



A New Cytotoxic Compound from Methanol Extract of *Koordersiodendron pinnatum* Merr. Leaves

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Abstract – Chemical investigation of the methanol extract of *Koordersiodendron pinnatum* Merr. leaves resulted a new naphthalene derivative, (*Z*)-4-(tetradec-3-enyl)naphthalene-1,2,7-triol (**1**), together with three known compounds, β -sitosterol (**2**), 20-epibryonolic acid (**3**), and scopoletin (**4**). The structure of the new compound was elucidated based on spectroscopic evidence. The isolated compounds (**1-4**) were tested their cytotoxic activities against the P-388 murine leukemia cell line and compound **1** has highest activity with IC_{50} 1.94 μ M.

Keywords – Naphthalene derivative, *Koordersiodendron pinnatum* Merr., cytotoxic activities, P-388 murine leukemia cell lines

Introduction

Koordersiodendron pinnatum Merr. (Anacardiaceae family), with synonyms *Koordersiodendron celebium* Engl., *K. papuanum* Kaneh & Hatus, is widely distributed in lowland forest throughout the Philippines, Borneo, Sulawesi, Moluccas and Papua New Guinea.¹ This plant is used in Philippines for treating chronic wounds, bruises and sprains, and in India to prepare lotions for treating impetigo.² This plant shows a diverse range of biological activities, including broad spectrum antibacterial activities against Gram-positive and Gram-negative bacteria and DPPH radical scavenger activity.^{3,4} In another pharmacological study, methanol extract of this plant was demonstrated to be practically no mortality for both male and female in ddY mice, with $LD_{50} > 15$ g/kg.² However, there is no report on the chemical constituents of *K. pinnatum*. As part of a program to search for chemical components of the Indonesian plant species, especially from Mekongga forest, Southeast Sulawesi, we report the isolation and structure elucidation of a new naphthalene derivative, along with three known compounds, and evaluated their cytotoxic activities.

Experimental

General experimental procedures – NMR spectra were recorded with JEOL JNM ECA-500 spectrometer, operating at 500 MHz (¹H-NMR) and 125 MHz (¹³C-NMR), using TMS (Tetra Methyl Silane) as an internal standard. LCMS were obtained with Mariner Biospectrometry spectrometer using ESI (Electrospray Ionisation) system with positive ion mode. LCMS/MS was carried out using XEVO-G2SQTOF (Waters). FTIR and UV/Vis spectrum were obtained from Shimadzu-Prestige 21, and Agilent Technologies Carry 60 UV Vis, respectively. Column chromatography was carried out using Merck Silica gel 60 (70 - 230 mesh ASTM) and Sephadex LH-20 (Sigma). TLC (Thin Layer Chromatography) analysis on pre-coated Silica gel plates (Merck Kieselgel 60 F 254, 0.25 mm).

Plant materials – Samples of *K. pinnatum* Merr. were collected from Mekongga forest, Southeast Sulawesi, Indonesia. The plant was determined and deposit at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences with voucher specimen UHA-49.

Extraction and isolation – The dried leaves of *K. pinnatum* (700 g) were successively extracted with *n*-hexane and methanol. The methanol extract (27.27 g) was partitioned with *n*-hexane and ethyl acetate afforded *n*-hexane fraction (6.71 g), ethyl acetate fraction (3.58 g)

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and residue (16.62 g). The ethyl acetate fraction was further fractionated on a silica gel column with gradient elution (*n*-hexane-ethyl acetate-methanol; each step is up to 10% from 100% *n*-hexane until 100% methanol with 200 mL v/v of solvent), yielding 11 fractions. Fraction 3 was further purified using Sephadex LH-20 with dichloromethane and methanol (1:1) as system solvents, to obtain compound **1** (20 mg). The *n*-hexane fraction was further purified using column chromatography on silica gel as stationary phase and *n*-hexane-ethyl acetate (elution gradient, each step is up to 10% from 100% *n*-hexane until 100% ethyl acetate with 100 mL v/v of solvent) to yield compound **2** (25 mg) and compound **3** (15 mg). The residual fraction was subjected to column chromatography on silica gel and Sephadex LH-20 affording compound **4** (5 mg).

(Z)-4-(Tetradec-3-enyl)naphthalene-1,2,7-triol (1): - gum; IR (KBr) ν_{\max} cm⁻¹: 3385, 2956, 2931, 2872, 2121, 1649, 1460, 1373, 1165, 1109, 1070, 1020, 952; UV (MeOH) λ_{\max} 251, 294 nm; ¹H, ¹³C NMR: see Table 1.; ESI-QTOFMS: *m/z* 370.32579 [M]⁺; 371.32579 [M+H]⁺.

β -Sitosterol (2) – White crystal; ¹H NMR (CDCl₃, 500 MHz): δ 5.33 (1H, dd, H-6), 3.50 (1H, m, H-3), 1.25 (2H, m, H-22), 1.23 (2H, m, H-23), 1.01 (3H, d, H-21), 1.00 (3H, s, H-19), 0.84 (3H, d, H-29), 0.80 (3H, d, H-27), 0.92 (3H, d, H-26), 0.69 (3H, s, H-18); ¹³C NMR (CDCl₃, 125 MHz): δ 39.8 (C-1); 31.8 (C-2); 72.0 (C-3); 40.6 (C-4); 140.9 (C-5); 121.9 (C-6); 32.1 (C-7, C-8, & C-25); 50.4 (C-9); 37.4 (C-10); 21.4 (C-11); 42.4 (C-12); 42.5 (C-13); 57.1 (C-14); 24.6 (C-15); 29.1 (C-16); 56.1 (C-17); 12.4 (C-18); 19.59 (C-19); 36.7 (C-20); 19.1 (C-21); 34.0 (C-22); 26.1 (C-23); 51.4 (C-24); 21.3 (C-26 & C-27); 25.6 (C-28); 12.2 (C-29);. ESI-MS *m/z* 414.

20-Epibryonolic acid (3) – White powder, ¹H NMR (DMSO-*d*₆, 500 MHz): δ 4.4 (1H, d, *J* = 4.5 Hz, 3-OH), 2.95 (1H, t, H-3), 2.23 (2H, d, *J* = 15.5), 2.00 & 1.48 (2H, m, H-7), 1.82 (2H, m, H-11), 1.82 & 1.61 (2H, m, H-6), 1.65 (2H, m, H-1), 1.62 (2H, m, H-22), 1.58 & 1.22 (2H, m, H-16), 1.46 (2H, m, H-2), 1.45 & 1.18 (2H, m, H-12), 1.43 & 1.22 (2H, m, H-21), 1.37 (1H, d, *J* = 8.45), 1.23 (2H, m, H-15), 1.05 (3H, s, H-29), 0.94 (3H, s, H-28), 0.90 (1H, d, *J* = 12.3 Hz, H-5), 0.87 (3H, s, H-26), 0.85 (3H, s, H-23), 0.84 (3H, s, H-25), 0.80 (3H, s, H-27), 0.63 (3H, s, H-24); ¹³C NMR (DMSO-*d*₆, 125 MHz): δ 180.3 (-COOH), 134.3 (C-9), 133.8 (C-8), 77.4 (C-3), 50.7 (C-5), 44.7 (C-18), 41.9 (C-14), 39.5 (C-20), 37.6 (C-4 & C-10), 37.2 (C-16), 35.2 (C-1), 35.3 (C-13), 34.5 (C-22), 33.1 (C-29), 31.5 (C-28), 31.0 (C-17), 30.5 (C-19), 30.3 (C-21), 29.9 (C-12), 28.7 (C-23), 28.1 (C-7), 27.6 (C-2), 25.1 (C-15), 22.4 (C-26), 20.7 (C-11), 20.2 (C-25), 19.4

Table 1. ¹H and ¹³C NMR data of **1** in CDCl₃. (δ in ppm, 500 MHz for ¹H and 125 MHz for ¹³C)^a

Position	1	
	δ_{H}	δ_{C}
1	-	149.9
2	-	161.2
3	6.27 s	101.9
4	-	105.6
4a	-	130.1
5	7.25 d (9.1)	111.2
6	6.70 dd (2.6; 9.1)	111.5
7	-	151.4
7-OH	4.59 s	-
8	6.89 d (2.6)	105.6
8a	-	130.1
1'	2.73 m	28.7
2'	1.72 m	27.8
3'	5.35 m	130.11
4'	5.35 t (5.2)	130.06
5'	2.00 m	27.4
6'-13'	1.32 bs	29.9
14'	0.88 t	14.3

^a*J* values are in parentheses and reported in Hz; the assignments were based on ¹H-¹H COSY, HMQC, and HMBC experiments.

(C-6), 17.7 (C-27), 16.5 (C-24). ESI-QTOFMS *m/z* 456.35480.

Scopoletin (4) – White powder, ¹H NMR (CDCl₃, 500 MHz): δ 7.59 (1H, d, *J* = 9.08 Hz, H-4), 6.92 (1H, s, H-5), 6.85 (1H, s, H-8), 6.26 (1H, d, *J* = 9.08 Hz, H-3), 3.95 (3H, s, H-11); ¹³C NMR (CDCl₃, 125 MHz): δ 56.7 (6-OCH₃), δ 160.7 (C-2), 151.9 (C-7), 151.1 (C-9), 148.8 (C-6), 144.8 (C-4), 113.6 (C-3), 112.4 (C-10), 110.8 (C-5), 103.4 (C-8). ESI-MS *m/z*: 192.04.

Cytotoxicity assay – The cytotoxicity of compounds **1-4** against murine leukemia P-388 cells was evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method.^{5,6} In brief, P388 cells were seeded into a 96 well plate (3 × 10⁴ cells/well). After 24 h of incubation for cell attachment and growth, samples were added at various concentrations prepared at six levels using PBS (phosphate buffer solution). The assay was terminated after 48 h of incubation by adding MTT reagent, and the incubation was continued for another 4 h, during which time MTT-stop solution containing SDS (sodium dodecyl sulphate) was added, and further incubation was carried out for 24 h. The optical density was read by using a microplate reader at 550 nm. IC₅₀ values were determined from the graph of percentage of survival cells compared to the control (%), which received

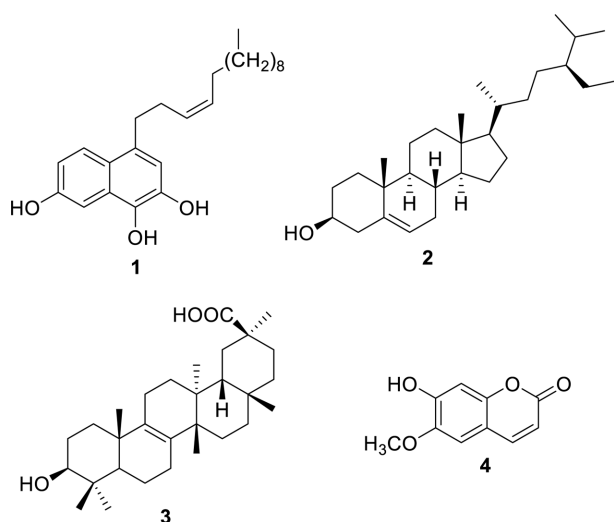


Fig. 1. The structures of 1 - 4 isolated from *K. pinnatum*.

only PBS and DMSO, versus the tested concentration of compounds (μM). Each assay and analysis were performed in triplicate, and the results were averaged. Artonin E was used as the positive control.

Result and Discussion

Fractionation of the methanolic extract of the leaves of *K. pinnatum* by sequential column chromatography on silica gel and Sephadex LH-20 resulted in the isolation of a new compound 1 and three known compounds 2 - 4 (Fig. 1). The known compounds were identified as β -sitosterol (2), 20-epibryonolic acid (3), and scopoletin (4) by comparison of their physical and spectral data with literature values.⁷⁻⁹ The structure of the new compound 1 was determined by a combination of ESI-QTOFMS, UV/Vis and FTIR together with 1D and 2D NMR experiments.

Compound 1 was obtained as brown pasta. The UV spectrum of 1 showed maxima at 251 and 294 nm, which are typical of naphthalene derivative.¹⁰ The IR spectrum of 1 suggested the presence of hydroxyl group (3385 cm^{-1}) and aliphatic group ($2956, 2931\text{ cm}^{-1}$). Aromatic compounds also showed skeletal vibrations (including C-C stretching within the ring) in the 1649 cm^{-1} region and weak overtone bands in the 2121 cm^{-1} region. The molecular formula of 1 was determined as $\text{C}_{24}\text{H}_{34}\text{O}_3$ by ESI-QTOFMS at m/z 370.32579 (M^+). The $^1\text{H-NMR}$ spectrum (CDCl_3 , 500 MHz) indicated the existence of three aromatic protons with chemical shifts δ_{H} 6.70 (1H, dd, $J=2.60$ and 9.1 Hz), 6.89 (1H, d, $J=2.60$ Hz) and 7.25 ppm (1H, d, $J=9.1$ Hz). The coupling constants indicated

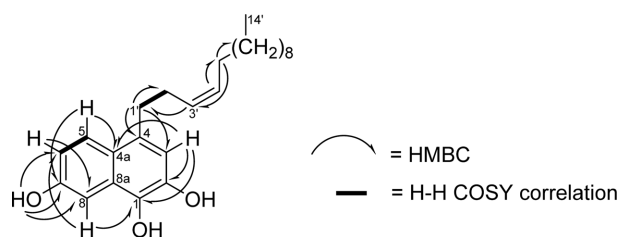


Fig. 2. Key HMBC (\rightarrow) and $^1\text{H-}^1\text{H}$ COSY (—) correlations of 1.

that the signal at δ 6.70 shows an *ortho* correlation with the proton at δ 7.25 and a *meta* correlation with proton at 6.89 ppm (ABX ring system). The spectrum also indicated the presence of an uncoupled aromatic proton at δ 6.27 (1H, s), which was assigned to ring B. Besides, there were signals attributable to the two methine protons of a *cis* double bond (H-C=C-H) at δ 5.35 (2H, t, $J=5.2$ Hz). Signals due to eleven methylene protons (CH_2) were also seen at δ 1.32 (16H, bs), 1.72 (2H, m), 2.00 (2H, m) and 2.73 ppm (2H, t). A signal due to methyl (CH_3) group appeared at δ 0.88 (3H, t, $J=7.2$ Hz). $^{13}\text{C-NMR}$ and DEPT 135 (125 MHz, CDCl_3) spectra showed signals for four aromatic methine groups (δ_{C} 101.9, 111.5, 105.6, and 111.2), and six quaternary carbons (δ_{C} 105.6, 130.1 (2C), 149.9, 151.4, and 161.2). The $^{13}\text{C-NMR}$ spectrum also indicated signals for two methine group at δ_{C} 130.0 and 130.1. The signals at δ_{C} 29.9, 27.8, 27.4, and 28.7 were assigned to methylenes with an sp^3 configuration, while the signal at δ_{C} 14.3 was assigned to a methyl group.

Correlation between the ^1H and $^{13}\text{C-NMR}$ signals were supported by the HMQC and HMBC spectra. The HMBC spectrum (Fig. 2) indicated the presence of a 1,2,7-trihydroxynaphthyl group, with long-range coupling between H-5 at δ 7.25 with C-4a and C-7, H-6 at δ 6.70 with C-8 and 7-OH, H-8 at 6.89 with C-6, C-7, and H-3 at δ 6.27 with C-4a, 2-OH and 3-OH. An alkenyl group was concluded to be present at C-4 based on the long-range correlation between H-1' and C-3. The spectrum also showed the important correlation at H-1' to C-4 as the junction between naphthalene group and alkyl group. The position of a double bond at C-3' - C-4' was confirmed by the presence of long-range correlations between H-3' and C-1', H-4' and C-5', and H-5' and C-3' in the HMBC spectrum. Finally, the chemical structure of 1 was characterized as (*Z*)-4-(tetradec-3-enyl)naphthalene-1,2,7-triol.

The cytotoxic properties of compounds 1 - 4 (Table 2) were evaluated against murine leukemia P-388 according to the method described previously.⁵⁻⁶ A new compound 1 was found to be the most active, while compounds 3 and 4 were moderately cytotoxic but compound 2 was weakly cytotoxic.

Table 2. Cytotoxic activity of compounds **1-4** against P-388 murine leukemia cell line

Compound	IC ₅₀ (mM)
(Z)-4-(Tetradec-3-enyl)naphthalene-1,2,7-triol (1)	1.94
β -Sitosterol (2)	>241.54
20-Epibryonolic acid (3)	126.94
Scopoletin (4)	90.71
Artonin E*	0.69

*positive control

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