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Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*

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Abstract – One new compound, macasiamenene V (**1**), and two known stilbenes (**2** - **3**) were isolated from *Macaranga inermis* Pax & K.Hoffm leaves. The structure of **1** was fully assigned based on the information on high-resolution MS and (1D, 2D) NMR spectra. The cytotoxic of compounds **1** - **3** was evaluated against 4T1 and HeLa cells. Compounds **2** - **3** showed high activity against HeLa cells with an IC₅₀ value of 1.09 and 0.88 µg/mL, respectively.

Keywords – Macasiamenene V, Stilbenoid, *Macaranga inermis*, Cytotoxicity

Introduction

The genus *Macaranga* (Euphorbiaceae) is one of the vanguard plants found in the damaged forest regions. Several species of *Macaranga* have used treatment for cancer, wounds, coughs, and diarrhea.¹⁻² The phenolic group reported previously on the *Macaranga* leaves exhibited stilbenoids and flavonoids.³⁻⁴ Piceatannol, resveratrol, and pinosylvin with terpenyl side chain are stilbene derivatives found in the *Macaranga*. Macasiamenenes A-U is resveratrol and piceatannol derivatives from *M. siamensis*, showing antioxidant and cytotoxic properties.⁵ Schweinfurthins A-Q, a stilbene-type analog from *M. schweinfurthin*, *M. tanarius*, *M. alnifolia* displayed potent toward leukemia cell (NCI 60) and lung cell (A549).⁶⁻⁸

Macaranga inermis Pax & K.Hoffm is one of the indigenous plants from Papua island, Indonesia. There is no information published on isoprenylated stilbene from *M. inermis*. Furthermore, we informed the isolation of a new isoprenyl resveratrol derivative, macasiamenene V (**1**), together with two known stilbenes derivatives, 2',6'-di-isoprenylresveratrol (**2**), and macasiamenene E (**3**) from *M. inermis* leaves. The cytotoxic of compounds **1** - **3** against breast cancer cells (4T1) and human cervical cells (HeLa) also reported.

Experimental

General experimental procedures – The maximum absorption (λ_{max}) of each compound was measured by the UV-VIS spectrophotometer (UV-1800-Shimadzu). The functional groups of compounds **1** - **3** were recorded by the FTIR spectrophotometer (IR Tracer-100- Shimadzu). The NMR spectra of compounds were measured on an FTNMR ECA 400 spectrometer (JEOL) in acetone-*d*₆. The high-resolution MS of isolated was determined by an LCT Premier™ XE (Waters) mass spectrometer. Si gel G₆₀ and Sephadex LH-20 undertook column chromatography (CC). The visualization of compounds on TLC using UV lamp and cerium sulfate reagent.

Plant materials – The collecting of *M. inermis* leaves come from Tomage Village, Fakfak, West Papua, Indonesia, in December 2018. The plant material with receipt specimens (FFK-IS9) was identified by Ismail R., Herbarium Bogoriense, Bogor, Indonesia.

Extraction and isolation – The extraction at room temperature of the powdered *M. inermis* leaves (2.0 kg) using MeOH for three days carried three times. The MeOH extract was added with water (composition 9:1 v/v) and then partitioned with hexane and EtOAc. The separation of EtOAc extract (13 g) by silica gel CC, eluting by mobile phase (hexane, hexane-EtOAc, EtOAc) with increasing polarity afforded seven fractions (A-G). The separation of fraction F (2.45 g) by Sephadex LH-20 CC with methanol as mobile phase afforded fractions F₁-F₄. The purification of fraction F₃ (735 mg) by silica gel planar radial chromatography, eluting by mobile phase (hexane, hexane- diisopropyl ether) afforded **1** (5 mg), **2**

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(31 mg) and **3** (14 mg).

Macasiamenene V (1) – Colorless oil, UV (MeOH) λ_{\max} nm (log ϵ): 215 (4.54), and 274 nm (3.79). IR (KBr) ν_{\max} cm^{-1} : 3413, 1562, and 1476. The NMR spectral data see Table 1. HRESIMS: m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{24}\text{H}_{29}\text{O}_4$ 381.2064, found 381.2066.

2',6'-Di-isoprenylresveratrol (2) – Light yellow solid, UV (MeOH) λ_{\max} nm (log ϵ): 215 (4.57), and 277 nm (3.81). The comparison of the NMR spectra of **2** very identically to the literature data.⁵

Macasiamenene E (3) – White solid, UV (MeOH) λ_{\max} nm (log ϵ): 210 (4.48), and 274 nm (3.89). The comparison of the NMR spectra of **3** very identically to the literature data.⁵

Cytotoxic activity – The cytotoxic activity of **1-3** against human cervical cells (HeLa) and human breast cells (4T1) were assessed by the MTT assay according to the experiment previously.⁹⁻¹¹ HeLa and 4T1 cells were cultured in the RPMI-1640 medium containing 10% FBS at 37 °C flowed with 5% CO_2 for 48 h. The HeLa and 4T1 cells were added compounds **1-3** in the 96-well and incubated at 37 °C flowed with 5% CO_2 for 24 h. The

active compound's ability to kill cancer cells was evaluated by the microplate reader spectrometer at λ 590 nm. Doxorubicin, using as the positive control for the cytotoxic assay.⁹⁻¹¹

Result and Discussion

Compound 1 (macasiamenene V) was isolated as a light yellow oil, showing the chemical formula $\text{C}_{24}\text{H}_{29}\text{O}_4$ by high-resolution MS at ion peak $[\text{M}+\text{H}]^+$ at m/z 381.2064 (calcd 381.2066). The maximum absorption of **1** at λ_{\max} (log ϵ): 215 (4.54), and 274 nm (3.79) characteristic for resveratrol skeleton by the UV spectra.⁵ The functional group of **1** consists of a hydroxyl group (3413 cm^{-1}) and aromatic C=C (1476 and 1562 cm^{-1}) by the IR spectra.¹ The ^1H NMR (Table 1) exhibited conformities for three aromatic protons, a set of *ortho*-coupled of 1,4 disubstituted benzene at δ_{H} 6.93 (2H, d, $J=8.7$ Hz, H-2/6) and 6.61 (2H, d, $J=8.7$ Hz, H-3/5) at ring A, and an isolated aromatic proton at δ_{H} 6.30 (1H, s, H-4') at ring B. A pair of a *cis*-olefinic proton at δ_{H} 6.61 (1H, d, $J=13.1$ Hz, H- α) and δ_{H} 6.27 (1H, d, $J=13.1$ Hz, H- β), connecting

Table 1. NMR data (400 MHz, acetone- d_6) of macasiamenene V (**1**)

No.C	δ_{H} (mult, J in Hz)	δ_{C}	HMBC
1	-	130.1	-
2/6	6.93 (d, 8.7)	130.5	C-2/6; C-4
3/5	6.61 (d, 8.7)	115.7	C-1; C-4
4	-	157.7	-
α	6.60 (d, 13.1)	131.7	C-2/6; C-1'
β	6.27 (d, 13.1)	125.9	C-1, C-2', C-6'
1'	-	139.4	-
2'	-	118.9	-
3'	-	155.1	-
4'	6.30 (s)	103.1	C-2', C-3', C-5', C-6'
5'	-	152.8	-
6'	-	110.5	-
1''	3.24 (d, 7.2)	26.5	C-1', C-2', C-3', C-2'', C-3''
2''	5.08 (t, 7.0)	124.5	C-4', C-5''
3''	-	129.9	-
4''	1.49 (s)	25.8	C-2'', C-3'', C-5''
5''	1.64 (s)	18.0	C-2'', C-3'', C-4''
1'''	-	-	-
2'''	-	77.2	-
3'''	3.53 (dd, 4.7; 10.1)	70.9	C-4'''
4'''	2.75 (d, 6.0) 2.70 (d, 6.0)	30.7	C-1', C-5', C-6', C-2'', C-3''
5'''	1.13 (s)	25.3	C-2''', C-3''', C-6'''
6'''	0.93 (s)	18.4	C-2''', C-3''', C-5'''

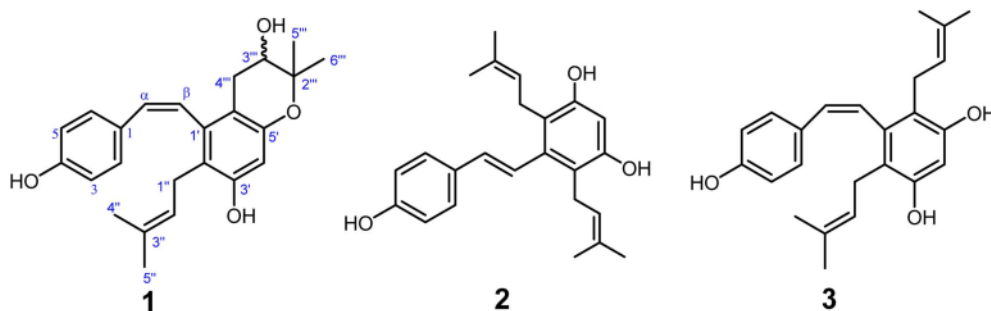


Fig. 1. Isoprenylated stilbenes (1 - 3) from *M. inermis* leaves.

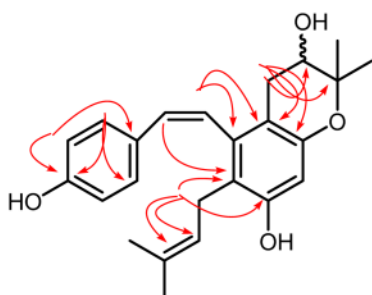


Fig. 2. HMBC corrections of macasiamenene V (1).

against two-unit aromatics showed that (*Z*)-stilbene skeleton.⁵ The presence of isoprenyl chain consists of two methyl protons [δ_{H} 1.49 (3H, s, H-4''), δ_{H} 1.64 (3H, s, H-5'')], a methylene proton at δ_{H} 3.24 (2H, d, $J = 7.2$ Hz, H-1''), and a vinylic proton at δ_{H} 5.08 (1H, t, $J = 7.2$ Hz, H-2''). The ^1H NMR of **1** also observed the presence of a 2,2-dimethyl-3-hydroxy-3,4-dihydro-2H-pyran ring consists of two methyl protons [δ_{H} 0.93 (3H, s, H-6'''), δ_{H} 1.13 (3H, s, H-5''')], methylene split proton at δ_{H} 2.75 (1H, d, $J = 6.0$ Hz, H-4a'''), δ_{H} 2.70 (1H, d, $J = 6.0$ Hz, H-4b'''), and a methine of alcohol at δ_{H} 3.53 (1H, dd, $J = 4.7$ and 10.1 Hz, H-3'''). The ^{13}C NMR (Table 1), showing twenty-four signals consistent with the total carbon. Three oxyaryl carbons of total carbon of **1** (δ_{C} 152.8, δ_{C} 155.1, and δ_{C} 157.7) recommended a resveratrol derivative. The HMBC spectrum established the isoprenyl and pyran ring location

in the resveratrol skeleton (Fig. 2). The HMBC spectrum described the isoprenyl and pyran ring location in the resveratrol skeleton. The HMBC spectrum, correlations of two the symmetric aromatic signals at δ_{H} 6.93 (H-2/6) and δ_{H} 6.61 (H-3/5) to an oxyaryl carbon at δ_{C} 157.7 (C-4) indicated a 1,4 disubstituted benzene at ring A. The correlation results indicated that the isoprenyl chain and the pyran ring bounded to the resveratrol structure's B ring. An olefinic at δ_{H} 6.61 (H- α), correlations to C-2/6 (δ_{C} 130.5), C-1' (δ_{C} 139.4), and other olefinic at δ_{H} 6.27 (H- β) correlated to C-1 (δ_{C} 130.1), C-2' (δ_{C} 118.9), C-6' (δ_{C} 110.5). These correlations indicated an isoprenyl chain, and the pyran ring bounded at the B ring. The methylene proton (a part of the isoprenyl chain) on δ_{H} 3.24 (H-1'') related to C-1', C-2', C-3' (δ_{C} 155.1), C-2'' (δ_{C} 124.5), and C-3'' (δ_{C} 129.9). Two methyl signals at δ_{H} 1.49 (H-4'') and δ_{H} 1.64 (H-5'') of the isoprenyl chain also described relations to C-2'' and C-3''. These correlations indicated that the isoprenyl chain attached at C-2'. A part of the pyran ring, the methylene split proton on δ_{H} 2.76 (H-4a'''), and 2.70 (H-4b''') connections to C-1', C-5' (δ_{C} 152.8), C-6', C-2''' (δ_{C} 77.2), C-3''' (δ_{C} 70.9). Another part of the pyran ring, two methyl protons at δ_{H} 0.93 (H-6'''), δ_{H} 1.13 (H-5''') connected to C-2''', and C-3'''. These connections indicated that the pyran ring is a 2,2-dimethyl-3-hydroxy-3,4-dihydro-2H-pyran ring fused at C-5' and C-6'. Therefore, the structure of **1** is described in Fig. 1, and namely as macasiamenene V.

Table 2. Cytotoxicity data of compounds 1 - 3

Compounds	IC ₅₀ ($\mu\text{g}/\text{mL}$)	
	HeLa	4T1
Macasiamenene V (1)	> 100	> 100
2',6'-Di-isoprenylresveratrol (2)	1.09 \pm 0.14	8.16 \pm 0.21
Macasiamenene E (3)	0.88 \pm 0.11	> 100
Doxorubicin	45.99 \pm 0.23	36.10 \pm 0.43

The cytotoxic activities of compounds **1-3** were assessed towards HeLa and 4T1 cells using MTT assay.¹²⁻¹³ Compounds **2-3** exhibited the highest activity towards HeLa (IC₅₀ = 1.09 and 0.88 µg/mL, respectively). However, Compound **3** was inactive towards 4T1 cells (Table 2). Compound **1** was inactive towards both of HeLa and 4T1. In terms of structure, compounds **2-3** are geometric isomers. Compound **2** has stereochemistry of the *trans* 2',6'-di-isoprenylresveratrol while compound **3** stereochemistry of the *cis* 2',6'-di-isoprenylresveratrol. The 2',6'-di-isoprenylresveratrol structure in the *cis* form showed higher activity than those in the *trans*. The cyclization of **3** afforded compound **1** with decreased cytotoxic activity.

Acknowledgments

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