

Nematicidal Activities of 4-Hydroxyphenylacetic Acid and Odiolactone D Produced by the Fungus *Oidiodendron* sp.

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Two nematicides, 4-hydroxyphenylacetic acid (4-HPA) (**1**) and odiolactone D (**2**), were isolated from cultures of the fungus *Oidiodendron* sp., and their structures were identified by spectroscopic analyses. Compound **2** showed nematicidal activities against the root-lesion nematode, *Pratylenchus penetrans*, and the pine wood nematode, *Bursaphelenchus xylophilus*. Compound **1** was also active against these two nematodes but to a lesser extent.

Key words: 4-Hydroxyphenylacetic Acid, Odiolactone D, Nematicide

Introduction

In connection with our program on the isolation of biologically active metabolites from fungi, we have previously investigated secondary metabolites such as aspyrone (Kimura *et al.*, 1996), peniprequinolone (Kusano *et al.*, 2000), $\beta\gamma$ -dehydrocurvularin (Kusano *et al.*, 2003), penipratynolene (Nakahara *et al.*, 2004), 5-hydroxymethyl-2-furoic acid (Kimura *et al.*, 2007), and fumiquinones A and B (Hayashi *et al.*, 2007) for their potential as nematicides against the root-lesion nematode *Pratylenchus penetrans*, which is a parasite of many crop plants and causes root necrosis (Pitcher *et al.*, 1963; Towshend 1963). Plant-parasitic nematodes are a serious threat for crop production, causing an estimated annual worldwide loss of 125 billion US Dollars (Abdel-Rahman *et al.*, 2008). In addition, 5-hydroxymethyl-2-furoic acid (Kimura *et al.*, 2007), fumiquinones A and B (Hayashi *et al.*, 2007), and beauvericin (Shimada *et al.*, 2010) were shown to have their potential as nematicides against the pine wood nematode *Bursaphelenchus xylophilus*, which is a parasite of Japanese black pine (*Pinus thunbergii* Parl.) and Japanese red pine (*P. densiflora* Sieb. et Zucc.), the main species in Japan, and causes

pine wilt disease (Fukuda, 1997; Kuroda *et al.*, 1991). Currently, plant-parasitic nematodes are generally controlled using synthetic nematicides, crop rotations, thermal treatments, and development of resistant cultivars. In particular, conventional control methods are based on the use of synthetic nematicides acting as nerve poisons, like carbamates and phosphorylated and halogenated organic compounds. Some of those compounds cause global environmental problems; for example, the wide use of methyl bromide has destructive effects on the ozone layer (Gonzalez and Estevez-Braun, 1997; Abdel-Rahman *et al.*, 2008).

Since it was necessary to develop effective nematicides with low risk for humans and wildlife, we have focused our attention on new nematicides from fungal metabolites that are valuable natural resources for agrochemical development, and we found the presence of the regulators in the culture filtrate of *Oidiodendron* sp. Our investigation about metabolites of this fungus has now led to the isolation of two active substances, 4-hydroxyphenylacetic acid (4-HPA) (**1**) and odiolactone D (**2**). The present paper describes the production, isolation, structural determination, and biological activities of **1** and **2**.

Material and Methods

General experimental procedures

Melting points were determined using a Yanagimoto (Kyoto, Japan) micromelting point apparatus and are uncorrected. Optical rotation data was determined with a Horiba (Kyoto, Japan) SEPA-200 polarimeter. The UV spectra were recorded on a Shimadzu (Kyoto, Japan) UV-2000 spectrophotometer and the IR spectra on a JASCO (Tokyo, Japan) FT IR-7000 spectrometer. The ^1H and ^{13}C NMR spectra were recorded with a JEOL (Tokyo, Japan) JNM-ECD 500 NMR spectrometer at 500 and 125 MHz, respectively. Chemical shifts are expressed in δ values with solvents as internal standards. HREIMS data was obtained with a JEOL JMS-SX 102 mass spectrometer. Silica gel (Wako Pure Chemical Industries, Ltd., Osaka, Japan; 75–150 μm) was used for column chromatography. Precoated silica gel plates (Merck, Darmstadt, Germany; Kieselgel 60 F254, 0.2 mm) were used for preparative TLC. 3-Nitropropionic acid (3-NPA), purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA), was employed as a positive control for nematocidal activity.

Fungal material and fermentation

Oidiodendron sp. was collected from the soil in the city of Tottori in April 2004, and identified by light microscopy and by the production of previously characterized, bioactive metabolites of this fungus. One hundred twenty 500-ml Erlenmeyer flasks, each containing 250 ml of malt extract medium supplemented with 3% polypeptone, were individually inoculated with 1-cm² agar plug taken from a stock culture of the fungus maintained at 20 °C on potato dextrose agar. The fungus was statically grown at 24 °C for 21 d.

Extraction and isolation

The culture filtrate (30 l) was adjusted to pH 2.0 with 2 M HCl and extracted twice with EtOAc. The combined solvents were partitioned twice with a saturated NaHCO_3 aqueous solution. The EtOAc-soluble neutral phases were combined and concentrated *in vacuo*. The NaHCO_3 phases were combined and adjusted to pH 2.0 with 2 M HCl. The acidic solution was extracted twice with EtOAc, and the EtOAc-soluble acidic phases were combined and concentrated *in vacuo*. The

resulting substance (3.0 g) was fractionated three times by column chromatography on silica gel (*n*-hexane/EtOAc). The third passage (0.16 g) obtained by eluting with 10% EtOAc was further purified by preparative TLC (benzene/acetone, 8:2, v/v) to yield 12 mg of **1** as colourless needles. The other fraction (0.37 g) obtained by eluting with 30% EtOAc was further purified by preparative TLC ($\text{CHCl}_3/\text{MeOH}$, 9:1, v/v) to yield 80 mg of **2** as colourless needles.

4-Hydroxyphenylacetic acid (1): M.p. 147–150 °C. – UV/Vis (EtOH): λ_{max} (lg ϵ) = 227 (3.89), 277 nm (3.38). – IR (KBr): ν = 3030, 2896, 1692 (C=O), 1586 (C=C), 1483, 1307, 1195 cm^{-1} . – ^1H NMR (500 MHz, acetone- d_6): δ = 3.51 (s, 2H, 7-H), 6.78 (dd, J = 8.7, 1.5 Hz, 2H, 2,6-H), 7.12 (dd, J = 8.7, 1.5 Hz, 2H, 3,5-H). – ^{13}C NMR (125 MHz, acetone- d_6): δ = 40.42 (t, C-7), 115.91 (d, C-2,6), 126.53 (s, C-1), 131.16 (d, C-3,5), 157.10 (s, C-4), 173.17 (s, C-8). – HREIMS: m/z = 152.0477 [M^+]; calcd. for $\text{C}_8\text{H}_8\text{O}_3$ 152.0474.

Oidiodendron D (2): M.p. 239–242 °C. – $[\alpha]_{\text{D}}^{20}$ = -53.0° (c 1.0, EtOH). – UV/Vis (EtOH): λ_{max} (lg ϵ) = 227 nm (3.85). – IR (KBr): ν = 3316 (OH), 2938 (alkane), 1787 (lactone), 1696 (lactone), 1462, 1094, 957 cm^{-1} . – ^1H NMR (500 MHz, acetone- d_6): δ = 0.96 (s, 3H, 16-H), 1.21 (s, 3H, 14-H), 1.39–1.50 (m, 2H, 1-H), 1.56–1.61 (m, 2H, 2-H), 1.67–1.72 (m, 1H, 3-H), 1.88 (d, J = 4.4 Hz, 1H, 5-H), 1.97–2.01 (m, 1H, 3-H), 4.15 (br.s, 1H, 7-H), 5.11 (d, J = 4.4 Hz, 1H, 6-H), 6.02 (s, 1H, 11-H). – ^{13}C NMR (125 MHz, acetone- d_6): δ = 17.15 (t, C-2), 23.47 (q, C-14,16), 27.97 (t, C-3), 28.77 (t, C-1), 35.21 (s, C-10), 41.42 (s, C-4), 43.12 (d, C-5), 53.40 (d, C-7), 57.21 (s, C-8), 71.59 (d, C-6), 117.17 (d, C-11), 155.52 (s, C-9), 162.91 (s, C-12), 180.24 (s, C-15). – HREIMS: m/z = 306.1100 [M^+]; calcd. for $\text{C}_{16}\text{H}_{18}\text{O}_6$ 306.1103.

Bioassay for nematocidal activity

Nematicidal activity was measured in microwell plates with the root-lesion nematode, *Pratylenchus penetrans*, and the pine wood nematode, *Bursaphelenchus xylophilus*, according to the method of Kusano *et al.* (2000). *P. penetrans* was cultured for about 2 weeks on a slant of alfalfa grown in the Krusberg medium. The cultured nematodes were separated from the callus by the Baermann funnel technique and counted under a microscope. *B. xylophilus* was cultured for about 2 weeks on a slant of *Botrytis cinerea* grown in potato dex-

trose agar medium. An aqueous suspension of adults and L₄ larvae (more than 90%) containing a definite number of each nematode (about 2000 nematodes/ml) was prepared by dilution. The test compounds and extracts were each dissolved in methanol and added to the nematode suspension (up to 3% volume of the suspension). The nematode suspension thus prepared was transferred to 24-well plates with the wells containing a definite amount of the test compound. After the plates had been kept at 24 °C for 3 d, the nematodes in the wells were examined under a microscope. The nematicidal activity is expressed as: mortality (%) = $(B - A)/B \cdot 100$, where *A* is the number of nematodes still alive after being treated with the test compound, and *B* is the number of nematodes alive in the control wells (3% methanol in distilled water).

Results and Discussion

The EtOAc-soluble acidic extract (3.0 g) from the culture filtrate of *Oidiodendron* sp. was purified by silica gel column chromatography and preparative TLC to yield **1** and **2**.

Compound **1** was obtained as colourless needles. The molecular formula of **1** was established as C₈H₈O₃ by HREIMS. The ¹H and ¹³C NMR spectra, and PFG-HMQC experiments indicated the presence of one methylene group, four aromatic methine groups, two aromatic quaternary and one carbonyl carbon atoms. The IR absorption band at 1692 cm⁻¹ and a signal at δ 173.2 ppm in the ¹³C NMR spectrum indicated the presence of a carboxy group. The IR absorption band at 1586 cm⁻¹ and four sp² carbon atoms in the ¹³C NMR spectrum indicated the presence of an 1,4-disubstituted benzene ring. The ¹H and ¹³C NMR spectra and the molecular mass ($[M^+] = 152$) led to the structure of **1**. From those results, **1** was identified as 4-hydroxyphenylacetic acid (Fig. 1) by comparing the physicochemical properties with those reported (Ko *et al.*, 2009).

Compound **2** was obtained as colourless needles. The molecular formula of **2** was established as C₁₆H₁₈O₆ by HREIMS. The IR spectrum showed the characteristic absorption bands for a γ -lactone at 1787 cm⁻¹ and a δ -lactone at 1696 cm⁻¹ in addition to a broad band at 3316 cm⁻¹, typical for a nonchelated hydroxy group. The ¹H and ¹³C NMR spectra, detailed analysis of PFG-HMBC experiments, and the molecular mass ($[M^+] = 306$) led

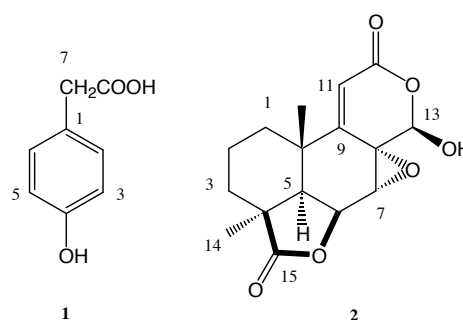


Fig. 1. Chemical structures of 4-hydroxyphenylacetic acid (**1**) and oidiolactone D (**2**).

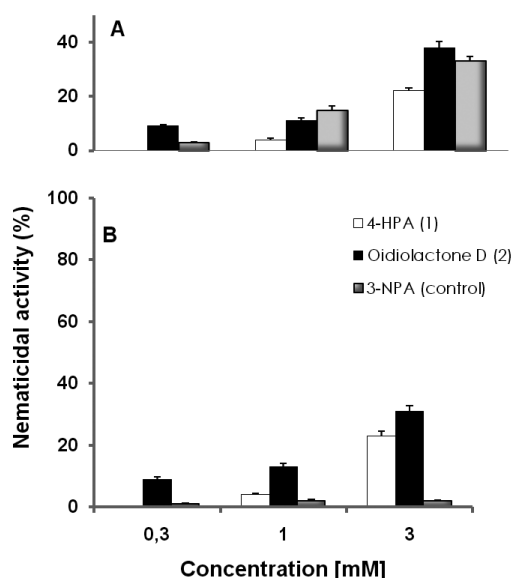


Fig. 2. Nematicidal activities of **1** and **2** against (A) *Pratylenchus penetrans* and (B) *Bursaphelenchus xylophilus*.

to the structure of **2** (Fig. 1). However, one signal in the ¹³C NMR spectrum for C-13 expected at ca. δ 100 ppm was missing, since an equilibrium between an open-chain form and the cyclized hemiacetal form is assumed (John *et al.*, 1999). From those results, **2** was identified as oidiolactone D by comparing the physicochemical properties with those reported (John *et al.*, 1999).

This is the first report on the nematicidal activities of **1** and **2**. These compounds are known to show antifungal activities (Ko *et al.*, 2009; John *et al.*, 1999). The nematicidal activities of **1** and **2** were examined against *P. penetrans* and *B. xylophilus* (Fig. 2). 3-Nitropropionic acid (3-NPA)

exhibiting antimycobacterial and nematicidal activities (Chomcheon *et al.*, 2005) was used as positive control. Compound **1** had nematicidal activities against *P. penetrans* and *B. xylophilus* of 22% and 23% at a concentration of 3 mM, respectively. Compound **2** showed nematicidal activities against *P. penetrans* and *B. xylophilus* of 38% and

31% at the same concentration. Compound **2** showed nematicidal activities against *P. penetrans* similar to those of 3-nitropropionic acid in the concentration range of 0.3–3 mM, and stronger nematicidal activities against *B. xylophilus* than 3-NPA at the same concentrations.

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