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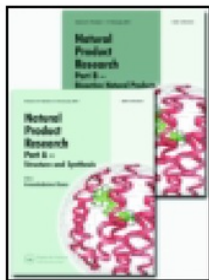
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1 Mesucalophylloidin, a new isoprenylated 4-phenylcoumarin from *Mesua calophylloides* (Ridl.) Kosterm

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ABSTRACT

A new isoprenylated 4-phenylcoumarin derivative, mesucalophylloidin (1) along with three known compounds, mamea BA cyclo F (2), calolongic acid (3) and isocalolongic acid (4) were isolated from the stem bark of *Mesua calophylloides* (Ridl.) Kosterm. Structures of all the compounds were elucidated using extensive spectroscopic methods, including UV, IR, HRESIMS, 1D and 2D NMR. Compounds 1–4 were evaluated for their cytotoxicity against P-388 cells, showing that compound 1 gave moderate activity with IC₅₀ 6.26 µg/mL.



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1. Introduction

Mesua calophylloides (Ridl.) Kosterm. locally known 'bitangur kunyit' with a commercial name ironwood belongs to the Clusiaceae family. This species of *Mesua* is endemic in Kalimantan Island and East Malaysia. Based on ethnomedicinal, the decoction of stem bark or leaves this plant has been used in the Dayak people to treat some diseases (Heyne 1987). The phytochemical survey from this plant until now has been not reported. The *Mesua* genus has been

1 own to be prolific a number of secondary metabolites, particularly xanthenes (Singh et al. 1993; Karunakaran et al. 2016), coumarins (Awang et al. 2010; Rouger et al. 2015; Tanjung

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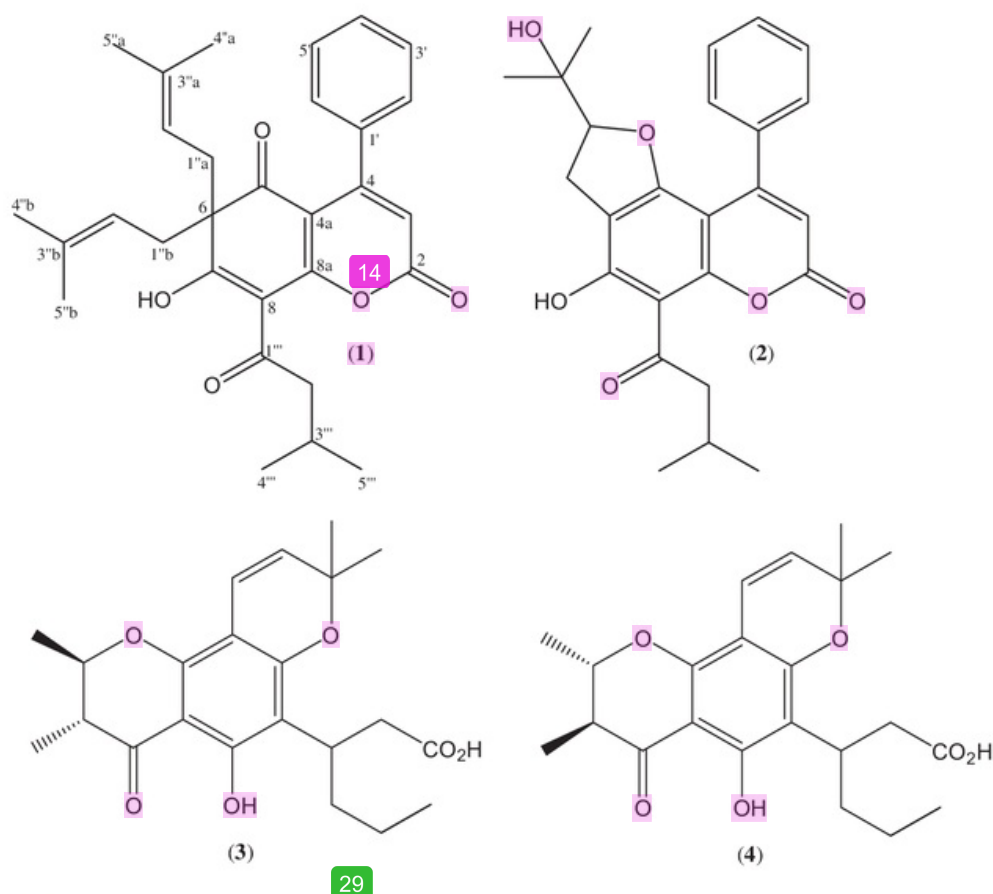


Figure 1. Compounds 1–4 isolated from the stem bark of *Mesua calophylloides*.

et al. 2016) and chromanone acids (Lim et al. 2015). In this article, we wish to report the isolation and structural elucidation of a new isoprenylated 4-phenylcoumarin, mesucalophylloidin (**1**) from the stem bark of *Mesua calophylloides*. The cytotoxic properties against murine leukaemia P-388 of isolated compounds from this plant are also reported.

2. Result and discussion

Phytochemical study on the ethyl acetate extract yielded two isoprenylated 4-phenylcoumarins, namely mesucalophylloidin (**1**), mamma A/BA cyclo F (**2**) (Awang et al. 2010), and chromanone acids, namely calolongic acid (**3**) and isocalolongic acid (**4**) (Lim et al. 2015) were isolated from the stem bark of *M. calophylloides*.

Mesucalophylloidin (**1**) was isolated as a yellow solid with a molecular formula of $C_{30}H_{33}O_5$ which was observed through HRESIMS $[M - H]^-$ ion at m/z 473.2339 (calcd. 473.2328). The UV maximum absorption at λ_{max} 236 (3.70), 296 (3.82) and 390 (3.90) nm supported the unusual type 4-phenylcoumarin chromophore (Karunakaran et al. 2013). The IR spectrum indicated absorptions for hydroxyl (3442 cm^{-1}), carbonyl (1728 and 1658 cm^{-1}) and aromatic (1606 and 1461 cm^{-1}) respectively.

The ^1H NMR spectrum showed the presence of singlet proton signal at δ_H 6.12 and two regions of multiplets phenyl group at δ_H 7.42 (3H) and δ_H 7.22 (2H) suggest that compound **1** is a typical for a 4-phenylcoumarin (Awang et al. 2010; Rouger et al. 2015). The proton

signals at δ_{H} 2.86 (4H, d, $J = 6.9$ Hz, H-1''a/1''b), 4.78 (2H, tm, $J = 7.2$ Hz, H-2''a/2''b), 1.56 (6H, s, H-4''a/4''b) and 1.55 (6H, s, H-4'''a/4'''b) indicated the presence of isoprenyl (3-methyl-2-butenyl) groups attached in the same carb⁷. Furthermore, the ^1H NMR spectrum also showed a 3-methylbutanoyl group at δ_{H} 2.90 (2H, d, $J = 7.8$ Hz, H-2'''), 2.07 (1H, m, H-3'''), 0.91 (6H, d, $J = 6.33$ Hz, H-4'''/5''') and a chelated hydroxyl group at δ_{H} 18.49 (Rouger et al. 2015).

The ^{13}C NMR spectrum of **1** showed signals of a modified coumarin nucleus (δ_{C} 203.2, 194.5, 169.0, 159.5, 156.3, 113.4, 115.2, 115.0, 54.2), a monosubstituted phenyl ring (δ_{C} 137.3, 129.0, 128.2, 127.0), two isoprenyl chains (δ_{C} 136.6, 116.6, 37.2, 26.0, 18.0; each 2C) and a 3-methyl-1-butanone chain (δ_{C} 205.6, 48.5, 26.3, 22.7). The COSY spectrum showed correlation between vinyl proton signal of isoprenyl group at 4.78 (H-2''a/2''b) with a methylene proton signal (δ_{H} 2.86; H-1''a/1''b) and two methyl proton signals (δ_{H} 1.56; H-4''a/4''b, δ_{H} 1.55; H-5''a/5''b). The COSY spectrum also showed correlation methine proton signal at 2.07 (H-3''') of 3-methylbutanoyl group with two methyl proton (δ_{H} 0.91; H-4'''/5'''). The placement of isoprenyl, 3-methylbutanoyl, carbonyl and hydroxy groups in 4-phenylcoumarin skeleton was established by HMQC and HMBC spectra. Long-range correlation was observed in HMBC spectrum of **1** between the proton signal at δ_{H} 6.12 (H-3) with an α -pyrone carbonyl carbon [δ_{C} 159.5 (C-2)] and two quaternary carbons [δ_{C} 137.3 (C-1'), 115.2 (C-4a)]. The methyne proton signal of phenyl at 7.22 (H-2'/6') showed correlations with a quaternary carbon (δ_{C} 156.3; C-4) and a methine carbon (δ_{C} 128.2; C-3'/5'). The methylene proton signal of isoprenyl group at 2.86 (H-1''a/1''b) showed long-range correlations with four quaternary carbons [δ_{C} 203.2 (C-5), 194.5 (C-7), 136.6 (C-3''a/3''b), 54.2 (C-6)] and a methine carbon [δ_{C} 116.6 (C-2''a/2''b)] showed that two isoprenyl attached at C-6. The presence of long-range correlations between the proton signal of a chelated⁹ hydroxyl group (δ_{H} 18.49, 7-OH) was correlated with four quaternary carbons [δ_{C} 205.6 (C-1'''); 194.5 (C-7), 113.4 (C-8); 54.2 (C-5)]. The presence of long-range correlations between the meth⁹ine proton signal (δ_{H} 2.90, H-2''') was correlated with four quaternary carbons [δ_{C} 205.6 (C-1'''); 194.5 (C-7), 113.4 (C-8); 54.2 (C-5)]. Furthermore, the proton signal of methylene (δ_{H} 2.90, H-2''') has correlation with a quaternary carbon [δ_{C} 205.6 (C-1''')], a methine carbon (δ_{C} 26.3, C-3''') and a gem dimethyl carbon (δ_{C} 22.7, C-4'''/C-5''') which showed that 3-methylbuta²⁶nyl group attached at C-8. Therefore, compound **1** was identified as 7-hydroxy-6,6-bis(3-methyl-2-butenyl)-8-(3-methylbutanoyl)-4-phenyl-chromene-2,5-dione and given the trivial name mesucalophylloidin.

The cytotoxic activity of compounds **1–4** were evaluated for their cytotoxicity using cell viability in murine leukaemia P-388 by MTT assay. These compounds exhibited IC_{50} values of 6.26 ± 0.4 , 59.10 ± 1.2 , 12.15 ± 0.6 and 10.45 ± 0.4 $\mu\text{g/mL}$, respectively. Those cytotoxic data suggested that compound **1** have moderate activity and compounds **2–4** were inactive. The cytotoxicity activity of isoprenylated 4-phenyl coumarin, compound **1** more than active compound **2**. Modification of coumarin structure of **1** in the ring B enhances activity. For chromanone acid, compound **4** slightly more than active compound **3**.

3. Experimental

3.1. General

UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-vis spectrophotometer (Kyoto, Japan). NMR spectra were measured on a JEOL JNM-ECA 400 MHz FTNMR

spectrophotometer (Tokyo, Japan) in CDCl_3 with TMS as the internal standard. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, $[\text{M}-\text{H}]^-$ negative ion mode (Santa Clara, CA, USA). Column chromatography and radial chromatography were carried out using silica gel 60 and silica gel 60 PF₂₅₄ (Merck, Darmstadt, Germany).

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3.2. Plant material

The stem bark of *M. calophylloides* was collected in Sungai Mendawak, anak Sungai Kapuas, District Kubu Raya, Kalimantan Indonesia on April 2015. The plant material was identified by Mr Ismail Rachman from the Herbarium Bogoriense, Bogor. A voucher specimen (PL 65795) was deposited in Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

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3.3. Extraction and isolation

The air-dried stem bark of *M. calophylloides* (2.0 kg) was successively twice (each for 48 h) by maceration in methanol, and then evaporated under reduced pressure to give a dark brown residue (150 g). The extract was redissolved in MeOH-water (9:1) and partitioned with *n*-hexane (101 g) and ethyl acetate (32 g) fractions. A part of ethyl acetate fraction (30 g) was subjected to vacuum liquid chromatography over silica gel eluted with *n*-hexane-ethyl acetate (from 9:1 to 3:7) to give fractions A-D. Fraction A was then subjected to column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 1:1) to produce sub-fractions A₁-A₃. Subfraction A₂ was purified by planar radial chromatography using *n*-hexane-acetone (from 9:1 to 4:1) to yielded compound **1** (8 mg). Fraction B was refracted using column chromatography and eluted *n*-hexane-chloroform (from 8:2 to 3:7) to give **3** (30 mg) and **4** (24 mg).

Fraction D was separated by column chromatography and eluted with *n*-hexane-ethyl acetate (from 4:1 to 1:1) to produce subfractions D₁-D₂. Subfraction D₁ was purified by planar radial chromatography using *n*-hexane-acetone (from 9:1 to 1:1) to yielded compound **2** (12 mg).

3.4. Spectral data

Mesucalophylloidin (**1**): yellow solid, m.p. 177–179 °C. UV/Vis (MeOH) λ_{maks} (nm) (log ϵ): 234 (3.94), 297 (3.90), and 334 (3.95). IR (KBr) ν_{max} (cm⁻¹): 3442, 2960, 2929, 2871, 1728, 1658, 1606, 1461 and 1286. ¹H NMR (400 MHz, CDCl_3) δ_{H} ppm: 6.12 (1H, s, H-3), 18.49 (1H, s, 7-OH), 7.42 (3H, m, H-3'/4'/5'), 3.12 (2H, m, H-2'/6'), 4.78 (1H, t, $J = 7.2$ Hz, H-2''a/H-2''b), 2.86 (15d, 6.9 Hz, H-1''a/H-1''b), 1.56 (6H, s, 20.4''a/H-4''b), 1.55 (6H, s, H-5''a/H-5''b), 2.90 (2H, d, 7.8 Hz, H-2''c), 0.07 (1H, m, H-3'''), 0.91 (6H, d, $J = 6.8$ Hz, H-4'''/5'''). ¹³C NMR (100 MHz, CDCl_3) δ_{C} ppm: 159.5 (C-2), 115.0 (C-3), 156.3 (C-4), 115.2 (C-4a), 203.2 (C-5), 54.2 (C-6), 194.5 (C-7), 113.4 (C-8), 169.0 (C-8a), 137.3 (C-1'), 127.0 (C-2'/6'), 128.2 (C-3'/5'), 129.0 (C-4'), 37.2 (C-1''a/C-21''b), 116.6 (C-2''a/C-2''b), 136.6 (C-3''a/C-3''b), 26.0 (C-4''a/C-4''b), 18.0 (C-5''a/C-5''b), 205.6 (C-1'''), 48.5 (C-2'''), 26.3 (C-3'''), 22.7 (C-4'''/5'''). HRESIMS: m/z $[\text{M}-\text{H}]^-$ calcd. for $\text{C}_{30}\text{H}_{33}\text{O}_5$ 473.2328, found 473.2339.

Mammea A/BA cyclo F (**2**): yellow solid. The ^1H and ^{13}C NMR spectral data are consistent with publish data (Awang et al. 2010).

Calolongic acid (**3**): yellow solid. The ^1H and ^{13}C NMR spectral data are consistent with publish data (Lim et al. 2015).

Isocalolongic acid (**4**): yellow solid. The ^1H and ^{13}C NMR spectral data are consistent with publish data (Lim et al. 2015).

3.5. Cytotoxic assay

Cytotoxic properties of the isolated compounds **1–4** against murine leukaemia P-388 cells was evaluated according to the MTT method as previously described (Tanjung et al. 2010; 2017). Artonin E was used as the positive control.

4. Conclusions

The phytochemical constituents of the stem bark of *Mesua calophylloides* (Ridl.) Kosterm. gave one new compound 4-phenylcoumarin, mesucalophylloidin (**1**) together with three known compounds, mammea A/BA cyclo F (**2**), calolongic acid (**3**) and isocalolongic acid (**4**). Compound **1** showed moderate activity against murine leukaemia P-388.

Supplementary material

HRESIMS, ^1H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra are reported in the supplementary materials as Figure S1–S7 and related to the following articles is available online.

Disclosure statement

No potential conflict of interest was reported by the authors.

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