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Antimalarial Activity of Furoquinoline Alkaloids from the Leaves of *Melicope moluccana*

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Abstract

Two furoquinoline alkaloids, leptanoine C (**1**) and haplopine-3,3´-dimethylallyl ether (**2**) were isolated from the leaves of *Melicope moluccana*. The chemical structure of both compounds was determined based on spectroscopic data, including UV, IR, HR-ESI-MS, 1D, and 2D NMR spectral data. The antimalarial activity of compounds **1-2** against *Plasmodium falciparum* 3D7 showing their IC_{50} values are 0.18 ppm and 2.28 µg/mL, respectively.

Keywords: *Melicope moluccana.*, alkaloid, leptanoine C, haplopine-3,3´-dimethylallyl ether, antimalarial

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■ Introduction

Melicope is a genus of the *Rutaceae* family, which consists of 230 species. *Melicope* plants are spread in tropical and subtropical regions. *Melicope* plants are often used by the community in traditional medicine, such as fever and high blood pressure [1]. The utilization of this plant is undoubtedly associated with secondary metabolites. Based on literature studies, secondary metabolites found in *Melicope* plants include alkaloids, coumarins, flavonoids, benzopirans, and terpenoids [2-7]. Recently, the *Plasmodium* parasites, showing resistance to malaria drugs such as chloroquine and artemisinin [8]. *Melicope moluccana* is one species of the family Rutaceae and is endemic in the Maluku Islands with the name 'Ki Sampang.' The decoction of the leaves of *M. moluccana* is used by the people of Maluku for the treatment of malaria [6]. This study aims to isolate the alkaloid compounds contained in *M. moluccana* leaves and determine the antimalarial activity against *Plasmodium falciparum* 3D7. Two furoquinolines, leptanoin C (1) and 7-O-isoprenyl-γ-fagarin (2), were isolated from the leaves of *M.*

moluccana. The antimalarial activity against *Plasmodium falciparum* 3D7 also reported.

■ Experimental

General Prosedure

Kieselgel 60 GF_{254} TLC Plates 0.25 mm (Merck) for thin-layer chromatography (TLC). Silica gel 60 GF_{254} for vacuum liquid chromatography (VLC) and compressed column chromatography, and 60 PF_{254} silica gel for radial chromatography purposes. The UV spectrum was measured with a Shimadzu 1800 UV-Vis spectrophotometer. The IR spectrum was recorded with the Perkin Elmer IR spectrophotometer. The mass spectrum was measured with the ESI-TOF (LPR XE Waters) spectrometer (Electro Spray Ionization-Time of Flight), as well as the ^{1}H-NMR Spectrum and ^{13}C-NMR determined by NMR JEOL JICA 400 spectrometers operating at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C-NMR). Determination of antimalarial activity using the Trager and Jensen method.

Plant Material

The fresh leaves of *M. moluccana* was collected in Jun 2016 in the Conservation Area, District Weda, Central Halmahera District, North Maluku Province, Indonesia [6]. Identification of *M. moluccana* was determined at Herbarium Bogoriense, Botanical Garden Biology, Bogor, Indonesia. The voucher specimen (CP 20160624) was deposited at Herbarium Bogoriense.

Extraction and Isolation

Extraction of the leaves of *M. moluccana* (3.0 kg) with methanol at room temperature. The methanol extract obtained was evaporated using a rotary vacuum evaporator and the thick methanol extract was obtained. The thick methanol extract was added with 5% sulfuric acid (pH 3-4) and then partitioned successively with n-hexane and ethyl acetate. The acid phase is then added with ammonia (pH 8-9) and partitioned with ethyl acetate. Ethyl acetate extract containing alkaloid compounds was washed with water and evaporated with a rotary vacuum evaporator to produce a crude extract of alkaloids. VLC fractionated the crude alkaloid extract (12.5 g) with a mixture of n-hexane: ethyl acetate (9:1. 8:2, and 7:3) to get three fractions, A-C. Separation of fraction C (469.8 mg) by compressed column chromatography with n-hexane: ethyl acetate (9: 1, 8: 2, and 7: 3) produces three subfractions of C1, C2, and C3. Purification and purification of C1 subfraction (124 mg) by radial chromato-graphy using a mixture of n-hexane: ethyl acetate (9:1. 8:2, and 7:3) eluents produced compound **2** (8.6 mg). Purification and purification of C2 subfraction (153.8 mg) by radial chromatography using a mixture of n-hexane: ethyl acetate (9: 1, 8: 2, and 7: 3) eluents produced compound **1** (11.2 mg).

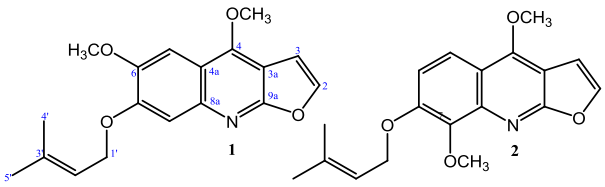

Figure 1. Furoquinoline alkaloids of **1**-**2**

Antimalarial Activity

The antimalarial activity test of the two isolated alkaloids was carried out in vitro using the Trager and Jensen method of *P. falciparum* strain 3D7, which was sensitive to chloroquine in a 96-microwell plate containing isolated compounds in various concentrations (100, 50, 10, 1, 0.1 µg/mL) and incubated at 37 °C for 48 hours. Inhibition of test compounds against the growth of parasitemia was observed with the aid of a microscope [8-9].

■ Results and Discussion

Phytochemical study on the ethyl acetate extract containing alkaloid compounds yielded two furoquinoline alkaloids, namely leptanoine C (**1**) and haplopine-3,3´-dimethylallyl ether (**2**) were isolated from the leaves of of *M. moluccana*.

Leptanoine C (**1**) was isolated as a light yellow solid. The mass spectrum of **1,** showing the positive quasimolecular ion peak $[M+H]^{+}$ at *m/z* 314.1407 which is in accordance with the molecular formula $C_{18}H_{20}NO_4$ (calculation

$[M+H]^+$ *m/z* 314,1392) based on the HRESIMS spectrum. The UV spectrum of **1** in methanol shows the maximum absorption at λ_{max} nm (log ε): 243 (4.5), 269 (3.6), 323 (3.8), and 334 (3.65), which are characteristic for furoquinoline chromophore [10]. The IR spectrum of **1,** showing the absorption band at ν (cm^{-1}): 1622-1587 (C = C aromatic) and 1236 (C-O-C ether). The proton of **1** consists of the furan ring, quinoline moiety, *O*-isoprenyl, and methoxy in the ^{1}H NMR. The resonances of the furan ring consist of a pair of the vinylic proton at δ_H 7.55 (1H, d, *J* = 2.8 Hz, H-2), and δ_H 7.02 (1H, d, *J* = 2.8 Hz, H-3). Two aromatic protons of quinoline moiety exhibited at δ_H 7.48 (1H, s Hz, H-5), and δ_H 7.33 (1H, s, H-8). The resonances of *O*-isoprenyl exhibited the presence of a vinyl proton at δ_H 5.59 (1H, tm, *J* = 6.7 Hz, H-2´), an oxy-methylene proton [δ_H 4.73 (2H, d, *J* = 6.6 Hz, H-1´), and two methyl proton [δ_H 1.79 (3H, s, H-4´), 1.77 (3H, s, H-5´)]. The ^{1}H NMR of **1** also showed two methoxy protons [δ_H 4.42 (3H, s, 4-OCH_3´), δ_H 4.00 (3H, s, 6-OCH_3´)]. The ^{13}C-NMR spectra of **1** consisted of 18 carbons distributed over eight quaternary carbon atoms (δ_C 163.0; 155.5; 151.8; 148.0; 142.5; 138.3; 112.8 and 102, 0), five methine carbons (δ_C 142.3; 119.2; 107.6; 104.6; and 101.1), one methylene carbon (δ_C 65.7) and four methyl carbons (δ_C 58.8; 56.0; 25.9 and 18.3). The placement of two methoxy and *O*-isoprenyl groups in the furoquinoline skeleton was determined based on the analysis of HMQC and HMBC spectra. Analysis of the HMBC spectrum, the furan ring proton signal at δ_H 7.55 (H-2) showed correlation with the furoquinoline skeleton at C-3 (δ_C 104.6), C-3a (δ_C 102.0), and C-9a (δ_C 163.0). The other furan ring proton signals at δ_H 7.02 (H-3) showed a correlation with C-2 (δ_C 142.3), C-3a, and C-9a. Two aromatic singlet signals at δ_H 7.48 (H-5), and δ_H 7.33 (H-8), showing two two methoxy and *O*-isoprenyl groups bound to C-4, C-5, and C-8 in the aromatic nucleus of the furoquinoline skeleton. The resonance of aromatic at δ_H 7.48 (H-5) correlated to C-4 (δ_C 104.6), C-4a (δ_C 112.8), C-6 (δ_C 148.0), C-7 (δ_C 151.8), and C-8a (δ_C 142.6). The resonance of methoxy at δ_H 4.42 (4-OCH_3) correlated to C-4. The other methoxy at δ_H δ_H 4.00 (6-OCH_3´) correlated to C-6. The resonance of oxy-methylene at δ_H 4.73 (H-1´) correlated to C-7, C-2´ (δ_C 119.2), and C-3´ (δ_C 138.3). The correlation of two methoxy and oxy-methlene signals with a carbon signal showed bound methoxy at C-4, C-5, and *O*-isoprenyl bound at C-7. The HRESIMS, 1D, and 2D NMR spectral data; the structure of **1** was identified as 4,6-dimethoxy-7-*O* –isoprenylquinoline. The structure of 4,6-dimethoxy-7-*O* –isoprenylquinoline as leptanoin C [11]. Based on HMBC correlations, supporting the structure of **2** are shown in Table 1, and Figure 2.

Haplopine-3,3´-dimethylallyl ether (**2**) was obtained as a yellow solid. The mass spectrum of **2,** showing the positive quasimolecular ion peak $[M+H]^+$ at *m/z* 314.1403 correspondings to the molecular formula $C_{18}H_{20}NO_4$ (calculation $[M+H]^+$ *m/z* 314,1392) based on the HRESIMS spectrum. The UV spectrum (λ_{max} (log ε) 250 (4.6), 269 (3.5), 320 (3.70), 333 (3.7) nm, and HRESIMS spectrum very similar to those of **1**. The proton of **2** consists of the furan ring [δ_H 7.52 (1H, d, *J* = 2.8 Hz, H-2)], aromatic of quinoline moiety [δ_H 7.92 (1H, d, *J* = 9.3 Hz, H-5), δ_H 7.17 (1H, d, *J* = 9.3 Hz, H-6)], *O*-isoprenyl [δ_H 5.53 (1H, tm, *J* = 6.7 Hz, H-2´), δ_H 4.72 (2H, d, *J* = 6.7 Hz, H-1´), δ_H 1.75 (s, H-4´), 1.74 (s, H-5´)], and two methoxy protons [δ_H 4.36 (3H, s, 4-OCH_3´), δ_H 4.00 (3H, s, 8-OCH_3´)]. The ^{13}C-NMR spectra of **2** consisted of 18 carbons very similar to those of **1**. The spectrum HMBC, the resonance of aromatic at δ_H 7.92 (H-5) correlated to C-4 (δ_C 157,0), C-7 (δ_C 151.2), and C-8a (δ_C 141.4) The resonance of methoxy at δ_H 4.36 (4-OCH_3) correlated to C-4. The resonance of oxy-methylene at δ_H 4.72 (H-1´) correlated to C-7, C-2´ (δ_C 120.0), and C-3´ (δ_C 137.8). The correlation of methoxy and oxy-methylene signals with a carbon signal showed bound methoxy at C-4, *O*-isoprenyl bound at C-7, and also showed the other methoxy attached at C-8. The 1D, and 2D NMR spectral data; the structure of **2** was identified as 4,8-dimethoxy-7-*O*–isoprenylquinoline or know as haplopine-3,3´-dimethyl-allyl ether [12]. Based on HMBC correlations, supporting the structure of **2** are shown in Table 2 and Figure 2.

Table 1. NMR data leptanoine C in $CDCl_3$

No	Leptanoine C (**1**)			Leptanoine C [11] in $CDCl_3$	
	δ_H	**δ_C**	**HMBC**	**δ_H**	**δ_C**
2	7.55 (*d*, 2.8)	142.3	C-3, C-3a, C-9a	7.59 (*d*, 2.6)	142.8
3	7.02 (*d*, 2.8)	104.6	C-2, C-3a, C-9a	7.06 (*d*, 2.6)	104.9
3a	-	102.0	-	-	103.4
4	-	155.5	-	-	156.0
4a	-	112.8	-	-	112.9
5	7.48 (*s*)	101.1	C-4, C-4a, C-6, C-7, C-8a	7.46 (*s*)	100.5
6	-	148.0	-	-	148.7
7	-	151.8	-	-	152.8
8	7.33 (*s*)	107.6	C-4a, C-6, C-7, C-8a	7.48 (*s*)	106.8
8a	-	142.5	-	-	139.0
9a	-	163.0	-	-	162.0
1’	4.73 (*d*, 6.6)	65.7	C-7, C-2’, C-3’	4.76 (*d*, 4.0)	66.2
2’	5.59 (*tm*, 6.7)	119.2	C-4’, C-5’	5.59 (*tm*, 6.6)	119.2
3’	-	138.3	-	-	138.8
4’	1.79 (*s*)	25.9	C-2’, C-3’, C-5’	1.80 (*s*)	26.0
5’	1.77 (*s*)	18.3	C-2’, C-3’, C-4’	1.80 (*s*)	18.5
4-OCH_3	4.42 (*s*)	58.8	C-4	4.46 (*s*)	59.3
6-OCH_3	4.00 (*s*)	56.0	C-6	4.00 (*s*)	56.2

Table 2. NMR data haplopine-3,3´-dimethyl-allyl ether in $CDCl_3$

No	Haplopine-3,3´-dimethyl-allyl ether (2)			Haplopine-3,3´-dimethyl-allyl ether [12] in DMSO-*d6*	
	δ_H	δ_C	HMBC	δ_H	δ_C
2	7.52 (*d*, 2.8)	142.8	C-3, C-3a, C-9a	7.80 (*d*, 2.0)	143.3
3	6.98 (*d*, 2.8)	104.5	C-2, C-3a, C-9a	6.75 (*d*, 2.0)	105.0
3a	-	101.8	-	-	102.3
4	-	157.0	-	-	157.5
4a	-	114.8	-	-	115.3
5	7.92 (*d*, 9.3)	117.8	C-4, C-7, C-8a	8.07 (*d*, 9.0)	118.2
6	7.17 (*d*, 9.3)	114.0	C-4a, C-7, C-8	6.95 (*d*, 9.0)	114.7
7	-	151.2	-	-	151.8
8	-	142.6	-	-	143.2
8a	-	141.4	-	-	141.9
9a	-	164.1	-	-	164.6
1’	4.72 (*d*, 6.7)	66.6	C-7, C-2’, C-3’	4.80 (*d*, 6.6)	67.2
2’	5.53 (*t*, 6.7)	120.0	C-4’, C-5’	5.57 (*t*, 6.8)	120.5
3’	-	137.8	-	-	138.2
4’	1.75 (*s*)	25.7	C-2’, C-3’, C-5’	1.98 (*s*)	26.1
5’	1.74 (*s*)	18.1	C-2’, C-3’, C-4’	1.98 (*s*)	18.7
4-OCH_3	4.36 (*s*)	58.8	C-4	4.47 (*s*)	59.3
8-OCH_3	4.07 (*s*)	61.4	C-8	4.01 (*s*)	61.9

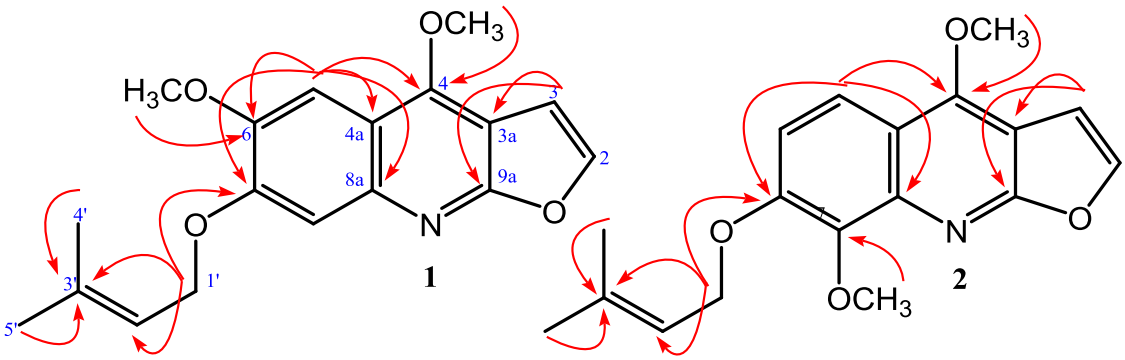

Figure 2. Selected HMBC correlations for **1-2**

Antimalarial activity of compounds **1-2** against *P. falciparum* strain 3D7 showed IC_{50} values of 2.28 and 0.18 µg/mL, respectively. Chloroquine was used as a positive control, showing the IC_{50} values of 1.03 µg/mL. Based on antimalarial test data showed that compound 1 was categorized as moderate, while compound 2 was categorized as very active [8]. Both of these alkaloid compounds, based on microscopic observations, showed that both compounds inhibited the growth of schizoids. Thus, both compounds are beneficial for malaria sufferers. Leptanoine C (**1**) and haplopine-3,3´-dimethyl-allyl ether (**2**) have potential as an antimalarial. Furthermore, compounds 1-2 suggested to cytotoxic assay, *in vivo* assay, preclinic assay for applied as malaria drug development.

Conclusion

Two furoquinolines, leptanoine C (**1**) and haplopine-3,3´-dimethylallyl ether (**2**) were isolated from the leaves of *Melicope moluccana*. Antimalarial activity of compounds **1-2** against *P. palcifarum*, showing against *Plasmodium falciparum* 3D7 showing their IC_{50} values are 0.18 ppm and 2.28 µg/mL, respectively.

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