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Pauciflorines A and B, Novel Melanin Biosynthesis Inhibitors from *Kopsia*

Toh-Seok Kam*, K. Yoganathan, Takashi Koyano[a] and Kanki Komiyama[b]

Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia.
[a]Terumo Corporation, Ashigarakami-gun, Kanagawa 259-01 Japan.
[b]The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108.

Abstract *: Two new pentacyclic indoles viz., Pauciflorines A and B from Kopsia pauciflora were found to exhibit potent inhibitory activity on melanin biosynthesis of cultutred B-16 melanoma cells.*
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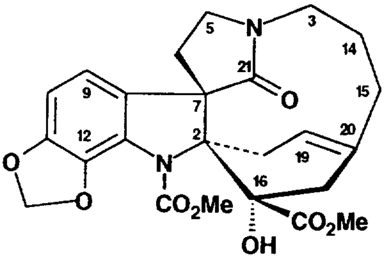
The Malaysian members of the genus *Kopsia* have yielded a prodigious harvest of new natural products possessing novel carbon skeletons as well as useful bioactivities[1-3]. We would like to report the structure and novel bioactivity of two new indoles *viz.*, pauciflorine A **1** and B **2**, isolated from the ethanol extract of the leaves of *Kopsia pauciflora* Hook. f. (Apocynaceae), a species native to North Borneo, which have been found to be potent inhibitors of melanin biosynthesis.

Pauciflorine A[4] and pauciflorine B[5] were obtained from the leaves of *Kopsia pauciflora* in yields of 0.22 g Kg^{-1} and 0.03 g Kg^{-1} respectively. Pauciflorine A **1**, was obtained in amorphous form, $[\alpha]_D$ - 50.7° ($CHCl_3$, *c* 2.0)[5]. The EIMS of pauciflorine A showed a molecular ion at *m/z* 470 ($C_{24}H_{26}N_2O_8$, 80%)) with other significant fragments at 452 (15%, M - H_2O), 438 (100%, M - OMe - H), 411 (20%, M - CO_2Me) and 394 (15%, M - CO_2Me - OH). The UV spectrum showed absorption maxima at 227, 248, 283 and 293 nm which indicate the presence of a dihydroindole chromophore. The ^{13}C NMR spectrum showed a total of 24 carbon resonances in agreement with the molecular formula deduced from the mass spectrum. The ^1H and ^{13}C NMR spectral data (Table 1) showed the presence of a methylenedioxy substituent at carbon-11 and 12 (a pair of aromatic AB doublets at δ_H 6.43 & 6.59 and another pair at δ_H 5.85, 5.97, δ_C 100.5), a CO_2Me substituent at N_1 (δ_C 156.9), an ester function at carbon-16 (δ_C 173.1), a hydroxyl function at carbon-16 (δ_H 8.43), a lactam carbonyl at carbon-21 (δ_C 174.9) and a trisubstituted double bond (δ_H 5.25; δ_C 121.6, 130.3). COSY and HMQC experiments revealed the presence of the following partial structures, *viz.*, an isolated methylene group, a CH_2-CH_2 unit, a CH_2-CH_2-CH_2 unit and a CH=C fragment. This information suggests that the lactam carbonyl should be placed at position 21 since the alternative location of the lactam carbonyl at position 3 or 5 would result in three isolated methylenes in the former case and two CH_2-CH_2 fragments in the latter which was clearly not the case. This would also account for the unusual deshielding observed for H-3α (δ_H 4.03) as a result of the anisotropy due to the proximate lactam carbonyl function. Finally the placement of the amide carbonyl at position 21 is also supported by the observed ^3J correlation between C-21 and H-6 in HMBC. The presence of a β-carbomethoxy function together with an α-OH substituent on carbon-16 is supported by the characteristic carbon NMR shifts which are characteristic of such a substitution pattern in related aspidofractinine-type alkaloids and furthermore this can be confirmed from the observed long range *W* coupling between the intramolecularly hydrogen-bonded 16-hydroxyl proton and H-17β as well as from the observed ^3J (C-17 to 16-OH) and ^2J (C-16 to 16-OH) interactions in HMBC. The molecular formula of pauciflorine yielded a DBE value of 13 and discounting the methylenedioxy function, suggests the presence of a pentacyclic ring system incorporating a trisubstituted double bond. HMBC and NOESY experiments allowed the molecule to be assembled as shown in 1. Key HMBC correlations include the following ^3J interactions : C-15 to H-19, C-20 to H-18, C-2 to H-19, C-7 to H-18 and C-16 to H-18. Key NOE interactions include the following : H17β-H5β, 16OH-H18β and H19-H3α. These observations provide a firm basis for the proposed structure of pauciflorine A and allow the assignment of pauciflorine B as the 11,12-dimethoxy congener of pauciflorine A[6].

Pauciflorines A and B inhibited melanin synthesis of B16 melanoma cells at a concentration of 13 and 25 μg ml^{-1} respectively without any cytotoxicity towards the cultured cells[7]. This level of inhibition is equivalent to that of the commercial compound, arbutin[8] and represents a rare example of melanin synthesis inhibition by indole compounds of plant origin.



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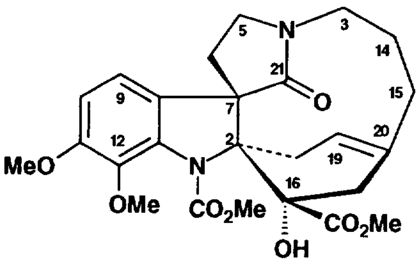

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Table 1. ^1H and ^{13}C NMR Spectral Data for **1** and **2**[a]

	1		**2**	
Position	δ_C	δ_H	δ_C	δ_H
2	82.1	-	83.2	-
3b	42.5	2.91 m	42.6	2.89 m
3a	-	4.03 br t (12.5)	-	4.01 br t (12.5)
5a	44.2	3.27 td (10, 2)	44.5	3.24 brt (10.5)
5b	-	3.66 m	-	3.62 m
6a	30.1	1.99 m	29.9	2.00 m
6b	-	2.61 ddd (16, 9, 2)	-	2.86 m
7	60.1	-	60.5	-
8	130.0	-	129.5	-
9	117.3	6.43 d (7.8)	119.2	6.64 d (8.3)
10	104.9	6.59 d (7.8)	108.8	6.67 d (8.3)
11	148.4	-	152.7	-
12	134.3	-	138.3	-
13	124.4	-	134.8	-
14a	21.5	1.51 br d (14.6)	21.7	1.50 br d (14.6)
14b	-	2.05 m	-	2.06 m
15a	35.7	2.24 m	35.7	2.22m
15b	-	2.24 m	-	2.22 m
16	75.3	-	75.4	-
17a	36.8	2.44 br d (19.5)	37.1	2.47 br d (19.5)
17b	-	2.85 br d (19.5)	-	2.84 br d (19.5)
18a	30.9	2.71 dd (16.6, 6.4)	32.1	2.67 dd (16.6, 6.4)
18b	-	3.07 d (16.6)	-	3.04 d (16.6)
19	121.6	5.25 d (6.4)	121.6	5.22 d (6.4)
20	130.3	-	130.2	-
21	174.9	-	175.3	-
16-OH	-	8.43 d (2)	-	8.03 d (2)
OCH_2O	100.5	5.97 d (1.5); 5.85 d (1.5)	-	-
$\mathrm{NCO_2\underline{Me}}$	53.5	3.88 s	53.8	3.84 s
$\mathrm{N\underline{C}O_2Me}$	156.9	-	158.4	-
$\mathrm{CO_2\underline{Me}}$	52.7	3.80 s	52.5	3.79 s
$\mathrm{\underline{C}O_2Me}$	173.1	-	173.2	-
11-OMe	-	-	60.2	3.70 s
12-OMe	-	-	56.1	3.84 s

[a] $CDCl_3$, 270 MHz; assignments based on COSY, HMQC, HMBC and NOESY.

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4. Compound **1**, Pauciflorine A, $[\alpha]_D$ - 50.7° ($CHCl_3$, *c* 2.0). EIMS, *m/z* (rel. int.) : 470 [M^+, $C_{24}H_{26}N_2O_8$] (80), 452 (15), 438 (100), 424 (10), 411 (20), 394 (15), 383 (20), 354 (10), 335 (15), 317 (10), 301 (80), 287 (22), 273 (30), 214 (25), 200 (15) and 109 (24). UV (EtOH), λ_{max} (log ε) 227 (4.62), 248 (4.17), 283 (3.14) and 293 (3.02). ^1H and ^{13}C NMR : see Table 1.
5. Compound **2**, Pauciflorine B, $[\alpha]_D$ = -25.0° ($CHCl_3$, *c* 0.4). EIMS, *m/z* (rel. int.) : 486 [M^+, $C_{25}H_{30}N_2O_8$] (60), 468 (5), 454 (100), 435 (20), 427 (10), 410 (10), 399 (10), 351 (10), 317 (70), 303 (15), 289 (10), 259 (10), 230 (20) and 109 (12). UV (EtOH), λ_{max} (log ε) 224 (4.49), 253 (3.83), 282 (3.04) and 290 (3.01). ^1H and ^{13}C NMR : see Table 1.
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