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Phenolic Nematicidal Compound from *Knema hookeriana* (Bark)

Yohannes Alen
Department of Pharmacy, Faculty of Sciences, Andalas University, Padang, Indonesia

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Abstract

The activity-guided chromatographic purification of the methanol extract of *Knema hookeriana* (bark), using pine wood nematodes *Bursaphelenchus xylophilus* has successfully led to the isolation and characterization of a phenolic nematicidal compound with the activity (MED) of 36 μg/cotton ball, (abbreviated to μg/bl). Using its chemical and spectral properties, this compound was determined to be 3-undecenylphenol. The compound is isolated for the first time from this species.

Keywords : *Knema hookeriana*, Cotton ball-fungal mat method, *Botrytis cinerea*, *Bursaphelenchus xylophilus*, 3-undecenylphenol.

Introduction

Little is known about the chemistry of the genus *Knema* (Myristicaceae) plants which comprises approximately 60 Southeast Asian species. This plants are tropical evergreen trees for which some species are described as having medical uses. The extract of stem bark of *K. furfuracea* Warb are used in Thailand as a remedy for sores and pimples. The bark of *K. tenuinervia*. yielded 3-(8Z-Pentadecenyl) -phenol, 2,4-Dihydroxy-6-(10-phenyldecyl)-acetophenon and 8-hydroxy-6-methoxy-3-n-pentyl-isocoumarin.[1] The active phenonic compound Kneglomeratanol was isolated from *Knema glomerata*. This compound showed significant toxicities to the three human tumor cell lines and inhibited the growth of crown gall tumor on disc of potato tuber.[2]

The methanol bark extract of *Knema hookeriana*, a sapwood evergreen plant from the Sumatran Rainforest, has been found to have nematicidal activity.[3] Herein, we report the isolation and characterization of a nematicidal compound from this species. Traditionally, in the West Sumatera the leaf of this plant is used for stomach remedy and the sapwood useful for dying casting nets and clothes.

The plant material was collected in August 1997 in "Panti" forest region, 120 Km north of capital city Padang, West Sumatra, Indonesia. Parts of this paper It was already presented on the seminar of *Nippon Nogeikagakukaishi*, in Kochi Japan 1998, *Abstr. Paper* 73 (2) 1999 pp. 118.[4] A literature survey revealed that the isolation work of nematicidal compounds from this species has not been reported so far.

Experimental Section

General Experimental Procedures

^{1}H and ^{13}C-NMR spectra were recorded by a Varian VXR-500 instrument using $CDCl_3$ at 500 and 125 MHz, respectively. HREI, EI, FAB-Mass spectra were measured with a JEOL JMS-D 300 mass spectrometer. GC-Mass were analyzed with Hitachi-3000 and detected by Hitachi D-2100 Chromato Integrator instruments, HPLC were recorded with HITACHI Model L-7100 Pump and D-7500 Integrator. Preparative HPLC carried out on double packing inertsil ODS-2. IR and UV spectra were obtained by a Nicolet 710 FT-IR and a Shimadzu UV-3000 spectrometer, respectively. Specific rotation was measured by a Jasco DIP-360 polarimeter. Silica gel column chromatography was carried out on Wakogel (C-100 and C-300), ODS

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*Corresponding author : Telp. 62-751-71682, Fax: 62-751-73118
E-mail : farmasi-unand@telkom.net

Rp-18, and ODS Millipore Prep. C18. Thin layer chromatography was carried out on Merck silica gel 60 F_{254} plates (0.25 mm) spots and bands were detected by UV irradiation (254 and 365 nm) through with a vanillin sulfuric acid spray reagent. The plant material was identified by Drs. Rusdi Tamin and voucher specimen is kept in the Herbarium Biology Andalas (AND) Padang, West Sumatera, and the Herbarium Bogoriense (BO), Bogor, Indonesia.

Test Nematodes

The pine wood nematodes, *Bursaphelenchus 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 the fungus, *Botrytis cinerea* grown on the Glucose-Czapek-Dox Agar medium.

Bioassay Procedure

The cultured nematodes (propagative form) were separated from the culture medium by the Baermann funnel technique and counted on a section under a microscope (x 20). An aqueous suspension of the nematode (*ca.* 15.000 heads/ml) was prepared by appropriate dilution.

Botrytis cinerea was cultured on 3 ml of the Czapeck-Dox agar medium (1.3 % agar) in a petri dish (4 cm in dia.) at 22^0 C for 4 days. At the center of the fungal mat in the dish was placed a cotton ball (5 mm in dia.) containing the test concentrate. The nematodes suspension (0.1 ml) prepared above was injected by a micropipette into the cotton ball, and the dish was kept at 26^0 C for 96 h. The nematicidal effect (inactive or active) was estimated by observing whether the mycelia were consumed by the nematode or not, and denoted by a sign - or +, respectively. For the convincing purpose, the living nematodes were counted as follows. The living nematodes were separated from the culture medium through double sheets of tissue paper (JK Wiper 150-S) in the Baermann funnel for 6 h. The nematodes were collected by centrifugation (650 x g, 3 min.), and the suspension of nematodes of appropriate concentration was poured into a flat dish placed on a section. The nematodes were counted under a microscope (x 20). The rate of propagation was expressed in term of percentage of the number of the nematodes to that from a control.

Extraction of Knema hookeriana

Chopped fresh bark (35 kg) of *Knema hookeriana* was macerated with MeOH (60 lt., one week). The MeOH soln. was concentrated *in vacuo* to give 350 g of crude extract 286-71-1. For preliminary isolation, a part (7 g) of crude extract was added with water (*ca* 300 ml), partitioned with EtOAc (100 ml x 4). The EtOAc fraction dried with Na_2SO_4 and concentrated *in vacuo* to give 2.68 g of EtOAc fraction (286-71-2) with MED 12 mg/bl. A part of 35 ml aqueous layer was partitioned with BuOH (50 ml X 4) to give 106 mg BuOH fraction and 374 mg most polar fraction in aqueous layer with MED >50 mg/bl and 6 mg/bl, respectively. The EtOAc fraction (286-71-2) was dry column chromatographic over silica gel 60 PF_{254} (1:15, Merck 7747) with hexane-EtOAc (7:3) to give six fractions. The fraction 286-78-2 (114 mg) showed the most active with MED 8.2 mg. The remaining 66 mg of active fraction 286-78-2 was chromatographic with medium pressure on Wakogel C-300 (6.6 g) using hexane-EtOAc (96:4) to give 42.2 mg active fraction 286-83-24 with MED 0.16 mg/bl. The active fraction exhibited only one spot on Kiesel gel tlc PF_{254} (hexane-EtOAc 9:1) detected with UV followed by vanillin-sulfuric acid, while afforded three spots on the ODS tlc (H_2O-MeOH 4:94) with Rf. values 0.45, 0.40 and 0.33 cm, respectively. The active fraction AY286-83-24 (42.2 mg) was subjected to ODS-LiChroprep RP-18 (Merck Art.9303) using eluent water and methanol (14 : 86) to give three oily fractions. The active fraction AY286-93-4 was the most active (1.65 mg, MED 13.3 μg/bl). The same fraction denoted AY286-129-39 (27.5 mg) was isolated in ten times large scale using the similar method as described earlier. Both of HPLC analysis and GC-Mass exhibited two peaks in close retention time. Further purification of the AY286-129-39 (27.5 mg) was carried out on preparative ODS HPLC (double packing inertsil ODS-2) using eluent 8% H_2O in MeOH to gave pure oily compound (5.91 mg) with the MED 36 μg/bl.

Ozonolysis of active compound

Ozone (O_3) reacts vigorously with alkenes to form unstable compounds called initial ozonides, which rearrange spontaneously to form ozonides. The substrate (7 mg) was dissolved in *n*-pentane (*ca.*10 ml) and cooled to minus 70 degree celcius. Ozon gas was passed slowly until the solution become blue color and reaction stop until 15 min. Then ozonides was reduced and react with dimethyl sulfide to form aldehydes. In a preliminary experiment, the reaction has been done succesfully on Methyl oleate using Ethyl acetate as solvent. Two major reaction products, nonanal and nonanal methyl ester were confirmed by GC and GC-Mass spectroscopy, and comparing with standard commercially of nonanal. Ozonolysis of isolated compound gave products pentanal.

Results and discussion

Positive reaction the isolated compound toward a series ferric chloride followed Gibbs reagent indicated that this compound had phenol group where the was no substituent on the *para*-position.

The active compound (5.84 mg, MED 36 µg/bl) obtained as clear oily was no optically active $[\alpha]^{23}_{D}$ 0^{O} (*c* 20, EtOAc). The IR spectrum (KBr) showed the presence of hydroxyl group (3455 cm^{-1}), and C=C aromatic ring (1589 cm^{-1}). The UV spectrum (MeOH) exhibited absorption maximal at 217 nm (ε 5204), 273 nm (ε 1614) and (sh) 280 (ε 1434). Its EIMS exhibited the molecular ion peak at *m/z* 274. The $(M+H)^{+}$ ion peak (*m/z* 275) in its CIMS (isobutane) also suggested the molecular weight of 274. The high resolution measurement of the molecular ion in its EIMS indicated the molecular formula of $C_{18}H_{30}O$. The fragmentation pattern at *m/z* 55, 77 and base peak 108 of the compound was similar to that of 3-undecylphenol, except at *m/z* 147, 121 and 274 would be due to a double bond in the monoolefinic side chain. The ^{1}H-^{1}H COSY correlated also suggest the proton double bond only coupling with two methylene protons at δ 2.00 ppm. Based on their carbons at allylic position chemical shifts of ^{13}C-NMR were observed at δ 27.20 and δ 26.9 ppm. the double bound could be assigned to be *cis*-configuration[4,6] By the chemical confirmation ozonolysis, the location of double bond in the side chain was established.

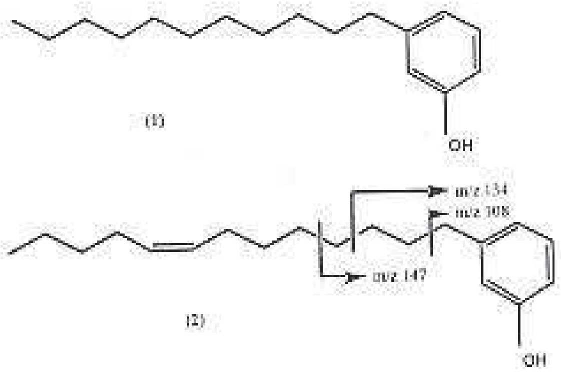

Fig. 1. Isolated active compound and 3-undecylphenol

Table 1. The ^1H, ^{13}C-NMR spectrum of active compound and 3-undecylpenol* were taken in d-$CDCl_3$ (500 and 125 Mhz)

Position	3-undecylpenol		3-undecenylphenol	
	C	H	C	H
		4.63 1H br.S(OH)		4.63 1H br.s (OH)
1	155.42		155.44	
2	115.27	6.64, 1H d (1,8	115.27	6.64, 1H d (1,8)
3	144,96		144.92	
4	120.94	6.75, 1H br d (7.3)	120.92	6.74, 1H br d (7.3)
5	129.34	7.13, 1H td (7.6, 1.8)	129.35	7.13, 1H td (7.6, 1.8)
6	112.42	6.62, 1H br d (7.3)	112.44	6.62, 1H br d (7.3)
7'	35.81	2.55, 2H dd (7.6, 7.9)	35.80	2.55, 2H dd (7.6, 7.9)
8'	31.90	1.25, 14H m	31.95	1.26, 8H m
9'	31.28	1.58, 4H m	31.26	1.60, 6H m
10'	29.70		29.73	2.03, 4H m
11'	29.68		29.53	
12'	29.62		29.37	
13'	29.57		27.16	
14'	29.50		129.88	5.34, 2H m
15'	29.31		130.05	
16'	14.12	0.88, 3H t (7.0)	26.90	
17'			29.25	
18'			22.33	
19'			13.99	0.88, 3H t (6.7)

* In ppm. The assignments were made on the basis of ^1H-^1H COSY correlations.

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