ANTI-PLASMODIAL ACTIVITY OF BISBENZYLISOQUINOLINE ALKALOIDS FROM *MICHELIA FIGO* LEAVES

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Abstract

Two bioactive bisbenzylisoquinoline namely, magnoline (**1**) and magnolamine (**2**), were isolated from the leaves of *Michelia figo* Sprenge. These compounds possessed potent *in vitro* anti-malarial activity both chloroquine-resistant (K1) and chloroquine-sensitive (FCR3) strains of *Plasmodium falciparum*. It is the first time for **2** to be reported this activity. Their structures were determined by spectroscopic methods and by comparison of previous reports.

Key words: *Michelia figo*, bisbenzylisoquinoline alkaloid, anti-malarial, magnoline, magnolamine

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ฤทธิ์ต้านเชื้อมาลาเรียของอัลคาลอยด์บิสเบนซิลไอโซควิโนลีน จากใบจำปีแขก

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บทคัดย่อ

จากการศึกษาองค์ประกอบทางเคมีของใบจำปีแขกโดยวิธีคัดเลือกจากสิ่งสกัดที่มีฤทธิ์ต้านเชื้อมาลาเรีย (Bioassay guided fractionation) สามารถแยกสารอัลคาลอยด์กลุ่มบิสเบนซิลไอโซควิโนลีนได้ 2 ชนิดคือ Magnoline (**1**) และ Magnolamine (**2**) ซึ่งแสดงฤทธิ์ต้านเชื้อมาลาเรียที่แรงต่อทั้งเชื้อ *Plasmodium falciparum* สายพันธุ์ที่ต้านต่อ Chloroquine (K1) และ สายพันธุ์ที่ไวต่อ Chloroquine (FCR3) โดย **1** แสดงฤทธิ์ต้านเชื้อมาลาเรียสายพันธุ์ที่ต้านต่อ Chloroquine (K1) และสายพันธุ์ที่ไวต่อ Chloroquine (FCR3) ด้วย ค่า IC_{50} 0.9 μg/ml และ น้อยกว่า 0.1 μg/ml ตามลำดับ ขณะที่ **2** ถูกรายงานถึงฤทธิ์ต้านเชื้อมาลาเรียเป็นครั้งแรกในงานวิจัยนี้โดยที่แสดงฤทธิ์ต้านเชื้อ K1 และ FCR3 ด้วย ค่า IC_{50} 0.8 μg/ml และน้อยกว่า 0.1 μg/ml ตามลำดับ ในการพิสูจน์สูตรโครงสร้างทางเคมีของสารทั้งสองอาศัยเทคนิคทางสเปคโตรสโคปีร่วมกับการเปรียบเทียบข้อมูลจากรายงานการวิจัยในอดีต

คำสำคัญ: จำปีแขก บิสเบนซิลไอโซควิโนลีนอัลคาลอยด์ สารต้านมาลาเรีย แมกโนลีน แมกโนลามีน

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Introduction

Malaria is one of the diseases for which even today not many sensible drugs are available. WHO estimates that throughout the world more than 300 million clinical cases of malaria occur every year, and over 1 million people die of malaria. At present the need for novel chemotherapeutic agents is therefore acute. Plants are potential source of new anti-plasmodial compounds and are thence the focus of much current interest[1-3].

Michelia figo Sprenge (Magnoliaceae) is native of the south of China. In traditional uses, flowers have been used for cardiac tonic, root barks for killing fish[4]. In this paper *Michelia figo* Sprenge was studied and reported the present of bisbenzylisoquinoline alkaloids **1** and **2**, which have been evaluated for anti-malarial activity against both chloroquine-resistant (K1) and chloroquine-sensitive (FCR3) strains of *Plasmodium falciparum*. These two alkaloids, however, had been isolated from *Magnolia fuscata* Blume[5], whereas **1** was also isolated from *Abuta grisebachii*[6] and *A. glandiflolia*[3] (Menispermaceae).

Materials and Methods

General experimental procedures

The UV and IR spectra were recorded on Analytic Jena Spekol 1200 and Thermo Nicolet (EZ OMNIC), repectively. The optical rotation was measured on ATAGO Polax-L. The HR-FAB MS spectra were obtained from JEOL JMS-700 Mstation spectrometer and NMR data were recorded from a Varian Mercury Plus-300 MHz NMR spectrometer.

Plant material

The leaves of *Michelia figo* Sprenge (Magnoliaceae) were collected in the Rayong province, Thailand in June 2004. A voucher specimen has been deposited in the Forest Herbarium (BKF), Royal Forest Department, Ministry of Agriculture and Cooperatives, Bangken, Bangkok, Thailand.

Extraction and isolation

A dried and powdered leaves of *Michelia figo* (900 gm) was macerated with 95% ethanol to give crude ethanolic extract (40 gm). This extract (30 gm) was then partitioned with order of polarity of hexane, ethyl acetate, butanol and water. After partition, the extracts of hexane, ethyl acetate, butanol and water were acquired. Among these, four extracts that were subjected to anti-malarial activity test, the water extract showed significant activity. The water extract (900 mg) was basified and extracted with chloroform to give

chloroform extract (550 mg) which was separated by silica column chromatography and reversed HPLC to obtain 14 mg (2.08 % based on dried weight of leaves) of Magnoline (1) and 86 mg (12.74 % based on dried weight of leaves) of Magnolamine (2). These compounds were also assayed for anti-malarial activity.

Bioassay: *In vitro* anti-malarial assay against *P. falciparum*[10-11]

P. falciparum strains were cultured in the human erythrocytes in RPMI medium supplemented with 10% human plasma at 37°C, under 93% N_2, 4% CO_2, and 3% O_2. Anti-malarial activity of the test compound have been achieved by dose response curve using the parasite lactate dehydrogenase (pLDH) assay. One hundred ninety µl of asynchronous parasites (2.0% hematocrit and 0.5 or 1% parasitemia) was seeded in a 96-well microplate, and 10 µl of a test compound solution (dissolved in 50% MeOH) was added. After incubation at 37°C for 72 hours under 93% N_2, 4% CO_2, and 3% O_2, the plate was immediately frozen at -20°C for 18 hours. The plate was then thawed at 37°C, and 20 µl of the haemolyzed parasite suspension was transferred to another plate containing 100 µl of Malstat reagent. The plate was further incubated for 15 minutes at room temperature, and 20 µl of a 1:1 mixture of nitroblue tetrazolium and phenazine ethosulfate (2 mg and 0.1 mg/ml, respectively) was added to each well. After incubation for 2 hours at room temperature in the dark condition, the blue formazan product was measured at 655 nm by an iEMS microplate reader MF. The 50% inhibitory concentration (IC_{50}) value was estimated from dose response curve.

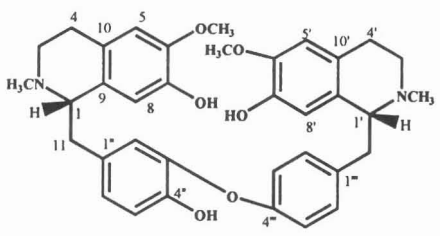
Cytotoxicity tests on MRC-5 cells[12-13]

A human diploid embryonic cell line, MRC-5 was a generous gift of Dr. L. Maes (Tibotec NV, Mechelen, Belgium). The cytotoxicity of the test compound was measured by the colorimetric MTT assay in 96-well microplates. In brief, 100 µl of MRC-5 cell suspension was added in 96-well microplates at $1x10^3$ cells/well, and cultivated for 24 hours. Then 90 µl of standard culture medium (MEM+10% FCS) with or without 10 µl of test compound solutions, which were dissolved in 50% MeOH were added to each well. The cultures were further incubated at 37°C under 5% CO_2-95% air for 7 days, and 20 µl of MTT-PBS solution (5 mg/ml) was added to each well. The plate was the incubated at 37°C for 4 hours under 5% CO_2-95% air. Then the incubation medium was aspirated, and 100 µl of DMSO was added to solubilise the MTT formazan product. After mixing,

absorbance at 540 nm was measured with an iEMS microplate reader MF. The 50% inhibitory concentration (IC_{50}) value was estimated from dose response curve.

Results and Discussion

The water extract from the leaves of *M. figo* exhibited anti-malarial activity *in vitro* against both chloroquine-resistant (K1) and chloroquine-sensitive (FCR3) strains of *P. falciparum*. Alkaloidal extract was then obtained from chloroform extraction of water extract. After silica column chromatography of alkaloidal extract, Magnolamine (**2**) was obtained. Furthermore, more polar fraction from silica column chromatography was separated by reversed HPLC to give Magnoline (**1**). Magnolamine (**2**) was major alkaloid as pale yellow amorphous, UV λ_{max} (ε) 211 nm (4.97), 289 (4.49) ; IR 3500 cm^{-1} (OH) and the molecular formula was confirmed to be $C_{37}H_{42}N_2O_7$ by the high resolution FAB MS which showed $[M+1]^+$ ion of m/z 627 as base peak. The complete structure was performed by NMR (**Table 1**), and spectropolarimeter to show its configuration as 1*S*, 1'*S* ($[\alpha]^{23}_D$ value of +111.4° (c 0.1, MeOH)) which correspondence with literatures[5]. Magnolamine showed significant inhibition concentration (IC_{50}) of 1.28 μM and less than 0.16 μM for *P. falciparum* K1 and FCR3, respectively. This is the first time to report anti-malarial activity for Magnolamine. Magnoline was obtained in small amounts as pale yellow amorphous, UV λ_{max} (ε) 211 nm (4.08), 286 (3.53) ; IR 3400 cm^{-1} (OH) and the molecular formula was also confirmed to be $C_{36}H_{40}N_2O_6$ by the high resolution FAB MS which showed $[M+1]^+$ ion of m/z 597 as base peak. The structure was elucidated by NMR and spectropolarimeter to show its configuration as 1*S*, 1'*R* ($[\alpha]^{23}_D$ value of -187.7° (c 0.3, MeOH)) which consistent with literatures[3,6-9]. Magnoline inhibited both *P. falciparum* K1 at the IC_{50} of 1.51 μM and *P. falciparum* FCR3 at the IC_{50} less than 0.16 μM (standard chloroquine: ID_{50} of 0.51 μM for K1 and 0.50 μM for FCR3, standard artemisinine: ID_{50} of 0.04 μM for K1 and 0.015 μM for FCR3).



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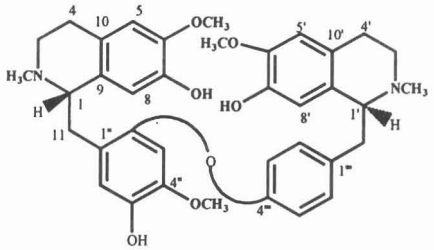

2

Table 1 The ^{1}H-and ^{13}C-NMR data of **2** in CD_3OD

C	δ_C (ppm)	δ_H (ppm), *J* (Hz)	C	δ_C (ppm)	δ_H (ppm), *J* (Hz)
1	64.0	3.75 (t, 6 Hz)	10'	124.9	-
3	46.6	2.75 (m)	11'	41.0	2.80 (m)
		3.18 (m)			3.15 (m)
4	25.9	2.58 (m)	1''	124.0	-
		2.92 (m)	2''	148.0	-
5	112.6	6.62 (s)	3''	106.1	6.55 (s)
6	148.0	-	4''	148.4	-
7	145.1	-	5''	144.0	-
8	115.8	6.05 (s)	6''	119.0	6.54 (s)
9	130.1	-	1'''	158.5	-
10	125.3	-	2'''	117.3	6.75 (d, 9 Hz)
11	35.0	2.65 (m)	3'''	132.0	7.00 (d, 9 Hz)
		3.03 (m)	4'''	134.2	-
1'	65.9	3.72 (t, 6 Hz)	5'''	132.0	7.00 (d, 9 Hz)
3'	47.5	2.75 (m)	6'''	117.3	6.75 (d, 9 Hz)
		3.18 (m)	2-NCH_3	42.4	2.37 (s)
4'	25.9	2.58 (m)	2'-	42.5	2.47 (s)
		2.92 (m)	NCH_3	56.5	3.80 (s)
5'	112.6	6.62 (s)	6-OCH_3	56.5	3.77 (s)
6'	148.0	-	6'-OCH_3	56.5	3.76 (s)
7'	145.1	-	4''-	-	-
8'	115.8	6.06 (s)	OCH_3	-	-
9'	129.9	-	7-OH	-	-
			7'-OH		
			5''-OH		

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