EDUCATUM - Journal of Science, Mathematics and Technology Vol. 1 No.2 (2014) 1 - 7
ISSN 2289-7070

Hernagine type of Aporphine Alkaloids from *Alseodaphne perakensis*

Alkaloid Aporfina jenis Hernagina dari Alseodaphne perakensis

Mahfuzah Md Nazimuddin[1], Muhammad Hafiz Husna Hasnan[2], Kartini Ahmad[1], Khalijah Awang[2] and Mohd Azlan Nafiah[1*]
[1]Chemistry Department, Faculty of Science and Mathematics, Sultan Idris Education University, Tanjung Malim, Perak, Malaysia.
[2]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.
[*]e-mail: azlan@upsi.edu.my

Abstract

A phytochemical study of the stem bark of *Alseodaphne perakensis* (*Lauraceae*) had been carried out. The dichloromethane crude extract from stem bark of *Alseodaphne perakensis* (*Lauraceae*) yielded two hernagine type of aporphine alkaloids namely oxohernagine and *N*-methylhernagine. The isolation and purification of these alkaloids were achieved using Column Chromatography (CC) and Preparative Thin Layer Chromatography (PTLC) techniques. The structural elucidations were performed by spectral methods notably Nuclear Magnetic Resonance (NMR) Spectroscopy one-dimentional (^{1}H, ^{13}C and DEPT) and two-dimentional (COSY, HMQC/HSQC and HMBC).

Keywords *Alseodaphne perakensis,* aporphine, oxohernagine, *N*-methylhernagine

Abstrak

Kajian fitokimia terhadap kulit batang *Alseodaphne perakensis* (*Lauraceae*) telah dijalankan. Ekstrak mentah diklоromethana dari batang kulit *Alseodaphne perakensis* (*Lauraceae*) telah menghasilkan dua jenis alkaloid aporfina yang dinamakan oxohernagina dan *N*-metilhernagina. Proses pengasingan dan penulenan alkaloid-alkaloid ini dijalankan dengan menggunakan teknik CC dan PTLC. Struktur sebatian diperincikan dengan menggunakan kaedah spektroskopi lain seperti resonan magnet nukleus satu dimensi (^{1}H, ^{13}C dan DEPT) dan dua dimensi (COSY, HMQC/HSQC dan HMBC).

Kata kunci *Alseodaphne perakensis,* aporfina, oxohernagina, *N*-methilhernagina

INTRODUCTION

Alseodaphne perakensis (Gamble) Kosterm known as Medang belongs to the family Lauraceae (Ng *et al.*, 1989). It is a small tree of about six meters in height with yellow-green flowers. It is distributed mainly in Peninsular Malaysia. Lajis *et al*., (1985) reported that in field tests, the leaves were found to be rich in alkaloids, however there has been no

EDUCATUM - Journal of Science, Mathematics and Technology Vol. 1 No.2 (2014) 1 - 7
ISSN 2289-7070

reported study on the use of this plant in traditional folk medicine practices (Lajis *et al.*, 1991). From previous studies, the genus *Alseodaphne* is well known to contain various compounds including alkaloid such as aporphine and bisbenzylisoquinoline (Li Hsi-wen *et al*., 1982). This paper reports the isolation and characterization of two hernagine types of aporphine alkaloids, oxohernagine and *N*-methylhernagine, from the stem bark of *Alseodaphne perakensis* collected at Sg. Merantor, Gua Musang, Kelantan, Malaysia.

MATERIALS AND METHODS

General

Melting points were determined on a melting point apparatus and were uncorrected. The ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded in $CDCl_3$ using TMS as internal standard on a JOEL ECA at 400 MHz. PTLC was carried out using Merck Silica Gel 60 PF254 containing Gypsum. Column Chromatography (CC) was prepared by using silica gel 60F, 70-230 mesh ASTM (Merck 1.07734.1000) ; silica gel 60F, 230-400 mesh ASTM (Merck 1.09385.1000) as stationary phase and Thin Layer Chromatography (TLC) analysis on precoated Si-gel plates (Merck Kieselgel 60 F254, 0.2 mm thickness).

Reagents

The organic solvents (dichloromethane, hexane, methanol, hydrochloric acid, sulphuric acid and ammonium hydroxide) and reagents (Dragendroff's reagent and Mayer reagent) used were of analytical and HPLC grade.

Plant Material

Samples of the stem bark of *Alseodaphne perakensis* was collected from KPK Sg. Merantor, Gua Musang, Kelantan, Malaysia.

Extraction and Separation

The stem barks of *Alseodaphne perakensis* were obtained from the Herbarium collection of University of Malaya (KL 5135). Five kilograms of the air-dried barks were moistened with 25% NH_4OH and soaked in CH_2Cl_2 for three days for cold extraction. The CH_2Cl_2 extract was evaporated to 500 ml followed by extraction using 5% 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 rinsed with distilled H_2O and dried over anhydrous sodium sulphate. Finally, the extract was evaporated to dryness to give crude alkaloid. The extract was subjected to gradient elution column chromatography over silica gel using mixtures of hexane, hexane/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/MeOH and MeOH as eluents. Then, the composition of individual chromatographic fraction was confirmed by TLC. Each series was then treated separately to isolate and purify its alkaloid content either by extensive CC or PTLC (Silica gel 60 F254). The purified alkaloids were controlled by a single spot on the TLC and were visualized by spraying with Dragendroff's reagent.

RESULTS & DISCUSSION

The first isolated compound was oxohernagine (Figure 1), a yellow amorphous solid (Nafiah, 2009 & Nafiah *et al*., 2011) with relatively simple mass spectral fragmentation. Its molecular ion peak at *m/z* 338.10771 (calc. 338.10285) and corresponded to a molecular formula of $C_{19}H_{16}NO_5$.

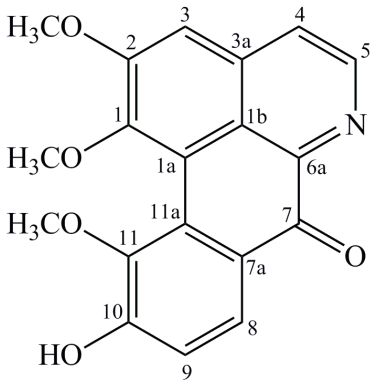

Figure 1 Oxohernagine

The ^{1}H NMR spectrum revealed a singlet at δ 7.20 corresponding to H-3. It also showed two distinct doublet signals in the aromatic region at δ 8.29 and 7.21 with the coupling constant of 8.05 and 8.0 Hz corresponding to the protons which were most probably positioned at C-8 and C-9, respectively. The small *J* value was due to the electronegative atom, nitrogen. Three singlets of three methoxyl protons appeared at δ 4.10, 3.75 and 3.53 were attached to C-1, C-2 and C-11, respectively. C-1 methoxy group of oxoaporphine was located at a higher field in the presence of an oxygenated group in C-11. Thus, the C-1 methoxy signal appeared at an upfield at δ 3.52. This could be reasonably considered due to the steric effect of the oxygenated groups in C-11. Besides, there was no signal for the aliphatic proton.

The ^{13}C indicated the presence of 19 carbon resonances: eleven quaternary carbons which were δ 115.3 (C-1a), δ 122.2 (C-1b), δ 134.6 (C-3a), δ 144.8 (C-6a), δ 154.7 (C-7a), δ 145.1 (C-10) and δ 126.2 (C-11a); one carbonyl group δ 181.7 (C-7); one hydroxyl group δ 145.1 (C-10); three methoxyl groups δ 56.5 (C-1), δ 62.4 (C-2) and δ 60.8 (C-11); and the rest were sp^2 carbons δ 105.7 (C-3), δ 122.6 (C-4), δ 144.3 (C-5), δ 126.7 (C-8) and δ 115.7 (C-9). A signal at most down fielded positions δ 181.6 reconfirmed that it was the oxoaporphine skeleton proven with position C-7 as carbonyl group. The full assignment of ^{1}H and ^{13}C NMR spectral data (Table 1) involved comparing data from the literature (Chen *et al.*, 1996) and all correlations observed (Figure 2), which hence determined the attribution of all the ^{13}C signals of oxohernagine.

Table 1 1D-NMR (^{1}H & ^{13}C) Spectral Data of oxohernagine and *N*-methylhernagine

Position	^{1}H, δ, $CDCl_3$ (*J*,Hz)		^{13}C, δ, $CDCl_3$ (*J*,Hz)	
	oxohernagine	*N*-methylhernagine	oxohernagine	*N*-methylhernagine
1			151.9	145.4
1-OCH_3	4.10 (s)	3.49 (s)	56.5	60.5
1-OH				
1a			115.3	125.0
1b			122.2	128.8
2			156.9	151.5
2-OCH_3	3.75 (s)	3.87 (s)	62.4	56.1
3	7.20 (s)	6.68 (s)	105.7	111.7
3a			134.6	127.7
4	7.77 (d, 4.6)	3.15 (m)	122.6	28.9
		2.70 (dd, 3.45, 16.1)		
5	8.86 (d, 4.0)	3.04 (d, 6.3)	144.3	53.0
		2.52 (m)		
N-CH_3		2.53 (s)		44.0
6a		2.90 (dd, 4.0, 13.8)	144.8	63.1
7		3.01 (dd, 4.0, 13.8)	181.7	35.6
		2.37 (t, 13.2)		
7a			154.7	129.8
8	8.29 (d, 8.05)	6.90 (d,10.0)	126.7	122.4
9	7.21 (d, 8.0)	6.86 (d, 10.0)	115.7	113.5
10			145.1	148.0
10-CH_3				
11			126.8	145.5
11-OCH_3	3.53 (s)	3.52 (s)	60.8	60.9
11a			126.2	123.0

Table 2 ^{1}H NMR Spectra Data comparison of oxohernagine and *N*-methylhernagine with the literatures (Chen *et al*., 1996 & Kozuka *et al*., 1986)

Position	^{1}H, δ, $CDCl_3$ (*J*,Hz)			
	oxohernagine	oxohernagine (Chen *et al*., 1996)	N-methylhernagine	N-menthylhernagine (Kozuka *et al*., 1986)
1-OCH_3	4.10 (s)	4.11 (s)	3.49 (s)	3.50 (s)
2-OCH_3	3.75 (s)	3.76 (s)	3.87 (s)	3.87 (s)
3	7.20 (s)	7.21 (s)	6.68 (s)	6.68 (s)
4	7.77 (d, 4.6)	7.77 (d, 5.3)		
5	8.86 (d, 4.0)	8.87 (d, 5.3)		
8	8.29 (d, 8.05)	8.31 (d, 8.5)	6.90 (d, 10.0)	6.87 (s)
9	7.21 (d, 8.0)	7.22 (d, 8.5)	6.86 (d, 10.0)	6.87 (s)
11-OCH_3	3.53 (s)	3.54 (s)	3.52 (s)	3.54 (s)
N-CH_3			2.53 (s)	2.55 (s)

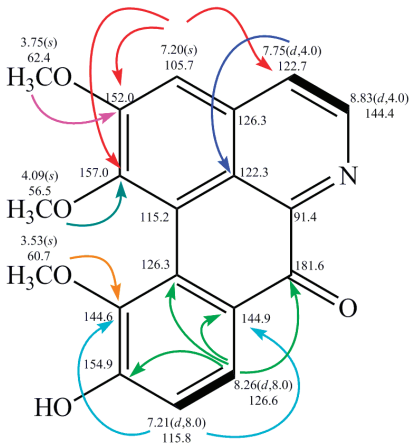

Figure 2 Attribution pf all ^{13}C signals of oxohernagine

The second compound isolated was *N*-methylhernagine, a brown amorphous solid from CH_2Cl_2. The mass spectrum exhibited a molecular ion peak at m/z 342.17122 $[M+H]^+$ (calc. 342.17053) that suggests a molecular formula of $C_{20}H_{23}NO_4$ (Figure 3).

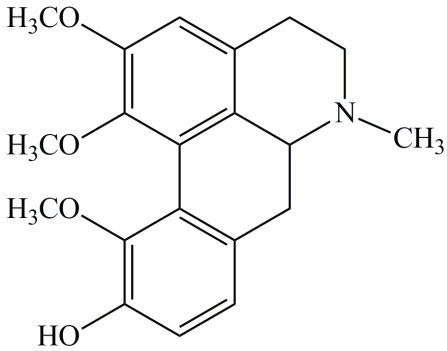

Figure 3 *N*-methylhernagine

The spectroscopy studies (^1H NMR and HMQC spectrum) showed three methoxyl group singlet signals at δ 3.49, 3.87 and 3.52 attached to C-1, C-2 and C-11, respectively. A strong *N*-methyl singlet peaked at δ 2.53 attached to N-6 methyl protons. A pair of doublet signals appeared in the aromatic regions at δ 6.90 and δ 6.86 with the vicinal coupling constant of 10.0 Hz that corresponded to the adjacent protons at H-8 and H-9, while another doublet signal led for H-4 aliphatic at δ 3.04 with coupling constant 6.3 Hz. A singlet signal also appeared at δ 6.68 that corresponded to aromatic proton at position C-3, while a triplet signal at C-7 appeared at δ 2.37 (J = 13.2 Hz). The remaining aliphatic signals that appeared as doublet of doublet at δ 2.70 (J = 3.5, 16.1 Hz), δ 2.90 (J = 1.2, 12.6 Hz) and δ 3.01 (J = 4.0, 13.8 Hz) where attached at positions C-4, C-6a and C-7, respectively.

The ^{13}C NMR spectrum revealed a N-methyl carbons signals at δ 44.0 and three methoxyl carbons signals that appeared at δ 60.5, δ 56.1 and δ 60.9 that coressponded to 1-OCH_3, 2-OCH_3 and 11-OCH_3, respectively. The aliphatic carbons consisting of three

EDUCATUM - Journal of Science, Mathematics and Technology Vol. 1 No.2 (2014) 1 - 7
ISSN 2289-7070

methylene carbon signals positioned at δ 28.9, δ 53.0 and δ 35.6 were assigned to C-4, C-5 and C-7 respectively, while a methine carbon signal positioned at δ 63.1 corresponded to C-6a. Three aromatic carbon signals were appeared at δ 111.7 (C-3), δ 122.4 (C-8) and δ 113.5 (C-9), respectively. The rest were nine quaternary carbons C-2, C-10, C-11, C-1, C-1b, C-7a, C-3a, C-1a and C-11a that appeared at regions δ 151.5 to δ 123.0. The ^{13}C NMR spectrum exhibited 20 signals; a N-methyl carbon, three aromatic carbons, four aliphatic carbons, three methoxyl carbons and nine quarternary carbons. The full assignment of ^{1}H and ^{13}C NMR spectral data (Table 1) involved comparing with data from the literature (Table 2) (Kozuka *et al*., 1984). All correlations observed (Figure 4) determined the attribution of all the ^{13}C signals of *N*-methylhernagine.

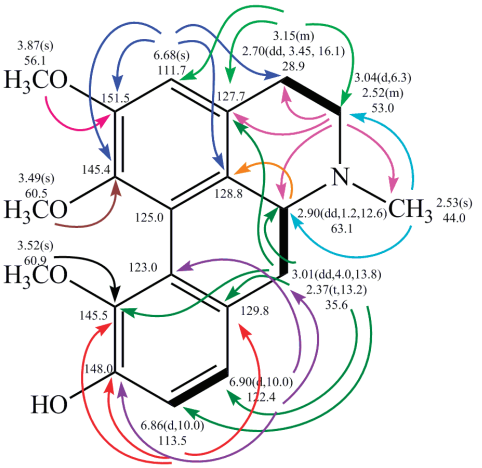

Figure 4 Attribution of all ^{13}C signals of *N*-methyhernagine

CONCLUSION

The bark of *Alseodaphne perakensis* yielded two aporphine alkaloids, namely oxohernagine and *N*-methylhernagine. The structures of both compounds were elucidated by using CC and PTLC techniques.

ACKNOWLEDGEMENT

The authors are very grateful to Sultan Idris Education University and University Malaya for the excellent technical assistance and laboratory facilities and the financial support for the grant; GPU (2010-0016-102-01).

REFERENCES

Chen, J., Tsai, I.-L., & Chen, I.-S. (1996). New Oxoaporphine Alkaloids from Hernandia nymphaeifolia. *Journal of Natural Product*, 59(2), 156–158.

Kozuka, M., Inada, A., Konoshima, T. & Sawada, T. (1984). Aporphine Alkaloids from Prabenzion praecoxb (SIEB. et ZUCC) NAKAI. *Chemical & pharmaceutical bulletin*, 32(12), 5055–5058.

Lajis, N. & Din, L. B. (1985). Phytochemical survey of Krau Game Reserve, Lancang. *In*: *Proceedings of a workshop*. Universiti Kebangsaan Malaysia 45 pp.

Lajis, N. H., Mahmud, Z. & Toha, R. F. (1991). N-methyl-2,3,6-trimethoxymorphinandien-7-one N-oxide: A Minor Alkaloid From *Alseodaphne perakensis*. *Journal of Natural Products*, 54(2), 612–614.

Li Hsi-wen, Li Jie, Huang Pu-hwa, Wei Fa'nam, Tsui Hung-pin & Henk van der Werff (1982) *Lauracea. Fl. Reipubl. Popularis Sin*., 31:1-463. Retrieved from http://flora.huh.harvard.edu/china/mss/volume07/Lauraceae.pdf.

Nafiah, M. A. (2009). *Alkaloids Isolated from Alseodaphne Species*. Kuala Lumpur: University of Malaya.

Nafiah, M.A., Mukhtar, M.R., Omar, H.; Ahmad, K., Morita, H., Litaudon, M., Awang, K., Hadi, A.H.A (2011). *N*-Cyanomethylnorboldine: A New Aporphine Isolated from *Alseodaphne perakensis (Lauraceae)*. *Molecules* 2011, 16, 3402-3409.

Ng, F. S. P. (1989) Malayan Forest Records. *Tree Flora of Malaya*. *Vol. 4*. Forest Research Institute Malaysia.

Rachmatiah, T., Mukhtar, M. T., Nafiah, M. A., M. Hanafi, Awang, K., Kosela, S., and A. Hamid A. H. (2009) *Molecules*, 114:2850-2856.