Biologically Active Aspidofractinine, Rhazinilam, Akuammiline, and Vincorine Alkaloids from *Kopsia*

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Eleven new indole alkaloids, in addition to the previously reported rhazinal (**1**), and 14 other known alkaloids, were obtained from the Malayan *Kopsia singapurensis*, viz., kopsiloscines A–F (**2**–**7**), 16-epikopsinine (**8**), kopsilongine-*N*-oxide (**9**), 16-epiakuammiline (**10**), aspidophylline A (**11**), and vincophylline (**12**). The structures of these alkaloids were determined using NMR and MS analyses. Rhazinal (**1**), rhazinilam (**17**), and rhazinicine (**18**) showed appreciable cytotoxicity toward drug-sensitive as well as vincristine-resistant KB cells, while kopsiloscines A (**2**), B (**3**), and D (**5**) and aspidophylline A (**11**) were found to reverse drug-resistance in drug-resistant KB cells.

The genus *Kopsia*, which is widely distributed in Southeast Asia,[1–3] is rich in indole alkaloids, and the Malaysian representatives in particular have proven to be rich sources of novel alkaloids with unusual or intriguing carbon skeletons and interesting biological activity.[4,5] In continuation of our studies on the Malaysian members of this genus,[6–24] we would like to report the isolation of new indole alkaloids from *K. singapurensis*. An early study by Thomas et al. gave kopsingine and kopsaporine.[25] A subsequent study by Awang et al. provided additional aspidofractinine-type alkaloids, including a kopsaporine derivative and the singapurensines,[26] congeners of the oxo-bridged kopsidine-type alkaloids.[24,27] When we first collected the sample, initial examination indicated that it was *K. teoi*. Accordingly we submitted a preliminary communication on the structure of the rhazinilam derivative rhazinal (**1**), which was isolated from the stem-bark extract of this sample of *Kopsia*, which was then attributed by us (erroneously it would appear) to *K. teoi*.[28] Since then a synthesis of rhazinal has also been published.[29] The sample was later sent to Dr. David Middleton, who eventually provided final confirmation of the identity of the sample upon completion of his revision of the genus.[3] The source of rhazinal as well as the alkaloids reported in the present paper can now therefore be attributed to *K. singapurensis*.

Results and Discussion

A total of 26 alkaloids (see Experimental Section) were isolated, 14 from the leaf extract and 15 from the stem-bark extract. Of these, nine are new alkaloids, while kopsingine, 16-epiakuammiline, and 16-epideacetylakuammiline were common in both the stem and leaf extracts.

The kopsiloscines (**2**–**7**) represent a group of aspidofractinine-type alkaloids, all sharing a common stereochemical feature, which is an α-oriented hydroxy group at C-17. The first member of this group was 17α-hydroxy-$\Delta^{14,15}$-kopsinine (**13**) reported from *K. teoi*.[30] As a result of some uncertainty concerning the configurational assignments,[31,32] a detailed NMR study was subsequently carried out which completely vindicated the original assignments.[33] In this study six additional members have been added to this group of aspidofractinine alkaloids, all possessing in common α-OH substitution at C-17.

Kopsiloscine A (**2**) was obtained as a yellowish oil, $[\alpha]_D$ +16 ($CHCl_3$, *c* 0.25). The UV spectrum showed absorption maxima at 206, 244, 279, and 285 nm (log ε 4.31, 3.96, 3.28, and 3.23, respectively), characteristic of a dihydroindole chromophore. The IR spectrum showed bands due to OH (3507 and 3288 cm^{-1}), ester (1736 cm^{-1}), and carbamate (1673 cm^{-1}) functions. The EIMS showed a molecular ion at *m/z* 426, and HREIMS measurements yielded the formula $C_{23}H_{26}N_2O_6$. The ^{1}H NMR spectrum (Table 1) of **2** showed four resonances in the aromatic region characteristic of an unsubstituted indole ring. The broad doublet at δ 7.18 can be assigned to H-9, which was confirmed from the observed NOE interaction with H-21. This then facilitated the assignment of H-10 (δ 7.03), H-11 (δ 7.20), and H-12 (δ 7.54). The low-field singlet at δ 8.10 is due to the C(16)-OH group, while the doublet at δ 3.53 was assigned to the C(17)-OH function.[30] Both signals were exchanged on addition of D_2O. The olefinic H-14 and H-15 resonated at δ 5.82 and 5.71, respectively. Two methoxy signals were observed at δ 3.76 and 3.98, which are associated with ester and carbamate methoxy groups, respectively, from the observation of the associated carbonyl resonances at δ_C 171.8 and 156.7. COSY and HMQC experiments showed the presence of the partial structures CH_2CHCH, corresponding to the C(3)–C(14)–C(15) unit, and two other CH_2CH_2 fragments, which were assigned to the C(5)–C(6) and C(18)–C(19) units, of an aspidofractinine-type molecule. The spectroscopic data (Tables 1 and 3) were similar to those of kopsaporine, except for the H-17 and H-6β resonances, which were shifted to lower field, and the C(17)-OH resonance, which was shifted to higher field. The COSY experiment also showed that H-17 (δ 4.78) coupled with C(17)-OH (J = 7.5 Hz) and also with H-19 (J = 2 Hz, *W* coupling), but not with H-21, the latter observation being possible only if H-17 has a β-orientation. These observations indicated the α-orientation of 17-OH. Further confirmation of this was provided by NOE experiments. Irradiation of the 17-OH resonance resulted in the enhancement of H-19β, while irradiation of H-17 resulted in enhancement of H-5β and H-6β. The ^{1}H and ^{13}C NMR data are summarized in Tables 1 and 3, respectively.

Kopsiloscine B (**3**) was obtained as a colorless oil, $[\alpha]_D$ +50 ($CHCl_3$, *c* 0.23). The UV and IR spectra of **3** were similar to those of kopsiloscine A (**2**). The EI mass spectrum showed a molecular ion at *m/z* 428, consistent with the molecular formula $C_{23}H_{28}N_2O_6$, differing from **2** by addition of two hydrogens. Comparison of the ^{1}H and ^{13}C NMR data (Tables 1 and 3) suggested that **3** is closely

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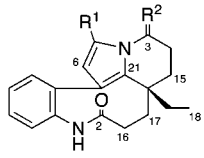

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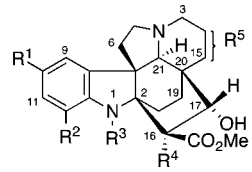
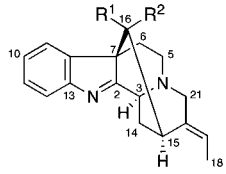
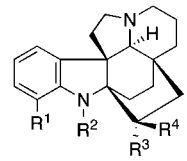
18 R = H, R^2 = O



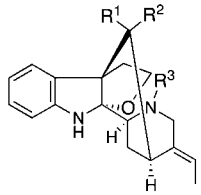
2 R^1 = R^2 = H, R^3 = CO_2Me, R^4 = OH, R^5 = $\Delta^{14,15}$
3 R^1 = R^2 = H, R^3 = CO_2Me, R^4 = OH, R^5 = Nil
4 R^1 = R^2 = H, R^3 = CO_2Me, R^4 = OH, R^5 = 15-αOH
5 R^1 = OMe, R^2 = H, R^3 = CO_2Me, R^4 = OH, R^5 = Nil
6 R^1 = OMe, R^2 = H, R^3 = CO_2Me, R^4 = OH, R^5 = 15-αOH
7 R^1 = H, R^2 = OMe, R^3 = CO_2Me, R^4 = OH, R^5 = 15-αOH
13 R^1 = R^2 = R^3 = R^4 = H, R^5 = $\Delta^{14,15}$



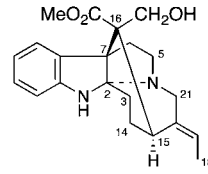
10 R^1 = CO_2Me, R^2 = CH_2OAc
15 R^1 = CH_2OAc, R^2 = CO_2Me



8 R^1 = R^2 = R^4 = H, R^3 = CO_2Me
9 R^1 = OMe, R^2 = R^4 = CO_2Me, R^3 = OH, *N*(4)→O
14 R^1 = R^2 = R^3 = H, R^4 = CO_2Me



11 R^1 = H, R^2 = CO_2Me, R^3 = CHO
16 R^1 = CH_2OH, R^2 = CO_2Me, R^3 = H



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related to **2**, except for the resonances of the 14,15-double bond, which were absent in the spectra of **3**. The 14,15-olefinic hydrogens have been replaced by methylenes showing the anticipated upfield shifts. The same behavior was also shown by the C-14 and C-15 carbon resonances, which were found at δ_C 17.8 and 31.2, respectively. As in the case of **2**, the observed $J_{17\beta-19\alpha}$ *W* coupling of 2 Hz as well as the observed H-17/H-5β, H-6β NOEs confirmed the α-OH substitution at C-17. Kopsiloscine B (**3**) is therefore the 14,15-dihydro derivative of **2**.

Kopsiloscine C (**4**) was isolated as light yellowish crystals with a melting point of 172–174 °C, $[\alpha]_D$ –40 ($CHCl_3$, *c* 0.28). It showed a molecular ion in the EIMS at *m/z* 444, consistent with the formula $C_{23}H_{28}N_2O_7$, differing from kopsiloscine A (**2**) by 18 mass units. Other significant fragments in the mass spectrum were observed at *m/z* 385 (M – CO_2Me) and 367 (M – CO_2Me – H_2O). Comparison of the NMR data of **4** with those of **2** (Tables 1 and 3) revealed that the two compounds have essentially similar structures, except for the 14,15-double bond, which was absent in **4**, being replaced instead by a hydroxy group in the piperidine ring D. Analysis of the COSY and HMQC spectra indicated that the OH group can be placed on C-3 or C-15. The ^1H and ^{13}C NMR shifts are, however, more consistent with hydroxy substitution at C-15.[34] Moreover, NOEs were observed between H-15β and C(17)-αOH, which not only confirmed OH substitution at C-15 but also allowed the orientation of the C(15)-OH group to be assigned as α. Unlike the case of **2** and **3**, H-17 in **4** was observed as a broad doublet without any splitting due to long-range coupling, while H-19α was a multiplet. Nevertheless, evidence for α-OH substitution at C-17 was provided by the NOEs observed between H-17 and H-5β, H-6β.

Kopsiloscine D (**5**) was isolated in minute amounts as a colorless oil, $[\alpha]_D$ −63 ($CHCl_3$, *c* 0.10). The UV spectrum (λ_{max} 201, 249, and 297) was characteristic of a dihydroindole chromophore, while the IR spectrum displayed bands due to hydroxy (3445 cm^{-1}), ester (1730 cm^{-1}), and carbamate (1672 cm^{-1}) groups. The EI mass spectrum showed a molecular ion at *m/z* 458, corresponding to the molecular formula $C_{24}H_{30}N_2O_7$, differing from **3** by addition of 30 mass units. The high-field region in the ^1H NMR spectrum (Table 1) of **5** was similar to that of **3**, except for the presence of the aromatic methoxy resonance at δ 3.80. The low-field region of the ^1H NMR spectrum differed from that of **3** and indicated substitution by an aromatic methoxy group (δ 6.86, br s; 6.67, dd, J = 9, 2.8 Hz; 7.41, d, J = 9 Hz). This coupling pattern indicated methoxy substitution at C(10) or C(11). Examination of the carbon chemical shifts showed that the values are more consistent with OMe substitution at C-10.[22] This was confirmed by NOE difference experiments. Irradiation of the broad singlet at δ 6.86 caused enhancement of H-21 (δ 3.00), which allowed the assignment of this resonance to H-9. This then facilitated the assignment of H-11 (δ 6.67) and H-12 (δ 7.41). Kopsiloscine D (**5**) is therefore the 10-methoxy derivative of **3**.

Kopsiloscine E (**6**) was obtained as a yellowish oil with $[\alpha]_D$ –25 ($CHCl_3$, *c* 0.15). The IR and UV spectra were similar to those of **5**. The EIMS of **6** showed a molecular ion at *m/z* 474, which analyzed for $C_{24}H_{30}N_2O_8$, differing from **5** by replacement of H with an OH. The presence of the M – H_2O fragment ion at *m/z* 456 indicated the presence of a hydroxy function. The ^1H NMR spectrum of **6** (Table 1) was similar to that of **5**, except for H-15 (δ 4.19), which has undergone a significant downfield shift. Likewise, the ^{13}C NMR spectrum of **6** (Table 3) was generally similar to that of **5**, except for the notable downfield shifts of the oxygenated C-15 and, to a lesser extent, the adjacent C-14, C-20, and C-21 resonances. Determination of the location of the hydroxy function was also supported by COSY and HMQC data, which confirmed the *N*CH_2CH_2CH(OH) fragment corresponding to the *N*-C(3)–C(14)–C(15) partial structure. The orientation of the 15-

Table 1. ^{1}H NMR Data (δ) for **2–7** (400 MHz, $CDCl_3$)[a]

H	2	3	4	5	6	7
3	3.45 m	2.91 td (13, 3)	2.87 ddd (13, 5, 2)	2.91 td (13, 3)	2.86 ddd (13, 5, 2)	2.88 ddd (13, 5, 2)
	3.45 m	3.09 m	3.37 td (13, 3)	3.09 m	3.36 td (13, 3)	3.28 td (13, 3)
5	2.89 td (8.5, 4.5)	2.97 ddd (8.5, 7.5, 5)	2.99 td (8.5, 5)	2.97 m	2.98 td (8.5, 5)	2.96 m
	2.94 ddd (8.5, 6.8, 1.5)	3.06 m	3.06 td (8.5, 6)	3.05 m	3.06 td (8.5, 6)	3.00 m
6	1.37 ddd (12.5, 4.5, 1.5)	1.53 ddd (14, 7.5, 6)	1.52 m	1.55 dt (14, 6)	1.55 m	1.72 m
	1.92 ddd (12.5, 8.5, 6.8)	2.02 ddd (14, 8.5, 5)	2.01 ddd (14, 8.5, 5)	2.03 ddd (14, 8.5, 5)	2.01 ddd (14, 8.5, 5)	2.14 ddd (14, 7.5, 6)
9	7.18 br d (7.5)	7.29 dd (7.5, 1.5)	7.29 dd (7.5, 1)	6.86 br s	6.86 d (2.8)	6.89 d (8)
10	7.03 td (7.5, 1)	7.03 td (7.5, 1)	7.03 br t (7.5)			7.00 t (8)
11	7.20 ddd (8, 7.5, 1)	7.16 td (7.5, 1.5)	7.16 td (7.5, 1)	6.67 dd (9, 2.8)	6.67 dd (9, 2.8)	6.78 d (8)
12	7.54 br d (8)	7.50 br d (7.5)	7.49 br d (7.5)	7.41 d (9)	7.40 d (9)	
14	5.82 ddd (10, 3.5, 2)	1.30 m	1.52 m	1.30 m	1.50 m	1.52 m
		1.91 qt (13, 4)	2.27 m	1.91 qt (13, 4)	2.26 m	2.23 m
15	5.71 dt (10, 2)	0.97 td (13, 4) 2.23 m	4.20 br s	0.97 td (13, 4) 2.23 m	4.19 t (3)	4.16 t (3)
17	4.78 dd (7.5, 2)	4.77 dd (7.5, 2)	4.81 br d (7.5)	4.75 br d (7)	4.79 br s	4.77 d (7.5)
18	1.43 ddd (13, 11, 8)	1.43 ddd (13, 11, 8)	1.42 ddd (13, 11, 8)	1.43 ddd (13, 11, 8)	1.42 ddd (13, 11, 8)	1.45 m
	2.22 ddd (13, 11, 2)	2.19 ddd (13, 11, 2)	2.22 ddd (13, 11, 2)	2.18 ddd (13, 11, 2)	2.20 ddd (13, 11, 2)	2.28 m
19	1.04 ddt (13, 11, 2)	0.85 ddt (13, 11, 2)	1.68 ddd (13, 11, 8)	0.85 br dd (13, 11) 1.83 ddd	1.55 m	1.48 m
	2.06 ddd (13, 11, 8)	1.83 ddd (13, 11, 8)	1.56 m	(13, 11, 8)	1.68 ddd	1.67 m
21	2.78 s	3.02 s	3.41 s	3.00 s	3.39 s	3.25 s
10-OMe				3.80 s	3.79 s	
12-OMe						3.89 s
CO_2Me	3.76 s	3.78 s	3.78 s	3.78 s	3.78 s	3.79 s
NCO_2Me	3.98 s	3.98 s	3.98 s	3.96 s	3.96 s	3.83 s
16-OH	8.10 s	8.20 br s	8.40 s	8.28 br s	8.39 br s	7.53 br s
17-OH	3.53 d (7.5)	3.43 d (7.5)	3.51 d (7.5)	3.42 d (7)	3.51 br s	3.44 d (7.5)

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

OH group was deduced to be α from the reciprocal NOEs observed for H-15/C(17)-αOH, indicating that the orientation of H-15 is β.

Kopsiloscine F (**7**) was isolated in minute amounts as a light yellowish oil, with $[\alpha]_D$ +9 ($CHCl_3$, *c* 0.28). The UV and IR spectra of kopsiloscine F were nearly identical to those of **6**. The MS showed a molecular ion at *m/z* 474 ($C_{24}H_{30}N_2O_8$), indicating that **7** is isomeric with **6**. The NMR data (Tables 1 and 3) of **7** in fact showed a close resemblance to those of **6** except for differences in the aromatic region, due to methoxy substitution at C-12 in **7** instead of at C-10 in **6**. This was further confirmed by NOE experiments. Irradiation of H-21 of **7** resulted in enhancement of H-9 (δ 6.89, d, J = 8 Hz), which in turn allowed the assignment of H-10 (δ 7.00, t, J = 8 Hz) and H-11 (δ 6.78, d, J = 8 Hz). In common with compounds **5** and **6**, the orientation of the OH group at C-17 in **7** was deduced to be α from the NOEs observed between H-17 and H-5β, H-6β, and H-14.

Compound **8** was obtained as a yellowish oil, with $[\alpha]_D$ +7 ($CHCl_3$, *c* 0.22). The EI mass spectrum showed a molecular ion at *m/z* 338 ($C_{21}H_{26}N_2O_2$), with other significant fragments at *m/z* 310 (M – CH_2=CH_2) and 279 (M – CO_2Me). The UV, IR, and MS of **8** were nearly identical to those of kopsinine (**14**), indicating that **8** and **14** are epimers. The ^{1}H NMR spectrum of **8** (Table 2) largely resembled that of **14** except for changes involving the H-16 resonance. In **8**, H-16 was observed as a doublet of triplets (dt) at δ 3.20 (J = 11.5, 3 Hz), while in kopsinine (**14**), H-16 was observed as a triplet of doublets at δ 2.89 (J = 9.3, 1 Hz). The ^{13}C NMR spectrum of **8** (Table 3) was also almost identical with that of kopsinine (**14**) except for C-18, which was shifted upfield by ca. 7.5 ppm. Further confirmation for the change in configuration at C-16 was provided by the observed NOE interaction between H-16 and H-6β, which was only possible if H(16) is β. Compound **8** is therefore 16-epikopsinine, which was also independently isolated from several other *Kopsia*.[35]

Compound **9**, the most polar alkaloid isolated from the leaf extract, was readily identified as the *N*-oxide of kopsilongine on the basis of the FABMS data ($[M + H]^+$ *m/z* 459, $C_{24}H_{31}N_2O_7$) and the characteristic downfield shifts of H-3, H-5, H-6, H-9, and H-21 in the ^{1}H NMR spectrum (Table 2) and C-3, C-5, and C-21 in the ^{13}C NMR spectrum (Table 3), when compared to kopsilongine. Additional confirmation was obtained by *m*-CPBA oxidation of kopsilongine, which gave a product identical to **9**.

Compound **10** was obtained as a yellowish oil with $[\alpha]_D$ +116 ($CHCl_3$, *c* 0.23). The UV spectrum showed absorption maxima at 210, 220, and 265 nm, indicating the presence of an unsubstituted indolenine chromophore, which was confirmed by the carbon resonance at δ_C 188.9. The IR spectrum (1738 cm^{-1}) indicated the presence of an ester function. The EIMS showed a molecular ion at *m/z* 394 corresponding to the molecular formula $C_{23}H_{26}N_2O_4$. The ^{1}H NMR spectrum (Table 2) showed the presence of four aromatic hydrogens (δ 7.57, br d, J = 7.5 Hz, H-9; 7.16, td, J = 7.5, 1 Hz, H-10; 7.31, td, J = 7.5, 1 Hz, H-11; 7.61, br d, J = 7.5 Hz, H-12), an ethylidene side chain (methyl group at δ 1.73, dd, J = 7, 2.5 Hz, and vinyl-H at δ 5.61, q, J = 7 Hz), and two 3H singlets at δ 3.09 and 2.10 due to methyl ester and acetyl groups, respectively. The ^{1}H NMR spectrum also showed the presence of a doublet at δ 3.15 and a doublet of triplets at δ 4.15, due to the C-21 methylene hydrogens, and a pair of AB doublets due to the C-17 oxymethylene hydrogens at δ 4.83 and 4.77. Application of COSY and HMQC allowed assignment of the other resonances (Tables 2 and 3). The ^{1}H and ^{13}C NMR data of **10** resemble those

Table 2. ^{1}H NMR Data (δ) for **8**–**12** (400 MHz, $CDCl_3$)[a]

H	8	9	10	11	12
3	2.94 m	3.53 td (13, 5)	4.64 d (5)	3.92 m	2.39 m
	3.10 m	3.81 m			2.39 m
5	2.94 m	3.35 t (11)	2.65 m	3.56 m	2.76 ddd (11, 9.5, 1.5)
	3.17 q (8)	3.81 m	2.65 m	3.96 m	3.49 m
6	1.65 m	2.44 dd (15, 7.5)	1.88 dd (14, 4)	2.70 m	1.85 ddd
				2.70 m	(13.5, 8.8, 1.5)
	2.62 ddd	2.59 ddd	3.02 ddd		2.61 dt
	(14, 8, 4)	(15, 11, 9)	(14, 12.5, 7)		(13.5, 9.5)
9	7.23 br d (7.5)	8.20 dd (7.7, 1)	7.57 br d (7.5)	7.11 d (7.5)	7.14 br d (7.5)
10	6.77 t (7.5)	7.04 dd (8.2, 7.7)	7.16 td (7.5, 1)	6.80 t (7.5)	6.59 br t (7.5)
11	7.02 td (7.5, 1)	6.01 dd (8.2, 1)	7.31 td (7.5, 1)	7.10 t (7.5)	6.94 td (7.5, 1)
12	6.66 d (7.5)		7.61 br d (7.5)	6.67 d (7.5)	6.50 br d (7.5)
14	1.28 m	1.85 m	2.45 ddd (14.5, 5, 2.5)	2.03 dt (13.5, 4)	1.62 m
	1.83 m	1.85 m	2.56 dd (14.5, 2.5)	2.20 dt (13.5, 2.8)	1.73 m
15	1.28 m	1.43 td (13.5, 5)	3.39 m	3.41 m	3.09 d (4.5)
	1.60 m	1.66 br d (13.5)			
16	3.20 dt (11.5, 3)			2.83 d (4.5)	
17	1.78 ddd (14.5, 3, 1.5)	1.46 br d (16)	4.77 d (11.5)		4.05 d (11.8)
	2.39 ddd (14.5, 11.5, 3.5)	2.99 dd (16, 3)	4.83 d (11.5)		4.33 d (11.8)
18	1.37 m	1.56 ddd (13.5, 11, 7.5)	1.73 dd (7, 2.5)	1.58 dt (7, 1.7)	1.68 dd (5, 2)
	1.70 m	2.49 ddd (13.5, 11, 2)			
19	1.13 m	1.27 m	5.61 q (7)	5.60 br q (7)	5.48 q (6.8)
	1.37 m	1.85 m			
21	2.96 d (1.5)	3.57 br s	3.15 d (17.5)	4.07 br d (17.8)	3.00 d (15.5)
			4.15 dt (17.5, 2.5)	4.29 br d (17.8)	3.99 br d (15.5)
12-OMe		3.90 s			
CO_2Me	3.80 s	3.80 s	3.09 s	3.70 s	3.46 s
NCO_2Me		3.83 s			
OCOMe			2.10 s		
*N*CHO				8.15 s	
*N*H	3.96 br s			4.48 br s	3.90 br s
16-OH		6.96 s			

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

of akuammiline (**15**),[36] except for chemical shift changes involving the ester methyl, *O*-acetyl, and H-17 resonances. These observations suggested that **10** and **15** are epimers due to different configurations at C-16. This was confirmed by the unusual shielding observed for the ester methyl in **10** (δ 3.09 versus 3.76 in **15**) due to anisotropy from the benzene ring, as a result of the change in configuration at C-16. Compound **10** is therefore 16-epiakuammiline.

Another new akuammiline alkaloid, aspidophylline A (**11**), was obtained as colorless crystals (mp 110–111 °C), with $[\alpha]_D$ −86 ($CHCl_3$, *c* 0.09). The UV spectrum showed absorption maxima at 206, 239, and 293 nm (log ε 4.52, 4.04, and 3.58, respectively), consistent with a dihydroindole chromophore. The IR spectrum showed absorption bands at 3305, 1740, and 1656 cm^{-1}, which are assigned to *N*H, ester, and formamide functions, respectively. The EIMS showed a molecular ion at *m/z* 368 (base peak) with other significant fragment peaks at *m/z* 337 (M – OMe), 309 (M – CO_2Me), and 108 ($C_7H_{10}N$). HREIMS measurements gave the formula $C_{21}H_{24}N_2O_4$. The ^{13}C NMR spectrum (Table 3) gave a total of 21 carbon resonances, in agreement with the molecular formula. The ^{1}H NMR spectrum of **11** (Table 2) is generally similar to that of aspidodasycarpine (**16**), which was also isolated, except for the following changes. First, the CH_2OH group at C-16 is replaced by H. This was confirmed by the absence of the signals normally attributable to H-17 in both the ^{1}H and ^{13}C NMR spectra (Tables 2 and 3), while H-16 was observed as a doublet at δ 2.83. Another significant change was the presence of a formyl function at N(4). The ^{1}H NMR spectrum showed the formamide-H as a singlet at δ 8.15 (δ_C 164.4). The presence of the formyl group has resulted in pronounced changes in the chemical shifts of H-3 and H-21 when compared with aspidodasycarpine (**16**), indicating that the site of substitution of the formyl group is at N(4). In addition, irradiation of N(1)-H resulted in NOE enhancement of H-12 and vice versa, furnishing additional proof that the formyl group is on N(4). The orientation of the ester function at C-16 and that of the ethylidene side chain were determined from NOE experiments. Irradiation of H-16 resulted in NOE enhancement of H-14, which places it above the dihydroindole moiety, while the ester group is directed away from the dihydroindole moiety. The NOEs seen for H-18/H-15 and H-19/H-21 indicated that the geometry of the 19,20-double bond is *E*.

A new vincorine alkaloid, vincophylline (**12**), was isolated in minute quantities from the stem-bark extract as a yellowish oil, with $[\alpha]_D$ +102 ($CHCl_3$, *c* 0.10). The UV spectrum showed absorption maxima at 206, 241, and 296 nm, characteristic of a dihydroindole chromophore, while the IR spectrum showed bands due to *N*H/OH (3329 cm^{-1}) and ester (1730 cm^{-1}) functions. The FABMS showed an $[M + H]^+$ peak at *m/z* 355, which analyzed for $C_{21}H_{27}N_2O_3$ + H (DBE 10). The ^{13}C NMR spectrum (Table 3) gave a total of 21 carbon resonances (two methyls, six methylenes, six methines, and seven quaternary carbons), in agreement with the molecular formula. The ^{1}H NMR spectrum (Table 2) showed the presence of four aromatic hydrogens (δ 7.14, 6.94, 6.59, and 6.50), an *N*H at δ 3.90, a methoxy group at δ 3.46, a pair of AB doublets at δ 4.33 and 4.05, and an ethylidene side chain (δ 5.48 q; 1.68 dd). COSY and HMQC experiments revealed the presence of the following partial structures: two isolated methylenes (NCH_2

Table 3. ^{13}C NMR Data (δ) for **2–12** (100 MHz, $CDCl_3$)[a]

C	2	3	4	5	6	7	8	9	10	11	12
2	73.8	74.2	74.1	74.2	74.2	74.7	65.2	73.8	188.9	102.1	93.5
3	49.0	47.7	40.9	47.7	41.0	41.4	47.6	65.8	54.6	53.6	28.1
5	48.8	50.0	50.3	50.1	50.4	50.4	50.2	65.4	51.1	69.1	52.9
6	39.2	37.6	37.3	37.5	37.4	37.6	35.0	34.1	35.3	34.3	40.6
7	54.4	55.5	55.5	55.7	55.8	56.3	58.0	59.4	57.2	53.6	56.8
8	138.8	140.0	139.6	141.4	141.1	142.8	139.2	140.3	138.9	135.7	137.2
9	121.6	121.7	121.7	107.8	107.6	114.4	121.7	118.6	124.2	122.9	123.5
10	123.4	123.9	123.9	156.6	156.7	125.0	119.5	125.2	125.0	120.2	118.2
11	127.6	127.3	127.1	112.1	112.4	111.9	126.8	111.8	127.8	128.4	126.7
12	115.8	115.4	115.1	116.3	116.2	148.1	110.5	147.4	120.7	110.0	108.7
13	140.2	140.0	139.6	133.5	133.3	128.8	149.1	128.9	155.6	146.3	146.8
14	127.2	17.8	24.9	17.8	25.1	26.1	17.4	19.4	31.8	30.1	23.4
15	131.4	31.2	66.5	31.2	66.9	67.2	35.6	33.6	34.9	30.6	37.9
16	74.6	74.2	73.9	74.2	74.1	74.3	41.9	73.8	60.8	53.8	60.4
17	72.8	68.5	69.3	68.4	69.3	69.7	31.1	41.0	65.2		65.7
18	24.3	24.0	23.5	24.1	23.7	24.5	26.1	23.5	13.9	12.8	14.3
19	22.7	25.9	19.0	25.9	19.1	18.8	33.8	33.0	121.3	124.2	123.8
20	40.8	37.5	41.4	37.5	41.6	41.8	31.6	34.1	146.0	129.1	138.8
21	65.5	67.6	61.8	67.5	61.9	62.2	68.1	84.7	53.3	44.5	57.8
10-OMe				55.7	55.7						
12-OMe						56.2		56.1			
CO_2Me	52.5	52.6	52.5	52.6	52.7	52.7	51.9	52.8	51.5	51.6	51.6
CO_2Me	171.8	172.2	171.9	172.3	172.1	172.2	175.5	173.0	171.2	172.0	174.9
NCO_2Me	53.6	53.5	53.5	53.4	53.4	53.1		53.0			
NCO_2Me	156.7	157.0	156.9	156.7	156.8	157.5		157.7			
OCOMe									21.0		
OCOMe									170.4		
*N*CHO										164.4	

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

and OCH_2), an NCH_2CH_2 unit, a CH_2CH_2CH unit, and a $C{=}CHCH_3$ fragment. The NMR data (Tables 2 and 3) indicated that **12** possesses a basic vincorine-type skeleton.[37,38] This was further confirmed by the C-2 resonance at δ 93.5, the significant downfield shift due to it being linked to two nitrogen atoms. The relative configuration at C-16 was established from the NOESY spectrum, which showed NOEs between H-17 and H-6. This interaction is only possible if the CH_2OH group is directed away from the dihydroindole moiety, while the ester group is placed above the dihydroindole moiety. The geometry of the ethylidene side chain was deduced to be *E* from the observed reciprocal NOEs between H-18 and H-15. This represents the first report of the isolation of a vincorine-type compound in the genus *Kopsia*.

Rhazinal (**1**), rhazinilam (**17**), and rhazinicine (**18**, from *K. dasyrachis*)[39,40] were found to show appreciable cytotoxicity toward both drug-sensitive and vincristine-resistant KB cells (IC_{50} 0.73, 0.65, 4.06 μM for **1**, **17**, and **18**, respectively, against KB, Table 4). The observed IC_{50} value of 0.65 μM for rhazinilam against KB is in excellent agreement with that of a previous determination (0.6 μM),[41] while rhazinal and rhazinicine, previously obtained from semisynthesis, have been tested for their tubulin-binding properties, which were found to be inferior to that of rhazinilam.[42,43] Kopsiloscines A (**2**), B (**3**), and D (**5**) and aspidophylline A (**11**) were found to reverse drug-resistance in drug-resistant KB cells (Table 4), while kopsiloscines C (**4**), E (**6**), and F (**7**), which are characterized by the presence of a C(15)-OH substituent, were ineffective (IC_{50} > 25 μg/mL).[17]

Table 4. Cytotoxic Effects of **1–8**, **10–12**, **17**, and **18**

compound	IC_{50}, μg/mL (μM)		
	KB	KB/VJ300[a]	KB/VJ300(+)[b,c]
rhazinal (**1**)	0.24 (0.73)	0.25 (0.76)	0.30 (0.93)
rhazinilam (**17**)	0.19 (0.65)	0.25 (0.83)	0.34 (1.16)
rhazinicine (**18**)	1.25 (4.06)	2.5 (8.12)	1.85 (6.01)
kopsiloscine A (**2**)	19.5 (45.8)	18.0 (42.3)	3.8 (8.9)
kopsiloscine B (**3**)	>25	>25	5.0 (11.7)
kopsiloscine D (**5**)	>25	>25	11.5 (25.1)
aspidophylline A (**11**)	>25	>25	12.0 (29.1)
kopsiloscine C (**4**)	>25	>25	>25
kopsiloscine E (**6**)	>25	>25	>25
kopsiloscine F (**7**)	>25	>25	>25
16-epikopsinine (**8**)	>25	>25	>25
16-epiakuammiline (**10**)	>25	>25	>25
vincophylline (**12**)	>25	>25	>25

[a] KB/VJ300 is a vincristine-resistant human oral epidermoid carcinoma cell line.[47] [b] With added vincristine, 0.1 μg/mL (0.121 μM), which did not affect the growth of the KB/VJ300 cells. [c] The IC_{50} values of vincristine against KB and KB/VJ300 strains are 0.0023 and 1.0 μg/mL (0.0028 and 1.2 μM), respectively.

Experimental Section

General Experimental Procedures. Optical rotations were determined at 25 °C on a JASCO P-1020 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrophotometer. UV spectra were obtained on a Shimadzu UV-3101PC spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a JEOL JNM-LA 400 spectrometer at 400 and 100 MHz, respectively. MS measurements were obtained, courtesy of Dr. Komiyama of the Kitasato Institute, Tokyo, Japan, and at Organic Mass Spectrometry, Central Science Laboratory, University of Tasmania, Tasmania, Australia.

Plant Material. Plant material was collected in Pahang, Malaysia (July 1996), and identification was confirmed by Dr. David Middleton, Herbarium, Royal Botanic Garden, Edinburgh, 20A Inverleith Row, EH3 5LR Scotland. Herbarium voucher specimens (K 634) are deposited at the Herbarium, University of Malaya, Kuala Lumpur, Malaysia, and at the Rijksherbarium, University of Leiden, Leiden, The Netherlands.

Extraction and Isolation. Extraction of the leaf and stem-bark material was carried out in the usual manner by partitioning the concentrated EtOH extracts with dilute acid, as has been described in detail elsewhere.[44] 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 TLC were Et_2O/hexanes, Et_2O/cyclohexane/NH_3-saturated, EtOAc/hexanes, $CHCl_3$, EtOAc, EtOAc/MeOH, Et_2O/MeOH, $CHCl_3$/MeOH, and $CHCl_3$/MeOH/NH_3-saturated. The yields (g kg^{-1}) of the alkaloids from the leaf extract were as follows: **2** (0.009), **3** (0.013), **4** (0.022), **5** (0.003), **6** (0.012), **7** (0.010), **8** (0.008), **9** (0.007), **10** (0.004), **12** (0.003), kopsingine (1.520), kopsidine D (0.005), kopsilongine

(0.005), and 16-epideacetylakuammiline (0.030). The yields (g kg^{-1}) of the alkaloids from the stem-bark extract were as follows: **1** (0.006), **10** (0.005), **11** (0.014), **13** (0.009), **14** (0.022), kopsingine (1.283), kopsaporine (0.004), kopsinganol (0.094), rhazinilam (0.005), leuconolam (0.003), 16-epideacetylakuammiline (0.006), aspidodasycarpine (0.021), lonicerine (0.007), akuammidine (0.019), and tetrahydroalstonine (0.005).

Kopsiloscine A (2): light yellowish oil; $[\alpha]_D$ +16 (*c* 0.25, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 206 (4.31), 244 (3.96), 279 (3.28), 285 (3.23) nm; IR (dry film) ν_{max} 3507, 3288, 1736, 1673 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 426 [M]$^+$ (100), 409 (12), 394 (25), 377 (70), 367 (34), 349 (7), 308 (68), 280 (16), 253 (27), 229 (22), 216 (32); HREIMS *m/z* 426.1795 (calcd for $C_{23}H_{26}N_2O_6$, 426.1791).

Kopsiloscine B (3): colorless oil; $[\alpha]_D$ +50 (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 204 (4.32), 244 (3.96), 281 (3.27), 287 (3.23) nm; IR (dry film) ν_{max} 3515, 3200, 1736, 1676 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 428 [M]$^+$ (84), 379 (100), 369 (87), 347 (13), 309 (9), 179 (58), 123 (36); HREIMS *m/z* 428.1942 (calcd for $C_{23}H_{28}N_2O_6$, 428.1947).

Kopsiloscine C (4): light yellow crystals from Et_2O; mp 172–174 °C; $[\alpha]_D$ −40 (*c* 0.28, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 206 (3.28), 243 (2.98), 279 (2.02), 288 (1.76) nm; IR (dry film) ν_{max} 3474, 3207, 1736, 1676 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 444 [M]$^+$ (96), 395 (98), 385 (100), 367 (18), 325 (11), 309 (9), 281 (12), 243 (19); HREIMS *m/z* 444.1889 (calcd for $C_{23}H_{28}N_2O_7$, 444.1897).

Kopsiloscine D (5): colorless oil; $[\alpha]_D$ −63 (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 201 (4.59), 249 (4.27), 297 (3.67) nm; IR (dry film) ν_{max} 3445, 1730, 1672 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 458 [M]$^+$ (100), 457 (13), 409 (65), 399 (64), 377 (10), 339 (8), 294 (31), 293 (12), 264 (8), 238 (8), 209 (7); HREIMS *m/z* 458.2069 (calcd for $C_{24}H_{30}N_2O_7$, 458.2053).

Kopsiloscine E (6): colorless oil; $[\alpha]_D$ −25 (*c* 0.15, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 202 (4.46), 249 (4.14), 298 (3.51) nm; IR (dry film) ν_{max} 3356, 1735, 1671 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 474 [M]$^+$ (100), 473 (14), 456 (8), 425 (48), 415 (55), 397 (9), 393 (8), 355 (7), 339 (6), 311 (5), 273 (7), 167 (7), 149 (16); HREIMS *m/z* 474.2014 (calcd for $C_{24}H_{30}N_2O_8$, 474.2002).

Kopsiloscine F (7): light yellowish oil; $[\alpha]_D$ +9 (*c* 0.28, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 216 (3.57), 252 (3.10), 283 (2.55) nm; IR (dry film) ν_{max} 3447, 3281, 1735, 1670 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3, respectively; EIMS *m/z* 474 [M]$^+$ (74), 444 (6), 425 (23), 415 (100), 393 (15), 355 (9), 311 (6), 273 (9), 205 (10), 168 (6); HREIMS *m/z* 474.2001 (calcd for $C_{24}H_{30}N_2O_8$, 474.2002).

16-Epikopsinine (8): colorless oil; $[\alpha]_D$ +7 (*c* 0.22, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 201 (4.23), 241 (3.45), 291 (2.89) nm; IR (dry film) ν_{max} 3377, 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; EIMS *m/z* 338 [M]$^+$ (100), 310 (78), 279 (10), 254 (21), 220 (16), 205 (37), 120 (66), 118 (73), 109 (52); HREIMS *m/z* 338.1986 (calcd for $C_{21}H_{26}N_2O_2$, 338.1994).

Kopsilongine-*N*-oxide (9): colorless oil; $[\alpha]_D$ –29 (*c* 0.21, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 216 (4.15), 252 (3.58), 287 (2.51) nm; IR (dry film) ν_{max} 3320, 1735, 1672 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; FABMS *m/z* 459 [MH]$^+$ (100), 441 (24), 383 (5), 351 (5), 339 (5), 176 (10), 154 (19), 136 (15); HRFABMS *m/z* 459.2177 (calcd for $C_{24}H_{31}N_2O_7$, 459.2131).

16-Epiakuammiline (10): colorless oil; $[\alpha]_D$ +116 (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 210 (3.99), 220 (4.05), 265 (3.35) nm; IR (dry film) ν_{max} 3378, 1738 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; EIMS *m/z* 394 [M]$^+$ (97), 335 (100), 321 (13), 275 (20), 246 (21), 232 (15), 180 (17), 167 (24), 154 (12); HREIMS *m/z* 394.1887 (calcd for $C_{23}H_{26}N_2O_4$, 394.1893).

Aspidophylline A (11): colorless crystals from $CHCl_3$; mp 110–111 °C; $[\alpha]_D$ –86 (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 206 (4.52), 239 (4.04), 293 (3.58) nm; IR (dry film) ν_{max} 3305, 1740, 1656 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; EIMS *m/z* 368 [M]$^+$ (100), 337 (23), 309 (12), 232 (9), 172 (17), 159 (12), 136 (70), 108 (38); HREIMS *m/z* 368.1743 (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).

Vincophylline (12): light yellowish oil; $[\alpha]_D$ +102 (*c* 0.10, $CHCl_3$); UV (EtOH) λ_{max} (log ε) 206 (4.36), 241 (3.75), 296 (3.30) nm; IR (dry film) ν_{max} 3329, 1730 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 3, respectively; FABMS *m/z* 355 [MH]$^+$ (100), 337 (10), 323 (8), 295 (9), 265 (10), 249 (10), 222 (11), 185 (14), 167 (24), 144 (27), 109 (28); HRFABMS *m/z* 355.2030 (calcd for $C_{21}H_{27}N_2O_3$, 355.2022).

Cytotoxicity Assays. Cytotoxicity assays were carried out following the procedure that has been described in detail previously.[45,46]

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