Two Nematicidal Substances from Roots of Cirsium japonicum

Kazuyoshi KAWAZU, Yoshiki NISHII* and Shuhei NAKAJIMA

Department of Agricultural Chemistry, Okayama University,
Tsushima, Okayama 700, Japan

Received November 20, 1979

Guided by the bioassay with Bursaphelenchus lignicolus described in the preceding paper, two nematicidal substances were isolated from roots of Cirsium japonicum and identified with tridec-1-ene-3,5,7,9,11-pentayne and 9,10-epoxy-heptadec-16-ene-4,6-diyne-8-ol. These two compounds completely inhibited propagation of the nematode at a dose of 16 μg and 250 μg, respectively. 1-Phenylhepta-1,3,5-triyne and 2-phenyl-5-(1'-propynyl)-thiophene (from Coreopsis lanceolata) and cis-dehydromatricaria ester (from Solidago altissima) also inhibited the propagation at a dose of 110 μg.

In the preceding paper¹ a convenient screening method for nematicidal activity was proposed, and by this method a methanol extract of roots of Cirsium japonicum was shown to have pronounced activity against Bursaphelenchus lignicolus. This paper deals with the isolation and identification of two nematicidal substances from its roots.

The methanol extract of the roots was concentrated and the aqueous concentrate was extracted successively with benzene and ethyl acetate. Only the benzene soluble material completely inhibited propagation of the nematode, Bursaphelenchus lignicolus, at a dose of 6 mg. The benzene soluble material was chromatographed on silica gel with benzene—ethyl acetate mixtures by stepwise elution. The fractions eluted with benzene and 10% ethyl acetate in benzene stopped propagation of the nematode at a dose of 2 mg, but the fraction eluted with 5% ethyl acetate showed no activity at the same level of dose. This suggested that the activities in these two fractions were due to different constituents.

The fraction eluted with benzene was chromatographed on a silica gel column with n-hexane and a successive silica gel dry column with n-hexane—ethyl acetate (19: 1) to give active constituent 1 in 5.5×10⁻⁴% yield. Constituent 1 completely inhibited propagation of the nematode at a dose of 16 μg. This constituent, pale yellow needles instantly turning black, M⁺, m/z 162 (base peak), no optical rotation, was identified with tridec-1-ene-3,5, 7,9,11-pentayne,² isolated as a fly ovicidal substance from Xanthium canadense by spectral comparison (UV, IR, ¹H-NMR and GC/MS).

The fraction eluted with 10% ethyl acetate in benzene was subjected to silica gel dry column chromatography, eluted with n-hexane—ethyl acetate (3: 1). The active fraction was further chromatographed on silica gel with n-hexane—acetone (9: 1) as the eluant. Active constituent 2 was obtained in 1.1×10⁻³% yield, and completely inhibited the propagation at a dose of 250 μg. Constituent 2, a colorless viscous oil, [α]D⁰ +102° (c=2.4, benzene), has the molecular formula, C₁₇H₂₄O₂ (M⁺, m/z 260.1786, calcd. 260.1776). Its UV spectrum [λmax nm (10⁻²×ε): 231 (7.4), 243 (6.8), 256 (4.6), 268 (3.2) and 284 (2.1)] suggested the presence of conjugated diyne,³ and its IR spectrum (KBr) indicated the presence of hydroxyl (3410 cm⁻¹), disubstituted acetylene (2256 cm⁻¹) and vinyl (3080, 1645, 995 and 910 cm⁻¹) groups. Its ¹³C-NMR spectrum in C₆D₆ revealed the presence of a vinyl group (139.3 d, 114.7 t) and two disubstituted acetyl-
TABLE I. NEMATICIDAL ACTIVITY$^a$ OF 1 AND 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, µg</th>
<th>250</th>
<th>125</th>
<th>64</th>
<th>16</th>
<th>8</th>
<th>4</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>81</td>
<td>78</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>23</td>
<td>83</td>
<td>75</td>
<td>95</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$ Nematode propagation percent of control.

es (82.3, 75.2, 71.8, 65.7, s each) as well as of a secondary hydroxyl and a disubstituted oxirane group (61.3, 58.4, 57.7, d each) accounting for the two oxygens in the molecule. The presence of the oxirane and secondary hydroxyl was also supported by the IR absorption bands at 1230 and 835 cm$^{-1}$ and by the $^1$H-NMR signals (2.09, 3H, s and 5.24, 1H, d, J=8 Hz) of the acetate of 2, respectively. The IR band at 835 cm$^{-1}$ suggested that the oxirane was cis-disubstituted. The $^1$H-NMR spectrum in CDCl$_3$ of 2 displayed signals ascribed to a primary methyl (1.02, 3H, t, J=6 Hz), 7 methylenes (1.3~1.8, 10H, 1.9~2.4, 4H), 2 epoxy methines (2.9~3.3, 2H), a carbinol (4.33, 1H, d, J=7 Hz) and a vinyl bond to a methylene (4.90, 1H, dd with a fine splitting, J=10, 2 Hz; 4.95, 1H, dd with a fine splitting, J=18, 2 Hz; 5.85, ddt, J=18, 10, 6 Hz) and a hydroxyl (2.62, 1H). The collapse of the carbinyl proton doublet to a singlet on irradiation at $\delta$ 3.10 (epoxy methine protons) suggested the partial structure, -C=C-C=C-C=CH-CH=CH-. To clarify the number of methylenes flanked by this partial structure and both terminal groups, i.e., methyl and vinyl groups, constituent 2 was subjected to acid-catalyzed hydrolysis followed by periodate cleavage to give a 1:1 mixture of two aldehydes, which was hydrogenated over Adams platinum oxide to n-octanol. GC/MS of the mixture of two aldehydes showed that the first aldehyde is C$_8$H$_{14}$O (M+, m/z 126.1042, calcld. 126.1043) and the second is C$_8$H$_8$O (M+, m/z 120.0577, calcld. 120.0576): the former should be CH$_3$-CH-(CH$_2$)$_2$-CHO and the latter CH$_3$-(CH$_2$)$_2$-C=C=C=CH-CHO. Therefore, constituent 2 was deduced to be 9,10-cis-epoxyheptadec-16-ene-4,6-diyn-8-ol. The stereochemistry of C-8 was shown to be the S-configuration by application of the Horeau-Brooks method.

The nematicidal activities of these two constituents isolated were shown in Table I. Constituent 1 showed activity stronger than constituent 2.

Since the acetylenic compounds were proved to be active, a few naturally occurring acetylenic compounds isolated as fly ovicidal substances were also tested. 1-Phenylhepta-1,3,5-triyne (3) and 2-phenyl-5-(1'-propynyl)-thiophene (4) isolated from Coreopsis lanceolata as well as cis-dehydromatricaria ester (5) from Solidago altissima completely stopped the propagation at a dose of 110 µg (Table II).

TABLE II. NEMATICIDAL ACTIVITY$^a$ OF 3, 4 AND 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose, µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>440</td>
</tr>
<tr>
<td>4</td>
<td>220</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
</tr>
</tbody>
</table>

$^a$ Nematode propagation percent of control.

The nematicidal activity of flowers of Coreopsis lanceolata observed in the screening test would be attributed to the former two compounds.

EXPERIMENTAL

Optical rotations were measured on a Yanaco automatic polarimeter OR-50, and UV spectra were taken in EtOH on a Shimadzu multipurpose recording spectrophotometer MPS-5000, IR spectra on a Hitachi...
EPI-G3 spectrometer. $^1$H-NMR spectra were run on a Hitachi R-24 spectrometer, and the $^13$C-NMR spectrum was obtained at 25.05 MHz in the Fourier transform mode on a JEOL FX-100 spectrometer. Chemical shifts are expressed from tetramethylsilane as internal standard, and singlet, doublet, triplet, quartet and multiplet are abbreviated to s, d, t, q and m, respectively. Gas chromatograms were obtained on a Yanaco G-80 gas chromatograph with an F.I.D., equipped with a glass column ($\phi$3 mm × 75 cm), and GC/MS spectra on a JEOL JMS-D 300 mass spectrometer combined with a JGC-20K gas chromatograph (glass column, $\phi$2 mm) on lined with a mass data analysis system, JMA-2000.

**Nematicidal test.** The test was carried out on *Bursaphelenchus lignicolus* according to the procedure in the preceding paper.1)

**Extraction and preliminary separation.** Roots (4.7 kg, fresh weight) of *Cirsium japonicum* were collected at Okayama on May 27, 1978 and soaked in methanol (14.5 liters) for one month. The methanol extract was concentrated in vacuo below 40°C, and the aqueous concentrate (1,250 ml) was extracted successively with benzene (300 ml × 4) and EtOAc (300 ml × 4). The combined benzene extract was washed with water, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo below 40°C, and the aqueous residue of the benzene extract was similarly chromatographed, and the respective fractions from the 2 runs were combined. Residues from these combined fractions weighed 2.1, 1.2, 1.4, 0.6 and 2.7 g, and propagation percentages of control were 0%, 52% and 100%, respectively, when assayed with 6 mg of each residue. The benzene soluble residue (4 g) dissolved in a small amount of benzene was applied on the top of a column (34.1 (t), 57.7 (d), 58.4 (d), 61.3 (d), 65.7 (s), 71.8 (s), 75.2 (s), 82.3 (s), 114.7 (t) and 139.3 (d). $^1$H-NMR $\delta$CDCl$_3$ Me$_4$Si: 1.02 (3H, t, J=6 Hz), 1.5 (10H), 2.2 (4H), 2.62 (1 OH), 3.10 (2H, m), 4.33 (1H, d, J=7 Hz), 4.90 (1H, dd with a fine splitting, J=10, 2 Hz), 4.95 (1H, dd with a fine splitting, J=18, 10 Hz) and 5.85 (1H, ddt, J=18, 10, 6 Hz). GC/MS: a peak given at t$_R$=2.4 min on 3% SE-30 column ($\phi$2 mm × 1 m) at 160°C with a helium flow (20 ml/min) was scanned at 70 eV from m/z 34 to m/z 500; m/z (relative abundance, %): 162 (M$^+$, 100), 136 (24), 110 (22).

**Isolation of active constituent 1.** The residue (1.4 g) from the 10% EtOAc eluate was equally divided into 7 parts, and each part was placed on the top of a silica gel dry column (Merck PF$_{254}$ 42 g in a quartz tubing, $\phi$2 mm) and developed with n-hexane–EtOAc (3:1). The UV absorbing zone was dug out and extracted with EtOAc. EtOAc extracts from the 7 runs were combined (760 mg) and the combined extract was divided into 4 parts. Each part was placed on the top of a silica gel column (Wakogel C-300 20 g, $\phi$1.2 × 42 cm) and eluted with n-hexane–EtOAc (3:1). The yellow eluates from the 4 runs were collected to give active constituent 2 (52 mg, 1.1 × 10$^{-3}$ % yield based on fresh weight of the root) as a colorless viscous oil.

**Active constituent 2, [\(\alpha\)]$_D$ +102° (c=2.4, benzene), UV $\lambda_{max}$ nm (10$^{-3}$ × e): 231 (7.4), 243 (6.8), 256 (4.6), 268 (3.2) and 284 (2.1). IR $\nu_{max}$ cm$^{-1}$: 3410, 3080, 2256, 1645, 1995, 910 and 835. $^{13}$C-NMR $\delta_{CDCl_3}$ Me$_4$Si: 13.4 (q), 21.2 (t), 21.8 (t), 26.7 (t), 27.8 (t), 29.2 (t × 2), 34.1 (t), 57.7 (d), 58.4 (d), 61.3 (d), 65.7 (s), 71.8 (s), 75.2 (s), 82.3 (s), 114.7 (t) and 139.3 (d). $^1$H-NMR $\delta_{CDCl_3}$: 1.02 (3H, t, J=6 Hz), 1.5 (10H), 2.2 (4H), 2.62 (1 OH), 3.10 (2H, m), 4.33 (1H, d, J=7 Hz), 4.90 (1H, dd with a fine splitting, J=10, 2 Hz), 4.95 (1H, dd with a fine splitting, J=18, 10 Hz) and 5.85 (1H, ddt, J=18, 10, 6 Hz). GC/MS: a peak given at t$_R$=3.1 min on 3% OV-1 (Uniport HP, 80/100 mesh) column ($\phi$2 mm × 2 m) at 222°C with a helium flow (20 ml/min) was scanned at 70 eV from m/z 25 to m/z 400; m/z (relative abundance, %): 260 (M$^+$, 1.5), 242 (2.4), 163 (6.1), 134 (16), 121 (73), 91 (100), 55 (77) and 41 (88).

**Acetylation of 2.** Constituent 2 (26 mg) was treated with acetic anhydride (0.5 ml) in pyridine (0.5 ml) at room temperature overnight. Methanol (1 ml) was poured to the reaction mixture under ice-cooling and evaporated to dryness until free of pyridine. The residue was chromatographed on Wakogel C-300 (1.5 g, $\phi$6 mm × 10 cm) with n-hexane–acetone (95:5)
to give the acetate of 2 (16 mg), [α]D +8.1° (c=1.6, benzene). UV λmax nm (ε): 221.5 (6.1), 232.5 (7.7), 244.5 (7.9), 255 (4.6), 258.5 (5.2), 269 (2.1) and 283 (1.4). IR νmax cm⁻¹: 3080, 2260, 1755, 1642, 1210, 910 and 835. 1H-NMR δCDCl₃ (Me₄Si): 0.96 (3H, t, J=7 Hz), 1.5 (10H), 2.09 (3H, s), 2.2 (4H), 3.1 (2H, m), 4.90 (1H, dd with a fine splitting, J=10, 2 Hz), 4.95 (1H, dd with a fine splitting, J=18, 2 Hz), 5.24 (1H, d, J=8 Hz) and 5.85 (1H, ddt, J=18, 10, 6 Hz). GC/MS (direct inlet, 70 eV) m/z (relative abundance, %): 302 (M⁺, 0.5), 260 (4), 205 (18), 163 (23), 134 (14), 91 (12), 55 (18) and 43 (100).

Oxidative cleavage⁵) of 2. Constituent 2 (300 µg) dissolved in dioxane (100 µl) was treated with 2 N H₂SO₄ (20 µl) at 60°C for 25 min and subsequently with a solution of NaIO₄ (1 mg) in 2 N H₂SO₄ (20 µl) for 30 min. The reaction mixture was extracted with ether and the ether solution was dried over Na₂SO₄ and evaporated to a volume of 100 µl. An aliquot (1 µl) of the solution was injected into a gas chromatograph with a 3% OV-1 on Uniport HP (80/100 mesh) column (φ3 mm × 75 cm) programmed at a rate of 6°C/min from 50°C to 150°C with a helium flow (30 ml/min) to show two peaks at tR=6.8 min and 9.6 min of the nearly equal area. GC/MS (direct inlet, 70 eV) m/z (relative abundance, %): the first peak, 126.1042 (126.1043, calcd. for C₈H₁₄O) (0.5), 111.0811 (C₇H₁₁O) (1.9), 108 (7.4), 98 (14), 93 (28), 67 (72), 55 (87) and 41 (100); the second peak, 120.0577 (120.0576, calcd. for C₈H₈O) (35), 119.0500 (C₇H₇O) (6.1), 91.0554 (C₆H₇) (100) and 63 (58).

The mixture of aldehydes dissolved in EtOH was hydrogenated over Adams platinum oxide. An aliquot of the reaction mixture was injected into a gas chromatograph with a 3% OV-1 column (Uniport HP 80/100 mesh) programmed at a rate of 6°C/min from 60°C to 150°C with a helium flow (25 ml/min) to give a peak at tR=5.1 min which was proved to represent n-octanol by co-injection with the authentic sample. GC/MS confirmed that the peak represented n-octanol, m/z (relative abundance, %): 112 (M⁺ -H₂O, 5.0), 84 (34), 70 (41), 56 (91), 43 (73) and 41 (100).

Determination of absolute configuration at C-8 by application of the Horeau-Brooks method.⁶) Constituent 2 (10 µmol) in dry pyridine (7 µl) was treated with (±)-α-phenylbutyric anhydride (1.0 molar excess) and kept in a sealed tube at 40°C for 1.5 hr. A parallel reaction was carried out with cyclohexanol. (+)-(R)-α-phenylethylamine (6 µl) was added and mixed thoroughly. After 15 min, the mixture was diluted with dry EtOAc (400 µl) and a sample was analyzed by GC at 200°C on a 1.5 m column packed with 1.5% OV-17 on Shimalite W. The relative proportions of the amides of (−)-(R)- and (+)-(S)-α-phenylbutyric acid were indicated by the areas of the respective peaks (tR=11.4 min and 12.8 min). The percentage area representing the (−)-(R)-acid was assessed. Subtraction of the corresponding value from the reaction with cyclohexanol gave the increment (+10.3). This value means that the absolute configuration at C-8 of 2 is S.

Acknowledgments. The authors express their sincere thanks to Professor Junkichi Iwasa of this Department for his encouragement and to Dr. Kenji Uneyama, Department of Industrial Chemistry of this University, for 13C-NMR measurement. Thanks are due to Miss Akemi Shiraishi for her assistance in experimental works. This work was partially supported by a grant from the Ministry of Education of Japan.

REFERENCES