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Short Note

5,7-Dihydroxy-3,6-Dimethoxy-3′,4′-Methylendioxyflavone

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Abstract: A new flavonoid derivative, namely 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxy flavone (**1**), was isolated from the leaves of *Melicope glabra* (Blume) T.G. Hartley. The structure of **1** was elucidated based on their UV, IR, HRESIMS, and 1D and 2D NMR spectral data.

Keywords: *Melicope glabra*; flavonol; 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone

1. Introduction

Melicope glabra is one species belonging to the Rutaceae family and is found in all of Indonesia Island. The leaves of *Melicope glabra* are used in Indonesia as traditional medicine for the treatment of fever and cough. According to previous phytochemical studies, the most common secondary metabolites isolated from the genus *Melicope* are alkaloids [1,2], coumarins [3], acetophenones [4], and flavonoids [5]. Flavonoid derivatives in the genus *Melicope* have demonstrated their value as a chemical marker. Secondary metabolites from the genus *Melicope* have shown a wide range of biological and pharmacological applications, owing to such properties as antioxidant [3], antimalarial [1], and anticancer [2]. In the present study, a phytochemical investigation is reported from leaves of *Melicope glabra* (Blume) T.G. Hartley, focused on the isolation and structural elucidation of a new flavonol derivative, 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone, shown in Figure 1. The cytotoxic activity against murine leukemia P-388 cells and the antioxidant radical scavenging activity toward 2,2-diphenyl-1-picrihydrazyl (DPPH) are also reported.

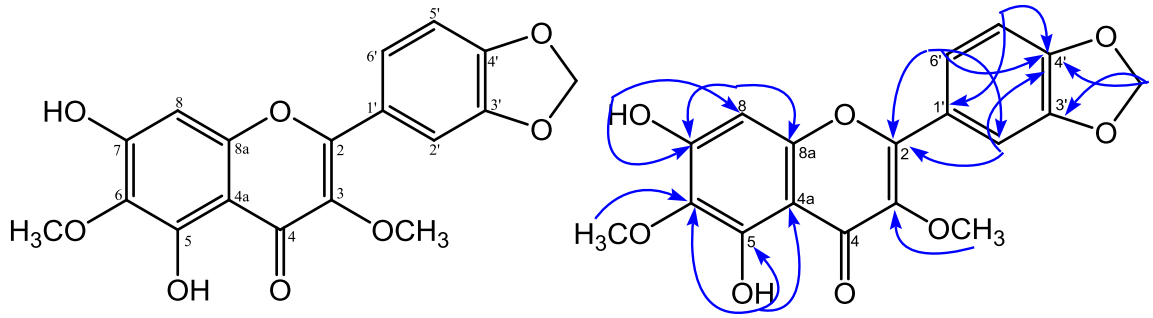

Figure 1. Structure and selected HMBC correlations for 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone.

2. Results and Discussion

5,7-Dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone was obtained as a yellow solid and showed an m.p. of 119–121 °C. HRESIMS measurement of **1** revealed a pseudomolecular ion peak $[M - H]^-$ at m/z 357.0610 (calcd. 357.0610), consistent with a molecular formula of $C_{18}H_{14}O_8$. The UV spectrum showed absorption maxima at λmax 245, 255, 296, and 344 nm, which is characteristic for a flavonol structure [6]. The IR spectrum showed an absorption for hydroxyl (3423 cm^{-1}), conjugated carbonyl (1645 cm^{-1}), aromatic (1577 and 1481 cm^{-1}), and ether (1132 cm^{-1}) groups, respectively [7]. The ^{13}C-NMR spectrum (Table 1) of **1** showed 18 carbon signals, 2 of them signals at δ_C 138.2 and δ_C 179.3, typical for a flavonol structure at C-3 and C-4 [6]. The ^{1}H-NMR spectrum (Table 1) of **1** showed the presence of a proton signal of two aromatic units an ABX system at δ_H 7.68 (H-6′), 7.59 (H-2′), 6.95 (H-5′) at ring B in the aromatic region, and a singlet of an isolated aromatic proton at δ_H 6.54 (H-8) at ring A. The ^{1}H-NMR spectrum of **1** also revealed the presence of two proton signals of a hydroxyl group at δ_H 12.88 (5-OH), δ_H 6.50 (7-OH); two methoxyls at δ_H 4.04 (6-OCH_3), 3.85 (3-OCH_3); and a methylenedioxy group at δ_H 6.08 (3′,4′-OCH_2-O). The position of hydroxyl, methoxyl groups, and methylenedioxy group were confirmed based on HMQC and HMBC spectra. The long-range correlations in the HMBC spectrum of **1** showed a proton signal of a chelated hydroxyl group (δ_H 12.88, 5-OH) with three quaternary carbons at δ_C 151.8 (C-5), 130.1 (C-6), and 106.3 (C-4a). A methoxyl group at δ_H 4.04 was correlated with a quaternary carbon at δ_C 130.1 (C-6), showing that a methoxyl group was placed at C-6. The proton signal of a hydroxyl group at δ_H 6.50 (7-OH) correlated with a quaternary carbon at δ_C 155.1 (C-7), and a methine carbon at δ_C 93.2 (C-8), indicating that a hydroxyl group was placed at C-7 and suggesting an isolated aromatic proton at δ_H 6.54 at H-8. From the ^{1}H-NMR spectrum, the presence of a proton signal of an ABX system at ring B indicated that a methylenedioxy group was placed at C-3′ and C-4′. The proton signal of a methylenedioxy group at δ_H 6.08 correlated with two oxyaryl carbons at δ_C 149.7 (C-3′) and at δ_C 150.0 (C-4′). Furthermore, the proton signal of a methoxyl group at δ_H 3.85 correlated to δ_C 138.2 revealed that a methoxyl group was placed at C-3. One proton signal of ABX at δ_H 7.59 (H-2′) showed correlations with two oxyaryl carbons—δ_C 155.8 (C-2), 150.0 (C-4′)—and one methine carbon at δ_C 123.8 (C-6′). The proton signal at δ_H 6.95 (H-5′) showed correlations with one quaternary carbon, δ_C 124.2 (C-1′), and one oxyaryl carbon at δ_C 149.7 (C-3′). Furthermore, the proton signal at δ_H 7.68 (H-6′) showed correlations with two oxyaryl carbons (δ_C 155.8 (C-2), 150.0 (C-4′)), and one methine carbon signal at δ_C 108.7 (C-2′). Based on the above spectral evidence, the structure of **1** was elucidated as 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone.

Table 1. NMR spectroscopic data of 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone in $CDCl_3$.

No.C	δ_H (Mult. *J* in Hz)	δ_C	HMBC
2	-	155.8	-
3	-	138.2	-
4	-	179.3	-
4a	-	106.3	-
5	-	151.8	-
6	-	130.1	-
7	-	155.1	-
8	6.54 (*s*, 1H)	93.2	C-4a, C-6, C-7, C-8a
8a	-	153.9	-
1′	-	124.2	-
2′	7.59 (*d*, 1.8, 1H)	108.7	C-2, C-4′, C-6′
3′	-	149.7	-
4′	-	150.0	-
5′	6.95 (*d*, 8.4, 1H)	108.6	C-1′, C-3′
6′	7.68 (*dd*, 8.4; 1.8, 1H)	123.8	C-2, C-2′, C-4′
5-OH	12.88 (*s*, 1H)	-	C-4a, C-5, C-6
7-OH	6.50 (*s*, 1H)	-	C-7, C-8
3-OCH_3	3.85 (*s*, 3H)	61.0	C-3
6-OCH_3	4.04 (*s*, 3H)	60.3	C-6
3′,4′-OCH_2-O-	6.08 (*s*, 2H)	101.8	C-3′, C-4′

The cytotoxic activity of **1** was evaluated using cell viability in murine leukemia P-388 cells by MTT assay, exhibiting IC_{50} values of 48.30 μg/mL. The antioxidant activity against DPPH radical of **1** showed IC_{50} values of 38.68 μg/mL, which suggests that it has moderate activity.

2.1. General

Column chromatography and planar radial chromatography were carried out using silica gel 60 G 1.07734.1000 and Si gel 60 PF_{254} 1.07749.1000 (Merck, Darmstadt, Germany). The UV spectra was measured with Shimadzu series 1800 spectrophotometer (Shimadzu, Kyoto, Japan).The IR spectra was recorded with Perkin-Elmer spectrum-100 FT-IR (Perkin-Elmer, Waltham, MA, USA). The mass spectra were recorded using a Waters LCT Premier XE (Waters, Santa Clara, CA, USA). NMR spectra were recorded on a JEOL 400 ECA spectrophotometer (JEOL, Tokyo, Japan) in $CDCl_3$ at 400 (^{1}H) and 100 (^{13}C) MHz using TMS as the internal standard.

2.2. Plant Material

The leaves of *M. glabra* were collected in Gunung Salak, Bogor, West Java, Indonesia on March 2017. The specimen was identified at the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

2.3. Extraction and Isolation

The leaves of *M. glabra* (1.7 kg) were macerated in MeOH twice for 2 days each. After evaporating the solvent in a rotary evaporator, 210 g of pale brown semisolid was obtained. The extract was redissolved in MeOH/water (9:1) and partitioned with *n*-hexane (95 g) and ethyl acetate (30 g). The EtOAc extract (29 g) was subjected to vacuum liquid chromatography over silica gel and eluted with *n*-hexane/ethyl acetate by increasing polarity (9:1, 4:1; 7:3, 1:1, and 1:4) to give three major fractions, A–C. Fraction A (4.68 g) was separated by column chromatography eluted with *n*-hexane-ethyl acetate (9:1 to 7:3) to produce subfractions A_1–A_3. Subfraction A_1 was purified by planar radial chromatography using *n*-hexane/$CHCl_3$ (from 4:1 to 1:4) to yield compound **1** (20 mg).

2.4. Cytotoxic Assay

The cytotoxic activity of **1** against murine leukemia P-388 cells was evaluated according to the MTT method as previously described [8–10]. Artonin E was used as the positive control.

2.5. DPPH Radical Scavenging

The antioxidant activity of **1** against DPPH (2,2-diphenyl-1-picrihydrazyl) radical measured at λ 517 nm by UV spectrometer as described previously [11–13]. The inhibition percentage (%) of radical scavenging activity was calculated using the following equation: Inhibition (%) = $(A_o - A_s/A_o) \times 100$, where A_o is the absorbance of the control reaction (containing all reagents except the active compound), and A_s is the absorbance of the active compound. Ascorbic acid was used as the positive control.

3. Conclusions

A new flavonol, 5,7-dihydroxy-3,6-dimethoxy-3′,4′-methylenedioxyflavone, was isolated for the first time from the leaves of *M. glabra*. The cytotoxic activity of **1** against murine leukemia P-388 cells showed IC_{50} values of 48.30 μg/mL, and the antioxidant activity against the DPPH radical showed IC_{50} values of 38.68 μg/mL.

Supplementary Materials: The following are available online. HRESIMS, ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC spectra are reported in the Supplementary Materials as Figures S1–S5, and structure refinement parameters are in Table S1.

Author Contributions: M.T. designed the whole experiment of bioactivity and wrote the manuscript. T.S.T. researched data, analyzed the NMR and HRESIMS spectra and contributed to the manuscript, R.D.S. and U.H.

designed the whole experiment. F.H., a botanist was identified of plant material. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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