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Extraction and Isolation of Alkaloids from The Leaves of *Alseodaphne corneri* Kosterm

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Abstract : The isolation and purification of the leaves extract of *Alseodaphne corneri* Kosterm yielded four alkaloids; norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**. This phytochemical study involves extraction, separation by using various chromatographic methods and structural determination by spectroscopic technique such as ultraviolet spectroscopy (UV), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR) including 1D-NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, NOESY, HMQC/HSQC, and HMBC) and mass spectrometry (MS). The IC_{50} value of antiplasmodial activity for isocorydine **2** and obamegine **4** are 0.50 µmolL^{-1} and 0.14 µmolL^{-1} respectively.

Keywords : Alkaloids, *Alseodaphne corneri*, *Lauraceae*.

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Introduction

The Lauraceae are nearly all woody trees and shrubs comprising of 30 to 50 genera with about 2,000 species. There is about fifty or more *Alseodaphne* species that can be found in Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Philipines, Sri Lanka, Thailand and Vietnam [1]. *Alseodaphne corneri* Kosterm of Lauraceae, grows as wild plant, 6-8 m high. In Malaysia, the plant is also known as Medang [2].

Based on literature review, both aporphine and bisbenzylisoquinoline alkaloids showed interesting biological bioactivities such as vasorelaxants effect [3], cytotoxic action [4] and cardiovascular pharmacological effects [5].

This paper reports the isolation and identification of four alkaloids which are aporphine and bisbenzylisoquinoline types from leaves extract of the plant species. The structural elucidation was performed by various spectroscopic methods; nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), ultraviolet spectroscopy (UV) and mass spectroscopy (MS).

In this study, the isolated compounds were then tested for *in vitro* inhibitory activity against *Plasmodium falciparum*.

Experimental

General methods

^{1}H and ^{13}C and 2D NMR were recorded in $CDCl_3$ with TMS as internal reference on a JEOL JNM-FX100 (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts were reported in ppm or δ scale and the coupling constant are given in Hz. Mass spectra obtained using JEOL JMS 700 TZ spectrometer. The infrared spectra were obtained with chloroform as a solvent on a Perkin Elmer 2000 spectrometer. UV spectra were recorded on a Shimadzu UV-310 IPC Ultraviolet-Visible NIR Scanning Spectrophotometer. All solvents used are AR grade except those that are used for bulk extraction (distilled). Column chromatography (CC) was carried out using Merck silica gel 230-400 mesh and TLC was performed on silica gel 60 F_{254}, Merck.

Plant material

The leaves of *Alseodaphne corneri* was obtained and identified by the team of the Herbarium of Chemistry Department, University of Malaya, Kuala Lumpur, Malaysia on 2008. Voucher specimens (KL 4928) were deposited at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation

4.0 kg of the air dried leaves of *Alseodaphne corneri* were moistened with 25% ammonia solution and soaked in dichloromethane (CH_2Cl_2) for 3 days (cold extraction). The CH_2Cl_2 extract was evaporated to 500 ml followed by extraction using 5% hydrochloric acid (HCl) until Mayer's test is negative. The HCl extract was basified with concentrated ammonia to pH 11 and re-extracted with CH_2Cl_2. The CH_2Cl_2 was washed with distilled water and dried over anhydrous sodium sulphate.

Finally, the extract was evaporated to dryness to give crude alkaloid (10.5g). The crude

alkaloid was introduced to column chromatography over silica gel with the solvent systems of CH_2Cl_2 (100%), CH_2Cl_2: MeOH (99:1, 98:2, 97:3, 95:5) and finally 100% MeOH. Further purification was done by using the preparative thin layer chromatography (PTLC). The purified alkaloids were indicated by a single spot on thin layer chromatography (TLC).

In vitro antiplasmodial activity

The antimalaria activity of isolated compounds was determined by the procedure described by Budimulya et al [6]. In brief, each sample was separately dissolved in dimethyl sulfoxide (DMSO; 10^{-2} mol L^{-1}) and kept at -20 ^{o}C until use. The malaria parasite *Plasmodium falciparum* 3D7 clone was propagated in a 24 well culture plate in the presence of wide range of concentrations of each sample. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa. The half maximal inhibitory concentration, IC_{50} value, used to measure the effectiveness of isolated compound in antiplasmodial activity was calculated.

Result and Discussion

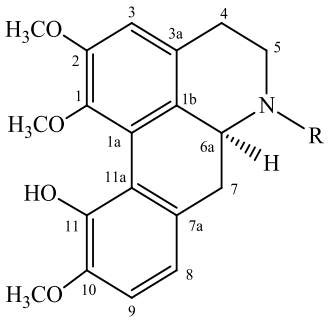
Four known alkaloids have been isolated from the leaves of *Alseodaphne corneri*. They are norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**.

Compound **1** was isolated as brownish amorphous solid. Its UV spectrum showed an absorption bands at 223, 267 and 308 nm, thus suggesting a 1,2,10,11-tetrasubstituted aporphine skeleton [7,8]. In addition, the IR spectrum gave a broad band between 3500 and 2936 cm^{-1} due to the presence of OH and NH groups [9,10]. In its mass spectrum, the base peak $[M-1]^+$, m/z 326 was formed by the loss of a hydrogen atom from the molecular ion. The $[M]^+$ occurred at m/z 327 suggesting a molecular formula of $C_{19}H_{21}NO_4$. In addition, the peak at m/z 312 $[M-CH_3]^+$ and m/z 296 $[M-OCH_3]^+$ suggested the fragmentation of a methyl and methoxyl groups, respectively. The ^{1}H NMR spectrum showed the presence of an aromatic proton appeared as a singlet at δ 6.65, attributable to H-3. The spectrum also revealed two doublets belonging toH-8 at δ 6.73 (*J*= 8.0 Hz) and H-9 at δ 6.78 (*J*= 8.0 Hz) which formed an AB spin system. Three singlets at δ 3.65 (1-OCH_3), 3.83 (2-OCH_3) and 3.84 (10-OCH_3) were detected, which corresponded to the three methoxyl groups.

The ^{13}C and DEPT experiments further confirmed the presence of nineteen carbons, which consisted of three aromatic methines, four sp^3 aliphatic carbons, nine sp^2 quaternary carbons and three methoxyl carbons. The full assignment of the 1D-NMR (^{1}H and ^{13}C) spectral data are given in Table 1. Finally comparison of this spectroscopic data with those reported in the literature, showed significantly that alkaloid obtained was norisocorydine [11].

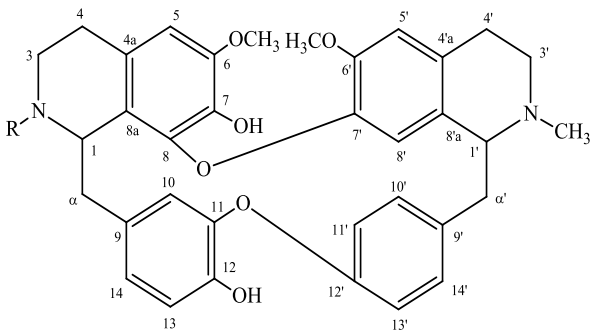
Compound **2** was obtained as a dark brown amorphous solid. The UV spectrum showed absorption bands at 283 and 304 nm, which were typical of the aporphine skeleton [7,8]. The IR spectrum showed the presence of hydroxyl group at about 3450 cm^{-1}. The molecular ion peak was observed at *m/z* 341 proposed the molecular formula of $C_{20}H_{23}NO_4$. The base peak at *m/z* 340 indicated the loss of a proton. The high intensity fragment ions at *m/z* 326 $[M-CH_3]^+$ and *m/z* 310 $[M-OCH_3]^+$ indicated the loss of a methyl and methoxyl group**,** respectively.

The ^{1}H NMR spectrum showed a singlet at δ 3.68 and another six proton singlet at δ 3.89, corresponding to the three methoxyl groups. The former was attributed to methoxyl on C-1 and the latter to C-2 and C-10, respectively. The C-1 methoxyl signal was rather shielded compared to the normal aromatic methoxyls since the protons of the methoxyl were forced to place themselves on top of ring A where the electron density was high. Another singlet at δ 2.51 was attributed to *N*-methyl group which differentiates compound **2** with compound **1** due to the absence of this peak in the ^{1}H NMR spectrum of compound **1**. The spectrum also revealed two doublets assigned to H-8 at δ 6.82 (*J*= 8.0 Hz) and H-9 at δ 6.83 (*J*= 8.0 Hz) which formed an AB system. In ring A, the C-3 aromatic proton served as singlet at δ 6.68.



1: R = H

2: R =CH_3



3: R = H

4: R =CH_3

Table 1 : ^{1}H and ^{13}C NMR Spectral Data of Compound **1** and **2**

Position	^{1}H δ, $CDCl_3$ (*J*, Hz)		^{13}C (δ, $CDCl_3$)	
	1	**2**	**1**	**2**
1			141.8	142.2
1-OCH_3	3.65 (*s*)	3.68 (*s*)	61.9	62.0
1a			125.4	125.9
1b			130.0	129.6
2			151.2	151.4
2-OCH_3	3.83 (*s*)	3.89 (*s*)	55.9	55.5
3	6.65 (*s*)	6.68 (*s*)	111.5	110.0
3a			130.1	128.5
4	2.63 (*d*, 13.4) 2.86 (*dd*, 11.2, 2.9)	2.66 (*d*, 16.0) 3.15 (*m*)	29.0	28.7
5	2.94 (*dd*, 15.8, 4.8) 3.27 (*t*, 5.4)	2.50 (*m*) 3.01 (*d*, 3.2)	42.5	52.5
N-CH_3		2.51 (*s*)		43.4
6a	3.58 (*dd*, 13.2, 3.9)	3.30 (*m*)	53.8	62.7
7	2.50 (*t*, 13.2) 2.70 (*dd*, 13.1, 4.1)	2.41 (*t*, 13.2) 3.04(*d*, 3.6)	38.1	35.4
7a			129.6	129.5
8	6.73 (*d*, 8.0)	6.82 (*d*)	118.7	119.0
9	6.78 (*d*, 8.0)	6.83 (*d*)	110.8	111.0
10			149.2	149.5
10-OCH_3	3.84 (*s*)	3.89 (*s*)	55.7	55.8
11			143.9	143.9
11a			119.9	120.0

The ^{13}C NMR and DEPT experiments further confirmed the presence of twenty carbons, which consist of three aromatic methines, four sp^3 aliphatic carbons; nine sp^2 quaternary carbons, three methoxyl carbons and one *N*-methyl carbon. The correlations of 2D NMR of compound **2** are similar to 2D NMR of norisocorydine. Comparison of the spectral data with the literature values confirmed that alkaloid obtained was isocorydine [12,13]. The full assignment of the ^{1}H and ^{13}C NMR spectral data is given in Table 1.

Compound **3** was obtained as a brownish amorphous solid with $[\alpha]_D^{27}$ +290.0^o (*c* = 0.5, MeOH). Its UV spectrum exhibited absorption maxima at 295 nm which is characteristic of a bisbenzylisoquinoline [14, 15].

The IR spectrum revealed absorption peaks at 3394, 2930, 1514 and 1264 cm^{-1} corresponding to the stretching of O-H, C-H, C=C ring and C-O-C; diphenyl ether groups, respectively [16]. It showed a molecular ion $[M+H]^+$ peak at m/z 581 corresponding to the molecular formula of $C_{35}H_{36}N_2O_6$.

The ^{1}H NMR spectrum revealed one *N*-methyl signal at δ 2.51. The spectrum also exhibited the presence of two methoxyl groups, with one in the upfield region, δ 3.53 which was the characteristic for methoxyl on C-6´ substituted and the other peaks at δ 3.73 were located at C-6. The presence of three protons singlet at δ 6.27, 6.31 and 6.64 were related to the H-5, H-5´ and H-8´, respectively. In addition, H-10 resonated as a broad singlet at δ 5.48. H-11´, H-14 and H-10´ each appeared as doublets at δ 6.21 (*J* = 6.8 Hz), 6.69 (*J* = 7.6 Hz) and 6.78 (*J* = 7.6 Hz), respectively; and another three signals corresponding to H-13, H-13´and H-14´ resonated as a doublet of doublets at δ 6.55 (*J* = 8.0 and 1.6 Hz), δ 6.87 (*J* = 8.4 and 2.8 Hz) and δ 7.38 (*J* = 10.4 and 1.6 Hz), respectively. A

broad singlet and a doublet signal corresponding to two protons, H-1 and H-1´ were observed at δ 4.09 and 4.13, respectively.

The ^{13}C NMR spectrum of this alkaloid showed thirty-five carbons. There were fourteen quarternary carbons, two methoxyl, twelve methines, six methylenes and one methyl group which attached to nitrogen atom (*N*´-CH_3), consistent with the structure proposed.

Compound **4** was obtained as a brownish amorphous state with $[\alpha]_D^{26}$ +140^o (*c* = 8.28, MeOH). The UV spectrum revealed absorbance band at 283 nm, while the IR spectrum exhibited absorption for aromatic ring and diphenyl ether at 1500 and 1220 cm^{-1} respectively. Another significant peak was also observed at 3400 cm^{-1} corresponding to the phenolic function [17,18]. The EIMS mass spectrum revealed the $[M+H]^+$ peak at m/z 594 thus suggesting a molecular formula of $C_{36}H_{38}N_2O_6$.

Table 2 : ^{1}H and ^{13}C NMR Spectral Data of Compound **3** and **4**

Position	^{1}H δ, $CDCl_3$ (*J*, Hz)		^{13}C (δ, $CDCl_3$)	
	3	**4**	**3**	**4**
1	4.09 (*br s*)	4.06 (*d*, 10.1)	54.7	60.6
N-CH_3	-	2.31 (*s*)		42.4
3	2.69 (*m*) 2.97 (*m*)	2.79 (*d*, 11.0) 3.28 (*m*)	42.1	44.1
4	2.29 (*m*) 2.29 (*m*)	2.42 (*m*) 2.82 (*m*)	29.2	23.0
4a			133.7	132.1
5	6.27 (*s*)	6.34 (*s*)	104.7	121.5
6			146.1	147.0
6-OCH_3	3.73 (*s*)	3.77 (*s*)	56.2	56.2
7			141.1	136.2
8			144.2	143.9
8a			122.1	124.2
α	2.72 (*d*, 2.8) 3.10 (*d*, 14.8)	2.66 (*d*, 15.5) 2.93 (*m*)	38.7	38.9
9			127.5	132.5
10	5.48 (*br s*)	6.21 (*br s*)	116.0	114.6
11			148.7	148.4
12			148.1	143.6
13	6.55 (*dd*, 8.0, 1.6)	6.75 (*d*, 8.2)	123.6	115.2
14	6.69 (*d*, 7.6)	6.67 (*d*, 7.3)	114.9	122.8
1′	4.13 (*d*, 4.8)	3.72 (*dd*, 11.8, 6.8)	61.1	64.8
N′-CH_3	2.51 (*s*)	2.53 (*s*)	41.4	42.7
3′	2.85 (*dd*, 12.8, 6.8) 3.15 (*m*)	2.88 (*m*) 3.51 (*m*)	44.8	45.5
4′	2.56 (*dd*, 16.4, 4.8) 3.93 (*m*)	2.96 (*m*) 2.96 (*m*)	23.8	24.9
4′a			130.6	130.3
5′	6.31 (*s*)	6.73 (*s*)	112.0	112.3
6′			148.7	149.6
6′-OCH_3	3.53 (*s*)	3.87 (*s*)	55.3	56.1
7′			144.1	143.5
8′	6.64 (*s*)	6.05 (*s*)	116.7	121.5
8′a			128.3	129.3
α′	2.78 (*d*, 5.6) 3.20 (*d*, 14.8)	2.83 (*m*) 3.37 (*dd*, 13.2, 4.1)	40.3	38.5
9′			139.3	135.1
10′	6.78 (*d*, 7.6)	6.42 (*dd*, 8.2, 1.8)	131.2	132.1
11′	6.21 (*d*, 6.8)	6.78 (*d*, 8.2)	120.8	122.8
12′			151.9	154.4
13′	6.87 (*dd*, 8.4, 2.8)	7.05 (*dd*, 8.2, 2.7)	122.3	122.7
14′	7.38 (*dd*, 10.4, 1.6)	7.32 (*dd*, 8.2, 2.2)	128.1	130.1

Table 3 : Results of *Plasmodium falciparum* Inhibition Screening Assay

Compound	Compound name	IC_{50} ($\mu molL^{-1}$)
1	Norisocorydine	*
2	Isocorydine	0.50
3	2-norobamegine	*
4	Obamegine	0.14

**Not available*

The ^{1}H-NMR spectrum particularly revealed two *N*-methyl singlets, which were at δ 2.31 and 2.53 corresponding to *N*-2 and *N*-2′ methyl protons, respectively. It also showed another two singlets attributed to two methoxyl groups appeared at δ 3.77 and 3.87 which were attached to C-6 and C-6′, respectively. The absence of signals positioned between δ 2.95 to δ 3.20 characteristic of a C-7′ methoxyl indicated that C-7′ was phenyl ether linkage instead substituted with hydroxyl or methoxyl group [19]. The spectrum also showed three singlets at δ 6.05, 6.34 and 6.73 which were assignable to H-8′, H-5 and H-5′, respectively. In addition, H-10 resonated as a broad singlet at δ 6.21. The spectrum also displayed three doublet of doublets attributable to H-10′, H-13′ and H-14′ were present at δ 6.42 (*J* = 8.2 and 1.8 Hz), 7.05 (*J* =8.2 and 2.7 Hz) and 7.32 (*J* = 8.2 and 2.2 Hz), respectively.

In addition, H-14, H-13 and H-11' appeared as a doublet at δ 6.67 (*J* = 7.3 Hz), 6.75 (*J* = 8.2 Hz) and 6.78 (*J* = 8.2 Hz), respectively. A doublet (*J* = 10.1 Hz) and doublet of doublets (*J* = 11.8 and 6.8 Hz) signals corresponding to two protons, H-1 and H-1′ were observed at δ 4.06 and 3.72, respectively.

The ^{13}C NMR spectrum revealed thirty-six carbons. There were fourteen quarternary carbons, two methoxyls, twelve methines, six methylenes, and two methyl groups attached to two different nitrogen atoms consistent with the structure proposed. Signals for C-1 (δ 60.6), C-3 (δ 44.1) and C-8a (δ 124.2) shifted to a lower field due to the presence of methyl group at *N*-2 position when compared with compound **3**. Furthermore, in the HMBC spectrum for compound **4**, long-range correlation at *N*-2 connected with C-1 and C-3 was observed and this is to further confirm the position of methyl. The full assignment of the 1D-NMR (^{1}H and ^{13}C) spectral data are given in Table 2.

The isolated alkaloids were tested for *in-vitro* inhibitory activity against *Plasmodium falciparum*. The IC_{50} value of compound **2** and **4** were tabulated in Table 3 and then compared with standard chloroquine (IC_{50}, 0.0069 $\mu molL^{-1}$) [20]. Both compounds showed weak inhibitory activity against *Plasmodium falciparum*.

Conclusions

Study on the leaves of *Alseodaphne corneri* has resulted in the isolation and the identification of norisocorydine **1**, isocorydine **2**, 2-norobamegine **3** and obamegine **4**. Antiplasmodial activity test showed that isocorydine **2** and obamegine **4** exhibited weak inhibitory activity against *Plasmodium falciparum* compared with standard chloroquine.

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