CHAPTER 3

RESULTS AND DISCUSSION

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3.1 Introduction

Six Malaysian plants from the family of Lauraceae have been studied. Three plants from the genus *Phoebe i.e. Phoebe grandis, Phoebe scortechinii* and *Phoebe lanceolata*, and three other species from the genus *Dehaasia i.e. Dehaasia longipedicellata, Dehaasia candolleana* and *Dehaasia incrassata* have been investigated. This thesis will discuss the alkaloids found in the leaves and barks of the *Phoebe* species (**Part A**) and the alkaloids isolated from the leaves and bark of the *Dehaasia* species (**Part B**).

PART A

Isolation and structural elucidation of alkaloids from *Phoebe* species (Lauraceae)

3.2 Alkaloids from *Phoebe grandis* (Nees) Merr.

Two plant samples of *Phoebe grandis* have been studied. This report will discuss the alkaloids found in the leaves and the bark of *Phoebe grandis*, which were collected from two different localities (Gunong Stong Forest Reserve, Kelantan, KL4224, and Kuala Tahan Forest Reserve, Pahang, KL4994). Two aporphines, five proaporphine-tryptamines, seven proaporphines, and one indoloquinolizidine were isolated from the leaves and the bark of *Phoebe grandis* as shown in Table 3.1. Previous studies on this genus showed the presence of aporphines, oxoaporphines, proaporphines and proaporphine-tryptamine type of alkaloids[42, 112, 113, 114].

The two leaf samples of *Phoebe grandis* yielded proaporphine-tryptamine dimer alkaloids; phoebegrandine A and phoebegrandine B. Previously, the sample of *Phoebe grandis* collected from the lowland, Kedah (KL4318) gave aporphine type alkaloids besides the tryptamine dimers[42]. On the other hand, samples collected from Kuala Tahan Forest Reserve, Pahang (KL4994) and Gunong Stong Forest Reserve, Kelantan (KL4224) produced the proaporphine types. Interestingly, the latter (KL4224) also produced three novel proaporphine-tryptamines; phoebescortechiniine A **175**, phoebegrandine C **173** and phoebegrandine D **174** together with a novel indole alkaloid, phoebegrandine E **176** and tetrahydropronuciferine **177** which is never been

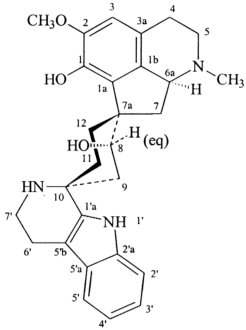
reported as a natural compound. This is the first report of such an indole alkaloid present in the *Phoebe* species.

Table 3.1: Alkaloids isolated from *Phoebe grandis*.

Herbarium Number	Plant Part (% Crude bases)	Alkaloids	Type of skeleton
KL4224	Leaves (0.480)	(-)-Phoebescortechiniine A **175**	Proaporphine-tryptamine
		(-)-Phoebegrandine A **28**	
		(-)-Phoebegrandine B, **29**	
		(+)-Phoebegrandine C **173**	
		(-)-Phoebegrandine D **174**	
		(-)-Phoebegrandine E **176**	Indole
	Bark (0.750)	Boldine **97**, Norboldine **98**,	Aporphine
		Grandine A **179**, Lauformine **116**	Proaporphine
		Grandine B **180**	
KL4994	Leaves (0.355)	(-)-Phoebescortechiniine A **175**	Proaporphine-tryptamine
		Phoebegrandine A **28**	
		Phoebegrandine B **29**	
		Tetrahydropronuciferine **177**	Proaporphine
		Tetrahydroglaziovine **178**	
	Bark (0.402)	Grandine C **183**	Proaporphine
		Grandine D **184**	
		Lauformine **116**	

3.2.1 Alkaloids Isolated from the leaves of *Phoebe grandis.*

3.2.1.1 *Phoebegrandine C* 173



173

Phoebegrandine C **173**, $[\alpha]_D^{23}$ +3.2° (*c* = 0.275, MeOH) was isolated as a brown amorphous. The UV spectrum showed absorptions at 238, 301 and 376 nm, characteristic of a β-carboline skeleton[115]. The IR spectrum showed a hydroxyl absorption band at 2918.72 cm^{-1}. The HRESI$^+$ spectrum showed an $[M+H]^+$ peak at 460.2616 thus, suggesting a molecular formula of $C_{28}H_{34}N_3O_3$ (calc. 460.2600).

The ^{1}H-NMR spectrum revealed four vicinal aromatic protons of the β-carboline moiety at δ 7.31 (1H, dd, *J, J'* = 1.2, 8.1 Hz), δ 7.15 (1H, dt, *J, J'* = 8.1, 1.2 Hz), δ 7.09 (1H, dt, *J, J'* = 8.0, 1.2 Hz), and δ 7.49 (1H, dd, *J, J'* = 8.0, 1.2 Hz) assignable to H-2', H-3', H-4' and H-5' respectively.

A singlet corresponding to one proton appeared at δ 6.54 which may be attributed to H-3 thus indicating that C-1 and C-2 are substituted. One methoxyl singlet revealed was at δ 3.81. The NOESY spectrum showed a correlation signal between the methoxyl protons and H-3, thus implying that the methoxyl group is attached to C-2. N-6 methyl protons resonated as a singlet at δ 2.35. A broad singlet corresponding to N-1' proton resonance appeared at δ 8.24. In the upfield region, the equatorial H-8 resonated as a broad singlet at δ 4.11. It was deshielded due to the presence of the adjacent hydroxyl group[116]. The H-6a signal appeared at δ 3.24 as dd (J, J' = 7.5, 7.9 Hz).

The ^{13}C-NMR DEPT experiment established the presence of 28 carbons; two methyls, eight methylenes, seven methynes and eleven quaternaries. The signals at δ 52.83 and δ 53.28 belonged to the two-spiro centers of the proaporphine-tryptamine skeleton, C-7a and C-10, respectively. In fact, the C-7a signal was more shielded as compared to those of phoebegrandines A **28** and B **29** (δ 47.8) due to the presence of the hydroxyl group at C-8 (β substituent effect)[116-118].

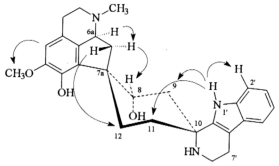


Figure 3.1: The main correlation of the protons in phoebegrandine C **173**

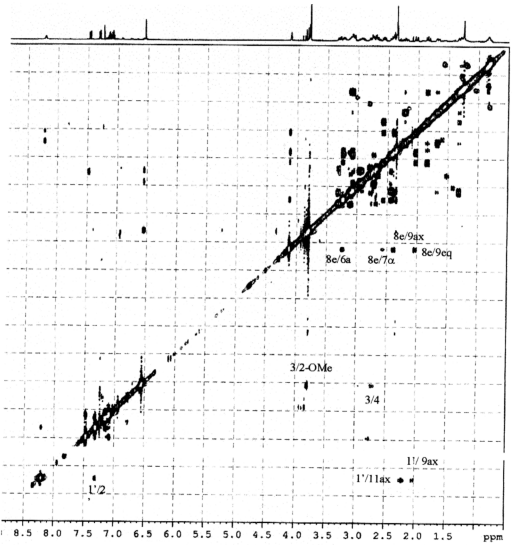


Figure 3.2: NOESY spectrum of phoebegrandine C **173**

2D NMR experiments (COSY, HMBC, HMQC and NOESY) allowed the complete assignments of the proton and carbon chemical shifts as shown in Table 3.2. The COSY experiment showed correlation peaks between CH-6a/CH_2-7, CH_2-11/CH_2-12, CH-8/CH_2-9, CH_2-4/CH_2-5 and CH_2-6'/CH_2-7'. The NOESY correlations between N-1' proton (δ 8.24) with H-2' (δ 7.31), H-9ax (δ2.02) and H-11ax (δ 2.35) confirmed that the stereochemistry at C-10 as *syn*. The following cross peaks; 6a/8eq, 6a/7α, 7β/12eq, established the relative stereochemistry at C-6a and C-7a[42, 43] (Figure 3.1 and Figure 3.2).

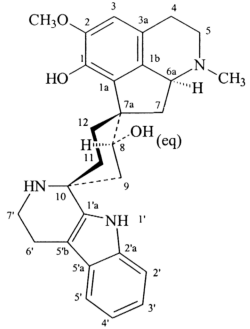
Finally, from the obtained spectra data, one may conclude that this novel alkaloid, phoebegrandine C **173** possesses the structure as shown above.

Table 3.2: ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data for phoebegrandine C **173**

Position	δ C ($CDCl_3$)	δ H (J Hz, $CDCl_3$)	HMBC	NOESY
1	141.96s			
1a	131.45s			
1b	132.69s			
2	149.33s			
3	110.55d	6.54 s	1, 1b, 2, 3a, 4	4α, 2-OMe
3a	121.55s			
4	27.06t	α 2.70 m	3, 3a	4β, 5β
		β 3.10 m	3a, 5	
5	55.18t	α 2.42 m	6a	5β, 6a
		β 3.15 m	3a, 4, 6a	
6a	65.45d	3.24 dd (7.5, 7.9)	1b, 7	4α, 7α, 8eq, N-Me
7	43.08t	α 2.58 m	1b, 6a, 8	6a, 8eq
		β 1.85 m	6a, 7a, 8, 12	12eq
7a	52.83s			
8	74.69d	eq 4.11 br s	7a, 9, 12	6a, 7α , 9ax, 11ax
9	35.35t	eq 2.35 m		
		ax 2.02 dd (12.6, 2.3)	8, 10	N-1'
10	53.28s			
11	34.66t	eq 1.63 m	7a, 10	
		ax 2.20 m		N-1'
12	27.13t	eq 1.38 br d	7a, 8, 10	11eq
		ax 3.10 m		
1'a	138.01s			
2'	110.97d	7.31 dd (8.1, 1.2)	4', 5'a	N-1', 3'
2'a	135.60s			
3'	122.11d	7.15 dt (8.1, 1.2)	2'a, 4', 5'	2', 4'
4'	119.54d	7.09 dt (1.2, 8.0)	2'a, 5'b, 5'	3', 5'
5'	118.23d	7.49 dd (8.0, 1.2)	2'a, 3', 5'a, 5'b	4'
5'a	127.10s			
5'b	108.7s			
6'	22.24t	2.78 m		7'
		3.18 m		
7'	38.74t	3.28 m		
$N\text{-}CH_3$	43.59q	2.35 s		
$2\text{-}OCH_3$	56.44q	3.84 s		
N-1'		8.24 br s	1'a, 2'a, 5'a, 5'b	2', 9ax, 11ax

eq = equatorial; ax = axial

3.2.1.2 *Phoebegrandine D* 174



174

Phoebegrandine D **174**, $[\alpha]_D^{23}$ = -22.16 (c=0.165, MeOH) was isolated as a brown amorphous. This rare proaporphine-tryptamine alkaloid exhibited an $[M+H]^+$ peak in HRESI$^+$ mass spectrum at m/z 460.2596 corresponding to the molecular formula of $C_{28}H_{34}N_3O_3$ (calc. 460.2600).

The UV and IR spectra were very close to those of phoebegrandine C **173**. Since the alkaloid was not very soluble in $CDCl_3$ and isolated in small amount (2mg), ^{1}H NMR of phoebegrandine D **174** was run in CD_3OD. The five aromatic protons of tryptamine and proaporphine moieties showed the same pattern of chemical shifts as those in phobegrandine C **173** (see Table 3.3). However, significant differences were observed in the chemical shifts of the H-6a and H-8 of ring D. H-6a of phoebegrandine D **174** resonated as a multiplet at δ 4.30. The chemical shift of H-8 appeared more downfield at δ 4.43 (dd, *J, J'* =11.0, 5.4 Hz) compared to that of

phoebegrandine C **173** (br s, δ 4.01) (see Figure 3.3). From the coupling constant of H-8 signal, it may be suggested that H-8 is in axial position[119]. In addition, Ricca analyzed that C-8 of proaporphine bearing hydroxyl group would resonate more upfield around δ 69.0 if the hydroxyl is equatorial. In the case of phoebegrandine D **174**, C-8 appeared at δ 69.88 in the ^{13}C NMR spectrum as compared to δ 74.69 in the case of axial[43,119]. The configuration of C-6a is only relative to phoebegrandine C **173**. It may be concluded that phoebegrandine D **174** is also having the same configuration at the spiro carbon, C-10, *i.e.* isolated as syn series[40-44].

The COSY experiment led to the assignments of the aliphatic protons with the significant correlation of H-6a (δ 4.30) to H-7 β (δ 1.85), H-6a (δ 4.30) to H-7α (δ 3.28), and H-8axial (δ 4.43) to H-9ax (δ 2.30) and H-8 axial (δ 4.43) to H-9eq (δ 2.35) respectively. Comparison of ^{1}H and ^{13}C NMR data of phoebegrandine C **173** and phoebegrandine D **174** is shown in Table 3.3.

Table 3.3: Comparison of ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of phoebegrandine C **173** and phoebegrandine D **174**.

Alkaloid	**173**		**174**	
Position	δ ^{13}C ($CDCl_3$)	δ ^{1}H (*J* Hz, CD_3OD)	δ ^{13}C ($CDCl_3$ + 2 drops of CD_3OD)	δ ^{1}H (*J* Hz, CD_3OD)
1	141.96		140.84	
1a	131.45		129.38	
1b	132.69		133.60	
2	149.33		147.86	
3	110.55	6.62 s	109.10	6.74 s
3a	121.55		121.21	
4	27.06		26.34	
5	55.18		55.18	
6a	65.45	3.30 m	66.54	4.30 m
7	43.08		47.66	
7a	52.83		54.80	
8	74.69	eq 4.01 br s	69.88	ax 4.43 dd (11.0, 5.6) (4.44 dd in $CDCl_3$)
9	35.35	eq 2.35 m ax 2.02 dd (12.6, 2.3)	31.90	eq 2.35 m ax 2.30 m
10	53.28		54.20	
11	34.66	eq 1.63 m ax 2.20 m	29.73	eq 2.02 m ax 2.20 m
12	27.13	eq 1.38 br d (13.9) ax 3.10 m	27.29	eq 1.38 br d (13.9) ax 3.10 m
1'a	138.01		138.89	
2'	110.97	7.30 dd (8.1, 1.2)	111.13	7.36 dd (8.6, 1.2)
2'a	135.60		135.92	
3'	122.11	7.15 dt (8.1, 1.2)	121.45	7.13 dt (8.6, 1.2)
4'	119.54	7.04 dt (1.2, 8.0)	118.98	7.03 dt (8.6, 1.2)
5'	118.23	7.40 dd (8.0, 1.2)	118.05	7.45 dd (8.6, 1.2)
5'a	127.10		126.98	
5'b	108.7		107.43	
6'	22.24		22.33	
7'	38.74		39.16	
N-CH_3	43.59	2.46 s	41.19	2.87 s (2.46 in $CDCl_3$)
2-OCH_3	56.44		56.45	

eq = equatorial; ax = axial

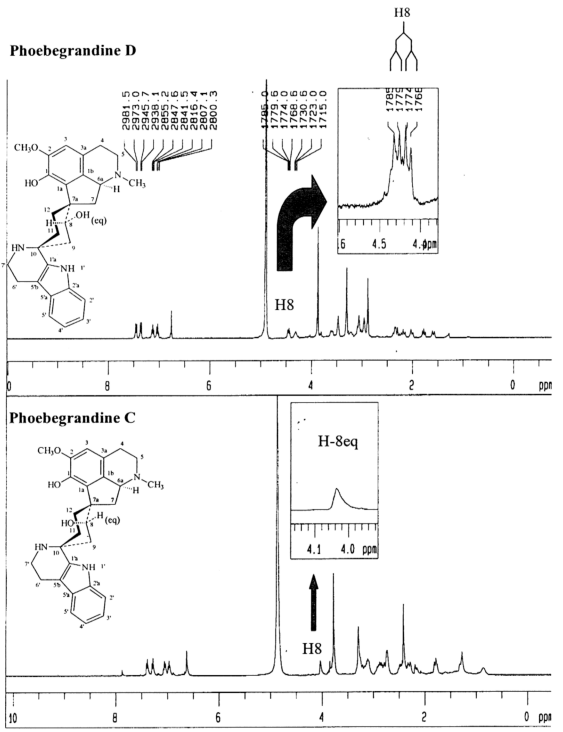
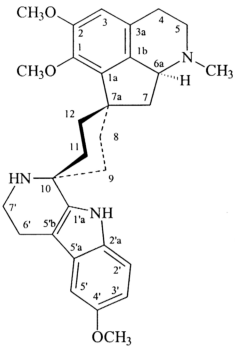
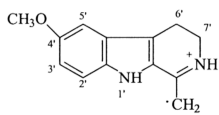


Figure 3.3: ^{1}H NMR spectrum of phoebegrandines C **173** and D **174** in CD_3OD.

3.2.1.3 *(-)-Phoebescortechiniine A* 175



175



175a

(-)–Phoebescortechiniine A **175**, $[\alpha]_D^{23}$ = -3.26 (c=0.525, MeOH) was afforded as a brown amorphous. HR MS showed an $[M+H]^+$ peak at 488.2924 (calc. 488.2913) corresponding to the molecular formula of $C_{30}H_{38}N_3O_3$. The high molecular weight suggested that the alkaloid might be a dimer. EIMS showed a fragmentation characteristic of β-carboline type moiety at m/z 214 **175a**[40]. The UV spectrum exhibited two maximum at 215.0 and 272.0 nm, which revealed an indole chromophore[42,44].

^{1}H NMR spectrum showed the signals of three aromatic methoxy at δ 3.86, 3.84 and 3.80, one of which belonged to the β-carboline moiety. The presence of three aromatic protons, two doublets at δ 7.20 and δ 6.93 (*J*=8.6 and 1.5 Hz respectively) and one doublet doublet at δ 6.78 (*J*= 8.6 and 1.5 Hz) suggested that the aromatic ring

of the β-carboline was monomethoxylated. A broad exchangeable proton at δ 7.75 is assignable to the NH-1'. A spin system of two multiplets at δ 3.75 m and δ 3.18 m is attributed to the 6' and 7' methylenes respectively. Ring A contains two methoxy and one aromatic proton at δ 6.54 singlet. The nitrogen atom of ring B was methylated (N-Me at δ 2.42, s).

The ^{13}C NMR spectrum showed there are 30 carbon resonances, which is in agreement with the molecular formula. The typical signals of the aromatic quarternary carbons of β-carboline[120] skeleton were observed in ^{13}C NMR as shown in Table 3.4. Moreover, the signals at δ 52.0 and δ 48.7 belonged to the two-spiro centers of the proaporphine-tryptamine skeleton, C-7a and C-10, respectively.

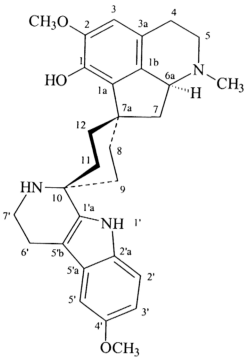
2D NMR experiments (COSY, HMBC, HMQC and NOESY) allowed the complete assignments of the proton and carbon chemical shifts as shown in Table 3. The COSY experiment showed correlation peaks between CH-6a/CH_2-7, CH_2-11/CH_2-12, CH-8/CH_2-9, CH_2-4/CH_2-5 and CH_2-6'/CH_2-7'. NOESY correlations were observed between N-1' proton (δ 7.75) with H-2' (δ 7.20), H-9ax (δ 1.90) and H-11ax (δ 1.90), thus confirming the stereochemistry at C-10 as *syn*. The following cross peaks; 6a/8eq, 6a/7α, 7β/12eq, established the relative stereochemistry at C-6a as *S* [40-43].

Table 3.4: ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data for alkaloid Phoebescortechiniine A **175** in $CDCl_3$

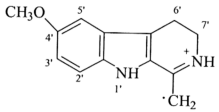
Position	δ C	δ H (J Hz)	HMBC	NOESY
1	144.50			
1a	140.10			
1b	133.30			
2	153.30			
3	108.60	6.54 s	1,1b, 2, 3a, 4	4α, 2-OMe
3a	126.60			
4	27.50	α 2.70 m	3,3a	4β, 5β
		β 2.90 m	5,3a	
5	55.20	α 2.45 dd (12, 5)	6a 3a, 4, 6a	5β, 6a
		β 3.15 m		
6a	65.40	3.23 m	1b, 5, 7	7α, N-Me
7	42.50	α 2.80 dd (11, 6.5)	6a	
		β 1.55 dd (11, 8.9)	6a, 7a, 8, 12	
7a	47.80			
8	28.90	eq 1.85 brd (13.5) ax 2.25 m	9, 7a	8ax, 9
9	33.60	1.90 m	7a, 8, 10, 11	1'
10	52.00			
11	33.90	1.85 m		1'
12	31.30	eq 1.38 brd (13.8) ax 3.01 m	7, 7a, 8, 11	12ax 1-OMe
1'a	141.70			
2'	111.50	7.20 d (8.6)	4' 5'a	1', 3'
2'a	130.80			
3'	111.40	6.78 dd (8.6, 1.5)	2'a, 4', 5'	4'-OMe
4'	154.20			
5'	100.70	6.93 d (1.5)	2'a, 4', 5'b	4'-OMe, 6'
5'a	128.10			
5'b	110.60			
6'	23.10	2.70 m	1'a, 5'a, 5'b, 7'	7'
7'	39.30	3.18 m	5'b, 6' 10	
N-CH_3	43.70	2.38 s		
1-OCH_3	61.20	3.86 s	1	
2-OCH_3	56.20	3.80 s	2	
4'-OCH_3	56.40	3.84 s	4'	
NH-1'		7.76 brs	1'a, 2'a, 5'a, 5'b	2', 9ax, 11ax

eq = equatorial; ax = axial

3.2.1.4 *Phoebegrandine A* **28**



28



175a

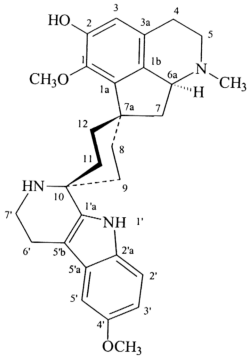
Phoebegrandine A **28**, $[\alpha]_D^{23}$= -10.56° was isolated as a brown gummy. The UV spectrum showed absorptions at 224, 277 and 298 nm. The IR spectrum showed OH absorption at 3270 cm^{-1}. Significantly, the MS spectrum of phoebegrandine A exhibited an M^+ peak at m/z 473 corresponding to the molecular formula of $C_{29}H_{35}N_3O_3$. The fragmentation peak m/z 214 ($C_{13}H_{14}N_2O$ ion **175a**) indicated that the carboline moiety was monomethoxylated.

The ^1H NMR spectrum showed the signals of two aromatic methoxyl groups at δ 3.77 and δ 3.78 corresponding to the 2-OMe and 4'-OMe, respectively. A broad exchangeable proton appears at δ 7.75 and can be assigned to NH-1'. The presence of three aromatic protons, two doublets at δ 6.87 and 7.15 (J= 2.4 and 8.8 Hz respectively) and one doublet doublet at δ 6.73 (J= 2.4, 8.8 Hz) corresponding to the

H-5', H-2' and H-3', respectively. One singlet at δ 6.44 (H-3) was observed in the spectrum confirming that C-1 and C-2 are substituted. A peak appeared at δ 2.36 is corresponding to the 6-NMe.

The ^{13}C-NMR spectrum of phoebegrandine A **28** showed the presented of a total of 29 carbon atoms as shown in Table 3.5. Finally, from the obtained spectra data[42,120], one may conclude that phoebegrandine A **28** possesses the structure as shown above.

3.2.1.5 Phoebegrandine B 29



29

Phoebegrandine B **29**, $[\alpha]_D^{23}$= -8.9° was isolated as a brown amorphous. The UV spectrum showed absorptions at 225, 278 and 308 nm. The IR spectrum showed OH absorption at 3270 cm^{-1}. The EIMS spectrum of phoebegrandine A exhibited an M^+ peak at m/z 473 corresponding to the molecular formula of $C_{29}H_{35}N_3O_3$. The fragmentation peak m/z 214 ($C_{13}H_{14}N_2O$) indicated that the carboline moiety was monomethoxylated.

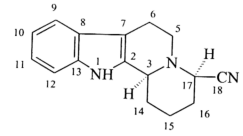
The ^1H NMR spectrum of phoebegrandine B **29** is very similar to that of phoebegrandine A **28**[5]. However, a significant difference is the presence of a sharp singlet corresponding to 1-OMe (δ 3.91), which is more downfield compared to 2-OMe proton resonance of phoebegrandine A (δ 3.80). The methoxyl carbon of alkaloid **29** resonated at δ 61.3 (1-OMe) compared to δ 56.2 (2-OMe) of

phoebegrandine A **28** as shown in Table 3.5. The signals at δ 48.0 and 54.2 belonged to the two-spiro centers of the proaporphine-tryptamine skeleton, C-7a and C-10, respectively. The relative configuration at C-6a is in accordance with that of phoebegrandine A **28** and determined as *S* [21,42,43].

Table 3.5: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of Phoebegrandine A **28** and Phoebegrandine B **29**

Position	**28** ^{1}H $CDCl_3$ (J, Hz)	^{13}C (δ, $CDCl_3$)	^{13}C (δ CD_3OD)	**29** ^{1}H $CDCl_3$ (J, Hz)	^{13}C (δ, CD_3OD)
1		144.50	140.50		144.40
1a		140.10	132.50		141.10
1b		*133.30*	*133.40*		*132.70*
2		*153.30*	*147.20*		*151.20*
3	6.54 s	108.60	108.40	6.56 s	115.10
3a		126.60	121.90		127.80
4	α 2.70 m β 2.90 m	27.50	27.30	α 2.70 m β 3.00 m	27.70
5	α 2.45 dd (12, 5) β 3.15 m	55.20	55.40	α 2.45 dd (12, 5) β 3.10 m	56.00
6a	3.22 m	65.40	65.80	3.23 m	66.60
7	α 2.80 dd (11, 6.5) β 1.55 dd (11, 8.9)	42.50	42.70	α 2.80 dd (11, 6.5) β 1.50 dd (11, 8.9)	43.10
7a		47.80	47.10		48.00
8	eq 1.60 br d (13.5) ax 2.25 m	28.90	27.90	eq 1.60 br d (13.5) ax 2.25 m	30.00
9	1.90 m	33.60	33.40	1.95 m	33.60
10		52.00	52.00		54.20
11	1.85 m	33.90	33.90	1.85 m	33.90
12	eq 1.38 brd (13.8) ax 3.01 m	31.30	31.00	eq 1.40 brd (13.8) ax 3.05 m	32.90
1'a		141.70	141.80		141.20
2'	7.20 d (8.6)	111.50	111.40	7.21 d (8.6)	112.40
2'a		130.80	130.80		132.90
3'	6.78 dd (8.6, 1.5)	111.40	115.50	6.79 dd (8.6, 1.5)	111.90
4'		154.20	154.20		155.00
5'	6.93 d (1.5)	100.70	100.50	6.93 d (1.5)	101.20
5'a		128.10	128.10		128.10
5'b		110.60	109.00		107.80
6'	2.70 m	23.10	23.10	2.70 m	22.80
7'	3.18 m	39.30	39.400	3.20 m	40.40
N-CH_3	2.35 s	43.70	43.80	2.35 s	43.30
1-OMe				3.91 s	61.30
2-OMe	3.80 s	56.20	56.20		
4'-OMe	3.84 s	56.40	56.60	3.84 s	56.30

3.2.1.6 *Phoebegrandine E* 176



176

Phoebegrandine E **176** was isolated as white amorphous, $[\alpha]_D^{23}$ = -38.44 (c=0.167, $CHCl_3$). The APCI$^+$ mass spectrum showed a molecular ion peak at m/z 252 thus suggesting a molecular formula of $C_{16}H_{18}N_3$. A fragmentation peak corresponding to a Retro-Diels Alder cleavage of ring D was observed at m/z 197[121]. Another fragmentation peak arising from the loss of CN (M^+ -26) was observed at m/z 225 (Scheme 3.1). The UV revealed maxima typical of an indole chromophore at 233 (3.95), 300 (3.60) and 339 (3.87)[115]. The IR spectrum showed the absorption of the nitrile group[122] at 2260 cm^{-1}.

The ^{1}H-NMR spectrum showed four vicinal aromatic protons signals at δ 7.45 (H-9, dd, *J*, *J'*= 1.2, 8.1 Hz), δ 7.08 (H-10, dt, *J*, *J'*= 1.2, 8.0 Hz), δ 7.14 (H-11, dt, *J*= 1.2, 8.0 Hz), and δ 7.30 (H-12, dd, *J*= 1.2, 8.1 Hz). H-3 showed a broad doublet at δ 3.75 (*J*= 11.5 Hz), while H-17 resonated at δ 4.01 as a dd (*J*, *J'*= 3.4). The methylene protons of C-5 (m) signals overlapped at δ 2.95. The COSY spectrum showed cross peaks between CH_2-5 and CH_2-6; CH_2-16 and CH_2-17.

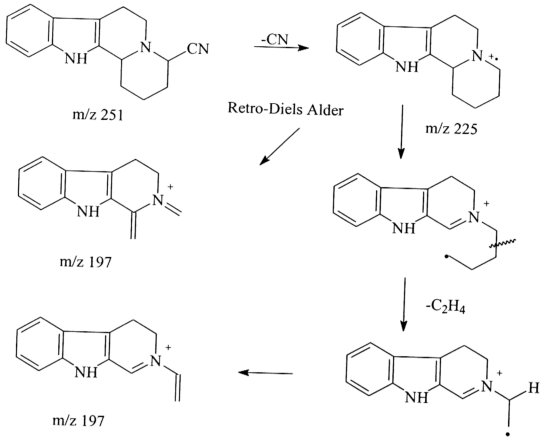
The ^{13}C-NMR and DEPT spectra established the presence of 16 carbons; six methylenes, six methynes and four quaternaries. The signal of C-17, which is attached

to N-4, appeared at δ 54.75 while the adjacent C-18 resonated at δ 116. The proximity of these two carbons was confirmed by the HMBC correlation peaks between H-3 and C-17. The connectivities of the other carbon atoms were established mainly by the HMBC correlations (Table 3.6). The N-4/C-17 ring closure was proven especially by the HMBC correlation of H-3 with C-17 and is further confirmed by a strong NOESY cross peak between H-5 and H-17.

The relative configuration of C-3 and C-17 was established from the NOESY spectrum, which showed cross peaks between H-3 and H-17, thus we deduce that the latter has the same spatial orientation as the former[123].

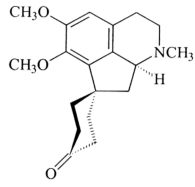
Table 3.6: ^{13}C NMR (100 MHz) and ^{1}H NMR (400 MHz) data for phoebegrandine E **176**

Position	^{13}C	^{1}H	NOESY	HMBC	DEPT	HMQC
1-NH		7.73 bs	3, 5, 14β	7, 8		
2	133.73					
3	54.85	3.75 bd	1, 7, 14β, 15, 16	2, 7, 14, 17	CH-3	H-3
5	51.64	2.95 m (2H)	6α	6, 7, 17	CH_2-5	2H-5
6	21.47	α 2.75 m		2, 5, 7	CH_2-6	2H-6
		β 2.95 m		5, 7		
7	107.84			5, 18		
8	127.05			1, 10, 12		
9	118.63	7.45 d		11, 12	CH-9	H-9
10	119.58	7.08 ddd		8, 9	CH-10	H-10
11	121.67	7.14 ddd		9, 13	CH-11	H-11
12	110.77	7.30 d	5, 6α	8, 10, 11	CH-12	H-12
13	136.08			9, 11		
14	29.77	α 1.55 dd		15, 16	CH_2-14	2H-14
		β 2.10 dd				
15	28.73	2.0 m (2H)		14, 16	CH_2-15	2H-15
16	20.58	α 1.90 m		18	CH_2-16	2H-16
		β 1.95 m				
17	54.75	4.01 t	5, 6α, 15	3, 15, 16, 18	CH-17	H-17
18	116.0			16, 17		



Scheme 3.1: The main fragmentation patterns of phoebegrandine E **176**.

3.2.1.7 *Tetrahydropronuciferine* 177



177

Tetrahydropronuciferine **177**, $[\alpha]_D^{23}$ -1.66° (MeOH, *c* 0.391), -50° ($CHCl_3$, *c* 1.0), was isolated as a brown amorphous. This proaporphine alkaloid exhibited an $[M+H]^+$ peak in the HRES (positive mode) mass spectrum at m/z 316.1957 (calc. 316.1913). The EI mass spectrum revealed the molecular ion peak as a base peak $[M]^+$ at m/z 315, thus referring to a molecular formula of $C_{19}H_{25}NO_3$. Another significant signal is m/z 314, which shows a fragmentation of $[M-H]^+$. The UV spectrum gave absorptions at 249 and 285 nm, which indicated the absorption of aromatic nucleus[124,125]. The IR spectrum revealed that absorption band at 1713 cm^{-1} was due to the C=O stretching vibration.

The ^1H NMR spectrum showed a singlet at δ 6.53, which can be ascribed to H-3, therefore indicating that C-1 and C-2 were substituted by the methoxyl groups. Two sharp singlets representing two methoxyl groups appeared at δ 3.74 and δ 3.76. The COSY spectrum showed the following correlation signals: CH_2-4/CH_2-5, CH_2-8/CH_2-9, CH_2-11/CH_2-12 and CH-6a/CH_2-7.

The DEPT spectrum showed that it consists of one tertiary carbon (-CH-) and seven secondary carbons (-CH_2-) in the molecule skeleton. Furthermore, the ^{13}C NMR spectrum showed 19 carbons resonance, which is in agreement with the molecular formula[120]. The spectrum revealed one carbonyl carbon, C-10 at δ 211.72. The rest of the ^{13}C NMR signals revealed a quaternary carbon (δ 47.30), two methoxy (δ 60.69 and δ 56.87) and an *N*-Methyl group (δ 42.52). HMQC and HMBC data aided the assignments of all the protons and carbons of **177** (Table 3.7 and Figure 3.4).

It is known that compounds of the C-6a *S* configuration possess a negative rotation, while those of C-6a *R* series are dextrorotatory[126-128]. Alkaloid **177** possesses a negative rotation, thus the configuration at C-6a is *S*.

The configuration of C-7a is determined through NOE differential experiment, which showed the enhancement of H-7α and H-8eq upon irradiation of H-6a.

Table 3.7: ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data# for tetrahydropronuciferine **177** and tetrahydroglaziovine **178**.

	177			**178**	
Position	δ C	^{1}H (Hz)	HMBC	δ C	^{1}H (Hz)
1	144.10			140.50	
1a	133.02			130.50	
1b	138.07			135.00	
2	153.02			147.30	
3	110.63	6.53 s	1, 1a, 2, 3a, 4	108.50	6.45 s
3a	126.49			121.40	
4	27.26	α 2.61 m	5	27.10	
		β 2.55 m	5		
5	54.91	α 2.38 m	4	41.40	
		β 3.07 m	4, 6a		
6a	65.05	3.23 dd (10.3, 6.3)	1a, 5, 7	65.30	3.17 m
7	43.40	α 2.72 m	6a, 8	46.80	
		β 1.65 dd (10.3, 11.3)	6a, 7a, 8, 12		
7a	47.30			54.90	
8	33.47	ax 2.22 m	7a	32.80	
		eq 1.98 m	7a, 10, 12		
9	38.62	2.48 m		38.60	
10	211.72			211.90	
11	39.13	eq 2.48 m		39.00	
		ax 2.80 m			
12	36.11	ax 2.82 m		35.50	
		eq 1.82 m	10		
1-OCH_3	60.69	3.74 s	1		
2-OCH_3	58.87	3.76 s	2	56.40	3.75 s
N-CH_3	42.52	2.38 s	5, 6a	42.90	2.37 s

Assignments based on 2D (HMBC and HMQC) experiments

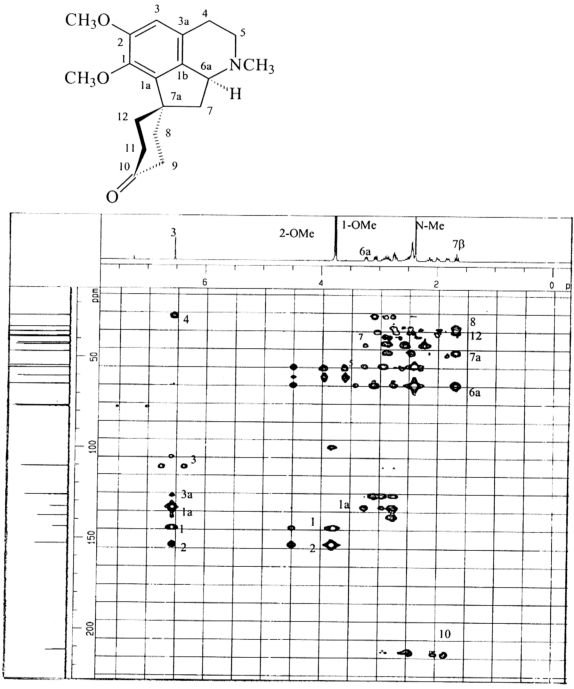
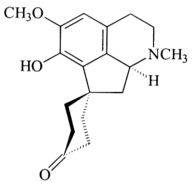


Figure 3.4: HMBC spectrum of tetrahydropronuciferine **177**.

3.2.1.8 *Tetrahydroglaziovine* 178



178

A proaporphine, tetrahydroglaziovine **178** was isolated from the leaves as an orange red amorphous. The UV spectrum showed absorptions at 226 and 283 nm, suggesting the absorption of the aromatic nucleus in the compound with its electron donating substituents (OH, OCH_3)[129]. The IR spectrum showed a very significant peak at 1672 cm^{-1}. This peak was due to stretching of a carbonyl group. In addition, a broad band at 3345 cm^{-1} was due to the presence of hydroxyl group.

Its mass spectrum gave a very significant molecular ion peak at m/z 301, giving the possibility of the molecular formula to be $C_{18}H_{23}NO_3$. The peak at m/z 300 indicated the loss of a proton and the peak at m/z 258 $[M\text{-}CH_2NCH_3]^+$ was consistent with that of a *N*-methylproaporphine[130-131].

The ^{1}H-NMR spectrum displayed one singlet at δ 3.75 corresponding to methoxyl group. In addition, a singlet corresponding to one proton was observed at δ 6.45, which is ascribed to H-3. This observation also indicated that C-2 is substituted. The *N*-methyl group resonated at δ 2.37 and the aliphatic protons gave a multiplet between δ 1.79 and δ 3.27 and the H-6a resonated at δ 3.17 m.

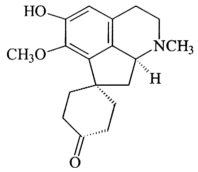
The ^{13}C-NMR spectrum of **178** showed the presence of eighteen carbon atoms. Analysis of the ^{13}C-NMR data allows the assignment of the carbonyl group (C-10) at δ 211.9. The characteristic proaporphine spirocarbon, C-7a appeared at δ 54.9, while C-2-methoxyl group peak appeared at δ 56.4. The recognition of the other peaks is summarized in Table 3.7. The stereochemistry of H-6a is only relative to **177**.

Finally, from the obtained spectra data and by comparison with those reported in the literature[132], one may conclude that the structure above is indeed the known alkaloid tetrahydroglaziovine **178**.

3.2.2 Alkaloids from bark of *Phoebe grandis* (Nees) Merr.

The bark of *Phoebe grandis* (KL 4224) yielded two aporphines: boldine **97**, norboldine **98**, and three proaporphines; lauformine **116**, grandine A **179** and grandine B **180** in which the last two are novel. On the other hand, the bark of *Phoebe grandis* collected at different location (KL 4994) afforded the known lauformine **116** and two novel proaporphines; grandine C **183** and grandine D **184** (see Table 3.1). The identities of known compounds; boldine **97**, norboldine **98** and lauformine **116** were confirmed by comparison of their spectrocopic data with the literature values[94,130, 131,147].

3.2.2.1 *Grandine A* 179



179

Grandine A **179**, $[\alpha]_D^{23}$ –50.0 (c =0.1, MeOH) was isolated as a white amorphous solid. The HRESI$^+$ mass spectrum showed the $[M+H]^+$ peak at m/z 302.1707 thus giving the possibility of the molecular formula to be $C_{18}H_{23}NO_3$ (calc. 302.1756). The peak at m/z 300 indicated the loss of a proton and the peak at m/z 258, $[M-CH_2NCH_3]^+$, were consistent with that of an *N*-methylproaporphine skeleton[130-134] (Scheme 3.2). The UV spectrum showed absorption maxima at 226 and 283 nm respectively. The IR spectrum showed a very significant absorption of a carbonyl at

1712 cm^{-1}. In addition, a broad band at 3345 cm^{-1} was due to the presence of a hydroxyl group[67].

The ^{1}H-NMR spectrum (Figure 3.5) displayed one singlet at δ 3.76 attributable to the methoxyl group attached to C-1. In addition, one proton singlet was observed at δ 6.41, which may be ascribed to H-3. This observation also indicated that C-2 is substituted. The *N*-methyl group resonated at δ 2.38 and the aliphatic protons gave a multiplet between δ 1.82 to δ 3.27. H-6a resonated at δ 3.23 (dd, *J, J'* = 11.0, 6.3 Hz) while H-7 appeared as multiplet at δ 2.72 and 1.65 as showed in COSY spectrum.

The ^{13}C-NMR spectrum of grandine A **179** showed the presence of eighteen carbon atoms, whereas the DEPT experiment displayed the presence of seven methylene and two methine signals. The quaternary C-7a peak appeared at δ 47.29, while the C-1 methoxyl group peak appeared at δ 61.00. The complete assignments of all protons and carbons were listed in Table 3.8.

The NOE differential measurements showed the interaction between H-6a/H-7α and H6a/H-8eq indicating that H-6a and those protons are *syn* to each other. The irradiation of the H-3 in the NOE's measurement only induced enhancement of H-4 (δ 2.55) suggested that the methoxyl group at C-1 instead of C-2. It is known that compounds of the C-6a *S* configuration possess a negative rotation, while those of C-6a *R* series are dextrorotatory[126-128]. Grandine A **179** possesses a negative rotation, thus the configuration at C-6a is *S*.

Table 3.8: ^{1}H (400 MHz), ^{13}C, DEPT (100 MHz) data (δ/ppm) for grandine A **179** in ($CDCl_3$ + two drops CD_3OD).

Position	δ ^{13}C	δ ^{1}H (Hz)	DEPT	HMBC
1	142.83			
1a	137.99			
1b	127.11			
2	149.84			
3	114.32	6.41 s	CH-3	1, 1b, 2, 4
3a	131.83			
4	26.72	α 2.61 m	CH_2-4	1b, 5
		β 2.55 m		
5	54.76	α 2.38 m	CH_2-5	3a, 6a, N-CH_3
		β 3.07m		
6a	65.12	3.23 dd	CH-6	1b, 7, 7a
		(11.0, 6.3)		
7	42.21	α 2. 72 m	CH_2-7	6a, 1b, 7a
		β 1.65 m		6a, 7a, 8, 12
7a	47.29			
8	33.70	ax, 2.22 m	CH_2-8	7
		eq, 1.98 m		
9	38.58	2.48 m	CH_2-9	8, 10
10	212.20			
11	39.13	ax, 2.48 m	CH_2-11	10, 12
		eq, 2.80 m		
12	36.23	ax, 2.82 m	CH_2-12	7a, 7
		eq, 1.82 m		
1-OCH_3	61.00	3.76 s		1
N-CH_3	43.06	2.38 s		5, 6a

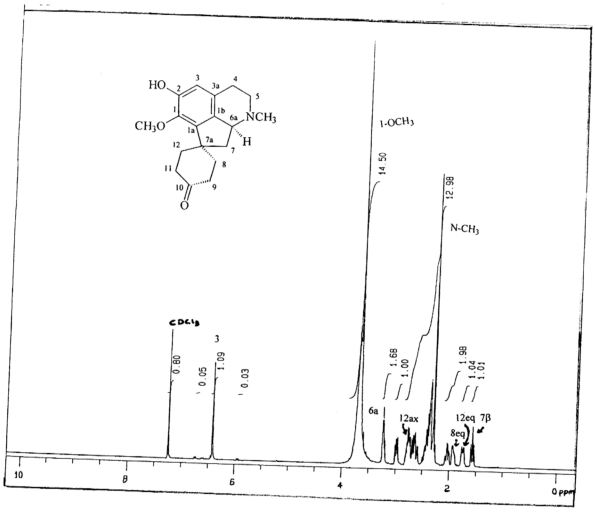
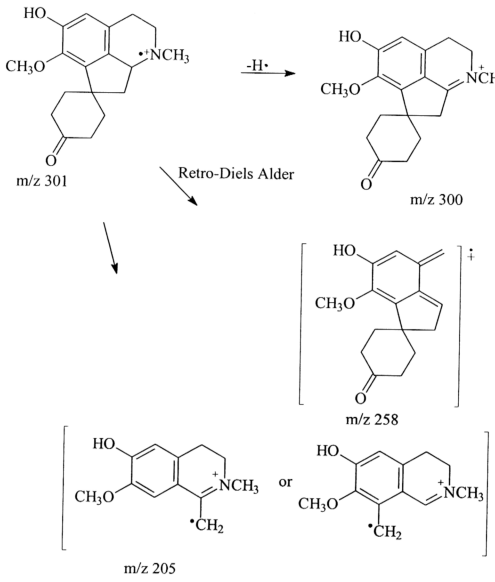
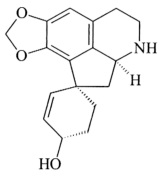


Figure 3.5: ^{1}H NMR spectrum (400 MHz) of grandine A **179**

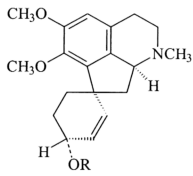


Scheme 3.2: Suggested fragmentation patterns of grandine A **179**

3.2.2.2 *Grandine B* 180



180



181 R = H

182 R = CH_2CH_3

Grandine B **180** was afforded as an amorphous solid, $[\alpha]_D^{23}$ +19.29° (c= 0.225, MeOH). The HRFAB mass spectrum revealed an $[M+H]^+$ peak at m/z 286.1439 and the EIMS showed an $[M]^+$ peak at m/z 285 (100%), thus suggesting a molecular formula of $C_{17}H_{20}NO_3$ (calc. 286.1431). The peak at m/z 256, $[M-29]^+$ was the product of the Retro-Diels Alder fission of the tetrahydroisoquinoline system, while the peak at m/z 189, $[M-96]^+$ was due to the methylenedioxy substituent (Scheme 3.3)[131,135]. The UV spectrum showed absorption bands at 240 and 298 nm. The IR spectrum revealed an absorption band of the hydroxyl group at 3285 cm^{-1}.

The ^{1}H-NMR spectrum (Figure 3.6) exhibited one aromatic proton singlet at δ 6.46 corresponding to H-3. Two doublets of AB system representing two protons centred at δ 5.78 and 5.80 (J = 10.3 Hz) was assignable to H-11 and H-12. The methylenedioxy protons appeared as a pair of doublets at δ 5.83 (J = 1.4 Hz) and δ 5.88 (J = 1.4 Hz). H-6a resonated at δ 4.07 (dd J, J' = 10.5, 6.6 Hz) and the signal of H-10 was seen as a dt at δ 4.11 (J, J' = 4.2, 1.3 Hz). Comparison of the ^{1}H NMR data

of **180** with those of two known alkaloids; amurolin **181** and *O*-acetylamurolin **182** is presented in Table 3.9[136].

Table 3.9: ^{1}H NMR data of Grandine B **180**, amurolin **181** and *O*-acetylamurolin **182** in $CDCl_3$ (δ in ppm, *J* in Hz).

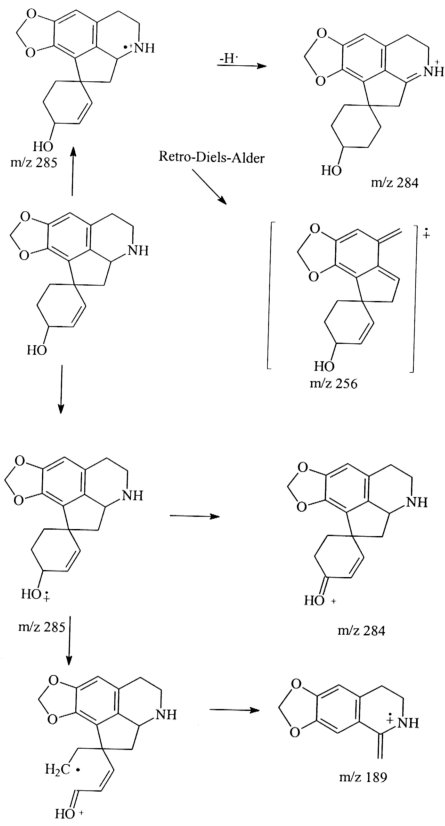
Position	**180** δ H (*J* Hz)	**181** δ H (*J* Hz)	**182** δ H (*J* Hz)
1			
1a			
1b			
2			
3	6.46 s	6.50 s	6.48 s
3a			
4	α 2.71 m		
	β 2.60 m		
5	α 3.38 m		
	β 3.08 m		
6a	4.07 dd (10.5, 6.6)		
7	α 2.39 m		
	β 1.68 dd (10.5, 11.0)		
7a			
8	eq 1.63 m	5.54 dd (10, 1.2)	5.68 s
	ax 2.35 m		
9	eq 1.95 ddd (13.6, 12.5, 3.6)	5.70 dd (10, 1.8)	5.68 s
	ax 2.10 m		
10	4.11 m		
11	5.78 d (10.3)		
12	5.80 d (10.3)		
OCH_2O	5.83 d (1.4)		
	5.88 d (1.4)		
1-OMe		3.70	3.68
2-OMe		3.78	3.76
NMe		2.38	2.31

The ^{13}C-NMR spectrum showed the presence of eight signals belonging to the aromatic and olefinic carbons (δ >110 ppm) of which three are protonated (CH), five are fully substituted, two oxygenated (δ >140 ppm) and three non-oxygenated. This suggested that grandine B contains an aromatic ring together with a double bond, which is part of an allylic alcohol. The rest of the ^{13}C-NMR signals revealed a quaternary carbon (δ 46.62), two methines (δ 57.46 and 64.52), five methylenes (δ 26-51) and one methylenedioxy (δ 100.4) (Table 3.10). The COSY experiment showed correlations between vicinal protons; CH-6a/CH_2-7, CH_2-11/CH_2-12, CH-10/CH_2-11 and CH-10/CH-9 respectively.

The relative stereochemistry of grandine B **180** at C-6a is determined as *S* in accordance with that of grandine A, and the H-6a is *anti* to the double bond in ring D. According to Guinaudeau *et. al.*[21] proaporphines with C-6a *S* configuration will have positive specific rotation when H-6a and the double bond is *anti* to each other, which is the case for grandine B. In addition, Ricca *et. al.*[26] has reported that the signal at C-7 in the *syn* and *anti* reduced proaporphines bearing an allylic alcohol in ring D is observed around δ 50 and δ 45 respectively. C-7 of grandine B resonated at δ 44.86, thus reinforcing the suggested *anti* positioning of the double bond.

Table 3.10: ^{1}H (400 MHz), ^{13}C, DEPT (100 MHz) data (δ/ppm) for grandine B **180** in $CDCl_3$.

Position	^{13}C	DEPT	δ H (J Hz, $CDCl_3$)	HMBC
1	140.48			
1a	136.23			
1b	126.58			
2	148.00			
3	106.64	CH-3	6.46 s	1, 2, 3a
3a	124.64			
4	26.31	CH_2-4	α 2.71 m	1a, 5, 3a
			β 2.60 m	3a
5	50.76	CH_2-5	α 3.38 m	1b, 4, 6a
			β 3.08 m	4,6a
6a	57.46	CH-6a	4.07 dd (10.5, 6.6)	1b, 3a
7	44.86	CH_2-7	α 2.39 m	
			β 1.68 dd (10.5, 11.0)	6a, 7a, 8
7a	46.62			
8	134.05	CH-8	eq 1.63 m	1a, 7, 7a, 10,
			ax 2.35 m	11
9	128.46	CH-9	eq 1.95 ddd (13.6, 12.5, 3.6)	7a, 9, 10,12
			ax 2.10 m	7a, 10
10	64.52	CH-10	4.11 m	11
11	30.00	CH_2-11	5.78 d (10.3)	10
12	29.41	CH_2-12	5.80 d (10.3)	7, 10, 12
OCH_2O	100.4	CH_2-1,2	5.83 d (1.4)	2
			5.88 d (1.4)	1



Scheme 3.3: Suggested fragmentation patterns of alkaloid B **180**.

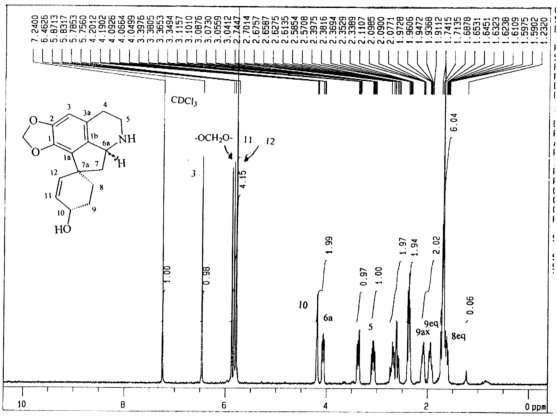
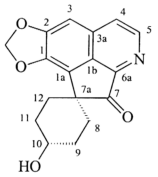


Figure 3.6: ^{1}H-NMR spectrum (400 MHz) of alkaloid grandine B **180**

3.2.2.3 *Grandine C* 183



183

The alkaloid grandine C **183**, $[\alpha]_D^{23}$ +100° (c= 0.5, $CHCl_3$), was obtained as a yellow amorphous mass. The HRFAB mass spectrum showed an $[M+H]^+$ peak at m/z 298.1079, thus giving a molecular formula of $C_{17}H_{16}NO_4$ (calc. 298.1078). Two major fragmentation peaks were observed at m/z 279 and m/z 251 which may be attributed to the loss of a water molecule, $[M-H_2O]^+$, and a carbonyl, $[M-CO]^+$ respectively. The UV spectrum showed absorption maxima at 330, 350 and 360 nm, which indicated the existence of a highly conjugated system[28]. The IR spectrum revealed absorption bands at 3378 and 1723 cm^{-1} caused by the OH and the pentacyclic C=O stretching vibrations respectively.

The ^{1}H-NMR spectrum (Figure 3.8) exhibited the dd peak of an unsaturated ring B, H-4 and H-5 protons centered at δ 7.56 and δ 8.66 (J = 5.4 Hz respectively). The small coupling constant is due to the close proximity to the adjacent nitrogen atom[137]. The spectrum showed a singlet at δ 7.04, ascribable to H-3. A two-proton singlet of a methylenedioxy group appeared at δ 6.13 thus suggesting that ring A and B were planar. H-10 resonated as a multiplet at δ 3.96.

The ^{13}C NMR spectrum of grandine C **183** showed a total of 17 carbons in the molecule, which is in agreement with the molecular formula of $C_{17}H_{16}NO_4$. The spectrum revealed the C-7 carbonyl peak at δ 206.02 and the quaternary spiro carbon, C-7a, at δ 50.57. The carbon bearing the hydroxyl group, C-10, resonated at δ 68.44. The methylene carbons of C-8 and C-12 resonated at δ 29.92 whereas the signals of C-9 and C-11 were seen overlapped at δ 29.38. The COSY, HMQC and HMBC experiments allowed the complete assignments of all protons and carbons (Table 3.11).

NMR NOE differential measurements, additionally attested to the chair conformation of ring D. Specifically, the interactions were observed between H-10 (δ 3.96), H-9 and H-11 at (δ 2.08) (Figure 3.7).

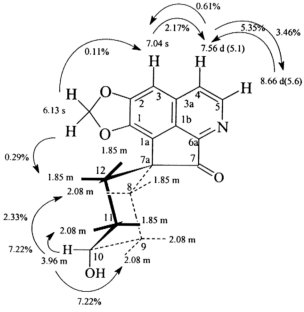


Figure 3.7: Partial NOE measurements of grandine C **183** showed the configuration of C-10 and C-7a.

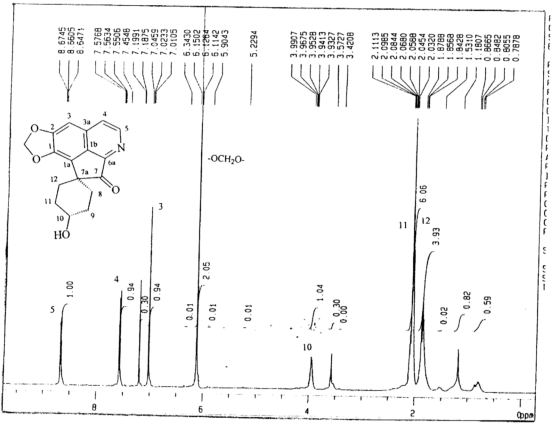
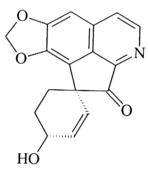


Figure 3.8: ^{1}H NMR spectrum (400 MHz) of grandine C **183**

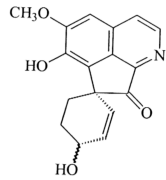
Table 3.11: ^{1}H (400 MHz), ^{13}C (100 MHz) data (δ/ppm) for grandine C **183** in $CDCl_3$

Position	^{13}C	δ H(J Hz, $CDCl_3$)	DEPT	HETCOR	HMBC
1	154.43				
1a	120.15				
1b	136.39				
2	142.06				
3	99.66	7.04 s	CH-3	H-3	1, 1a, 1b, 2
3a	151.23				
4	121.58	7.56 d (5.4)	CH-4	H-4	3a, 1b
5	146.96	8.66 d (5.4)	CH-5	H-5	3a, 4, 6a
6a	133.18				
7	206.02				
7a	50.57				
8	29.38	1.85 m (1H) 2.08 m (1H)	CH_2-8	2H-8	7, 7a, 10, 12
9	29.92	2.08 m	CH_2-9	2H-9	7, 10,11
10	68.44	3.96 m	CH-10	H-10	
11	29.92	2.08 m	CH_2-11	2H-11	10, 12
12	29.38	1.85 m	CH_2-12	2H-12	7, 7a, 8, 10
OCH_2O	102.11	6.13 s	CH_2	2H	1, 2

3.2.2.4 Grandine D 184



184



6

Alkaloid grandine D **184**, $[\alpha]_D^{23}$ + 55° (c= 1.0, $CHCl_3$), was isolated as brown amorphous solid. The HRESI$^+$ mass spectrum showed an $[M+Na]^+$ peak at m/z 318.0768 and the EI mass spectrum revealed the molecular ion peak at m/z 295 which is in accord with the molecular formula of $C_{17}H_{13}NO_4$ (calc. 318.0742 for $C_{17}H_{13}NO_4Na$). Fragmentation peaks were observed at m/z 277; $[M-H_2O]^+$, and m/z 249 $[M-CO]^+$. The UV spectrum gave absorptions at 250 and 320 nm, and the IR spectrum is similar to that of **183** indicating the presence of the pentacyclic C=O group (1729 cm^{-1})[138].

Similarly, the ^{1}H-NMR spectrum is also comparable to that of **183.** A major difference is the presence of two olefinic proton signals corresponding to H-8 and H-9, which appeared at δ 5.43 (d, J = 10.0 Hz) and δ 6.14 (dd, J, J' = 10.0, 2.9 Hz) respectively. Another significant difference is the more downfield multiplet of H-10 at δ 4.45 compared to δ 3.96 in **183**. This H-10 showed correlation to H-9 in the COSY spectrum. The COSY spectrum confirmed the assignments of all these protons by revealing the following correlation signals: CH-4/CH-5, CH-8/CH-9, CH-10/CH9 and

CH_2-11/CH_2-12. Comparison of the ^{1}H-NMR of grandine D **184** and the known alkaloid prooxocryptochine **6**[36] is shown in Table 3.13.

Table 3.12: ^{1}H (400 MHz), ^{13}C (100 MHz) data (δ/ppm) for grandine D **184** in $CDCl_3$

Position	^{13}C	δ ^{1}H (J Hz)	DEPT	HMQC	HMBC	NOESY
1	154.59					
1a	118.11					
1b	133.11					
2	150.86					
3	99.76	7.19 s	CH-3	H-3	1, 2, 3a	OCH_2O, H-4
3a	136.78					
4	121.66	7.60 d (5.4)	CH-4	H-4	3a	H-5
5	146.92	8.68 d (5.4)	CH-5	H-5	3a, 4, 6a	H-4
6a	142.62					
7	204.37					
7a	52.73					
8	126.28	5.43 d (10.0)	CH-8	H-8	7, 7a	H-9
9	134.75	6.14 dd (10.0, 2.9)	CH-9	H-9	7, 10	H-8, H-10
10	65.05	4.45 br s	CH-10	H-10		H-9, H-11
11	28.14	2.38 m 2.12 m	CH_2-11	2H-11	10, 12	H-12
12	28.60	2.12 m (2H)	CH_2-12	2H-12	7, 7a, 11	H-7 H-11
OCH_2O	102.37	6.16 br s 6.12 br s	CH_2-1, 2	2H	1, 2	

The ^{13}C NMR spectrum showed the signals of the olefinic C-8 and C-9 at δ 126.28 and δ 134.75 while the pentacyclic carbonyl carbon resonance at δ 204.37. The assignments of all the carbons were established through the aid of the HMBC and HMQC experiments (Table 3.12).

Table 3.13: ^{1}H (400 MHz) data (δ/ppm) for grandine D **184** and prooxocyprochine **6**[36] in $CDCl_3$

Position	Grandine D **184**	Prooxocryptochine **6**
1		
1a		
1b		
2		
3	7.19 s	7.15 s
3a		
4	7.60 d (5.4)	7.67 d (5.6)
5	8.68 d (5.4)	8.77 d (5.6)
6a		
7		
7a		
8	5.43 d (10.0)	5.49 dd (10, 3.2)
9	6.14 dd (10.0, 2.9)	6.25 dd (10, 3.2)
10	4.45 br s	4.44 m
11	2.38 m	2.29 m
12	2.12 m (2H)	2.06 m 2.26 m
OCH_2O	6.16 s 6.12 s	
1-OMe		3.72 s
2-OMe		4.02 s

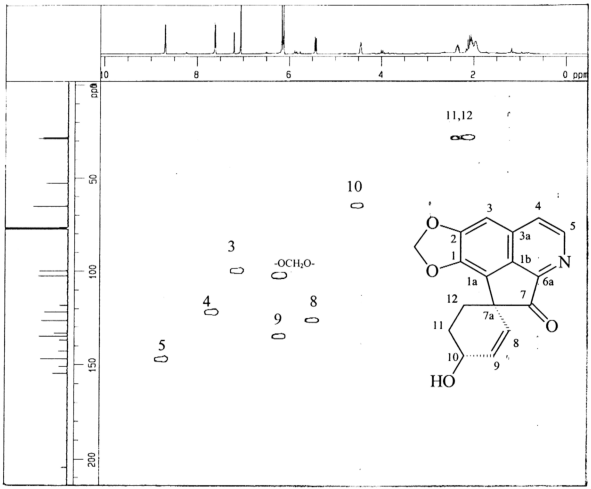
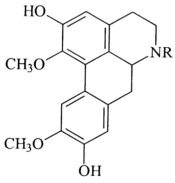


Figure 3.9: HMQC spectrum of grandine D **184**.

3.2.2.5 Boldine 97 and norboldine 98



97 R = CH_3
98 R = H

Boldine **97** and norboldine **98** isolated from this species were also found in the bark of *Phoebe lanceolata*. Their structural elucidation will be explained in section 3.4 (see page 148).

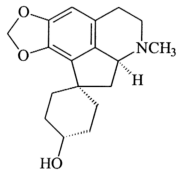
3.3 Alkaloids isolated from *Phoebe scortechinii.*

Three proaporphine-tryptamine dimers were isolated from the leaves *i.e.* phoebegrandine A **28**, phoebegrandine B **29** and phobescortechiniine A **175**. One proaporphine alkaloid, tetrahydropronuciferine **178** was also isolated from this species. This is the first natural product reported on this species.

The bark of *Phoebe scortechinii* produced five proaporphine alkaloids, grandine B **180**, grandine C **183**, (-)-hexahydromecambrine A **186**, and (-)-norhexahydromecambrine A **187** together with one aporphine type, norboldine **98**.

The structural elucidation of the alkaloids isolated from the leaves and bark of *Phoebe scortechinii* have been previously discussed except for (-)-Hexahyromecambrine A **186** and (-)-norhexahyromecambrine A **187**.

3.3.1 *(-)-Hexahydromecambrine A* **186**



186

(-)-Hexahyromecambrine A **186**, $[\alpha]_D^{23}$ –7.2 (c= 0.15, MeOH) was isolated from the bark of *Phoebe scortechinii* as an amorphous solid. The EI mass spectrum showed a molecular ion peak at m/z 301. In addition, ESI^+ spectrum revealed an

$[M+H]^+$ peak at m/z 302.09 and APCI$^+$ spectrum produced an $[M+H]^+$ peak at m/z 302.13. All these data suggested a molecular formula of $C_{18}H_{25}O_3N$. The UV spectrum showed three peaks at 300, 247 and 267 nm, which indicated the existence of the conjugated system[28]. The IR spectrum showed absorption bands at 3420 cm^{-1}, which indicated the presence of a secondary hydroxyl group in the molecule[141]. The presence of a methylenedioxy group was proven by its characteristic absorption at 1245 and 934 cm^{-1}, which indicate asymmetric C-O-C stretching.

The ^{1}H-NMR spectrum showed a doublet doublet at δ 5.81 and δ 5. 82 (d, J = 1.3 Hz.) representing the methylenedioxy protons. A singlet corresponding to H-3 appeared at δ 6.45. The aliphatic protons of ring D resonated as multiplets between δ 1.50 to 3.80. H-6a resonated as doublet doublet at δ 3.18 (J, J' =10.3, 6.3 Hz). The *N*-methyl protons appeared at δ 2.35 as a sharp singlet. H-10, which is in proximity to a hydroxyl group resonated further upfield at δ 3.71 as a broad multiplet suggesting the possibility of an axial configuration (Figure 3.10). Interestingly, Guinaudeau *et. al*[21,39]. reported that H-10 of propaorphines appeared at δ 4.11 when equatorial and δ 3.75 when axial, thus substantiate the hypothesis that H-10 of **186** is axial. In addition Ricca *et. al.*[116] analyzed that C-10 of reduced proaporphine bearing hydroxyl group would resonate more downfield around δ 69.1 if the hydroxyl is equatorial. In the case of **186**, C-10 appeared at δ 70.38 as compared to δ 63.2 in the case of axial.

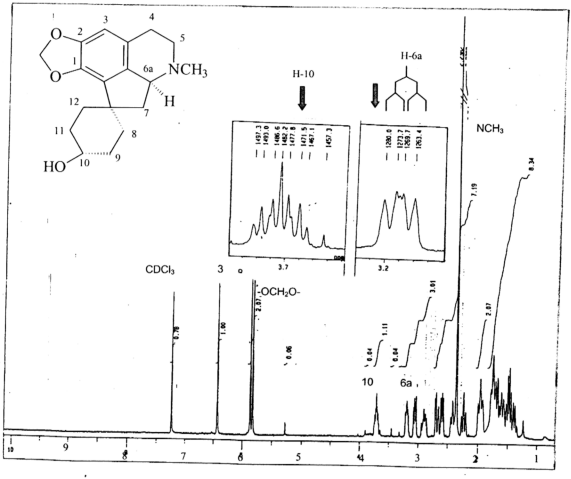


Figure 3.10: ^{1}H NMR spectrum (400 MHz) of hexahydromecambrine A **186**.

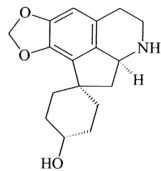
The ^{13}C-NMR DEPT spectrum showed the presence of one *N*-methyl signal, eight methylenes and three methynes and six quaternary carbons. The characteristic propaorphine spirocarbon, C-7a appeared at δ 46.01. Apparently, C-10 resonated at δ 70.38 further strengthen the hypothesis of H-10 being axial. The complete assignments of all protons and carbons (Table 3.14) were aided by the HMBC, HMQC and COSY experiments.

It seems that hexahydromecambrine A can exist as a natural compound and this is the first report of hexahydromecambrine A found in plant. It was communicated previously as a synthesized compound by Nakasato[142] from *Litsea* species.

Table 3.14: ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for alkaloid hexahydromecambrine A **186.**

Position	^{13}C	^{1}H (Hz)	DEPT
1	147.98		
1a	128.75		
1b	135.81		
2	140.37		
3	105.90	6.45 s	C-3
3a	124.05		
4	27.45	2.61 m	C-4
		2.55 m	
5	55.11	3.38 m	C-5
		3.07 m	
6a	65.99	3.18 dd	C-6
		(6.3, 10.3)	
7	42.91	β 1.45 m	CH_2-7
		α 2.55 m	
7a	46.01		
8	34.96	2.25 m	CH_2-8
		1.60 m	
9	32.10	1.75 m	CH_2-9
10	70.38	3.71 br m	CH-10
11	33.09	1.94 m	CH_2-11
12	31.99	1.74 m	CH_2-12
		1.76 m	
OCH_2O	100.55	5.87 d (1.3)	CH_2
		5.82 d (1.3)	
NCH_3	43.46	2.35 s	CH_3

3.3.2 *(-)-Norhexahydromecambrine A* 187



187

(-)-Norhexahydromecambrine A **187**, $[\alpha]_D^{23}$ –2.15 (c= 0.325, MeOH) was isolated from the bark of *Phoebe scortechinii* as an amorphous solid. The HR-ESI$^+$ mass spetrum showed the $[M+H]^+$ peak at m/z 288.1634 (calc. 288.1600). The mass spectrum (EI) showed a molecular ion peak at m/z 287 which correlated to the molecular formula of $C_{17}H_{21}O_3N$. In UV spectrum three peaks revealed at 300, 247 and 267 nm, which indicated the existence of the conjugated system[28]. The IR spectrum indicated the presence of hydroxyl group[141] by exhibiting absorption at 3420.9 cm^{-1}.

The ^{1}H-NMR spectrum (Figure 3.11) of **187** is similar to that of **186** except for the absence of N-Methyl protons signal in **187**. Another slight difference is the resonance of H-6a (δ 4.01), which is more downfield compared to that of **186** (δ 3.18). In the ^{13}C NMR spectrum, C-6a appeared at δ 58.29. The complete assignment of ^{13}C NMR together with DEPT and HMBC data is summarized in Table 3.15.

Table 3.15: ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for alkaloid norhexahydromecambrine A **187** in $CDCl_3$.

Position	δ C	DEPT	δ ^{1}H (Hz)	HMBC
1	148.36			
1a	128.85			
1b	135.81			
2	140.88			
3	106.67	CH-3	6.63 s	1, 1b, 2
3a	125.11			
4	26.48	CH_2-4	β, 2.55 m	1b, 3a, 5,
			α, 2.61 m	
5	45.36	CH_2-5	β, 3.07 m	6a
			α, 3.38 m	
6a	58.29	C-H	4.01 dd (9.9, 7.1)	1b
7	44.49	CH_2-7	β 2.68 m	
			α 1.45 m	6a, 7a, 8
7a	46.33			
8	35.29	CH_2-8	ax, 2.28 m	7, 9
			eq, 1.60 m	9
9	33.52	CH_2-9	1.78 m	8, 10
10	70.85	CH-10	3.70 br m	
11	32.34	CH_2-11	1.99 m	12
12	34.45	CH_2-12	1.76 m	11
OCH_2O	100.79	CH_2-1, 2	5.81 d (1.3)	1
			5.82 d (1.3)	2

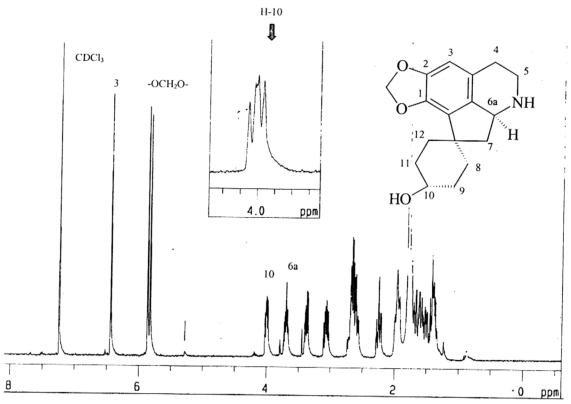


Figure 3.11: ^{1}H NMR spectrum (400 MHz) of norhexahydromecambrine A **187**.

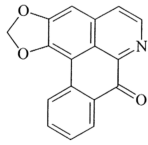
In conclusion, we observed that *Phoebe scortechinii* produces alkaloid closely related to *Phoebe grandis,* which was discussed earlier (Section 3.2.2). Both produced proaporphines, aporphines and proaporphine-tryptamines.

3.4 **Alkaloids isolated from *Phoebe lanceolata***

One oxoaporphine, four aporphines and one morphinandienone alkaloid were isolated from the stem bark of *Phoebe lanceolata i.e.* liriodenine **135**, boldine **97**, norboldine **98**, asimilobine **57**, roemerine **47** and N-methyl-2,3,6-trimethoxymorphinandien-7-one. The leaves of *Phoebe lanceolata* produced liriodenine **135**, roemerine **47** and laurotetanine **99**. Interestingly, N-methyl-2,3,6-trimethoxymorphinandien-7-one is the first morphinandienone alkaloid reported present in the bark of *Phoebe* species. It has been found in *Dehaasia candolleana* and this type of alkaloid will be discussed in Part B section 3.7.

3.4.1 Alkaloids isolated from the bark of *Phoebe lanceolata*

3.4.1.1 *Liriodenine* 135.



135

Liriodenine **135** was obtained as fine yellow needles from chloroform with m.p. 278 - 280°C. An oxoaporphines nature was deduced for the major alkaloid of **135**, based on its intense yellow colour, strongly fluorescent chloroform solution and the deep red colouration it produced in acid medium. This was supported by data

obtained from UV-Vis and IR spectroscopy. The former showed absorption bands at 262, 248, 310 and 415 nm. The latter showed a very significant peak at 1658 cm^{-1}. This peak was due to the stretching of a highly conjugated carbonyl group. In addition an absorption characteristic of a methylenedioxy was also observed at 969 cm^{-1}. Finally a peak at 865 cm^{-1} was present as a result of the C-H out of plane deformation of a single isolated aromatic proton, which is attached to C-3 [143]. All these data signified the presence of a highly conjugated chromophore with a ketone group enwrapped within the system. Its mass spectrum gave a very significant molecular ion peak at m/z 275, giving the possibility of the molecular formula to be $C_{17}H_9NO_3$. Other fragmentation peaks observed were m/z 247 [M^+ - CO] and m/z 246 [M^+ - CHO][144,145].

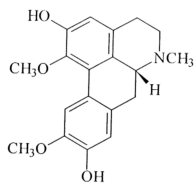
The ^1H NMR spectrum revealed the characteristic AB quartet typical of H-4 and H-5 at δ 7.5 and δ 8.87 respectively with a coupling constant of 5.3 Hz. A one proton singlet was observed which is attributable to H-3 at δ 7.17. In addition a two proton singlet at δ 6.36 indicative of a methylenedioxy group was also present. More over two sets of multiplets at δ 7.56 - 7.80 and δ 8.55 - 8.63 corresponding to four protons suggested that ring D is not substituted. The ^{13}C NMR data is shown in Table 3.16.

Table 3.16 : ^{13}C NMR data of liriodenine.

Position	Liriodenine
1	163.20
2	165.30
3	105.60
3a	146.20
4	129.00
5	140.00
6a	161.40
7	179.90
7a	129.80
8	131.20
9	132.40
10	135.40
11	130.40
11a	152.20
1a	124.50
1b	139.60
O-CH_2-O	107.60

Comparison with literature values[146,147] and comparative tlc with the authentic sample (base of Rf 0.85, silica gel $CHCl_3$-MeOH, 7:1) confirmed that alkaloid **135** is liriodenine. It was isolated as major alkaloids in both bark and leaves of *Phoebe lanceolata.*

3.4.1.2 *Boldine* **97**



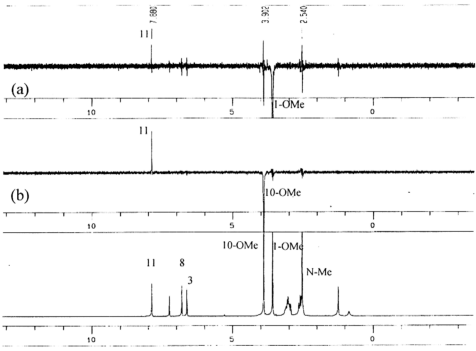
97

Alkaloid **97,** boldine was isolated as a white amorphous from methanol. The UV spectrum showed absorption typical of aporphine at 283 nm and 304 nm. These absorption peaks were due to the degree of resonance in the biphenyl system and any bands in the region above 305 nm eliminated the possibility of a 9,10 -disubstitution [86, 148]. Moreover, IR spectrum showed the presence of a highly conjugated hydroxyl group at about 3533 cm^{-1}.

The characteristic $[M-1]^+$ peak which appeared as the base peak in the mass spectrum of **97** further supported its aporphinic nature [149]. Molecular ion peak observed at m/z 327 gave a possible molecular formula of $C_{19}H_{21}O_4N$ and the peak at m/z 284 $[M-CH_2=NCH_3]^+$ was consistent with that of an *N*-methylaporphine. The low intensity fragment ions at m/z 312 $[M-CH_3]$ and m/z 296 $[M-OCH_3]^+$ indicated the presence of a methoxyl substituent at C-1.

The ^{1}H-NMR spectrum showed the presence of a singlet peak resonating at δ 2.52 was due to N-CH_3 group. Two singlets corresponding to two methoxyl groups appeared at δ3.91 and δ3.60 respectively. The former was attributed to C-10 and the

latter was assigned to C-1, which was more shielded since it experienced the anisotropic effect of the ring D. Three singlets representing three aromatic protons were observed at δ 6.63, δ 6.83 and δ 7.89, which can be ascribed to H-3, H-8 and H-11 respectively.



^{1}H NMR spectrum

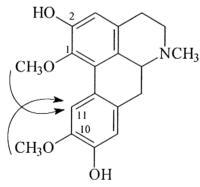
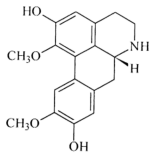


Figure 3.12: ^{1}H NOE differential spectrum for alkaloid **97**.

The positions of the C-1 and C-10 methoxyls were also proven by NOE differential experiments. Irradiation of 1-OMe and 10-OMe unsimultaneously at δ 3.60 and δ 3.91 only enhanced the H-11 singlet at δ 7.89, thus C-1 and C-10 are bonded to methoxyls (see Figure 3.12a and 3.12b).

The ^{13}C NMR spectrum for alkaloid **97** showed eighteen carbon signals as well as DEPT experiment which showed three methyls, three methylenes, four methines and eight quaternary carbon signals, which were agreement with the molecular formula of $C_{19}H_{21}NO_4$. Finally, comparison of its spectroscopic data with those reported in the literature [148-149], confirmed that alkaloid **97** is indeed boldine.

3.4.1.3 *Norboldine* **98**



98

(-)-Norboldine **98** was isolated as a solid mass, $[\alpha]_D^{23}$ –86.77° (c= 0.167, MeOH). Its UV spectrum showed absorption bands at 284 and 304 nm, thus suggesting a 1,2,9,10-tetrasubstituted aporphine skeleton[85]. The maxima were due to the resonance of the biphenyl system that existed in ring A and D. In addition, the IR spectrum gave a broad band between 3500 and 2936 cm^{-1} due to the presence of OH and NH groups. The UV and IR spectra of **98** were typical of an aporphine carrying two hydroxyl groups.

Alkaloid **98** showed an M^+ (70%) at m/z 313 suggesting a molecular formula of $C_{18}H_{19}NO_4$. The base peak at m/z 312 $[M-1]^+$ (100%) indicated the loss of a proton. In addition, the peaks at m/z 298 $[M-CH_3]^+$ and m/z 282 $[M-OCH_3]^+$ confirmed the presence of a methoxyl group at C-1.

The ^{1}H-NMR spectrum showed three aromatic proton singlets at δ 7.90, δ 6.73 and δ 6.57 due to H-11, H-8 and H-3 respectively. Two aromatic methoxyl groups attached to the C-2 and C-10 appeared at δ 3.88 and δ 3.56.

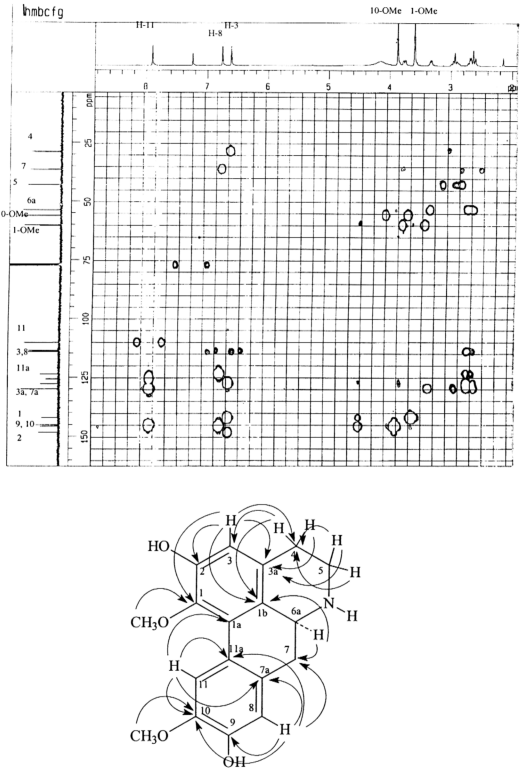
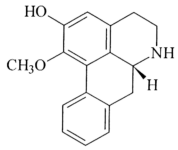


Figure 3.13: Homonuclear Multiple Bond Connectivity (HMBC) experiment of alkaloid **98** (δ H correlations).

The HMBC spectrum is shown in Figure 3.13. The proton carbon conectivities were established from the HMQC experiment (Table 3.17). Comparison of **98** with the authentic sample and its spectroscopic data with the literature values[150, 151] confirmed that alkaloid **98** is norboldine.

3.4.1.4 *Asimilobine* 57



57

Asimilobine **57**, $[\alpha]_D^{23}$ −36.13° (*c* 0.075, MeOH) was isolated and crystallized from acetone to give a colourless prism. It was also characterized by the development, on exposure to iodine vapour, of an orange-coloured spot that gradually darkened to a green colour when the plate was allowed to stand exposed to the atmosphere.

The mass spectrum showed a molecular ion peak at m/z 267 corresponding to a molecular formula of $C_{17}H_{17}O_2N$. The peak at m/z 238 $[M-29]^+$ due to the loss of methylene imine and a base peak at m/z 266 $[M-1]^+$ was also present.

In the UV region, it absorbed at 273 and 308 nm and in alkaline medium it experienced a bathochromic shift, hence suggested that hydroxyl substituent may be present. Moreover, the IR spectrum showed a strong absorption at 3255 cm^{-1} and 3550 cm^{-1} due to the stretching of OH and N-H. An absorption by the methoxyl was observed at 1035 cm^{-1}.

The ^1H NMR spectrum exhibited four aromatic proton signals as a series of multiplets. The doublet centred at δ 8.03 and those multiplets between δ 7.20-δ6.80, were integrated for one proton and three protons respectively. The former was attributed to H-11 since in aporphines this proton would always be found more downfield than the other aromatic protons due to the deshielding effect caused by the facing ring A and the latter was attributed to H-8, H-9 and H-10. One methoxy singlet was observed at δ 3.60, which is rather shielded compared to the normal aromatic methoxyls since the protons of the methoxy were forced to place themselves on top of ring D where the electron density is high. A singlet at δ 6.70 was also observed which is attributable to H-3.

The aromatic methoxyl group substituted at C-1 in ring A was confirmed by corresponding NOE differential experiment (Figure 3.14). Irradiation of 1-OMe singlet at δ 3.60 enhanced the aromatic doublet at δ 7.89 (H-11), thus confirming the position of OMe at C-1. Comparison of the spectral data obtained from the literature values[152,153] with those of **57** confirmed that it was asimilobine.

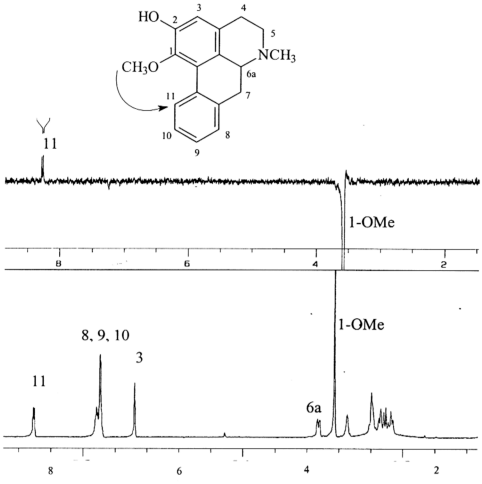
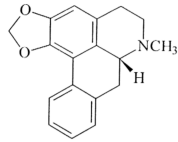


Figure 3.14: ^{1}H NMR spectrum (400 MHz) for alkaloid **57** and the NOE diff. Spectrum (irradiation on OMe-1).

3.4.1.5 *(-)-Roemerine* 47



47

(-)-Roemerine **47** $[\alpha]_D^{23}$ –21.92° (c= 0.075, MeOH) was isolated as a white amorphous. The UV spectrum showed maxima at 273, 295 and 318 nm. The IR spectrum showed a methylenedioxy group at 940 cm^{-1}. In addition the MS spectrum revealed the molecular ion peak at m/z 279 corresponding to a molecular formula of $C_{18}H_{17}NO_2$. Another significant peak observed was m/z 278 $[M-H]^+$. The peak at m/z 236 was observed due to the loss of the methylene imine group ($-CH_2NCH_3$).

The ^1H NMR spectrum showed a sharp peak representing two protons of methylenedioxy group resonated at δ 6.02. Other peaks were resonating of H-3 (δ 6.55) in ring A, four aromatic protons in ring D (doublet centred at δ8.04, H-11 and multiplets those between δ 7.20 - δ 6.80, H-8, H-9 and H-10 respectively) and *N*-methyl group at δ 2.53. The chemical shift values of the ^{13}C-NMR carbon signals are given in Table 3.17. Comparison of the spectral data obtained from the literature values[152] with those of **47** confirmed that it was roemerine.

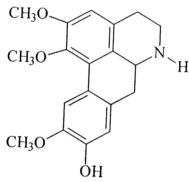
Table 3.17: ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data (δ/ppm in $CDCl_3$) for (-)-norboldine **98**, (-)-roemerine **47** and (-)-laurotetanine **99**

Position	**98**		**47**		**99**	
	^{13}C	^{1}H (Hz)	^{13}C	^{1}H (Hz)	^{13}C	^{1}H (Hz)
1	142.10		142.30		144.20	
1a	127.60		116.10		128.10	
1b	125.70		126.20		126.70	
2	148.30		146.40		152.10	
3	113.90	6.57	107.40	6.55	111.30	6.57
3a	129.90		127.80		129.00	
4	28.80	2.58-3.34 m	28.80	2.68 m	29.10	2.68 m
				2.98 m		2.98 m
5	43.10	2.58-3.34 m	53.20	2.98-3.34 m	43.10	2.98-3.34 m
6a	53.40	3.75 dd	61.80	3.78 d (12.5)	53.70	3.78 d (12.5)
		(5.0, 7.6)				
7	36.50	2.65 dd	34.30	2.72 m	36.60	2.72 m
		(7.6, 12.5)		2.68 m		2.68 m
		2.60 dd				
		(12.5, 5.0)				
7a	129.90		135.10		129.80	
8	114.40	6.73	126.50	]	113.80	6.77
9	145.20		126.70	] 7.27 m	145.30	
10	145.70		126.80	]	144.90	
11	110.40	7.90	127.20	8.04 d	110.80	8.01
11a	123.60		130.80		124.00	
1-OMe	60.20				60.10	3.55
2-OMe					56.00	3.85
10-OMe	56.10				55.80	3.86
OCH_2O			100.40	6.06		
				5.92		
N-Me			43.50	2.53		

3.4.2 Alkaloids isolated from the leaves of *Phoebe lanceolata*

Liriodenine **135**, roemerine **47**, asimilobine **57** and laurotetanine **99** were isolated from the leaves of *Phoebe lanceolata*. The structures of these known alkaloids were confirmed by direct comparison of the data from the literature review[143-155].

3.4.2.1 *Laurotetanine* 99



99

Laurotetanine **99**, $[\alpha]_D^{23} = 0$ was isolated as a rasemic. The UV spectrum exhibited maxima at 221 nm, 278 nm and 305 nm and any bands in the region above 300 nm eliminated the possibility of a 9,10-disubstitution[145]. The IR spectrum showed absorption at 3350 cm^{-1} indicating the presence of a hydroxyl group. The mass spectrum exhibited a molecular ion peak at m/z 327 suggesting a molecular formula of $C_{19}H_{21}NO_4$. Other significant fragmentation peaks were revealed at m/z 326 $[M-1]^+$ and m/z 312 $[M-15]^+$ indicating the loss of $H^{\cdot}$ and $CH_3^{\cdot}$ respectively. Moreover, the presence of a strong $[M-31]^+$ fragmentation peak at m/z 296 in the mass spectrum suggested that C-1 was substituted by a methoxyl group.

The ^{1}H NMR spectrum of alkaloid **99** exhibited three methoxyl singlets at δ 3.55, δ 3.85 and δ 3.86. The singlet at δ 3.55 is assigned to the methoxyl at C-1 since the protons were shielded by the anisotropic effect caused by ring D. A one proton singlet at δ 6.57 was observed in the spectrum, confirming that H-3 is unsubstituted. A singlet appeared at δ 6.85, which can be attributed to H-8. The downfield signal of H-11 at δ 7.95 suggested that C-1 was substituted by a methoxyl group. The aliphatic protons gave a multiplet between δ 3.70 - 2.30. The chemical shift values of the ^{13}C NMR carbon signals are given in Table 3.17.

The positions of C-1, C-2 and C-10 methoxyls in ring A and C were determined by NOE differential experiments. Irradiation of H-3 enhanced the methoxyl singlet at δ 3.85 while the irradiation of H-11 singlet at δ 8.01 enhanced both the methoxyls singlet at δ 3.58 and δ 3.86. Therefore, methoxyls are bonded to C-1, C-2 and C-10. On the other hand, irradiation of H-8 no methoxyl singlet was enhanced, hence suggesting that a hydroxyl attached to C-9.

Comparison with the authentic sample and its data with the literature values[153-155] confirmed that alkaloid **99** is indeed laurotetanine.

PART B

ISOLATION AND STRUCTURAL ELUCIDATION OF ALKALOIDS FROM *DEHAASIA* SPECIES (LAURACEAE)

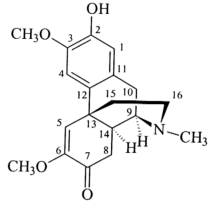
3.5 Introduction

In our continuing research on alkaloids from Malaysian plants, we have investigated the alkaloidal constituents from the leaves of *Dehaasia longipedicellata* (Ridl.) Kosterm, *Dehaasia candolleana* (Meisn.) Kosterm and *Dehaasia incrassata* (Jack) Kosterm, which belong to the Lauraceae family. Previous studies on plant belonging to the genus *Dehaasia* showed that it contain aporphines, bisbenzylisoquinolines, oxoaporphines, bisaporphines and dioxoaporphines [88,89,156-158]. The occurrence of morphinoid alkaloids from this genus had never been reported before.

3.6 Alkaloids isolated from the leaves of *Dehaasia longipedicellata*

Five morphinoid alkaloids have been isolated from *Dehaasia longipedicellata*, namely (-)-pallidine **189**, a new alkaloid (+)-pallidinine **188**, (+)-milonine **190**, (-)-8,14-dehydrosalutaridine **191** and (-)-sinoacutine **81**.

3.6.1 *(+)-Pallidinine* 188

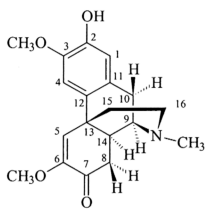


188

(+)-Pallidinine **188**, $[\alpha]_D^{27} = +45^o$ (c= 1.0, $CHCl_3$) was obtained as a yellow amorphous solid. The FAB^+ mass spectrum showed an $[M+H]^+$ peak at m/z 330.1700 corresponding to the molecular formula of $C_{19}H_{22}NO_4$ (calc. 330.1688). The UV spectrum showed absorptions at 228 and 260 nm characteristic of an α,β-unsaturated carbonyl chromophore[28]. Absorption bands observed at 3515 and 1685 cm^{-1} in the IR spectrum signified the presence of hydroxyl and carbonyl groups respectively.

The ^{1}H-NMR spectrum revealed two OMe peaks at δ 3.68 and δ 3.89 attached to C-6 and C-3. The presence of three downfield protons singlet at δ 6.72, δ 6.82 and δ 6.20 were related to the H-1, H-4, and H-5 respectively. The H-9 proton appeared as a doublet at δ 2.89 (*J*=5.8 Hz). In addition, two signals appeared at δ 3.08 (d, *J*= 18.0 Hz) and δ 2.64 (dd, *J*=5.9, 18.0 Hz) were attributable to Hax-10 and Heq-10. Meanwhile, the multiplet signals at δ 1.50 and δ 2.12 were the resonance of C-15 protons. An *N*-methyl singlet was observed at δ 2.34. The ^{13}C-NMR spectrum showed a signal at δ 194.70 (C-7) for α,β -unsaturated carbonyl[159], δ 42.78 (N-Me), δ 54.97 (C-6-OMe) and δ 56.16 (C-3-OMe). These information, coupled with those of HMBC

and HMQC led to the complete assignment of protons and carbons as shown in Table 3.18.



188

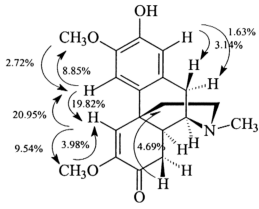


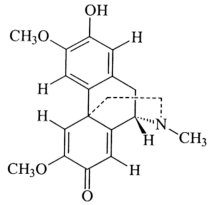
Figure 3.15: The numbering of pallidinine **188** and its important NOE measurement showing the relative configuration of the structure.

The NOE differential experiment showed a strong NOE signal between H-8ax (δ 3.36 m) and one of the protons on C-15 (δ 1.50 m), thus indicating that the junction between the rings B and C must be *trans* and not *cis* (Figure 3.15). The positions of the C-3 and C-5 methoxyls and the C-2 hydroxyl were also determined by NOE differential experiments. Irradiation of H-5 enhanced the methoxyl singlet at δ 3.68 while the irradiation of the methoxyl singlet at δ 3.89 enhanced the H-4 signal. Thus C-3 and C-5 are bonded to methoxyls. Meanwhile the irradiation of H-1 enhanced the signals of C-10 protons and no methoxyl singlet was enhanced, therefore suggesting that C-2 is attached to a hydroxyl group. From the analysis of the collected data and comparison with literature values [160-162,166], it is confirmed that alkaloid **188** is indeed the new alkaloid namely (+)- pallidinine.

Table 3.18: Spectral data of (+) pallidinine **188** (^{1}H NMR, 400 MHz in $CDCl_3$)

Position	δ ^{1}H (Hz)	δ ^{13}C	DEPT	HMQC (^1J)	HMBC (^2J, ^3J)
1	6.72 s	114.21	CH-1	H-1	3, 12, 10
2		144.21			
3		145.08			
4	6.82 s	106.22	CH-4	H-4	2, 11, 13
5	6.20 s	121.49	CH-5	H-5	12, 13, , 7, 14
6		150.88			
7		194.70			
8	ax 3.36 dd (17.6, 14.0)	39.11	CH_2-8	Hax-8	7, 14
	eq 2.44 dd (17.6, 4.4)			Heq-8	
9	2.89 brd (5.8)	56.59	CH-9	H-9	10, 11, 13, 14, 16
10	ax 3.08 d (18.0)	26.97	CH_2-10	Hax-10	1, 11, 14
	eq 2.64 dd (18.0, 5.9)			Heq-10	
11		130.33			
12		132.07			
13		36.27			
14	2.43 m	40.08	CH-14	H-14	
15	1.50 m (1H)	36.35	CH_2-15	2H-15	12, 13, 14
	2.12 m (1H)				
16	2.12 m (1H)	45.80	CH_2-16	2H-16	12
	2.48 m (1H)				
3-OMe	3.89 s	56.16	OCH_3-3	3H-3	3
6-OMe	3.68 s	54.97	OCH_3-6	3H-6	6
N-Me	2.34 s	42.78	N-CH_3	N-CH_3	9, 16

3.6.2 *(-)-Pallidine* 179



189

(-)-Pallidine **189**, $[\alpha]_D^{23}$ = -9.6° (c = 0.08, $CHCl_3$) was obtained as a yellow amorphous solid. The mass spectrum showed a molecular ion peak at m/z 327 corresponding to the molecular formula of $C_{19}H_{21}NO_4$. The UV spectrum showed two absorption bands at 245 and 295 nm characteristic of morphinandienone moiety[166]. The IR spectrum exhibited a conjugated carbonyl absorption at 1666 cm^{-1}.

The ^{1}H-NMR spectrum revealed two OMe peaks at δ 3.71 and δ 3.84 attached to C-6 and C-3 respectively. The presence of four downfield protons singlet at δ 6.66 (H-1), δ 6.75 (H-4), δ 6.32 (H-5) and δ 6.29 (H-8) were related to the aromatic ring and the cross-conjugated cyclohexadienone protons[5]. An ABX system was observed between H-9, Heq-10 and Hax-10. The H-9 proton appeared as a doublet at δ 3.65 (J = 6.3 Hz). The Heq-10 and Hax-10 signals were observed at δ 3.29 (d, J = 6.3 Hz) and δ 2.94 (dd, J, J' = 6.3, 17.8 Hz). The C-16 protons resonated at δ 2.55 and δ 2.58 as multiplet. Another set of multiplet belonging to H-15/H-15' appeared at δ 1.80 and δ 2.90 respectively. The *N*-methyl singlet was observed at δ 2.41.

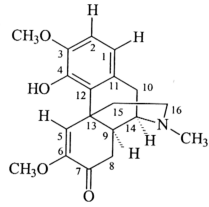
Table 3.19: Spectral data of pallidine **189** (^{1}H NMR, 400 MHz in $CDCl_3$)

Position	δ ^{13}C	δ ^{1}H (Hz)	DEPT	HMQC	NOESY
1	133.54	6.66 s	CH-1	H-1	Heq-10
2	144.85				
3	145.73				
4	107.50	6.75 s	CH-4	H-4	H-5, OMe-3
5	118.90	6.32 s	CH-5	H-5	Heq-15, OMe-6
6	151.37				
7	180.92				
8	122.19	6.29 s	CH-8	H-8	H-9
9	60.82	3.65 d (6.1)	CH-9	H-9	H-8, Hax-10, N-Me
10	32.35	ax 2.94 dd	CH_2-10	Hax-10	Heq-10
		(6.3, 17.8)		Heq-10	Hax-10, N-Me
		eq 3.29 d (6.3)			
11	129.35				
12	129.58				
13	42.31				
14	161.77				
15	41.32	ax 1.80 m	CH_2-15	Hax-15	Heq-16
		eq 2.90 m		Heq-15	Hax-15. H-5
16	45.66	ax 2.55 m	CH_2-16	Hax-16	H-9, Hax-10
		eq 2.58 m		Heq-16	Hax-15, NMe
3-OMe	56.12	3.84 s	CH_3	3-Ome	H-4
6-OMe	55.08	3.71 s	CH_3	6-Ome	H-5
N-Me	41.67	2.41 s	CH_3	N-Me	Heq-10, H-9

The ^{13}C-NMR spectrum showed signals at δ 180.92 (C-7 carbonyl), δ 41.67 (N-Me), δ 55.08 (C-6-OMe) and δ 56.12 (C-3-OMe). A complete assignment of

protons and carbons is given in Table 3.19. Finally, from the analysis of the collected data and comparison with literature values [159,162-167], it is confirmed that alkaloid **189** is indeed the known alkaloid namely (-)- pallidine.

3.6.3 *(+)-Milonine* 190



190

Milonine **190**, $[\alpha]_D^{23}$ +40° (c = 1.0, $CHCl_3$) was isolated as a pale yellow amorphous. It showed a molecular ion peak in the EI mass spectrum at m/z 329 giving a possible molecular formula of $C_{19}H_{23}NO_4$. Moreover, Retro-Diels Alder fragmentation peak revealed at m/z 286 $[M\text{-}CH_2{=}NCH_3]^+$ confirmed the existence of an *N*-methyl group[168-169]. The UV spectrum showed an αβ-unsaturated carbonyl chromophore and an aromatic ring absorption bands at 210 and 264 nm[28].

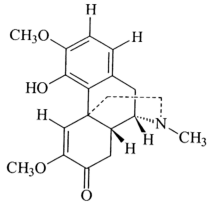
^{1}H-NMR showed two distinct methoxyl peaks observed at δ3.59 and δ3.85, which were positioned at C-6 and C-3 respectively. These were proven by HMBC and HMQC field gradient correlation as stated in Table 3.20. Furthermore, a pair of doublets (*J* = 8.3 Hz) revealed was at δ 6.62 and δ 6.71 indicating the presence of CH aromatic at C-1 and C-2. The *N*-methyl group resonated as a singlet at δ 2.29.

Meanwhile multiplet signals observed between δ 2.00 and 3.48 were assignable to the aliphatic protons, which were determined through 'C-H correlated' (HMQC) and DEPT experiments. A broad peak of the hydroxyl was observed at δ 6.30.

The strong NOE interaction between H-8ax (δ 1.94 m) and one of the protons in C-15 in the NOESY experiment proved that the junction between rings B and C is *trans* and not *cis*. The strong cross peak also can be found between H-2 at δ 6.71 doublet with the downfield C-3 methoxyl singlet at δ 3.85. The interaction between H-5 and 4-OH is possible only in B/C-trans structures. Furthermore, the mass spectrum showed two important features; the lack of the fragment at m/z 59, [-CH_2CH_2-N-CH_3]$^+$, and the strong intensity of M^+, which was 55% of the parent peak, m/z 214. Both properties were characteristic of trans-morphinane structure[168,170].

Table 3.20: Spectral data of (+)-milonine **190** (^{1}H NMR, 400 MHz in $CDCl_3$)

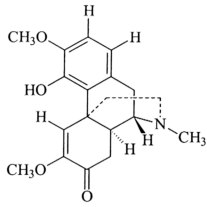
Position	^{13}C (δ)	^1H (Hz)	DEPT	HMBC	NOESY
1	118.9	6.62 d (8.3)	CH-1	2, 11, 12	H-2, Heq-10
2	108.6	6.71 d (8.3)	CH-2	1, 3, 11	H-1, 3-OMe
3	144.3				
4-OH	142.6	6.30 br s			H-5
5	123.3	7.68 s	CH-5	6, 7, 13, 14	4-OH, 6-OMe, H-15
6	150.4				
7	194.5				
8	46.3	Hax 1.94 m	CH_2-8	7, 9, 14	H-9, H-15
		Heq 2.41 m			Hax-10, NMe
9	56.2	2.82 br d (6.3)	CH-9	8, 10, 11, 13, 14	Hax-10, N-Me
10	27.3	Hax 2.71 dd (6.3, 17.8)	CH_2-10	1, 9, 11, 12	Heq-10
		Heq 3.05 d (17.8)		1, 9, 10, 11	Hax-10, NMe
11	130.9				
12	125.8				
13	40.6				
14	38.8	3.38 dd	CH-14	8, 9, 10, 13	H-16
15	31.7	2H 1.90 m	CH_2-15	12, 13	H-16, H-5
16	37.4	2H 2.45 m	CH_2-16	N-Me	H-9, Hax-10, H-15, NMe
3-OMe	55.8	3.85 s	CH_3	3	H-2
6-OMe	54.4	3.59 s	CH_3	6	H-5
N-Me	42.3	2.29 s	CH_3		Heq-10



69

Comparison of the spectra data with the literature values[171-173] confirmed that alkaloid **190** is indeed milonine. (+)-milonine **190** was reported in 1995 from the leaves of *Cissampelos sympodialis* and its enantiomer (-)-ocobotrine **69** was isolated in 1976 from *Ocotea brachybotra*[171,174]. Both compounds were isolated as trans-morphinane alkaloids.

3.6.4 *(-)-8,14-dihydrosalutaridine* 191.

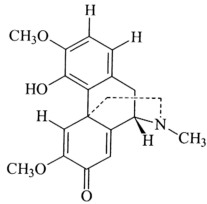


191

(-)-8,14-dihydrosalutaridine **191**, $[\alpha]_D^{23}$ - 41.14° (c = 0.07, MeOH) was isolated as a pale yellow amorphous. It showed a molecular ion peak at m/z 329 in the EI mass spectrum suggesting a possible molecular formula of $C_{19}H_{23}NO_4$. Its UV spectrum showed absorptions at 241 and 278 nm, meanwhile the IR spectrum revealed the presence of a carbonyl group at 1676 cm^{-1}.

^{1}H-NMR indicated the presence of two methoxyl groups by revealing two singlets at δ 3.51 and δ 3.75. An *N*-methyl singlet appeared at δ 2.64 and a pair of doublets (J = 8.0 Hz) was revealed at δ 6.67 and δ 6.55 corresponding to H-1 and H-2 respectively. The data reported for alkaloid **191** were almost similar to those of milonine **190**[171], except for the H-5 vinyl proton signal at δ 6.84. A similar value of vinyl proton H-5 found in milonine **190** was downfield at δ7.68. Comparison with literature values [168,175], it is confirmed that alkaloid **191** is indeed the known alkaloid namely (-)-8,14-dihydrosalutaridine **191**.

3.6.5 *(-)-Sinoacutine* 81



81

(-)-Sinoacutine **81**, $[\alpha]_D^{23}$ –5.75° (c = 0.12, $CHCl_3$) exhibited a $[M+H]^+$ peak at m/z 328.1574 in $HRES^+$ mass spectrum corresponding to the molecular formula of $C_{19}H_{22}NO_4$ (calc. 328.1549).

The ^{1}H-NMR spectrum showed the presence of two downfield singlet protons at δ7.53 and δ6.25 representing H-5 and H-8 respectively, and two downfield doublets protons at δ6.70 (H-1) and δ6.61 (H-2) (J = 8.3 Hz) *orto* to each other, which were related to the aromatic ring and the cross conjugated cyclohexadienone protons[176-177]. Two methoxyl singlets resonated at δ3.84 and δ3.71 assignable to 3-OMe and 6-OMe respectively.

Table 3.21: Specral data of sinoacutine **81** (^{1}H NMR, 400 MHz in $CDCl_3$)

Position	δ ^{13}C	δ ^{1}H (Hz)	DEPT	HMBC	HMQC	NOESY
1	109.45	6.70 d (8.3)	CH-1	2, 3, 10, 11, 12	H-1	H-2, Heq-10
2	118.76	6.61 d (8.3)	CH-2	1, 3, 4, 11	H-2	H-1, 3-OMe
3	145.3					
4-OH	143.30	6.30 br s				H-5
5	120.43	7.53 s	CH-5	6, 13, 14	H-5	4-OH, 6-OMe, Heq-15
6	150.94					
7	181.38					
8	122.11	6.25 s	CH-8	7, 14, 9	H-8	H-9
9	61.00	3.67 d (6.3)	CH-9	8, 10, 14	H-9	H-8, Hax-10, N-Me
10	32.60	ax 2.92 dd	CH_2-10	9, 11	Hax-10	Heq-10
		eq 3.27 d		9, 10, 11	Heq-10	Hax-10, NMe
11	129.73					
12	123.94					
13	43.62					
14	161.55					
15	37.69	ax 1.73 m	CH_2-15	5, 13	Hax-15	Heq-16
		eq 2.31 m		5, 13, 16	Heq-15	H-ax-15, H-5
16	46.96	ax 2.38 m	CH_2-16	15	Hax-16	H-9, Hax-10
		eq 2.40 m		15	Heq-16	H-ax-15, NMe
3-OMe	56.24	3.84 s	CH_3	2, 3, 4	3-OMe	H-2
6-OMe	54.77	3.71 s	CH_3	5, 6, 7	6-OMe	H-5
N-Me	41.60	2.41 s	CH_3		N-Me	Heq-10

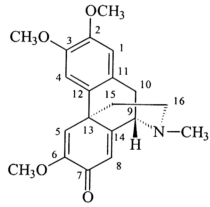
The NOESY experiment showed strong interactions between H-2/3-OMe, and H-5/6-OMe, thus confirming methoxyl's position at C-3 and C-6 respectively. With the aid of DEPT, HMQC and HMBC experiments, a full assignment of the protons and carbons was made possible (Table 3.21). Finally, after comparison of spectral

data obtained from the literature values[70,75,178-180], one may conclude that alkaloid **81** could none other than (-)-sinoacutine **81**.

3.7 Alkaloids isolated from the leaves of *Dehaasia candolleana*

Chemical investigation on the leaves of this species afforded three morphinandienones; (-)-sebiferine **71**, its enantiomer, (+)-sebiferine **192**, and (-)-pallidine **189** besides one non-alkaloidal, phenantrenoid type; perakensol **193**.

3.7.1 *(-)-Sebiferine* 71



71

Sebiferine (*N*-methyl-2,3,6-trimethoxymorphinandien-7-one) **71**, $[\alpha]_D^{23}$ –8.47 (c = 0.3 $CHCl_3$) was isolated as pale yellow amorphous solid. The mass spectrum of sebiferine afforded a molecular ion peak at m/z 341 consistent with a molecular formula of $C_{20}H_{23}NO_4$. The other prominent fragmentation peaks were found at m/z 326, 313 and 298. Significant peaks of α-methoxy cross-conjugated cyclohexadienone system were observed at 1662, 1639 and 1616 cm^{-1} in the IR spectrum and the absorptions at 209, 238 and 280 nm were observed in UV spectrum[28,166].

The ^{1}H-NMR spectrum revealed three OMe peaks at δ 3.71, δ 3.84 and δ 3.86. The spectrum also showed the same pattern as those peaks in pallidine **189** except that sebiferine **71** has one extra methoxy group at C-2 (δ 3.86). The presence of three downfield singlet protons at δ 6.51 (H-1), δ 6.71 (H-4) and δ 6.29 (H-5) were related to the aromatic ring and one proton singlet at δ 6.17 (H-8) was α-conjugated cyclohexadienone proton[68,76]. The H-9 proton appeared as a doublet at δ 3.60 (J = 6.3 Hz). Furthermore, the ABX system signals resonated at δ 3.24 (d, J = 17.8 Hz) and δ 2.94 (dd, J = 6.3, 17.8 Hz) corresponding to Hax-10 and Heq-10 respectively were observed as prominent peaks for morphinandienone alkaloids. A signal resonated as multiplets at δ 2.48 was assigned to the C-16 protons. Another set of multiplets belonging to C-15 protons appeared at δ 1.80. The *N*-methyl singlet was observed at δ 2.41. The complete assignment of protons and carbons was aided by 2D NMR (COSY, HMQC, HMBC and NOESY) as shown in Table 3.22.

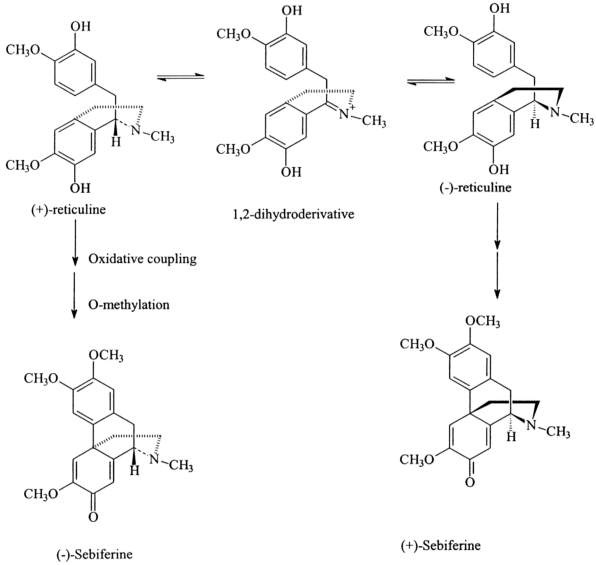
The physical constant and spectral data of the base were identical with reported data of sebiferine. It was isolated by Sivakumaran *et. al.* from the stem bark of *Litsea sebifera* (Luraceae)[181,182].

Table 3.22: ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) data for (-)-sebiferine **71** and (+)-sebiferine **192**.

No. Carbon	(-)-sebiferine **71**		(+)-sebiferine **192**
	^{13}C	^{1}H (Hz)	^{13}C
1	110.43	6.51 s	110.20
2	147.95		147.70
3	148.26		148.50
4	108.72	6.71 s	108.30
5	118.90	6.28 s	118.70
6	151.23		150.70
7	180.82		180.30
8	122.02	6.17 s	121.40
9	60.72	3.60 d (6.1)	61.00
10	32.47	Hax 2.94 dd (6.3, 17.8) Heq 3.24 d (18.0)	31.90
11	128.61		128.40
12	129.87		122.90
13	42.19		42.90
14	161.77		161.80
15	41.54	2H, 1.80 m	41.20
16	45.55	2H, 2.48 m	45.10
2-OMe	56.23	3.86 s	55.80
3-Ome	55.79	3.84 s	55.50
6-Ome	55.08	3.71 s	55.40
N-Me	41.67	2.41 s	41.70

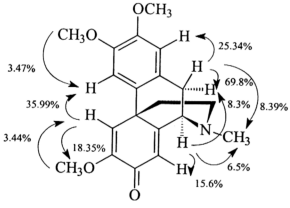
3.7.2 *(+)-Sebiferine* 192

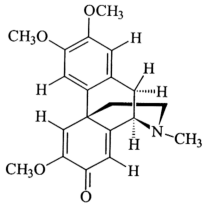
The data collected from MS, UV, ^{1}H-NMR and ^{13}C-NMR are very similar to (-)-sebiferine **71** which has been discussed earlier. The main different is that the optical rotatory values of (-)-sebiferine **71** is $[\alpha]_D^{27}$ = –8.47, and (+)-sebiferine is +1.38 for **192**. This indicated that these two compounds are enantiomers.



Scheme 3.4: Biosynthesis (+/-) sebiferine.

Reticuline is one of the specific benzyltetrahydroisoquinoline precursors of biosynthesis of morphinandienone alkaloids, sebiferine (Scheme 3.4)[183]. Reticuline had been isolated from opium and found to be present in both enantiomeric forms with an excess of the *S*(+)-isomer over the *R*(-)-isomer [184,185].The existence of enantiomers of the reticuline can be explained by their biosynthesis. (+)-Reticuline was incorporated almost as the (-)-reticuline. The results are interpreted as showing that (+) and (-)-reticuline are undergoing interconversion in the plant by oxidation and reduction presumably *via* the 1,2-dihydro-derivative.



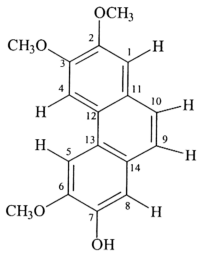


(+)-sebiferine

Figure 3.16: NOE differential measurement of (+)-sebiferine **192**

The C-3 and C-6 methoxyls signal were determined by NOE differential experiment. The strong interactions are observed between H-4/3-OMe, H-5/6-OMe, H-9/H-10, H-9/H-8 and H-10/H-1 (Figure 3.16).

3.7.3 *Perakensol* 193



193

In continuing investigation on alkaloidal constituents of *Dehaasia condolleana*, the author isolated perakensol (7–hydroxy–2,3,6-trimethoxyphenantrene) **193**, a known phenantrenoid as a minor constituent coextracted with the basic fraction. A major alkaloid as mentioned earlier is (-)-sebiferine **71**. Perakensol **193** has a molecular formula of $C_{17}H_{16}O_4Na$ as proven by HRESI (positive mode) mass spectrum with $[M+Na]^+$ 307.0956 (calc. 307.0946). The EIMS spectrum showed the molecular ion peak (100%) at m/z 284. The other prominent fragmentation peaks were found at m/z 269 and m/z 253. The UV spectrum showed the absorption bands at 300, 322, 338 and 346 nm, which is a characteristic pattern of substituted phenantrene[186,187]. The absorption of a hydroxyl group was observed at 3400 cm^{-1} in the IR spectrum.

The ^{1}H-NMR showed three aromatic methoxyl singlets resonated at δ 3.96, δ 4.02 and δ 4.05 respectively. The spectrum also displayed signals for six aromatic

protons, two of which appearing as a pair doublets (J = 8.0 Hz) centred at δ 7.45 and are typical of 9- and 10- protons of phenantrene derivatives[187-193]. The remaining four aromatic protons resonated at δ 7.14 (1H, s), δ 7.70 (2H, s) and δ 7.18 (1H, s) corresponding to four isolated aromatic protons attached to C-1, C-4, C-5 and C-8 respectively. From the preliminary results we proposed that the structure for compound **193**, is most probably the tetraoxygenated phenantrene compound.

In addition, the ^{13}C NMR spectrum clearly showed the absence of methylene carbon and the presence of three methyls, six methynes and eight quaternary carbons. Furthermore, the absence of a carbonyl function and the presence of three methoxyl groups in the compound suggested that the fourth substituent must be a hydroxyl group[194]. In fact, the phenolic hydroxyl proton appeared at δ 5.80 (disappering on deuterium exchange)[190].

2D NMR (COSY, HMQC, HMBC and NOESY) was performed in order to assign the positions of the aromatic protons. A complete assignment of protons and respective carbons are shown in Table 3.23. The NOESY spectrum showed a strong cross peak between the upfield H-1 singlet at δ 7.14 and 2-OMe at δ 3.96. On the other hand, H-4 and H-5, which have the same chemical shift at δ 7.70 showed a strong interaction with each adjacent methoxyl protons suggesting that the methoxyl groups are attached to C-3 and C-6 respectively. The hydroxyl group assigned at C-7 since H-8 at δ 7.18 showed no correlation with methoxyl proton and only interacted with H-9 in the NOESY spectrum.

Table 3.23: ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) data for perakensol **193**.

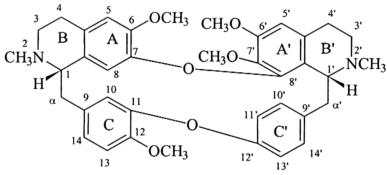
No. Carbon	^{13}C	^{1}H (Hz)	HMBC	NOESY
1	109.10	7.14 s	2,3,10,11,12	2-OMe, 10
2	149.70			
3	149.30			
4	102.60	7.70 s	2,3,11, 12,13	3-OMe, 5
5	103.50	7.70 s	6,7, 12,13,14	6-OMe, 4
6	147.50			
7	145.50			
8	112.50	7.18 s	6,7,9, 14	9
9	125.01	7.45 d (8.0)	10, 8, 13,14	8
10	125.01	7.45 d (8.0)	1, 12,14	1
11	127.50			
12	125.01			
13	125.10			
14	112.50			
2-Ome	56.70	3.96 s	2	
3-OMe	56.70	4.02 s	3	
6-Ome	56.50	4.05 s	6	
7-OH		5.8 br s		

Perakensol **193** was fist reported as a natural compound in 1992 by Zurinah *et.al*[186] form *Alseodapne perakensis* and extracted from both the alkaline and acidic aqueous solution. These findings together with the spectral data have confirmed the compound is indeed the known phenantrenoid, perakensol **193**.

3.8 Alkaloids Isolated From *Dehaasia incrassata*

Four bisbenzylisoquinoline alkaloids namely (-)-gyrolidine **194**, (-)-3', 4'-dihydrostephasubine **198**, (-)-norstephasubine **201** and stephasubine **202** were isolated from the bark of *Dehaasia incrassata* (KL4640). (-)-3', 4'-Dihydrostephasubine **198** and (-)-norstephasubine **201** are new alkaloids. The molecular weights of these alkaloids were detected by high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) analysis and comparison with reported data of bisbenzylisoquinoline alkaloids[195-198]. Structural elucidation was carried out using spectroscopic techniques, notably NMR.

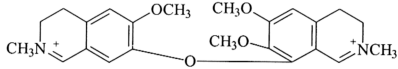
3.8.1 *(-)-Gyrolidine* 194



194

(-)-Gyrolidine **194**, $[\alpha]_D^{23}$ –53.0 (c =0.02, MeOH) was isolated as a brownish amorphous state. In the UV spectrum, maxima were observed at 208, 261 and 284 nm, which indicated the existence of the conjugated system[199]. From UV, ^{1}H NMR and accurate mass spectrum measurement of the molecular ion, it appeared likely that

the alkaloid is bisbenzylisoquinoline type[200,201]. The EIMS spectrum showed a molecular ion peak at m/z 622 corresponding to a molecular formula of $C_{38}H_{42}N_2O_6$. Thus, peaks at m/z 198 (ion **194a**), 515 and 516 are characteristic of head to head and tail to tail bisbenzylisoquinoline alkaloid (two ether linkages between 7-8', 11-12')[201-205].



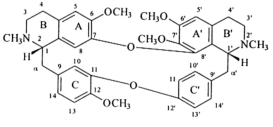
m/z 198

194a

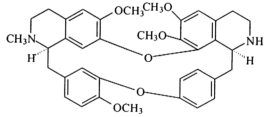
The ^{1}H NMR spectrum showed two *N*-methyl singlets, which were very close to each other at δ 2.49 and δ 2.58 assignable to *N*-2 and *N*-2' methyl protons respectively. Bick *et. al.* reported[206] that in alkaloids of berbamine series (8-7', 11-12') the well separated peaks will appear near δ 2.60 and δ 2.30, whereas for repandine series [199] (7-8', 11-12'), both *N*-methyl-resonances occur near δ 2.55. In the case of **194** the difference between the two *N*-methyl chemical shifts is very small (δ 0.09) compared to δ 0.30 in berbamine type; therefore alkaloid **194** is from repandine series. H-8 signal was observed at δ 6.57, which is also characteristic of repandine type[207-208]. In addition, H-10 resonated as a broad singlet at δ 5.39.

The singlet for 7'-methoxyl was observed further upfield at δ 3.12 due to the presence of bulky substituents near C-7'-methoxyl group. Another signal for methoxyl group appeared further downfield at δ 3.89 indicated that ring C has a C-12 methoxyl. The resonances for H-10', H-11', H-13' and H-14' were observed between

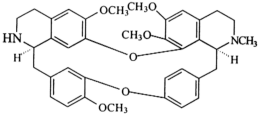
δ 6.32 to δ 7.36. Of these four protons, H-14' is the furthest downfield near δ 7.36. All aromatic proton signals were found in the usual range of δ 6.30 to δ 7.40. The peak for H-1 appeared as a broad doublet at δ 4.19 meanwhile H-1' was near δ 3.25. The COSY spectrum showed the main correlations of vicinal protons between CH-1/CH_2-α, CH-1'/CH_2-α', CH_2-3/CH_2-4, CH_2-3'/CH_2-4', CH-13/CH-14, CH-10'/CH-11' and CH-13'/CH-14'.



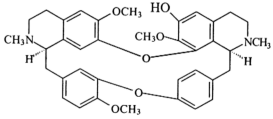
194



195



196



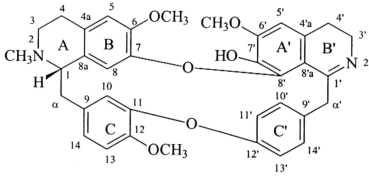
197

A comparison of ^{1}H NMR data of gyrolidine **194** with those of three known alkaloids[136,196,200]; *(+)*-2'-norobaberine **195**, (+)-2-norobaberine **196** and (+)-stepibaberine **197** is presented in Table 3.24.

Table 3.24: ^{1}H NMR spectral data for (-)-gyrolidine **194**, *(+)*-2'-norobaberine **195**, *(+)*-2-norobaberine **196** and (+)-stepibaberine **197**[136,196,200]

Position	**194**	**195**	**196**	**197**
H-1	4.19 br d (11.0)	3.65 m	4.23 m	3.75 m
H-5	6.31 s	6.34 s	6.37 s	6.37 s
H-8	6.57 s	6.79 s	5.69 s	6.71 s
H-10	5.39 br s	5.44 br s	5.61 br s	5.46 br s
H-13	6.69 br s	6.79 br s	6.81 br s	6.79 br s
H-14	6.69 br s	6.79 br d	6.81 br s	6.79 br s
H-α'				
H-1'	3.25 br d (11.0)	4.70 m	4.23 m	4.26 m
H-5'	6.24 s	6.36 s	6.36 s	6.46 s
H-1O'	6.37 br d (8.1)	6.96 m	6.87 dd (8.3, 2.2)	6.91 dd (8.3, 2.1)
H-11'	6.37 br d (8.1)	6.36 m	6.31 dd (8.3, 2.2)	6.45 dd (8.3, 2.1)
H-13'	6.88 br d (8.1)	6.97 m	6.99 dd (8.3, 2.1)	6.87 dd (8.3, 2.1)
H-14'	7.36 br d (8.1)	7.62 dd (8.2, 2.2)	7.47 dd (8.3, 2.1)	7.44 dd (8.3, 2.1)
2-NCH_3	2.49 s	2.60 s		2.58 s
2'-NCH_3	2.58 s		2.69 s	2.67
6-OCH_3	3.54 s	3.67 s	3.64 s	3.61 s
12-OCH_3	3.89 s	3.90 s	3.92 s	3.90 s
6'-OCH_3	3.71 s	3.80 s	3.79 s	
7'-OCH_3	3.12 s	3.20 s	3.23 s	3.26 s

3.8.2 *(-)-3', 4'-Dihydrostephasubine* 198



198

(-)-3', 4'-Dihydrostephasubine **198**, $[\alpha]_D^{23}$ -196.0 (c = 0.01, MeOH) was isolated as a brown amorphous. The EIMS spectrum showed a strong molecular ion peak at m/z 592.1 (90%) with m/z 591 as the base peak. The fact that the upper part of the dimer is not observed in the spectrum suggested that an imine or aromatic ring B' was present[201,209]. This suspicion was reinforced by IR absorption at 1464 cm^{-1}, which showed the presence of the imine chromophore of a dihydroisoquinoline moiety. In addition, APCI (positive mode) spectrum revealed an $[M+H]^+$ peak at m/z 593.30 and APCI (negative mode) spectrum produced an $[M-H]^-$ peak at m/z 591.15. All these data suggested a molecular formula of $C_{36}H_{36}N_2O_6$. The UV spectrum showed three peaks at 215, 287 and 337 nm, which indicated the existence of the conjugated system[28].

The ^1H NMR spectrum indicated the presence of a highly functionalized bisbenzylisoquinoline system and displayed *N*-2 methyl signal at δ 2.46 rather upfield than *N*-2' methyl signal, which normally appeared around δ 2.60 [200,206]. The peaks for three methoxyls singlet appeared at δ 3.88, δ 3.91 and δ 3.95 corresponding to C-6,

C-6' and C-12 respectively. The absence of peak positioned between δ 2.95 and δ 3.20 characteristic of a C-7' methoxyl indicated that the hydroxyl group was at C-7' which, leads to downfield shift of the 6'-methoxyl signal[206]. Another prominent peak in the spectrum was a pair of doublets resonated at δ 4.52 and δ 4.08 (1H each, J = 14 Hz) representing the two geminal protons of the methylene attached to the imine function (C-α')[210]. Meanwhile, ten aromatic protons were also observed in the spectrum, three of which appeared as singlets at δ 6.48, δ 6.39 and δ 6.03 assignable to H-5', H-5 and H-8 respectively.

The upfield signal of H-10 appeared as a broad singlet at δ 4.91. Another broad singlet appeared at δ 6.61 corresponding to the ovelapped resonances H-13 and H-14 respectively, which were part of an *ortho-para* trisubstituted benzene ring system. The spectrum also established two broad doublet signals of H-10' and H-11' resonated at δ 7.31 and δ 6.41 (J = 8.1 Hz) respectively. Another two broad doublet peaks representing H-13' and H-14' were also observed at δ 6.70 and δ7.35, therefore indicating that ring C' was *para* disubstituted. The above observations were reinforced by COSY experiment which showed correlations between CH-1/CH_2-α, Ha-α'/Hb-α', CH_2-3/CH_2-4, CH_2-3'/CH_2-4', CH-13/CH-14, CH-10'/CH-11' and CH-13'/CH-14'.

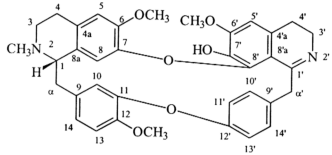
The ^{13}C NMR spectrum showed there are 36 carbon resonances, which is in agreement with the molecular formula. Thus, the signals at δ 64.03 and δ 164.78 belonged to the chiral center C-1 and imine group C-1' respectively. 2D NMR experiments (COSY, HMQC and HMBC) allowed the complete assignments of the proton and carbon chemical shifts as shown in Table 3.25.

Table 3.25: ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of (-)-3', 4'-dihydrostephasubine **198**, (-)-cycleapeltine **199** and (+)-homoaromoline **200**[196-200]

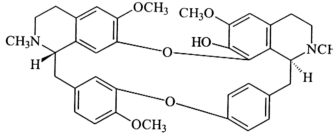
Alkaloid	**199**	**200**	**198**			
Position	$\delta^{13}C$	$\delta^{13}C$	$\delta^{13}C$	$\delta^{1}H$	HMQC	HMBC
1	65.31	64.26	64.03	3.42 br s	H-1	9
3	46.78	51.10	50.54		2H-3	4a, 4
4	26.59	28.45	27.35		2H-4	3
4a	127.92	130.56	135.41			
5	112.38	111.10	111.96	6.39 s	H-5	6, 7, 8a
6	149.12	148.50	147.58			
7	144.15	143.96	144.42			
8	120.73	116.93	113.57	6.08 s	H-8	4a, 6, 7
8a	131.31	128.02	128.24			
9	133.90	130.95	130.85			
10	120.46	117.00	117.38	4.91 s	H-10	9, 11, 12, 14
11	148.58	148.70	149.94			
12	148.50	146.64	146.46			
13	112.77	110.71	110.90	6.61 br s	H-13	11, 12, 14
14	123.45	123.65	123.47	6.61 br s	H-14	11, 12, 13
α	40.38	38.32	38.49		2H-α	
1'	60.22	60.46	164.78			
3'	44.26	44.96	47.22		2H-3'	
4'	22.70	24.96	29.99		2H-4'	
4'a	122.95	122.99	117.02			
5'	105.82	104.50	105.83	6.48 s	H-5'	4'a, 6', 7'
6'	146.42	147.61	149.41			
7'	134.91	133.39	135.79			
8'	143.06	142.37	140.83			
8'a	122.95	122.91	131.20			
9'	136.46	138.17	135.41			
10'	131.69	131.49	132.25	7.31 br d (8.1)	H-10'	α', 12', 14'
11'	120.38	121.12	122.27	6.41 br d (8.1)	H-11'	9', 12', 13'
12'	155.42	152.74	153.16			
13'	121.55	121.90	122.82	6.70 br d (8.2)	H-13'	9', 11', 12'
14'	129.78	128.34	128.63	7.35 br d (8.2)	H-14'	10', 12'

Table 3.25 [Continued]

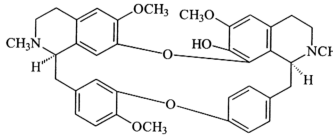
Alkaloid	**199**	**200**			**198**	
Position	δ ^{13}C	δ ^{13}C	δ ^{13}C	δ ^{1}H	HMQC	HMBC
α'	43.99	38.20	45.16	4.08 d, 4.52 d (each d, 14.0)	2H-α'	1', 8'a, 9', 14'
2-NMe	42.36	43.72	43.92	2.40 s	3H-N-2	1, 3
2'-NMe	41.54	41.50				
6-OMe	55.21	55.21	56.05	3.88 s	3H-6-OMe	6
12-OMe	56.22	55.79	56.52	3.95 s	3H-12-OMe	12
6'-OMe	55.82	55.68	56.17	3.91 s	3H-6'-OMe	6'



198



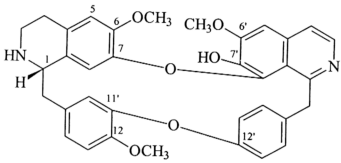
199



200

Finally, from the obtained spectra data and comparison of ^{13}C NMR with those of two known alkaloids[196-209]; (-)-cycleapeltine **199** and (+)-homoaromoline **200**, one may conclude that the structure above is indeed the new alkaloid, (-)-3', 4'-dihydrostephasubine **198**.

3.8.3 *(-)-Norstephasubine* 201



201

(-)-Norstephasubine **201**, $[\alpha]_D^{23}$ - 46.0 (c = 0.1, MeOH) was isolated as a brown amorphous solid. Its EIMS spectrum showed the M^+ at m/z 576 corresponding to the molecular formula of $C_{35}H_{32}N_2O_6$. The fact that the upper part of the dimer is not observed in the spectrum similarly that suggested an imine or aromatic ring B' was present[209]. It was further supported by IR absorption band of a dihydroisoquinoline imine chromophore at 1454 cm^{-1}. The UV spectrum showed the peaks at 219, 260 and 337 nm, which indicated the existence of a highly conjugated system[28,199].

Table 3.26: ^{1}H NMR (400 MHz, in $CDCl_3$) spectral data for (-)-3', 4'-dihydrostephasubine **198**, (-)-norstephasubine **201** and stephasubine **202**.

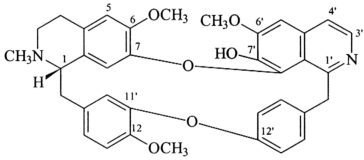
	^{1}H (Hz)		
Position	**198**	**201**	**202**
H-1	3.42 br s	3.75 br s	3.42 br s
H-5	6.39 s	6.50 s	6.47 s
H-8	6.03 s	5.97 s	5.91 s
H-10	4.91 br s	4.82 br s	4.72 br s
H-13	6.61 br s	6.64 br s	6.61 br s
H-14	6.61 br s	6.64 br s	6.61 br s
H-α			
H-α'	4.08 d, 4.52 d	4.43 d, 5.30 d	4.44 d, 5.28 d
	(each d, 14.0)	(each d,13.9)	(each d, 13.9)
H-1'			
H-3'		8.38 d (5.6)	8.36 d (5.6)
H-4'		7.41 d (5.6)	7.40 d (5.6)
H-5'	6.48 s	6.90 s	6.92 s
H-1O'	7.31 br d (8.1)	6.97 dd	6.95 dd
		(8.3, 2.2)	(8.3, 2.2)
H-11'	6.41 br d (8.1)	6.61 dd	6.58 dd
		(8.3, 2.2)	(8.3, 2.2)
H-13'	6.70 br d (8.2)	6.40 dd	6.40 dd
		(8.3, 2.1)	(8.3, 2.1)
H-14'	7.35 br d (8.2)	7.33 dd	7.35 dd
		(8.3, 2.1)	(8.3, 2.1)
2-NCH_3	2.40 s		2.41 s
2'-NCH_3			
6-OCH_3	3.88 s	3.81 s	3.79 s
12-OCH_3	3.95 s	3.98 s	3.98 s
6'-OCH_3	3.91 s	3.97 s	3.97 s

The ^{1}H NMR spectrum displayed the similar pattern in rings C and C' as those of (-)-3', 4'-dihydrostephasubine **198** (Table 3.26). However, the different was

observed for the resonances of C-3' and C-4' protons, which appeared as a pair of doublets at δ 7.41 and δ 8.38 (J = 5.6 Hz) respectively. In fact, *N*-methyl signal was absent in the spectrum.

Two doublets at δ 4.43 and δ 5.30 were also present with a large coupling constant (J = 13.9 Hz), which representing two geminal protons of a characteristic benzylic methylene adjacent to the pyridine ring[210]. (+)-Norstephasubine was isolated from *Stephania hernandifolia* by Amarendra *et. al*[210] in 1986. Finally, from the analysis of the collected data[196,210], it is confirmed that alkaloid **201** is indeed the new alkaloid namely (-)-norstephasubine **201**.

3.8.4 *Stephasubine* 202



202

Stephasubine **202** was obtained as a brown amorphous. The APCI (positive mode) mass spectrum revealed an $[M+H]^+$ peak at m/z 591.13 meanwhile APCI (negative mode) spectrum produced an $[M-H]^-$ peak at m/z 589.14. All these data

suggested a molecular formula of $C_{36}H_{34}N_2O_6$, which showed 14 mass unit more than for norstephasubine **201**.

Similarly, the UV, IR and ^{1}H-NMR spectra are also comparable to that of **201.** However a major difference in ^{1}H NMR spectrum is the presence of upfield *N*-2 methyl signal at δ 2.41 compared to *N*-2' methyl signal, which normally appeared around δ 2.60 [200,206]. Another significant difference is more upfield broad singlet of H-1 at δ 3.42 compared to δ 3.75 in **201**. The important of ^{1}H NMR values for (-)-3', 4'-dihydrostephasubine **198**, (-)-norstephasubine **201** and stephasubine **202** are quoted in Table 3.26.

The assignment of protons was further ascertained by a complete COSY experiment. The spectrum showed the correlations between vicinal protons; CH-1/CH_2-α, Ha-α'/Hb-α', CH_2-3/CH_2-4, CH-3'/CH-4', CH-13/CH-14, CH-10'/CH-11' and CH-13'/CH-14'. Finally, comparison of the spectra data with the literature values[206,209-212] confirmed that alkaloid **200** is indeed stephasubine.

In conclusion, having known the configuration at C-1 and C-1' of the (-)-gyrolidine **194** is *S* and *R* respectively, the relative configuration at C-1 of (-)-3', 4'-dihydrostephasubine **198**, (-)-norstephasubine **201** and stephasubine **202** must also incorporate the same configuration at C-1 as *S*. As a result, the two ether linkages between the benzyltetrahydroisoquinoline moieties are determined to be at 7-8', 11-12' as in the type VI BBIQ[206-210].