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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; $^{1}H/^{13}C$-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; ^{1}H 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;

^{1}H 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; ^{1}H 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

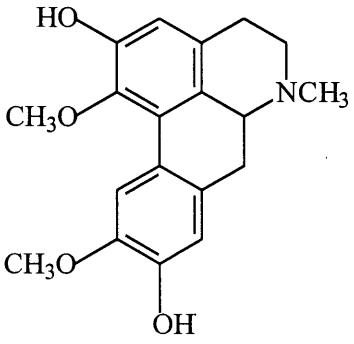
that C-1 was substituted by a methoxy group. The aliphatic protons gave a multiplet between 3.70-2.30 ppm.

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 $[M^+-1]$ and $[M^+-29]$ 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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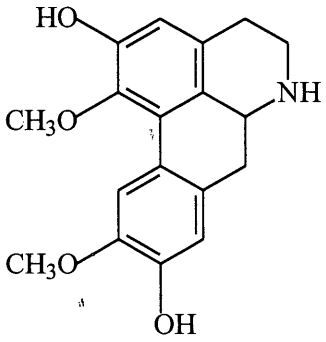

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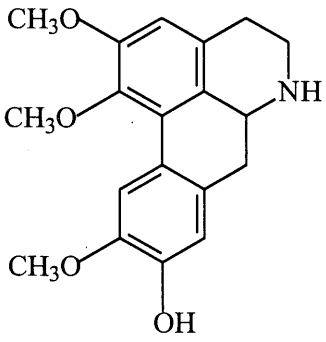

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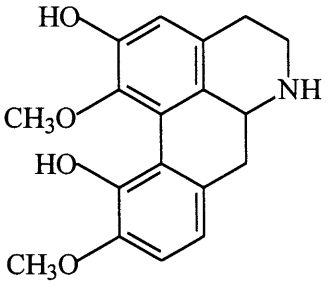

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