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A NEW SESQUITERPENE FROM *Knema patentinervia*

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A new widdrane sesquiterpene was isolated from Knema patentinervia *by chromatographic methods. The structure was elucidated by spectroscopic methods, including* ^1H–^1H *COSY, HMQC, HMBC, and NOESY NMR experiments. The compound showed no inhibition of leukemia cancer cell growth and no inhibition towards bacterial growth of* Staphylococcus aureus*,* Bacillus anthracis*,* Pseudomonas aeruginosa*, and* Escherichia coli.

Keywords: *Knema patentinervia*, Myristicaceae, sesquiterpene.

Knema is a genus of trees of the family Myristicaceae found in Southeastern Asia. Burkill [1] reported that at least 10 species are found in Malaysia, where they are commonly known to the Malays as "penarahan," meaning blood, referring to the red resin secreted in the bark. Previous phytochemical studies reported the presence of diterpenes in the fruits of *K. glauca* [2], arylnaphthalene lignans in the leaves of *K. furfuracea* [3], and stilbenes in the wood of *K. austrosiamensis* [4]. The extract from *K. malayana* was reported to have a broad spectrum of activity against microbes [5]. Alcoholic extracts of *K. laurina* were found to have high anti-inflammatory and neuroprotective effects in cell culture by reducing NO- and IL-6 release [6]. Alen et al. [7] reported antinematodal activity from *K. hookeriana* against *Bursaphelenchus xylophilus*. In this paper, we report the isolation of a novel sesquiterpene from the stem bark of *Knema patentivervia* (J. Sinclair) W.J. de Wilde that is locally also called as *penarahan*, which produces a red exudate on the stems.

Purification of dichloromethane extract (25 g) by vacuum liquid chromatography (VLC) on silica gel with hexane (Hex), Hex–dichloromethane (DCM) (1:1), DCM, DCM–acetone (1:1), and acetone yielded five fractions. The Hex–DCM (1:1) fraction (0.9 g) was chromatographed with Hex and Hex–DCM (9:1→1:9) and DCM to give compound **1**. Compound **1** is a white powder whose infrared spectrum showed the presence of OH stretching at 3340 cm^{-1} and a trisubstituted alkene (C=C) band at 809 cm^{-1}. The ^1H NMR spectrum of **1** showed an overlapping signal for protons, suggesting that they have a similar electronic environment. The olefinic proton appeared at δ 5.53 as a doublet (J = 6.8 Hz) corresponding to H-2. Two doublets at δ 0.90 (J = 7.2 Hz) and δ 0.83 (J = 6.8 Hz) were assigned to protons H-14 and H-13, respectively. A singlet at δ 1.67 was assigned to methyl proton H-12, which has strong correlation with C-4. The COSY spectrum showed strong correlation between H-4 with H-6 and H-5. The ^{13}C NMR, DEPT, and HMQC spectra displayed the occurrence of 15 carbons with two quaternary carbons at δ 134.4 and δ 72.6, five methines at δ 124.6, 45.5, 44.1, 36.8, and 26.4, and four methylene carbons at δ 35.3, 31.1, 21.5, and 18.5. Confirmation of the partial structural units and their connectivity were made by analysis of the HMBC spectrum. It was found that there was a mutual long correlation between the olefinic proton at δ 5.53 (H-2) and the methyl and methylene carbon at δ 23.6 (C-12) and δ 31.1 (C-4), respectively. The methylene proton (H-9a) at δ 2.01–1.89 was coupled to the methyl carbon at δ 21.7 (C-14). The methyl protons (H-12) showed long-range coupling to the methylene carbon at δ 31.1 (Table 1).

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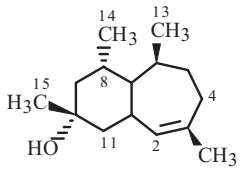
TABLE 1. NMR Data for Compound **1** in $CDCl_3$

C atom	δ_H (δ, ppm, multiplicity, J/Hz)[a]	δ_C (δ, ppm)[b]	HMBC	NOESY
1	1.67–1.44 (1H, m)	45.5	C-11, C-5	
2	5.53 (1H, d, J = 6.8)	124.6	C-8, C-1, C-4, C-12	H-12
3	–	134.4		
4	2.01–1.89 (2H, m)	31.1	C-5	H-12
5	1.67–1.44 (2H, m)	21.5	C-8	H-7
6	2.01–1.89 (1H, m)	26.4	C-5	
7	1.30 (1H, s)	44.1	C-9, C-11, C-1	H-9b, H-5
8	2.01–1.89 (1H, m)	36.8	C-5	H-13
9a	2.01–1.89 (1H, m)	18.5	C-14	H-15
9b	1.67–1.44 (1H, m)		C-8	H-7
10	–	72.6		
11	1.67–1.44 (2H, m)	35.3	C-8	
12 CH_3	1.67 (3H, s)	23.6	C-11, C-4	H-4, H-2
13 CH_3	0.83 (3H, d, J = 6.8)	15.3	C-7, C-6, C-5	H-8
14 CH_3	0.90 (3H, d, J = 7.2)	21.7	C-7, C-6, C-13	
15 CH_3	1.3 (3H, s)	27.9	C-9, C-11, C-1	H-9a

[a]Recorded at 400 MHz; [b]recorded at 100 MHz.



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The relative configuration of **1** was determined via analysis of the NOESY experiment (Table 1). The H-9b proton correlated to H-8, requiring a *cis* ring fusion and placing them on the same face of the ring. Therefore, the orientation of the methyl groups at 3, 6, 8, and 10 were determined to be β, β, α, and β, respectively.

The molecular formula of $C_{15}H_{26}O$ gives a double bond equivalent (DBE) value of 3, which suggested the presence of one C=C group and two cyclics. A seven-membered ring presence in the structure was suggested. Compound **1** had the molecular formula $C_{15}H_{26}O$ as shown by ESI-MS with $(M + 1)^+$ at *m/z* 223.20. To the best of our knowledge, the compound has never been reported before. The bioactivities of the compound were tested against leukemia cells and microbes, and, unfortunately, no inhibition activity was exhibited by the compound.

EXPERIMENTAL

General Procedures. The melting point was measured on a Buchi B545 melting point apparatus and uncorrected. The ^{13}C NMR and ^1H NMR were recorded on a Bruker 400 MHz in $CDCl_3$. Chemical shifts are reported in ppm and in the δ scale, and coupling constants are given in Hz. The ultraviolet (UV) spectrum was recorded using a Secomam Uvi Light XT2 spectrophotometer in ethanol. The infrared (IR) spectrum was obtained with a Perkin–Elmer infrared spectrophotometer. Optical rotation was measured on a Polax-2L polarimeter. Silica gel (70–230 and 230–400 mesh, Merck) were used for chromatography.

Plant Material. The stem bark of *K. patentinervia* was collected from Taman Pertanian Kuantan, Pahang in March 2010. The plant was identified and confirmed by Dr. Shamsul Khamis (Institute of Bioscience, Universiti Putra Malaysia). A voucher specimen (MT17) of this plant was deposited at the Herbarium of the Faculty of Pharmacy of the International Islamic University Malaysia.

Extraction and Isolation. Dried and powdered *K. patentinervia* (758 g) was extracted with hexane, ethyl acetate (EtOAc), and methanol (MeOH), succesively. Each solvent was evaporated under reduced pressure at 60°C to obtain hexane (1.2 g, 0.15%), EtOAc (29.9 g, 3.9%), and MeOH (81 g, 10.6%) extracts. A portion of the EtOAc extract (25 g) was fractionated by VLC silica gel with Hex, DCM–Hex (1:1), DCM, DCM–acetone (1:1), and acetone fractions. The Hex–DCM (1:1) fraction

(0.9 g) was chromatographed with Hex, Hex–DCM (9:1→1:9), and DCM, yielding 22 fractions. Fractions 8–10 were combined to give compound **1** (189 mg, 0.02%).

3β,6β,8α,10β-Tetramethylwiddrane-2(3)-en-10α-ol (1). White amorphous solid, mp 119.2–123.5°C, $[\alpha]_D^{27}$ +111.1° (*c* 0.0162, $CHCl_3$), R_f 0.49 (silica gel 60 F_{254}, dichloromethane). ESI-MS *m/z*: 223.20 $[M + 1]^+$, (calcd for $C_{15}H_{26}O$, 222.1984). UV (EtOH, λ_{max}, nm): 286. IR (KBr, ν_{max}, cm^{-1}): 3340, 2898, 1487, 1240, 1038, 925, 809. ^1H NMR and ^{13}C NMR (Table 1).

Cell Lines and Microorganisms. K562 leukemia cells were purchased from ATCC (CCL-243), and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus anthracis* ATCC 14578) and two Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218) were used for the antibacterial test.

Cytotoxicity Assay. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorometric assay was performed as described by Mosmann [8]. K562 cells were cultured in a 96-well culture plate (2×10^5 cells/mL). The sample was dissolved in absolute ethanol (1 mg/mL). The cells were treated with the compound ranging from 3 μg/mL to 100 μg/mL for 24 h. The activity of the sample towards cell proliferation was measured by adding 2 μL MTT. The formazan crystals that formed were dissolved with dimethylsulfoxide (DMSO) and measured in a microplate reader (Tecan) at 570 and 630 nm (reference).

Antimicrobial Assay. The agar disc diffusion method was employed according to the literature [9] for the determination of antimicrobial activity. An inoculum containing 10^6–10^7 CFU/mL was spread on Mueller-Hinton agar plates. Using sterile forceps, the sterile filter papers (6 mm diameter) containing compound **1** (20 μg), standard antibiotic (30 μg of chloramphenicol), and the negative control (DMSO-d_6) were laid down on the surface of the inoculated agar plate. The plates were incubated at 37°C for 24 h. Each sample was tested in triplicate, and the diameter of the zone of inhibition, in millimeters, was measured.

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