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

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Z. Naturforsch. **63c**, 843–847 (2008); received June 17, 2008

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; Towshend, 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 Estevez-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 δ val-

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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 1 l *in vacuo*. The aqueous concentrates were reextracted twice with EtOAc. The EtOAc-soluble phases were combined and partitioned twice with a saturated $NaHCO_3$ 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 ($CHCl_3$) 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}. – ^{1}H NMR (500 MHz, $CDCl_3$): 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). – ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ = 4.7 (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 $C_{13}H_8$ 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}. – ^{1}H NMR (500 MHz, $CDCl_3$): 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 (d, J = 7.3 Hz, 2H, 2″-H and 6″-H). – ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ = 4.7 (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 $C_{13}H_{10}S$ 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}. – ^{1}H NMR (500 MHz, $CDCl_3$): 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). – ^{13}C{^{1}H} NMR (125 MHz, $CDCl_3$): δ = 20.8 (q, C-2‴), 52.9 (t, C-3′), 80.0 (s, C-1′), 87.6 (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 $C_{15}H_{12}SO_2$ 256.0558.

Bioassay to test the nematicidal activity

Nematicidal activities were measured in microwell 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, raddish, 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-

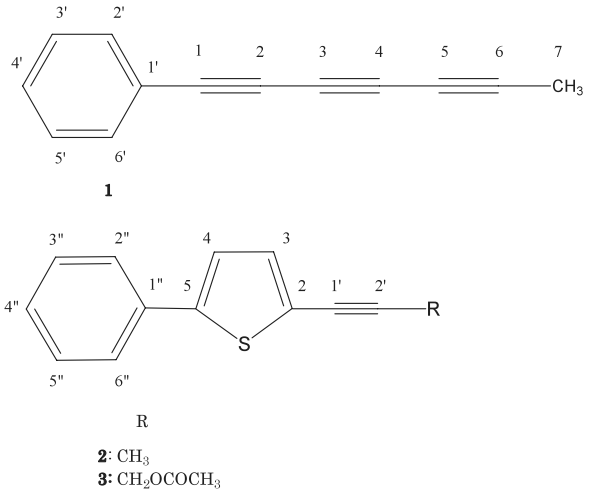

Fig. 1. Chemical structures of 1-phenylhepta-1,3,5-triyne (**1**), 5-phenyl-2-(1′-propynyl)-thiophene (**2**), and 2-(3′-acetoxy-1′-propynyl)-5-phenylthiophene (**3**).

pynyl)-5-phenylthiophene (Sörensen and Sörensen, 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 ovicidal 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.

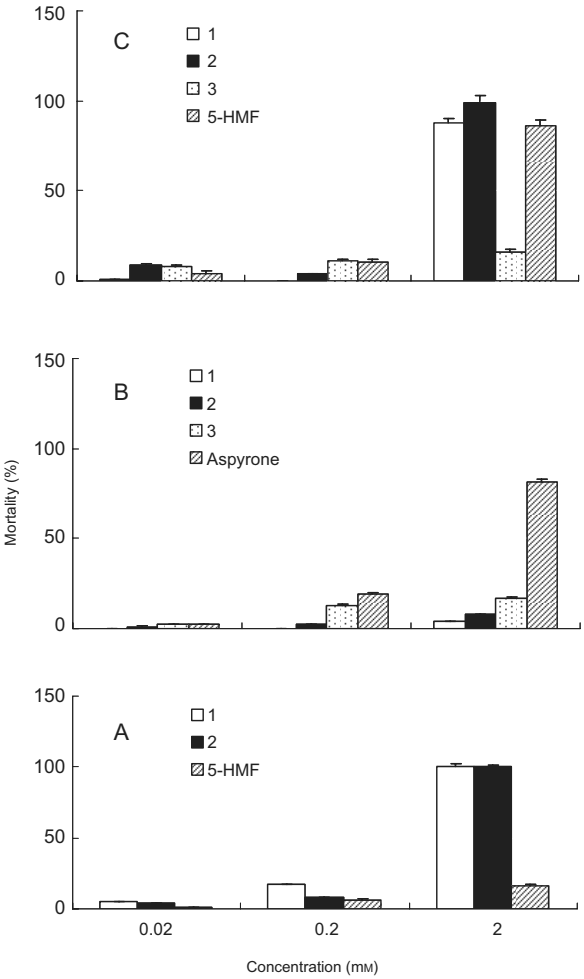

Fig. 2. Nematicidal activities of **1**–**3** against (A) *Bursaphelenchus xylophilus*, (B) *Pratylenchus penetrans*, and (C) *Caenorhabditis elegans*. Each value is presented as mean ± SE (n = 3). 5-Hydroxymethyl-2-furoic acid is abbreviated as 5-HMF.

The effects on plant growth of **1** and **2** were examined with lettuce, carrot, raddish, 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, raddish, 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

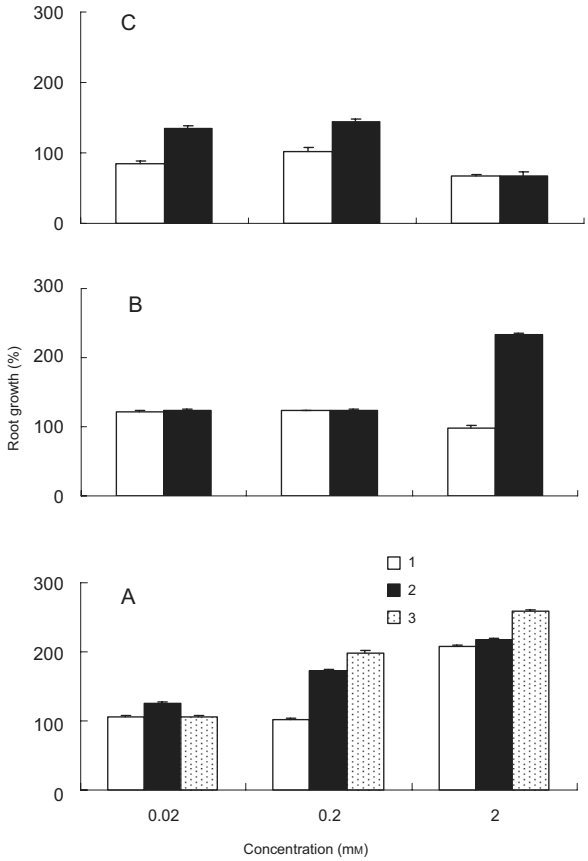

Fig. 3. Plant growth activities of **1**–**3** against root of (A) lettuce, (B) carrot, and (C) raddish seedlings. Each value is presented as mean ± SE (n = 3).

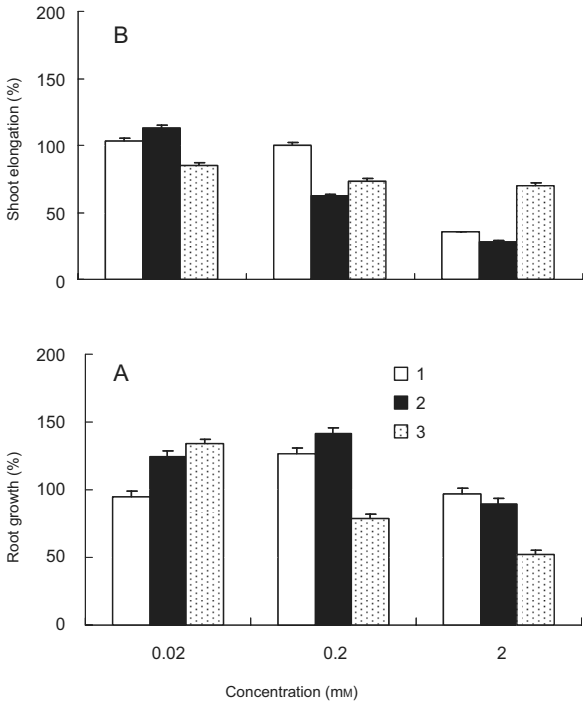

Fig. 4. Plant growth activities of **1**–**3** against (A) root and (B) shoot of barnyard millet seedlings. Each value is presented as mean ± SE (n = 3).

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 raddish seedlings analogous to its concentration from 0.02 to 0.2 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*.

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