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Leishmanicidal alkaloids from *Kopsia griffithii*

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Abstract

Thirteen alkaloids were isolated from the stem-bark extract of *Kopsia griffithii*, of which three were new. These were the *N*(4)-oxides of akuammiline, 16-*epi*-deacetylakuammiline and 11,12-methylenedioxykopsinaline. Harmane, pleiocarpine and buchtienine showed antileishmanial activity. © 1998 Published by Elsevier Science Ltd. All rights reserved.

Keywords: *Kopsia griffithii*; Apocynaceae; Stem-bark; Antileishmanial alkaloids

1. Introduction

The genus *Kopsia* comprises some 30 species found mostly in tropical Asia (Markgraf, 1972; Sevenet et al., 1994). There are ca. 17 species which occur in Malaysia. The Malaysian members of this genus have received considerable attention which has resulted in several novel alkaloid structures and some important bioactivities (Awang, Sevenet, Hadi, David, & Pais, 1992; Kam, Yoganathan, & Chuah, 1995; Kam, Yoganathan, & Li, 1996; Kam, Yoganathan, Koyano, & Komiyama, 1996; Kam, Yoganathan, & Chen, 1996a,b; Uzir et al., 1997). We have previously reported the alkaloidal composition of the leaf extract of *Kopsia griffithii* King and Gamble (Kam & Sim, 1998). We now report the alkaloidal composition of the stem-bark extract, including the isolation of three new alkaloids. Preliminary screening of extracts from this species showed strong anti-leishmanial activity which was previously traced to the basic fraction from the ethanol extract of the leaves (Kam & Sim, 1998). We have now identified the active principles responsible for this activity.

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2. Results and discussion

The ethanol extract of the leaves of *K. griffithii* furnished the following alkaloids: harmane **1**, harmicine **2**, kopsilongine **3**, kopsamine **4**, kopsamine-*N*(4)-oxide **5**, pleiocarpine **6**, 12-methoxypleiocarpine **7**, leuconolam **8**, leuconoxine **9**, kopsinine **10**, (+)-eburnamonine **11**, *N*(1)-methoxycarbonyl-12-methoxy-Δ-^{16,17}-kopsinine **12**, *N*-carbomethoxy-11-hydroxy-12-methoxykopsinaline **13**, *N*-carbomethoxy-11,12-dimethoxykopsinaline **14**, tetrahydroalstonine **15**, 12-methoxy-10-demethoxykopsidasinine **16**, deacetylakuammiline (rhazimol) **17**, 16-(*R*)-19,20-*E*-isositsirikine **18** and buchtienine **19** (Kam & Sim, 1998). From the stem-bark extract, a total of 13 alkaloids were isolated including **1**, **8**, **9**, **10**, **17**, **19**, (−)-eburnamine **20** (Kam, Tan, & Chuah, 1992; Kam, Tan, & Chen, 1993), kopsinine *N*(4)-oxide **21** (Thomas, Achenbach, & Biemann, 1966), 16-*epi*-deacetylakuammiline **22** (Kan, Deverre, Sevenet, Quirion, & Husson, 1995), rhazinaline *N*(4)-oxide **23** (Abe & Yamauchi, 1993), akuammiline *N*(4)-oxide **24**, 16-*epi*-deacetylakuammiline *N*(4)-oxide **25** and 11,12-methylenedioxykopsinaline *N*(4)-oxide **26**; the last three are new alkaloids. Harmane **1** and buchtienine **19** are the major alkaloids in the leaves, while the major alkaloids in the stem-bark are deacetylakuammiline **17** and kop-

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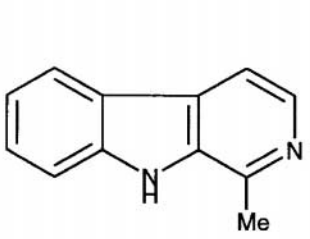

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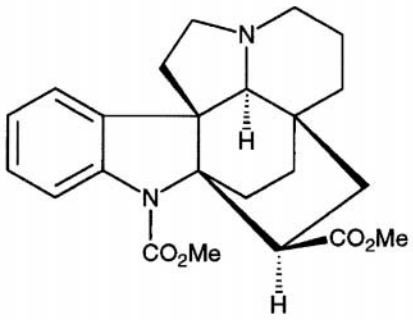

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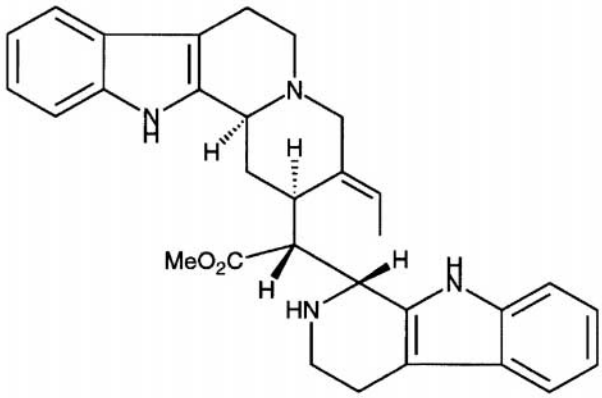

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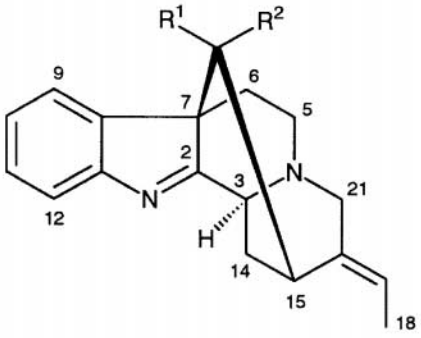
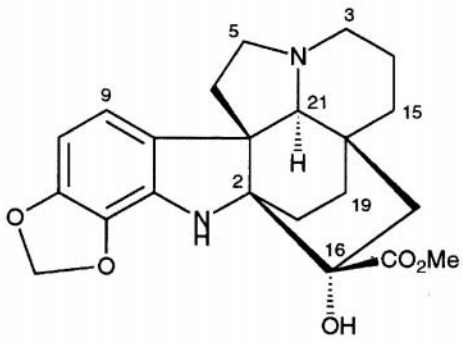


24 $R^1 = CH_2OAc$, $R^2 = CO_2Me$, *N*(4)→O

25 $R^1 = CO_2Me$, $R^2 = CH_2OH$, *N*(4)→O



26 *N*(4)→O

sinine *N*(4)-oxide **21**. Compounds **24–26** are readily deduced to be the *N*(4)-oxides of akuammiline (Dugan, Hesse, Renner, & Schimd, 1969), 16-*epi*-deacetylakuammiline (Kan et al., 1995) and 11,12-methylenedioxykopsinaline **27** (Feng, Kan, Potier, Kan, & Lounasmaa, 1983), respectively, from the ^1H and ^{13}C NMR spectral data (Tables 1 and 2), in particular the characteristic downfield shifts of the C-3, -5 and -21 resonances when compared with the parent compounds (Table 2). *Kopsia griffithii* is notable for furnishing a remarkable array of alkaloidal types which include, simple *β*-carbolines (**1**, **2**), corynantheine alkaloids (**15**, **18**), eburnane alkaloids (**11**, **20**), sarpagine alkaloids (**17**, **22**, **23**, **24**, **25**), aspidosperma alkaloids (**8**, **9**), aspidofractinine alkaloids (**3**, **4**, **5**, **6**, **7**, **10**, **12**, **13**, **14**, **16**, **21**, **26**) and the quasidimer, buchtienine

Table 1
^{1}H NMR spectral data for compounds (**22**, **24**–**26**) (400 MHz, $CDCl_3$)

H	**24**	**22**	**25**	**26**
3	5.05 d (4.5)	4.87 d (4.5)	5.07 br s	3.57 m
3	–	–	–	3.93 m
5	3.17 m	2.79 m	3.13 m	3.57 m
5	3.42 m	3.02 m	3.58 br dd (13, 5)	3.93 m
6	2.12 br dd (15, 5)	1.97 br dd (15, 5)	1.93 br dd (15, 5)	2.35 m
6	3.42 m	2.79 m	2.86 m	2.47 br dd (15, 8)
9	7.62 dd (7.5, 1)	7.72 br d (7.5)	7.76 br d (7.5)	7.50 d (8)
10	7.24 td (7.5, 1)	7.18 td (7.5, 1)	7.22 td (7.5, 1)	6.15 d (8)
11	7.37 td (7.5, 1)	7.33 td (7.5, 1)	7.35 td (7.5, 1)	–
12	7.66 dd (7.5, 1)	7.62 br d (7.5)	7.64 br d (7.5)	–
14	2.17 m	2.44 ddd (14, 4.5, 2)	2.81 m	1.82 m
14	2.91 ddd (15, 4.5, 2.5)	2.79 m	2.81 m	2.94 m
15	3.57 br s	3.20 br s	3.38 br s	1.75 m
15	–	–	–	2.04 m
17	3.59 m	4.34 m	4.26 d (12)	1.26 d (15)
17	3.59 m	4.34 m	4.30 d (12)	3.16 dd (15, 2)
18	1.72 dd (7,2)	1.77 dd (7,2)	1.85 dd (7, 2)	1.45 m
18	–	–	–	1.67 m
19	5.73 q (7)	5.69 q (7)	5.88 q (7)	1.33 m
19	–	–	–	1.82 m
21	4.07 d (16)	3.33 d (17)	4.60 dd (17, 2)	3.62 br s
21	4.53 dd (16, 2)	4.30 dd (17, 2)	4.28 d (17)	–
CO_2Me	3.79 s	3.11 s	3.18 s	3.83 s
OCOMe	1.59 s	–	–	–
OCH_2O	–	–	–	5.51 d (1.5)
OCH_2O	–	–	–	5.71 d (1.5)
NH	–	–	–	5.29 br s

Table 2
^{13}C NMR spectral data for compounds (**22**, **24**–**27**) (100 MHz, $CDCl_3$)

C	**24**	**22**	**25**	**27**	**26**
2	178.4	189.5	178.4	71.9	71.1
3	77.1	54.8	77.2	47.9	64.7
5	69.0	51.2	67.5	50.7	64.8
6	27.6	33.5	25.2	35.6	32.2
7	56.2	57.3	55.2	58.5	58.7
8	129.5	137.9	128.5	130.9	131.2
9	125.8	125.2	125.5	114.4	117.7
10	126.7	125.4	126.5	101.1	100.1
11	129.0	128.0	128.6	147.4	147.4
12	122.2	120.6	121.6	137.9	134.0
13	155.0	155.9	155.1	133.2	131.4
14	28.7	32.0	29.6	17.4	19.6
15	34.5	34.5	31.8	26.7	25.0
16	58.0	62.3	62.3	76.9	75.6
17	64.8	64.2	62.7	40.9	40.0
18	13.7	14.4	14.9	36.1	33.7
19	125.1	121.6	126.8	33.5	34.0
20	143.0	145.4	144.3	33.0	34.7
21	71.8	52.8	70.6	67.9	84.5
CO_2Me	52.1	51.7	52.1	53.2	53.0
CO_2Me	170.5	173.6	171.8	174.8	175.1
OCOMe	19.9	–	–	–	–
OCOMe	169.0	–	–	–	–
OCH_2O	–	–	–	100.9	100.6

(**19**). Since antileishmanial activity was detected in preliminary screening, we have carried out bioassays on the alkaloids isolated in order to determine the compounds responsible for this activity. Only three compounds were found to show activity against *Leishmania donovani*, viz., harmane **1** and pleiocarpine **6** ($6.25 < IC_{50} < 25$ μg ml^{-1}), and the quasidimer, buchtienine **19** ($0.39 < IC_{50} < 1.56$ μg ml^{-1}), with the highest level of activity being shown by buchtienine **19**. This result, which identifies buchtienine as the principal compound responsible for the leishmanicidal activity, is in agreement with the recent isolation of buchtienine along with other indole alkaloids from the Bolivian plant *Peschiera buchtieni* (Syn. *Tabernaemontana buchtieni*), which is also used locally for the treatment of leishmaniasis (Azoug et al., 1995).

3. Experimental

3.1. Plant material

Details of the collection of plant material and the deposition of voucher specimens are given in Kam and Sim (1998).

3.2. Extraction and isolation

Extraction of alkaloids was carried out as described previously to obtain the crude alkaloidal mixt. from the stem-bark (Kam & Sim, 1998; Kam & Tan, 1990). Alkaloids were isolated by CC and centrifugal TLC on silica gel. The solvent system used for CC was $CHCl_3$–MeOH. 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 stem-bark were: **1** (0.01), **8** (0.01), **9** (0.003), **10** (0.002), **17** (0.02), **19** (0.001), **20** (0.001), **21** (0.027), **22** (0.0008), **23** (0.0008), **24** (0.002), **25** (0.002) and **26** (0.003).

3.3. Akuammiline N(4)-oxide **24**

$[\alpha]_D = -144^\circ$ ($CHCl_3$, *c* 0.13). UV (EtOH), λ_{max} (log ε) 222 (4.35), 270 (3.77). EIMS, *m/z* (rel. int.): 410 $[M]^+$ (17), 394 (100), 378 (42), 335 (30), 321 (70), 310 (92), 282 (57), 267 (47) and 168 (13). HREIMS, $[M]^+$, found 410.1848, calcd for $C_{23}H_{26}N_2O_5$, 410.1842. ^1H and ^{13}C NMR: Tables 1 and 2.

3.4. 16-Epideacetylakuammiline N(4)-oxide **25**

$[\alpha]_D = -66^\circ$ ($CHCl_3$, *c* 0.15). UV (EtOH), λ_{max} (log ε) 222 (4.12), 272 (3.57). EIMS, *m/z* (rel. int.): 368 $[M]^+$ (23), 352 (100), 321 (35), 293 (75), 168 (28); HREIMS, $[M]^+$, found 368.1739, calcd for $C_{21}H_{24}N_2O_4$, 368.1736. ^1H and ^{13}C NMR: Tables 1 and 2.

3.5. 11,12-Methylenedioxykopsinaline N(4)-oxide **26**

$[\alpha]_D = -16^\circ$ ($CHCl_3$, *c* 0.19). UV (EtOH), λ_{max} (log ε) 221 (4.35), 246 (3.77), 282 (2.97). EIMS, *m/z* (rel. int.): 414 $[M]^+$ (15), 398 (100), 370 (36), 339 (22), 124 (25) and 109 (23); HREIMS, $[M]^+$, found 414.1799, calcd for $C_{22}H_{26}N_2O_6$, 414.1791. ^1H and ^{13}C NMR: Tables 1 and 2.

3.6. Leishmanicidal assay (Roy, Bhattacharya, Siddiqui, & Bhadra, 1990)

Leishmania donovani (promastigote) was maintained in 199 medium supplemented with 10% foetal calf serum and kanamycin. In a 96 well round-bottom plate were placed 200 μl each of the cell suspension (10000 cells ml^{-1}), followed by the addition of 5 μl each of the test solns. The initial concn of the test soln was 25 μg ml^{-1} in a well and four different concns of each sample were examined for their growth rate. Cells were cultured for 3 days and then examined using the MTT staining method (Alley et al., 1988).

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