Anti-Fungal Sesquiterpenoid from the Root Exudate of Solanum abutiloides

Toshiyuki Yokose, Kozue Katamoto, Sun Park, Hideyuki Matsuura,¹ and Teruhiko Yoshihara

Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan

Received August 5, 2004; Accepted September 21, 2004

The Solanum abutiloides plant is highly resistant to soil-borne pathogens such as Fusarium oxysporum f. sp. melongenae, Verticillium dahliae, and Ralstonia solanacearum. This species is utilized as a mating source of resistant cultivars and also as a rootstock. The root exudate of Solanum abutiloides was extracted from a soil system composed of charcoal and vermiculite. Anti-fungal activity was found in the extract, and an active ingredient was isolated. The chemical structure of the active compound was determined to be 3-β-acetoxy-solavetivone, a new sesquiterpenoid. The anti-fungal activity of 3-β-acetoxy-solavetivone examined by the inhibition of spore germination of Fusarium oxysporum was close to that of lubimin, and higher than that of solavetivone.

Key words: Solanum abutiloides; Fusarium oxysporum f. sp. melongenae; root exudate; soil-borne pathogen; 3-β-acetoxy-solavetivone

In respect of solanaceous rootstock which was resistant to soil-borne pathogens, Nagaoka et al.,¹,² Nagase et al.,³ and Yoshihara et al.⁴ have reported anti-fungal alkaloids, fatty acids, sesquiterpenes, and phenolic compounds from solanaceous rootstock, and suggested the possible roles of sesquiterpenes which might lead to plants having resistance to soil-borne pathogens. We were also interested in whether the anti-fungal compounds are released from the roots to control soil-borne pathogens. In order to prove this hypothesis, we developed a method to collect the root exudate from a soil system composed of charcoal and vermiculite. Nagaoka et al.⁵ used this method and reported the isolation of five known sesquiterpenoids from the root exudate of Solanum aethiopicum. Solanum abutiloides, a relative of the eggplant and native to Southeast Asia, is highly resistant to such soil-borne pathogens as Fusarium oxysporum f. sp. melongenae, Verticillium dahliae, and Ralstonia solanacearum. This species is utilized as a mating source of resistant cultivars and also as rootstock against Ralstonia solanacearum.⁶ We report here the isolation and structural elucidation of a novel sesquiterpene from the root exudate of Solanum abutiloides.

The components absorbed by the soil system were eluted by using hexane, ethyl acetate, and ethanol as solvents. Anti-fungal activity was found in the eluate of ethyl acetate. This eluate was concentrated under reduced pressure to give a dark brown residue which was subjected to a silica gel column chromatography and HPLC to give compound 1 (Fig. 1).

Compound 1 gave a molecular ion at m/z 276, and its molecular formula was deduced to be C₁₇H₂₄O₃ (m/z: 276.1695) by HR-FD–MS (calcd. for C₁₇H₂₄O₃: 276.1726). The ¹H- and ¹³C-NMR spectra showed strong resemblance to those of 3-β-hydroxysolavetivone (2), except for the resonances derived from the acetyl moiety. Acetylation of 2 gave 3-β-acetoxy-solavetivone. Since the data from ¹H-NMR and EI–MS, including the optical rotation value, were identical between 1 and 3-β-acetoxy-solavetivone, the structure of 1 was determined to be that of 3-β-acetoxy-solavetivone. This structure was substantiated by the results of HMQC, HMBC, and ¹H–¹H COSY experiments. The absolute configuration of 3-β-hydroxysolavetivone (2) has been discussed by Anderson et al.,⁷ and they determined it by an X-Ray experiment.⁷ We could not discuss the absolute configuration of 2 any further, because they did not provide the optical rotation value in their report. The absolute configuration of 2 that was isolated during our study therefore remains unconfirmed, so that the absolute configuration of 1 is still unclear. However, biological considerations make it likely that the absolute configurations of 1 and 2 are those shown in Fig. 1, because the compounds isolated by our group and Anderson’s

¹ To whom correspondence should be addressed. Tel: +81-11-706-2495; Fax: +81-11-706-2505; E-mail: matsuura@chem.agr.hokudai.ac.jp
group were obtained from the same family. Compound 1 has been isolated from the root and the root exudate of *S. aethiopicum*, and has been reported to have anti-fungal properties. To investigate the effect of the compounds on spore germination, the spores of *F. oxysporum* were incubated with the compounds at 25 °C for 19 hours. Solavetivone (3) and lubimin (4) were used as positive controls. As shown in Fig. 2, suppression of the spore germination of *F. oxysporum* induced by 1 and lubimin was dose-dependent, and solavetivone showed the lowest activity.

In our series of studies, we isolated the anti-fungal sesquiterpene from the root and root exudate of solanaceous rootstock, and it is likely that the sesquiterpenoids played an important role in controlling the pathogen in soil. We mentioned in a recent paper the effect of crop rotation on corn (*Zea mays*). Anti-fungal compounds from the root and root exudate were isolated and discussed for their potential to control soil-borne pathogens. However, further studies should be carried out to clarify the roles of sesquiterpenoids and other anti-fungal compounds for building up resistance to soil-borne diseases.

**Experimental**

**General.** FD–MS and EI–MS data were respectively recorded by Jeol JMS-O1SG-2 and JMS-DX-300 mass spectrometers. NMR spectra were recorded by a Bruker AM-500 instrument (1H at 500 MHz; 13C at 125 MHz). Chemical shifts in the 1H-NMR spectra are reported relative to TMS as an internal standard. Chemical shifts in the 13C-NMR spectra are reported as δ (ppm) values relative to the carbon signal (δ 77.0) of CDCl3. IR spectra were recorded by a Perkin Elmer system 2000 instrument. Silica gel 60 F254 TLC plates (0.25 mm thickness; Merck, Darmstadt, Germany) were used for analytical TLC, and silica-gel column chromatography was carried out with C-200 silica gel provided by Wako Pure Chemical Industries (Japan). Compounds 2–4 had already been isolated by our group, and their structures were confirmed by comparing the spectral data with reported data. The plants of *S. abutiloides* were provided by Field Science Center for Northern Biosphere at Hokkaido University. The fungus, *F. oxysporum* f. sp. *melongenae*, was purchased from Department of Biotechnology at National Institute of Technology and Evaluation.

**Bioassay on spore germination.** The spores of *F. oxysporum* f. sp. *melongenae* were collected from the sterile distilled irrigating water (10 ml) over each individual culture grown in potato dextrose agar slants. The spores were collected by filtration through gauze and then centrifuged (1500 rpm, 10 min). The supernatant was removed, and the spores at the bottom were suspended in 1 ml of sterile distilled water for spore germination tests. Each test compound was dissolved in 0.4% glucose/EtOH (9:1 v/v). A spore suspension (2.5 μl) and a compound solution (5 μl) were mixed, and 7.5 μl of the mixture was pipetted on to a microscope slide. Each slide was put into a Petri dish and incubated at 25 °C for 19 h in the dark. Moistened filter papers were affixed to the inside of the lid and the bottom of the Petri dish to avoid dryness. After this incubation, the percentage of germinated spores was determined, and the spore-germination rate was calculated by comparing with that observed in the absence of a test compound. All treatments were duplicated.

**Isolation of compound 1.** The anti-fungal activities of each extract and fraction were evaluated by their inhibition of spore germination. Four-month-old seedlings of *S. abutiloides* were grown for 1 month in 15 pots each filled with 10 liters of a mixture of charcoal and vermiculite (1:2, v/v). A 500-times-diluted solution of Hyponex (Hyponex Japan Corp.) was applied once a week as a nutrient source. After cultivating each plant, the mixture of charcoal and vermiculite was carefully separated from the roots in a plastic container filled with tap water. This mixture of vermiculite and charcoal was loaded into three stainless steel columns (φ39 cm × 39 cm length). Each mixture of charcoal and vermiculite was rinsed with water, and successively eluted with hexane, ethyl acetate, and ethanol. Each eluate was collected and evaporated to dryness in vacuo to give a dark brown residue (3.76 g). This residue was subjected to silica gel column chromatography, using 5% MeOH/CHCl3, 20% MeOH/CHCl3, and MeOH as solvents. The eluate from 5% MeOH/CHCl3 (1.30 g) was further subjected to silica gel column chromatography to give active fractions which were finally purified by HPLC (Wakosil 5 SIL, φ6.0 × 250 mm; solvent, CHCl3; flow rate, 1 ml/min; detection, 254 nm) to give 1 (2.3 mg) as a colorless oil; [α]D20 +115° (c 0.23, CHCl3); FD–HR–MS *m/z*: 276.1695 (calcd. for C17H22O5, 276.1726); EI–MS (rel. int.): 276 [M]+ (30), 261 [M – Me]+ (15), 234...
H-NMR (CDCl₃/C14H): 5.72 (1H, s, H-1), 5.06 (1H, d, J = 12.5 Hz, H-3), 4.68 (2H, s, H-12), 2.54 (1H, m, H-7), 2.32 (1H, m, H-4), 2.15 (1H, m, H-8a), 2.11 (3H, s, -O-Ac), 1.98 (1H, m, H-9a), 1.95 (3H, s, H-15), 1.92 (1H, m, H-6a), 1.69 (3H, s, H-13), 1.68 (1H, m, H-9b), 1.59 (1H, m, H-6b), 1.56 (1H, m, H-8b), 1.59 (1H, m, H-15), 1.56 (1H, m, H-9b), 1.52 (1H, m, H-9b), 1.00 (3H, d, J = 6.6 Hz, H-14). ¹³C-NMR (CDCl₃/C14C): 193.1 (C-2), 170.5 (–O–Ac), 170.2 (C-10), 146.8 (C-11), 123.6 (C-1), 109.1 (C-12), 75.7 (C-3), 51.6 (C-5), 47.9 (C-7), 44.0 (C-4), 41.0 (C-9), 32.7 (C-6), 31.9 (C-8), 22.4 (C-15), 21.4 (C-13), 20.8 (–O–Ac), 12.3 (C-14).

Conversion of compound 2 to 3-β-acetoxy solavetivone. To a stirred solution of 2 (3.7 mg, 0.015 mmol) in pyridine (1 ml) was added a solution of acetic anhydride (1.6 mg, 0.015 mmol), and the reaction mixture was further stirred for 18 hour at room temperature. The usual work up was employed to give a residue which was purified by HPLC (Wakosil 5SIL, ϕ6.0 × 250 mm; solvent, CHCl₃; flow rate, 1 ml/min; detection, 254 nm) to give 3-β-acetoxy solavetivone (0.7 mg, 2.5 µmol, 17%); [α]D²⁶ +112° (c 0.09, CHCl₃).

Acknowledgments

The authors thank Mr. K. Watanabe and Dr. E. Fukushi of the GC-MS and NMR Laboratory in Faculty of Agriculture of Hokkaido University for MS and NMR measurements.

References