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NOTE

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Grandilodines A−C, Biologically Active Indole Alkaloids from *Kopsia grandifolia*

Wai-Sum Yap,[†] Chew-Yan Gan,[†] Yun-Yee Low,[†] Yeun-Mun Choo,[†] Tadahiro Etoh,[‡] Masahiko Hayashi,[‡] Kanki Komiyama,[§] and Toh-Seok Kam[*,†]

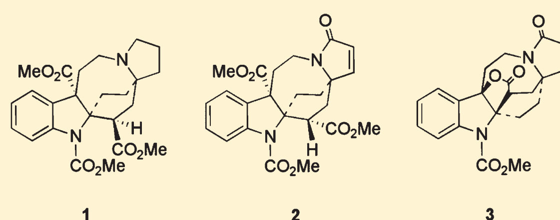
[†]Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia

[‡]Faculty of Pharmacy, Iwaki Meisei University, 5-5-1 Iino, Chuo-dai, Iwaki, Fukushima 970-8551, Japan

[§]Center for Basic Research, Kitasato University, 5-9-1, Shirokane, Minato-ku, Tokyo, 108-8642, Japan

Supporting Information

ABSTRACT: Three new indole alkaloids (**1**−**3**), named grandilodines A−C, and five known ones were obtained from the Malayan *Kopsia grandifolia*. The structures were established using NMR and MS analyses and, in the case of **1** and **2**, were confirmed by X-ray diffraction analyses. Alkaloids **1**, **3**, and lapidilectine B (**8**) were found to reverse multidrug resistance in vincristine-resistant KB cells.



The genus *Kopsia*, which is widely distributed in Southeast Asia,[1−3] is rich in alkaloids, and the Malaysian representatives in particular are fertile sources of novel alkaloids with unusual carbon skeletons and interesting biological activity.[3,4] In continuation of our studies on the Malaysian members of this genus,[3−17] we now report the structures of three new indole alkaloids (**1**−**3**), which we named grandilodines A−C, from *Kopsia grandifolia* D. J. Middleton (Apocynaceae).[2]

Grandilodine A (**1**) was initially obtained as a light yellowish oil, and subsequently as light yellowish block crystals from MeOH−CH_2Cl_2, mp 120−122 °C, $[\alpha]^{25}_{D}$ −76 (*c* 1.46, $CHCl_3$). The IR spectrum showed carbonyl bands due to ester (1731 cm^{-1}) and carbamate (1704 cm^{-1}) functions, and the UV spectrum had absorption maxima at 209, 253, and 289 nm, indicative of a dihydroindole chromophore. The ESIMS of **1** showed a quasi molecular ion at *m*/*z* 443, which analyzed for $C_{24}H_{30}N_2O_6$ + H. The ^{13}C NMR spectrum (Table 1) showed a total of 24 resonances, comprising three methyl, eight methylene, five methine, and eight quaternary carbon atoms, in agreement with the molecular formula. The quaternary carbon resonances at δ 174.3 and 173.0 were consistent with the presence of two ester groups, and the resonance at δ 154.0 was in agreement with the presence of a carbamate functionality. The ^{1}H NMR spectrum (Table 1) showed the presence of four aromatic hydrogens (δ 6.88−7.51) and three OCH_3 singlets (δ 2.94, 3.51, 3.91) associated with the methyl ester and carbamate groups. The relatively shielded OCH_3 signal associated with one of the ester functions (δ 2.94) suggested the possibility of anisotropy from the aromatic ring of the indole moiety. Furthermore, the ^{1}H NMR spectrum showed a general similarity to that of the lapidilectines (especially lapidilectine A **4**), the group of pentacyclic indoles characterized by the presence of a 1-azabicyclo[6.3.0]decane unit, previously isolated from *Kopsia*.[18,19] Comparison of the ^{1}H NMR spectrum of **1** with that of **4** showed that the main difference was the absence of the signals due to the 14,15-double bond seen at δ 5.49 and 5.71 in lapidilectine A.[19] These were replaced in the spectrum of **1** by signals due to an ethylene (CH_2CH_2) group (Table 1). The same was true of the ^{13}C NMR spectrum, where the signals due to an ethylene fragment (δ 22.1, 39.8) replaced signals due to the two olefinic carbons (δ 125.7, 138.4) in the spectrum of **4**.[19] These observations were also consistent with the 2-D NMR data, which showed the presence of a $NCH_2CH_2CH_2$ fragment in place of a $NCH_2CH{=}CH$. In the previous report,[18,19] the relative configurations at the various stereogenic centers in **4** were assigned on the basis of biogenetic reasoning by reference to venalstonine, as well as on the NOESY spectrum. Since **1** is a congener of **4**, we could conclude that the relative configuration of **1** is similar to that of **4** on the basis of the similarity of the NMR data. We were however uneasy with this assumption, since the ^{1}H NMR spectra of this group of compounds tended to show broadened signals, which render interpretation of the NOESY spectra difficult. In order to obtain unambiguous confirmation of the relative configuration of **4** (and hence **1**), suitable crystals of **1** were obtained, and X-ray diffraction analysis was carried out (Figure 1), which provided vindication of the original stereochemical assignment. In addition, catalytic hydrogenation of **4** gave a product identical ($[\alpha]^{25}_{D}$, ^{1}H and ^{13}C NMR, MS) to **1**.

Grandilodine B (**2**) was isolated as a white, amorphous solid and subsequently crystallized from CH_2Cl_2−hexanes as colorless block crystals, mp 204−206 °C, $[\alpha]^{25}_{D}$ +66 (*c* 0.39, $CHCl_3$).

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Table 1. ^{1}H and ^{13}C NMR Data (δ) for **1**−**3** (400 MHz, $CDCl_3$)[a]

position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	75.5		74.1		72.8	
3	56.3	2.73 m	172.8		171.6	
		2.99 m				
5	49.4	3.13 m	36.6	3.29 dd (15.4, 11.8)	34.9	3.15 dd (16, 12)
		3.13 m		4.46 dd (15.5, 5.5)		4.52 dd (16, 6)
6	28.8	2.78 t (5)	30.8	1.91 dd (16, 12)	29.2	2.25 m
		2.85 dd (15.4, 7.7)		3.15 dd (16, 6.5)		2.91dd (16, 6)
7	62.4		61.5		91.5	
8	131.7		131.8		126.9	
9	122.9	7.00 d (7.7)	123.3	7.18 dd (7.7, 1)	124.4	7.40 m
10	122.7	6.88 td (7.7, 1)	123.9	7.07 td (7.7, 1)	123.8	7.12 t (7.5)
11	128.8	7.14 td (7.7, 1)	129.5	7.28 td (7.7, 1)	132.0	7.40 m
12	115.4	7.51 br s	117.9	7.53 br d (7.7)	115.9	7.58 br s
13	144.0		143.0		142.0	
14	22.1	1.73 m	124.1	6.00 d (5.9)	124.9	6.07 d (5.9)
		1.73 m				
15	39.8	1.54 m	156.3	6.98 d (5.9)	155.1	6.82 d (5.9)
		1.70 m				
16	39.8	3.17 m	42.9	3.03 t (8.6)	39.6	3.97 m
17	32.8	1.85 dd (15, 10)	33.6	1.43 dd (14, 9)	31.4	2.02 d (15.4)
		2.40 m		2.75 dd (14.9, 9)		2.64 m
18	31.5	1.54 m	23.6	2.46 dd (14.9, 5.4)	21.2	1.91 m
		1.70 m		3.45 m		2.40 m
19	32.8	1.85 dd (15, 10)	23.5	1.67 m	28.7	1.60 m
		2.54 m		2.31 ddd (16, 8.6, 1.8)		2.23 m
20	60.3		62.8		64.2	
21	173.0		171.7		178.2	
21-O*Me*	51.9	3.51 s	52.6	3.57 s		
CO_2Me	174.3		172.9			
CO_2*Me*	51.7	2.94 s	52.1	3.52 s		
NCO_2Me	154.0		153.3		153.1	
NCO_2*Me*	52.4	3.91 s	52.7	3.77 s	52.8	3.90 s

[a] Assignments based on COSY, HMQC, and HMBC data.

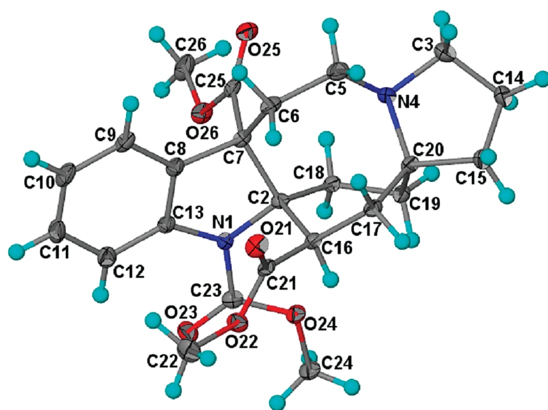
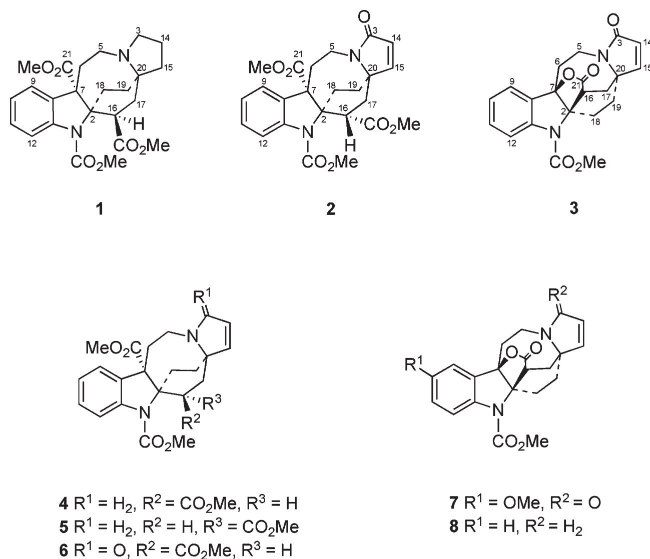


Figure 1. X-ray crystal structure of **1**. Thermal ellipsoids are shown at the 50% probability level.

The IR spectrum showed carbonyl bands due to ester (1734 cm^{-1}) and carbamate/lactam (1690 cm^{-1}) functions, and the UV spectrum showed dihydroindole absorption maxima at 210, 252, and 286 nm. The ESIMS of **2** showed a quasi molecular ion at m/z 455, which analyzed for $C_{24}H_{26}N_2O_7 + H$, indicating that **2** differed from **1** by the loss of four hydrogens and the addition of an oxygen atom. The ^{13}C NMR spectrum of **2** showed 24 resonances, comprising three methyl, five methylene, seven methine, and nine quaternary carbon atoms. The ^{13}C NMR spectrum (Table 1) of **2** differed from that of **1** by the presence of an additional lactam carbonyl at δ 172.8, in addition to the two esters and one carbamate signal at δ 171.7, 172.9, and 153.3, respectively. The ^{1}H NMR spectrum (Table 1) of **2** was also generally similar to that of **1** except for the signals due to H-3, H-14, and H-15. The signals due to H-3 in **1** were absent in **2**, having been replaced by a lactam carbonyl, while those due to the CH_2CH_2 unit corresponding to H-14 and H-15 were replaced by signals due to an olefinic CH═CH fragment (δ 6.00, 6.98; d, J = 5.9; δ_C 124.1, 156.3). The configuration at C-16 in **2** was deduced to be S on the basis of the NOESY spectrum, which showed a NOE between H-16 and H-6β. This assignment

receives additional support from consideration of the chemical shifts of the C-16 ester methyl signals.



Comparison of the chemical shifts of the C-16 ester methyl group for compounds **1**, **2**, **4**, **5**, and **6** showed that the C-16 ester methyl chemical shifts are of diagnostic significance for the assignment of C-16 configuration. When the C-16 methyl ester group is β-oriented, as in **1**, **4**, and **6**, the ester methyl signal was found at a somewhat shielded chemical shift value of ca. δ 3.0. In compounds **2** and **5**, with an α-oriented methyl ester group, the ester methyl signal was shifted downfield to the "normal" value of ca. δ 3.5, since the ester function is presumably no longer within the anisotropic influence of the aromatic ring. The same behavior of the C-16 ester methyl signal was previously noted in the ^{1}H NMR spectrum of lapidilectine versus that of isolapidilectine.[19] On the basis of these considerations, the C-16 ester methyl group in grandilodine B (**2**) was deduced to be α-oriented (16*S*). In any case, since we were able to obtain suitable crystals of grandilodine B (**2**), an X-ray diffraction analysis was carried out (Figure 2), which confirmed the structure and relative configuration deduced based on the spectroscopic data.

Grandilodine C (**3**) was obtained as a light yellowish oil, $[\alpha]^{25}_{D}$ +61 (*c* 0.55, $CHCl_3$). The IR spectrum showed carbonyl bands due to lactone (1772 cm^{-1}) and lactam/carbamate (1691 cm^{-1}) functions, and the UV spectrum showed dihydroindole absorption maxima at 209, 241, and 286 nm. The ESIMS of **3** showed a quasi molecular ion at *m*/*z* 381, which analyzed for $C_{21}H_{20}N_2O_5$ + H. The ^{13}C NMR spectrum of **3** (Table 1) showed 21 resonances, comprising one methyl, five methylene, seven methine, and eight quaternary carbon atoms. The ^{13}C NMR spectrum of **3** also confirmed the presence of lactone (δ 178.2), lactam (δ 171.6), and carbamate (δ 153.1) functionalities. The ^{1}H NMR spectrum (Table 1) showed the presence of four aromatic hydrogens (δ 7.40−7.58), one OCH_3 singlet (δ 3.90) associated with the carbamate group, and two olefinic hydrogens (δ 6.07, 6.82, d, J = 5.9 Hz). The COSY spectrum disclosed the following partial structures: NCH_2CH_2, CH_2CH_2, CH_2CH, and CH═CH. The NMR spectra were similar to those of **4** except for the loss of two methyl ester signals, the appearance of lactone (δ 178.2) and lactam carbonyl

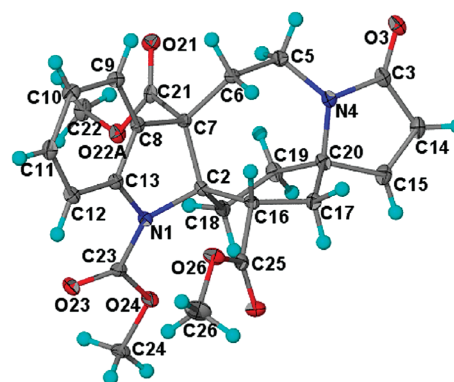


Figure 2. X-ray crystal structure of **2**. Thermal ellipsoids are shown at the 50% probability level.

(δ 171.6) resonances, and a downfield shift of the C-7 resonance to δ 91.5, indicating its attachment to the lactone oxygen. These features were reminiscent of alkaloids of the lapidilectine B[18] or tenuisine groups,[16] which are characterized by a hexacyclic dihydroindole skeleton incorporating a five-membered lactone ring and which were also found previously in *Kopsia*.[3] The NMR data were in fact similar to those of tenuisine C (**7**)[16] except for the aromatic region, where the 10-methoxy substituent of tenuisine C was absent in compound **3**. Grandilodine C (**3**) is therefore the 10-demethoxy derivative of tenuisine C, which is also in agreement with the HMBC data.

In addition to the above, the known alkaloids lapidilectine A (**4**), isolapidilectine A (**5**), lapidilectam (**6**), lapidilectine B (**8**), and kopsinine were also isolated, but venalstonine, lapidilectinol, and epilapidilectinol were not detected. Comparison of the results of the present study with those of the previous one carried out on a sample (under the old name *K. lapidilecta*) collected from the same locality but at a different date[19] revealed some differences that could be due to a seasonal dependence of the alkaloidal composition.

Grandilodine A (**1**), grandilodine C (**3**), and lapidilectine B (**8**) were found to reverse multidrug resistance in vincristine-resistant KB (VJ300) cells (IC_{50} 4.35, 4.11, and 0.39 μg/mL, respectively, in the presence of 0.1 μg/mL vincristine) without showing any cytotoxicity against both drug-sensitive and drug-resistant cells in the absence of added vincristine (IC_{50} > 25 μg/mL).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a hot stage Leitz-Wetzlar melting point apparatus and were uncorrected. Optical rotations were determined on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin-Elmer RX1 FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on JEOL JNM-LA 400 and JNM-ECA 400 spectrometers at 400 and 100 MHz, respectively. X-ray diffraction analyses were carried out on a Bruker SMART APEX II CCD area detector system equipped with a graphite monochromator and a Mo Kα fine-focus sealed tube (λ = 0.71073 Å), at 100 K. The structures were solved by direct methods (SHELXS-97) and refined with full-matrix least-squares on F^2 (SHELXL-97). ESIMS and HRESIMS were obtained on an Agilent 6530 Q-TOF mass spectrometer.

Plant Material. Samples of *K. grandifolia* were collected in Mersing, Malaysia, in May 1989 (GK401) and 1990 (GK541) and initially identified by L. E. Teo as *K. lapidilecta*. These samples were identical to that of KL3632, which was collected from the same location and deposited at the Herbarium (Chemistry Department) of the University of Malaya. The identification was subsequently revised to *K. grandifolia* following Middleton's revision of the genus.[2,3]

Extraction and Isolation. Extraction of the leaf and stem bark material and partitioning of the concentrated EtOH extracts with dilute acid were carried out as described in detail elsewhere.[20] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH, followed by rechromatography of the appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal preparative TLC were Et_2O−hexanes, EtOAc−hexanes, $CHCl_3$−hexanes, and $CHCl_3$−MeOH. Yields (g kg^{-1}) of the alkaloids from the stem-bark extract were as follows: **1** (0.0021), **2** (0.0007), **4** (0.0318), **5** (0.0190), **6** (0.0002), and kopsinine (0.0010). The yields (g kg^{-1}) of alkaloids from the leaf extract were as follows: **3** (0.0160) and **8** (0.4040).

Grandilodine A (1): light yellowish oil and subsequently light yellowish block crystals from MeOH−CH_2Cl_2; mp 120−122 °C; $[\alpha]^{25}_D$ −76 (*c* 1.46, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (3.04), 253 (2.71), 289 (2.13) nm; IR (dry film) ν_{max} 1731, 1704 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 443 $[M + H]^+$; HRESIMS *m/z* 443.2188 (calcd for $C_{24}H_{30}N_2O_6$ + H, 443.2182).

Grandilodine B (2): white, amorphous solid and subsequently colorless block crystals from CH_2Cl_2−hexanes; mp 204−206 °C; $[\alpha]^{25}_D$ +66 (*c* 0.39, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (3.80), 252 (3.48), 286 (2.78) nm; IR (dry film) ν_{max} 1734, 1690 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 455 $[M + H]^+$; HRESIMS *m/z* 455.1836 (calcd for $C_{24}H_{26}N_2O_7$ + H, 455.1813).

Grandilodine C (3): light yellowish oil; $[\alpha]^{25}_D$ +61 (*c* 0.55, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 209 (4.08), 241 (3.99), 286 (3.25) nm; IR (dry film) ν_{max} 1772, 1691 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 381 $[M + H]^+$; HRESIMS *m/z* 381.1454 (calcd for $C_{21}H_{20}N_2O_5$ + H, 381.1445).

Catalytic Hydrogenation of Lapidilectine A (4). Lapidilectine A (**4**) (15.3 mg, 0.035 mmol) was dissolved in MeOH (5 mL) and then stirred over 10% Pd/C (3 mg) under hydrogen atmosphere at room temperature for 1 h. The catalyst was removed by filtration over Celite. Evaporation of the solvent in vacuo, followed by chromatography of the resulting residue (silica gel, $CHCl_3$−MeOH), provided **1** (10.6 mg, 69%).

Crystallographic data of grandilodine A (1): light yellowish block crystals, $C_{24}H_{30}N_2O_6$, M_r = 442.50, orthorhombic, space group $P2_12_12_1$, a = 8.0067(2) Å, b = 11.2455(3) Å, c = 24.1247(7) Å, V = 2172.17(10) $Å^3$, T = 100 K, Z = 4, D_{calcd} = 1.353 g cm^{-3}, crystal size 0.15 × 0.20 × 0.61 mm^3, $F(000)$ = 944. The final R_1 value is 0.0465 (wR_2 = 0.1028) for 2913 reflections [$I > 2\sigma(I)$].

Crystallographic Data of Grandilodine B (2). colorless block crystals, $C_{24}H_{26}N_2O_7$, M_r = 454.47, monoclinic, space group $P2_1$, a = 8.6505(2) Å, b = 8.0985(2) Å, c = 15.3926(3) Å, $\alpha = \gamma$ = 90°, β = 90.057(10)°, V = 1078.34(5) $Å^3$, T = 100 K, Z = 2, D_{calcd} = 1.400 gcm^{-3}, crystal size 0.08 × 0.28 × 0.47 mm^3, $F(000)$ = 480. The final R_1 value is 0.0335 (wR_2 = 0.0790) for 2351 reflections [$I > 2\sigma(I)$].

Crystallographic data for the structures **1** and **2** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 794391 and 794392, respectively). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that was described in detail previously.[16,21]

ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra for compounds **1**−**3** and X-ray crystallographic data in CIF format for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel: +603-79674266. Fax: +603-79674193. E-mail: tskam@um.edu.my.

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