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Isolation and Antibacterial Activity of Alkaloids from *Phaeanthus opthalmicus*

M.S. ATAN*, K.A. DZULKEFLY, S. MOHD ASPOLLAH, K. ANUAR and S. VIJAY

Department of Chemistry, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

*Corresponding author: E-mail: atan@science.upm.edu.my

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Four alkaloids; O-methyldauricine (**1**), limacine (**2**), corydaldine (**3**) and oxostephanine (**4**) were isolated from *Phaeanthus opthalmicus* and their structures have been fully characterized by spectral methods. Antibacterial activities of the pure compounds **1**, **2** and **3** have also been assessed. This is the first report of the presence of compounds **1**, **3** and **4** in this genus.

Key Words: *Phaeanthus opthalmicus*, Annonaceae, Alkaloids, Antibacterial activity.

INTRODUCTION

Plants of genus *Phaeanthus*, family Annonaceae, comprising some 20 species are small trees about 15 m high and are distributed throughout tropical Asia. Only three species of the genus have previously been studied for their alkaloids contents. They are *P. ebracteolatus*[1,2], *P. macropodus*[3] and *P. crassipetalus*[4] and two species (*P. ebracteolatus* and *P. macropodus*) were reported to be found in East Malaysia and *P. opthalmicus* was endemic to Peninsular Malaysia[5]. *P. ebracteolatus* has been known to posses some medicinal properties and has been used as a remedy for sore eyes[6] and *P. opthalmicus* has been used by traditional healer for treatment of wound and ulcer[5]. *P. opthalmicus* was selected for investigation because of its strong alkaloid content and also because no previous work has been carried out on this plant. In present investigation on the alkaloids constituents of this plant we report the isolation of two bisbenzylisoquinoline alkaloids, O-methyldauricine (**1**), limacine (**2**), an isoquinoline alkaloid, corydaldine (**3**) and oxostephanine (**4**).

EXPERIMENTAL

Melting points were uncorrected and were determined on Kofhler melting points apparatus. The IR spectra were recorded using KBr disc on Perkin-Elmer FTIR spectrophotometer model 1650. ^{1}H and ^{13}C NMR spectra were obtained on Bruker WPT (90 MHz for ^{1}H and 20.1 MHz for ^{13}C) and Jeol NMR ECA at 400 and 125 MHz for ^{1}H and ^{13}C, respectively with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on an AE1-MS 12 spectrometer. The column and mini column chromatography were carried on silica gel Merck 1.07749 and 1.07734, respectively.

The bark of *P. opthalmicus* was collected from Sungkai Forest Reserve in Peninsular Malaysia and the plant was identified by botanist Mr. Shamsul Khamis from Institut Biosains, Universiti Putra Malaysia.

Isolation of the compounds: The air-dried bark (110 g) of the plant was extracted successfully at room temperature with petroleum ether (b.p. 40-60 ºC) (2 x 2 L) followed by methanol (7 × 2 L). The methanol extract was concentrated under reduced pressure to give a black oily residue. The residue was dissolved in chloroform (500 mL) and extracted exhaustively with 1 % H_2SO_4 (5 × 600 mL). The acid fraction was basified with Na_2CO_3 and was extracted with chloroform (5 × 200 mL) and the combined extracts were evaporated under reduced pressure to give 2.0 g of crude alkaloids. The crude alkaloids (2.0 g) was fractionated using column chromatography. The components were eluted with chloroform:methanol (9.5:0.5) to give alkaloidal fractions. The fractions were further purified by thin layer chromatography using chloroform: methanol (9:1) as eluting solvent to give two major alkaloids **1** (10 mg) and **2** (15 mg) and two minor alkaloids **3** (8 mg) and **4** (2 mg).

Antibacterial activity: The compounds were screened for antibacterial activity by using standard petri disc method. The gram positive and gram negative bacteria used for the test were *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, *Proteus vulgaris*, respectively. All of the bacteria used in the test are from a stock culture of the Department of Biochemistry and Microbiology, Universiti Putra Malaysia. All plates were prepared with an equal thickness of nutrient agar. A filter paper disc (5 mm diameter) was impregnated with the compound (at specific concentration) and the disc was then placed on the nutrient agar in a petri disc and left for

24 h at 37 ºC. Ampicilin was used as the control and the activity was based on the diameter (in mm) of the clear zone around the disc. The number of replications in the experiment being three.

Characterization of alkaloids

O-Methyldauricine (1): An amorphous brown solid, UV λ_{max} nm (EtOH), 285, 228, 208, $[\alpha]_D$- 90º (c = 0.06 in MeOH). MS m/z (% abundance), 638 (2), 327 (8), 312 (3), 307 (1), 206 (100). ^{1}H NMR (80 MHz, $CDCl_3$), δ (ppm), 2.49 (s, N'-methyl), 2.53 (s, N-methyl), 3.57 (s, 7-O-methyl), 3.61 (s, 7'-O-methyl), 3.80 (s, 6-O-methyl), 3.81 (s, 6'-O-methyl), 3.83 (s, 12-O-methyl), 6.01 (s, 8-H-aromatic) and 6.06 (s, 8'-H-aromatic). ^{13}C NMR (20.1 MHz, $CDCl_3$), 26.1 (C-4), 26.1 (C-4'), 40.9 (C-α), 41.2 (C- α'), 43.3 (N-methyl), 43.3 (N-methyl) 47.4 (C-3), 47.5 (C-3'), 56.2 (2 × OCH_3), 56.3 (2 × OCH_3), 56.7 (OCH_3), 65.3 (C-1), 111.4 (C-5), 111.4 (C-8), 111.7 (C-8'), 111.7 (C-5'), 113.0 (C-11'), 117.3 (C-13), 123.1 (C-13'), 126.4 (C-4α'), 126.6 (C-4α), 126.7 (C-14), 129.6 (C-8α), 129.7 (C-8α'), 131.4 (C-14'), 133.5 (C-9), 134.4 (C-9'), 145.1 (C-11), 146.9 (C-6'), 146.8 (C-7'), 147.8 (C-7, C-6), 150.3 (C-12), 156.9 (C-12').

Limacine (2): Colourless crystal (crystallized from benzene), m.p. 172-174 ºC (Lit. 173-174°)[8], UV λ_{max} (MeOH) 284 nm, $[\alpha]_D$ -240º (c = 0.20 in $CHCl_3$). MS m/z (% abundance), 609 (22), 608 (44, M^+), 607 (24), 471 (1), 382 (23), 381 (70), 367 (26), 192 (60), 191 (100), 174 (13) and 168 (14). ^{1}H NMR (80 MHz, $CDCI_3$), δ (ppm) 2.30 (s, 2-N-methyl), 2.60 (s, 2'-N-methyl), 3.30 (s 6'-O-methyl) 3.74 (s, 6-O-methyl), 3.90 (s, 12-O-methyl) and 6.05-7.40 (10H, aromatic). ^{13}C NMR (20.1 MHz, $CDCl_3$), 22.1 (C-4), 25.2 (C-4), 38.0 (C-α), 42.2 (C-α'), 42.4 (N-CH_3), 42.6 (N-CH_3), 44.3 (C-3), 45.4 (C-3'), 56.1 (3 × O-CH_3), 61.4 (C-1), 64.0 (C-1'), 104.8 (C-5), 112.0 (C-13), 113.0 (C-5'), 116.3 (C-10), 120.4 (C-8'), 121.5 (C-13'), 123.0 105.1 (C-11'), 123.2 (C-8a and C-14), 128.1 (C-8a') 128.3 (C-4a), 128.3 (C-4a), 130.2 (C-10'), 132.4 (C-14'), 134.7 (C-9), 135.0 (C-9'), 135.9 (C-7), 142.0 (C-7'), 143.7 (C-11), 146.0 (C-8), 147.0 (C-6'), 148.9 (C-12), 149.0 (C-6) and 153.6 (C-12').

Corydaldine (3): White needles (crystallized from benzene), m.p. 156-158 °C (Lit. 173-174 °C)[9], UV λ_{max} nm (MeOH), 300, 289, MS m/z (% abundance), 207 (m^+., 100) 178 (57), 150 (75) ,107 (15). IR (KBr, ν_{max}, cm^{-1}, disc) 3191.8, 1658.3, 127.4, ^{1}H NMR, (400 MHz, $CDCl_3$), δ (ppm), 2.96 (t, 2H, H-4), 3.56 (m-2H, H-3), 3.93 (s, 6H, H-6, -OCH_3, H-7, -OCH_3), 6.22 (s, 1H, H-2), 6.67 (s, 1H, H-5), 7.57 (s, 1H, H-8). ^{13}C NMR (125 MHz, $CDCl_3$) (as in the table).

Oxostephanine (4): An amorphous brown solid, UV λ_{max} nm 312, 274, MS m/z (% abundance), 305 (100), 290 (7), 276 (28), 275 (28), 247 (21), 234 (34). IR (KBr, ν_{max}, cm^{-1} (disc) 3436, 1662, 1606, 1020. ^{1}H NMR, (400 MHz, $CDCl_3$), δ (ppm), 4.00 (s, 3H, -OCH_3), 6.36 (s, 2H, -O-CH_2-O-), 7.16 (d, 1H, H-9), 7.30 (s, 1H, H-3), 7.77 (t, 1H, H-10), 8.03 (d,1H, H-4), 8.57 (d, 1H, H-11), 8.89 (d, 1H, H-5). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm), 55 (-OCH_3), 102 (C-3, C-9), 110 (C-7a), 122 (C-11, C-1a), 124 (C-4), 126 (C-10), 129 (C-1b), 132 (C-11a), 135 (C-5), 144 (C-3a), 145 (C-8), 147 (C-6a), 151 (C-1), 159 (C-2), 182 (C-7).

RESULTS AND DISCUSSION

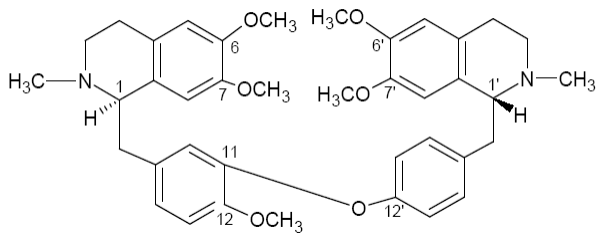
The alcoholic extraction of the ground stem bark of the plant followed by acid-base partition and the usual work-up procedure yielded the crude alkaloidal mixture. The crude extract was subjected to collumn chromatography and thin layer chromatography to afford four alkaloids, O-methyl-dauricine (**1**), limacine (**2**), corydaldine (**3**) and oxostephanine (**4**).

The first major alkaloid **1** from stem bark of *P. opthalmicus* was obtained as an amorphous brown solid and it has molecular ion M^+ at m/z 638 consistent with the molecular formula $C_{39}H_{46}N_2O_6$. The UV absorption of the alkaloid (λ_{max} at 285 and 209 mμ) indicated that it belonged to the *bis*benzyliso-quinoline group. The ^{1}H NMR spectrum of the alkaloid showed two three proton singlets for N-methyl group appeared at δ 2.49 and δ 2.53 and the methyl signals from five methoxyl groups have the chemical shift at δ 3.57, 3.61, 3.80, 3.81 and 3.83 which correspond to methoxyl groups at C-7, C-7', C-6, C-6' and C-12, respectively[7]. The assignment of the structure was further supported by the ^{13}C NMR spectrum which agreed with the reported value for O-methyldauricine[7]. This is the first report of the presence of *bis*benzylisoquinoline alkaloid in this genus.

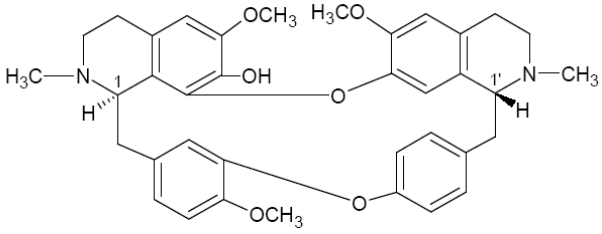
The mass spectrum of alkaloid **2** showed the molecular ion at m/z 608 which was consistent with the molecular formula $C_{39}H_{40}N_2O_6$. The ^{1}H NMR spectrum showed the presence of two N-methyl groups at δ 2.30 and δ 2.58 and 10 aromatic protons signals were observed in between δ 6.00 and δ 7.15. The 16 aliphatic protons signals were further observed in the region 2-4 ppm. The three methoxyl peaks at δ 3.30, 3.70 and δ 3.88 can be assigned to carbons position at 6',6 and 12, respectively and the absence of a peak near δ 3.20 suggests that there is no methoxyl group attached to the C-7 atom. The ^{13}C NMR of **2** was consistent with that of limacine[8].

The alkaloid **3** was crystallized in $CHCl_3$ as white needles with melting point 156-158 °C[9]. The UV absorption (λ_{max} MeOH 300 and 289 nm) was characteristic of isoquinoline alkaloid and the IR spectrum showed the presence of carbonyl amide group (ν_{max} 1657 cm^{-1}). The mass spectrum showed the molecular ion at m/z 207 which was consistent with the molecular formula $C_{11}H_{13}NO_3$. The ^{1}H NMR showed a chemical shift at δ 3.93 for two methoxyl groups. The triplet for 2 protons at δ 2.96 and a multiplet for 2 protons at δ 3.56 can be assigned for methylene protons at carbons 4 and 3, respectively. The aromatic protons appeared as singlet at δ 6.67 and δ 7.57 for carbons 6 and 7, respectively. The amino proton was resonated as a broad singlet at d 6.22. Thus, these data is consistent with that of tetrahydroisoquinoline alkaloid, corydaldine (**3**)[9]. The structure of **3** was further supported by ^{13}C NMR which has not been reported, so it is described here (Table-1)[10].

The alkaloid **4** was obtained as an amorphous brown solid and the MS spectrum of alkaloid **4** showed the molecular ion peak at m/z 307 corresponding to the molecular formula of $C_{18}H_{11}NO_4$. A base peak was observed at m/z 305. The peaks at m/z 290 $[M\text{-}CH_3]^+$ and 275 $[M\text{-}OCH_3]^+$ show that the methoxyl group is also presence in this structure. The IR spectrum gave absorption peaks at 3436 and 1662 indicated the presence of NH and carbonyl groups respectively. The absorption



1



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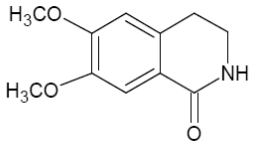

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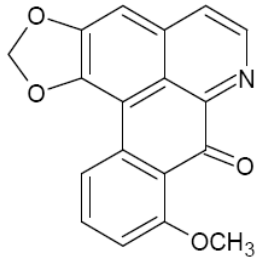

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TABLE-1
^{13}C NMR SPECTRAL DATA OF CORYDALDINE (**4**)

Carbons	δ (ppm)	Carbons	δ (ppm)
C-1	16645	C-6	147.97
C-3	40.37	C-7	152.11
C-4	27.91	C-87	132.62
C-4a	109.53	C-8a	110.06
C-5	121.26		

at 1020 cm^{-1} due to the presence of methylene dioxy group. The ^{1}H NMR spectrum of **4** indicated the presence of methoxyl group at δ 4.0 ppm, the signals for methylene dioxy was observed at δ 6.36. A singlet at δ 7.30 ppm can be assigned for proton at C-3. Observation of two signals appeared in AB system at δ 8.03 and 8.89 ppm (J = 6Hz) are attributed to protons at carbons **4** and **5**, respectively. The three adjacent aromatic protons resonate at δ 8.57, 7.77 and 7.16(d) (J_{ortho} = 8Hz) for protons at carbon 11, 10 and 9, respectively. The ^{13}C NMR spectrum showed the presence of 19 carbons signals which comprising of one methylene dioxy, one methyl carbon, six methines and nine quaternaries. These spectra agreed with the published data for oxostephanine[9].

All pure compounds except **4** were screened for antibacterial activity using standard disc method. Two species of gram positive (*Bacillus subtilis* and *Staphilococcus aureus*) and two species of gram negative (*Escherichia coli* and *Proteus vulgaris*) have been utilized.The activity was measured in terms of growth inhibition (in mm) and ampicillin was used as standard. At 103 ppm only compound **2** was weakly active against the gram negative bacteria and at 10^4 ppm, **1** was slightly active against the gram negative bacteria while limacine (**2**) was more active against both strains of bacteria.

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