



Flavonoids from *Macaranga lowii*

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Abstract. A new isoprenylated dihydroflavonol derivative, macalowiinin (**1**), together with two known flavonoids 4'-*O*-methyl-8-isoprenylnaringenin (**2**) and 4'-*O*-methyl-5,7,4'-trihydroxyflavone (**3**) (= acasetin), have been isolated from the methanol extract of the leaves of *Macaranga lowii*. The structures of these compounds were determined based on UV, NMR, and mass spectral data, and optical rotation. Preliminary cytotoxic evaluation of compounds **1** – **3** against P-388 cells showed that compound **3** is the most active with IC₅₀ was 58.7 μM.

Keywords: *acasetin; cytotoxicity; isoprenylated dihydroflavonol; macaranga lowii; macalowiinin; 4'-O-methyl-8-isoprenylnaringenin; P-388 cells.*

1 Introduction

Macaranga is a large genus of Euphorbiaceae consisting of about 250 species and is distributed in the tropical region of the world, including Indonesia [1,2]. Phytochemical investigation has revealed that this genus is a rich source of phenolic compounds, particularly the isoprenylated and geranylated flavonoids and stilbenes [1,3]. In the course of our phytochemical study on Indonesian *Macaranga*, recently we reported the isolation of isoprenylated flavanones and dihydrochalcones from *M. trichocarpa* [4], isoprenylated, geranylated and farnesylated flavonols from *M. rhizinoides* [5], *M. pruinosa* [6], and *M. gigantea* [7], respectively, and a unique stilbene and dihydroflavonol derivatives containing an irregular sesquiterpenyl side chain from *M. pruinosa* [6]. As part of this study, we have also examined a species collected from Kalimantan island of Indonesia, *M. lowii* King ex. Hook.f., and successfully isolated three flavonoids, including a new isoprenylated dihydroflavonol derivative, named macalowiinin (**1**), together with two known flavonoids 4'-*O*-methyl-8-isoprenylnaringenin (**2**) [8] and 4'-*O*-methyl-5,7,4'-trihydroxyflavone (**3**) (= acasetin) [9] (Figure 1), from the methanol extracts of the leaves of the plant. This paper reports the isolation and structure elucidation of the new compound and cytotoxic properties of compounds **1** - **3** against murine leukemia P-388 cells.

2 Results and Discussion

Macalowiinin (1) was isolated as an optically active pale yellow powder, and its UV spectrum exhibited absorption maxima (296, 334 [sh] nm) typical for a dihydroflavonol [6]. The UV absorption showed a bathochromic shift (37 nm) on addition NaOH solution, indicating that the compound contains one or more free –OH phenolic groups. More specifically, the presence of a free –OH phenolic group at C-5 was also disclosed from the observation of a large bathochromic shift (22 and 60 nm) on addition AlCl₃ solution. However, on addition of HCl, following AlCl₃ addition, the UV spectrum was unchanged indicating that the compound does not bear an 1,2-dihydroxyl group in the aromatic rings. The HR-ESI-MS spectrum (negative mode) of 1 showed a quasimolecular [M-H]⁻ ion (m/z 369.1340) consistent with a molecular formula C₂₁H₂₂O₆ (calculated [M-H]⁻ 369.1338, Δ 0.5 ppm), suggesting that 1 is a 2,3-dihydroflavonol derivative containing an isoprenyl and a methoxyl groups. In the ¹H NMR spectrum (Tab. 1.) the presence of three proton signals at δ H 5.09, 4.73, and 4.61, with multiplicities d (J = 11.5 Hz), d (J = 4.0 Hz), and dd (J = 11.5, 4.0 Hz), respectively, confirmed for the 2,3-dihydroflavonol skeleton in 1. The ¹H NMR spectrum of 1 also showed signals for an isoprenyl (δ H 5.16, 1H; 3.19, 2H; 1.59 and 1.54, each 3H) and a methoxyl (δ H 3.82, 3H) groups, and a proton singlet signal at δ H 11.64 that is consistent with an OH-phenolic at C-5. Further analysis of the ¹H spectrum in the aromatic region revealed the presence of a pair of doublets of two-proton signals (δ H 7.52 and 6.99) and a singlet of one-proton signal (δ H 6.06), suggesting that the isoprenyl group is either at C-6 or C-8. By analysis of HMQC and HMBC spectra of 1, the 5-OH phenolic signal (δ H 11.64) exhibited ¹H-¹³C long range correlations with the signals of two aromatic quarternary (δ C 162.6, C-5; 101.5, C-4a) and an aromatic methine (δ C 96.6, C-6) carbon atoms, and consequently these correlations assign the isoprenyl group at C-8. Furthermore, the methoxyl proton signal (δ H 3.82) displayed a long range correlation with an oxyaryl carbon signal (δ C 160.8, C-4') that does not have a correlation to the methylene proton signal (δ H 3.19) of an isoprenyl group, confirming that the methoxyl group is at C-4'. From these NMR data analysis, macalowiinin (1) was assigned as 4'-O-methyl-5,7,4'-trihydroxy-8-isoprenyl-2,3-dihydroflavonol. Other HMQC and HMBC correlations, as well as ¹³C NMR data assignment, that are consistent with the structure 1 are shown in Tab. 1. The absolute stereochemistry at C-2/C-3 was determined as shown in the structure 1, based on the coupling constant (J = 11.5 Hz, *trans*) between H-2/H-3 and the optical rotation (+5.5°) [1].

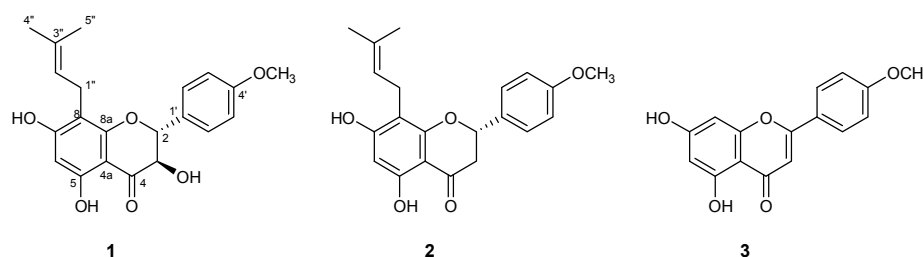


Figure 1 Structures of the flavonoids from *M. lowii*.

The occurrence of dihydroflavonol and flavone derivatives in the genus *Macaranga* is very limited. To our knowledge the dihydroflavonol derivatives have been isolated and identified only in three species, *M. alnifolia* [1], *M. conifera* [10], and *M. pruinosa* [6], while the presence of the flavone is the second time after a similar compound has been isolated from *M. gigantea* [7].

Table 1 NMR (^1H , 500 MHz; ^{13}C 125 MHz) data of macalowiinin (**1**).

No C	δ_{H}	δ_{C}	HMBC ($^1\text{H} \leftrightarrow ^{13}\text{C}$)
2	5,09 (d, 11,5)	84,0	C-3, C-4, C-1', C-2'/C-6'
3	4,61 (dd, 11,5, 4,0)	73,2	C-2, C-4, C-1',
3-OH	4,73 (d, 4,0)	-	-
4	-	198,4	-
4a	-	101,5	-
5	-	162,6	-
6	6,06 (s)	96,6	C-4a, C-5, C-7,C-8
7	-	165,4	-
8	-	108,6	-
8a	-	160,9	-
1'	-	131,3	-
2'/6'	7,52 (d, 9,0)	130,0	C-2, C-3'/5', C-4', C-6'/2'
3'/5'	6,99 (d, 9,0)	114,4	C-1', C-2'/6', C-4', C-5'/3'
4'	-	160,8	-
1''	3,19 (d, 7,5)	22,0	C-7, C-8, C-8a, C-2'', C-3''
2''	5,16 (tm, 7,5)	123,3	C-1'', C-4'', C-5''
3''	-	130,5	-
4''	1,59 (s)	25,8	C-2'', C-3'', C-5''
5''	1,54 (s)	17,8	C-2'', C-3'', C-4''
5-OH	11,64 (s)	-	C-4a, C-5, C-6
4'-OCH ₃	3,82 (s)	55,5	C-4'

Thus, the presence of these flavonoids could have a significant as a marker of a certain group of *Macaranga*.

Compounds **1** – **3** were evaluated for their cytotoxicities against murine leukemia P-388 cells, showing their IC₅₀ were 119.3, 166.6, and 58.7 μM, respectively.

3 Experimental

3.1 General

Optical rotation was measured with Polarimeter Perkin Elmer 341, while UV spectra were acquired with Varian 100 Conc spectrometer. ¹H and ¹³C NMR spectra were recorded with a Bruker Avance 500 spectrometer (¹H, 500 MHz; ¹³C, 125 MHz), and mass spectra were measured with an ESI-TOF Water LCT Premier XE (negative mode). VLC (vacuum liquid chromatography) and PCC (planar centrifugal chromatography) were carried out using Merck silica gel 60 GF₂₅₄, respectively, and for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF₂₅₄, 0.25 mm thickness) were used. Solvents used for extraction and preparative chromatography are technical grades that were distilled before use.

3.2 Plant Materials

The leaves of *M. lowii* were collected from Kalimantan island, Indonesia, in August 2008. The plant was identified by Mr. Ismail, Herbarium Bogoriense, Bogor, Indonesia, and the voucher specimen was deposited in the herbarium.

3.3 Extraction and Isolation

The powdered and dried leaves of *M. lowii* (0.8 kg) were macerated in methanol at room temperature (3x 5 L), and after evaporation of the solvent gave a methanol extract as a semisolid residue (130 g). A portion of the extract (50 g) was divided into acetone-soluble (22 g) and acetone-insoluble (28 g) fractions. The acetone soluble fraction was fractionated through a VLC column, eluted with *n*-hexane-EtOAc (17:3, 4:1, 7:3, and 1:1, each 450, 300, 300, and 600 mL, respectively) to give ten fractions A-J. TLC analysis, monitored with UV lamp 254 nm, showed that the suspected flavonoid spots were contained in the fraction D and H. Refractionation of the fraction D (1.08 g) by using the same method (19:1, 9:1, 17:3, and 4:1, each 150, 150, 200, and 200 mL, respectively) afforded 14 fractions, and the fractions rich with flavonoids (175 mg) were purified with sephadex LH-20 column eluted with MeOH to give a fraction which on crystallization yielded compound **2** (50 mg) [8]. Fraction H (720 mg)

was also refractionated using PCC eluted with *n*-hexane-EtOAc (4:1 to 3:2) to give two major fraction H1 and H2 containing flavonoids. Purification of fraction H1 (140 mg) using the same method (*n*-hexane-EtOAc, 4:1) afforded compound **1** (25 mg). Fraction H2 (215 mg) was purified using PCC technique (*n*-hexane-EtOAc, 9:1) and sephadex LH-20 (MeOH) to give compound **3** (5 mg) [9].

Macalowiinin (1)

Pale yellow powders; $[\alpha]_D = +5.5^{\circ}$ (*c* 0.15, MeOH); UV (MeOH) λ_{\max} nm: 296, 334 (sh); UV (MeOH+NaOH) λ_{\max} nm: 333; UV (MeOH+AlCl₃) λ_{\max} nm: 318, 394 (sh); UV (MeOH+AlCl₃+HCl) λ_{\max} nm: 318, 394 (sh); ¹H NMR (500 MHz, acetone-*d*₆) δ ppm: see Tab. 1.; ¹³C NMR (125 MHz, acetone-*d*₆) δ ppm: see Table 1.; HR-ESI-MS *m/z*: [M-H]⁻ 369.1340 (calculated [M-H]⁻ for C₂₁H₂₂O₆ 369.1338).

3.4 Cytotoxic Assay

The cytotoxic properties of compounds **1** – **3** were evaluated against murine leukemia P-388 cells, and were carried out by MTT assay according to the method previously described [11].

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