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Melixyloidin, A New Acridone Alkaloid from *Melicope xanthoxyloides* Leaves

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Abstract – A new acridone alkaloid, melixyloidin (**1**), and two known alkaloids (**2-3**) were isolated from *Melicope xanthoxyloides* (F. Muell) T.G. Hartley leaves. The structure of melixyloidin were elucidated using NMR spectra and high-resolution ESIMS data. Acridone alkaloids **1-3** were evaluated against MCF-7 and HeLa cells. 1,3,4-Trimethoxy-10-methylacridin-9-one (**2**) showed potent cytotoxic activity against MCF-7 cells with an IC_{50} value of 5.31 μM.
Keywords – Melixyloidin, Acridone alkaloid, *Melicope xanthoxyloides*, Cytotoxic

Introduction

Melicope xanthoxyloides (F. Muell) T.G. Hartley is one of the endemic plants in Papua Island, Indonesia, belonging to the Rutaceae family. The *Melicope* genus produces alkaloids,[1-3] coumarins,[4] benzopyran,[5] and flavonoids[6-7] with an isoprenyl side chain. Many alkaloids from plants of the genus *Melicope* showed biological activities such as antimalaria,[7] anticancer,[1-3] and antioxidant[8] activities. Acridones, furoquinolines, and quinolinones are among the alkaloids found from the *Melicope* plants. Melicodenines A-H are hybrid compounds formed via the [2+2] cycloaddition and the Diels-Alder reaction in quinolinone-quinolinone, benzopyran-quinolinone, and phenylpropanoid-quinolinone.[2-3] Melicodenine G is highly cytotoxic to human colon cancer cells (DLD-1).[2-3] The objective of this research was to determine the cytotoxicity of acridone alkaloids from the leaves of *M. xanthoxyloides.* A new acridone alkaloid, melixyloidin (**1**), along with two known compounds, 1,3,4-trimethoxy-10-methylacridin-9-one (**2**), and melicopidine (**3**), were isolated from the *M. xanthoxyloides* leaves (Fig. 1). The cytotoxic activities of the isolated compounds (**1-3**) against MCF-7 and HeLa cell lines were evaluated in this research study.

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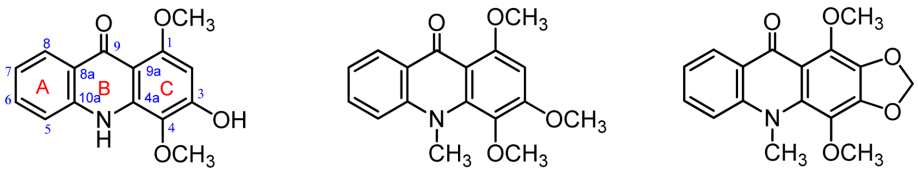
Experimental

General experimental procedures – The maximum absorption of alkaloids was measured using a Shimadzu UV-VIS 1800 spectrophotometer. The 1D and 2D NMR spectra of the alkaloids in $CDCl_3$ were measured using an FTNMR JEOL ECA-400 spectrometer. The exact mass and chemical formula of the new compound were determined using an LCT Waters Premier™ XE. Column chromatography was performed using Si gel G_{60} and Sephadex LH-20. The UV lamp and cerium sulfate reagent were used to visualize chemicals on TLC. To visualize alkaloids on TLC, a UV lamp and Dragendorff reagent were utilized.

Plant materials – The leaves of *M. xanthoxyloides* were collected in Siboru Village, Fakfak, West Papua, Indonesia, in December 2017. The plant material with specimens (FFK-IS9) was authenticated by Dr. Ismail R. of Herbarium Bogoriense, Indonesia.

Extraction and isolation – The air-dried *M. xanthoxyloides* leaves (2.0 kg) were pulverized and extracted twice with 95% MeOH at room temperature for two days. The methanol extract was partitioned with hexane (54 g) and then EtOAc (30 g) in that sequence. Separation of the EtOAc extract (25 g) on a silica gel column chromatography eluted with hexane-EtOAc (from 9:1 to 1:1 v/v) yielded three fractions A-C. Fraction B was separated using Sephadex LH-20 column chromatography eluted with MeOH to provide subfractions B_1-B_2. After purification by silica gel radial chromatography eluted with



1 2 3

Fig. 1. Structures of the isolated alkaloids (**1-3**) from *M. xanthoxyloides* leaves.

hexane-$CHCl_3$ (3:7 to 7:3 v/v), alkaloids **1** (17 mg), **2** (11 mg), and **3** (9 mg) were obtained from fraction B_2 (780 mg).

Melixyloidin (**1**) – Yellow powder, m.p. 170-172 °C, UV (MeOH) λ_{max} nm (log ε): λ_{maks} nm (log ε): 272 (3.84), 293 (3.31), 333 (2.66), and 392 (2.81). The NMR spectra see Table 1. HRESIMS: *m/z* $[M+H]^+$ calculated for $C_{15}H_{13}NO_4$ 272.0923, found 272.0926.

1,3,4-Trimethoxy-10-methylacridin-9-one (**2**) – White powder, m.p. 144-146° C, UV (MeOH) λ_{max} nm (log ε): 272 (4.46), 289 (4.27), 313 (3.94), and 393 (4.24). The comparison of the NMR spectra of **2** has similarities to the literature data.[9]

Melicopidine (3) – Yellow powder, m.p. 162-164 °C, UV (MeOH) λ_{max} nm (log ε): 279 (4.39), 284 (4.35), 311 (4.31), and 382 (3.13). The comparison of the NMR spectra of **3** has similarities to the literature data.[9]

Cytotoxic activity – The cytotoxicity assays of alkaloids (**1-3**) were evaluated against human breast cells (MCF-7) and human cervical cells (HeLa) by the MTT assay. [10-12] Doxorubicin was used as a positive control. The MCF-7 and HeLa cells were cultivated in the RPMI-1640 medium containing 10% fetal bovine serum at 37 °C flowed with 5% CO_2 in the air for 48 h. An ELISA reader measured the inhibition of acridone alkaloids (**1-3**) at λ 590 nm. Different IC_{50} values for **1-3** were noted by regression analysis.[10-12]

Result and Discussion

Compound **1** (melixyloidin) was isolated as a yellow powder with an ion peak $[M+H]^+$ at *m/z* 272.0926 (calcd 272.0923), which was confirmed by high-resolution MS to be compatible with the chemical formula $C_{15}H_{13}NO_4$. The maximum absorption was observed in the UV spectrum of **1** at λ_{max} (log ε): 272 (3.84), 293 (3.31), 333 (2.66), and 392 (2.81), which is a typical UV pattern of the acridone alkaloid.[9] The ^1H NMR spectrum of melixyloidin (Table 1) revealed protons for two aromatic units and two methoxy groups. At ring A, four aromatic protons [δ_H 8.25 (1H, dd, J = 1.3; 8.2 Hz, H-8), δ_H 7.67 (1H, dt, J = 1.3; 7.3 Hz, H-6), δ_H 7.49 (1H, d, J = 8.4 Hz, H-5), and δ_H 7.26 (1H, t, J = 7.3 Hz, H-7)] were indicative of a 1,2-disubstituted benzene.[13-14] At ring C, there was an aromatic signal at δ_H 6.48 (1H, s, H-2) that was a typical

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for melixyloidin (**1**)

No.C	δ_H (mult, *J* in Hz)	δ_C	HMBC
1	-	158.5	-
2	6.48 (*s*)	88.5	C-1; C-4; C-9a
3	-	151.5	-
4	-	129.4	-
4a	-	138.1	-
5	7.49 (*d*, 8.2)	117.6	C-7; C-8a
6	7.67 (*dt*, 1.3; 7.3)	134.4	C-8; C-10a
7	7.26 (*t*, 7.3)	122.2	C-5; C-8a
8	8.25 (*dd*, 1.3; 8.2)	126.2	C-6; C-9; C-10a
8a	-	118.8	-
9	-	182.0	-
9a	-	105.6	
10a	-	139.9	
1-OCH_3	3.90 (*s*)	56.2	C-1
4-OCH_3	3.75 (*s*)	60.4	C-4

Table 2. Cytotoxic activities of alkaloids **1-3**

Compounds	IC_{50} (μM)	
	MCF-7	HeLa
Melixyloidin (**1**)	> 100	> 100
1,3,4-Trimethoxy-10-methylacridin-9-one (**2**)	5.31 ± 0.12	39.18 ± 0.27
Melicopidine (**3**)	59.92 ± 0.46	74.32 ± 0.37
Doxorubicin	10.48 ± 0.38	0.15 ± 0.70

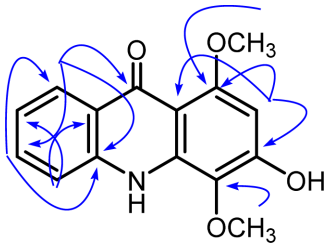


Fig. 2. HMBCs of melixyloidin (**1**).

signal of 1,2,3,4,5-pentasubstituted benzene and it was confirmed by HMBC data[15-16] Two methoxy groups [δ_H 3.90 (3H, s, 1-OCH_3), and δ_H 3.75 (3H, s, 4-OCH_3)] were also detected in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum of melixyloidin (Table 1) showed 15 signals, including five methine carbons, two methoxy carbons, four quaternary carbons, one carbonyl carbon, and three oxyaryl carbons. In the HMQC spectrum, four aromatic protons at ring A ([δ_H 8.25 to δ_C 126.2 (C-8), δ_H 7.67 to δ_C 134.4 (C-6), δ_H 7.49 to δ_C 117.6 (C-5), and δ_H 7.26 to δ_C 122.2 (C-7)]) showed a direct connection to the corresponding carbons, supporting the presence of a 1,2-disubstituted benzene. An aromatic proton at δ_H 8.25 (H-8) showed HMBC correlations with C-6, a carbonyl carbon at δ_C 182.0 (C-9), and a quaternary carbon at δ_C 139.9 (C-10a), while an aromatic proton at δ_H 7.67 (H-6) correlated to C-10a, and C-8 (Fig. 2). An aromatic signal at δ_H 7.49 (H-5) correlated to C-7, a quaternary carbon at δ_C 118.8 (C-8a), and δ_H 7.26 (H-7) correlated to C-5, and C-8a in the HMBC correlations of **1**. The isolated aromatic proton at δ_H 6.48 (H-2) at ring C showed HMBC cross-peak with a quaternary carbon at δ_C 105.6 (C-9a), and two oxyaryl carbons [δ_C 158.5 (C-1), δ_C 129.4 (C-4], while a methoxy group at δ_H 3.90 correlated C-1, and another methoxy group at δ_H 3.75 correlated to C-4, reinforcing the position of a hydroxy group at C-3 in the structure of melixyloidin. Therefore, the structure of melixyloidin (**1**) was assigned as 3-hydroxy-1,4-dimethoxyacridin-9-one.

The structures of the known acridone alkaloids, 1,3,4-trimethoxy-10-methylacridin-9-one (**2**) and melicopidine (**3**), were assigned by comparing their NMR spectra with those reported in the literature.[9]

The MTT assay was used to assess the cytotoxicity of the alkaloids (**1-3**) against two cancer cell lines (MCF-7 and HeLa).[17-18] 1,3,4-Trimethoxy-10-methylacridin-9-one (**2**) showed potent activity with an IC_{50} value of 5.31 μM against MCF-7 but was inactive against HeLa with an IC_{50} value of 39.18 μM (see Table 2). Furthermore, compound **2** was more active against MCF-7 cell line than doxorubicin, a positive control, with an IC_{50} value of 10.48 μM.[4,19] Compounds **1** and **3** were inactive against MCF-7 and HeLa cell lines. In compound **2** (Table 2), the methoxy group at C-3 is important in enhancing the cytotoxic activity than the hydroxy group at C-3 in compound **1**. The inclusion of a methylenedioxy group linked at C-3 and C-4 reduced the cytotoxic activity of compound **3**.

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