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Two New Flavanones from the Leaves of *Flemingia lineata* (L.) Aiton

Mulyadi Tanjung[1,*], Tjitjik Srie Tjahjandarie[1], Shola Mardhiyyah[1], Ghinsha Zakatina Rahman[1], Muhammad Fajar Aldin[1], Ratih Dewi Saputri[2], and Norizan Ahmat[3]

[1]*Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Surabaya 60231, Indonesia*
[3]*Faculty of Applied Sciences, Universiti Teknologi MARA 40450 Shah Alam, Selangor, Malaysia*

Abstract – Three isoprenylated flavanones were isolated from the leaves of *Flemingia lineata* (L.) Aiton. Among them are two new flavanones, flemilineatins A and B (**1-2**), along with 6-isoprenyl eridioctyol (**3**). Their structures were determined using HRESIMS data and NMR spectra. Flavanones **1-3** were assayed in the HeLa cancer cells. Compound **1** showed moderate activity with an IC_{50} value of 11.2 μM.
Keywords – *Flemingia lineata*, flemilineatins A-B, flavanone, cytotoxicity

Introduction

Flemingia lineata (L.) Aiton, belonging to the Fabaceae family, is a fast-growing shrub native to Southeast Asia and Australia. *F. lineata*, commonly called as otok-otok in Java, is used to soil erosion control and provide organic manure. The leaves also cure scabies, fever, and inflammation.[1] The *Flemingia* genus produces flavonoids and isoflavonoids with a terpenyl side chain.[2-3] The presence of these terpenyl side chains enhances biological activities such as cytotoxic, antioxidant, and estrogenic properties.[4-7] Flemilineatins A (**1**) and B (**2**), two new flavanones, along with 6-isoprenyl eridioctyol (**3**) (Fig. 1), were isolated from *F. lineata* leaves, and their cytotoxic effects on HeLa cells were reported here.

Experimental

General experimental procedures – UV spectra were performed with a UV-VIS spectrophotometer (Shimadzu series 1800). The functional groups were recorded using an FT-IR spectrophotometer (Shimadzu IR Tracer 100). The mass molecule and the chemical formula were recorded using an ESI-TOF (Waters- LCT Premier XE). NMR spectra (JEOL JNM ECA-400) at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR were measured using TMS as the internal standard and acetone-d_6 (δ_H 2.04 and δ_C 29.8, respectively) as reference standards. For column and radial chromatography, silica gel 60 and silica gel 60 PF_{254} were employed, respectively. The optical rotation was measured with a polarimeter (Perkin Elmer Model 341).

Plant material – In February 2020, the fresh leaves of *F. lineata* were collected in Kemiren Village, Banyuwangi, East Java, Indonesia. A senior botanist (Ismail Rachman) from the Bogoriense Herbarium identified the plant. As a reference, a specimen (FL 20200218) was deposited.

Extraction and isolation – The dried leaves of *F. lineata* (8.1 kg) were extracted for a week with 90% methanol and then successively partitioned with *n*-hexane and ethyl acetate. The ethyl acetate extract (98 g) was separated on silica gel by column chromatography, using an *n*-hexane-ethyl acetate gradient (from 19:1 to 7:3 v/v) to yield three fractions A–C. Sephadex LH-20 column chromatography, eluting with methanol, divided fraction B into two subfractions, B_1–B_2. Compounds **1** (28 mg), **2** (9 mg) and **3** (18 mg) were obtained from fraction B_1 after separation by silica gel radial chromatography, eluting with *n*-hexane-chloroform gradient (from 19:1 to 3:7 v/v).

Flemilineatin A (**1**) – yellowish oil, $[\alpha]^{20}_D = -3.2^o$ (*c* 0.05, MeOH): UV (MeOH) λ_{max} (log ε) 224 (4.52); 292 (4.17) and 343 nm (3.52). Table 1 shows the NMR data of **1**. HRESIMS *m/z* $[M+H]^+$ calculated for $C_{30}H_{37}O_6$ *m/z*

*Author for correspondence
Mulyadi Tanjung, Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya 60115, Indonesia
Tel: +62-31-5936501; E-mail: mulyadi-t@fst.unair.ac.id

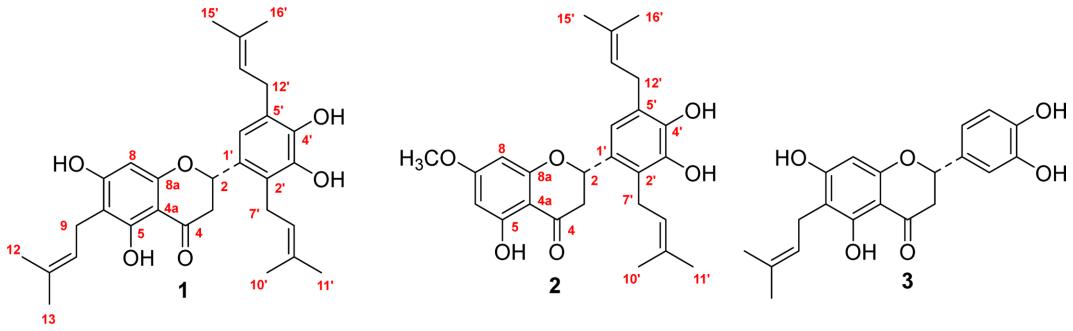

Fig. 1. Structures of flavanones isolated from *F. lineata* leaves.

493.2649, found 493.2643.

Flemilineatin B (**2**) – yellowish oil, $[\alpha]^{20}_{D} = -3.1^{\circ}$ (*c* 0.05, MeOH): UV (MeOH) λ_{max} (log ε) 226 (4.50); 289 (4.20) and 341 nm (3.51). Table 1 shows the NMR data of **2**. HRESIMS *m/z* $[M+H]^+$ calculated for $C_{26}H_{31}O_6$ *m/z* 439.2121, found 439.2112.

6-Isoprenyl eridioctyol (**3**) – yellowish oil, UV (MeOH) λ_{max} (log ε) 226 (4.34); 295 (4.27) and 339 nm (3.45). The NMR data of **3** have been compared with the literature data.[8]

Cytotoxicity assay – In 96-well plates, HeLa cells were cultivated at a density of 3×10^4 cells/cm^3. For growth, the cells were incubated at 37ºC for 24 hours. The isolates (**1–3**) were added to each well in triplicate with varying concentrations (100, 30, 10, 3, 1, 0.3, and 0.1 µM) and incubated at 37ºC for 48 hours. After incubation, the MTT reagent was applied to the culture cells and allowed to sit for four hours. A microplate reader spectrometer set to λ 540 nm was used to measure the inhibition of cells by the isolates (**1–3**) was recorded.[9-11] Regression analysis was used to get the IC_{50} values of the isolates (**1–3**).

Results and discussion

Flemilineatin A (**1**) was isolated as a yellowish oil. The HRESIMS revealed the $[M+H]^+$ ion at *m/z* 493.2643 (calcd 493.2649), corresponding to the chemical formula $C_{30}H_{37}O_6$. The UV spectrum in MeOH (λ_{max} 224, 292, and 343 nm) revealed the presence of a flavanone core.[12] The NMR data revealed the presence of an oxymethine proton [δ_H 5.66 (1H, *dd*, *J* = 13.0, 3.0 Hz, H-2), δ_C 77.1 (C-2)], distinct methylene protons [δ_H 3.13 (1H, *dd*, *J* = 17.1, 13.0 Hz, H-3_{ax}), 2.62 (1H, *dd*, *J* = 17.1, 3.0 Hz, H-3_{eq}), δ_C 43.3 (C-3)], and a conjugated carbonyl at δ_C 197.7 (C-4), which are characteristics for flavanone moiety.[13] Compound **1** also featured two protons of two aromatic units at δ_H 6.00 (1H, *s*, H-8) for ring A, δ_H 6.91 (1H, *s*, H-6′) for ring B, and a hydroxy proton at δ_H 12.47 (1H, *s*, 5-OH). In addition, three isoprenyl protons were visible, including three olefinic protons [δ_H 5.32 (1H, *t*, *J* = 7.3 Hz, H-13′), 5.22 (1H, *t*, *J* = 7.3 Hz, H-10), 5.11 (1H, *t*, *J* = 6.6 Hz, H-8′)], three methylene protons [δ_H 3.48 (2H, *d*, *J* = 6.9 Hz, H-7′), 3.35 (2H, *d*, *J* = 7.2 Hz, H-12′), 3.24 (2H, *d*, *J* = 7.1 Hz, H-9)], and six methyl protons [δ_H 1.74 (3H, *s*, H-11′), 1.69 (6H, *s*, H-12/H-15′), 1.65 (3H, *s*, H-10′), 1.63 (3H, *s*, H-16′), 1.62 (3H, *s*, H-13)]. The ^{13}C NMR spectrum of flemilineatin A showed the signal of 28 carbons in the structure, including five oxyaryl carbons [δ_C 164.6, 162.2, 162.1, 144.1, 143.9], indicating that the flavanone structure is an eriodictyol derivative. The isoprenyl groups in the flavanone structure were identified using the HMBC spectrum (Fig. 2). The chelated proton at δ_H 12.47 (5-OH) correlated to an oxyaryl at δ_C 162.2 (C-5) and two carbon signals at δ_C 103.0 (C-4a), and 108.9 (C-6). A methylene proton at δ_H 3.24 (H-9) correlated to C-5, C-6, δ_C 164.6 (C-7), a methine carbon at δ_C 123.5 (C-10), and a carbon signal at δ_C 131.5 (C-11), indicating an isoprenyl chain was located at C-6. Two methyl protons at δ_H 1.69 (H-12) and 1.62 (H-13) from the isoprenyl chain correlated to C-10 and C-11 in favor of isoprenyl bound at C-6. An aromatic proton at δ_H 6.00 (H-8) correlated to C-4a, C-6, C-7, and oxyaryl at δ_C 162.1 (C-8a), was supported the location of an isoprenyl at C-6. An aromatic proton (ring B) at δ_H 6.91 (H-6′)

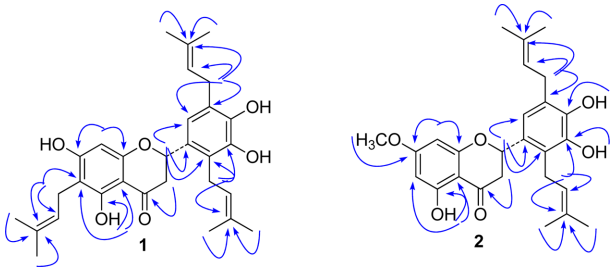

Fig. 2. HMBC of flemilineatins A (**1**) and B (**2**).

Table 1. ^{1}H (400 MHz),^{13}C NMR (100 MHz) data of **1** and **2** in acetone-d_6

	1			**2**		
No. C	δ_H (mult, *J* in Hz)	δ_C	HMBC	δ_H (mult, *J* in Hz)	δ_C	HMBC
2	5.66 (*dd*, 13.0; 2.8)	77.1	C-1′, C-2′, C-6′	5.61 (*dd*, 13.0; 2.9)	77.3	C-1′, C-2′, C-6′
3	3.13 (*dd*, 17.1; 13.0) 2.62 (*dd*, 17.1; 3,0)	43.3	C-2. C-4	3.18 (*dd*, 17.2; 13.0) 2.67 (*dd*, 17.2; 2.9)	43.2	C-2, C-4
4	-	197.7	-	-	198.0	-
4a	-	103.0	-	-	103.7	-
5	-	162.2	-	-	165.0	-
6	-	108.9	-	6.01 (*d*, 2.2)	94.5	C-4a, C-7, C-8
7	-	164.6	-	-	168.7	-
8	6.00 (*s*)	95.2	C-4a, C-6, C-7, C-8a	6.02 (*d*, 2.2)	95.4	C-4a, C-6, C-7, C-8a
8a	-	162.1	-	-	-	-
9	3.24 (*d*, 7.1)	21.6	C-5, C-6, C-7, C-10, C-11	-	-	-
10	5.22 (*t*, 7.3)	123.5	C-12, C-13	-	-	-
11	-	131.5	-	-	-	-
12	1.69 (*s*)	25.7	C-10, C-11, C-13	-	-	-
13	1.62 (*s*)	17,8	C-10, C-11, C-12	-	-	-
1′	-	129.4	-	-	129.2	-
2′	-	125.8	-	-	125.8	-
3′	-	143.9	-	-	144.0	-
4′	-	144.1	-	-	144.1	-
5′	-	127.0	-	-	127.0	-
6′	6.91 (*s*)	119.6	C-2, C-2′, C-4′, C-12′	6.92 (*s*)	119.6	C-2, C-1′, C-4′, C-1′′′
7′	3.48 (*d*, 6.9)	25.3	C-2′, C-3′, C-8′	3.49 (*d*, 6.7)	25.3	C-3’, C-7′, C-8′
8’	5.11 (*t*, 6.6)	124.3	C-10′, C-11′	5.12 (*t*, 6.7)	124.3	C-10′, C-11′
9’	-	131.2	-	-	132.8	-
10’	1.65 (*s*)	25.8	C-8′, C-9′, C-11′	1.69 (*s*)	17,8	C-8′, C-9′, C-11′
11’	1.74 (*s*)	17.8	C-8′, C-9′, C-10′	1.63 (*s*)	25.7	C-8′, C-9′, C-10′
12’	3.35 (*d*, 7.2)	29.1	C-5′, C-6′, C-13′, C-14′	3.35 (*d*, 7.2)	29.1	C-4′, C-13′, C-14′
13’	5.32 (*t*, 7.3)	123.3	C-15′, C-16′	5.32 (*t*, 7.3)	123.3	C-15′, C-16′
14’	-	132.8	-	-	131.5	-
15’	1,69 (*s*)	25.8	C-13′, C-14′, C-16′	1.70 (*s*)	17.9	C-13′, C-14′, C-16′
16’	1.63 (*s*)	18.0	C-13′, C-14′, C-15′	1.64 (*s*)	25.8	C-13′, C-14′, C-15′
3′-OH	-	-	-	7.33 (*s*)	-	C-2′, C-3′
4′-OH	-	-	-	7.52 (*s*)	-	C-4′, C-5′
5-OH	12.47 (*s*)	-	C-4a, C-5, C-6	12.15 (*s*)	-	C-4a, C-5, C-6
7-OCH_3	-	-	-	3.83 (*s*)	56.2	C-7

Table 2. Cytotoxic data of compounds **1**-**3**

Compounds	IC_{50} (μM)	
	HeLa	MCF-7
Flemilineatin A (**1**)	11.2 ± 0.3	36.4 ± 1.5
Flemilineatin B (**2**)	>100	80.5 ± 1.2
6-Isoprenyl eriodictyol (**3**)	>100	>100
Doxorubicin	4.5 ± 0.1	5.0 ± 0.2

correlated to C-2, an oxyaryl at δ_C 144.1 (C-4′), a carbon signal at δ_C 103.0 (C-2′), and methylene at δ_C 29.1 (C-12′), indicating two isoprenyl chains were located at C-2′ and C-5′ in the ring B of eriodictyol structure. A methylene proton of 2-methyl-2-butenyl at δ_H 3.48 (H-7′) correlated to an oxyaryl carbon at δ_C 143.9 (C-3′), a methine carbon at δ_C 124.3 (C-8′), and a carbon signal at δ_C 131.2 (C-9′), indicating that isoprenyl chain attached at C-2′. The long-range correlation between two methyl protons [δ_H 1.65 (H-10′), 1.74 (H-11′)] to C-8′, C-9′, and an olefinic proton at δ_H 5.11 (H-8′) to signals δ_C 25.8 (C-10′) and δ_C 17.8 (C-11′), showing the isoprenyl located at

C-2′. The methylene carbon at δ_C 29.1 (C-12′) in the HMQC spectrum exhibits direct connectivity with the proton signal at δ_H 3.35. The methylene proton at δ_H 3.35 (H-12′) linked to C-4′, two methine carbons [δ_C 119.6 (C-6′), 123.3 (C-13′)], and two carbon signals [δ_C 127.0 (C-5′), 132.8 (C-14′)], indicating that an isoprenyl located at C-5′ (ring B). The stereochemistry of flemilineatin A at C-2 was determined to be *S* due to the negative specific rotation.[12-13] In summary, the structure of flemilineatin A was determined to be (*S*)-6,2′,5′-triisoprenyl-5,7,3′,4′-tetrahydroxyflavanone.

Flemilineatin B (**2**) was isolated as a yellowish oil. The HRESIMS exhibited the $[M+H]^+$ ion at *m/z* 439.2112 (calcd 439.2121), consistent with the molecular formula $C_{26}H_{31}O_6$. The ^1H and ^{13}C NMR data of flemilineatin B were very similar to those of compound **1**, particularly at ring C [δ_H 5.61 (H-2), 3.18 (H-3_{ax}), 2.67 (H-3_{eq}), δ_C 77.3 (C-2), 43.2 (C-3), 198.0 (C-4)]. The presence of an aromatic group [δ_H 6.92 (H-6′), δ_C 119.6 (C-6′)] and two isoprenyl chains [δ_H 5.32 (H-13′), 5.12 (H-7′), 3.49 (H-8′), 3.35 (H-12′), 1.70 (H-15′), 1.69 (H-10′), 1.64 (H-16′), 1.63 (H-11′), δ_C 132.8 (C-9′), 131.5 (C-14′), 124.3 (C-8′), 123.3 (C-13′), 29.1 (C-12′), 25.8 (C-16′), 25.7 (C-11′), 25.3 (C-7′), 17.9 (C-15′), 17.8 (C-10′)] at ring B very similar to that of flemilineatin A. A pair of doublet protons ($J = 2.2$ Hz) at δ_H 6.01 (H-6), 6.02 (H-8), a chelated proton at δ_H 12.15 (5-OH), and a methoxy proton at δ_H 3.83 (7-OCH_3) in the ring A were striking differences in the ^1H NMR data of compound **2**. Signals for 28 carbons were identified in the ^{13}C NMR spectrum. The HMBC spectrum (Fig. 2), a chelated signal at δ_H 12.15 (5-OH) correlated to a methine at δ_C 94.5 (C-6), an oxyaryl at δ_C 165.0 (C-5), and a carbon signal at δ_C 103.7 (C-4a). The aromatic proton at δ_H 6.01 (H-6) correlated to C-4a, an oxyaryl at δ_C 164.6 (C-7), and a methine at δ_C 95.4 (C-8), while a methoxy group at δ_H 3.83 corresponded to C-7. The location of a methoxy group at to C-7 supports the long-range correlation between H-8 to C-4a, C-6, C-7, and an oxyaryl at δ_C 164.4 (C-8a). Due to the negative specific rotation, the stereochemistry of compound **2** at C-2 was also verified to be *S*.[12-13] As a result, the structure of flemilineatin B was determined to be (*S*)-2′,5′-diisoprenyl-5,3′,4′-trihydroxy-7-methoxyflavanone.

The MTT technique was used to investigate the cytotoxicity of compounds **1–4** against HeLa cells. Negative controls included cells that had not been exposed to the active compound, while positive controls included doxorubicin.[14-15] Flemilineatin A (**1**) showed moderate activity with an IC_{50} value of 11.2 μM, and compounds (**2-3**) did not affect the HeLa cells. The presence of the isoprenyl chain at C-6 of **1** increases activity than compound **2**, and the existing two 2-methyl-2-butenyl chains at C-2′ and C-5′ of **1** more than active with compound **3** against HeLa cells.[15-16]

In conclusion, two new flavanones, flemilineatins A and B (**1**–**2**), along with 6-isoprenyl eriodictyol (**3**), were isolated from the leaves of *F. lineata*. Compound **1** showed moderate activity against HeLa cells, and compounds (**2-3**) were inactive.

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References

(1) Heyne, K. The useful Indonesian Plants; Research and Development Agency, Ministry of Forestry: Indonesia, **1987**, p 2114.

(2) Tjahjandarie, T. S.; Tanjung, M.; Saputri, R. D.; Aldin, M. F.; Susanti, R. A.; Pertiwi, N. P.; Wibawa, R. S.; Halizah, I. N. *Phytochem. Lett.* **2021**, *44*, 78-81.

(3) Rahman, M. M.; Sarker, D. S.; Byres, M.; Gray, A. I. *J. Nat. Prod.* **2004**, *67*, 402-406.

(4) Fu, M.; Deng, D.; Huang, R.; Zhang, N.; Su, Z.; Qiu, S. X. *Nat. Prod. Res.* **2013**, *27*, 1237-1241.

(5) Gumula, I.; Alao, J. P.; Ndiege, I. O.; Sannerhagen, P.; Yenesew, A.; Erdelyi, M. *J. Nat. Prod.* **2014**, *77*, 2060-2067.

(6) Li, L.; Deng, X.; Zhang, L.; Shu, P.; Qin, M. *Fitoterapia* **2011**, *82*, 615-619.

(7) Xie, G.; Lin, B.; Qin, X.; Wang, G.; Wang, Q.; Yuan, J.; Li, C.; Qin, M. *Fitoterapia*, **2015**, 110, 97-101.

(8) Marliana, E.; Astuti, W.; Kosala, K.; Hairani, R.; Tjahjandarie, T. S.; Tanjung, M. *Asian J. Chem*. **2018**, *30*, 795-798.

(9) Saputri, R. D.; Tjahjandarie, T. S.; Tanjung, M. *Nat. Prod. Res*. **2021**, *35*, 1256-1261.

(10) Tanjung, M.; Tjahjandarie, T. S.; Saputri, R. D.; Kurnia, B. D.; Rachman, M. F.; Syah, Y. M. *Nat. Prod. Res.* **2021**, *35*, 407-412.

(11) Tjahjandarie, T. S.; Tanjung, M.; Saputri, R. D.; Rahayu, D. O.; Gunawan, A. N. I.; Aldin, M. F. *Nat. Prod. Res.* **2021**, *35*, 5637-5642.

(12) Tanjung, M.; Tjahjandarie, T. S.; Sentosa, M. H. *Asian Pac. J. Trop. Dis.* **2013**, *3*, 401-404.

(13) Syah, Y. M.; Hakim, E. H.; Achmad, S. A.; Hanafi, M.; Ghisalberti, E. L. *Nat. Prod. Commun*. **2009**, *4*, 63-67.

(14) Tanjung, M.; Aldin, M. F.; Tjahjandarie, T. S.; Rahayu, D. O.; Gunawan, A. N. I.; Saputri, R. D. *Nat. Prod. Sci*. **2021**, *27*, 172-175.

(15) Aldin, M. F.; Tjahjandarie, T. S.; Saputri, R. D.; Tanjung, M. *Nat. Prod. Sci*. **2021**, *27*, 45-48.

(16) Saputri, R. D.; Tanjung, M.; Tjahjandarie, T. S. *Nat. Prod. Sci*. **2021**, *27*, 183-186.

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