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ALKALOIDS FROM LEAVES AND STEM BARK OF *ERVATAMIA PEDUNCULARIS*

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Key Word Index—*Ervatamia peduncularis*; Apocynaceae; leaves; stem bark; indole alkaloids; NMR.

Abstract—Chemical study of the leaves and stem bark of *Ervatamia peduncularis* afforded two novel *bis*indole alkaloids, pedunculine and peduncularidine, together with seven known alkaloids, coronaridine, coronaridine hydroxyindolenine, eglandine, heyneanine, eglandulosine, heyneanine hydroxyindolenine and *N*(1)-methyl-aspidospermidine. Structural elucidation of the new alkaloids was based on their spectral data.

INTRODUCTION

In the framework of our chemical studies on the genus *Ervatamia* [1–3], and of our collaborative research program between C.N.R.S. and the University of Malaya [4], we report herein our results on the alkaloidal content of *Ervatamia peduncularis* [5]. This species is a shrub 2.5 m tall whose roots are used in traditional medicine for the treatment of syphilis and of ulcerations of the nose [6]. Plant material was collected at Genting Simpah 'Malaysia' and identified by two of the authors (H. S. and H. A. H.).

RESULTS AND DISCUSSION

The crude alkaloids were extracted and purified using a classical acid–base method. Isolation of alkaloids was performed by means of column chromatography and preparative TLC. Six known alkaloids were obtained from the crude alkaloid mixture (AM) of the stem bark. They are in increasing order of polarity, coronaridine (**1**) (10% AM), coronaridine hydroxyindolenine (**2**) (0.3% AM), eglandine (**3**) (2% AM), heyneanine (**4**) (44% AM), eglandulosine (**5**) (0.5% AM) and heyneanine hydroxyindolenine (**6**) (0.5% AM). In the AM of the leaves, we have isolated the known *N*(1)-methyl aspidospermidine (**7**) (2% AM) and two new bases for which we propose the trivial names, pedunculine (**8**) (17.9% AM) and peduncularidine (**9**) (15% AM). Compounds **1–5** and **7** were identified by comparison of their spectra (UV, IR, mass, NMR) and by co-TLC with reference samples.

The structure of **6** was elucidated by comparison of its spectral data with those in the literature [7]. However, assignments of carbons in the fragment C-9:C-12 differ; they were established by analysis of COSY and HMQC data. Inverting atoms at positions 9 and 12, and 10 and 11 also leads to coherent assignments. Structure **6** was proven by chemical correlation with heyneanine (**4**); oxidation of **4** with H_2O_2 [8] gave heyneanine hydroxyindolenine identical to **6**.

Isolation of **7** provided an opportunity for completing previous ^1H and ^{13}C NMR assignments [9, 10] by means of 2D NMR experiments. Its structure was confirmed by methylation of aspidospermidine [11], which gave a compound identical to **7**.

The new alkaloids **8** and **9** were obtained as purple amorphous solids and determination of their specific rotations was precluded due to the colour of their solutions. These two compounds possess many spectral analogies suggesting closely-related structures. Their complex UV spectra (λ_{max} *ca* 220, 260, 308, 333 nm) suggest the superimposition of anilinoacrylic and indoline chromophores. Their IR spectra display absorptions for a conjugated carbonyl group (*v ca* 1660 and 1600 cm^{-1}) and for OH and/or NH (*v ca* 3350 cm^{-1}). The *bis*indolic nature of **8** and **9** was deduced from mass and ^{13}C NMR spectra which gave 43 resonances for the two compounds in the latter (Table 2). Furthermore, ^1H NMR spectra (Table 1) showed two ethyl side-chains (triplets at δ0.8 and 0.75), three methyl singlets between δ3.75 and 3.9 corresponding to one methyl ester and two aromatic methoxyl groups. Another methyl singlet at δ2.8 is ascribable to a *N*-methylgroup. The low-field region of these spectra displays one deshielded NH, at δ8.75, the signals of an AMX system (*br s*: H-9′, *dd*: H-11′ and *d*: H-12′) and one shielded proton singlet at δ5.75 in **8** and 5.85 in **9**. These data suggest that one indole ring is substituted at C-10′ while the other is trisubstituted.

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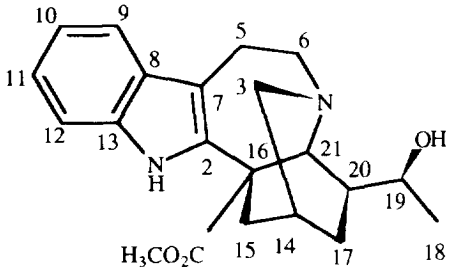
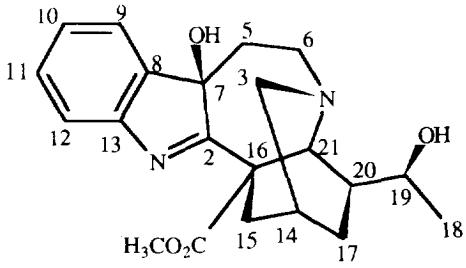
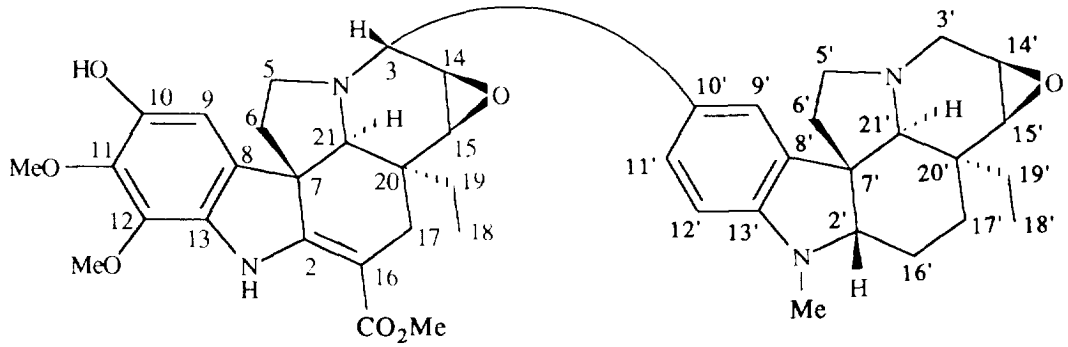


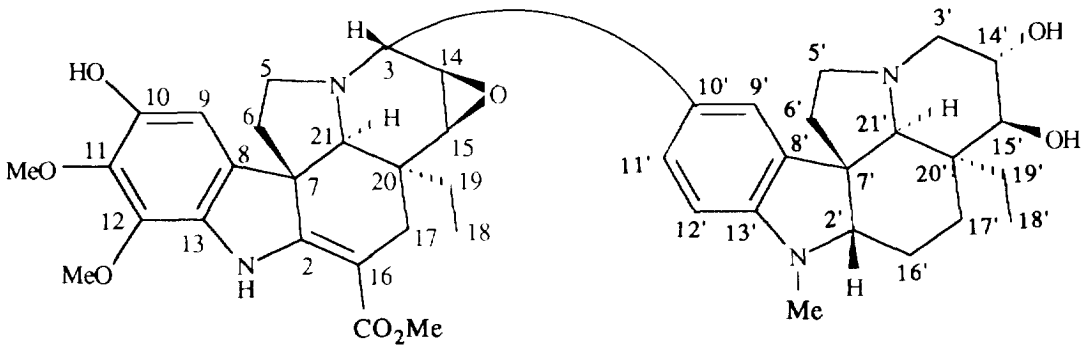
Heyneanine (**4**)



Heyneanine hydroxyindolenine (**6**)



Pedunculine (**8**)



Peduncularidine (**9**)

Thorough analysis of COSY, HMQC, and HMBC spectra of **8** and **9** allowed the identification of the two dimers units as a 14,15-epoxytabersonine and a *N*-methylaspidospermidine. This second unit is substituted at C-14′ and C-15′ showing correlations in COSY spectra for two H-3′, one H-14′ and one H-15′. The position of the branching points between the monomeric units was fully supported by the presence of a single proton at position C-3 and of a quaternary carbon at position 10′ (δ122.4 in **8** and 123.1 in **9**), as well as by HMBC correlations of C-3 with H-9′ and H-11′. The β-orientation of the epoxide ring in the tabersonine part is deduced from chemical shift comparison with data from tabersonine epoxides of α- and β-configurations [12], especially in what concerns positions C-19 and C-20. The strong shielding of C-21 (δ61.6 in **8** and 61.8 in **9**), instead of δ70.9 [12], was interpreted as an effect of the proximity of the aspidospermidine aromatic ring. This observation favoured an α-orientation of the substituent at C-3, as found in pandicine [13] and cryophylline [14]. Moreover, for steric reasons, the α-side of the tabersonine unit is more prone to attack by a bulky reagent. The substitu-

Table 1. ^{1}H NMR spectral data of **8** and **9** (300 and 500 MHz, $CDCl_3$)

H	8	9
3	4.5 *s*	4.5 *s*
5	2.6 *m*	2.65 *m*
5	2.9 *m*	2.9 *m*
6	1.55 *m*	1.6 *m*
6	2.05 *dt* (7, 11)	2.0 *dt* (7, 12)
9	5.75 *s*	5.85 *s*
14	3.4 *dd* (1, 4)	3.4 *d* (3.5)
15	3.25 *d* (4)	3.25 *d* (3.5)
17	2.6 *d* (15)	2.6 *d* (15)
17	2.7 *d* (15)	2.7 *d* (15)
18	0.8 *t* (7.5)	0.8 *t* (7.5)
19	1.0 *q* (7.5)	1.0 *q* (7.5)
21	2.75 *s*	2.85 *s*
11-OMe	3.85 *s*	3.8 *s*
12-OMe	3.9 *s*	3.85 *s*
N(1)-H	8.75 *s*	8.75 *s*
CO_2CH_3	3.8 *s*	3.75 *s*
2′	3.45 *d* (5)	3.45 *dd* (2, 4)
3′	2.4 *m*	2.1 *m*
3′	3.6 *d* (13)	3.2 *m*
5′	2.25 *m*	2.4 *m*
5′	3.2 *m*	3.1 *m*
6′	1.65 *ddd* (1, 8.5, 9.5)	1.6 *m*
6′	2.35 *m*	2.45 *m*
9′	7.0 *br s*	7.05 *br s*
11′	7.05 *dd* (1.5, 8)	7.02 *d* (8)
12′	6.45 *d* (8)	6.4 *d* (8)
14′	3.35 *d* (4)	3.9 *m*
15′	2.95 *d* (4)	3.45 *d* (9)
16′	1.2 *m*	1.35 *q* (13)
16′	1.8 *m*	1.85 *m*
17′	1.8 *m*	1.5 *br d* (13)
17′	1.5 *m*	1.8 *t* (13)
18′	0.75 *t* (7.5)	0.75 *t* (7.5)
19′	1.3 *dq* (15, 7.5)	1.4 *q* (7.5)
19′	1.4 *dq* (15, 7.5)	1.4 *q* (7.5)
21′	2.3 *s*	2.6 *s*
N(1′)-CH_3	2.8 *s*	2.8 *s*

Table 2. ^{13}C NMR spectral data of **8** and **9** (75 MHz, $CDCl_3$)

C	8	9
2	165.1	165.2
3	58.2	58.2
5	47.7	48.2
6	42.1	42.2
7	54.5	54.8
8	135.5	133.7
9	104.0	104.2
10	143.7	143.7
11	138.6	138.7
12	136.7	136.8
13	128.2	128.5
14	54.3	54.6
15	56.5	56.7
16	90.4	90.5
17	23.5	23.9
18	7.3	7.5
19	26.8	27.1
20	36.5	36.5
21	61.6	61.8
22	168.6	168.9
11-OMe	60.8	61.0
12-OMe	60.3	60.4
CO_2CH_3	50.8	51.0
2′	73.2	71.3
3′	52.7	56.5
5′	53.5	51.6
6′	40.8	39.7
7′	51.2	52.5
8′	136.5	135.4
9′	122.8	122.7
10′	122.4	123.1
11′	128.0	128.4
12′	105.9	106.2
13′	149.7	150.0
14′	52.6	68.9
15′	57.0	78.4
16′	19.7	21.0
17′	23.6	29.9
18′	7.4	8.6
19′	27.9	26.1
20′	34.5	39.7
21′	67.2	66.7
N(1′)-CH_3	31.4	31.4

tion pattern of the tabersonine aromatic part, i.e. OH on C-10 and OMe on C-11 and C-12, is in full agreement with correlation by HMBC spectra of **8** and **9** and with ^{13}C NMR δ values observed for pandicine [13] and polyervine [15].

Pedunculine (**8**) gives no significant $[M]^+$ in its EI mass spectrum but its FAB mass spectrum shows an intense $[M + H]^+$, at *m/z* 737. This corresponds to an M_r of 736 and molecular formula, $C_{43}H_{52}O_7N_4$. Besides the oxygenated groups previously located in this molecule, an additional oxygen atom belongs to another epoxide ring between C-14′ and C-15′. The NMR δ values of protons H-14′ and H-15′ and of carbons C-3′, C-14′, C-15′ and C-20′ are reminiscent of those of hazuntiphyllidine [16] and thus, indicate a β-orientation of the 14′-15′ epoxide group.

Peduncularidine (**9**) exhibited a quasi $[M]^+$ 18 mu higher than that of **8** (FAB mass spectrum $[M + H]^+$ *m/z* 755) suggesting additional hydration. Analysis of NMR spectra led to the assignment of C-14′ and C-15′ to signals at δ68.9 and 78.4, respectively. Therefore, **9** was considered to be the *trans*-diol (J(H-14′, H-15′) = 9 Hz) obtained from **8** by the epoxide ring-opening of the aspidospermidine unit (threo isomer). Nucleophilic substitution by a water molecule would preferentially proceed at position 14′, position 15′ being sterically hindered by the α-ethyl side-chain. Thus, a 14′β-H, 15′α-H configuration is proposed. This was confirmed by analysis of the ROESY spectrum of **9**, the proton H-2′ being β-axial, as well as the triplet proton H-17′ at δ1.8. H-17′ correlates with H-14′, thus proving that the latter is in a β-position. Moreover, correlation between CH_3-18′ from

the α-ethyl side-chain with H-15′ proves the α-orientation of the latter. The α-orientation of the aspidospermidine substituent on C-3 is confirmed by H-3/H-5 correlation. These data also support the structure of **8**. The complete NMR analysis of **8** and **9** establishes the relative configuration of each part of these molecules.

In conclusion, *E. peduncularis* contains two novel dimeric bases, pedunculine and peduncularidine. They are *bis*-tabersonine derivatives containing a highly oxygenated chromophore reminiscent of that of kisantine [17] and polyervine [15]. It should be noted that association of the two moieties of biogenetic type II in *Ervatamia* species is only found in *E. aurantiaca* [18], *E. pandacaqui* [19] and *E. polyneura* [15]. In contrast, the monomeric alkaloids from *E. peduncularis* are common in the genus.

Pedunculine could have been independently isolated by another group (Dr Kam, personal communication) from *Tabernaemontara divaricata* (double flower variety) from Malaysia.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively. ^{1}H proton multiplets of **8** and **9** were described from spectra recorded at 500 MHz. Chemical shifts are reported in δ from TMS. 1D (^{1}H, ^{13}C) and 2D (COSY, ROESY, HMBC, HMQC) expts were performed using standard Bruker microprograms.

Plant material. Ervatamia peduncularis King and Gamble was collected in December 1989 at Genting Simpah, Malaysia. A voucher specimen is kept at the herbarium of the Department of Phytochemistry, University of Malaya, under number K.L. 3799.

Extraction. From 2.7 kg of stem bark submitted to continuous Soxhlet extraction by CH_2Cl_2, a crude extract (9 g) was isolated. This was purified using a classical acid–base method. Its soln, in $CHCl_3$, was extracted with 0.33 M H_2SO_4. The aq. layer was basified with 12 M aq. NH_3 and extracted with Et_2O. The Et_2O layers were dried (Na_2SO_4) and evapd *in vacuo* to give 2.4 g of alkaloid mixt. (AM, 0.89 g kg^{-1} dried plant). Leaf (1.3 kg) extraction was performed following an identical process yielding 1.57 g of AM (1.2 g kg^{-1}).

Isolation. Stem bark AM was purified by flash-CC. Elution was performed initially with CH_2Cl_2 and then with CH_2Cl_2–MeOH mixts of increasing polarity. Frs (20 ml) were collected, analyzed by TLC and grouped according to their composition. Further purifications were achieved by prep. TLC. Alkaloid **1** was in frs 1–11, **2**–**4** in frs 12–18, **5** in frs 18–20, **6** in following frs eluted with MeOH. Compound **4** recrystallized from MeOH. An identical process was used for the AM from leaves. Coronaridine (**1**) was identified from frs 1–13 by comp. TLC. Alkaloid **7** was isolated in frs 17–19, **8** in frs 26–31 and **9** in frs 98–100, all of them as pure compounds. Other frs and complex polar mixtures obtained were not studied.

Heyneanine hydroxyindolenine (**6**). Ceric-spray: grey–yellow. $[\alpha]_D$ −4.3° ($CHCl_3$; *c* 0.49). UV λ_{max}^{MeOH} nm: 222, 258, 285 (sh), 290. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3480, 1730. MS *m/z* (rel. int.): 370 $[M]^+$ (80), 353 (100), 335 (12), 325 (20), 311 (10), 309 (10), 230 (12), 188 (70), 160 (40). ^{1}H NMR ($CDCl_3$): δ7.45 (*d*, *J* = 7 Hz, H-12), 7.35 (*d*, *J* = 7 Hz, H-9), 7.3 (*t*, *J* = 7 Hz, H-11), 7.25 (*t*, *J* = 7 Hz, H-10), 5.9 (*bs*, OH), 4.15 (*s*, H-21), 4.05 (*dq*, *J* = 1, 6 Hz, H-19), 3.7 (*s*, CO_2CH_3), 3.6 (*ddd*, *J* = 15, 12, 3 Hz, H-5), 3.5 (*bs*, OH), 2.9 (*ddd*, *J* = 15, 4, 1.5 Hz, H-5′), 2.85 (*m*, H-15 and H-3), 2.7 (*d*, *J* = 9 Hz, H-3′), 2.5 (*dt*, *J* = 13, 3.5 Hz, H-15′), 2.1 (*m*, H-6 and H-14), 1.9 (*m*, H-17), 1.8 (*m*, H-6′), 1.6 (*tdd*, *J* = 12, 2, 1 Hz, H-17′), 1.5 (*m*, H-20), 1.05 (*d*, *J* = 6 Hz, H-18). ^{13}C NMR ($CDCl_3$): δ188.7 (C-2), 172.7 (C-22), 151.2 (C-13), 142.0 (C-18), 129.3 (C-11), 126.9 (C-10), 121.4 (C-9), 120.9 (C-12), 87.7 (C-7), 71.1 (C-19), 59.7 (C-21), 57.4 (C-16), 53.3 (CO_2CH_3), 48.1 (C-3), 47.7 (C-5), 38.2 (C-20), 35.6 (C-15), 32.8 (C-6), 26.4 (C-14), 22.7 (C-17), 20.2 (C-18).

Synthesis of **6** *by oxidation of heyneanine* (**4**). A soln of 149 mg (0.42 mmol) of heyneanine (**4**) in a mixt. of 1.5 ml HOAc and 1.5 ml of 30% H_2O_2 was left for 24 hr at 20°. The reaction mixt. was diluted with 10 ml H_2O and basified by 12 M aq. NH_3 in the presence of Et_2O (10 ml). The organic layer was washed with H_2O, dried (Na_2SO_4) and evapd. The residue (96 mg) was recrystallized from Et_2O (mp 197°). The product was identical to **6** (co-TLC, IR and NMR).

N(1)-*Methyl-aspidospermidine* (**7**). Ceric-spray: orange. UV λ_{max}^{MeOH} nm: 257, 308. ^{1}H NMR ($CDCl_3$): δ7.05 (*dt*, *J* = 1, 7.5 Hz, H-11), 7.0 (*d*, *J* = 7.5 Hz, H-9), 6.6 (*dt*, *J* = 1, 7.5 Hz, H-10), 6.35 (*d*, *J* = 7.5 Hz, H-12), 3.4 (*dd*, *J* = 5.5, 9 Hz, H-2), 3.1–3.05 (*m*, H-5 and H-3), 2.75 (*s*, NMe), 2.3 (*m*, H-6 and H-5′), 2.2 (*s*, H-21), 1.8–2 (*m*, H-3′ and H-17), 1.75 (*m*, H-14, H-16), 1.65 (*m*, H-15), 1.4–1.55 (*m*, H-14′, H-19 and H-6′), 1.25 (*m*, H-16′), 1.1–1.2 (*m*, H-15′, H-17′), 0.85 (*dq*, *J* = 14.5, 7.5 Hz, H-19), 0.6 (*t*, *J* = 7.5 Hz, H-18). ^{13}C NMR ($CDCl_3$): δ150.5 (C-13), 137.0 (C-8), 127.2 (C-11), 122.0 (C-9), 117.0 (C-10), 106.4 (C-12), 71.6 (C-2), 71.1 (C-21), 53.7 (C-3), 52.9 (C-5), 52.4 (C-7), 38.9 (C-6), 35.5 (C-20), 34.4 (C-15), 31.4 (NCH_3), 30.0 (C-19), 28.8 (C-17), 21.9 and 21.6 (C-14 and C-16), 6.7 (C-18).

Synthesis of **7** *by methylation of aspidospermidine.* To a soln of aspidospermidine (100 mg) in 1.5 ml of 30% aq. HCHO, was added 0.5 ml of HOAc followed by 110 mg of $NaBH_3CN$, in small portions, within 15 min. The reaction mixt. was stirred for 1 hr, diluted with H_2O, basified with 12 M aq. NH_3 and extracted with $CHCl_3$. The organic layer was washed with H_2O and dried (Na_2SO_4). The residue (96 mg) contained 5% of starting material and was purified by prep. TLC, (CH_2Cl_2–MeOH, 97:3) to obtain a product identical to **7** (co-TLC and NMR).

Pedunculine (**8**). Ceric-spray: purple. UV λ_{max}^{MeOH} nm: 208, 204 (sh), 263, 307, 337; + HCl: 216, 277, 300, 331; + NaOH: 219, 262, 296. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340, 1660, 1600. FAB MS *m/z* (rel. int.): 737 $[M + H]^+$ (85), 702 (10), 683 (18), 588 (20), 307 (22), 289 (18), 197 (20), 170 (10),

136 (75), 107 (30). ^{1}H and ^{13}C NMR ($CDCl_3$) in Tables 1 and 2.

Peduncularidine (**9**). Ceric-spray: purple. UV λ_{max}^{MeOH} nm: 221, 234 (sh), 264, 308, 333; + HCl: 221, 235 (sh), 278, 306, 329; + NaOH: 217, 264, 316. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1670, 1600. FAB MS *m/z* (rel. int.): 755 $[M + H]^+$ (90), 723 (10), 693 (18), 588 (3), 341 (15), 307 (20), 289 (15), 170 (10), 136 (75), 107 (25). ^{1}H and ^{13}C NMR ($CDCl_3$) in Tables 1 and 2.

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