Melimoluccanin, A new isoprenylated quinolone alkaloid from the leaves of *Melicope moluccana* T.G. Hartley

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Abstract. A new quinolone alkaloid, melimoluccanin (1) along with two known alkaloids, leptanoine C (2) and 7-O-isoprenyl-γ-fagarin (3) were isolated from the leaves of *Melicope moluccana* T.G. Hartley. Structures all of compounds were elucidated using spectroscopic methods such as UV, IR, HRESIMS, 1D and 2D NMR. Compounds 1-3 were evaluated for their cytotoxicity against P-388 cells and for antimalaria activity using *giemsa* methods. The cytotoxic data of compounds 1-3 showed IC₅₀ of 0.63; 5.85 and 2.09 μg/mL, respectively. The antiplasmodial activity of compound 1-3 showed IC₅₀ value of 4.28; 2.28 and 0.18 μg/mL, respectively. Based on the data, it can be concluded that compound 1 has high activity against P-388 cells and compound 3 is potential as antimalaria.

Keywords: Melimoluccanin, *Melicope moluccana*, antimalaria

1. Introduction

Malaria is a major cause of death in the world caused by a protozoan of the genus *Plasmodium* and transmitted by *Anopheles* mosquito vectors, especially in tropical countries. Recently, chloroquine and artemisinin have been used as antimalarial drug and showed resistance against *Plasmodium* parasites in Indonesia.

*Melicope* is one of the genus of family Rutaceae, consisting of about 280 species. The plants have been known produce a variety of alkaloids [1,2], flavonoids [3], coumarins [4], acetophenones [5] and lignans [4], which exhibit various biological activities including antioxidant [4], anticancer [1,2], and antiinflammatory [6].
This study is part of our research on the chemical constituents of Melicope species found in Indonesia. In continuation of our research for alkaloid compound in this medicinal plant, we report the isolation of 4-methoxy-3-(3-methylbut-2-en-1-yl)-7-((3-methylbut-2-en-1-yl)oxy)quinolin-2(1H)-one (1) from the methanol extract of the leaves of Melicope moluccana T.G. Hartley. The chemical structure of compound 1 were established by UV, IR, HRESIMS, 1D and 2D NMR, as well as by comparison with those related compounds previously reported. The cytotoxic and antioxidant activities of isolated compound from this species are also briefly described.

In this paper we report the isolation of melimoluccanin (1) melineurine (2) and 7-O-isoprenyl-γ-fagarin (3) from the leaves of Melicope moluccana T.G. Hartley. In addition, preliminary cytotoxic and antiplasmodial activities of the isolated compounds cells will also be described.

2. Materials and Methods

2.1 General experimental

$^1$H and $^{13}$C NMR spectra were measured on a JEOL JNM-ECA spectrophotometer operating at 400 ($^1$H) and 100 ($^{13}$C) MHz using residual and deuterated solvent peaks ($\delta_{\text{H}}$ 7.26 and $\delta_{\text{C}}$ 77.1 for CDCl$_3$) as reference standards. High-resolution mass spectra were obtained with an ESI-TOF Waters LCT Premier XE mass spectrometer. UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-VIS spectrophotometer. IR spectra were recorded in KBr on a One Perkin Elmer instrument. Column chromatography and planar radial chromatography were carried out using silica gel 60 and silica gel 60 PF$_{254}$.

2.2 Plant Material

The leaves of Melicope moluccana T.G. Hartley were collected in July 2015 from the conserved forest of Weda, Central Halmahera, North Maluku, Indonesia. The plant was identified at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia.

2.3 Extraction and isolation

The dried and powdered leaves of M. moluccana T.G. Hartley (2.0 kg) was macerated with methanol at room temperature for 24 h two times to give a dark methanol extract (108 g) after solvent evaporation. The methanol extract was partitioned into n-hexane (15 g) soluble fractions. The methanol extract was added 3% sulfuric acid and adjusted to pH 3-4. Furthermore, the acid extracts partitioned with ethyl acetate and then evaporated by rotavapor to yield ethyl acetate extract (8 g). The acid extracts were basified with ammonia solution and adjusted to pH 8-9 and then partitioned with ethyl acetate to give crude alkaloid. The crude alkaloid (5 g) of M. moluccana was separated by column chromatography on silica gel. Elution with n-hexane-ethyl acetate mixture containing increasing amount of ethyl acetate (9:1, to 1:1) to give four fractions A-D. On TLC analysis, fraction A (250 mg) showed two major spots. On separation of this fraction using column chromatography eluted with n hexane-EtOAc (from 4:1 to 1:1) gave three subfractions, A$_1$-A$_3$. Purification of subfraction A$_2$ by planar radial chromatography with eluent n hexane-CHCl$_3$ (from 7:3 to 1:4) yielded compound 1 (12 mg). Fraction C (470 mg), purification using planar radial chromatography with eluent n hexane-CHCl$_3$ (from 9:1 to 7:3) yielded compound 2 (18 mg) and 3 (19 mg).

2.4 Cytotoxic assay

Cytotoxic properties of the isolated compounds 1-3 against murine leukemia P-388 cells was evaluated according to the MTT method as previously described [7-9]. Artonin E was used as the positive control.
2.5 Plasmodial activity

Antiplasmodial activity of the isolated compounds 1-3 against *Plasmodium falciparum* strain 3D7 was evaluated according to Trager and Jensen method as previously described [10,11]. Chloroquine was used as the positive control.

3. Results and discussion

Phytochemical study on the MeOH extract from the leaves of *M. moluccana* yielded melimoluccanin (1), leptanoine C (2) and 7-O-isoprenyl-γ-fagarin (3).

![Figure 1. Alkaloid compounds 1-3 isolated from the leaves of M. moluccana.](image)

Melimoluccanin (1), isolated as yellow solid has a molecular formula of C$_{20}$H$_{25}$NO$_{3}$ based on HRESIMS spectrum ([M+H]$^+$ 328.1928, calcd m/z 328.1918). The UV spectrum exhibited absorption maxima $\lambda_{\text{max}}$ (nm) (log $\varepsilon$): 227 (4.57), 269 (3.86), 282 (3.85), 322 (4.09) and 336 (3.98) typical for a 2-quinolone structure [1]. The IR absorptions showed vibrations for conjugated carbonyl (1645 cm$^{-1}$), aromatic (1562, 1512, 1461 cm$^{-1}$) and ether (1093 cm$^{-1}$) groups, respectively. The $^1$H NMR spectrum of 1 (Table 1) showed the presence of ABX systems at $\delta_{\text{H}}$ 7.65 (d, $J = 8.8$ Hz; H-5), 6.83 (dd, $J = 8.8$; 2.4 Hz; H-6) and 6.79 (d, $J = 2.4$ Hz; H-8) characteristic for 2-quinolone with three substituents. Furthermore, the $^1$H NMR spectrum also showed an isoprenyl (3-methyl-2-butenyl) group [$\delta_{\text{H}}$ 1.83 (3H, s, H-4’), 1.79 (3H, s, H-5’), 3.39 (2H, d, $J = 6.8$ Hz, H-1’), 5.29 (1H, t, $J = 6.9$ Hz, H-2’)], an oxyisoprenyl (3-methylbut-2-en-1-yl)oxy) group [$\delta_{\text{H}}$ 1.83 (3H, s, H-4”), 1.70 (3H, s, H-5”), 4.59 (2H, d, $J = 6.8$ Hz, H-1”), 5.52 (1H, t, $J = 6.9$ Hz, H-2”)] and a singlet proton of methoxy group at $\delta_{\text{H}}$ 3.93. The $^{13}$C NMR spectrum
(Table 1) of 1 showed 20 carbon signals. The assignment of $^{13}$C NMR spectrum was confirmed by HMQQC and HMBC spectra. The placement of isoprenyl, oxyisoprenyl and methoxy groups in 2-quinolone skeleton were established by HMQQC and HMBC spectra. Based on HMBC spectrum of 1, a methoxyl signal at $\delta_{H}$ 3.93 correlated to $\delta_{C}$ 162.5 (C-4). The methylene proton signal of isoprenyl group at 3.39 (H-1’) correlated to a carbonyl of amide carbon ($\delta_{C}$ 166.0; C-2), an oxyaryl carbon ($\delta_{C}$ 162.5; C-4), two quarternary carbons [(δC 132.2, C-3’), (δC 119.3, C-3)] and a methine carbon signal (δC 121.8, C-2’), indicating that isoprenyl group attached at C-3 and methoxyl at C-4. The proton signal of aromatic region at $\delta_{H}$ 7.65 (H-5) showed long-range correlations with an oxyaril carbon signals (δC 160.6) and a quarternary carbon (δC 139.1). The result revealed δC 160.6 has position at C-7 and δC 139.1 at C-8a. Furthermore, the methylene proton signal of an oxyisoprenyl group at δH 4.59 (H-1”) correlated to a oxyaryl carbon (δC 160.6, C-7), a quarternary carbons (δC 139.0, C-3’”) and a methine carbon signal (δC 118.9, C-2’”), showing that oxyisoprenyl group attached at C-7. On the basis of the above spectral evidence, the structure of 1 was elucidated as melimoluccanin (1). Other HMBC correlations consistent with the structure 1 are shown in Table 1 and Figure 2. The structure of 1 is a novel compound.

**Table 1.** NMR spectroscopic data of melimoluccanin (1) in CDCl$_3$.

| No | C  | $\delta_{H}$ (mult, J Hz) | $\delta_{C}$ | HMBC |
|----|----|-------------------------|-----------|------|
| 2  | -  | 166.0                   | -         | -    |
| 3  | -  | 119.3                   | -         | -    |
| 4  | -  | 162.5                   | -         | -    |
| 4a | -  | 111.0                   | -         | -    |
| 5  | 7.65 (d, 8.8) | 124.2 | C-7, C-8a |
| 6  | 6.83 (dd, 8.8; 2.4) | 112.4 | C-4a, C-8 |
| 7  | -  | 160.6                   | -         | -    |
| 8  | 6.79 (d, 2.4) | 98.8 | C-4a, C-7, C-8a |
| 8a | -  | 139.1                   | -         | -    |
| 1’ | 3.39 (d, 6.8) | 23.2 | C-2, C-3, C-4, C-2’, C-3’ |
| 2’ | 5.29 (t, 6.9) | 121.8 | C-4’, C-5’ |
| 3’ | -  | 132.2                   | -         | -    |
| 4’ | 1.83 (s) | 18.0 | C-2’, C-3’, C-5’ |
| 5’ | 1.79 (s) | 25.7 | C-2’, C-3’, C-4’ |
| 1’’| 4.59 (d, 6.8) | 65.1 | C-7, C-2”’, C-3”’ |
| 2’’| 5.52 (t, 6.9) | 118.9 | C-4”, C-5”’ |
| 3’’| -  | 139.0                   | -         | -    |
| 4’’| 1.83 (s) | 18.3 | C-2”’, C-3”, C-5”’ |
| 5’’| 1.70 (s) | 25.9 | C-2”, C-3”, C-4”’ |
| 4-OCH$_3$ | 3.93 (s) | 61.8 | C-4 |
| NH | 11.29 (s) | - | - |
Leptanoine C (2) was isolated as a yellow solid, showed a quasimolecular ion [M+H]+ at m/z 314.1407 corresponding to the molecular formula C_{18}H_{20}NO_{4}. The UV spectrum exhibited absorption maxima λ_{max} (nm) (log ε): 243 (4.50), 323 (3.77) and 334 (3.65) typical for a furoquino line alkaloid [6]. The IR absorptions showed vibrations for aromatic (1622, 1587 cm\(^{-1}\)) and ether (1236 cm\(^{-1}\)) groups, respectively. The \(^1\)H NMR spectrum (Table 2) of 1 showed the presence of a pair (J = 2.8 Hz) of proton signal of furo at δ\(H\) 7.55 (H-2), δ\(H\) 7.02 (H-3) and two singlets of isolated aromatic proton at δ\(H\) 7.48 (H-5) and δ\(H\) 7.33 (H-8) which is typical for a furoquino line alkaloid with three substituents [6]. The \(^1\)H NMR spectrum also showed an oxyisoprenyl group [δ\(H\) 1.79 (3H, s, H-4'), 1.77 (3H, s, H-5'), 4.73 (2H, d, J = 6.6 Hz, H-1'), 5.59 (1H, t, J = 6.7 Hz, H-2')], and two singlets of methoxyl group at δ\(H\) 4.42 (4-OCH\(_3\)), and 4.00 (6-OCH\(_3\)). The \(^13\)C NMR spectrum (Table 1) of 2 showed 18 carbon signals were confirmed by HMQC and HMBC spectra. The placement of oxyisoprenyl at C-7, and two methoxyl groups at C-4 and C-6 in furoquino line skeleton were established by HMQC and HMBC spectra. Based on HMBC spectrum of 2, a methoxyl signal at δ\(H\) 4.42 correlated to δ\(C\) 155.5 (C-4) and a methoxy signal at δ\(H\) 4.00 correlated to δ\(C\) 148.0 (C-6). The methylene proton signal of an oxyisoprenyl group at δ\(H\) 4.73 (H-1') correlated to a oxyaryl carbon (δ\(C\) 151.8, C-7), a quarternary carbons (δ\(C\) 138.3, C-3') and a methine carbon signal (δ\(C\) 119.2, C-2'), showing that oxyisoprenyl group attached at C-7. Other HMBC correlations consistent with the structure 2 are shown in Table 2 and Figure 3.

Table 2. NMR spectroscopic data of leptanoine C (2) and 7-O-isoprenyl-γ-fagarin (3).

| No. | δ\(H\) (mult, J in Hz) | δ\(C\) | HMBC | δ\(H\) (mult, J in Hz) | δ\(C\) | HMBC |
|-----|-------------------------|--------|------|-------------------------|--------|------|
| 2   | 7.55 (d, 2.8)           | 142.3  | C-3, C-3a, C-9a | 7.52 (d, 2.8)           | 142.8  | C-3, C-3a, C-9a |
| 3   | 7.02 (d, 2.8)           | 104.6  | C-2, C-3a, C-9a | 6.98 (d, 2.8)           | 104.5  | C-2, C-3a, C-9a |
| 3a  | -                       | 102.0  | -    | -                       | 101.8  | -    |
| 4   | -                       | 155.5  | -    | -                       | 157.0  | -    |
| 4a  | -                       | 112.8  | -    | -                       | 114.8  | -    |
| 5   | 7.48 (s)                | 101.1  | C-4, C-4a, C-6 | 7.92 (d, 9.3)           | 117.8  | C-4, C-7, C-8a |
| 6   | -                       | 148.0  | -    | 7.17 (d, 9.3)           | 114.0  | C-4a, C-7, C-8 |
| 7   | -                       | 151.8  | -    | -                       | 151.2  | -    |
7-O-Isoprenyl-γ-fagarin (3) was isolated as a yellow solid, showed a quasi-molecular ion [M+H]+ at m/z 314.1403 corresponding to the molecular formula C_{18}H_{20}NO_{4}. The UV spectrum (λ_{max} 250, 269, 320 and 333 nm) and IR spectrum (1616, 1502 and 1263 cm\(^{-1}\)) absorptions were very similar to those of 2.

The \(^{13}\)C NMR spectrum of 3 showed 18 carbon signals and the structure of 3 were confirmed by HMQC and HMBC spectra (Table 2 and Figure 4). The 1D and 2D NMR spectra data are consistent for 7-O-isoprenyl-γ-fagarin (3).

The cytotoxic activity (Table 3) of compounds 1-3 were evaluated for their cytotoxicity by MTT assay against murine leukemia P-388. Artonin E was used as the positive control. The cytotoxic activity of 1 showed high activity with IC\(_{50}\) 0.63 µg/mL. Those cytotoxic data for
alkaloids 2-3 suggested to have moderate activity. Methoxyl group at C-8 of compound 3 showed higher activity than 2 (methoxyl group at C-6). The antiplasmodial activity (Table 3) of compounds 1-3 were evaluated according to Trager and Jensen method against against *Plasmodium falciparum*. Chloroquine was used as the positive control. The antiplasmodial activity of 2 showed high activity with IC$_{50}$ 0.18 µg/mL. Those antiplasmodial for alkaloids 1 and 3 suggested to have moderate activity.

**Table 3.** Cytotoxic and antiplasmodial activities of compounds 1-3

| No. | Compound                      | P-388 cells | Antiplasmodial |
|-----|-------------------------------|-------------|---------------|
|     |                               | IC$_{50}$ (µg/mL) | IC$_{50}$ (µg/mL) |
| 1   | Melimoluccanin (1)            | 0.63        | 4.28          |
| 2   | Leptanoine C (2)              | 5.85        | 2.28          |
| 3   | 7-O-Isoprenyl-γ-fagarin (3)    | 2.09        | 0.18          |
| 4   | Artonin E                     | 1.33        | -             |
| 5   | Chloroquine                   | -           | 1.03          |

4. Conclusions

The phytochemical investigation of the leaves of *M. moluccana* to yielded compounds, melimoluccanin (1), leptanoine C (2) and 7-O-isoprenyl-γ-fagarin (3). Melimoluccanin (1) showed high activity against murine leukemia P-388 cells. 7-O-Isoprenyl-γ-fagarin (3) showed high activity against *Plasmodium falciparum* strain 3D7.

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