Nematicidal Activities of Acetylene Compounds from *Coreopsis lanceolata* L.

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1-Phenylhepta-1,3,5-triyne (1), 5-phenyl-2-(1′-propynyl)-thiophene (2), and 2-(3′-acetoxy-1′-propynyl)-5-phenylthiophene (3) were isolated from *Coreopsis lanceolata* L., and their structures identified by spectroscopic methods. Compounds 1 and 2 showed effective nematicidal activities against *Bursaphelenchus xylophilus* and *Caenorhabditis elegans*, but had hardly any effect against *Pratylenchus penetrans*. Compound 3 did not show any effective nematicidal activity.

**Key words:** *Coreopsis lanceolata* L., Acetylene, Nematicide

**Introduction**

We had previously investigated fungal 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), and 5-hydroxymethyl-2-furoic acid (Kimura et al., 2007) for their potential to act 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; Towsend, 1963). In addition, Japanese black pine (*Pinus thunbergii* Parl.) and Japanese red pine (*P. densiflora* Sieb. et Zucc.), the main species in Japan, have the highest susceptibility to pine wilt disease caused by the pine wood nematode *Bursaphelenchus xylophilus* (Fukuda, 1997; Kuroda et al., 1991). Plant parasitic nematodes cause crop losses that have been estimated to be 9% of the world’s crop yield each year. Conventional control methods are currently based on the use of low-specific biocidal compounds acting as nerve poisons, like carbamates and halogenated organic compounds. Some of these compounds cause global environmental problems. Methyl bromide has a destructive effect on the ozone layer, and its production is restricted (Gonzalez and Estevaz-Braun, 1997). Since it was necessary to develop effective nematicides with low risk for humans and wildlife, we have focused our attention on new nematicides from plant secondary metabolites that are valuable natural sources for the agrochemical development.

In the course of our screening search for bioactive compounds, we investigated the metabolites of *Coreopsis lanceolata* (Sörensen and Sörensen, 1958a, b; Nakajima and Kawazu, 1980; Bohlmann and Zdero, 1968; Bohlmann et al., 1983). Our investigation of the metabolites of this plant has now led to the isolation of three acetylenic compounds. Naturally occurring acetylenes have been isolated from a wide variety of plant species (Kimura et al., 1981; Mori et al., 1982), and have proven to be important bioactive compounds due to their pesticidal properties. We report here the isolation, structures, and nematicidal and plant growth activities of 1–3.

**Material and Methods**

**General experimental procedures**

Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. The IR spectra were recorded on a JASCO FT IR-7000 spectrometer. The \(^1\)H and \(^13\)C NMR spectra were recorded with a JEOL JNM-ECD 500 NMR spectrometer at 500 and 125 MHz, respectively. Chemical shifts are expressed in ppm val-
ues with solvents as internal standards. HREIMS data were obtained with a JEOL JMS-SX 102 mass spectrometer. Silica gel (Wako Pure Chemical Industries, Ltd., Osaka, 75–150 μm) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F254, 0.2 mm) were used for preparative TLC.

**Extraction and isolation**

Yellow flowers of *C. lanceolata* were collected in Tottori City in June 2002. The fresh petals and sepals (ca. 1.4 kg) were immersed in MeOH for 4 months, and the extracted solution was concentrated to 11 in vacuo. The aqueous concentrates were reextracted twice with EtOAc. The EtOAc-soluble phases were combined and partitioned twice with a saturated NaHCO₃ solution. The EtOAc-soluble neutral phases were combined and concentrated in vacuo. The resulting neutral residue (22.6 g) was first fractionated by column chromatography on silica gel (*n*-hexane/acetone). The individual fractions were monitored by TLC. The fraction (2.4 g) obtained by eluting with *n*-hexane was fractionated by column chromatography on silica gel (*n*-hexane). The fraction (657.6 mg) was purified by preparative TLC (petroleum ether) to afford 26.5 mg of 1. The other fraction (100.8 mg) was purified by preparative TLC (*n*-hexane/acetone, 95:5, v/v) to afford 34.6 mg of 2. The first-column fraction (4.53 g) obtained by eluting with 10% EtOAc was fractionated by column chromatography on silica gel (*n*-hexane/acetone). The fraction (409.1 mg) obtained by eluting with 2% acetone was purified by preparative TLC (*n*-hexane/acetone, 93:7, v/v), and the fraction (30.7 mg) was further purified by preparative TLC (CHCl₃) to afford 7.2 mg of 3.

1-Phenylhepta-1,3,5-triyne (1): M. p. 45–48 °C. – IR (KBr): ν = 2361 (C≡C), 2218 (C≡C), 1561 (C= C), 1491, 1441, 1026, 756, 691 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 2.00 (s, 3H, 7-H), 7.31 (dd, J = 7.7, 7.2 Hz, 2H, 3'-H and 5'-H), 7.37 (dd, J = 7.2, 1.2 Hz, 1H, 4'-H), 7.50 (dd, J = 7.7, 1.2 Hz, 2H, 2'-H and 6'-H). – ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 47.4 (q, C-7), 58.9 (s, C-2-6), 64.9 (s, C-2-6), 67.4 (s, C-2-6), 74.6 (s, C-2-6), 75.2 (s, C-1), 78.3 (s, C-2-6), 121.1 (s, C-1'), 128.4 (d, C-4'), 129.5 (d, C-3' and 5'), 132.9 (d, C-2' and 6'). – HREIMS: m/z = 164.0635 (M⁺); calcd. for C₁₃H₈ 164.0626.

5-Phenyl-2(1'-propynyl)-thiophene (2): M. p. 36–37 °C. – IR (KBr): ν = 2368 (C≡C), 1597, 808, 750, 683 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 2.09 (s, 3H, 3'-H), 7.07 (d, J = 3.7 Hz, 1H, 4-H), 7.13 (d, J = 3.7 Hz, 1H, 3-H), 7.27 (br. d, J = 7.8 Hz, 1H, 4'-H), 7.36 (dd, J = 7.3, 7.8 Hz, 2H, 3'-H and 5'-H), 7.55 (dd, J = 7.3 Hz, 2H, 2'-H and 6'-H). – ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 47.4 (q, C-3'), 73.1 (s, C-1'), 90.8 (s, C-2'), 122.7 (d, C-3), 123.4 (s, C-5), 125.8 (d, C-2' and 6'), 127.7 (d, C-4'), 128.9 (d, C-3' and 5'), 132.0 (d, C-4), 133.9 (s, C-1'), 144.4 (s, C-2). – HREIMS: m/z = 198.0475 (M⁺); calcd. for C₁₃H₁₀S 198.0503.

2(3'-Acetoxy-1'-propynyl)-5-phenylthiophene (3): M. p. 72–76 °C. – IR (KBr): ν = 2228 (C≡C), 1740 (C=O), 1495, 1456, 1381, 1354, 1250, 1026, 932, 760, 687 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): δ = 2.14 (s, 3H, 2''-H), 4.93 (s, 2H, 3'-H), 7.17 (d, J = 3.7 Hz, 1H, 4-H), 7.22 (d, J = 3.7 Hz, 1H, 3-H), 7.31 (d, J = 7.3 Hz, 1H, 4'-H), 7.38 (dd, J = 7.3, 8.0 Hz, 2H, 3'-H and 5'-H), 7.57 (d, J = 8.0 Hz, 2H, 2'-H and 6'-H). – ¹³C[¹H] NMR (125 MHz, CDCl₃): δ = 20.8 (q, C-2''), 52.9 (t, C-3''), 80.0 (s, C-1''), 127.6 (s, C-2''), 121.0 (s, C-2''), 122.9 (d, C-4), 125.9 (d, C-2'' and 6''), 128.2 (d, C-4''), 129.0 (d, C-3'' and 5''), 133.5 (s, C-1''), 134.1 (d, C-3), 146.5 (s, C-5), 170.3 (s, C-1''). – HREIMS: m/z = 256.0562 (M⁺); calcd. for C₁₃H₁₂SO₂ 256.0558.

**Bioassay to test the nematicidal activity**

Nematicidal activities were measured in micro-well plates with the pine wood nematode *Bursaphelenchus xylophilus*, the rootlesion nematode *Pratylenchus penetrans*, and the free living nematode *Caenorhabditis elegans* according to the method of Kimura et al. (2007).

**Bioassay to test the plant growth activity**

Plant growth activities were measured with lettuce, carrot, radish, barnyard millet, and rice seedlings according to the method of Kimura et al. (2007).

**Results and Discussion**

The EtOAc-soluble neutral fraction (22.6 g) from MeOH extracts of *C. lanceolata* was purified by silica gel column chromatography and preparative TLC to afford 1–3. Compounds 1–3 were identified as 1-phenylhepta-1,3,5-triyne, 5-phenyl-2(1'-propynyl)-thiophene, and 2-(3'-acetoxy-1'-pro-
pynyl)-5-phenylthiophene (Sorensen and Sorensen, 1958a, b; Nakajima and Kawazu, 1980; Bohlmann and Zdero, 1968), respectively, by comparing their physicochemical properties with those reported (Fig. 1). Compounds 1 and 2 showed ovi-cidal activity against Drosophila melanogaster (Nakajima and Kawazu, 1980) but their nematicidal and plant growth activities are not known. Compound 3 was found in Coreopsis nuecensis A. Heller (Bohlmann and Zdero, 1968; Yoke Marchant et al., 1984) but the nematicidal and plant growth activities are not known. This is the first report on the nematicidal activities and effects on plant growth of compounds 1–3.

The nematicidal activities of 1 and 2 were examined against the pine wood nematode B. xylophilus, the root lesion nematode P. penetrans, and the free living nematode C. elegans (Fig. 2). The activities of 3 were examined against P. penetrans and C. elegans but could not be examined against B. xylophilus because of its low yield. 5-Hydroxymethyl-2-furoic acid from Aspergillus sp. and aspyrone from A. melleus were used as positive controls (Kimura et al., 1996, 2007). Compounds 1 and 2 completely inhibited the growth of B. xylophilus at a concentration of 2 mM. However, 1–3 had hardly any effect on P. penetrans in the concentration range 0.02–2 mM. Compounds 1–3, respectively, had nematicidal activities against C. elegans of 89%, 99%, and 16% of the control value at a concentration of 2 mM.

The effects on plant growth of 1 and 2 were examined with lettuce, carrot, radish, barnyard millet, and rice seedlings (Figs. 3 and 4). The activities of 3 were examined with lettuce and barnyard millet but could not be examined against carrot, radish, and rice because of its low yield. Compounds 1–3 increased the root growth of lettuce seedlings analogous to their concentration from 0.02 to 2 mM. Compounds 1–3, respectively, elongated the root growth to 207%, 218%, and 258% of the control length at a concentration of 2 mM. Compound 2 inhibited the hypocotyl elongation to 54% of the control length at a concentration of...
2 mM and elongated the root growth of carrot seedlings to 233% of the control length at the same concentration, but 1 did not show any effect on the root growth in the concentration range 0.02–2 mM. Compound 1 inhibited the hypocotyl elongation to 52% of the control length at a concentration of 2 mM. Compound 2 increased the root growth of radish seedlings analogous to its concentration from 0.02 to 2 mM. Compound 1 did not show any inhibitory activity against the root growth in the concentration range 0.02–2 mM. Compounds 1–3 had inhibitory activities against shoot elongation of barnyard millet seedlings analogous to their concentration from 0.02 to 2 mM. Compounds 1 and 2, respectively, inhibited the shoot elongation to 35% and 28% of the control length at a concentration of 2 mM, but 1 and 2 showed weak promotive activities against the root growth at a concentration of 0.2 mM. Compounds 1 and 2 did not show any inhibitory activity against shoot elongation of rice seedlings in the concentration range 0.02–2 mM. Compound 1 increased the root growth analogous to its concentration from 0.02 to 2 mM, and 1 elongated the root growth to 193% of the control length at a concentration of 2 mM. Compound 2 elongated the root growth to 189% of the control length at a concentration of 0.2 mM.

The difference in the nematicidal activities of 1–3 against the three test nematodes should be attributable to the chemical composition and membrane permeability (Ellenby, 1946; Bird, 1958; McRae et al., 1985). Furthermore, the triple bond conjugated to an aryl function and a thienyl function may play an important role in the emergence of the nematicidal and plant growth activities (Mori et al., 1982); an ester function such as the acetoxy group was insufficient. Compounds 1 and 2 may be useful nematicides against B. xylophilus.


