituent Effects on the Propionic Acid Moiety. Even though it was previously found that neither the carboxylic acid function nor the length of the side-chain moiety could be modified to obtain greater

Table 1. Antibacterial Activity of IPA Derivatives with a Substituent on the Propionic Acid Moiety


compd no.	R α	R β	isomer	MIC ($\mu\text{g/mL}$)
I	H	H		2.5
IV	CH ₃	H	<i>RS</i>	75
V	H	CH ₃	<i>RS</i>	2.5
V	H	CH ₃	<i>R</i>	10
V	H	CH ₃	<i>S</i>	1.0
VI	H	CF ₃	<i>RS</i>	10
VII	H	C ₂ H ₅	<i>RS</i>	<100 ^a

^a Could not be determined because of limited solubility of the compound.

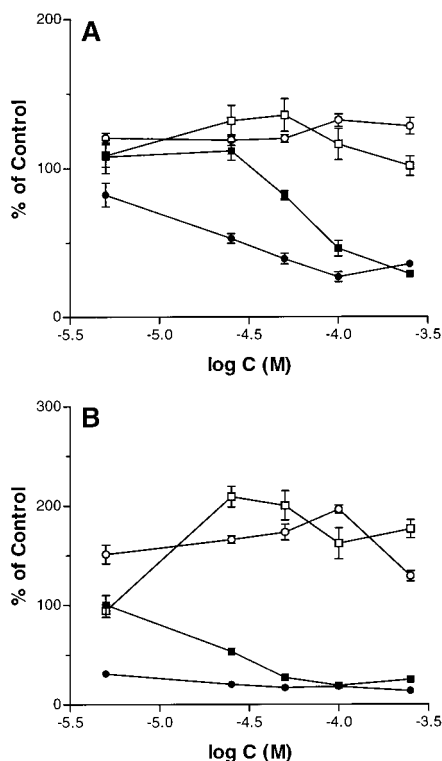


Figure 2. Effects of the compounds on the length of hypocotyls (A) and radicles (B) of tomato seedlings. Seedlings were soaked in aqueous solutions of IAA (●), IPA (■), 3-IBA (○), and TFIBA (□) for 3 days as described under Materials and Methods. Each plot represents the mean \pm SEM of the length expressed as a percentage of control.

antibacterial action, several compounds having a small alkyl substituent were capable of inhibiting bacterial growth (Table 1). The order of the activity of compounds I (H), V (CH₃) > VI (CF₃) > VII (C₂H₅) appears to reversely depend on steric bulkiness of the substituents. 3-IBA (V) was more active than 2-MIPA (IV). This may be explained by assuming that the α -substituent interferes with the binding of the carboxylic acid group to the target molecule.

Addition of an alkyl branch to the propionic acid moiety generates an asymmetric carbon. The *S* isomer of 3-IBA was separated from its counterpart by hydrolyzing the ethyl ester of 3-IBA with lipase AK. The *S* isomer was 10-fold more active than the *R* isomer (Table 1).

Table 2. Stem-Winding Effects of IAA, IPA, and 3-IBA on Tomato Seedlings

compd	concn				
	5 μM	25 μM	50 μM	100 μM	250 μM
IAA	0.5 \pm 0.3 ^a	1.5 \pm 0.3	2.8 \pm 0.3	2.8 \pm 0.3	2.8 \pm 0.3
IPA	0.5 \pm 0.3	1.8 \pm 0.5	2.0 \pm 0.0	2.0 \pm 0.0	2.5 \pm 0.3
3-IBA	0	0	0	0	0.3 \pm 0.3

^a Scores of the stem-winding of tomato seedlings were measured as described under Materials and Methods. Data are given as the mean \pm SEM of the scores of quadruplicated experiments.

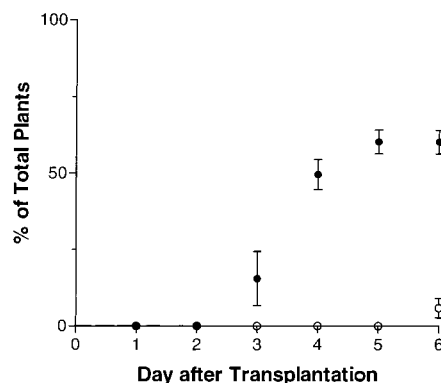


Figure 3. Wilt of tomato plants caused by *R. solanacearum* in the absence (●) or presence (○) of 10 $\mu\text{g/mL}$ 3-IBA. Each plot represents the mean \pm SEM of the wilted plants expressed as a percentage of total plants.

3. Phytotoxicity of Antibacterial Indolepropionoids. Because IPA is a homologue of indole-3-acetic acid (IAA), it could exhibit auxinic activity toward plants. IAA inhibited the growth of tomato seedlings, especially in the elongation of radicles at all concentrations used in the test (Figure 2). IPA also inhibited the growth of the seedlings at concentrations >50 μM in the hypocotyls (Figure 2A) and >25 μM in the radicles (Figure 2B). IAA has been shown to promote the growth of plants at lower concentrations; however, at greater concentrations, inhibition was observed. On the basis of the inhibitory effects observed, IPA may exhibit growth-promotive effects at lower concentrations than those tested. In contrast, IPA derivatives, 3-IBA and TFIBA, slightly promoted the growth of the seedlings, suggesting that the introduction of substituents to the β -carbon lowers the auxinic activity.

3-IBA and IPA were tested for their activity toward 1-month-old tomato seedlings to further compare their phytotoxicities. IPA, as well as IAA, caused stem winding of the seedlings due to auxinic effects, whereas 3-IBA had little effect even at 250 μM (Table 2).

4. Control of Bacterial Wilt by 3-IBA. To examine the protective effect of 3-IBA on bacterial wilt, we tested it in a hydroponic culture of the tomato plants. In the absence of the compound, the wilt was first observed 3 days after the plants were transferred to the culture and >60% of total plants were wilted at the end of the test period (Figure 3). In the presence of 10 $\mu\text{g/mL}$ 3-IBA, in contrast, bacterial wilt was effectively suppressed and only <10% of the plants were slightly wilted even 6 days after the transplantation.

Because Hikichi (1993) reported that oxolinic acid, which is used for controlling *Burkholderia glumae*, was strongly antibacterial to *R. solanacearum* in vitro, we also tested it in the same assay system. Application of oxolinic acid at concentrations of ≥ 5 $\mu\text{g/mL}$, however, resulted in strongly inhibiting the growth of the plants

(data not shown). It is therefore difficult to use this compound for wilt management.

In conclusion, we have for the first time found that a methylated derivative of IPA effectively protected tomato plants from bacterial wilt at low concentrations. Due to the current situations in Japan, where there are no chemical measures other than fumigants for managing bacterial wilt and some tomato species are in fact grown in hydroponic cultures, 3-IBA is expected to be used for management.

ABBREVIATIONS USED

IPA, 3-indolepropionic acid; 2-MIPA, 2-methyl-3-(3-indolyl)propionic acid; 3-IBA, 3-(3-indolyl)butanoic acid; TFIBA, 4,4,4-trifluoro-3-(3-indolyl)butanoic acid.

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