

Isolation of Aporphine Alkaloid From The Leaves of *Alseodaphne corneri* Kosterm

Pemencilan Alkaloid Aporfina daripada Daun Alseodaphne corneri Kosterm

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Abstract

A phytochemical study on the leaf extract of *Alseodaphne corneri* Kosterm involving extraction, separation by using various chromatographic methods and structural determination by spectroscopic technique such as ultraviolet spectra (UV), infrared spectra (IR), nuclear magnetic resonance (NMR) and mass spectra (MS) was carried out. The isolation and purification from the leaves of *Alseodaphne corneri* Kosterm yielded two aporphines: norlirioferine and lirioferine.

Keywords *Alseodaphne corneri*, aporphines, lirioferine, norlirioferine

Abstrak

Satu kajian fitokimia ke atas ekstrak daun *Alseodaphne corneri* Kosterm yang melibatkan pengekstrakan dan pemisahan menggunakan pelbagai kaedah kromatografi dan penentuan struktur kimia dengan teknik spektroskopi seperti spektra ultraviolet (UV), spektra inframerah (IM), resonan magnetik nuklear (RMN) dan spektra jisim (MS) telah dijalankan. Penyaringan dan penulenan sebatian tulen daripada ekstrak daun itu telah menghasilkan dua aporfina: norlirioferina dan lirioferina.

Kata kunci *Alseodaphne corneri*, aporfina, lirioferina, norlirioferina

Introduction

Located near the Equator, Malaysia confers on it a typically tropical climate which contributes to the lush tropical rainforests. This country is rich in flora and is estimated to have 17,631 species of plants including 61 gymnosperm, 337 algae, 1,387 bryophyte, 1,600 ferns and 4,180 families of monocotyledons and 10,026 dicotyledons. The 2001 Global Diversity Outlook has recognized Malaysia as one of the 12 mega diversity countries in the world (Department of Statistics, Malaysia, 2012)

The genus *Alseodaphne* is distributed through Yunnan, China to West Malaysia. Out of 56 recorded species 23 are found in Malaysia (Nafiah, 2009; Thakur *et al.*, 2012). *Alseodaphne corneri* from the Lauraceae family is locally known as Medang is one of

these species. It grows as a wild plant to a height 6-8 m. This paper reports the isolation and identification of two aporphine alkaloids from leaves extract of Medang, *A. corneri*.

Materials and Methods

A 4.0 kg air-dried sample of *Alseodaphne corneri* (KL 4928) obtained from the University of Malaya was first defatted with hexane for three days at room temperature (28° C) followed by filtration of hexane extract. The samples were then dried and moistened with 28% of ammonia solution and left to soak for 2 h before re-extracted with dichloromethane solvent for three days. The latter extract was evaporated to about 500 ml and re-extracted with 5% hydrochloric acid. The combined extract was then basified using 10% of ammonia solution to pH 11. A re-extraction with dichloromethane was made until a negative Mayer test was obtained. The dichloromethane extract was washed with distilled water and dried with anhydrous sodium sulphate followed by evaporation to dryness to give a crude alkaloid paste (10.5 g). The crude alkaloid was introduced to column chromatography over silica gel with dichloromethane and methanol solvents. The composition of each chromatographic fraction was visualized by thin layer chromatography (TLC) under UV lamp (254 and 365 nm). The alkaloids were isolated using preparative TLC and purified alkaloids were controlled by a single spot on TLC.

Structural elucidation was performed with the aid of spectroscopic methods; NMR including 1D-NMR (¹H, ¹³C and DEPT), 2D-NMR (COSY, HMQC, and HMBC), IR, UV and MS.

Results and Discussion

Two alkaloids, namely norlirioferine (1) and lirioferine (2), as shown in Figure 1 were successfully isolated from the leaves of *Alseodaphne corneri*.

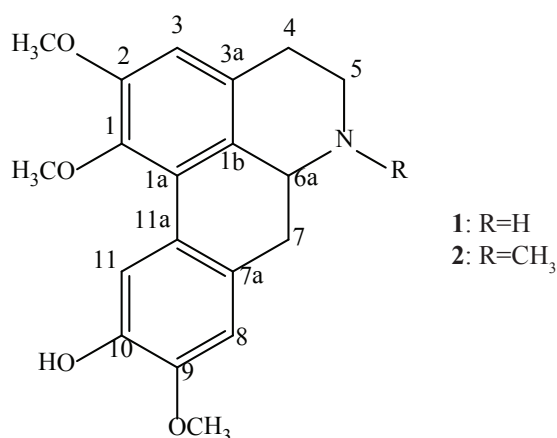


Figure 1 Alkaloids isolated from the leaves of *Alseodaphne corneri* (KL4928)

Compound 1 was isolated as dark brown amorphous. The UV spectrum assigned the absorption bands at 219, 281 and 302 nm as typical of aporphine skeleton (Azimova & Yunusov, 2013). The IR spectrum revealed absorptions at 3323 cm^{-1} , represented a highly conjugated hydroxyl group in the molecule. The mass spectrum of compound **1** exhibited a molecular ion peak at m/z 341 suggesting a molecular formula of $\text{C}_{20}\text{H}_{23}\text{NO}_4$.

Data obtained from 1D and 2D NMR was almost the same as compound **1**, except for the absence of one peak at ^{13}C NMR spectrum suggesting that this compound contained 19 carbons. Three methoxyl groups (δ 3.66, 3.89 and 3.90) were attached to aromatic carbons at C-1, C-2 and C-9. Three deshielded aromatic proton signals in ^1H NMR spectrum appeared as singlets at δ 8.08 (H-11), 6.80 (H-8) and 6.60 (H-3). The aliphatic protons appeared as doublet of doublet and multiplet at high field region.

From the ^{13}C and DEPT experiment, it was further proposed that there were nine quaternary carbons, three methyls, three methylenes, and four methines in this structure. The positions of these carbon resonances were further supported by 2D NMR, i.e. HMQC and HMBC spectra, respectively. Furthermore, correlations were observed as illustrated in Figure 2. After a review in previous study, this compound was confirmed as norlirioferine (Nafiah *et al.*, 2009). Compound **1** was previously found in the genus *Alseodaphne*, but this is the first report of leaf extract from Medang, *Alseodaphne corneri*.

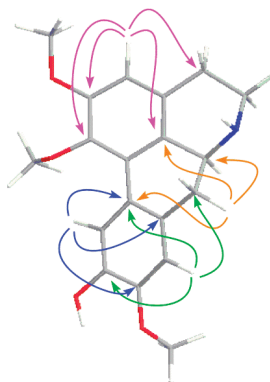


Figure 2 ^1H - ^{13}C Correlations observed in HMBC spectrum of the compound norlirioferine

Compound 2 was obtained as light brown amorphous. The IR spectrum showed a broadband at 3327 cm^{-1} while the UV spectrum showed absorption band at 281 and 302nm. The mass spectrum of compound **2** exhibited a molecular ion peak at m/z 327 suggesting a molecular formula of $\text{C}_{19}\text{H}_{21}\text{NO}_4$.

The ^1H NMR spectrum of this compound exhibited three aromatic proton signals at δ 6.57, 6.80 and 8.05 assignable to H-3, H-8 and H-11, respectively. A singlet at δ 3.64 and an integrated six proton singlet peak at δ 3.89, corresponding to three methoxyl groups, attached to C-1, C-2 and C-9, respectively. One *N*-methyl singlet was observed at δ 2.54 and aliphatic protons appeared between δ 2.51 to 3.16.

The ^{13}C and DEPT experiments further confirmed the presence of 20 carbons, which consisted of three aromatic carbons, four aliphatic carbons, three methoxyl group, nine

quarternary carbons and one *N*-methyl group. The positions of these carbon resonances were further supported by HMQC and HMBC spectra. In addition, correlations were observed as in Figure 3. This compound was then compared to the literature and confirmed that it was lirioferine (Saidi *et al.*, 2009). The full assignment and literature values of ^1H NMR and ^{13}C NMR spectral data for both compounds are given in Table 1.

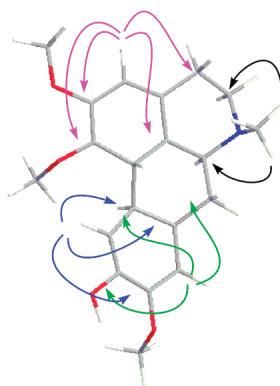


Figure 3 ^1H - ^{13}C Correlations observed in HMBC spectrum of the compound lirioferine

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

Position	δ , CDCl_3 (J , Hz)			
	^1H (1)	^1H (2)	^{13}C (1)	^{13}C (2)
1			144.5	144.2
1-OCH ₃	3.66 (s)	3.64 (s)	60.3	60.2
1a				127.1
1b			126.9	126.9
2			152.5	152.0
2-OCH ₃	3.89 (s)	3.88 (s)	55.9	55.8
3	6.60 (s)	6.57 (s)	110.7	110.2
3a			128.3	128.8
4	2.75 (<i>dd</i> , 15.0, 5.0)	2.68 (<i>dd</i> , 16.1, 2.9)		
	3.19 (<i>m</i>)	3.16 (<i>m</i>)	28.2	29.1
5	3.08 (<i>m</i>)	2.51 (<i>dd</i> , 12.1, 4.0)		
		3.03 (<i>m</i>)	42.7	53.3
<i>N</i> -CH ₃		2.54 (s)		43.9
6a		3.00 (<i>s, br</i>)	53.6	62.6
7	2.90 (<i>m</i>)	2.56 (<i>m</i>)	35.8	34.1
		2.97 (<i>dd</i> , 13.8, 4.0)		
7a			129.1	130.0
8	6.80 (s)	6.80 (s)	114.1	114.1
9			145.1	145.0
9-OCH ₃	3.90 (s)	3.88 (s)	56.1	56.0
10			145.5	145.4
11	8.08 (s)	8.05 (s)	111.4	111.2
11a			123.8	123.9

Conclusion

Extrants from the leaves of *Alseodaphne corneri* have resulted in the isolation and identification of the alkaloids norlirioferine and lirioferine.

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