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PHOEBEGRANDINES A AND B, PROAPORPHINE-TRYPTAMINE DIMERS, FROM *PHOEBE GRANDIS*

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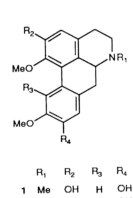
Key Word Index—*Phoebe grandis*; Lauraceae; aporphine alkaloids; proaporphine-tryptamine dimer alkaloids.

Abstract—Four known aporphine alkaloids, boldine, norboldine, laurotetanine and lindecarpine were isolated from the bark of *Phoebe grandis*. The leaves yielded two new alkaloids belonging to the proaporphine-tryptamine dimers series, phoebegrandines A and B. The structure of the new compounds was elucidated by spectral methods. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In the course of our research on Malaysian plants,§ we have investigated the alkaloid extracts of the bark and leaves of *Phoebe grandis* (Nees) Merr. It is a tree of 20 m high with yellowish brown flowers. The plant material was collected from Sik, Kedah in the Northern part of Peninsula Malaya. The bark afforded four known aporphine alkaloids boldine (**1**), norboldine (**2**), lauretanine (**3**) and lindecarpine (**4**). The leaves, however, yielded exclusively two new alkaloids, phoebegrandine A (**5**) and B (**6**) of the rare proaporphine-tryptamine type. Such alkaloids have been found previously only in the *Roemeria hybrida* species (Papaveraceae) [1–3]. Structural elucidation was done mainly by 2D NMR.



	R_1	R_2	R_3	R_4
1	Me	OH	H	OH
2	H	OH	H	OH
3	H	OMe	H	OH
4	H	OH	OH	H

RESULTS AND DISCUSSION

The alkaloids were extracted using conventional methods. The bark alkaloids (**1–4**), as well as phoebegrandines A (**5**) and B (**6**), were separated by column chromatography on silica gel.

Phoebegrandine A (**5**), $[\alpha]_D$ 0°, showed a molecular ion peak in the HREI mass spectrum at m/z 473.2715 (Δ 3.65 mmu) corresponding to the molecular formula $C_{29}H_{35}N_3O_5$. The UV spectrum exhibited two maxima at 224 and 277 nm which revealed an indole chromophore. The high M_r indicated that compound **5** is probably a dimeric alkaloid. An intense peak was observed in the mass spectrum at m/z 214 (214.1103, $C_{13}H_{14}N_2O$, Δ −0.28 mmu), suggesting a β-carboline type moiety (ion **7**). The ^{1}H NMR showed the signals of two aromatic methoxy groups at δ 3.80 and 3.84 with one belonging to the β-carboline part. 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 double doublet at δ 6.78 (J = 8.6 and 1.5 Hz) indicated that the aromatic ring of the β-carboline was methoxylated either at C-3′ or C-4′. A broad exchangeable proton at δ 7.75 was assignable to the NH-1′ and a spin system of two multiplets at δ 2.70 and δ 3.18 was attributed to the CH_2-6′ and CH_2-7′, respectively. In the ^{13}C NMR, the typical signals of the aromatic

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Table 1. ^{13}C (62.5 MHz) and ^{1}H (400 MHz) NMR data[a] for phoebegrandines A (**5**) and B (**6**)

	5[b]				**6**			
Positon	δ C[c]	δ H (*J* Hz)	HMBC	NOESY	δ C[c]	δ H (*J* Hz)[b]	HMBC[c]	NOESY[b]
1	140.5				144.4			
1a	132.5				141.1			
1b	133.4				132.7			
2	147.2				151.2			
3	108.4	6.48 *s*	1,1b,2,3a	4α, OMe-2	115.1	6.56 *s*	1,1b,2,4	4$\alpha\beta$
3a	121.9				127.8			
4	27.3	α 2.70 *m*	3a	4β, 5β	27.7	α 2.70 *m*	1b,3,3a	4β, 5α
		β 2.90 *m*	3a,5			β 3.00 *m*	1b,3,3a	
5	55.4	α 2.45 *dd* (12, 5)	6a	5β, 6a	56.0	α 2.45 *m*	6a	5β, 6a
		β 3.15 *m*	3a,4,6a			β 3.10 *m*		
6a	65.8	3.22 *m*	1a	7α, 8e	66.6	3.23 *m*	1b	7α,8e,NMe
7	42.7	α 2.80 *dd* (11, 6.5)	1a,6a,7a,8	7β,9,11	43.1	α 2.80 *m*	3a,6a,7a,8	7β,9,11
		β 1.55 *dd* (11, 8.9)	6a,7a,8,12	12e		β 1.50 *ddd* (10.5, 11)	6a,7a,8,12	
7a	47.1				48.0			
8	27.9	e 1.60 *br d* (13.5)		8ax,9	30.0	e 1.60 *br d* (13.5)		8ax, 9
		ax 2.25 *m*	7,7a,9			ax 2.25 *m*		9,OMe-1
9	33.4	1.95 *m*	7a,8,10,11	1′	33.6	1.95 *m*		1′
10	52.0				54.2			
11	33.9	1.85 *m*		1′, 12e,ax	33.9	1.85 *m*	7a	1′
12	31.0	e 1.38 *br d* (13.8)	7,8a,11	12ax	32.9	e 1.40 *br d* (12)		12ax
		ax 3.10 *m*				ax 3.05 *m*	1a,1b	OMe-1
1′a	141.8				141.2			
2′	111.4	7.20 *d* (8.6)	4′,5′a	1′,3′	112.4	7.21 *d* (8.6)	4′,5′a	
2′a	130.8				132.9			
3′	115.5	6.78 *ddd* (8.6, 1.5)	2′a,4′,5′	OMe-4′	111.9	6.79 *ddd* (8.6, 1.5)	2′a,4′,5′	
4′	154.2				155.0			
5′	100.5	6.93 *d* (1.5)	2′a,3′,4′,5′b	6′, OMe-4′	101.2	6.93 *d* (1.5)	2′a,3′,4′,5′	6′, OMe-4′
5′a	128.1				128.1			
5′b	109.0				107.8			
6′	23.1	2.70 *m*	1′a,5′a,5′b,7′	7′	22.8	2.70 *m*		7′
7′	39.4	3.18 *m*	5′b,6′,10		40.4	3.20 *m*	10, 6′,5′b	
OMe-1					61.3	3.91 *s*	1	
OMe-2	56.2	3.80 *s*	2					
OMe-4′	56.6	3.84 *s*	4′		56.3	3.84 *s*	4′	
NMe	43.8	2.42 *s*	5,6a		43.3	2.42 *s*	5,6a	
NH-1′		7.74 *br s*				7.67 *br s*		

[a] Assignments based on 2D experiments.

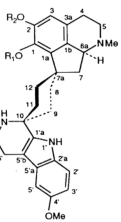
[b] In $CDCl_3$.

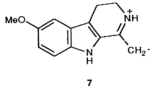
[c] In CD_3OD.

quaternary carbons of the β-carboline skeleton [4] were observed (Table 1). The HMBC experiment (Table 1) showed that the methoxy was located at C-4′ and confirmed the assignments of all the carbons.

In addition, the presence of seven methylene peaks between δ 27.3 and δ 55.4 in the ^{13}C NMR was reminiscent of a proaporphine skeleton. Ring A contained an OH, a methoxy and one aromatic proton (δ 6.48, *s*). The ring B nitrogen was methylated (NMe group at δ 2.42). The 2D NMR spectra showed that ring D contained four methylene groups and was attached to the β-carboline moiety by C-10 (δ 52.0) as in previously reported known proaporphine-tryptamine dimers. Four spin systems were observed in the COSY experiment: CH_2-4 (δ 2.90 and 2.70, 2*m*) and CH_2-5 (δ 3.15 and 2.45, 2*m*): CH-6a (δ 3.22, *m*) and CH_2-7 (δ 3.80 and 1.55, 2*m*); CH_2-8 (δ 2.25 and 1.60, 2*m*) and CH_2-9 (δ 1.95 *m*); CH_2-11 (δ 1.85, *m*) and CH_2-12 (δ 3.10 and 1.38, 2*m*). The HMBC spectrum (Table 1) verified the relative position of the substituents of the aromatic ring and further supported the connectivities and the assignments of all the carbons of the proaporphine moiety.

The relative configuration at C-6a, C-7a and C-10 of alkaloid **1** was deduced from the NOESY spectrum (Table 1). The cross peaks 6a/8e, 6a/7α and 7β/12e established the relative stereochemistry between C-6a and C-7a as shown. The spectrum further exhibited correlations between NH-1′ and the protons of both CH_2-9 and CH_2-11. H-11e and H-11ax were superimposed, as well as H-9e and H-9ax. However, the Dreiding models show that the correlations were with H-9ax and H-11ax and could be observed only if NH-1′ lies on the same side of ring D as the aporphine nitrogen, that is the alkaloid belongs to the *syn* series [2].





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5 $R_1 = H$, $R_2 = Me$
6 $R_1 = Me$, $R_2 = H$

Phoebegrandine B (**6**), $[\alpha]_D$ 0°, exhibited a M^+ peak in the HREI mass spectrum at *m/z* 473.2663 (Δ −1.57 mmu) corresponding to the molecular formula $C_{29}H_{35}N_3O_3$, which is isomeric to alkaloid **5**. The base peak *m/z* 214 (214.1087, $C_{13}H_{14}N_2O$, Δ −1.95 mmu) indicated that the *β*-carboline moiety was monomethoxylated. The UV, 1D and 2D NMR spectra were very close to those of compound **5**. However, significant differences were observed in the chemical shifts of the aporphine methoxy and aromatic signals (Table 1). The HMBC spectrum revealed clearly that the methoxy was attached to C-1 instead of C-2 as in phoebegrandine A, while the OH was at C-2.

The NOESY spectrum (Table 1) indicated that the stereochemistry of phoebegrandine (**6**) was similar to the one of alkaloid **5**. The correlations 6a/8e and 6a/7a established the stereochemistry of C-6a and C-7a, which was further confirmed by the cross peaks OMe-1/8ax and OMe-1/12ax. Alkaloid **6** also belonged to the *syn* series as shown by the correlations NH-1′/H-9 and NH-1′/H-11.

The absolute configuration of the dimeric alkaloids of *Roemeria hybrida* was determined by the fact that all aporphine alkaloids isolated from the same species possess an *S* configuration at C-6a [1]. The configuration shown for the alkaloids **5** and **6** is only relative. Both compounds appear as racemates, since no optical rotation and no CD curve were observed.

To our knowledge, *Phoebe grandis* and *Roemeria hybrida* are the sole plants containing proaporphine-tryptamine dimers. The substitution pattern of the aromatic rings of all those alkaloids are very similar. However two methoxy groups were found in the aromatic ring of the tryptamine part of some *Roemeria* alkaloids. The main difference between the dimers of these two species is that phoebegrandines A and B lack the aliphatic methoxy group, which is present in ring D of all *Roemeria* alkaloids.

EXPERIMENTAL

General. UV: MeOH; ^{1}H NMR: 250 or 400 MHz; ^{13}C NMR: 62.5 MHz; 2D experiments: 400 MHz; CC: Merck silica gel H 60.

Plant material. Bark and leaves of *Phoebe grandis* (Nees) Merr. (Lauraceae) were collected at Sik, Kedah (1994) by G. Perromat (Institut de Chimie des Substances Naturelles, CNRS, Gif sur Yvette). Identification was made by Dr K. M. Kochummen (Forest Research Institute of Malaysia, Kepong, Malaysia). Voucher specimens (KL 4318) are deposited at the Laboratoire de Phanérogamie, Muséum National d'Historie Naturelle in Paris, at the Herbarium of Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and isolation of the alkaloids. The alkaloids were extracted by the classical method after alkalinisation of the plant material. A total of 11.5 g of crude alkaloids was obtained from the bark (1 kg). The crude product underwent column chromatography on silica gel with CH_2Cl_2 containing increasing amounts of MeOH. The alkaloids purified were boldine (**1**) (15 mg after subsequent purification by prep. TLC), norboldine (**2**)(20 mg), laurotetanine (**3**) (4 mg) and lindcarpine (**4**) (3.2 mg). The crude alkaloids (2.5 g) from the leaves (500 g) were purified by a similar column chromatography yielding phoebegrandine B (**6**) (15 mg) CH_2Cl_2–MeOH, 49:1 and phoebegrandine A (**5**) (12 mg) CH_2Cl_2–MeOH, 24:1. Identification of the known compounds was made by comparison of their spectral data with lit. data [5].

Phoebegrandine A (**5**). Amorphous gum, $[\alpha]_D$ 0° ($CHCl_3$, *c* 0.5) UV λ_{max} nm (log ε) 224 (4.55), 277 (3.97), 308 sh (3.51). EIMS: *m/z* (rel. int.) 473 $[M]^+$ (23), 230 (88), 229 (100), 227 (87), 214 (81). ^{1}H and ^{13}C NMR, see Table 1.

Phoebegrandine B (**6**). Amorphous gum, $[\alpha]_D$ 0°

($CHCl_3$, *c* 0.5) UV λ_{max} nm (log ε) 225 (4.55), 278 (3.97), 308 sh (3.51). EIMS: *m/z* (rel. int.) 473 $[M]^+$ (42), 230 (42), 227 (40), 214 (100). ^1H and ^{13}C NMR: see Table 1. Since the alkaloid was not very soluble in $CDCl_3$ the HMQC and HMBC spectra were run in CD_3OD; ^1H NMR (CD_3OD): δ 6.54 (*s*, H-3), 2.77 (*m*, H-4α), 2.95 (*m*, H-4β), 2.56 (*m*, H-5α), 3.18 (*m*, H-5β), 3.38 (*m*, H-6a), 3.16 (*m*, H-7α), 1.55 (*m*, H-7β), 1.69 (*br d*, J = 13.5 Hz, H-8e), 2.10 (*m*, H-8ax), 2.10, 2.20 (*m*, H-9), 1.90, 2.15 (*m*, H-11), 1.53 (*m*, H-12e), 2.90 (H-12ax), 7.20 (*d*, J = 8.6 Hz, H-2'), 6.74 (*dd*, J = 8.6 and 1.5 Hz, H-3'), 6.92 (*d*, J = 1.5 Hz, H-5'), 2.80 (*m*, H-6'), 3.30 (*m*, H-7'), 3.81 (*s*, OMe-1), 3.88 (*s*, OMe-4'), 2.48 (*s*, NMe).

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Chemical constituents and bioactive compounds of *Goniothalamus tortilipetalus* Hend (Annonaceae)

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ABSTRACT A Malaysian plant, *Goniothalamus tortilipetalus* Hend (Annonaceae) was studied for its chemical constituents and biological activities. The stem bark of *Goniothalamus tortilipetalus*, produced two isoquinolines namely discretamine (**1**) and liriodenine (**2**). The leaves yielded asimilobine (**3**), liriodenine (**2**) and lanuginosine (**4**). In addition, non-alkaloidal aromatic compounds were also purified from the petroleum ether extract. They were 6-styryl-2-pyrone (**5**) and goniothalamin (**6**). The pharmacological study revealed that 6-styryl-2-pyrone possesses a vasorelaxant effect on rat aorta and it is cytotoxic to KB cells (ED_{50} = 48.5 μg/ml).

ABSTRAK Satu tumbuhan Malaysia, *Goniothalamus tortilipetalus* Hend., (Annonaceae) telah dikaji kandungan kimia dan aktiviti biologinya. Kulit batang *Goniothalamus tortilipetalus*, menghasilkan dua alkaloid isokuinolina: diskretamina (**1**) dan liriodenina (**2**). Manakala asimilobina (**3**), liriodenina (**2**) dan lanuginosin (**4**) telah dipisahkan daripada daun *G. tortilipetalus*. Sebagai tambahan, sebatian bukan alkaloid juga telah ditulinkan daripada ekstrak petroleum eter; 6-stiril-2-piron (**5**) dan goniothalamin (**6**). Kajian farmakologikal juga mendapati 6-stiril-2-piron memberikan kesan positif terhadap pengenduran otot licin aorta tikus dan ianya sitotoksik terhadap sel KB (ED_{50} = 48.5 μg/ml).

(Alkaloid, Annonaceae, Cytotoxicity, *Goniothalamus tortilipetalus*, Vasorelaxant effect).

INTRODUCTION

Chemical constituents of *Goniothalamus tortilipetalus*, were investigated which yielded four alkaloids; diskretamine (**1**), liriodenine (**2**), asimilobine (**3**) and lanuginosine (**4**) and two non-alkaloidal compounds; 6-styryl-2-pyrone (**5**) and goniothalamin (**6**). Structural elucidation was performed with the aid of spectroscopic methods; $^1H/^{13}C$ - NMR, IR, UV, MS.

EXPERIMENTAL

Goniothalamus tortilipetalus Hend., was collected at Grik, Perak. 1kg of the dried and milled stembark of *Goniothalamus tortilipetalus* Hend., were moistened with 15 % NH_4OH, soaked in CH_2Cl_2 for 3 days (cold extraction) or extracted with soxhlet apparatus (17 hours). The CH_2Cl_2 extract were reduced to 500 ml followed by basic extraction using 5 % HCl until Mayer's test is negative. The aqueous solution obtained was basified to pH ≈ 10 and reextracted with CH_2Cl_2 followed by washing with distilled H_2O and dried over anhydrous sodium sulphate. Finally, the extract was concentrated to give crude alkaloids of 3.0 g in weight. The same procedure was followed for the dried leaves of *G. tortilipetalus* Hend., except for the stembark of *Goniothalamus tortilipetalus* Hend., which was previously defatted with petroleum ether. The petroleum extract afforded two non-alkaloidal aromatic compounds.

Discretamine (**1**): UV λ_{max} (log ε) nm: 207 (4.20), 230 (4.10), 282 (3.19); IR υ_{max} cm^{-1}: 3450, 1350, 950, 1025; Mass spectrum m/e (%): 327 (M^+), 312, 176, 162, 150, 135; ^1H NMR ($CDCl_3$) ppm: 3.82 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.18 (1H, d, J = 16 z, H - 8eq), 6.68 (1H, s, H -1 or H - 4), 670 (1H, s, H -1 or H - 4), 6.83 (1H, d, J =

6 Hz, H - 11, or H - 12), 6.81 (1H, J = 6 Hz, H - 11, or H - 12).

Liriodenine (**2**): mp 278 - 280°C (chloroform dec.) lit [1]. 275 - 276°C); UV λ_{max} (log ε) nm: 248 (3.91), 275 (4.1), 310 (3.18) sh, 415 (3.10); IR υ_{max} cm^{-1}: 1660, 960, 860; Mass spectrum m/e (%): 275 (100), 247, 219, 189, 188, 162; ^1H NMR ($CDCl_3$) ppm: 6.34 (2H, s, -OCH_2O-), 7.17 (1H, s, H - 3), 7.62 - 7.80 (2H, m, H - 9, 10), 8.63 (1H, dd, J = 8 Hz, J' = 1 Hz, H - 8), 8.71 (1H, dd, J'' = 8 Hz, J''' = Hz, H - 11), 7.77 (1H, d, J = 5.4 Hz, H - 4), 8.96 (1H, d, J = 5.4 Hz, H - 5).

Asimilobine (**3**): UV λ_{max} (log ε) nm: 273 (4.21), 308(3.51); IR υ_{max} cm^{-1}: 3255, 3550, 1035.; Mass spectrum m/e (%): 267 (70), 266 (100), 238, 223 252, 236, 194, 177.;^1H NMR (270 MHz) ppm: 3.59 (3H, s, 1-OCH_3), 6.70 (1H, s, H-3), 8.3 (1H, m, H-11), 2.8 3.2 (3H, m, H - 8, H - 9, H - 10); ^{13}C NMR ($CDCl_3$): 48.3 (C-1), 142 - 9 (C -2), 125 (C - 1a), 132 (C - 1b), 114.6 (C - 3), 127 (C - 3a), 28.9 (C - 4), 43.2 (C - 5), 53.6 (C - 6a), 36.1 (C - 7), 136.1 (C - 7a), 129.8 (C - 8), 128.0 (C - 9), 128.3 (C -10), 127.2 (C - 11), 131 (C - 11a), 60.4 (OCH_3 - C - 1).

Lanuginosine (**4**): mp 318-320°C (decomp) (lit [2]. 315-317°C); UV λ_{max} (EtOH) nm: 211, 247, 273, 432 (log ε: 421, 4.30, 420 and 3.68); λ_{max} (EtOH - HCl) nm: 258, 283, 396 (log ε 4.31, 4.23, 3.66); IR υ_{max} cm^{-1}: 1655 (conjungated C = O) and 945); Mass spectrum m/e (%): 305 (M^+ 100), 290 (12), 277 (4.3), 276 (10), 275 (11), 262 (8), 247 (5); ^1H NMR ($CDCl_3$) ppm: 4.0 (3H, s, 9 - OCH_3), 6.35 (2H, s, OCH_2O), 7.15 (1H, s, H - 3), 7.33 (1H, dd, J_m = 3 Hz J_o = 9 Hz, H - 10), 7.78 (1H, d, J = SH_2, H - 4), 8.03 (1H, d, J = 3 Hz, H - 8), 8.57 (1H, d, J = 9 Hz, H - 11), 8.89 (1H, d, J = 5 Hz, H - 5).

6-Styryl-2-pyrone (dehydrogoniothalamin) (**5**):, mp 115 - 116°C (lit [3]. 116°C); UV λ_{max} (log ε) nm: 363, 269 nm; IR υ_{max} cm^{-1}: 1726.5, 965; Mass spectrum m/e (%):198 (100), 170, 141, 131, 103, 95, 77, 39, 28; ^1H NMR ($CDCl_3$) ppm: 6.58 (1H, d, J = 16.2 Hz), 6.19 (1H, dd, J = 9.6 Hz and 0.9 Hz, H - 3), 6.12 (1H, dq, J = 6.7, 0.9 and 0.4 Hz, H - 5).

Goniothalamin (**6**): UV λ_{max} (log ε) nm: 207, 255 and 284; IR υ_{max} cm^{-1}: 1720, 1577, 1663, 1492,970, 760; Mass spectrum m/e (%): 200, 172, 131, 115, 104, 91, 77, 68 (100); ^1H NMR ($CDCl_3$) ppm: 2.45 (2H, m, CH_2 -5), 5.03 (1H, m, H - 6), 6.05 H, dt, J = 9.7, J' = 1.8 Hz, H - 4), 6.85 (1H, dt, J = 9.7 Hz, J'' = 4.2 Hz, H - 3), 6.21 (1H, dd, J = 15.7 Hz, J''' = 1 Hz, H - 8), 6.68 (1H, dd, J = 15.7 Hz, J'' + 1 Hz, H - 8), 7.35 (5H, m, 5 H aromatic).

RESULTS AND DISCUSSION

Alkaloids

The crude alkaloid (0.3 % of bark) and (0.35 % of leaves) were subjected to chromatography over silice gel, followed by subsequent purification of the fractions collected.

Two alkaloids were isolated from the bark of *Goniothalamus tortilipetalus*; discretamine (**1**) and liriodenine (**2**) while the leaves produced three alkaloids; liriodenine (**2**) asimilobine (**3**) and lanuginosine (**4**). The petroleum ether extract yielded two non-alkaloidal aromatics; 6-styryl-2-pyrone (**5**) and goniothalamin (**6**).

A tetrahydroprotoberberine alkaloid, discretamine (**1**) was isolated from the bark as brownish amorphus solid. The UV spectrum showed a maximum at 284, 262 and 208 nm. The IR spectrum showed absorption at 3543cm^{-1} typical of an intramolecular hydrogen bonded hydroxyl group. Another peak was also observed at 1332 cm^{-1} which is due to the in plane O-H bend.

The mass spectrum revealed a molecular ion peak at m/e 327 which corresponded to a molecular formula of $C_{19}H_{21}NO_4$.Two important peaks were also observed in the mass spectrum at m/e 149 (48%) and m/e 178 (97%). The former peak was indicative of a tetrahydroprotoberberine bearing a hydroxyl group in ring D, while the latter was formed by an expulsion of a proton which is typical for the 9-methoxy, 10-hydroxy, substitution. Other peaks observed were m/e 326, 312, 176, 162, 150 and 135.

Interestingly, the ^1H NMR revealed the downfield resonances in the region of 6.60 - 7.00 ppm corresponding to a tetrahydroprotoberberine skeleton with substitutions at C-2, C-3, C-9 and C-10. An AB doublet of doublet (dd) centred at 6.82 ppm with a coupling constant of 6 Hz was observed which is attributable to H-11 and H-12. Furthermore a singlet corresponding to two aromatic protons, which may be ascribed to H-1 and H-4 was observed at 6.68 ppm. A half quartet was observed at 4.18 ppm (J = 16 Hz) due to the

resonance of the C-8 equatorial proton. Moreover, ^{1}H NMR spectrum also showed two sharp singlets, belonging to two methoxyl groups appeared at 3.82 ppm and 3.92 ppm.

Liriodenine (**2**) was obtained as fine yellow needles from chloroform with m.p. 278 - 280°C. An oxoaporphines nature was deduced for the major alkaloid, based on its intense yellow colour, strongly fluorescent chloroform solution and the deep red colouration it produced in acid medium [5]. 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 [6]. 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 [4]. 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/e 275, giving the possibility of the molecular formula to be $C_{17}H_9NO_3$.

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

Asimilobine (**3**), was isolated and crystallized from acetone to give a colourless prism, with m.p 177 – 179 °C. 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 [7].

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

The ^{1}H NMR spectrum exhibited four aromatic proton signals as a series of multiplets. The multiplets centred at 8.30 ppm and those between 7.20 - 6.80 ppm, was integrated for one proton and for three protons, respectively. The former was attributed to H-11 since in aporphine this proton would always be found more down field than the other aromatic protons due to the deshielding effect caused by the facing ring A. One methoxyl singlet was observed at 3.59 ppm which is rather shielded compared to the normal aromatic methoxyls since the protons of the methoxyl were forced to place themselves on top of ring D where the electron density is high. A singlet at 6.70 ppm was also observed which is attributable to H-3.

The last alkaloid, lanuginosine (**4**) was isolated as an orange red needles with m.p 318 - 320°C. Rf: 0.67 [$CHCl_3$: MeOH, 9:1]. It formed strong red solution in mineral acids and showed a strong green fluorescence in $CHCl_3$ solution [8]. The UV spectrum showed maxima at 211 nm (4.21), 247 (4.30), 273 (4.20) and 432nm (3.68). Its IR spectrum revealed absorption bonds at 1655 cm^{-1} assignable to conjugated ketone.

Furthermore, the mass spectrum showed a base peak at m/e 305 corresponded to a molecular formula of $C_{18}H_{11}NO_4$. Other significant peaks were observed at m/e 290 and m/e 275 due to the $[M-CH_3]^+$ and $[M^+-CH_2O]^+$ fragment, respectively.

The ^{1}H NMR spectrum showed a distinct methoxyl peak at 4.00ppm and a strong methylenedioxy peak of oxoaporphine at 6.35 ppm. The chemical shifts of methoxyl and methylenedioxy protons vary with their location [8]. The spectrum showed a singlet at 7.15 ppm which can be ascribed to H-3. The characteristic A-B doublet of doublet of the ring B protons (H-4, H-5) centered at 7.78 ppm and 8.89 ppm with a coupling constant 5 Hz was also present Furthermore the spectrum revealed a doublet belonged to H-8 at 8.03 ppm ($J_{8,10}$ = 3 Hz) and a doublet of doublet at 7.33 ppm of H-10. The highly deshielded proton, H-11 resonated as a doublet doublet at 8.55 ppm ($J_{10,11}$ = 9 Hz, $J_{11,8}$ = 3

Hz), which proved that C-9 was substituted by methoxyl group.

Non Alkaloidal Aromatic Compounds

The aromatic derivatives of monocyclic 2-pyrones are phytochemically associated with *goniothalamus* species [9,10] and *aniba* species [11]. 6-Styryl-2-pyrones (**5**) have previously been reported as constituents of the Lauraceae [12] Piperanceae and Basidiomycetes [13]. In this study, the author isolated two major compounds from *Goniothalamus tortilipetalus* Hend labelled as compound (**5**) and (**6**).

6-Styryl-2-pyrones (**5**) was crystalized from chloroform to form yellow hexagonal crystal with m.p. 115 - 116°C. The UV spectrum showed maxima at 363 and 269 nm. Its IR spectrum showed strong absorption at 1726 cm^{-1} indicating a 2-pyrone functionality [14,15]. The peak at 965 cm^{-1} was reminiscent of a trans-disubstituted ethylene associated with a styrenoid residue [16].

The mass spectrum showed a strong peak at m/e 198 thus suggesting the molecular formula of $C_{13}H_{10}O_2$. It also showed strong ion peaks at m/e 170 [M - 2CO]$^+$, 141 [M - CO - CHO]$^+$, 131 [C_8H_7CO]$^+$, 103 [C_8H_7]$^+$, 95 [M - C_8H_7]$^+$, 77 [C_6H_5]$^+$, 39 [C_3H_3]$^+$ and 28[CO].

Such a structure was also evident from the ^{1}H NMR data. The ^{1}H NMR spectrum showed that the lactone ring had been dehydrogenated. No resonance was observed in the 0-6 ppm region of the spectrum. One of the styryl olefinic protons was observed at 6.58 ppm (1H, d, J = 16.2 Hz) while H-3 and H-5 were observed at 6.19 ppm (1H, dd, J = 9.6 and 0.9 Hz) and 6.12 (1H, dq, J = 6.7, 0.9 and 0.4 Hz), respectively.

The ^{13}C NMR data indicate that all the 13 carbons resonated in the aromatic region. The high field signal of C-2 was observed at 161.8 ppm and the low field signal of C-8 was observed at 104.9 ppm. A peak at 135.2 ppm was assigned to the quartenary carbons C-6 and C-9.

The last non-alkaloidal compound, goniothalamine (**6**) was isolated in white crystals from chloroform with mp 83°C. It showed strong bands in IR spectrum at 1722, 1249, 752 cm^{-1} corresponding to the resonance of the α, β-unsaturated γ-lactone moiety. Additional bands were detected at 1494 and 965 cm^{-1} which belong to the styryl group.

In addition its mass spectrum showed a molecular ion peak at m/e 200 giving possibility to a molecular formula of $C_{13}H_{12}O_2$. The base peak was observed at m/e 68 corresponding to the ionized furan [17].

The ^{1}H-NMR spectrum revealed aromatic protons at 7.3 ppm as multiplet, and olefinic proton peaks at 6.68 (dd, 1H, J = 15.7 and 1Hz) and 6.21 ppm (dd, 1H, J = 15.7 and 6Hz), respectively with a trans configuration. ^{1}H-NMR were also indicative of an α,β-unsaturated δ-lactone moiety. Two olefinic protons were observed at 6.05 (1H, dt, J = 9.6 and 1.7 Hz) and 6.85 ppm (1H, dt, J = 9.6 and 4.2 Hz) which were assigned to H-3 and H-4, respectively. An allylic methylene was observed as a multiplet at 2.45 ppm and a proton on a carbon bearing the oxygen of the lactone group appeared as a multiplet at 5.03 ppm.

Bioactivity

1. Vasorelaxant activity on rat aorta

In anaesthetized rats, intravenous administration of 6-styryl-2-pyrone (10mg/kg) caused a transient drop in systolic (-50mm of Hg) and diastolic (-30 mm of Hg) blood pressure. A slight drop in heart rate was also observed but with variable results. In the isolated rat thoracic aorta [18] 6-styryl-2-pyrone caused a dose-dependent inhibition of the contractile responses to the phenylephrine (0.1μM) and high K (80 μM). The vasorelaxant effect of 6-styryl-2-pyrone is probably responsible for the hypotensive effect observed in anaesthesized rats. The mechanism of the vasorelaxant effect of the 6-styryl-2-pyrone may be due to the inhibition of calcium influx through both receptor and voltage operated calcium channels which were activated by phenylephrine and high potassium concentration, respectively.

2. Cytotoxic activity on KB cells

Two of the compounds isolated from *Goniothalamus tortilipetalus* Hend., were tested for their activity on KB cells [19] and positive results were obtained. They are liriodenine and 6-styryl-2-pyrone. Figure 1 illustrates the mortality curve of liriodenine and 6-styryl-2-pyrone. The ED_{50} of liriodenine is below 13 μg/ml and the ED_{50} for 6-styryl-2-pyrone is 48.5 μg/ml.

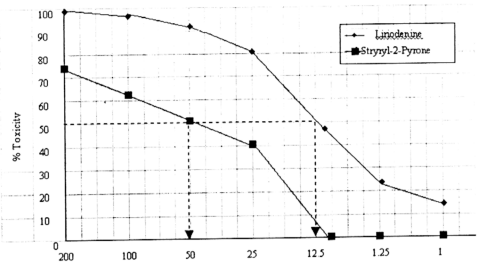
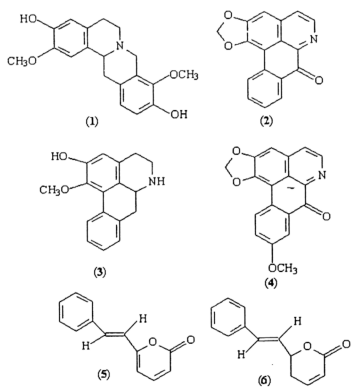


Figure 1: % Toxicity versus concentrations of liriodenine and 6-Styryl-2-pyrone



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Chemical constituents of *Phoebe grandis* (Nees) Merr. (Lauraceae)

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ABSTRACT A Malaysian plant, *Phoebe grandis* (Nees) Merr. (Lauraceae) was studied for its chemical constituents. Four aporphine alkaloids were isolated from the stem bark, namely, boldine (**1**), norboldine (**2**), laurotetanine (**3**), and lindcarpine (**4**).

ABSTRAK Satu spesis tumbuhan Malaysia, *Phoebe grandis* (Nees) Merr., (Lauraceae) telah dikaji kandungan kimianya. Empat aporfina alkaloid telah diperolehi daripada kulit batang iaitu boldina (**1**), norboldina (**2**), laurotetanina (**3**), dan lindkarpina (**4**).

(*Phoebe grandis*, alkaloid)

INTRODUCTION

In continuation of our research on Malaysian Plants, we have extracted the alkaloids from the bark of *Phoebe grandis*. The compounds isolated from the bark of *Phoebe grandis* are boldine (**1**), norboldine (**2**), laurotetanine (**3**), and lindcarpine (**4**). All alkaloids were isolated as wihite amorphous [1] from methanol. Structural elucidation was performed with the aid of spectroscopic methods; $^1H/^{13}C$-NMR, IR, UV, MS.

MATERIALS AND METHODS

Phoebe grandis (Nees) Merr., was collected at Sik, Kedah. 1kg of the dried and milled stembark of *Phoebe grandis* (Nees) Merr., were moistened with 15% NH_4OH and soaked in CH_2Cl_2 for 3 days (cold extraction). The CH_2Cl_2 extract was evaporated to 500ml followed by extraction using 5% HCl until Mayer's test is negative. The HCl extract was basified with concentrated ammonia to pH $\approx$ 11 and reextracted with CH_2Cl_2 The CH_2Cl_2 extract was washed with distilled H_2O and dried over anhydrous sodium sulphate. Finally, the extract was evaporated to dryness to give crude alkaloid (11.5g). Further purification by preparative TLC (Silica gel 60 F_{254}, CH_2Cl_2: MeOH; 97:3, 95:5, 90:10) afforded boldine (**1**), norboldine (**2**), laurotetanine (**3**) and lindcarpine (**4**).

Boldine (**1**): UV λ_{max} (MeOH) nm: 283 (4.21), 304 (4.23); IR υ_{max} cm^{-1}: 3533.4 (OH); Mass spectrum m/e (%): 327 (70%), 326 (100), 312, 310, 296, 284 253; ^1H NMR ($CDCl_3$) ppm: 87.89 (s, 1H, H-11), 6.83 (s, 1H, H-8), 6.63 (s, 1H, H-3), 2.52 (3H, s, N-CH_3), 258-320 (a complex pattern, C-4, 2H; C-5, 2H; C-6a, 1H; C-7, 2H): ^{13}C NMR: 142 (C-1), 148 (C-2), 113.3 (C-3), 130 (C-3a), 126 (C-1a), 125 (C-1b), 28.9 (C-4), 53.4 (C-5), 62.6 (C-6a), 34.2 (C-7), 130.2 (C-7a), 114.2 (C-8), 145.1 (C-9), 145 (C-10), 110 (C-11), 123 (C-119), 56.1 (OMe-C-10), 60.2 (OMe-C-1).

Norboldine (**2**): UV λ_{max} nm (log ε): 284 (4.13), 304 (3.17);IR υ_{max} cm^{-1} 3500, 2936;Mass spectrum m/e (%): 313 (70), 312 (100), 282, 298, 269, 284, 253;

^1H NMR ($CDCl_3$) ppm: 7.91 (s, 1H, H-11), 6.67 (s, 1H, H-8), 6.65 (s, 1H, H-3), 3.60 (s, 3H, OMe), 3.80 (s, 3H, OMe) 2.8-3.20 (aliphatic protons).

Laurotetanine (**3**): UV λ_{max} nm: 278 (3.83), 221 (4.31), and 305 (4.17);IR υ_{max} cm^{-1}: 3350, (OH); Mass spectrum m/e (%): 327 (100), 326, 312, 269, 298, 253; ^1H NMR ppm: 7.95 (1H, s, H - 11), 6.85 (1H, s, H-8), 6.69 (1H, s, H-3), 3.58 (3H, s, 1-OCH_3), 365 (3H, s, 10-OCH_3), 3.93 (3H, s, 2-OCH_3).

Lindcarpine (**4**): UV λ_{max} (log ε) nm: 262 (4.57), 208 (4.14) and 303 (3.82); IR υ_{max} cm^{-1}: 3490, 3350 and 3125;Mass spectrum m/e (%): 313 (100),

312, 298, 284, 282, 283; ^{1}H NMR ppm: 6.84 (1H, s, H-9), 6.84 (1H, s, H-8), 6.79 (1H, s, H-3), 3.65 (3H, s, 1-OCH_3), 3.92 (3H, s, 10-OCH_3).

RESULTS AND DISCUSSION

The first alkaloid boldine (**1**), was isolated as a white amorphous from methanol. The UV spectrum showed absorption typical of aporphine at 283 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 pattern [2]. 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 (**1**) further supported its aporphinic nature [3]. Molecular ion peak observed at m/e 327 gave a possible molecular formula of $C_{19}H_{21}O_4N$ and the peak at m/e 284 $[M-CH_2{=}NCH_3]^+$ was consistent with that of an N-methylaporphine. The low intensity fragment ions at m/e 312 $[M-CH_3]^+$ and m/e 296 $[M-OCH_3]^+$ indicated the presence of a methoxy substituent at C-1.

Furthermore, ^{1}H-NMR spectrum displayed two singlets corresponding to two methoxy groups at 3.91 and 3.60 ppm . The former is attributed to C-10 and the latter is assigned to C-1 which is more shielded since it experiences the anisotropic effect of the ring D. Three singlets representing three aromatic protons were observed at 6.63, 6.83 and 7.89 ppm which can be ascribed to H-3, H-8, and H-11. The N-methyl group resonated as a singlet at 2.52 ppm and the aliphatic protons appeared as multiplets at the region of 2.58 - 3.20 ppm.

The ^{13}C-NMR spectrum supported the hypothesis of alkaloid (**1**) bearing two methoxy groups at C-1 and C-10. The former resonated at 60.2 ppm while the latter resonated at 56.1 ppm. Moreover, the C-3 of boldine resonated at 113.3 ppm due to the fact that C-3 is ortho to a hydroxy group. The hydroxy group exhibited a lesser ortho shielding effect with respect to the methoxy group.

Norboldine (**2**) was isolated in its amorphous form. Its UV spectrum showed absorption bands at 284 and 304 nm, thus suggesting a 1,2,9, 10-tetrasubstituted aporphine skeleton [4]. The maxima was 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 (**2**) were typical of an aporphine carrying two hydroxyl groups.

Alkaloid (**2**) showed an M^+ (70 %) at m/e 313 suggesting a molecular formula of $C_{18}H_{19}NO_4$. The base peak at m/e 312 $[M-1]^+$ (100%) indicated the loss of a proton. In addition the peaks at m/e 298 $[M-CH_3]^+$ and m/e 282 $[M-OCH_3]^+$ confirmed the presence of a methoxy group at C-1.

Furthermore, the ^{1}H NMR spectrum also proved the existance of two methoxy by revealing two singlets at 3.60 and 3.80 ppm. These methoxy groups belonged most probably to C-1 and C-10. In addition a singlet corresponding to one proton was observed at 6.65 ppm which may be ascribed to H-3. This observation also indicated that C-2 is substituted.

In addition, the aromatic ring D were substituted by hydroxyl and methoxy groups at C-9 and C-10 respectively. Hence, H-8 and H-11 resonated as two singlets at 6.67 and 7.91 ppm, respectively. H-11 is more deshielded due to the anisotropic effect caused by ring A. The aliphatic protons of C-4, C-5, C-6a and C-7 resonated between 2.80-3.20 ppm.

The third alkaloid, laurotetanine (**3**) was afforded as white amorphous from methanol. The UV spectrum exhibited maxima at 221, 278 nm and 305 nm and any bands in the region above 300 nm eliminated the possibility of a 9,10-disubstitution. 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/e 327 suggesting a molecular formula of $C_{19}H_{21}NO_4$. Other significant fragmentation peaks were revealed at m/e 326 $[M-1]^+$ and m/e 312 $[M-15]^+$ indicating the loss of H and CH_3, respectively.

Moreover, the presence of a strong $[M-31]^+$ fragmentation peak at m/e 296 in the mass spectrum suggested that C-1 was substituted by a methoxy group.

The ^{1}H NMR spectrum exhibited three methoxy singlets at 3.58, 3.65 and 3.93 ppm. The former is assigned to the methoxy at C-1 since the protons were shielded by the anisotropic effect caused by ring D. A one proton singlet at 6.69 ppm was observed in the spectrum, confirming that H-3 is unsubstituted. Furthermore, the singlet at 6.85 ppm can be attributed to H-8. This value is typical of a 9,10-substitution pattern [5,6]. It was clear that, the low field signal of H-11 at 7.95 ppm suggested

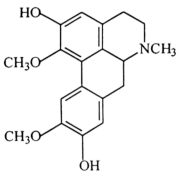
that C-1 was substituted by a methoxy group. The aliphatic protons gave a multiplet between 3.70-2.30 ppm.

The last alkaloid lindcarpine (**4**) was isolated as white amorphous solid. It was unstable, and tend to darken when exposed to air or light. Alkaloid (**4**) also showed OH and NH absorptions at 3330 and 3125 cm^{-1}, respectively and no carbonyl absorption was observed. The ultraviolet spectrum showed maxima at 208, 262 and 305 nm which were characteristic of 1,2,10,11-tetrasubstituted noraporphines [7,8].

Its electron impact mass spectrum (EIMS) showed a molecular ion peak at m/e 313 (100%) and chemical ionization mass spectrum (CIMS) also gave a peak at 313 (77%), thus giving a possible molecular formula of $C_{18}H_{19}NO_4$.

Fragmentation peaks at m/e 298 and 282 indicated losses of CH_3 and OCH_3 groups, respectively. The aporphine structure of (**4**) was further supported by the characteristic $[M^+\text{-}1]$ and $[M^+\text{-}29]$ peaks due to the losses of H and CH_2 = NH moiety [9], respectively.

The ^1H NMR spectrum showed a one proton singlet attributable to H-3 at 6.79 ppm which indicated that C-1 and C-2 are substituted [10]. The spectrum also showed two singlets of two methoxy groups at 3.65 and 3.92 ppm most probably belonged to C-1 and C-10, respectively. A sharp singlet corresponding to two-protons at 6.84 ppm was assigned to H-8 and H-9 protons. In addition a multiplet representing H-4, H-5, H-6a and H-7 appeared at the region of 2.70-3.30 ppm.



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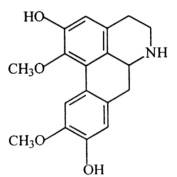

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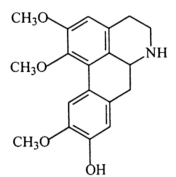

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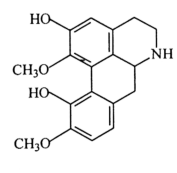

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ATURAL PRODUCT RESEARCH

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Dear Prof. Awang

I am happy to inform you that your Ms. No. 07032003-H-IN-517 has been accepted for publication subject to some minor modifications (referees reports enclosed). I shall be grateful if you could kindly send your manuscript modified according to the enclosed comments as soon as possible.

With kind regards,

Yours sincerely,

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PHOEBEGRANDINE C, A NOVEL PROAPORPHINE-TRYPTAMINE DIMER, FROM *PHOEBE GRANDIS* (NEES) MERR.

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A novel proaporphine-tryptamine dimer alkaloid, named phoebegrandine C **1**, was isolated from the leaves of *Phoebe grandis* (Nees) Merr. Its structural elucidation was carried out using spectroscopic techniques, notably 2D NMR.

Keywords; proaporphine-tryptamine dimer, phoebegrandine C, NMR, *Phoebe grandis*, alkaloid

INTRODUCTION

The genus *Phoebe* (Lauraceae) of the tribe Perseae is found mainly in Asia and America. There are 50 species found in Pantropic and in Malaysia, of which about six species were reported [1]. *Phoebe* is most abundant in Borneo and the Malaysian Peninsula. In the course of our continuing studies on Malaysian

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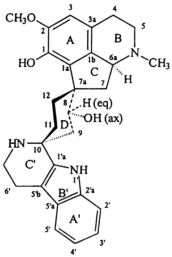
Lauraceae, a chemical investigation on *Phoebe grandis* was undertaken. The sample studied was collected at Gunung Stong Forest, Sg. Terang Dam, Kelantan (KL 4224). *Phoebe grandis* is an evergreen timber tree with alternate leaves, often fascicle at the end of the twigs. The tree is medium size to 121 m tall and 150 cm girth. The bark is lenticellate and scaling off in thin papery flakes with strongly aromatic smells. The inner bark is dark brown. In the present paper, we shall report the alkaloids isolated from the leaves, which yielded one novel proaporphine-tryptamine dimer named (+)-phoebegrandine C **1**, two known proaporphine-tryptamine dimers; (±)-phoebegrandine A **2** and (±)-phoebegrandine B **3** and a proaporphine, tetrahydroglaziovine **4**.

RESULTS AND DISCUSSION

The dichloromethane extract of the leaves was subjected to the usual alkaloidal acid-base extraction to obtain the crude alkaloids, which was then subjected to extensive column chromatography and phoebegrandine C **1**, (CH_2Cl_2: MeOH; 97:3), phoebegrandine A **2**, (96:4), phoebegrandine B **3**, (90:10) and tetrahydroglaziovine **4**, (95:5), were obtained. The identified known structures of compounds were confirmed by comparison of their spectroscopic data with the literature values [5-6].

Phoebegrandine C **1**, $[\alpha]_D^{23}$ +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 [7]. The IR spectrum showed a hydroxyl absorption band at 2918 cm^{-1}. The HRESI (positive mode) spectrum showed an $[M+H]^+$ peak at 460.2616 (calc. 460.2600) thus, suggesting a molecular formula of $C_{28}H_{34}N_3O_3$.

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.



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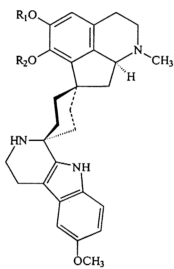
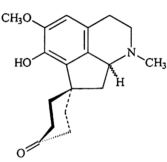


2 R_1 = Me, R_2 = H
3 R_1 = H, R_2 = Me



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

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

2D NMR experiments (COSY, HMBC, HMQC and NOESY) allowed to propose the complete assignment of the proton and carbon chemical shifts as shown in Table 1. 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 spectrum showed correlations between N-1' proton (δ 8.24) with H-2' (δ 7.31), H-9ax (δ2.02) and H-11ax (δ 2.35) thus confirmed 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 [3, 5].

A previous study on the leaves of *Phoebe grandis* has been reported before in which the sample was collected from Kedah (West Coast of Malaysian Peninsula) [5]. The alkaloids communicated in this paper were isolated from *Phoebe grandis* collected from a different site; Kelantan (East Coast of Malaysian Peninsula). One observes that both leaves produced tryptamine dimers but the latter also produced

Table 1: ^{13}C NMR (75 MHz) and ^{1}H NMR (400 MHz) data for phoebegrandine C **1**

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	e 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	e 1.63 m	7a, 10	
		ax 2.20 m		N-1'
12	27.13t	e 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-CH_3	43.59q	2.35 s		
2-OCH_3	56.44q	3.84 s		
N-1'		8.24 br s	1'a, 2'a, 5'a, 5'b	2', 9ax, 11ax

proaporphine type alkaloid; tetrahydroglaziovine **4**. Therefore, this observation showed that plants from different sites might exhibit some different chemical components, which may be due to environmental variations. At present, fourteen proaporphine-tryptamine dimers have been isolated and structurally identified from the genus *Phoebe* and *Roemeria*. The genus *Roemeria* produced both *syn* and *anti* types but the *Phoebe* only produced the *syn* type [2-5].

EXPERIMENTAL

General Experimental Procedure

The optical rotations were recorded on Jasco (Japan) P1010 with tungsten lamp. HRMS was obtained on Automass Multi Thermofinnigan. The ultraviolet spectra were obtained in MeOH on Shimadzu UV-160A ultraviolet-visible spectrometer. The infrared spectra were taken on a Perkin Elmer 1600 Double-Beam recording Spectrometer, using chloroform. The ^{1}H NMR were recorded in deuterated chloroform on a JEOL 400 MHz (unless stated otherwise); chemical shifts are reported in ppm on δ scale, and the coupling constants are given in Hz. Aluminium supported silica gel 60 F_{254} plates were used for TLC. The plates were activated at 100°C for one hour and stored in a dessicator until needed. TLC spots were visualized under ultra-violet light (254 nm and 365 nm) followed by spraying with the Dragendorff's reagent.

Silica gel 60, 70-230 mesh ASTM (Merck 7734) and silica gel 60, 230-400 Mesh ASTM (Merck 9385) were used for column and flash chromatography, respectively. Silica gel 604 F_{254} was used for preparative TLC. Meyer's reagent was used for alkaloid screening.

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