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Cover Page Footnote

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A Known Naphthalene, Isoeleutherol, from the Herb of *Lygodium microphyllum*

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Abstract

A known naphthalene, isoeleutherol (**1**), was isolated from the herb of *Lygodium microphyllum.* The chemical structure of **1** was determined on the basis of spectroscopic data mainly UV, IR, HRTOFMS, 1D- and 2D-NMR spectroscopy, as well as by comparing with compounds previously reported. Isoeleutherol was isolated from this plant for the first time and showed moderate antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) with IC_{50} value of 53.96 ± 2.87 µg/mL.

Abstrak

Senyawa Naftalena yang telah dikenal, Isoeleutherol, dari Herba *Lygodium microphyllum*. Senyawa naftalena yang telah dikenal, isoeleutherol (**1**) telah diisolasi dari herba *Lygodium microphyllum*. Struktur kimia senyawa 1 telah ditentukan berdasarkan data spektroskopi terutama UV, IR, HRTOFMS, NMR 1D- dan 2D- serta perbandingan dengan senyawa yang mirip dari laporan sebelumnya. Isoeleutherol diisolasi dari tanaman ini untuk pertama kalinya dan menunjukkan aktivitas antioksidan yang sedang terhadap DPPH (2,2-difenil-1-pikrilhidrazil) dengan nilai IC_{50} 53.96 ± 2.87 µg/mL.

Keywords: Naftalena, Lygodium microphyllum, isoeleutherol, Lygodeceae, antioxidant activity

Introduction

Lygodium is only one genus of the Lygodiaceae family consisting of more than 50 species, and has been reported for the treatment of kidney stones [1] and as an expectorant [2], as well as for its antiplasmodial [3], antibacterial [4], antiviral [5], and antidiarrheal activities [6]. Phytochemical studies on the *Lygodium* have reported it to contain unique secondary metabolites with diverse biological activities. These metabolites include flavonoids [7-9], phenolic glycosides [10], naphthoquinones [11], ecdysteroids [12], and steroids [13]. As part of our studies on the Indonesian *Lygodium* species, we have performed a phytochemical examination of the herb of *L. microphyllum*. The plant, known as "krokot" in Indonesia, is a perennial fern that typically grows in the rain forest and can be found on Kalimantan island [14]. This plant is used in Indonesia folk medicine for the treatment of fever and kidney stones [1-3]. In previous papers, we reported the isolation of flavonoids from the herb of *L. microphyllum* [8-9]. In this paper, we present the isolation of a known naphthalene derivative, isoeleutherol, and its antioxidant activity.

Materials and Methods

General. The melting point was measured on electrothermal melting point apparatus and not corrected. The

UV-Visible spectrum was obtained on a Shimadzu series 1800 spectrophotometer (Shimadzu, Kyoto, Japan). The IR spectrum was measured on a Perkin-Elmer 1760X spectrophotometer (Waltham, MA, USA) in KBr. Mass spectra recorded with a Waters, QToF HR-MS XEVotm mass spectrometer (Waters, Milford, MA, USA). ^{1}H and ^{13}C NMR spectra are obtained with a JEOL NMR A-500 MHz using tetramethylsilane (TMS) as an internal standard (JEOL, Tokyo, Japan). Chromatographic separations were carried out on silica gel 60 (Merck, Darmstadt, Germany), ODS (Fuji Silysia, Kyoto, Japan). TLC plates were precoated with silica gel GF_{254} and RP-18 (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating and under ultraviolet-visible light at wavelength of 257 and 364 nm. Preparative MPLC using a Buchi Pump Controller C-610, Buchi Pump Modules C-605, and a FLH-R10030B SiliCycle column- ISO04 Siliasep^{TM} (Buchi, Swizerland).

Plant material. The herb of *L. microphyllum* was collected from forest areas in Samarinda, East Kalimantan in June 2016. The plant was identified by staff at the Faculty of Forestry, University of Mulawarman, Samarinda and sample specimens (No. 02042013) were stored at the Faculty of Forestry, University of Mulawarman, Samarinda, Indonesia.

Extraction and Isolation. The dried herbs (2.5 kg) of *L. microphyllum* were extracted with methanol (12 L) at room temperature for 4 days. After removal of the solvent under vacuum, the viscous concentrated MeOH extract (210 g) was suspended in H_2O and partitioned with n-hexane, EtOAc, and n-butanol, successively. Evaporation of the solvents resulted in n-hexane (59 g), EtOAc (72 g), and n-butanol (54 g) extracts. The n-hexane soluble fraction (30 g) was fractionated by vacuum liquid chromatography on silica gel 60 using a gradient of n-hexane and EtOAc to give ten fractions (A-J). Fraction C (3.4 g) was chromatographed on a column of silica gel, using a gradient of n-hexane-EtOAc (10:0-1:1), to give eight fractions (C1-C8). Subfraction C6 (450 mg) was separated by using MPLC on silica gel, eluted with $CHCl_3$-EtOAc (9:1) to give five fractions (C6.1-C6.5). Subfraction C6.3 (86 mg) was separated on preparative TLC, eluted with n-hexane:EtOAc:HOAc = 9:1:0.5, R_f value of 0.42, to give **1** as a brown crystals. Compound **1** had an R_f value of 0.45 on silica gel using n-hexane:EtOAc (7:3) and 0.38 on silica RP-18 using MeOH:H_2O (3:2).

Antioxidant assay. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) method was used to evaluate radical scavenging activity. A 10 µL aliquot of each extract sample was added to 990 µL of DPPH solution (0.002% in methanol). The mixture was incubated for 30 minutes at room temperature, the absorbance was measured at 517 nm against a corresponding blank, and the antioxidant activity was calculated as:

$$AA\% = (A_{DPPH} - A_{sample}) / A_{DPPH} \times 100$$

where AA is the antioxidant activity, A_{DPPH} is the absorption of DPPH against the blank, and A_{sample} is the absorption of the extract or control against the blank. Ascorbic acid was used as positive control. All tests were carried out in triplicate [15].

Results and Discussion

Isoeleutherol (**1**) was isolated as brown crystals, m.p. 203-204 °C, optical rotation $[\alpha]^{20}_{D}$ -64° (*c* 0.10, $CHCl_3$) (Figure 1). The molecular formula of compound **1** is designated as $C_{14}H_{12}O_4$ based on the HRTOF-MS spectrum (*m/z* 245.0817 $[M-H]^+$, calcd. for $C_{14}H_{13}O_4$, *m/z* 245.0814) and NMR data (Table 1), thus requiring nine degrees of unsaturations. IR spectrum of **1** showed the presence of hydroxyl (3420 cm^{-1}), ester (1710 cm^{-1}), benzene (1610 and 780 cm^{-1}), and ether (1210 cm^{-1}) groups, while the UV spectrum showed absorption maxima at 320, 275, and 150 nm, indicating the presence of aromaticity in **1**. The ^{1}H-NMR (acetone-d_6) spectrum of **1** revealed the ABC-type signals at δ_H 7.70 (1H, d, *J*=8.5 Hz), 7.51 (1H, dd, *J*=8.5, 7.8 Hz), and 7.17 ppm (1H, d, *J*=7.8 Hz), which implied the presence of a trisubstituted benzene ring in **1**. A resonance signal at δ_H 7.88 ppm (1H, s) in the ^{1}H-NMR spectrum of **1** indicated the presence of a pentasubstituted benzene ring. The ^{1}H-NMR spectrum also showed a signal for an alkyl group at [δ_H 1.69 (3H, d, *J*=6.5 Hz), 5.71 ppm (1H, q, *J*=6.5 Hz)], a methoxyl group at δ_H 4.21 ppm (3H, s), and a hydroxyl proton at δ_H 9.88 ppm (1H, s). The ^{13}C-NMR spectrum showed 14 carbon resonances, which were classified by their chemical shifts and DEPT spectra as one methyl, one methoxy, four sp^2 methines, six sp^2 quaternary carbons, one sp^3 methine and one lactone carbon.

These functionalities accounted for six out of the total nine degrees of unsaturation. The remaining of three degrees of unsaturation were consistent with a naphthalene skeleton-[13]. The gross structure of **1** was deduced from the ^{1}H-^{1}H COSY and HMBC spectra (Figure 2). The hydroxyl proton at δ_H 9.88 ppm was correlated to δ_C 118.3 (C-12)

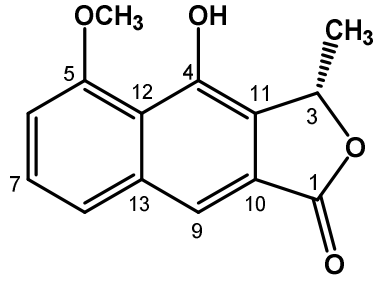

Figure 1. Structure of Isoeleutherol (1)

Table 1. NMR for 1, Eleutherol [14] and Isoeleutherol [15]

Position of C	**1** (acetone-d_6)		Eleuthero [14] (DMSO-d_6)		Isoeleutherol ($CDCl_3$) [15]	
	^{1}H-NMR δ_H (int., mult., *J* Hz)	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (int., mult., *J* Hz)	^{13}C-NMR δ_C (mult.)	^{1}H-NMR δ_H (int., mult., *J* Hz)	^{13}C-NMR δ_C (mult.)
1	-	170.3 (s)	-	170.1 (s)	-	170.5 (s)
3	5.71 (1H, q, 6.5)	77.5 (d)	5.77 (1H, m)	77.2 (d)	5.70 (q, 6.5)	76.6 (d)
4	-	150.2 (s)	-	116.2 (s)	-	149.2 (s)
5	-	157.7 (s)	-	156.7 (s)	-	156.6 (s)
6	7.17 (1H, d, 8.5)	107.5 (d)	7.12 (1H, d, 7.8)	107.3 (d)	6.93 (1H, s, 7.8)	106.3 (d)
7	7.51 (1H, dd, 8.5, 7.8)	123.9 (d)	7.48 (1H, dd, 8.3, 7.8)	123.2 (d)	7.54 (1H, d, 8.3)	123.7 (d)
8	7.70 (1H, d, 7.8)	128.7 (d)	7.69 (1H, d, 8.3)	127.7 (d)	7.54 (1H, d, 8.3)	127.6 (d)
9	7.88 (1H, s)	116.6 (d)	7.93 (1H, s)	149.4 (d)	7.84 (1H, s)	116.5 (d)
10	-	126.8 (s)	-	125.5 (s)	-	125.9 (s)
11	-	128.0 (s)	-	128.1 (s)	-	127.9 (s)
12	-	118.3 (s)	-	117.5 (s)	-	117.5 (s)
13	-	138.3 (s)	-	137.3 (s)	-	137.2 (s)
3-CH_3	1.69 (3H, d, 6.5)	19.5 (q)	1.64 (1H, d, 1.2)	19.4 (q)	1.73 (1H, d, 6.5)	19.2 (q)
5-OCH_3	4.21 (3H, s)	57.1 (q)	4.04 (s)	56.4 (q)	4.11 (1H, s)	56.9 (q)
4-OH	9.88 (1H, s)	-	-	-	9.63 (1H, s)	-

and 128.0 ppm (C-11), and the aromatic proton at δ_H 7.88 ppm was correlated to δ_C 126.8 (C-10) and 138.3 ppm (C-13), which indicated that the hydroxyl group and aromatic proton were located at C-4 and C-9, respectively. The methoxyl proton at δ_H 4.21 ppm was correlated to δ_C 157.7 ppm (C-5), which indicated that the methoxy group was located at C-5. The methyl proton at δ_H 1.69 ppm was correlated to δ_C 77.5 (C-3) and 128.0 ppm (C-11), which indicated that the methyl group was located at C-3. The signal of an oxygenated sp^3 methine at δ_H 5.71 ppm was correlated to the carbonyl lactone at δ_C 170.3 ppm (C-1), which indicated that the lactone ring was formed between C-10, C-1, and C-3. Aromatic signals at δ_H 7.17 and 7.70 ppm were correlated to δ_H 157.7 (C-5) and 138.3 ppm (C-13), whereas another aromatic signal at δ_H 7.51 ppm was correlated to δ_C 107.5 (C-6) and 128.7 ppm (C-8), which suggested the presence of ABC aromatic protons in **1**.

In addition, the ^{1}H-^{1}H COSY spectrum showed a correlation of H-6, H-7, and H-8, which supported the presence of ABC aromatic protons from a trisubstituted benzene ring.

A detailed comparison of the NMR data of **1** to those of eleutherol and isoeleutherol [14,15], revealed that the structure of the compound **1** is more closely related to isoeleutherol rather than eutherol. The assignment as isoeleutherol was supported also by comparing the measured specific optical rotation of **1** $[\alpha]^{20}_D$ -64^o (*c* 0.10, $CHCl_3$) to that of isoeleutherol ($[\alpha]^{20}_D$ -60.5^o (*c* 0.5, $CHCl_3$)] [14] and eleutherol ($[\alpha]^{18}_D$ +83^o (*c* 0.373, $CHCl_3$)] [15,16]. Consequently, compound **1** was identified as isoeleutherol.

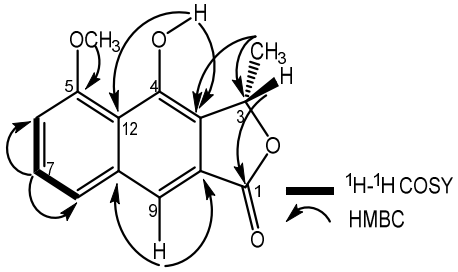

Figure 2. Selected ^{1}H-^{1}H COSY and HMBC Correlations for 1

Isoeleutherol (**1**) was evaluated for its radical scavenging activity using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, using ascorbic acid as a positive control [17]. Isoeleutherol showed moderate antioxidant activity with an IC_{50} value of 53.96 ± 2.87 µg/mL.

Conclusions

A known naphthalene, isoeleutherol (**1**) has been isolated from the herb of *Lygodium microphyllum.* Isoeleutherol **1** was isolated from this plant for the first time and showed moderate antioxidant activity in a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Because it was previously unknown that *Lygodium microphyllum* contained isoeleutherol, it may be of interest to test isoeleutherol for activity in a variety of additional assays, such antiplasmodial, antibacterial, and antiviral assays."

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References

[1] Lee, S., Xiao, C., Pei, S. 2008. Ethnobotanical survey of medicinal plants at periodic markets of Honghe Prefecture in Yunnan Province, China. J. Ethnopharmacol. 117(2): 362–377. Doi: 10.1016/j.jep.2008.02.001.

[2] Upreti, K., Jalal, J.S., Tewari, L.M., Joshi, G.C., Pangtey, Y.P.S., Tewari, G. 2009. Ethnomedicinal uses of Pteridophytes of Kumaun Himalaya, Uttarakhand, India. J. Am. Sci. 5(4): 167–170.

[3] Zheng, X.L., Xing, F.W. 2009. Ethnobotanical study on medicinal plants around Mt. Yinggeling, Hainan Island, China. J. ethnopharmacol. 124(2): 197-210. Doi: 10.1016/j.jep.2009.04.042.

[4] Guo-Gang, Z., Ying-Cui, H., Hong-Xia, L., Lin-Xia, Z., Li-Juan, C. 2012. The Research of Lygodium. Intechopen. Shanghai. pp. 78-106.

[5] Ma, C.M., Nakamura, N., Miyashiro, H., Hattori, M., Komatsu, K., Kawahata, T., Otake, T. 2002. Screening of Chinese and Mongolian herbal drugs for anti- human immunodeficiency virus type 1 (HIV- 1) activity. Phytother. Res. 16(2): 186-189. Doi: 10.1002/ptr.922.

[6] Cao, H., Chai, T.T., Wang, X., Morais-Braga, M.F.B., Yang, J.H., Wong, F.C., Wang, R., Yao, H., Cao, J., Cornara, L., Burlando, B. 2017. Phytochemicals from fern species: potential for medicine applications. Phytochem. Rev. 16(3): 379-440. Doi:10.1007/s11101-016-9488-7.

[7] Zhang, L., Yin, Z., Ye, W. 2006. Flavonoids from Lygodium japonicum. Biochem. Syst. Ecol. 34: 885-886. Doi: 10.1016/j.bse.2006.04.009.

[8] Kuncoro, H., Farabi, K., Julaeha, E., Rijai, L., Shiono, Y., Supratman, U. 2017. Flavonols From The Leaves Lygodium microphyllum (Lygodiaceae). Jurnal Kimia. 11(1): 10-14.

[9] Ye, W., Fan, C., Zhang, L., Zhiqi, Yin., Zhao, S. 2007. A new phenolic glycoside from the roots of *Lygodium japonicum*. Fitoterapia. 78(7–8): 600–601. Doi: 10.1016/j.fitote.2007.04.003.

[10] Chen, L., Zhang, G., He, J., Guan, J., Pan, C., Mi, W., Wang, Q. 2010. New naphthoquinone from the root of *Lygodium japonicum* (Thunb.) Sw. J. Nat. Med. 64(1): 114–116. Doi: 10.1007/s11418-009-0376-y.

[11] Zhu, L., Zhang, G., Chen, L., Wang, S., Li, P., Li, L. 2009. A new ecdysteroside from *Lygodium japonicum* (Thunb.) Sw. J. Nat. Med. 63(2): 215–219. Doi: 10.1007/s11418-008-0310-8.

[12] Kuncoro, H., Farabi, K., Julaeha, E., Rijai, L., Supratman, U. 2015. Stigmast-5(6)-en-3-ol dari Herba Tumbuhan Krokot (*Lygodium microphyllum*). Jurnal Kimia Valensi. 1(1): 50-54. Doi:10.15408/jkv. v0i0.3152.

[13] Arung, E.T., Kusuma, I.W., Kim, Y., Shimizu, K., Kondo, R. 2012. Antioxidative compounds from leaves of Tahongai (*Klienhovia hospita*). J. Wood Sci. 58(1): 77–80. Doi: 10.1007/s10086-011-1217-7.

[14] Zhengxiong, C., Huizhu, H., Chengrui, W., Yuhui, L., Jianmi, D., Sankawa, U., Noguchi, H., Iitaka, Y. 1986. Hongconin, a new naphthalene derivative from Hong-Cong, the Rhizome of *Eleutherine Americana* Merr (Iridaceae). Chem. Phar. Bull. 34(7): 2743-2746. DOI: doi: 10.1055/s-0029-1186143

[15] Chen, D.L., Hu, M.G., Liu, Y.Y., Li, R.T., Yu, M., Xu, X.D., Ma, G.X. 2018. New Naphthalene Derivatives from the Bulbs of *Eleutherine americana* with Their Protective Effect on the Injury of HUVECs. Molecules. 223: 1-9. DOI: 10.3390/molecules23092111

[16] Hara, H., Moruyama, N., Yamashita, S., Hayashi, Y., Lee, K.H., Bastow, K.F., Chairul., Marumoto, R., Imakura, Y. 1997. Elecanin, a novel new naphthoquinone from the bulb of *Eleutherine americana*. Chem. Pharm. Bull. 45(10): 1714-1716.

[17] Rijo, P., Falé, P.L., Serralheiro, M.L., Simões, M.F., Gomes, A., Reis, C. 2014. Optimization of medicinal plant extraction methods and their encapsulation through extrusion technology, Measurement. 58: 249–255. Doi: 10.1016/j.measurement.2014.08.045.