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ALKALOIDS FROM *KOPSIA GRIFFITHII*

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Key Word Index—*Kopsia griffithii*; Apocynaceae; leaves; alkaloids.

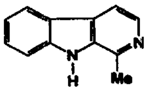
Abstract—Nineteen alkaloids were isolated from the leaf extract of *Kopsia griffithii*, of which two were new, *viz.*, 12-methoxypleiocarpine and the tetrahydro-β-carboline derivative, harmicine. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

The genus *Kopsia* include some 30 species found mostly in tropical Asia [1, 2]. There are about 17 species which occur in Malaysia. The phytochemistry of this genus has been quite well investigated and our own studies of this genus have resulted in several novel structures and some important bioactivities [3–7]. We now wish to report the alkaloidal composition of the leaf extract of *Kopsia griffithii* King and Gamble, which has not been previously investigated, including the isolation of two new alkaloids. Preliminary screening showed strong anti-leishmania activity, which was traced back to the basic fraction from the ethanol extract of the leaves.

RESULTS AND DISCUSSION

The ethanol extract of the leaves of *K. griffithii* furnished the following alkaloids: harmane (**1**), kopsilongine (**2**), kopsamine (**3**) [8], kopsamine-N_4-oxide (**4**) [9], pleiocarpine (**5**), leuconolam (**6**) [10], kopsinine (**7**), (+)-eburnamonine (**8**), buchtienine (**9**) [11], N_1-methoxycarbonyl-12-methoxy-Δ-^{16,17}-kopsinine (**10**) [12], N-carbomethoxy-11-hydroxy-12-methoxykopsinaline (**11**) [9], N-carbomethoxy-11,12-dimethoxykopsinaline (**12**) [8], tetrahydroalstonine (**13**), 12-methoxykopsidasinine (**14**) [13], rhazimol (deacetylakuammiline) (**15**) [14], 16-(R)-19,20-E-isositsirikine (**16**) [15–17], leuconoxine (**17**) [18], 12-methoxypleiocarpine (**18**) and harmicine (**19**). The last two are new alkaloids. Among the known alkaloids, the β-carboline alkaloid harmane (**1**), the quasidimer buchtienine (**9**) [11] and 16R-19,20-E-isositsirikine (**16**) [15–17], are reported for the first time from *Kopsia*. Harmane (**1**) and buchtienine (**9**) constitute the major alkaloids in the leaves.



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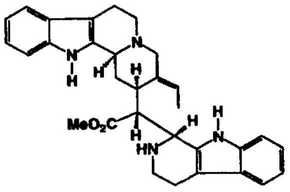

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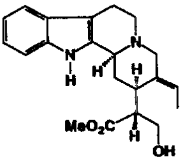

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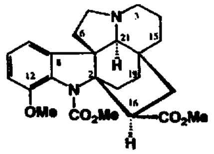

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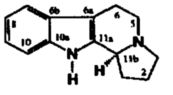

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Compound **18** showed a $[M]^+$ at m/z 426 ($C_{24}H_{30}N_2O_5$) in the EI mass spectrum, with other major fragments at m/z 411 $[M\text{-}Me]^+$, 398 $[M—CH_2{=}CH_2]^+$, 367 $[M—CO_2Me]^+$ and 339 $[M—CH_2{=}CH_2—CO_2Me]^+$. The presence of the retro Diels–Alder fragment at m/z 398 provided early indication of an aspidofractinine-type compound. The ^{13}C NMR spectrum is in agreement with the molecular formula deduced from the mass spectrum, accounting for all 24 carbons. The UV spectrum showed absorption maxima at 216, 253, 282, and 288 nm (log ε 4.23, 3.78, 3.17 and 3.20, respectively) indicating the presence of a dihydroindole chromophore. The ^1H NMR spectrum showed the presence of three aro-

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matic hydrogens from δ 6.78 to 6.98, three methoxy groups (δ 3.70, 3.75, 3.83) and a one-H singlet attributable to H-21 (δ 2.91). The three methoxy signals are associated with ester, carbamate and an aromatic methoxy substituent from the carbon resonances at δ 174.3, 153.7 and 56.3, respectively. The coupling pattern of the aromatic hydrogens, together with the aromatic carbon resonances, suggest placement of the methoxy substituent at C-12 [12]. The NMR spectra can be assigned from 2-D COSY and HETCOR experiments and reveals a pleiocarpine-type derivative [19]. The ^{13}C NMR spectra, in fact, resembles that of pleiocarpine, except for changes due to additional methoxy substitution in the aromatic ring. Compound **18** is therefore 12-methoxypleiocarpine.

Compound **19** was obtained in an amorphous form and its EI mass spectrum showed a $[M]^+$ at *m/z* 212 ($C_{14}H_{16}N_2$), with other major fragments at *m/z* 211 $[M\text{-}H]^+$ (base) and 184 $[M—CH_2{=}CH_2]^+$. The strong $[M\text{-}1]^+$ peak, together with characteristic fragments at *m/z* 170, 169, 168 and 156, are typical of tetrahydro-β-carboline compounds [20]. The UV spectrum showed absorption maxima at 225, 281 and 292 nm (log ε 3.98, 3.30 and 3.18, respectively), which are also typical of tetrahydro-β-carboline alkaloids. Analysis of the ^{1}H and ^{13}C NMR spectra showed that compound **19** is the indoloindolizine compound 2,3,5,6,11,11b-hexahydro-1H-indolizino [8, 7-b] indole, for which we propose the trivial name harmicine. Compound **19** has been previously synthesized in racemic form [21–24], but is isolated for the first time as an optically active natural product. The observation of Wenkert–Bohlmann bands in the IR spectrum (2780 and 2835 cm^{-1}) suggests that the stereochemistry of H-11b is α and, also, that the C/D ring junction is *trans* [25–28]. Further confirmation of the *trans*-ring junction is obtained from NOE experiments. Thus, irradiation of the α H-11b signal at δ 4.26 resulted in NOE enhancements of NH (δ 8.19), H-1α (δ 2.29), H-2α (δ 1.91), H-3α (δ 2.92) and H-5α (δ 3.09). Likewise, irradiation of the NH signal causes enhancement of the H-11b signal and *vice versa*. The observed NOE interaction between H-11b with H-3α as well as H-5α, confirms the *trans*-C/D ring junction in compound **19** [29].

EXPERIMENTAL

Plant material. Plant material was collected in Selangor, Malaysia, and voucher specimens are deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and isolation. Extraction of alkaloids was carried out in the usual manner, as described in detail elsewhere [12]. Alkaloids were isolated by CC and centrifugal TLC on silica gel. Solvent systems used for CC were $CHCl_3$–MeOH and Et_2O. Solvent systems used for centrifugal TLC were Et_2O, Et_2O–hexane and MeOH–$CHCl_3$. The yields (g kg^{-1}) of the alkaloids isolated from the leaves were: **1** (0.035), **2** (0.015), **3** (0.010), **4** (0.003), **5** (0.003), **6** (0.005), **7** (0.003), **8** (0.005), **9** (0.032), **10** (0.008), **11** (0.003), **12** (0.002), **13** (0.001), **14** (0.002), **15** (0.006), **16** (0.011), **17** (0.003), **18** (0.009) and **19** (0.009).

12-*Methoxypleiocarpine* (**18**). $[\alpha]_D = -79°$ ($CHCl_3$, *c* 0.097). UV λ_{max}^{EtOH} (log ε) 216 (4.23), 253 (3.78) 282 (3.17), 288 (3.20). EIMS, *m/z* (rel. int.): 426 [$[M]^+$, $C_{24}H_{30}N_2O_5$] (100), 411 (5), 398 (35), 380 (21), 367 (15), 354 (28), 339 (8) 124 (19), 109 (25). ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.16 (1H, *m*, H-19), 1.26 (1H, *m*, H-14), 1.31 (1H, *m*, H-19), 1.43 (1H, *m*, H-15), 1.52 (1H, *m*, H-15), 1.60 (1H, *m*, H-18), 1.68 (1H, *m*, H-17), 1.77 (1H, *m*, H-6), 1.83 (1H, *m*, H-14), 1.87 (1H, *m*, H-18), 2.50 (1H, *ddd*, $J = 13$, 9 and 2 Hz, H-17), 2.69 (1H, *ddd*, $J = 14$, 8 and 4 Hz, H-5), 2.91 (1H, *br s*, H-21), 2.94 (1H, *m*, H-3), 3.01 (1H, *m*, H-6), 3.04 (1H, *m*, H-3), 3.14 (1H, *t*, $J = 8$ Hz, H-5), 3.56 (1H, *t*, $J = 9$ Hz, H-16), 3.70 (3H, *s*, CO_2Me), 3.75 (3H, *s*, NCO_2Me), 3.83 (3H, *s*, ArOMe), 6.78 (1H, *dd*, $J = 7.5$ and 2 Hz, H-9), 6.96 (1H, *dd*, $J = 7.5$ and 2 Hz, H-11), 6.98 (1H, *t*, $J = 7.5$ Hz, H-10). ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 174.3 (CO_2Me), 153.7 (NCO_2Me), 148.2 (C-12), 143.3 (C-13), 129.5 (C-8), 124.3 (C-10), 114.4 (C-11), 112.1 (C-9), 69.9 (C-2), 68.6 (C-21), 58.0 (C-7), 56.3 (ArOMe), 52.2 (NCO_2Me), 51.8 (CO_2Me), 50.4 (C-5), 47.7 (C-3), 41.8 (C-16), 37.1 (C-15), 36.1 (C-6), 33.5 (C-18), 33.1 (C-19), 32.1 (C-20), 30.1 (C-17), 17.5 (C-14).

Harmicine (**19**). $[\alpha]_D = +119°$ ($CHCl_3$, *c* 0.086). UV λ_{max}^{EtOH} (log ε) 225 (3.98), 281 (3.30), 292 (3.18). EIMS, *m/z* (rel. int.): 212 [$[M]^+$, $C_{14}H_{16}N_2$] (75), 211 (100), 184 (33), 168 (9), 169 (9), 170 (9), 156 (17), 106 (6). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86 (1H, *m*, H-1), 1.89 (1H, *m*, H-2), 1.91 (1H, *m*, H-2), 2.29 (1H, *m*, H-1), 2.68 (1H, *br dt*, $J = 15$ and 2 Hz, H-6), 2.92 (1H, *m*, H-3), 2.94 (1H, *m*, H-3), 2.99 (1H, *m*, H-6), 3.09 (1H, *td*, $J = 13$ and 5 Hz, H-5), 3.31 (1H, *ddd*, $J = 13$, 5 and 2 Hz, H-5), 4.26 (1H, *br s*, H-11b), 7.09 (1H, *td*, $J = 7$ and 1.2 Hz, H-8), 7.14 (1H, *td*, $J = 7$ and 1.2 Hz, H-9), 7.31 (1H, *dd*, $J = 7$ and 1.2 Hz, H-10), 7.48 (1H, *dd*, $J = 7$ and 1.2 Hz, H-7), 8.19 (1H, *br s*, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 136.2 (C-10a), 133.5 (C-11a), 126.8 (C-6b), 121.7 (C-9), 119.4 (C-8), 118.0 (C-7), 111.0 (C-10), 107.0 (C-6a), 57.5 (C-11b), 49.6 (C-3), 46.0 (C-5), 29.5 (C-1), 23.1 (C-2), 17.6 (C-6).

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