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Harman and isoquinoline alkaloids from *Litsea petiolata Hk.f* (Lauraceae)

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ABSTRACT A phytochemical investigation on the alkaloids of the barks of *Litsea petiolata Hk.f* obtained from Reserved Forest Teloi, Sik, Kedah has resulted in the isolation of 5 compounds namely harman or aribine **(1)** , norharman **(2)** , reticuline **(3)** , isoboldine **(4)** and thalifoline **(5)** . Their structures were established through the spectroscopic techniques such as UV, IR, GC-MS, 1D and 2D-NMR.

(**Keywords:** *Litsea petiolata,* harman, aribine, , indole and isoquinoline)

INTRODUCTION

Malaysia is known as the richest and the oldest rain forest in the world because it's characterized by high rainfall. Malaysian forest consists of many varieties of plants. At least 500 genera and more than 5000 species were found in the flora of Malaysia and 900 species had being used in traditional medicines. Lauraceae is known as "Medang" or "Tejur" in Malay; consists of 35 genera and 2500 species in the world and about 16 genera and 213 species in Malaysia. *Litsea* is the second biggest genera of Lauraceae.

The total number of species belonging to *Litsea* genera is 478 species, with 22 that occur in Indonesia [1,2]. The majority of alkaloid isolated from the *Litsea* genera are the aporphinoid type of alkaloids. At present, there is no report on the medicinal value of *Litsea petiolata.* This paper reports the isolation and structural elucidation of β-carboline alkaloid, harmane and norharmane type [3], which were isolated for the first time from *Litsea*; and 3 known isoquinoline alkaloids.

The β-carboline Harman **1** (sometimes also referred to Aribine) is the parent compound of an important class of heterocyclic compounds that are the basic structural unit of some indole alkaloids possessing a wide range of bioactive effects [4,5]. The β-carboline, harman and norharman, occur naturally in the cerebral cortex and other brain tissues, as well as in the liver and adrenal tissues [6]. The β-carboline alkaloids also occur in natural plant- derived foods and are often associated with fermentation process such as in the production of wines and beer, soya bean products and vinegar [7]. Although β-carbolines such harman and norharman at endogenous concentrations are not mutagenic but at high concentration they are also reported to having potential cytotoxic and carcinogenic activities, causing renal toxicity in male F344 rats at the dietary level of 1000 ppm [8,9].

EXPERIMENTAL DESIGN

General Methods

^{1}H and ^{13}C and 2D NMR were recorded in $CDCl_3$ with TMS as internal standard 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 constants are given in Hz. Mass spectra were obtained using Hewlett Packard HP 6890 Series Mass Selective Detector. Solvent used was Chloroform ($CHCl_3$).All solvents, except those used for bulk extractions are AR grade. 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

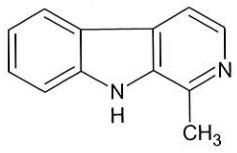
The barks of ***Litsea petiolata Hk.f*** (Lauraceae), were collected from Hutan Simpan Rimba Teloi, Sik, Kedah , Malaysia by the Phytochemical group of the Chemistry Department, Faculty of Science, University of Malaya. The voucher specimen (KL 4678) was deposited in the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and in the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

3kg of dried and milled stem barks were first defatted with hexane for 3 days at room temperature then filtered. After that they were moistened with 15% NH_4OH, and exhaustively extracted with CH_2CI_2 by Soxhlet extractor for about 18 hours. The CH_2CI_2 extract were reduced to 500 ml followed by acidic extraction using 5% HCI until Mayer's test is negative. The combined extracts were then basified with concentrated ammonia solution to pH 10 -11 and reextracted with CH_2CI_2. The CH_2Cl_2 fractions were washed with distill H_2O and dried over anhydrous sodium sulphate. The solvents were evaporated to dryness to yield 37.38 g of crude alkaloid. This crude alkaloid was subjected to column chromatography over silica gel using various ratio of CH_2CI_2 and MeOH (100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 93:7, 92:8, 91:9, 90:10, 88:12) and finally with pure MeOH. TLC was used to monitor the fractions collected which to be grouped into series of fractions. Extensive column chromatography was used to isolated the pure alkaloid.

RESULTS AND DISCUSSION

Five alkaloids have been isolated from the barks of ***Litsea petiolata Hk.f*** : the β-carboline alkaloids harman or aribine **1** and norharman **2** and the isoquinolines, reticuline **3** , isoboldine **4** and thalifoline **5.**



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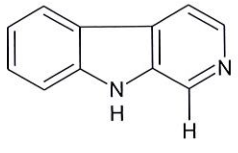

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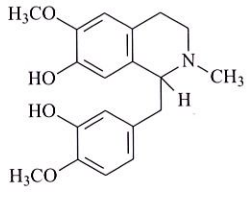

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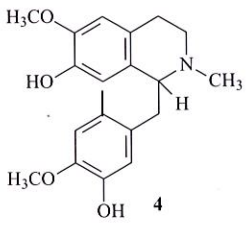

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5

Alkaloid 1 was isolated as brownish amorphous showed The IR spectrum of this alkaloid showed absorption peak at 3125 cm^{-1} indicated the presence of N-H group [10]. UV spectrum of **1** showed the characteristic of non-oxygenated indole alkaloids as indicated by the absorption maxima at 347, 334, 287 (sh), 239 (sh), 234 and 212 nm [11]. The mass spectral fragmentation for alkaloid 1 was relatively simplewith molecular ion peak at m/z 182 corresponding to the molecular formula $C_{12}H_{10}N_2$.

The ^{1}H NMR spectrum showed four vicinal aromatic hydrogens δ 8.04 (d, J = 8.08 Hz, H-9), δ 7.48 (br d, J = 2.5 Hz, H-12), δ 7.24 (m, H-10), δ 7.47 (m, H-11)] characterising the indole moiety or β carboline skeleton moiety. A pair of doubles at δ 8.27 and 7.75 (H-5 and H-6, J = 5.4 Hz, respectively) was consistent with two hydrogens in a pyridine-like skeleton. ^{1}H NMR spectrum also showed the presence of pyrrole structure. It shows an extreme example of quadropole broadening, where the NH absorption extends from δ 8.30-8.50 ppm, (NH, br s). The COSY spectrum showed cross peaks between ; H-5 and H-6; H-9 and H-10; and H-10 and H-11.

The ^{13}C NMR spectrum is in agreement with the molecular formula deduced from the mass spectrum, accounting for all 12 carbons. One of which was very upfield at δ 20.3 which belongs to the methyl group. In the DEPT spectrum, the typical signals of the aromatic quarternary carbons of the β-carboline skeleton were observed, the peaks revealed at δ 141.7, 140.2, 134.6, 128.5 and 122.1. The other 6 peaks represent CH aromatics and one CH_3 group is also present

The ^{13}C NMR data together with one-bond ^{1}H— ^{13}C correlations observed in the HSQC experiments of alkaloid **1** indicated the presence of 6 aromatic carbons, comprising 5 quartenary and 1 methyl. The long- range 1H— 13C correlations observed in the HMBC experiment allowed assignment of carbon atoms and confirmed the Harman type moiety.

Based on the above features and comparing their data with those of literature values, alkaloid 1 was deduced to be a Harmane or Aribine. Complete spectral data for 1D and 2D NMR were summarized in the Table 1 and Table 2 below. COSY correlations also shown in Figure 1 while HMBC correlations shown in Figure 2.

Table 1. Comparison ^{1}H (δ ppm) Spectral Data of Alkaloid **1**, Harmane (observed) with literature values(Claude *et al.* 1980).

No. C/H	δ ^{1}H (observed)	δ ^{1}H (Claude *et al.* 1980)	$^{2}J_{H-H}$ Hz (observed)	$^{2}J_{H-H}$ Hz (Claude *et al.* 1980)
2	-	-	-	-
3	-	-	-	-
5	8.27 *d*	8.38	5.4	6
6	7.75 *d*	7.83	5.4	6
7	-	-	-	-
8	-	-	-	-
9	8.04 *d*	8.13	8.0	8
10	7.24 *m*	7.29	-	*
11	7.47 *m*	7.50	-	*
12	7.48 *br d*	7.51	2.5	*
13	-	-	-	-
14	2.78 *s*	2.83	-	-

* J_{H-H} non measurable with CDCl

Table 2. ^{1}H and ^{13}C- NMR chemical shift (δ ppm) values and coupling patterns of the protons in HSQC, COSY, DEPT and HMBC techniques for Harmane.

No. C/H	δ ^{1}H (observed)	δ ^{13}C (observed)	^{1}H-^{13}C HSQC	DEPT	^{1}H-^{1}H COSY	HMBC Correlations
2	-	141.7	-	-	-	-
3	-	134.6	-	-	-	-
5	8.27 (*d, J=5.4*)	138.6	H-5	CH	H-5/H-6	6, 7
6	7.75 (*d, J=5.4*)	113.0	H-6	CH	H-6/H-5	3
7	-	128.5	-	-	-	-
8	-	122.1	-	-	-	-
9	8.04 (*d, J=8.0*)	122.0	H-9	CH	H-9/H-10	7, 13
10	7.24 *m*	120.3	H-10	CH	H-10/H-9, H-10/H-11	11
11	7.47 *m*	111.7	H-11	CH	H-11/H-10	9
12	7.48 (*br d, J* =2.5)	128.4	H-12	CH	-	13
13	-	140.2	-	-	-	-
14	2.78 *s*	20.3	H-14	CH_3	-	2

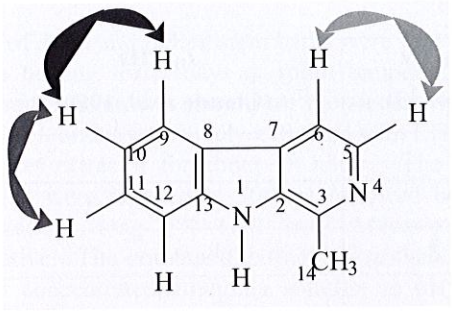

Figure 1. COSY correlations of Harmane

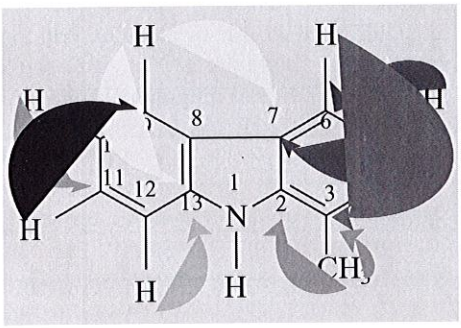

Figure 2 . ^{1}H-^{13}C long range correlations in HMBC spectrum of alkaloid 1, Harmane.

Alkaloid 2 was isolated as brownish amorphous. The IR spectrum of this alkaloid, the absorption peak at 3219 cm^{-1} indicated the presence of N-H group. The UV spectrum of **2** has the similar pattern with alkaloid **1**. characteristic of non-oxygenated indole alkaloids. The mass spectral fragmentation for **2** was relatively simple, with the molecular ion peak at m/z 168 corresponding to molecular formula $C_{11}H_8N_2$. It was identified as Norharman [12].

The ^{1}H NMR spectrum revealed four adjacent aromatic protons of the β-carboline moiety at δ 8.14 (1H, d, J = 8.08 Hz, H-9), δ 7.58-7.51 (2H, m, H-11 and H-12) and δ 7.32 - 7.28 (1H, m, H-10),]. A pair of doubles at δ 8.46 and δ 7.97 Hz, (H-5, J = 5.04 Hz; H-6 J'=5.48Hz) was consistent with two hydrogens in a pyridine-like skeleton. ^{1}H NMR spectrum also showed the presence of pyrrole structure. It shows an extreme example of quadropole broadening, where the NH absorption extends from δ 1.95 - 1.70 ppm, (NH, br, s). A proton singlet was observed at δ 8.92 which attributed to H-3. It showed that C-3 in **2** was unsubstituted.

The assignment of the aromatic protons was also confirmed by analysis of the COSY data. The COSY spectrum showed that H-5 (δ 8.46) was only correlated with H-6 (δ 7.97), while H-9 was coupled to H-10 and H-11 was coupled to H-10.

^{13}C-NMR spectrum gave 11 absorption peaks representing the presence of 11 carbons. Peaks showed the presence of four quarternary carbons δ 140.47, δ 135.87 , δ 129.12 and δ 121.55 , while another 7 peaks were assigned as CH aromatic. The DEPT spectrum also indicated the presence of 7 CH aromatic and the absence of CH_2 peak which further supported the structure of **2**.

Direct correlations between carbon-hydrogen were found from the HSQC spectrum and the results were supported by other data. The structure of alkaloid 2 was finally assigned by ^{1}H-^{13}C long range correlations in the HMBC spectrum (Figure 3).

The full assignments for the protons, carbons signal and comparison with literature values [13] was tabulated in Table 3.

Alkaloid 2 was identified as norharmane or 9H-pyrido[3,4-b]indole based on the 1D and 2D NMR spectral data (Table 4) and comparison with literatures [13]

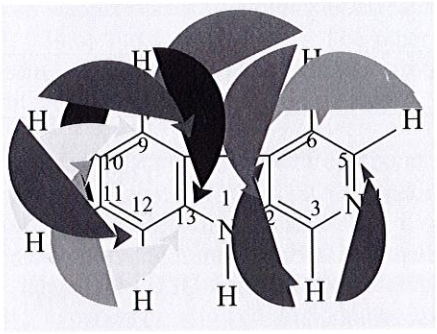

Figure 3. ^{1}H-^{13}C long range correlations in HMBC of alkaloid 2, Norharmane.

Alkaloid 3 was obtained as a brownish amorphous. The UV spectrum displayed an absorption maximum at 293 nm. Its IR spectrum showed a strong absorption at 3371 cm^{-1} and 2934 cm^{-1} due to the stretching of O-H and C-H aromatic, respectively. The spectrum also showed the absorption at 1513 cm^{-1}, 1252 cm^{-1} and 1445 cm^{-1} which indicated the C-H aromatic, C-O and CH_2 respectively.

The mass spectrum revealed the molecular ion peak at m/z 329 which corresponded to the molecular formula of $C_{19}H_{23}NO_4$. Another two important peaks

Table 3. Comparison ^{1}H (δ ppm) and coupling patterns of the protons of Alkaloid **1**, Harmane (observed) and alkaloid **2**, Norharmane (observed) with literature values (Claude *et al.*; 1980).

No. C/H	δ ^{1}H Harmane (observed)	δ ^{1}H Norharmane (observed)	δ ^{1}H Harmane (Claude *et.al.*; 1980)	$^{2}J_{H-H}$ Hz Harmane (observed)	$^{2}J_{H-H}$ Hz Norharmane (observed)	$^{2}J_{H-H}$ Hz Harmane (Claude *et al.* 1980.)
2	-	-	-	-	-	-
3	-	8.92 *s*	-	-	-	-
5	8.27 *d*	8.46 *d*	8.38	5.4	5.04	6
6	7.75 *d*	7.97 *d*	7.83	5.4	5.48	6
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	8.04 *d*	8.14 *d*	8.13	8.0	8.24	8
10	7.24 *m*	7.32 - 7.28 *m*	7.29	-	-	*
11	7.47 *m*	7.58 - 7.51 *m*	7.50	-	-	*
12	7.48 *br d*	7.58 - 7.51 *m*	7.51	2.5	-	*
13	-	-	-	-	-	-
14	2.78 *s*	-	2.83	-	-	-

* J_{H-H} non measurable with $CDCl_3$

Table 4: 1D NMR (^{1}H and ^{13}C) and 2D NMR (HSQC, COSY, DEPT and HMBC) spectral data of alkaloid **2**, Norharmane.

No. C/H	δ ^{1}H Norharmane (observed)	δ ^{13}C Norharmane (observed)	^{1}H-^{13}C HSQC	DEPT	^{1}H-^{1}H COSY	HMBC Correlations
2	-	135.87	-	-	-	-
3	8.92 *s*	133.64	H-3	CH	-	2,5,7
5	8.46 *d*	139.17	H-5	CH	H-5/H-6	6,7
6	7.97 *d*	114.85	H-6	CH	H-6/H-5	2,9
7	-	129.12	-	-	-	-
8	-	121.55	-	-	-	-
9	8.14 *d*	121.93	H-9	CH	H-9/H-10	11,13
10	7.32 - 7.28 *m*	120.33	H-10	CH	H-10/H-9, H-10/H-11	8,11,12
11	7.58 - 7.51 *m*	128.70	H-11	CH	H-11/H-10	9,13
12	7.58 - 7.51 *m*	111.65	H-12	CH	-	10
13	-	140.47	-	-	-	-

were also observed in the mass spectrum at m/z 192 and m/z 137. The former was due to the loss of the isoquinoline moiety while the latter was indicated to $C_8H_9O_2$ (benzyl substituent). The loss of a methyl from the isoquinoline moiety gave a peak at m/z 177.

The benzyl substituent loss a methyl as well which gave a peak at m/z 122. The ^{1}H NMR spectrum exhibited five aromatic proton signals at the lower region. There were two singlets at δ6.50 and δ6.26 attributable to H-5 and H-8 respectively. H-8 was shielded compared to H-5 because of the anisotropic effect caused by ring C (facing the ring C). Another two of the proton signals appeared at δ6.77 (*d*, J_m=1.92 Hz, H-2') and δ6.69 (*dd*, J_o=8.24 Hz, J_p =1.36 Hz; H-5') as doublet of doublets.

The last aromatic proton signal belongs to H-6' which was a doublet of doublets (*dd*) and the signal appears at δ6.52 (*dd*, J_m=1.68 Hz, J_o=8.68 Hz, H-6'). The ^{1}H NMR spectrum revealed two methoxyl groups overlapped to each other at δ3.80 corresponded to 6-OMe and 4'-OMe. Furthermore, a singlet at δ2.44 was characteristic of the *N*-methyl (N-CH_3) group. A total of seven proton signals were observed at a higher region between δ2.50-δ3.66 attributable to the aliphatic protons of H-1α, H-1, H-3 and H-4.

The ^{13}C NMR spectrum showed the presence of nineteen carbons in the molecule. The respective positions of the protons at C-3 and C-4; and C-5' and C-6' were confirmed by ^{1}H-^{1}H correlations in the Cosy spectrum (Figure 4). The ^{1}H-^{13}C direct correlations were determined by using HSQC spectrum and the results were supported by other data. The DEPT spectrum indicated the presence of three methylene carbons, one methyne, one methyl carbon attached to a nitrogen atom, five aromatic carbons, seven quaternary carbons and two methoxyl carbons in the molecule which further supported the structure of **3**.

The structure was finally confirmed by ^{1}H – ^{13}C long range correlations in the HMBC spectrum (Figure 5). Complete spectral data for ^{1}H NMR and ^{13}C NMR were summarized in the Table 5, whilst spectral data of ^{1}H - ^{1}H COSY, DEPT, ^{1}H – ^{13}C HSQC and HMBC in the table 6.

Comparison with the authentic sample and its data with the literature values confirmed that alkaloid 3 is indeed reticuline [14,15,16,17].

Alkaloid 4 was obtained as a yellowish amorphous. The UV spectrum showed maximum absorption at 219, 268, 280, 304 and 313 nm which are typical of an aporphinic nature at 280 and 304 nm, thus suggesting a 1,2,9,10-tetrasubstituted aporphine skeleton [18]. Its IR spectrum showed a strong absorption at 3300-3500 cm^{-1} due to the stretching of O-H, respectively.

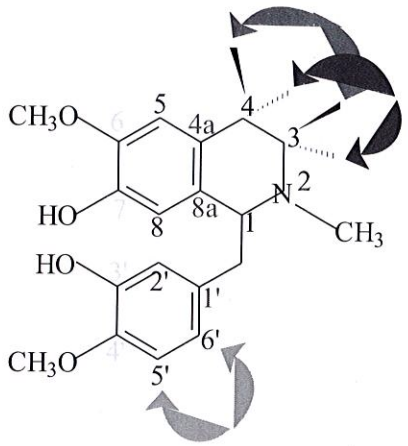

Figure 4 . The ^{1}H - ^{1}H COSY Correlations of alkaloid 3, Reticuline

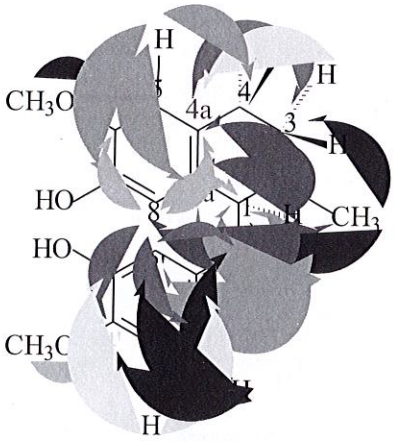

Figure 5 . The ^{1}H – ^{13}C HMBC long range correlations of alkaloid 3, Reticuline.

This region also indicated the presence of phenolic hydroxyl moiety. Others peak of absorptions are at 1515 cm^{-1}, 1250 cm^{-1} and 1415 cm^{-1} which indicated the C-H aromatic, C-O and CH_2 respectively. The molecular ion peak appeared at m/z 327 corresponding to the molecular formula $C_{19}H_{21}O_4N$, and the pattern of peaks was identical to the MS reported for thalifoline by Doskotch R.W. et al. (1969) and Azziz, S.A.S.S. (2006). Result (Calc. Mass = 230.0793) corresponding to the molecular formula of $C_{11}H_{13}NO_3Na$.

Table 5: ^{1}H NMR and ^{13}C-NMR chemical shift values (δ ppm) and coupling patterns of the protons of alkaloid 3, reticuline

Positions	δ ^{1}H (observed) Nita	δ ^{1}H (Nurdin,S.2007)	δ ^{1}H (Castro et *al.*1985)	δ ^{13}C (observed) Nita	δ ^{13}C (Nurdin,S.2007)	δ ^{13}C (Castro *et al.*1985)
1	3.66 (t)	3.66-3.69, t		64.41	64.45	64.51
3	2.80-3.20, m	2.74-2.79, m		46.42	46.48	46.81
4	2.50-2.85, m	2.55-2.60, m		24.72	24.65	25.83
4a	-	-		124.69	128.75	124.88
5	6.50 (s)	6.51,s	6.54 (s)	110.59	110.54	113.67
6	-	-		145.45	145.31	145.79
7	-	-		143.48	143.41	143.33
8	6.26 (s)	6.32, s	6.39 (s)	113.97	113.70	110.51
8a	-	-		129.70	124.71	132.88
1α	2.75-3.05, m	2.99-3.04, m		40.85	40.85	40.98
1'	-	-		132.83	132.72	129.84
2'	6.77 d (J_m=1.92 Hz)	6.73, d (J=1.72Hz)	6.77 (d)	115.82	115.61	110.51
3'	-	-		145.27	145.05	145.19
4'	-	-		145.51	145.26	145.59
5'	6.69 dd (J_o=8.24 Hz, J_p=1.36Hz)	6.69, d (J=8.32)	6.73 (d)	110.67	110.43	113.67
6'	6.52 dd (J_o=8.68Hz J_m=1.68,)	6.54, dd (J_1=8.28, J_2=1.96)	6.60 (dd)	120.98	120.90	120.79
6-OCH_3	3.80 (s)	3.81, s	3.85 (s)	55.82	55.84	55.92
4'-OCH_3	3.80 (s)	3.81, s	3.85 (s)	55.91	55.79	55.92
N-CH_3	2.44 (s)	2.44 (s)	2.47 (s)	42.15	42.12	42.38

The assignments of the ^{1}H and ^{13}C-NMR signals for this **4** were based on direct comparison with isoboldine [19,20].

The ^{1}H-NMR spectrum of this alkaloid **4**, showed three singlets aromatic protons at δ 7.99, δ 6.79 and δ 6.52 which were assigned to H-11, H-8 and H-3 respectively. Two singlets resonance at δ 3.90 and δ 3.88 were attributable to the methoxyl protons of C-2 and C-10. Aliphatic protons appeared as multiplet at the region of δ 2.55-3.17. The remaining signals at δ 2.53 indicated the presence of N-CH_3.

The ^{13} C-NMR spectrum gave a total of nineteen carbons, one of which was very downfield at δ 146.50 . The methoxyl carbon was observed at δ 55.80. Moreover, the C -3 of **4** resonated at δ 109.2 compare to δ 113.35 in the boldine [21] due to the fact that C-3 in the former is ortho to a methoxyl group, whereas in the latter it is ortho to a hydroxyl group.

Due to the limitation of the sample amount, we were unable to get its 2-D-NMR spectrum.

Furthermore, the spectroscopic data of **4** were identical with those reported for isoboldine [19,20], and it is confirmed that **alkaloid 4** was isoboldine or N-methyllaurelliptine.

Alkaloid 5 was obtained as colorless amorphous. The IR absorption spectrum indicated the presence of an OH stretching at 3418 cm^{-1} and a strong sharp band at 1638 cm^{-1} corresponding to the carbonyl group (tertiary δ-lactam) [22]. The UV spectrum showed absorption at 382 nm typical of an isoquinolone structure [23].

Using high-resolution electrospray ionisation (ESI) mass spectrometry (positive mode), spectrum of alkaloid 5 gave a molecular ion peak at m/z 208 $[M+1]^{+}$ giving a possible molecular formula of $C_{11}H_{13}NO_3$.

Table 6: ^{1}H - ^{1}H Cosy, DEPT, ^{1}H – ^{13}C HSQC and HMBC correlations of alkaloid 3, reticuline from *Litsea petiolata.*

Positions	^{1}H - ^{1}H Cosy	DEPT	^{1}H – ^{13}C HSQC	^{1}H – ^{13}C HMBC correlations
1		CH	H-1	N-CH_3, 3, 8, 4a, 1' 8a
3	H-3α / H-4α	CH_2	H-3	4a,
4	H-3β / H-4β	CH_2	H-4	3, 4a,
4a		C	-	
5		CH	H-5	4, 8[a], 7,
6		C	-	
7		C	-	
8		CH	H-8	1, 4a, 6
8a		C	-	
1α		CH_2	H-1α	1, 2', 6', 1', 8a
1'		C	-	
2'		CH	H-2'	1α, 6', 4',
3'		C	-	
4'		C	-	
5'	H-5' / H-6'	CH	H-5'	1', 3',
6'	H-6' / H-5'	CH	H-6'	1α, 2', 4'
6-OCH_3		CH_3	-	6,
4'-OCH_3		CH_3	-	4',
N-CH_3		CH_3	-	1, 3,

Table 7. Spectroscopic 1H-NMR data for alkaloid **4**, isoboldine.

Position	δ 1H ppm (J Hz) isoboldine (observed)	δ 1H ppm (J Hz) isoboldine (Ropi Mukhtar M. 1996)
1	-	-
1a	-	-
1b	-	-
2	-	-
3	6.52 (s)	6.49 (s)
3a	-	-
4	2.55-3.17 (m)	
5		
6a		
7		
7a		
8	6.79 (s)	6.77 (s)
9	-	-
10	-	-
11	7.99 (s)	7.98 (s)
11a	-	
2-OMe	3.90 (s)	3.85 (s)
10-OMe	3.88 (s)	3.85 (s)
N-Me	2.53 (s)	2.50 (s)

The ^{1}H NMR spectrum displayed the existence of one methoxyl group at δ 3.91 which most probably belong to the methoxyl attached to C-6. In addition a singlet corresponding to one proton was observed at δ 6.60 which may be ascribed to H-5. This observation also indicated that C-7 was substituted. The ^{1}H NMR also showed a singlet which was assignable to H-8 at δ 7.68. The chemical shift of H-8 was further downfield because of it was situated near the carbonyl group; C-1 thus, the proton was more deshielded compare to H-5. In conclusion, H-5 and H-8 were assigned to *para* position aromatic protons because of the absence of any observed spin coupling. A singlet at δ 3.12 was characteristic of the *N*-methylated (*N*-Me) group.

Table 8. Spectroscopic ^{13}C NMR data for isoboldine, (N-methyllaurelliptine)

Position	^{13}C isoboldine (observed)	^{13}C isoboldine (Jackman et al. 1979)	^{13}C boldine (Guinaudeau et al. 1979)
1	140.60	140.80	142.00
1a	119.70	119.60	126.80
1b	123.50	126.70	125.90
2	146.50	146.50	148.10
3	109.20	109.3	113.30
3a	126.70	123.10	129.90
4	28.40	28.60	28.90
5	52.90	53.10	53.40
6a	62.40	62.60	62.60
7	33.70	33.90	34.20
7a	129.10	129.20	130.20
8	114.90	115.10	114.20
9	145.40	145.40	145.10
10	145.30	145.20	145.60
11	113.60	113.70	110.10
11a	123.00	123.60	123.60
1-OMe	-	-	60.20
2-OMe	55.80	#	-
10-OMe	55.80	#	56.10
N-Me	43.60	43.80	44.00

have not reported

Table 9. Comparison spectral data of ^{1}H and ^{13}C NMR (400 and 100 MHz, $CDCl_3$) of alkaloid 5, thalifoline and literature values.

Position	δ^{13}C **(observed)**	δ^{1}H (multiplicity) **(observed)**	δ^{13}C **(Azziz,S.A.S.S.2006)**	δ^{1}H (multiplicity) **(Azziz,S.A.S.S.2006)**
1	164.9	-	164.5	-
3	48.6	3.53 (*t*, *J* = 6.72Hz)	48.4	3.52 *t*
4	27.8	2.91 (*t*, *J* = 6.72 Hz)	27.6	2.91 *t*
4a	131.03	-	130.8	-
5	108.9	6.60 *s*	108.7	6.60 *s*
6	149.6	-	149.3	-
7	144.8	-	144.4	-
8	114.1	7.68 *s*	114.1	7.63 *s*
8a	122.5	-	122.6	-
2-NCH_3	35.35	3.12 *s*	35.1	3.12 *s*
6-OCH_3	56.2	3.91 *s*	55.9	3.92 *s*

Moreover, the aliphatic protons appeared as two sets of triplets at δ 2.91 and δ 3.53 which were attributable to H-4 and H-3 respectively. The assignment of the aromatic protons was also confirmed by analysis of the COSY data.

The COSY spectrum showed that H-3 (δ 3.53) was only correlated with H-4 (δ 2.91). Correlations of COSY and HMBC of alkaloid **5** are shown in Figure 6 and 7 below.

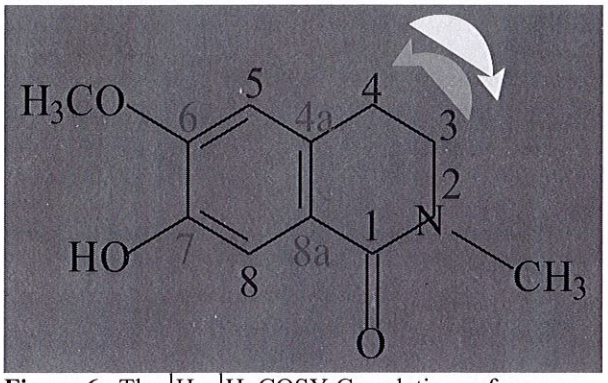

Figure 6 . The ^{1}H - ^{1}H COSY Correlations of **alkaloid 5**, thalifoline .

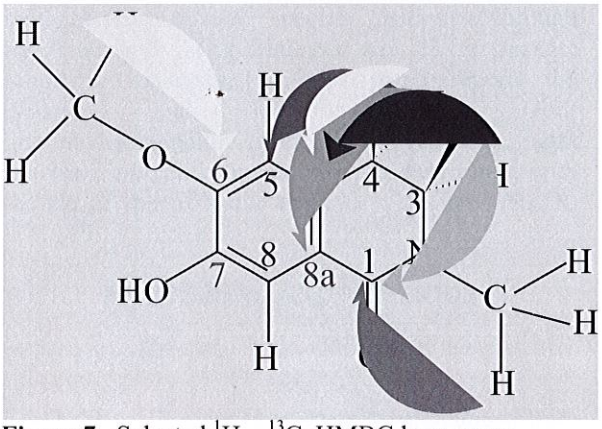

Figure 7 . Selected $^{1}H - ^{13}C$ HMBC long range correlations of **alkaloid 5**, thalifoline.

Detailed analysis on the ^{13}C NMR spectrum supported the spectroscopic data from the ^{1}H NMR. The signal resonated at the lower field (δ 164.9) indicated the presence of a carbonyl carbon which attached directly to the nitrogen atom (or amide group).

The spectrum also revealed one methyl at δ 35.35, two methylene at δ 48.6 and δ 27.8, two methine at δ 108.9 and δ 114.1, four quartenary at δ 122.5, 130.03, 144.4 and 149.3 and one methoxyl at δ 55.9. On the basis of the above results and comparison with the literature values [24] **alkaloid 5** was deduced as **thalifoline.**

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