Biologically Active Indole Alkaloids from *Kopsia arborea*

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Received May 11, 2007

Nine new indole alkaloids, rhazinoline (**1**), 19(*S*)-methoxytubotaiwine (**2**), 19(*R*)-methoxytubotaiwine (**3**), kopsamidine A (**4**), kopsamidine B (**5**), kopsinidine A (**6**), kopsinidine B (**7**), paucidactine C (**8**), and pericine *N*-oxide (**9**), in addition to several recently reported novel indoles and 34 other known ones, were obtained from the stem-bark extract of the Malayan *Kopsia arborea*. The structures were determined using NMR and MS analysis. Valparicine (**12**) showed pronounced cytotoxic effects against KB and Jurkat cells (IC_{50} 13.0 and 0.91 μM, respectively).

The genus *Kopsia* (Apocynaceae) comprises some 23 species of shrubs and trees distributed mainly over Southeast Asia, India, and China, of which about 16 species occur in Malaysia.[1] Plants belonging to this genus are prolific producers of a wide variety of indole alkaloids, including many with intriguing carbon skeletons as well as interesting biological activities.[2−16] In continuation of our ongoing studies of these plants, we wish to report the full alkaloidal composition of the stem-bark extract of *Kopsia arborea* Blume (an ornamental *Kopsia*, widely cultivated in gardens in Malaysia), including the isolation, structure determination, and biological activity of several new alkaloids.

Results and Discussion

Rhazinoline (**1**) was obtained as a colorless oil, $[\alpha]_D$ +136 (*c* 0.07, $CHCl_3$). The UV spectrum showed absorption maxima at 211 and 260 nm, characteristic of an indolenine chromophore, which was confirmed by the observed carbon resonance at δ 187.3 due to the imine carbon. The IR spectrum indicated the presence of an aldehyde function (1718 cm^{-1}). The EIMS spectrum showed a molecular ion at *m*/*z* 292, which was also the base peak, with other significant fragment ions observed at *m*/*z* 277 (M − CH_3) and 263 (M − CHO). HREIMS measurements gave the molecular formula $C_{19}H_{20}N_2O$. The ^{13}C NMR spectrum (Table 1) showed a total of 19 carbon signals (one methyl, four methylenes, eight methines, and six quaternary carbons). The methine signal observed at δ 200.6 was readily attributed to an aldehyde carbonyl carbon. The ^1H NMR spectrum (Table 2) of **1** showed the presence of an unsubstituted aromatic moiety and an ethylidene side chain. In addition, signals observed at δ 4.82 (d, J = 5.1 Hz), 3.31 (br s), and 4.13 (br d, J = 16.4 Hz), corresponding to H-3, H-15, and H-21β, respectively, are characteristic of an akuammiline-type alkaloid.[17] The COSY and HMQC spectral data also disclosed partial structures that are reminiscent of an akuammiline skeleton, except that the $NCHCH_2$-CH partial structure, corresponding to C(3)−C(14)−C(15), has been extended to include a CHCHO fragment, corresponding to C(16)−C(17). These observations revealed the similarity of rhazinoline to the akuammiline-type alkaloid strictalamine, previously reported from *Rhazya stricta*.[18] However, the *W*-coupling observed between H-14α and H-16 requires the stereochemistry at C-16 to be assigned as *S*, thus indicating that compound **1** is the C-16-epimer of strictalamine.[18] This was further supported by the NOEs observed between H-6β/H-16, H-16/H-18, and H-14β/H-17. The latter NOE also indicated that the formyl H-17 is located within the shielding zone of the imine double bond, which accounted for the unusually shielded low-field resonance observed for the formyl H-17 at δ 8.60.

Compound **2** was obtained as a colorless oil, $[\alpha]_D$ +429 (*c* 0.24, $CHCl_3$). The UV spectrum showed absorption maxima characteristic of a β-anilinoacrylate chromophore (229, 297, and 327 nm), while the IR spectrum showed absorption bands due to NH (3360 cm^{-1}) and conjugated ester (1674 cm^{-1}) functions. The EIMS of **2** showed a molecular ion at *m*/*z* 354, and HRMS measurements gave the molecular formula $C_{21}H_{26}N_2O_3$. Other major fragments were observed at *m*/*z* 339 (M − CH_3), 323 (M − OCH_3), and 295 (M − CO_2Me). The ^1H NMR spectrum (Table 2) showed the presence of an unsubstituted aromatic moiety, an indolic NH, and two methyl groups at δ 2.99 and 3.78, corresponding to OMe and CO_2Me, respectively. The ^{13}C NMR spectrum (Table 1) showed a total of 21 carbon signals (three methyls, four methylenes, eight methines, and six quaternary carbons). The signals at δ 96.3 and 169.7 were readily assigned to C-16 and C-2, respectively, of the β-anilinoacrylate chromophore. The NMR data of **2** showed a remarkable resemblance to those of lagunamine {19(*S*)-hydroxytubotaiwine},[19−21] except for the replacement of the C-20-hydroxyethyl side chain by a methoxyethyl group. Compound **2** is therefore 19(*S*)-methoxytubotaiwine. The large coupling constant (J = 10 Hz) observed between H-19 and H-20 suggested that the conformation adopted about the C(19)−C(20) bond is one that places the two hydrogens at C-19 and C-20 *anti* to one another. The preferred *anti* conformation was due to the presence of both the OMe and Me groups at C-19, which apparently causes steric hindrance to free rotation about the C(19)−C(20) bond. This was further supported by the observed NOEs between H-18/H-21 and 19-OMe/H-15 and the absence of NOEs between H-18/H-15 and 19-OMe/H-21 (Figure 1). Another noteworthy observation is that the chemical shift of H-15 in **2** was relatively deshielded (δ 3.50) compared to the chemical shift of H-15 in the 19(*R*)-epimer **3** (δ 3.13) (*vide infra*). This is attributed to paramagnetic deshielding caused by the proximity of the OMe oxygen atom to H-15, which is only possible if rotation about the C(19)−C(20) bond is indeed restricted (Figure 1).

Compound **3**, 19(*R*)-methoxytubotaiwine, was obtained as a colorless oil, $[\alpha]_D$ +442 (*c* 0.14, $CHCl_3$). The UV and IR spectra were similar to those of **2**, suggesting a tubotaiwine-type compound with similar functionalities. The EIMS showed a molecular ion at *m*/*z* 354, and HRMS measurements established the molecular formula as $C_{21}H_{26}N_2O_3$, indicating that compound **3** is isomeric with **2**. The ^1H and ^{13}C NMR data of **3** (Tables 2 and 1, respectively) are similar in all respects to those of **2** except for the chemical shifts of H-15, H-21, and 19-OMe in the ^1H NMR spectrum. This suggested that **3** is the C-19-epimer of **2**. The 19(*R*) configuration

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10.1021/np0702234 CCC: $37.00
Published on Web 08/01/2007

Table 1. ^{13}C NMR Data (δ) for **1**−**9** (100 MHz, $CDCl_3$)[a]

C	1	2	3	4	5	6	7	8	9
2	187.3	169.7	170.9	72.3	66.7	71.7	77.2	70.8	135.5
3	55.2	45.2	44.9	49.5	40.9	46.6	46.5	40.5	66.2
5	51.5	53.7	53.6	49.6	51.3	52.8	53.9	166.7	74.9
6	38.7	44.2	43.2	36.8	34.4	53.5	52.6	82.9	22.6
7	53.9	54.8	54.7	56.6	58.0	60.7	60.3	48.5	108.3
8	137.8	136.0	136.2	137.2	141.3	130.0	131.8	129.9	132.3
9	122.5	119.8	120.3	113.8	121.9	115.4	116.9	115.7	118.1
10	126.5	121.1	121.2	100.6	120.0	100.0	109.6	109.1	120.2
11	128.9	127.4	127.3	147.5	126.7	148.0	153.1	154.4	123.2
12	121.7	109.8	109.7	133.8	110.6	132.5	140.5	140.4	110.8
13	155.2	143.8	143.5	130.6	148.9	132.0	134.6	133.7	135.5
14	32.3	27.5	28.0	126.9	18.6	15.0	15.4	19.7	26.6
15	30.6	27.6	28.9	133.0	80.8	33.6	34.9	33.5	40.4
16	62.0	96.3	95.5	76.1	43.7	81.5	81.9	74.9	142.0
17	200.6			40.0	32.2	42.1	43.3	39.3	
18	12.7	16.5	16.0	26.7	33.4	20.8	20.5	21.9	13.7
19	120.9	74.8	75.5	28.9	27.3	35.9	31.9	29.9	127.1
20	143.3	46.4	45.5	36.8	36.1	33.5	33.1	33.7	128.1
21	53.5	62.6	62.0	66.9	63.3	69.7	69.6	64.6	71.9
22						215.4	213.9	169.4	119.0
CO_2*Me*		51.1	51.2	52.7	52.1				
CO_2Me		168.9	168.3	174.4	174.7				
NCO_2*Me*							53.9	54.0	
N*C*O_2Me							156.2	154.6	
OCH_2O				100.7		100.7			
11-OMe							56.3	56.2	
12-OMe							59.9	60.0	
15-OMe					56.9				
19-OMe		56.4	55.5						

[a] Assignments based on HMQC and HMBC.

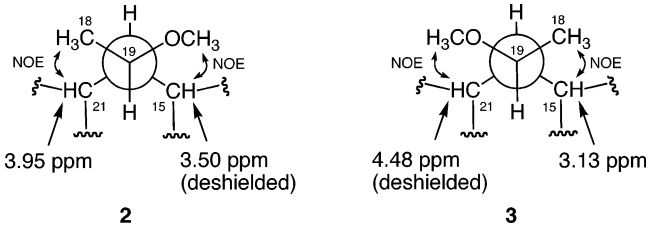
Table 2. ^1H NMR Data (δ) for **1**−**3**, **8**, and **9** (400 MHz, $CDCl_3$)[a]

H	1	2	3	8	9
3	4.82 d (5.1)	2.55 m	2.59 td (12.2, 5.6)	2.86 dt (13, 3)	3.65 dd (14.1, 7)
3′		3.10 m	3.17 m	4.28 dd (13, 4)	3.90 m
5	2.79 dd (14, 5.1) (α)	2.90 m	2.90 m		3.72 m
5′	2.85 dd (14, 6.3) (*β*)	3.13 m	3.22 m		3.86 m
6	2.10 dd (13.9, 5.1) (*β*)	1.89 m	1.96 m	4.75 br s	3.10 br d (14)
6′	3.11 td (13.9, 6.3) (α)	2.92 m	2.93 m		3.84 m
9	7.37 d (7.3)	7.16 d (7.5)	7.23 d (7.6)	6.80 d (8.2)	7.42 d (8)
10	7.25 td (7.6, 1.0)	6.90 td (7.5, 1.0)	6.93 t (7.6)	6.69 d (8.2)	7.13 ddd (8, 7.1, 1.0)
11	7.38 td (7.6, 1.2)	7.14 td (7.8, 1.2)	7.13 td (7.6, 1.0)		7.22 ddd (8, 7.1, 1.0)
12	7.68 d (7.8)	6.83 d (7.8)	6.83 d (7.8)		7.37 d (8)
14	1.94 dd (14.5, 2.9) (*β*)	1.85 m	1.86 m	1.47 m	1.71 m
14′	2.61 ddt (14.5, 5.1, 2) (α)	1.85 m	1.86 m	1.47 m	2.62 tt (14.1, 7)
15	3.31 br s	3.50 br s	3.13 br s	1.35 m	3.94 m
15′				1.60 m	
16	3.20 dt (3.5, 2)				
17	8.60 d (3.5)			1.65 d (15) (α)	
17				2.33 dd (15, 2) (*β*)	
18	1.77 dd (6.8, 2.4)	0.96 d (6.1)	0.91 d (5.8)	1.60 m	1.75 ddd (7.1, 2.4, 1.7)
18′				2.51 ddd (13, 12, 2)	
19	5.53 q (6.8)	2.52 dq (10, 6.1)	2.43 dq (10, 5.8)	1.27 m	5.95 br q (7.1)
19′				1.92 ddd (13, 12, 7)	
20		2.11 dt (10, 2.4)	2.10 dt (10, 2.4)		
21	3.20 d (16.4) (α)	3.95 br s	4.48 br s	3.58 br s	4.16 d (15)
21′	4.13 br d (16.4) (*β*)				4.43 dt (15, 2.0)
22					5.43 s
22′					5.53 s
NH		8.95 br s	8.84 br s		8.62 br s
19-OMe		2.99 s	2.66 s		
CO_2Me		3.78 s	3.78 s		
16-OH				6.14 br s	
11-OMe				3.88 s	
12-OMe				3.81 s	
NCO_2Me				3.84 s	

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

of **3** can be readily verified by applying the same analysis carried out for compound **2** (Figure 1). In compound **3**, NOEs were observed between H-18/H-15 and 19-OMe/H-21, while NOEs between H-18/H-21 and 19-OMe/H-15 were not seen. In addition, the paramagnetic deshielding experienced by H-15 in **2** was now observed for H-21 instead in **3** (Figure 1).

Kopsamidine A (**4**) was obtained as a colorless oil, $[\alpha]_D$ +97 (*c* 0.15, $CHCl_3$). The IR spectrum showed bands at 3447, 3350, and



2 **3**

Figure 1

1720 cm^{-1} due to OH, NH, and ester functions, respectively. The UV spectrum showed absorption maxima at 220, 245, and 281 nm, suggesting the presence of a dihydroindole chromophore. The EIMS of **4** showed a molecular ion at *m*/*z* 396, with peaks due to loss of CH_2CH_2 and CO_2Me observed at *m*/*z* 368 and 337, respectively. HRMS measurements gave the molecular formula $C_{22}H_{24}N_2O_5$. The ^{1}H and ^{13}C NMR data of **4** (Tables 3 and 1, respectively) are generally similar to those of the aspidofractinine alkaloid, 11,12-methylenedioxykopsinaline,[22] except for the presence of an additional disubstituted double bond. The two olefinic hydrogens (δ_H 5.72 and 5.54) were readily assigned to H-14 and H-15 from the COSY spectrum, which showed the presence of the $NCH_2CH{=}CH$ partial structure, attributed to *N*-C(3)−C(14)−C(15). Kopsamidine A is therefore 11,12-methylenedioxy-$\Delta^{14,15}$-kopsinaline.

Kopsamidine B (**5**) was obtained as a colorless oil, $[\alpha]_D$ −46 (*c* 0.22, $CHCl_3$). The UV spectrum was similar to that of kopsinine[23] with absorption maxima observed at 212, 245, and 295 nm, while the IR spectrum showed peaks due to NH (3348 cm^{-1}) and ester (1729 cm^{-1}) functions. The EI-mass spectrum showed a molecular ion at *m*/*z* 368, and HRMS measurements established the molecular formula as $C_{22}H_{28}N_2O_3$. Other significant fragments were observed at *m*/*z* 337 (M − OCH_3) and 309 (M − CO_2Me). The ^{1}H and ^{13}C NMR spectral data (Tables 3 and 1, respectively) were similar to those of kopsinine,[23] except for the presence of an additional OMe group. Analysis of the COSY and HMQC spectral data indicated that the OMe group was substituted at C-15. The correlations observed between H-15 and H-17α as well as H-19β in the NOESY spectrum established the stereochemistry of the 15-OMe group as α. This was further confirmed by the significant downfield shift of H-21 (from ca. δ 3.00 in kopsinine to δ 3.44 in **5**) observed in the ^{1}H NMR spectrum (Table 3) due to its proximity to the α-oriented OMe group at C-15. Kopsamidine B is therefore 15α-methoxykopsinine.

Kopsinidine A (**6**) was obtained as a colorless oil, $[\alpha]_D$ −15 (*c* 0.17, $CHCl_3$). The UV spectrum showed absorption maxima at 221, 242, and 277 nm, suggesting the presence of a dihydroindole chromophore, while the IR spectrum showed absorption bands due to OH (3447 cm^{-1}), NH (3350 cm^{-1}), and ketone (1748 cm^{-1}) functions. The EIMS of **6** showed a molecular ion at *m*/*z* 366, in addition to a significant fragment ion at *m*/*z* 338, which can be attributed to loss of CO or ethene. HREIMS measurements (*m*/*z* 366.1579) gave the formula $C_{21}H_{22}N_2O_4$ (calcd 366.1580), differing from 11,12-methylenedioxykopsine[23] by 58 mass units, suggesting replacement of a CO_2Me group with H. The ^{1}H and ^{13}C NMR spectra (Tables 3 and 1, respectively) showed the presence of a methylenedioxy substituent at C-11 and C-12 (a pair of aromatic AB doublets at δ_H 6.84 and 6.33 and another pair at δ_H 5.88, 5.83, δ_C 100.7), and the presence of a ketone function was indicated by a carbonyl resonance at δ 215.4. The NMR data (Tables 1 and 3) revealed **6** to be an alkaloid of the kopsine type and are generally similar to those of 11,12-methylenedioxykopsine,[23] except for the absence of peaks associated with the carbamate group. Kopsinidine A is therefore *N*(1)-decarbomethoxy-11,12-methylenedioxykopsine.

Another new kopsine derivative obtained is kopsinidine B (**7**), $[\alpha]_D$ +113 (*c* 0.18, $CHCl_3$). The UV spectrum is characteristic of a dihydroindole chromophore (219, 239, and 285 nm), while the IR spectrum indicated the presence of OH (3323 cm^{-1}), ketone (1756 cm^{-1}), and carbamate (1679 cm^{-1}) functions. The mass spectrum showed a molecular ion at *m*/*z* 440, which analyzed for $C_{24}H_{28}N_2O_6$. The ^{1}H and ^{13}C NMR spectral data (Tables 3 and 1, respectively) were similar to those of 11,12-methylenedioxykopsine,[23] except that the aromatic methylenedioxy substituent has been replaced by two methoxy groups (δ_H 3.87 and 3.77).

Compound **8**, paucidactine C, was obtained in minute amounts as a colorless oil, $[\alpha]_D$ +250 (*c* 0.01, $CHCl_3$). The IR spectrum showed bands at 3373, 1768, 1706, and 1686 cm^{-1}, suggesting the presence of OH, lactone, lactam, and carbamate functions, respectively. The UV spectrum showed absorption maxima at 220, 247, and 280 nm, indicating the presence of a dihydroindole chromophore. The ESIMS of **8** showed the MH^+ ion at *m*/*z* 471, and HRMS measurements gave the molecular formula $C_{24}H_{26}N_2O_8$. Analysis of the NMR data readily revealed **8** to be a congener of the paucidactine-type alkaloids.[23] In common with the other paucidactines, the ^{1}H and ^{13}C NMR spectra (Tables 2 and 1, respectively) displayed characteristic signals due to a carbamate function (δ_H 3.84, δ_C 154.6), a lactam carbonyl at C-5 (δ_C 166.7), a lactone carbonyl function (δ_C 169.4), an oxymethine (δ_H 4.75, δ_C 82.9), an oxygenated quaternary carbon (δ_C 74.9), and a hydroxyl group at C-16 (δ_H 6.14). The NMR data of **8** (Tables 1 and 2) are essentially the same as those of paucidactine B,[23] except that the aromatic methylenedioxy substituent in paucidactine B has been replaced by two methoxy groups (δ_H 3.88, 3.81 and δ 56.2, 60.0, respectively).

Compound **9**, pericine *N*-oxide, was obtained as colorless crystals (mp 205−208 °C), with $[\alpha]_D$ +71 (*c* 0.21, $CHCl_3$). The UV spectrum (227 and 301 nm) showed absorption maxima characteristic of an indole chromophore, while the IR spectrum showed the presence of an NH (3378 cm^{-1}) function. The ESIMS of **9** showed an M^+ at *m*/*z* 294, analyzed for $C_{19}H_{22}N_2O$, 16 mass units higher than that of pericine.[24−25] Compound **9** was readily identified as the *N*(4)-oxide of pericine[24,25] from its NMR data (Tables 1 and 2), in particular the characteristic downfield shifts of the carbon resonances for C-3, C-5, and C-21, when compared with those of pericine.

A total of 50 alkaloids (see Experimental Section) were thus obtained from the present investigation of the stem-bark extract of the Malayan *K. arborea*. Of these, several, such as mersicarpine (**10**),[2] arboflorine (**11**),[3] valparicine (**12**),[4] arboricine (**13**),[5] arboricinine (**14**),[5] arboloscine (**15**),[6] and pericidine (**16**),[6] which are notable for incorporating novel or intriguing molecular skeletons, have been reported in preliminary communications. The unusual cage indole, arbophylline (**17**), postulated to derive from an akuammiline-type precursor, has also been reported from the leaf extract,[7] while the tetracyclic dihydroindole mersicarpine (**10**) was also found in another *Kopsia*, viz., *K. singapurensis*.[2]

We previously reported the structure and partial synthesis from pericine of the new pericine-type alkaloid valparicine (**12**).[4] Valparicine was found to display strong cytotoxicity toward drug-sensitive (IC_{50} 13.0 μM) as well as drug-resistant KB cells (IC_{50} 2.72 μM against KB/VJ300), as well as Jurkat cells (IC_{50} 0.91 μM). Other alkaloids such as arboloscine (**15**) and vincadifformine showed moderate cytotoxicity toward KB cells, while arboricine (**13**), arboricinine (**14**), 19(*S*)-methoxytubotaiwine (**2**), and 19(*R*)-methoxytubotaiwine (**3**) showed moderate activity in reversing multidrug resistance in vincristine-resistant KB (VJ300) cells (Table 4).

Experimental Section

General Experimental Procedures. Optical rotations were determined on a JASCO P-1020 digital polarimeter or an Atago Polax-D 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 a JEOL JNM-LA 400

Table 3. ^{1}H NMR Data (δ) for **4**−**7** (400 MHz, $CDCl_3$)[a]

H	4	5	6	7
3α	3.44 ddd (16.4, 3.8, 2.0)	3.38 m	3.05 m	3.02 m
3*β*	3.37 br d (16.4)	2.86 br d (11)	3.05 m	3.02 m
5α	2.79 td (9, 4.6)	3.08 td (9, 3)	3.06 m	3.16 dd (10, 5)
5*β*	2.96 ddd (9, 6.4, 1.5)	3.40 m	3.48 t (10)	3.56 t (10)
6α	1.29 ddd (12.0, 4.6, 1.5)	1.64 m	2.60 dd (10, 5)	2.68 dd (10, 5)
6*β*	2.21 ddd (12.0, 9, 6.4)	2.66 dd (14, 3)		
9	6.59 d (7.7)	7.25 br d (7)	6.84 d (8)	7.06 d (8)
10	6.35 d (7.7)	6.75 td (7.5, 1)	6.33 d (8)	6.71 d (8)
11		6.98 td (7.5, 1)		
12		6.64 d (7.5)		
14α	5.72 ddd (9.8, 3.8, 2.0)	1.64 m	1.26 m	1.26 m
14*β*		2.03 br d (14)	1.79 m	1.80 m
15	5.54 dt (9.8, 2.0)	3.10 br s	1.36 m	1.31 m
15′			1.55 m	1.31 m
16		2.92 t (10)		
17α	1.44 dd (14.8, 1.4)	1.42 ddd (14, 10, 1.5)	1.46 m	1.58 d (15)
17*β*	3.02 dd (14.8, 2.7)	2.66 td (14, 3)	2.22 dd (15, 3)	2.38 dd (15, 4)
18α	1.69 m	1.95 m	1.72 td (12, 4)	1.67 ddd (14, 12, 5)
18*β*	2.00 m	1.24 m	2.00 td (12, 3)	2.46 ddd (14, 12, 4)
19α	1.24 m	1.98 m	1.50 m	1.50 m
19*β*	1.76 m	1.20 m	1.36 m	1.31 m
21	2.60 s	3.44 br s	3.30 s	3.13 d (2)
OCH_2O	5.85 d (1.4)		5.83 s	
	5.93 d (1.4)		5.88 s	
NH	4.45 br s	3.70 br s	3.80 br s	
CO_2*Me*	3.82 s	3.77 s		
16-OH	4.14 br s			6.97 br s
11-OMe				3.87 s
12-OMe				3.77 s
NCO_2*Me*				3.76 s
15α-OMe		3.34 s		

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

spectrometer at 400 and 100 MHz, respectively. ESIMS were obtained on a Perkin-Elmer API 100 instrument. EIMS and HREIMS were obtained at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia.

Plant Material. Plant material was collected in Petaling Jaya, Malaysia, and identification was confirmed by Dr. David Middleton, Herbarium, Royal Botanic Garden, Edinburgh, 20A Inverleith Row, EH3 5LR Scotland. Herbarium voucher specimens (K 668) are deposited at the Herbarium, University of Malaya, Kuala Lumpur, Malaysia, and at Edinburgh.

Extraction and Isolation. Extraction of the ground stem-bark (9 kg) was carried out in the usual manner by partitioning the concentrated EtOH extract with dilute acid, as has been described in detail elsewhere.[26] The alkaloids were isolated by initial column chromatography on silica gel using $CHCl_3$ with increasing proportions of MeOH followed by rechromatography of appropriate partially resolved fractions using centrifugal TLC. Solvent systems used for centrifugal TLC were Et_2O (NH_3-saturated), Et_2O/hexane (1:1; NH_3-saturated), Et_2O/hexane (1:3; NH_3-saturated), Et_2O/hexane (2:1; NH_3-saturated), Et_2O/MeOH (100:1; NH_3-saturated), Et_2O/MeOH (25:1; NH_3-saturated), Et_2O/MeOH (5:1; NH_3-saturated), EtOAc/hexane (1:1; NH_3-saturated), EtOAc/hexane (1:3; NH_3-saturated), EtOAc/hexane (5:1; NH_3-saturated), Me_2CO/hexane (1:3), $CHCl_3$, MeOH/$CHCl_3$ (1:100; NH_3-saturated), and MeOH/$CHCl_3$ (1:50; NH_3-saturated). The yields (g kg^{-1}) of the alkaloids were as follows: **1** (0.0015), **2** (0.0037), **3** (0.0075), **4** (0.0034), **5** (0.0032), **6** (0.0012), **7** (0.0025), **8** (0.00032), **9** (0.204), **10**[2] (0.00054), **11**[3] (0.00043), **12**[4] (0.0022), **13**[4] (0.0016), **14**[5] (0.0011), **15**[5] (0.0016), **16**[6] (0.081), prunifoline E (0.0011), venalstonine[27] (0.022), kopsiflorine[23] (0.0011), venalstonidine[28] (0.0032), kopsamine[23] (0.054), 11,12-methylenedioxykopsine[23] (0.0022), kopsilongine[23] (0.00065), kopsanone[29] (0.0054), kopsamine *N*-oxide[23] (0.0022), *N*(1)-methoxycarbonyl-11,12-dimethoxykopsinaline[30] (0.00075), kopsifine[23] (0.00043), kopsinine[23] (1.216), paucidactine B[23] (0.0011), pleiocarpamine[23] (0.0032), 15α-hydroxykopsinine[29] (0.0011), *N*(1)-decarbomethoxykopsamine[23] (0.039), aspidofractinine[31] (0.0032), dasyrachine[23] (0.00075), methyl-11,12-methylenedioxy-*N*(1)-decarbomethoxy-$\Delta^{14,15}$-chanofruticosinate[32] (0.059), methyl-11,12-methylenedioxy-*N*(1)-decarbomethoxychanofruticosinate[32] (0.024), methyl-11,12-methylenedioxychanofruticosinate[32] (0.0011), methyl-*N*(1)-decarbomethoxychanofruticosinate[32] (0.00054), methyl-12-methoxychanofruticosinate[33] (0.0012), *O*-methylleuconolam[34] (0.0011), vincadifformine[35] (0.0027), rhazimal[36] (0.0031), akuammidine[37] (0.00054), tetrahydroalstonine[23] (0.00054), norflourocurarine[38] (0.027), leuconoxine[23] (0.0011), pericine[24−25] (0.155), rhazinicine[23] (0.00086), rhazinilam[39] (0.012), and 5,21-dihydrorhazinilam[39] (0.0094).

Rhazinoline (1): colorless oil; $[\alpha]_D$ +136 (*c* 0.07, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 211 (4.27), 260 (3.70) nm; IR (dry film) ν_{max} 1718 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; EIMS, *m*/*z* 292 $[M]^+$ (100), 277 $[M - CH_3]^+$ (5), 263 $[M - CHO]^+$ (85), 249 (16), 234 (39), 220 (23), 194 (22), 180 (36), 167 (24), 154 (13), 122 (39), 40 (36); HREIMS *m*/*z* 292.1574 (calcd for $C_{19}H_{20}N_2O$, 292.1576).

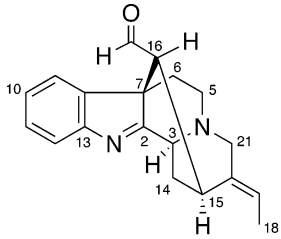
19(*S*)-Methoxytubotaiwine (2): colorless oil; $[\alpha]_D$ +429 (*c* 0.24, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (3.95), 297 (3.89), 327 (3.99) nm; IR (dry film) ν_{max} 3360, 1674 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 354 $[M]^+$ (71), 339 $[M - Me]^+$ (45), 323 $[M - OMe]^+$ (67), 295 $[M - CO_2Me]^+$ (44), 263 (53), 252 (41), 235 (39), 208 (39), 194 (61), 180 (82), 167 (63), 88 (81), 71 (100), 59 (31) 47 (40); HREIMS *m*/*z* 354.1937 (calcd for $C_{21}H_{26}N_2O_3$, 354.1943).

19(*R*)-Methoxytubotaiwine (3): colorless oil; $[\alpha]_D$ +442 (*c* 0.14, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 229 (3.88), 297 (3.75), 329 (3.87) nm; IR (dry film) ν_{max} 3360, 1676 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 354 $[M]^+$ (100), 339 $[M - Me]^+$ (46), 323 $[M - OMe]^+$ (65), 297 (40), 279 (44), 252 (39), 235 (33), 220 (44), 194 (55), 180 (76), 167 (58), 95 (45), 84 (69), 71 (91), 59(31), 49(61), 40 (67); HREIMS *m*/*z* 354.1940 (calcd for $C_{21}H_{26}N_2O_3$, 354.1943).

Kopsamidine A (4): colorless oil; $[\alpha]_D$ +97 (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (4.46), 245 (3.88), 281 (3.11) nm; IR (dry film) ν_{max} 3447, 3350, 1720 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 396 $[M]^+$ (100), 368 $[M - CH_2CH_2]^+$ (11), 337 $[M - CO_2Me]^+$ (8), 308 (20), 279 (16), 261 (13), 215 (32), 187 (7), 136 (13), 122 (22), 107 (18), 93 (8); HREIMS *m*/*z* 396.1685 (calcd for $C_{22}H_{24}N_2O_5$, 396.1685).

Kopsamidine B (5): colorless oil; $[\alpha]_D$ −46 (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ): 212 (4.05), 245 (3.73), 295 (3.36) nm; IR (dry film) ν_{max} 3348, 1729 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 368 $[M]^+$ (34), 337 $[M - OMe]^+$ (13), 309 $[M - CO_2Me]^+$ (5), 249 (5), 221 (5), 194 (4), 168 (5), 154 (100), 139 (18), 124 (7), 109 (25), 57 (14), 40 (10); HREIMS *m*/*z* 368.2100 (calcd for $C_{22}H_{28}N_2O_3$, 368.2100).

Chart 1



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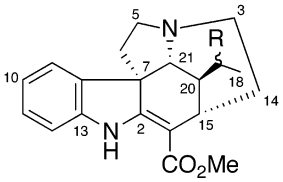
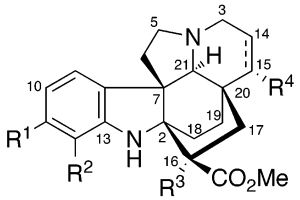
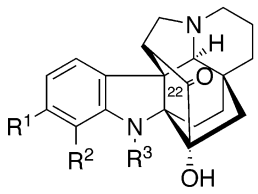


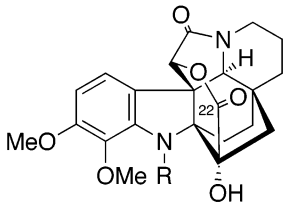
2 R = β-OMe
3 R = α-OMe



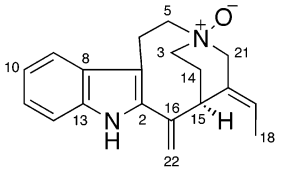
4 R^1, R^2 = OCH_2O, R^3 = OH, R^4 = H, $\Delta^{14,15}$
5 R^1 = R^2 = R^3 = H, R^4 = OMe



6 R^1, R^2 = OCH_2O, R^3 = H
7 R^1 = R^2 = OMe, R^3 = CO_2Me



8 R = CO_2Me



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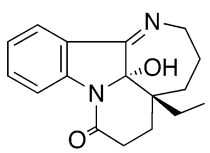

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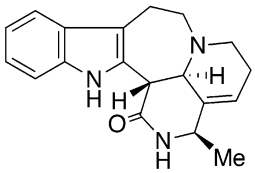

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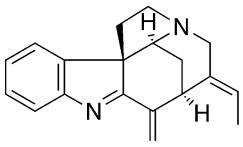

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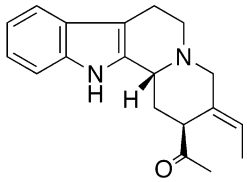

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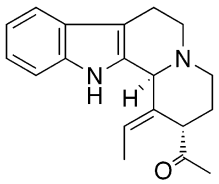

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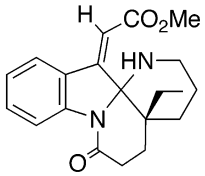

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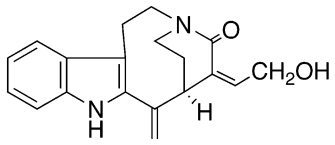

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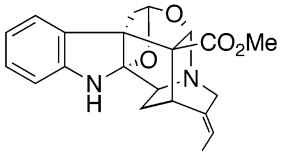

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Table 4. Cytotoxic Effects of **2**, **3**, **12**−**15**, and Vincadifformine

	IC$_{50}$, μg/mL (μM)			
compound	KB/S[a]	KB/VJ300[a]	KB/VJ300[b]	Jurkat[c]
19(*S*)-methoxytubotaiwine (**2**)	>25	>25	9.5 (26.8)	>25
19(*R*)-methoxytubotaiwine (**3**)	>25	>25	9.6 (27.1)	>25
valparicine (**12**)	3.6 (13.0)	0.75 (2.72)	0.46 (1.67)	0.25 (0.91)
arboricine (**13**)	>25	>25	10.8 (36.7)	>25
arboricinine (**14**)	>25	>25	9.2 (31.3)	>25
arboloscine (**15**)	15 (44.1)	11 (32.4)	3.8 (11.2)	*d*
vincadifformine	10.2 (30.2)	6.3 (18.6)	4.5 (13.3)	21.8 (64.5)

[a] KB/S and KB/VJ300 are vincristine-sensitive and -resistant human oral epidermoid carcinoma cell lines, respectively.[42] [b] With added vincristine, 0.1 μg/mL (0.121 μM), which did not affect the growth of the KB/VJ300 cells. [c] Jurkat is a human leukemic T-cell line. [d] Not determined.

Kopsinidine A (6): colorless oil; $[\alpha]_D$ −15 (*c* 0.17, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 221 (4.48), 242 (3.99), 277 (3.23) nm; IR (dry film) ν_{max} 3447, 3350, 1748 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 366 $[M]^+$ (74), 338 $[M - CH_2CH_2]^+$ (10), 319

(13), 294 (18), 281 (8), 268 (100), 251 (23), 225 (18), 188 (5), 128 (7), 83 (12), 70 (15), 57 (9), 40 (27); HREIMS *m*/*z* 366.1579 (calcd for $C_{21}H_{22}N_2O_4$, 366.1580).

Kopsinidine B (7): colorless oil; $[\alpha]_D$ +113 (*c* 0.18, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 219 (4.28), 239 (3.79), 285 (3.01) nm; IR (dry film) ν_{max} 3323, 1756, 1679 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 3; EIMS *m*/*z* 440 $[M]^+$ (34), 412 $[M - CH_2CH_2]^+$ (15), 342 (100), 283 (10); HREIMS *m*/*z* 440.1939 (calcd for $C_{24}H_{28}N_2O_6$, 440.1947).

Paucidactine C (8): colorless oil; $[\alpha]_D$ +250 (*c* 0.01, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 220 (4.64), 247 (3.93), 280 (3.36) nm; IR (dry film) ν_{max} 3373, 1768, 1706, 1686 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 471 $[MH]^+$; HREIMS *m*/*z* 470.1690 (calcd for $C_{24}H_{26}N_2O_8$, 470.1689).

Pericine *N*-oxide (9): colorless crystals; mp 205−208 °C; $[\alpha]_D$ +71 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 227 (4.41), 301 (4.23) nm; IR (dry film) ν_{max} 3378 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 294 $[M]^+$; EIMS *m*/*z* 278 $[M - O]^+$ (100), 263 $[M - O - Me]^+$ (78), 249 (34), 235 (51), 220 (67), 209 (62), 194 (43), 180 (30), 167 (31), 122 (46), 108 (15), 43 (23).

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described in detail previously.[40,41]

Acknowledgment. We thank the University of Malaya, ASM (SAGA), and MOSTI, Malaysia (ScienceFund) for financial support.

References and Notes

(1) Middleton, D. J. *Harvard Pap. Bot.* **2000**, *9*, 89−142.
(2) Kam, T. S.; Subramaniam, G.; Lim, K. H.; Choo, Y. M. *Tetrahedron Lett.* **2004**, *45*, 5995−5998.
(3) Lim, K. H.; Kam, T. S. *Org. Lett.* **2006**, *8*, 1733−1735.
(4) Lim, K. H.; Low, Y. Y.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 5037−5039.
(5) Lim, K. H.; Komiyama, K.; Kam, T. S. *Tetrahedron Lett.* **2007**, *48*, 1143−1145.
(6) Lim, K. H.; Kam, T. S. *Helv. Chim. Acta* **2007**, *90*, 31−35.
(7) Lim, K. H.; Kam, T. S. *Tetrahedron Lett.* **2006**, *47*, 8653−8655.
(8) Kam, T. S.; Subramaniam, G. *Tetrahedron Lett.* **2004**, *45*, 3521−3524.
(9) Kam, T. S.; Choo, Y. M. *Helv. Chim. Acta* **2004**, *87*, 991−998.
(10) Kam, T. S.; Choo, Y. M. *Tetrahedron Lett.* **2003**, *44*, 1317−1319.
(11) Kam, T. S.; Subramaniam, G.; Lim, T. M. *Tetrahedron Lett.* **2001**, *42*, 5977−5980.
(12) Kam, T. S.; Lim, T. M.; Choo, Y. M. *Tetrahedron* **1999**, *55*, 1457−1468.
(13) Kam, T. S. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: Amsterdam, 1999; Vol. 14, pp 285−435.
(14) Kam, T. S.; Subramaniam, G.; Sim, K. M.; Yoganathan, K.; Koyano, T.; Toyoshima, M.; Rho, M. C.; Hayashi, M.; Komiyama, K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2769−2772.
(15) Mok, S. L.; Yoganathan, K.; Lim, T. M.; Kam, T. S. *J. Nat. Prod.* **1998**, *61*, 328−332.
(16) Kam, T. S.; Yoganathan, K.; Koyano, T.; Komiyama, K. *Tetrahedron Lett.* **1996**, *37*, 5765−5768.
(17) Kan, C.; Deverre, J. R.; Sevenet, T.; Quirion, J. C.; Husson, H. P. *Nat. Prod. Lett.* **1995**, *7*, 275−281.
(18) Ahmad, Y.; Fatima, K.; Atta-ur-Rahman; Occolowitz, J. L.; Solheim, B. A.; Clardy, J.; Garnick, R. L.; Le Quesne, P. W. *J. Am. Chem. Soc.* **1977**, *99*, 1943−1946.
(19) Yamauchi, T.; Abe, F.; Padolina, W. G.; Dayrit, F. M. *Phytochemistry* **1990**, *29*, 3321−3325.
(20) Kuehne, M. E.; Brook, C. S.; Frasier, D. A.; Xu, F. *J. Org. Chem.* **1995**, *60*, 1864−1867.
(21) Nkiliza, J.; Vercauteren, J. *Tetrahedron Lett.* **1991**, *32*, 1787−1790.
(22) Feng, X. Z.; Kan, C.; Potier, P.; Kan, S. K.; Lounasmaa, M. *Planta Med.* **1983**, *48*, 280−282.
(23) Kam, T. S.; Subramaniam, G.; Chen, W. *Phytochemistry* **1999**, *51*, 159−169.
(24) Arens, H.; Borbe, H. O.; Ulbrich, B.; Stockigt, J. *Planta Med.* **1982**, *46*, 210−214.
(25) Kobayashi, J.; Sekiguchi, M.; Shimamoto, S.; Shigemori, H.; Ishiyama, H.; Ohsaki, A. *J. Org. Chem.* **2002**, *67*, 6449−6455.
(26) Kam, T. S.; Tan, P. S. *Phytochemistry* **1990**, *29*, 2321−2322.
(27) Awang, K.; Sevenet, T.; Pais, M.; Hadi, A. H. A. *J. Nat. Prod.* **1993**, *56*, 1134−1139.
(28) Linde, H. H. A. *Helv. Chim. Acta* **1965**, *48*, 1822−1842.
(29) Kam, T. S.; Choo, Y. M. *Phytochemistry* **2004**, *65*, 2119−2122.
(30) Kam, T. S.; Sim, K. M. *Phytochemistry* **1998**, *47*, 145−147.
(31) Djerassi, C.; Budzikiewicz, H.; Owellen, R. J.; Wilson, J. M.; Kump, W. G.; Le Count, D. J.; Battersby, A. R.; Schmid, H. *Helv. Chim. Acta* **1963**, *46*, 742−751.
(32) Kam, T. S.; Tan, P. S.; Hoong, P. Y.; Chuah, C. H. *Phytochemistry* **1993**, *32*, 489−491.
(33) Husain, K.; Jantan, I.; Kamaruddin, N.; Said, I. M.; Aimi, N.; Takayama, H. *Phytochemistry* **2001**, *57*, 603−606.
(34) Goh, S. H.; Ali, A. R. M.; Wong, W. H. *Tetrahedron* **1989**, *45*, 7899−7920.
(35) Smith, G. F.; Wahid, M. A. *J. Chem. Soc.* **1963**, 4002−4004.
(36) Atta-ur-Rahman; Habib-ur-Rehman. *Planta Med.* **1986**, *3*, 230−231.
(37) Jokela, R.; Lounasmaa, M. *Heterocycles* **1996**, *43*, 1015−1020.
(38) Clivio, P.; Richard, B.; Deverre, J. R.; Sevenet, T.; Zeches, M.; Le Men-Olivier. *Phytochemistry* **1991**, *30*, 3785−3792.
(39) Goh, S. H.; Ali, A. R. M.; Wong, W. H. *Tetrahedron* **1989**, *45*, 7899−7920.
(40) Kam, T. S.; Lim, K. H.; Yoganathan, K.; Hayashi, M.; Komiyama, K. *Tetrahedron* **2004**, *60*, 10739−10745.
(41) Hosoya, T.; Yamamoto, Y.; Uehara, Y.; Hayashi, M.; Komiyama, K.; Ishibashi, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2776−2780.
(42) Kohno, K.; Kikuchi, J.; Sato, S.; Takano, H.; Saburi, Y.; Asoh, K.; Kuwano, M. *Jpn. J. Cancer Res.* **1988**, *79*, 1238−1246.

NP0702234