Two Antinematodal Phenolics from *Knema hookeriana*, a Sumatran Rainforest Plant

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Antinematodal Compounds, *Knema hookeriana*, *Bursaphelenchus xylophilus*

The activity-guided chromatographic purification of the methanol extract of *Knema hookeriana*, using pine wood nematodes *Bursaphelenchus xylophilus* has successfully led to the isolation and characterization of two phenolic antinematodal compounds with minimun effective dose (MED) of 4.5 and 20 μg/cotton ball (μg/ bl.) or 0.018 and 0.073 μM/cotton ball (μM/bl.), respectively. Based on their chemical and spectral properties, these compounds were determined to be 3-undecylphenol (**1**) and 3-(8*Z*-tridecenyl)-phenol (**2**). These compounds were isolated for the first time from this species, and **2** seems to be a novel compound.

Introduction

In the previous paper, a convenient screening method for antinematodal activity was proposed (Kawazu *et al*., 1980). By using this method, methanolic extracts of 63 tropical rainforest plants were assessed and 27 plants were proved to contain active component(s) against the phytopathogenic nematode, *Bursaphelenchus xylophilus*. Among them, the extract of the sap of *Knema hookeriana* was shown to have pronounced activity (Alen *et al*., 2000). A literature survey revealed that the isolation work of antinematodal compounds from this species has not been reported so far. Herein, we will report the isolation and characterization of two antinematodal compounds from *K. hookeriana*.

Materials and Methods

General experimental procedures

^{1}H and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument at 500 and 125 MHz, respectively. EI and CIMS spectra were measured on a JEOL HX-110 and GC-MS on Automass 20 coupled with GC (HP 5980, capillary column: DB Wax 0.25 mm × 30 m). IR and UV spectra were obtained by a Nicolet 710 FT-IR and a Shimadzu UV-3000 spectrometer, respectively. Optical rotation was measured by a Jasco DIP-360 polarimeter. GC was recorded by Hitachi G-3000 with Hitachi D-2100 Chromato-Integrator, and analytical or preparative HPLC was done on Hitachi Model L-7100 Pump with tandem of inertsil ODS-2 columns and D-7500 Integrator. Column chromatography (c.c.) was carried out on silica gel (Wakogel C-300, Merck Kiesel gel 60 Art. 15111 and LiChroprep RP-18 Art. 9303). Thin layer chromatography (TLC) was on silica gel 60 F_{254} plates (Merck Art. 5554, 0.25 mm). Spots and bands were detected by UV irradiation (254 nm) through with a vanillin sulfuric acid spray reagent.

Test plant and nematode

The plant materials were collected in August 1997 in "Panti" forest region, 120 km north of Padang, the capital of West Sumatra, Indonesia. *K. hookeriana* was identified by Dr. Rusdi Tamin and voucher specimen is kept in the Herbarium Biology Andalas (AND) Padang, West Sumatra, and the Herbarium Bogoriense (BO), Bogor, Indonesia.

The pine wood nematodes, *B. xylophilus*, were collected by the Baermann funnel method from wood chips of the trunk or stem of wilted pine trees in the Okayama University Experimental Forest, and subcultured by being fed on the fungus, *Botrytis cinerea* grown on the glucose-Czapek-Dox agar medium.

Antinematodal bioassay

Bioassay method previously reported (Kawazu *et al*., 1980) was used with some modifications.

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Extraction and isolation

Among the 63 plants assayed, the extract of the sap of *K. hookeriana* exhibited pronounced antinematodal activity. However, due to difficulties in collecting enough amounts of it, we used the bark of this plant for the extraction and isolation of active constituents, because it is easy to collect and contains plenty of the sap.

Chopped fresh bark (35 kg) of *K. hookeriana* was macerated with MeOH (60 liter, one week). After filtration, the MeOH solution was concentrated *in vacuo* to give 350 g of a methanolic extract. For preliminary isolation, a part (7 g) of the crude extract was added with water (*ca.* 300 ml) and partitioned with EtOAc (100 ml × 4). The EtOAc solution was dried with Na_2SO_4 and concentrated *in vacuo* to give 2.68 g of the EtOAc soluble fraction with MED of 12 mg/bl. The fraction was chromatograped over a silica gel dry column (hexane-EtOAc, 7:3 v/v) to give six fractions. The fraction 2 (R_f 0.6–0.8, 114 mg) showed the highest activity with MED of 8.2 mg/bl. Work up of the active fraction and repeated medium pressure c.c. over Wakogel C-300 (hexane-EtOAc, 96:4 v/v) and LiChroprep RP-18 (water-methanol, 14 : 86 v/v) gave the active fraction (1.65 mg), which was used as a reference for the succeeding large scale isolation. Monitored by the reference in repeated chromatography of the crude extract, 27.5 mg of the active fraction was isolated. HPLC analysis (Inertsil ODS-2 × 2, 8%H_2O in MeOH, flow rate: 0.8 ml/min) suggested this fraction contained two components with close retention time (t_R 23.2 and 24.9 min). Further purification of the active fraction (27.5 mg) was carried out on preparative HPLC (Inertsil ODS-2 × 2) using 8% H_2O in MeOH as eluent to give pure oily compound **1** (7.6 mg) and **2** (6.0 mg) with the MED of 4.5 and 20 μg/bl. or 0.018 and 0.073 μM/bl., respectively.

Compound **1**. colorless oil; $[\alpha]_D^{23}$ 0° (*c* 20, EtOAc); EIMS *m/z* (rel. Int.%): 248 (M^+, 32), 149 (5), 121 (11), 108 (100), 107 (30), 77 (4); CIMS *m/z* (isobutane): 249 $(M+H)^+$; HREIMS *m/z* 248.2138 for $C_{17}H_{28}O$ (calcd. 248.2141); IR ν_{max} (KBr) cm^{-1}: 3357, 2926, 2855, 1592, 1463, 1254, 696 and 671; UV λ_{max} (MeOH) nm: 216 (ε 5480), 274 (ε 1790) and 280 (sh. ε 1600); ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.88 (3H t, *J* = 7.0 Hz), 1.20~1.35 (14H, m), 1.60 (4H, m), 2.55 (2H, dd, *J* = 8, 8 Hz), 4.63 (1H, br s), 6.64 (1H, br dd, *J* = 8, 2.5 Hz), 6.66 (1H, br s), 6.75 (1H, br d, *J* = 8 Hz) and 7.13 (1H, t, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.1 ($-CH_3$), 22.7 ($-CH_2-$), 29.3 ($-CH_2-$), 29.5 ($-CH_2-$), 29.6 ($-CH_2-$), 29.6 ($-CH_2-$), 29.7 ($-CH_2-$), 29.7 ($-CH_2-$), 31.3 ($-CH_2-$), 31.9 ($-CH_2-$), 35.8 ($-CH_2-$), 112.4 (-CH=), 115.3 (-CH=), 120.9 (-CH=), 129.3 (-CH=), 145.0 (-C=), 155.4 (-C=).

Compound **2**. colorless oil; $[\alpha]_D^{23}$ 0° (*c* 20, EtOAc); EIMS *m/z* (rel. Int.%): 274 (M^+, 2), 121 (24), 149 (2), 134 (6), 108 (100), 107 (45) and 77 (11); CIMS *m/z* (isobutane): 275 $(M+H)^+$; HREIMS *m/z* 274.2299 for $C_{19}H_{30}O$ (calcd. 274.2343); IR ν_{max} (KBr) cm^{-1}: 3455, 2926, 2855, 1589, 1264, 1156 and 693; UV λ_{max} (MeOH) nm: 217 (ε 5200), 273 (ε 1610) and 280 (sh. ε 1400); ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.88 (3H, t, *J* = 7 Hz), 1.25~1.35 (8H, m), 1.60 (6H, m), 2.03 (4H, m), 2.55 (2H, dd, *J* = 8, 8 Hz), 4.63 (1H, br s), 5.34 (2H, m), 6.64 (1H, br dd, *J* = 8, 2.5 Hz), 6.66 (1H, br s), 6.75 (1H, br d, *J* = 8 Hz) and 7.13 (1H, t, *J* = 8 Hz). ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.0 ($-CH_3$), 22.3 ($-CH_2-$), 26.9 ($-CH_2-$), 27.2 ($-CH_2-$), 29.3 ($-CH_2-$), 29.4 ($-CH_2-$), 29.5 ($-CH_2-$), 29.7 ($-CH_2-$), 31.3 ($-CH_2-$), 32.0 ($-CH_2-$), 35.8 ($-CH_2-$), 112.4 (-CH=), 115.3 (-CH=), 120.9 (-CH=), 129.4 (-CH=), 129.9 (-CH=), 130.1 (-CH=), 144.9 (-C=) and 155.4 (-C=).

Ozonolysis of compound 2

A part (2 mg) of compound **2** was dissolved in *n*-pentane (*ca.* 5 ml) and cooled to minus 70 °C with dry ice-acetone. Ozone gas was passed slowly into the solution until it turned became blue (15 min). Reduction of the ozonides with dimethylsulfide gave *n*-valeraldehyde detected by GC-MS [t_R 5.09 min, temp.; programmed from 30 °C (5 min) to 250 °C at a rate of 10 °C /min].

Results and Discussion

Compound **1** (MED 4.5 μg/bl.) was obtained as clear oil and optically inactive. Its EIMS exhibited the molecular ion peak at *m/z* 248. The $(M+H)^+$ ion (*m/z* 249) in its CIMS also suggested the molecular weight of 248. The high resolution (HR)EIMS indicated the molecular formula of $C_{17}H_{28}O$. The IR spectrum of compound **1** suggested the presence of a hydroxyl group (3340 cm^{-1}) and a benzene ring (1591 cm^{-1}). Positive reaction of compound **1** with ferric chloride and Gibbs reagents indicated a phe-

nol moiety in the molecule. The UV spectrum with the absorption maxima at 216 and 274 nm as well as the fragment ion at *m/z* 77 and 108 in EIMS supported the presence of such functional groups. The coupling pattern of the ^{1}H-NMR signals at δ 6.64 (br dd, J = 8 and 2.5 Hz), δ 6.66 (br s), δ 6.75 (br d, J = 8 Hz) and δ 7.13 (br t, J = 8 Hz) indicates a *meta*-disubstituted benzene ring. The mass difference of 140 amu between 248 and 108 should be correspond to $(-CH_2-)_{10}$. The methylene proton signals from δ 1.20 to 2.55 (20 H) and one terminal methyl signal at δ 0.88 (t, J = 7 Hz) suggested *n*-C_{11} alkyl group. All results suggested that compound **1** is *meta*-*n*-C_{11} alkylphenol. The spectral data of **1** is closely resembled to those of isolated from the liverwort *Schistochila apendiculata* (Asakawa *et al.*, 1987). Therefore, compound **1** was identified as 3-undecylphenol (Fig. 1).

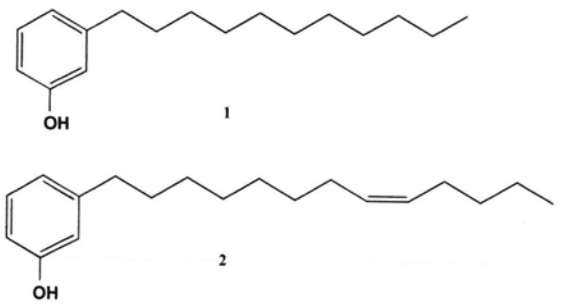

Fig. 1. Antinematodal Compounds **1** and **2**.

Compound **2** (MED 40 μg/bl.) was also obtained as clear oil and optically inactive. The molecular ion peak at *m/z* 274 in EIMS and the $(M+H)^+$ ion at *m/z* 275 in its CIMS suggested the molecular weight of 274. The HREIMS revealed that compound **2** had the molecular formula of $C_{19}H_{30}O$. A similarity of IR, UV and ^{1}H-NMR spectra of compound **2** with those of **1,** and the positive ferric chloride test suggested that compound **2** is also a *meta*-substituted phenol. In EIMS, the fragment ion peaks of **2** was similar to those of **1** at *m/z* 55, 77 and 108, except at *m/z* 147, 121 and 274 due to a double bond in the side chain. The ^{1}H-^{1}H COSY spectrum showed that two olefinic protons (δ 5.34, 2H m) were coupled with the methylene protons (δ 2.03, 4H) adjacent to the C=C double bond. The fact that chemical shifts of their carbons at allylic position in ^{13}C-NMR were observed at δ 26.9 and 27.2 indicated that the double bond could be assigned to be *cis*-configuration (Asakawa *et al.*, 1987 and Vysotskii *et al.*, 1990). By ozonolysis and the following detection of valeraldehyde, the location of double bond in the side chain of **2** was established at C-8. Therefore, the structure of compound **2** was determined as 3-(8*Z*-tridecenyl)-phenol (Fig. 1).

Little is known about the chemistry of the genus *Knema* plants (Myristicaceae) which comprises approximately 60 Southeast Asian species (Corner *et al.*, 1952; Zahir *et al.*, 1993 and Gonzales *et al.*, 1996). The extract of stem bark of *K. furfuracea* Warb are used in Thailand as a remedy for sores and pimples, and yielded isocoumarin as well as β-sitosterol (Pinto *et al.*, 1990). The bark of *K. laurina* and of *K. tenuinervia* also yielded 3-(8*Z*-pentadecenyl)-phenol, 2,4-dihydroxy-6-(10-phenyldecyl)-acetophenone and 8-hydroxy-6-methoxy-3-*n*-pentylisocoumarin (Kijjoa *et al.*, 1991), and none of them was reported for its biological activity so far. Furthermore, the active phenolic compound, Kneglomeratanol, was isolated from *K. glomerata*. It showed significant toxicities to three human tumor cell lines and inhibited the growth of crown gall tumor on disc of potato tuber (Zeng *et al.*, 1994). *K. hookeriana* used in the present study is a sapwood evergreen the Sumatran Rainforest plant. Traditionally, the leaf of this plant is used for stomach remedy. The sap is also useful for dying casting nets and cloths. Additionally, this plant is used as preservative surface coating material for valuable wooden, porcelain and metallic wares. However, the isolation work of biologically active components from this species has not been reported.

Thus, in the present study, compound **1** was isolated for the first time as the pronounced antinematodal component. Compound **2** also showed strong activity and seems to be novel because of no report on the isolation and structure elucidation of this compound from this species.

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