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LAHADININES A AND B, NEW CYANO-SUBSTITUTED INDOLE ALKALOIDS FROM *KOPSIA PAUCIFLORA*

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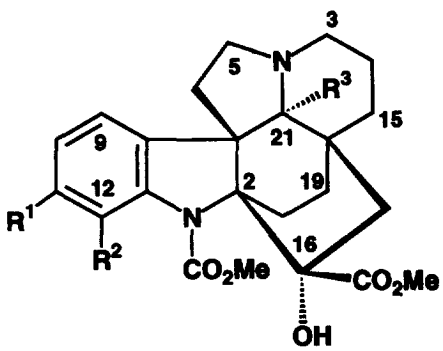
Key Word Index—*Kopsia pauciflora*; Apocynaceae; leaves; cyano-substituted indole alkaloids.

Abstract—Two new alkaloids of the aspidofractinine-type bearing cyano substituents at carbon-21 were obtained from *Kopsia pauciflora*, together with two other new alkaloids with 21-hydroxy-substitution. The structures of these alkaloids were deduced from their spectral data. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Kopsia* includes some 30 species found mostly in tropical Asia [1, 2]. There are *ca* 17 species which occur in Malaysia. The phytochemistry of this genus has been quite well investigated. Our own studies of this genus have resulted in several novel structures and some important bioactivities [3–6]. *Kopsia pauciflora* Hook f. is endemic to Sabah in North Borneo and in continuation of our studies of this species [7], we now report four new alkaloids from the leaf extract.



1 R^1, R^2 = -O-CH_2-O-, R^3 = CN

2 $R^1 = R^2$ = OMe, R^3 = CN

3 R^1, R^2 = -O-CH_2-O-, R^3 = OH

4 R^1, R^2 = -O-CH_2-O-, R^3 = OH, N(4)→O

5 R^1, R^2 = -O-CH_2-O-, R^3 = H

6 $R^1 = R^2$ = OMe, R^3 = H

RESULTS AND DISCUSSION

Lahadinine A (**1**) was obtained as a colourless oil. The UV spectrum showed absorption maxima at 227, 248, 255 (sh), 285 and 290 nm (log ε 4.36, 3.89, 3.84, 2.97 and 2.92, respectively) typical of a dihydroindole chromophore and is similar to that of other aspidofractinine-type compounds [8, 9]. The EI mass spectrum of **1** showed a $[M]^+$ at *m/z* 481, which was also the base peak, the odd mass indicating the presence of a third nitrogen ($C_{25}H_{27}N_3O_7$). This was further supported by the 25 separate carbon resonances in ^{13}C NMR, as well as the observation of the fragments at *m/z* 455 and 454 attributable to loss of CN and HCN, respectively, in addition to peaks at *m/z* 463 $[M-H_2O]^+$, 422 $[M-CO_2Me]^+$ and 394 $[M-CO_2Me-CH_2=CH_2]^+$. The IR spectrum showed a weak band at 2252 cm^{-1}. The ^1H and ^{13}C NMR spectral data (Tables 1 and 2) showed the presence of a methylenedioxy substituent at C-11 and C-12 (a pair of aromatic AB doublets at δ_H 6.57 and 6.99 and another pair at δ_H 5.87 and 5.93, δ_C 100.5), a urethane function at N_1 (δ_C 155.7), one hydroxyl group (16-OH, δ_H 6.94) and an ester group (δ_C 172.3). The spectra can be assigned with the aid of COSY and HETCOR and showed that **1** is an aspidofractinine-type alkaloid [8]. The NMR spectral data resembles those of the aspidofractinine derivative *N*-methoxycarbonyl-11,12-methylenedioxykopsinaline **5** [10, 11], which was also isolated from the stem extract of the same plant [7], except for the conspicuous absence of the H-21 signal and the appearance instead of an additional resonance in the ^{13}C NMR spectrum at δ_C 117.9. This resonance

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Table 1. ^{1}H NMR spectral data for compounds (**1–4**) (270 MHz, $CDCl_3$)*

H	1	2	3	4
3	3.05 *m*	3.04 *m*	2.81 *m*	3.21 *m*
3	3.05 *m*	3.04 *m*	3.15 *m*	3.51 *br d* (14)
5	2.94 *ddd* (9, 7, 2)	2.94 *ddd* (9, 7, 2)	3.01 *m*	3.31 *m*
5	3.07 *m*	3.06 *m*	3.15 *m*	3.77 *m*
6	1.51 *m*	1.60 *m*	1.65 *m*	2.13 *m*
6	1.93 *m*	2.00 *m*	2.05 *m*	2.13 *m*
9	6.99 *d* (8)	7.17 *d* (8)	6.75 *br s*	7.60 *d* (8)
10	6.57 *d* (8)	6.63 *d* (8)	6.54 *d* (8)	6.51 *d* (8)
14	1.43 *m*	1.45 *m*	1.25 *m*	1.71 *m*
14	1.72 *m*	1.74 *m*	1.76 *m*	1.71 *m*
15	1.60 *m*	1.62 *m*	1.46 *m*	1.56 *br d* (12)
15	1.60 *m*	1.62 *m*	1.70 *m*	1.65 *m*
17	1.49 *br d* (15)	1.51 *br d* (15)	1.56 *br d* (15)	1.46 *br d* (15)
17	3.05 *m*	3.04 *m*	2.95 *br d* (15)	2.87 *br d* (15)
18	1.80 *m*	1.86 *m*	1.67 *m*	1.67 *m*
18	2.37 *m*	2.40 *m*	2.29 *m*	2.26 *m*
19	1.51 *m*	1.60 *m*	1.65 *m*	1.66 *m*
19	1.89 *m*	1.94 *m*	1.65 *m*	1.66 *m*
16-OH	6.94 *s*	6.60 *s*	6.98 *s*	7.23 *s*
OCH_2O	5.93 *d* (1.5)	—	5.93 *d* (1.5)	5.93 *d* (1.5)
OCH_2O	5.87 *d* (1.5)	—	5.87 *d* (1.5)	5.85 *d* (1.5)
NCO_2Me	3.87 *s*	3.89 *s*	3.88 *s*	3.87 *s*
CO_2Me	3.73 *s*	3.75 *s*	3.75 *s*	3.76 *s*
11-OMe	—	3.75 *s*	—	—
12-OMe	—	3.82 *s*	—	—

* Assignments based on COSY and HETCOR.

Table 2. ^{13}C NMR spectral data for compounds (**1–4**) (67.5 MHz, $CDCl_3$)*

C	1	2	3	4
2	73.7	73.9	74.6	73.6
3	44.2	44.4	43.0	62.2
5	49.0	49.2	47.7	60.2
6	39.0	39.3	36.6	33.4
7	59.8	59.8	59.7	58.8
8	130.7	133.8	131.9	129.7
9	116.8	117.9	116.3	120.1
10	103.6	107.1	103.8	104.1
11	149.0	153.7	148.4	148.4
12	134.3	138.0	134.8	133.5
13	123.2	133.8	124.1	123.9
14	19.9	20.4	17.4	18.5
15	32.4	32.5	30.3	29.7
16	74.3	75.4	75.2	74.7
17	39.1	39.5	41.8	43.1
18	23.9	24.9	23.8	22.6
19	28.4	28.4	28.9	28.9
20	36.6	36.7	36.4	36.9
21	68.4	68.5	90.6	103.2
NCO_2Me	53.3	53.5	53.2	53.2
CO_2Me	52.5	52.5	52.5	52.8
$\underline{C}O_2Me$	172.3	172.6	173.0	172.8
$N\underline{C}O_2Me$	155.7	157.1	155.9	156.1
$O\underline{C}H_2O$	100.5	—	100.3	100.2
11-OMe	—	60.2	—	—
12-OMe	—	56.1	—	—
CN	117.9	118.6	—	—

* Assignments based on HETCOR and HMBC.

is due to a quaternary carbon and can be attributed to a cyano group [12] (the molecular formula of **1** shows that it differs from **5** by replacement of H by CN). The cyano substituent can *a priori* be placed on either of the two quaternary centres, 21 or 16. Placement of the OH at position 16 is consistent with the NMR data and is in agreement with the many aspidofractinine compounds reported, which have the same substitution pattern and stereochemistry at position 16 of the aspidofractinine skeleton. Furthermore, the assignment of the carbon-21 resonance is confirmed by HMBC; from the observed ^3J interactions between C-21 and H-5, H-17 and H-19. The observed chemical shift of carbon-21 (δ_C 68.4) firmly rules out the alternative placement of the hydroxyl substituent on C-21 and the cyano substituent on C-16, especially since the 21-OH substituted compound was also obtained (compound **3**, *vide infra*) where the 21-carbon resonance was observed at δ 90.6. Based on the above considerations, lahadinine A is therefore the 21-cyano derivative of **5**. Similarly, inspection of the spectral data and comparison with compound **6** [10, 11], which also occurs in the leaf extract, revealed lahadinine B **2** to be the 11,12-dimethoxy congenor of compound **1**. In addition to the cyano-substituted compounds **1** and **2**, two other related alkaloids were also obtained, *viz.*, paucifinine (**3**) and paucifinine *N*-oxide (**4**). Paucifinine is similar in all respects to **1** except for replacement of the 21-cyano group by a hydroxyl group. This is supported by its molecular

formula (EI mass spectrum, *m/z* 472, $C_{24}H_{28}N_2O_8$) and the NMR data (Tables 1 and 2), in particular the presence of an oxygenated quaternary carbon attributable to C-21 (δ_C 90.6). The identification of compound **4** as the *N*-oxide of **3** is based on the characteristic downfield shifts of the carbon resonances for C-3, C-5 and C-21 when compared with those of **3**. Further confirmation is also provided by the ready oxidation (*m*CPBA) of **3** to give **4**. The stereochemistry of the 21-substituent in all four compounds (**1**–**4**) is assumed to be α, in common with the stereochemistry at position 21 of the parent (unsubstituted) compounds **5** and **6** (21-αH), which also occur in the same plant [7], as well as with the many other aspidofractinine alkaloids occurring in *Kopsia*, assuming they share a common biogenetic origin [7–13]. The isolation of the rare cyano-substituted alkaloids **1** and **2** represents the first isolation of this class of compounds in *Kopsia*, although a cyano-substituted indole alkaloid has been previously reported from *Alstonia angustiloba* [12].

EXPERIMENTAL

Plant material. Plant material was collected in Sabah, Malaysia. Herbarium voucher specimens are deposited at the Herbarium of the Sabah Forest Department, Sandakan, Sabah, Malaysia.

Extraction and isolation. Extraction of alkaloids was carried out in the usual manner, as described in detail elsewhere [9]. 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–hexane (1:1), Et_2O–EtOAc (10:1) and MeOH–$CHCl_3$ (1:9). The yields (g kg^{-1}) of the alkaloids from the leaf extract were: **1** (0.009), **2** (0.006), **3** (0.011) **4** (0.017), **5** (0.019) and **6** (0.009).

Lahadinine A (**1**). $[\alpha]_D$ −184° ($CHCl_3$, *c* 0.15). UV (EtOH), λ_{max} nm (log ε): 227 (4.36), 248 (3.89), 255 (3.84), 285 (2.97), 290 (2.92). IR (dry film) ν_{max} cm^{-1} 3303, 2252, 1737, 1686. EIMS *m/z* (rel. int.): 481 [M^+ $C_{25}H_{27}N_3O_7$] (100), 463 (15), 455 (10), 454 (10), 422 (40), 394 (20), 379 (10), 352 (75) 322 (10). ^1H and ^{13}C NMR: Tables 1 and 2.

Lahadinine B (**2**). $[\alpha]_D$ −123°C ($CHCl_3$, *c* 0.03). UV (EtOH), λ_{max} nm (log ε) 222 (4.39), 253 (3.79), 284 (3.06), 289 (3.03). IR (dry film) ν_{max} cm^{-1}: 3327, 2252, 1737, 1678. EIMS *m/z* (rel. int.): 497 [M^+, $C_{26}H_{31}N_3O_7$] (100), 482 (50), 471 (5), 470 (5), 467 (10), 438 (25), 410 (20), 395 (15), 380 (15), 368 (40). ^1H and ^{13}C NMR: Tables 1 and 2.

Paucifinine (**3**). $[\alpha]_D$ −91° ($CHCl_3$, *c* 0.08). UV (EtOH), λ_{max} nm (log ε): 227 (4.51), 248 (4.08), 255 (4.03), 285 (3.13), 290 (3.09). IR (dry film) ν_{max} cm^{-1}: 3302, 1733, 1681. EIMS *m/z* (rel. int.): 472 [M^+, $C_{24}H_{28}N_2O_8$] (35), 454 (100), 446 (10), 413 (10), 395 (10), 364 (15), 351 (10), 323 (8), 262 (10), 246 (6), 124 (4), 109 (5). ^1H and ^{13}C NMR: Tables 1 and 2.

Paucifinine-N-*oxide* (**4**). $[\alpha]_D$ −36° ($CHCl_3$, *c* 0.30). UV (EtOH), λ_{max} nm (log ε): 227 (4.50), 248 (4.06), 255 (3.97), 285 (3.21), 290 (3.17). IR (dry film) ν_{max} cm^{-1} 3291, 1733, 1681. EIMS *m/z* (rel. int.): 488 [M^+, $C_{24}H_{28}N_2O_9$] (25), 470 (100), 454 (70), 428 (10), 411 (35), 395 (15), 369 (10), 341 (10), 246 (15), 124 (5) 109 (5). ^1H and ^{13}C NMR: Tables 1 and 2.

Oxidation of **3** to **4**. mCPBA (2 mg, 0.011 mmol) was added to a stirred soln of **3** (5 mg, 0.01 mmol) in CH_2Cl_2 (10 ml) at 4°. After *ca* 10 min, satd Na_2CO_3 soln (10 ml) was added and the mixt. extracted with CH_2Cl_2 (3 × 10 ml). The extract was then dried (Na_2SO_4) and the solvent evapd off. Centrifugal chromatography over silica gel (MeOH–$CHCl_3$) afforded 3 mg (58%) of **4**.

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