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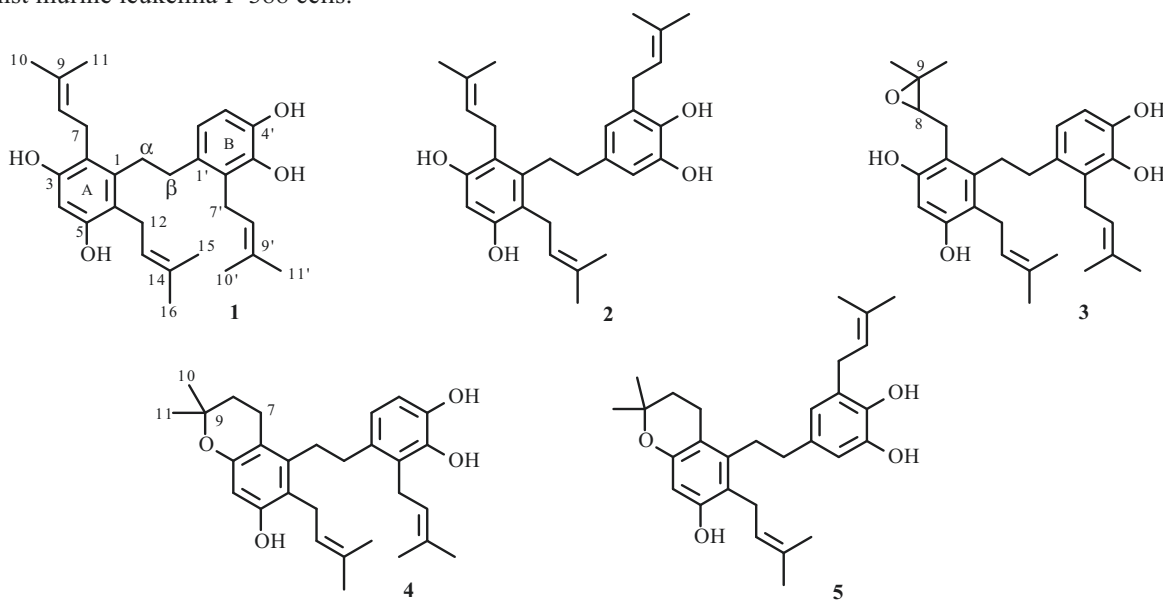
PRENYLATED DIHYDROSTILBENES FROM *Macaranga rubiginosa*

Mulyadi Tanjung,¹ Euis H. Hakim,² and Yana M. Syah^{2*}

Phytochemical isolation of the methanol extract of Macaranga rubiginosa leaves afforded five prenylated dihydrostilbenes. Two of them were known dihydrostilbenes laevifolins A (1) and B (2), while the other three were new compounds, trivially named macarubiginosins A–C (3–5). The structures of the new compounds were elucidated based on their UV, 1D and 2D NMR, and HR-ESI-MS spectral data. Compounds 1–5 were tested for their cytotoxicity against P-388 cells, showing that compound 1 was the most active with IC₅₀ 4.3 μM.

Keywords: macarubiginosins A–C, laevifolins A and B, prenylated dihydrostilbenes, *Macaranga rubiginosa*, Euphorbiaceae, cytotoxicity, P-388 cells.

Macaranga is a large genus of Euphorbiaceae (ca. 260 species) distributed in the tropical region of Africa, India, South East Asia, and Pacific islands [1]. The species of this genus are important plants found in the secondary forests of Kalimantan and Sumatra Islands of Indonesia [2, 3]. Our interest in the phytochemistry of *Macaranga* species is motivated by the fact that they contain flavonoid and stilbene derivatives bearing various terpenyl groups, including prenyl, geranyl, farnesyl, and labdanyl groups [4]. Recently, we reported our phytochemical investigation of some *Macaranga* plants growing in Kalimantan Island [5–9]. As further work on Indonesian *Macaranga*, in this paper we report the presence of prenylated derivatives of dihydrostilbenes, trivially named macarubiginosins A–C (3–5), from the leaf extract of *Macaranga rubiginosa* Ridl. collected from Sumatra Island. Along with these compounds, two known prenylated dihydrostilbens, laevifolins A and B (1, 2), were also isolated. As a preliminary biological test, this paper also briefly describes the cytotoxic properties of compounds 1–5 against murine leukemia P-388 cells.



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TABLE 1. NMR Data of Compounds 3–5 (δ , ppm, J/Hz)

C atom	δ_H			δ_C		
	3 ^a	4 ^b	5 ^b	3	4	5
1	–	–	–	140.8	139.3	139.5
2	–	–	–	119.7	117.7	112.0
3	–	–	–	155.1	153.7	153.2
4	6.21 (s)	6.22 (s)	6.21 (s)	102.3	102.7	102.9
5	–	–	–	152.6	153.1	153.8
6	–	–	–	110.9	111.9	117.7
7	2.87 (dd, J = 16.0, 5.7) 2.51 (dd, J = 16.0, 8.3)	2.55 (t, J = 6.8)	2.60 (t, J = 6.8)	29.9	20.1	20.3
8	3.70 (dd, J = 8.3, 5.7)	1.73 (t, J = 6.8)	1.76 (t, J = 6.8)	70.7	33.0	35.8
9	–	–	–	76.6	73.2	73.4
10	1.15 (s)	1.27 (s)	1.29 (s)	19.9	26.6	26.8
11	1.29 (s)	1.27 (s)	1.29 (s)	25.4	26.6	26.8
12	3.33 (d, J = 6.8)	3.31 (d, J = 6.7)	3.34 (d, J = 6.7)	25.5	25.8	25.5
13	5.09 (br.t, J = 6.8)	5.10 (br.t, J = 6.7)	5.14 (br.t, J = 6.7)	125.8	123.3	123.3
14	–	–	–	130.2	133.6	133.5
15	1.73 (br.s)	1.74 (br.s)	1.78 (br.s)	18.1	17.9	18.1
16	1.62 (br.s)	1.70 (br.s)	1.70 (br.s)	25.8	25.7	25.9
α	2.76 (m)	2.79 (m)	2.80 (m)	31.5	31.1	31.8
β	2.67 (m)	2.70 (m)	2.62 (m)	33.3	33.2	33.4
1'	–	–	–	133.0	132.2	136.8
2'	–	–	6.63 (d, J = 2.4)	127.3	125.8	113.3
3'	–	–	–	144.1	142.4	144.4
4'	–	–	–	143.2	142.2	140.2
5'	6.65 (d, J = 8.6)	6.72 (d, J = 8.0)	–	113.3	112.8	121.3
6'	6.36 (d, J = 8.6)	6.66 (d, J = 8.0)	6.48 (d, J = 2.4)	120.2	121.3	122.0
7'	3.39 (d, J = 6.8)	3.34 (d, J = 6.7)	3.33 (d, J = 6.7)	26.1	25.3	30.2
8'	5.09 (br.t, J = 6.8)	5.10 (br.t, J = 6.7)	5.14 (br.t, J = 6.7)	124.6	121.1	122.1
9'	–	–	–	131.2	134.8	134.7
10'	1.75 (br.s)	1.78 (br.s)	1.82 (br.s)	18.2	18.0	18.2
11'	1.64 (br.s)	1.71 (br.s)	1.80 (br.s)	25.8	25.6	26.0

^aIn acetone- d_6 ; ^bin $CDCl_3$.

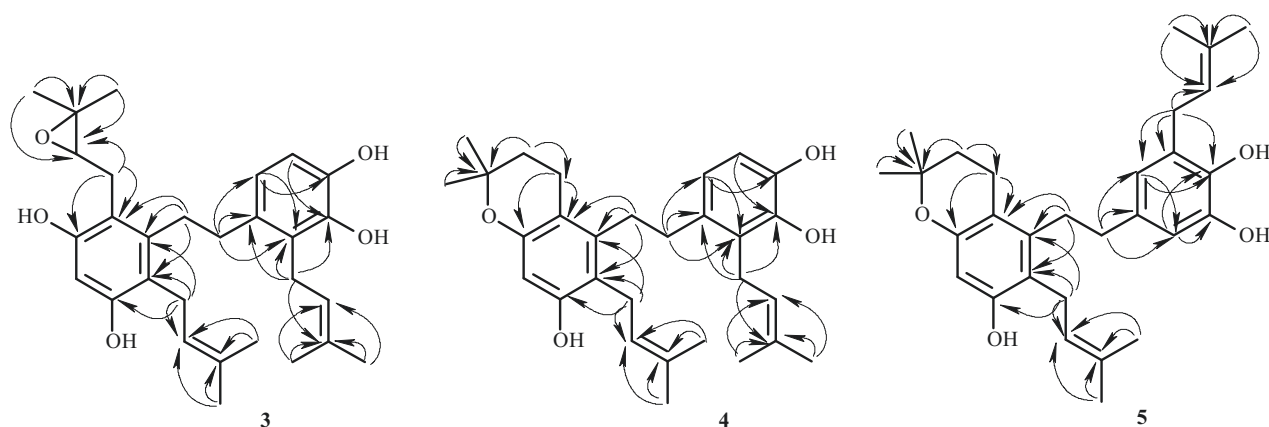


Fig. 1. Selected important HMBC correlations of compounds 3–5.

Compounds **1** and **2** were determined to have the structures of laevofilins A and B, respectively, based on comparison of their NMR spectral data with those reported in the literature [10].

Compound **3** (macarubiginosin A) was isolated as a pale yellowish semisolid, and its UV absorption characteristics were very close to those **1** and **2** (see Experimental). Based on high resolution electrospray ionization mass spectrometry (HR-ESI-MS) data, **3** has the molecular formula $C_{29}H_{38}O_5$ (found $[M - H]^-$ at m/z 465.2643, calcd 465.2641), suggesting that

it could be a monooxygenated derivative of either **1** or **2**. The ^{13}C NMR spectrum of **3** (Table 1), however, showed 29 carbon signals, 16 of which were sp^2 -carbon atoms, indicating that the symmetry of ring A in **1** or **2** is lost in **3**. The ^1H NMR spectrum (Table 1) disclosed two mutually coupled methylene signals (δ 2.76 and 2.67) for C- α and C- β , and signals characteristics for only two prenyl groups (δ 3.39 and 3.33, each 2H; 5.09, 2H; 1.75, 1.73, 1.64, and 1.62, each 3H). Accordingly, the third prenyl group in **3** should be oxygenated to form a 3-methyl-2,3-epoxybutyl group (δ 3.70, 2.87, and 2.51, each 1H and dd; 1.29 and 1.15, each 3H and s; δ_{C} 19.9, 25.4, 29.9, 70.7, and 76.6). The aromatic region of the ^1H NMR spectrum showed a singlet at δ 6.21 and a pair of *ortho*-coupled doublets at δ 6.65 and 6.36. From these NMR data, structure **3** was assigned to macarubiginosin A (**1**) as an epoxy derivative of laevofilin A. HSQC and HMBC spectra of **3** were consistent with the structure of macarubiginosin A, and the important HMBC correlations are as shown in Fig. 1. The stereochemistry of the epoxy group in **3** was not determined.

Compounds **4** (macarubiginosin B) and **5** (macarubiginosin C) have the same molecular formula, namely $\text{C}_{29}\text{H}_{38}\text{O}_4$, as determined by their HR-ESI-MS data (see Experimental), suggesting that these compounds are isomers of either laevofilins A (**4**) or B (**5**). Looking at their aromatic signals in their ^1H NMR spectra (Table 1), **4** contained a pair of *ortho*-coupled doublets (δ 6.72 and 6.66), while **5** had a pair of *meta*-coupled doublets (δ 6.63 and 6.48), in addition to singlet signals at δ 6.22 and 6.21, respectively. Therefore, **4** and **5** must have the same arrangements in the aromatic parts as those of **1** and **2**. Further spectral analysis disclosed that the ^1H and ^{13}C NMR data of **4** and **5** were very similar to those of **3**, except that the signals of the 3-methyl-2,3-epoxybutyl group in **3** were replaced by signals characteristic of a dihydrodimethylpyran ring in **4** and **5** (see Table 1). Because the ^1H and ^{13}C NMR spectra of **4** and **5** indicated no symmetry in the molecules, the dimethylpyran ring should be in ring A of **4** and **5**. Therefore, structures **4** and **5** can be assigned to macarubiginosins B and C, respectively. The HMBC correlations found in both compounds, as shown in Fig. 1, confirmed the structural assignments for these two compounds. In addition, the NMR chemical shifts and coupling constants of the dihydrodimethylpyran ring in **4** and **5** were in agreement with those reported in the literature [11, 12].

Preliminary cytotoxic evaluation of compounds **1–5** against murine leukemia P-388 was carried out by the method previously described [13]. These compounds exhibited IC_{50} values of 4.3 ± 0.6 , 12.3 ± 0.6 , 10.8 ± 3.0 , 52.9 ± 3.4 , and 45.8 ± 2.6 μM , respectively. Modification of one of the prenyl groups in ring A of **1** or **2** to an epoxy (compound **3**) or a dimethylpyran (compounds **4** or **5**) derivative reduces its cytotoxicity.

EXPERIMENTAL

General Experimental Procedures. UV spectra were measured with a Varian 100 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a JEOL ECA500 spectrometer operating at 500 (^1H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks of CDCl_3 (δ_{H} 7.26 and δ_{C} 77.0, respectively) or acetone- d_6 (δ_{H} 2.04 and δ_{C} 29.8, respectively) as reference standards. Mass spectra were obtained with an ESI-TOF Waters LCT Premier XE mass spectrometer (negative mode). Vacuum liquid chromatography (VLC) and centrifugal planar chromatography (CPC) were carried out using Merck Si gel 60 GF₂₅₄ and, for TLC analysis, precoated Si gel plates (Merck Kieselgel 60 GF₂₅₄, 0.25 mm) were used. Visualization of TLC chromatogram was done under 254 nm UV light and by spraying with ceric sulfate solution and heating. Solvents used for extraction and separation were technical grades that were distilled before used.

Plant Material. The leaves of *M. rubiginosa* were collected in April 2008 from Cinta Damai Village, Banyuasin District, South Sumatra, Indonesia. The specimen was identified at the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and Isolation. Extraction and isolation of the compounds from *M. rubiginosa* were done according to the method previously described [6] with some modifications. The dried and powdered leaves of *M. rubiginosa* (3.0 kg) were macerated in MeOH at room temperature (3×10 L) to give a semisolid extract (300 g) after solvent evaporation under reduced pressure. The extract was redissolved in MeOH–water (9:1) and partitioned into *n*-hexane (228 g) and EtOAc (50 g) fractions. A part of the EtOAc fraction (20 g) was fractionated using VLC (Si gel 160 g) eluted with *n*-hexane–EtOAc (4:1, 7:3, and 1:1) and EtOAc, giving four major fractions A–D. TLC analysis of fraction A (165 mg), detected under UV light 254 nm, showed no phenolic spots, and therefore analysis was not continued. Fraction C (1.3 g) was refractionated using CPC eluted with CHCl_3 and CHCl_3 –EtOAc (9:1) into two subfractions C1 and C2. Fraction B and subfraction C1 were combined into subfraction BC1 (450 mg), and this subfraction was repeatedly purified using PCC eluted with *n*-hexane– CHCl_3 (3:7, 1:4), and CHCl_3 to give macarubiginosins B (**4**, 15 mg) and C (**5**, 3.5 mg). Subfraction C2 (650 mg) was also purified using the

same method [eluent of *n*-hexane–CHCl₃ (3:7, 1:4), CHCl₃, CHCl₃–EtOAc (9:1)] to afford laevifolins A (**1**, 250 mg) and B (**2**, 60 mg). Using the same method, purification of fraction D (250 mg) with eluents of *n*-hexane–EtOAc (4:1 and 7:3) gave macarubiginosin A (**3**, 25 mg).

Laevofilin A (1). Pale yellowish semisolid. UV (MeOH, λ_{\max} , nm) (log ϵ): 209 (4.98), 228 sh (4.52), 284 (4.02), 316 (3.16); (MeOH + NaOH): 208 (4.98), 227 sh (4.71), 290 (4.10), 340 (3.98). ¹H and ¹³C NMR data were in agreement with those reported in [10].

Laevofilin B (2). Pale yellowish semisolid. UV (MeOH, λ_{\max} , nm) (log ϵ): 214 (4.98), 229 sh (4.82), 284 (4.32), 318 (3.35); (MeOH + NaOH): 215 (4.99), 229 sh (4.86), 286 (4.32), 332 (3.98). ¹H and ¹³C NMR data were in agreement with those reported in [12].

Macarubiginosin A (3). Pale yellowish semisolid. UV (MeOH, λ_{\max} , nm) (log ϵ): 215 (4.99), 232 sh (4.91), 284 (4.52), 315 (3.97); (MeOH + NaOH): 217 (4.99), 253 sh (4.69), 292 (4.55), 330 (3.98). For ¹H (500 MHz, acetone-d₆) and ¹³C (125 MHz, acetone-d₆) NMR data, see Table 1. HR-ESI-MS *m/z* 465.2643 [M – H][–] (calcd for C₂₉H₃₇O₅, 465.2641).

Macarubiginosin B (4). Pale yellowish semisolid. UV (MeOH, λ_{\max} , nm) (log ϵ): 215 (4.99), 231 sh (4.87), 280 (4.64), 317 (4.14); (MeOH + NaOH): 214 (4.99), 232 sh (4.90), 282 (4.63), 348 (4.11). For ¹H (500 MHz, CDCl₃) and ¹³C (125 MHz, CDCl₃) NMR data, see Table 1. HR-ESI-MS *m/z* 449.2672 [M – H][–] (calcd for C₂₉H₃₇O₄, 449.2692).

Macarubiginosin C (5). Pale yellowish semisolid. UV (MeOH, λ_{\max} , nm) (log ϵ): 215 (4.95), 232 sh (4.80), 283 (4.30), 318 (3.37); (MeOH + NaOH): 215 (4.96), 230 sh (4.86), 291 (4.16), 332 (3.96). For ¹H (500 MHz, CDCl₃) and ¹³C (125 MHz, CDCl₃) NMR data, see Table 1. HR-ESI-MS *m/z* 449.2688 [M – H][–] (calcd for C₂₉H₃₇O₄, 449.2692).

Cytotoxic Evaluation. Cytotoxic properties of the isolated compounds **1–5** against murine leukemia P-388 cells was evaluated according to the method of MTT assay as previously described [13].

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