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Flavestin K, An Isoprenylated Stilbene from the Leaves of *Macaranga recurvata* Gage

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Abstract – A new isoprenylatedstilbene, flavestinK (**1**) together with two known isoprenylatedstilbenes, flavestin B (**2**), flavestin G (**3**), and two isoprenilated flavanones, 4-*O*-methyl-8-isoprenylnaringenin (**4**) and 8-isoprenyl-5,7-dihydroxyflavanone (**5**) were isolated from the leaves of *Macaranga recurvata* Gage. All of the structures have been determined based on HRESIMS, 1D and 2D NMR spectral data. All of the isolated compounds were evaluated for their cytotoxicity against three human cancer cells (HeLa, T47D and WiDr). Compound **1** showed higher activity than doxorubicin against HeLa cells with IC_{50} value of 13.1 μg/mL.
Keywords – Flavestin K, Stilbene, *Macaranga recurvata*, Cytotoxicity

Introduction

Macaranga recurvata Gage (Euphorbiaceae), locally known as 'Mahang merah' is one pioneer plant and found endemic in Kalimantan Island, Indonesia. The genus *Macaranga* have been showed a number of phenolic compounds, predominantly flavonoids and stilbenes with terpenylated side chain (isoprenyl, geranyl and farnesyl) in aromatic ring.[1-4] Based on previously report, two isoprenylated dihydroflavonols, macarecurvatins A and B from the leaves of *M. recurvata* showed cytotoxicities against murine leukemia.[5] Isoprenylation of flavonoids and stilbenes seems to be a key factor to enhance their cytotoxicity.

In this research paper, we desiderate to report the isolation of a new isoprenylated stilbene, flavestin K (**1**) along with four known compounds, flavestin B (**2**), flavestin G (**3**), 4′-*O*-methyl-8-isoprenylnaringenin (**4**) and 8-isoprenyl-5,7-dihydroxyflavanone (**5**) from the leaves of *M. recurvata* (Fig. 1). The cytotoxic activities of compounds **1-5** against three human cancer cells (HeLa, T47D and WiDr) are also reported.

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Experimental

General experimental procedures – 1D NMR (^{1}H and ^{13}C), 2D NMR (HMQC and HMBC) spectra were recorded with a JEOL JNM-ECA 400 FT NMR spectrometer operating at 400 MHz usingdeuterated solvent (peaks: δ_H 2.04 and δ_C 29.8 for acetone-d_6 as reference standard). High resolution mass spectra were measured on an ESI-TOF Waters LCT Premier X Emass spectrometer. All of compounds were dissolved in methanol and were measured by UV spectrophotometer Shimadzu 1900. The functional group of compounds in KBr were measured by IR Tracer-100 Shimadzu FT IR spectrophotometer. Column chromatography (CC) was performed using Si gel 60 G and centrifugal planar chromatography (CPC) was performed using Si gel 60 PF_{254}. TLC analysis was performed using on pre-coated Si gel 60 GF_{254} 0.25 mm thickness plates.

Plant materials – The leaves of *M. recurvata* were collected from Muara Teweh, North Barito Districts, East Kalimantan, Indonesia on Feb. 2018, and identified by senior botanist Mr. Ismail Rachman from the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and isolation – The air-dried and powdered leaves of *M. recurvata* (3.5 kg) were extracted with MeOH (2 × 15 L) at room temperature for two days, and

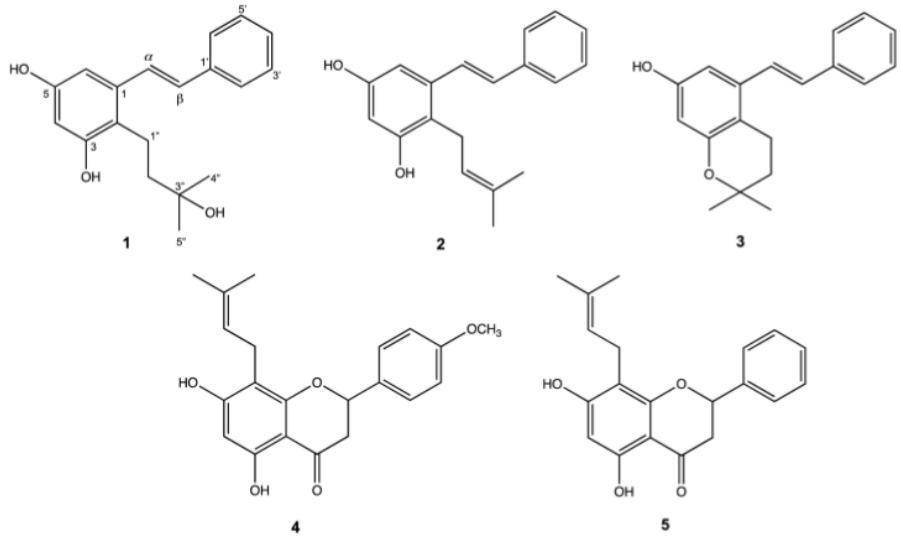

Fig. 1. Stilbenes and flavonoids **1 - 5** isolated from the leaves of *M. recurvata*.

after evaporation gave the viscous concentrated of MeOH extract (210 g). The suspended solids was redissolved in MeOH-H_2O (9:1) and then partitioned with C_6H_{14} (45 g) and EtOAc (22 g), successively.

The EtOAc extract (20 g) was further fractionated by column chromatography on silica gel (800 g) eluted with C_6H_{14}-EtOAc (from 9:1 to 1:1) by gradient elutionto give five major fractions A-E. Fraction B (2.10 g) was separated by sephadex LH-20 eluted with MeOH gave subfractions B_1-B_2. Subfraction B_2 was purified by centrifugal planar chromatography using C_6H_{14}-$CHCl_3$ (from 7:3 to 3:7) to yield compound **4** (30 mg), and **5** (19 mg). Fraction C (2.8 g) was subjected to sephadex LH-20 and eluted with MeOH gave three subfractions C_1-C_3. Subfraction C_1 was purified by CPC using C_6H_{14}-EtOAc (from 9:1 to 4:1) gave compound **2** (40 mg) and purification of subfraction C_2 by the same methods using *n*-hexane-diisopropylether (from 1:1 to 1:4) to yield compound **1** (5 mg), and **3** (21 mg).

Flavestin K (**1**) – Yellow solid, UV (MeOH) λ_{max} nm (log ε): 212 (4.49), and 293 (4.26). IR (KBr) νcm^{-1}: 3400, 1604, 1521, 1438 and 1033. ^1H and ^{13}C NMR see Table 1. HRESIMS: *m/z* $[M\text{-}H]^-$ calcd. for $C_{19}H_{21}O_3$ 297.1491, found 297.1489.

Flavestin B (**2**) – Amorphous powder, UV (MeOH) λ_{max} nm (log ε) : 215 (4.39), and 303 (4.10). IR (KBr) νcm^{-1}: 3368, 1608, 1530, 1463 and 1028. HRESIMS: *m/z* $[M\text{-}H]^-$ calcd. for $C_{19}H_{19}O_2$ 279.1523, found 279.1519. The ^1H and ^{13}C NMR spectral data were compared and consistent with the published data.[6]

Flavestin G (3) – Amorphous powder, UV (MeOH) λ_{max} nm (log ε): 214 (4.42), and 295 (4.17). IR (KBr) νcm^{-1}: 3340, 1602, 1548, 1459 and 1033. HRESIMS: *m/z* $[M\text{-}H]^-$ calcd. for $C_{19}H_{19}O_2$ 279.1489, found 279.1480. The ^1H and ^{13}C NMR spectral data were compared and consistent with the published data.[6]

4′-*O*-Methyl-8-isoprenylnaringenin (**4**) – White solid, m.p. 169 - 170 °C. UV (MeOH) λ_{max} nm (log ε): 214 (4.46), and 293 (4.67). IR (KBr) νcm^{-1}: 3425, 1640, 1515, 1448 and 1170. HRESIMS: *m/z* $[M\text{-}H]^-$ calcd. for $C_{21}H_{21}O_5$ 353.1457, found 353,1452. The ^1H and ^{13}C NMR spectral data were compared and consistent with the published data.[7]

8-Isoprenyl-5,7-dihydroxyflavanone (**5**) – White solid, m.p. 165 - 167 °C. UV (MeOH) λ_{max} nm (log ε) : 224 (4.49), and 294 (4.73). IR (KBr) νcm^{-1}: 3418, 1638, 1520, 1446 and 1168. HRESIMS: *m/z* $[M\text{-}H]^-$ calcd. for $C_{20}H_{19}O_4$ 323.1362, found 323.1365. The ^1H and ^{13}C NMR spectral data were compared and consistent with published data.[8]

Cytotoxic activity – Compounds (**1 - 5**) were appraised for their cytotoxicity toward HeLa (human cervical carcinoma), T47D (human breast cancer), and WiDr (human colon carcinoma) according to the MTT method as well as doxorubicin as the positive control.[9-12] Briefly, before the compounds were added, cells were cultured in 96-well at a density of 3×10^4 cells/well and incubated at 37 °C for 24 h. Compounds **1 - 5** with variations in concentration (100, 30, 10, 3, 1, 0.3, and 0.1 μg/mL) with triplicate were

Table 1. NMR data of flavestin K (**1**)

No. C	δ_H (mult, *J* in Hz)	δ_C	HMBC (H⇔C)
1	-	138.8	-
2	-	120.2	-
3	-	156.9	-
4	6.35 (*d*, 2.4)	103.2	C-2; C-3; C-6
5	-	156.7	
6	6.69 (*d*, 2.4)	104.4	C-α; C-2; C-4; C-5
α	7.52 (*d*, 16.1)	127.5	C-2; C-6; C-1'
β	6.98 (*d*, 16.1)	130.2	C-1; C-2'/6'
1'	-	138.2	-
2'/6'	7.57 (*m*)	127.3	C-β; C-4'
3'/5'	7.34 (*m*)	129.5	C-1'; C-3'/5'
4'	7.24 (*m*)	128.2	C-3'/5'
1"	2.83 (*m*)	20.7	C-1; C-2; C-3; C-2"; C-3"
2"	1.66 (*m*)	45.0	C-2; C-1"; C-3"; C-4"; C-5"
3"	-	70.5	-
4"	1.25 (*s*)	29.9	C-2"; C-3"; C-5"
5"	1.25 (*s*)	29.9	C-2"; C-3"; C-4"
3-OH	8.19 (*s*)	-	C-2; C-3; C-4
5-OH	7.99 (*s*)	-	C-5; C-6
3"-OH	3.58 (*s*)	-	C-2"; C-3"; C-4"; C-5"

added to each well and incubated at 37 °C for 48 h. After incubation, it was added MTT and let for 4 h, and the inhibition of cells by each of compounds **1** - **5** were recorded with microplate reader spectrophotometer at λ 570 nm. The IC_{50} values of all compounds were calculated by regression analysis.

Result and Discussion

Flavestin K (**1**) obtained as yellow solid, and the chemical formula $C_{19}H_{22}O_3$ was deduced by HRESIMS spectra with ion peak $[M-H]^-$ at *m/z* 297.1489 (calcd. for 297.1491). The UV spectrum showed two maximum absorption at λ_{max} (log ε) 212 (4.49), and 293 (4.26) nm characteristic for a stilbene chromophore.[13] The IR spectrum displayed absorption band for hydroxyl (3400 cm^{-1}), and aromatic ring (1521 - 1438 cm^{-1}), respectively. The ^1H NMR spectrum of compound **1** (Table 1) exhibited two doublets (J = 16.1 Hz) at δ_H 7.52 (H-α) and δ_H 6.98 (H-β) provided the evidence for a *trans* 1,2-disubstituted ethene connecting with two aromatic rings, revealed that compound **1** was (*E*)-stilbene.[14] The existence of *meta*-coupling doublets (J = 2.4 Hz) at δ_H 6.35 (H-4) and δ_H 6.69 (H-6) is characteristic for a 1,2,3,5-tetrasubstituted benzene. The presence of three multiplet signals at δ_H 7.57 (H-2'/H-6'), δ_H 7.34 (H-3'/H-5') and δ_H 7.24 (H-4') is consistent for a monosubstituted benzene. ^1H NMR spectrum of **1** also disclosed information two phenolic hydroxyl groups at δ_H 8.19 (3-OH), and δ_H 7.99 (5-OH). The proton signals for a 2-methylbutan-2-ol unit were observed at δ_H 3.58 (3"-OH), 2.83 (H_2-1"), 1.66 (H_2-2") and 1.25 (H_3-4" and H_3-5") in the ^1H NMR spectrum. Sixteen carbon signals were observed in ^{13}C NMR spectrum. The carbon NMR signals including two oxyaryl carbon signals (δ_C 156.9 and δ_C 156.7) recommended that compound **1** has a structure of pinosylvin (*E*-3,5-dihydroxystilbene) bearing a 2-methylbutan-2-ol group.[15] Location of a 2-methylbutyl-2-ol group in pinosylvin structure was established by 2D NMR spectra (HMQC and HMBC) (Fig. 2). The H-α proton

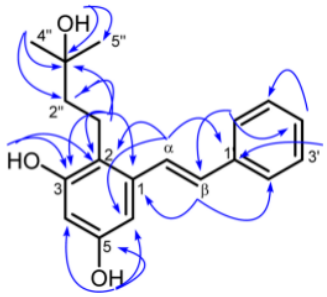

Fig. 2. Selected HMBC correlations for flavestin K (**1**).

Table 2. Cytotoxicity of compounds **1** - **5** of *M. recurvata*

Compounds	IC_{50} (μg/mL)		
	HeLa	T47D	WiDr
Flavestin K (**1**)	13.10 ± 0.87	51.12 ± 1.21	46.41 ± 1.25
Flavestin B (**2**)	54.80 ± 1.15	>100	>100
Flavestin G (**3**)	55.84 ± 2.68	73.04 ± 2.71	70.35 ± 2.58
4'-*O*-Methyl-8-isoprenylnaringenin (**4**)	>100	>100	>100
8-Isoprenyl-5,7-dihydroxyflavanone (**5**)	>100	>100	>100
Doxorubicin	46.11 ± 0.45	23.18 ± 0.45	12.44 ± 0.45

(δ_H 7.52) of the ethene group correlated with a methine carbon at δ_C 104.4 (C-6) and two quaternary carbons at δ_C 120.2 (C-2) and at δ_C 138.2 (C-1′) in the HMBC spectrum of **1**. The HMBC correlations of the methylene proton signal at δ_H 2.83 (H-1") to δ_C 138.8 (C-1), 120.2 (C-2), 156.9 (C-3), 45.0 (C-2"), and 70.5 (C-3") indicated that a 2-methylbutyl-2-ol chain was located at C-2. The presence of 2-methylbutan-2-ol chain was further supported by HMBC correlations between the hydroxyl proton signal at δ_H 3.58 (3"-OH) and the carbon signals at δ_C 29.9 (C-4" and C-5"), 45.0 (C-2") and 70.5 (C-3"). The structure of compound **1** was thus identified as 2-(3-hydroxy-3-methylbutyl)-pinosylvin. We designated the compound **1** as flavestin K.

The cytotoxicity activityof compounds **1** - **5** were appraised towards HeLa (human cervical carcinoma), T47D (human breast cancer), and WiDr (human colon carcinoma) by MTT method as well as doxorubicin (positive control).[16] Isoprenylated stilbenes (**1**, **2** and **3**) more active than isoprenylated flavonoids (**4** and **5**) (Table 2). Compound **1** exhibited higher activity than doxorubicin against HeLa with IC_{50} value of 13.1 μg/mL. However, compounds **4** - **5** were inactive against HeLa, T47D, and WiDr cells.

Acknowledgments

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