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# Biologically Active Aspidofractinine Alkaloids from *Kopsia singapurensis*

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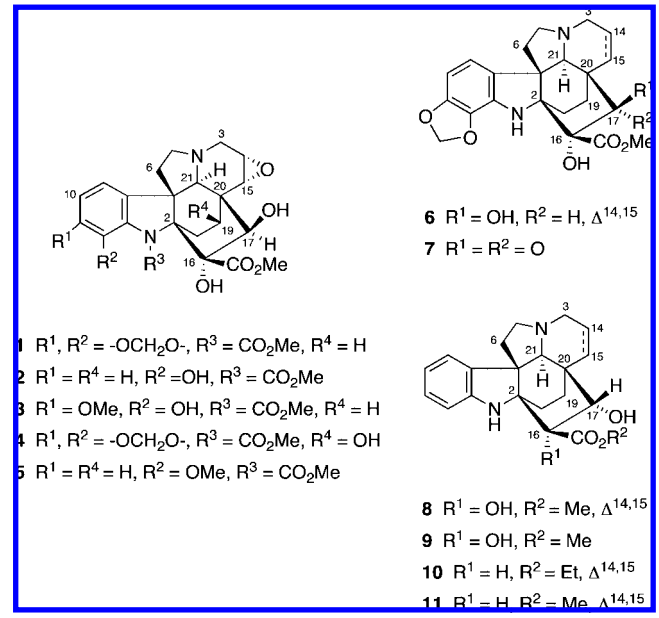
Ten new indole alkaloids of the aspidofractinine type, in addition to several recently reported indole alkaloids and 20 other known alkaloids, were obtained from the leaf and stem-bark extract of the Malayan *Kopsia singapurensis*, viz., kopsimalines A−E (**1**−**5**), kopsinicine (**6**), kopsofinone (**7**), and kopsiloscines H−J (**8**−**10**). The structures of these alkaloids were determined using NMR and MS analysis. Kopsimalines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) and kopsiloscine J (**10**) were found to reverse multidrug-resistance in vincristine-resistant KB cells, with **1** showing the highest potency.

The genus *Kopsia* (Apocynaceae), which is widely distributed in Southeast Asia,[1–3] is especially rich in indole alkaloids. The Malaysian representatives in particular have proven to be prolific producers of alkaloids with unusual carbon skeletons and interesting biological activities.[4,5] In continuation of our studies on the Malaysian members of this genus,[6–24] we now report the isolation of new indole alkaloids from *K. singapurensis*. Initial examination of the plant material indicated that it was probably a new *Kopsia* species. This was to some extent justified when preliminary studies yielded mersinine-type alkaloids, which had not been previously encountered in the genus *Kopsia*. The sample was sent to Dr. David Middleton at the Rijksherbarium, Leiden, who at the time provided only a tentative assignment of the sample as *K. fruticosa*. Accordingly we submitted preliminary communications on the structures of several novel alkaloids, including mersinines A and B,[15] mersilongine,[12] and mersicarpine,[11] isolated from this sample of *Kopsia*. Subsequently, Dr. Middleton, upon completion of his revision of the genus *Kopsia*,[3] amended the identity of this sample to *K. singapurensis*. Herein we report the structures and biological activity of 10 additional new alkaloids of the aspidofractinine type from this plant.




## Results and Discussion

The kopsimalines (**1**−**5**) represent a group of aspidofractinine-type alkaloids, all possessing an α-oriented epoxy function at C-14, C-15. The presence of the epoxide function was indicated by the characteristic signals due to H-14 and H-15 at $\delta_H$ 3.5 and 3.2, respectively, and the corresponding C-14 and C-15 carbon signals at $\delta_C$ 54 and 57, respectively. The orientation of the epoxide function in these compounds was deduced to be α from the observed reciprocal NOEs between H-17 and H-15.

Kopsimaline A (**1**) was obtained as a colorless oil, $[\alpha]_D = +159$ ($CHCl_3$, *c* 0.13). The UV spectrum showed absorption maxima at 225, 255, and 286 nm (log ε 4.43, 3.97, and 3.24, respectively) typical of a dihydroindole chromophore, while the IR spectrum showed absorption bands at 3376, 1742, and 1679 $cm^{-1}$, corresponding to hydroxyl, ester, and carbamate functions, respectively. The EI-mass spectrum showed a molecular ion at *m/z* 486, and HREIMS measurements gave the molecular formula $C_{24}H_{26}N_2O_9$.

The $^1H$ and $^{13}C$ NMR data (Tables 1 and 3, respectively) showed the presence of a methylenedioxy substituent at C-11 and C-12 (a pair of aromatic AB doublets at $\delta_H$ 6.51 and 6.56 and another pair at $\delta_H$ 5.91 and 5.95; $\delta_C$ 100.7), two hydroxyl groups (16-OH, $\delta_H$ 6.09; 17-OH, $\delta_H$ 8.30), a carbamate function at N-1 ($\delta_C$ 154.5), and an ester group ($\delta_C$ 171.6). The NMR signals, assigned with the aid of COSY and HMQC, indicated that **1** was an aspidofractinine-type alkaloid. The NMR data resembled that of 14,15-epoxykopsingine, which was also isolated from the leaf extract of the same plant, except for the absence of the aromatic methoxy signal and the presence of a methylenedioxy substituent at C-11 and C-12.[25] The orientation of the 17-OH in **1** was determined to be *β* from the *W* coupling observed between H-17α and H-21α.[26] The orientation of the 14,15-epoxide function was deduced to be α from the NOESY spectrum, which revealed a NOE between H-17 and H-15.

Kopsimaline B (**2**) was obtained as a colorless oil, $[\alpha]_D = -5$ ($CHCl_3$, *c* 0.53). The UV and IR spectra of **2** were nearly identical to those of **1**. The EI-mass spectrum showed an $M^+$ at *m/z* 458, consistent with the molecular formula $C_{23}H_{26}N_2O_8$. The $^{13}C$ NMR spectrum (Table 3) showed a total of 23 carbon resonances, in agreement with the formula derived from HRMS. Comparison of the $^1H$ NMR spectrum of **2** with that of **1** (Table 1) revealed that these two compounds were similar, except in the aromatic region,

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**Table 1.** $^1$H NMR Data for **1**−**5** (400 MHz, $CDCl_3$)[a]

| H | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 3 | 2.97 d (12.5) | 2.99 d (12.5) | 2.97 d (12.5) | 2.97 m | 2.96 d (12.5) |
| | 3.44 dd (12.5, 5) | 3.46 dd (12.5, 5) | 3.45 dd (12.5, 5) | 3.44 dd (12.5, 5) | 3.45 dd (12.5, 5) |
| 5 | 2.43 ddd (12.5, 8.5, 4) | 2.48 ddd (12.5, 8.5, 4) | 2.45 ddd (12.5, 8.5, 4) | 2.50 ddd(12.5, 8.5, 4) | 2.48 ddd (12.5, 8.5, 4) |
| | 2.76 dd (8.5, 6) | 2.78 dd (8.5, 6) | 2.77 dd (8.5, 6) | 2.82 dd (8.5, 6) | 2.79 dd (8.5, 6) |
| 6 | 1.58 dd (12.5, 4) | 1.63 dd (12.5, 4) | 1.62 dd (12.5, 4) | 1.57 dd (12.5, 4) | 1.70 m |
| | 2.97 m | 3.09 td (12.5, 6) | 3.05 td (12.5, 6) | 2.97 m | 3.10 td (12.5, 6) |
| 9 | 6.56 d (7.8) | 6.65 d (7.8) | 6.56 d (8) | 6.53 m | 6.73 d (7.8) |
| 10 | 6.51 d (7.8) | 7.04 t (7.8) | 6.63 d (8) | 6.53 m | 7.03 t (7.8) |
| 11 | | 6.86 d (7.8) | | | 6.85 d (7.8) |
| 14 | 3.54 dd (5, 4) | 3.55 dd (5, 4) | 3.55 dd (5, 4) | 3.58 m | 3.54 dd (5, 4) |
| 15 | 3.19 d (4) | 3.19 d (4) | 3.19 d (4) | 3.56 m | 3.18 d (4) |
| 17 | 4.02 br s | 4.03 br s | 4.02 br s | 4.45 br s | 4.01 br s |
| 18 | 1.49 m | 1.51 m | 1.51 m | 2.02 m | 1.42 m |
| | 2.07 m | 1.97 m | 2.00 m | 2.02 m | 2.10 t (12) |
| 19 | 1.49 m | 1.51 m | 1.51 m | 3.76 br d (10.5) | 1.46 m |
| | 1.74 m | 1.74 dd (10.5, 4.8) | 1.73 m | | 1.72 m |
| 21 | 2.85 d (1.5) | 2.91 d (1.5) | 2.86 d (1.8) | 2.75 br s | 2.86 br s |
| 11-OMe | | | 3.88 s | | |
| 12-OMe | | | | | 3.84 s |
| $CO_2Me$ | 3.81 s | 3.84 s | 3.84 s | 3.83 s | 3.80 s |
| $NCO_2Me$ | 3.82 s | 3.84 s | 3.85 s | 3.84 s | 3.81 s |
| 12-OH | | 9.28 br s | | | |
| 16-OH | 6.09 br s | | 6.00 br s | 6.72 br s | 5.81 s |
| 17-OH | 8.30 br s | 8.54 br s | 8.47 br s | 8.43 br s | 8.27 br s |
| $OCH_2O$ | 5.91 d (1.5) | | | 5.92 d (1.5) | |
| | 5.95 d (1.5) | | | 5.95 d (1.5) | |

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

**Table 2.** $^1$H NMR Data for **6**–**10** (400 MHz, $CDCl_3$)[a]

| H | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|
| 3 | 3.16 dt (16, 2) | 3.07 m | 3.38 br d (16.5) | 2.94 m | 3.44 br d (16.7) |
| | 3.49 ddd (16, 4.5, 2) | 3.07 m | 3.50 ddd (16.5, 4, 2) | 3.15 m | 3.50 ddd (16.7, 3.7, 2) |
| 5 | 2.91 dd (8.5, 6) | 2.69 m | 2.77 m | 2.94 m | 2.81 m |
| | 2.59 ddd (12, 8.5, 4) | 2.75 td (11, 5) | 2.94 t (7) | 3.05 t (7.5) | 2.97 ddd (8, 6.5, 1.5) |
| 6 | 1.61 dd (12, 4) | 1.60 m | 1.30 dd (12.5, 4) | 1.49 m | 1.32 m |
| | 3.11 td (12, 6) | 2.32 dd (14.5, 5) | 2.28 ddd (12.5, 10, 7) | 2.30 ddd (13.5, 7.5, 5.5) | 2.50 ddd (12, 9.8, 6.5) |
| 9 | 6.57 d (7.7) | 6.77 d (7.8) | 7.05 d (7.5) | 7.15 d (7.5) | 7.05 br d (7.5) |
| 10 | 6.32 d (7.7) | 6.35 d (7.8) | 6.74 t (7.5) | 6.74 td (7.5, 1) | 6.74 td (7.5, 1) |
| 11 | | | 7.03 td (7.5, 1) | 7.00 td (7.5, 1) | 7.03 td (7.5, 1) |
| 12 | | | 6.68 d (7.5) | 6.65 d (7.5) | 6.70 br d (7.5) |
| 14 | 5.97 ddd (10, 4.5, 2) | 1.28 m | 5.85 ddd (10, 4, 2) | 1.36 br d (13) | 5.89 ddd (10, 3.7, 2) |
| | | 1.93 m | | 1.85 m | |
| 15 | 5.67 td (10, 2) | 1.08 td (13.5, 4) | 5.64 dt (10, 2) | 1.02 td (13, 4) | 5.67 dt (10, 2) |
| | | 2.27 br d (13.5) | | 2.10 br d (13) | |
| 16 | | | | | 2.75 dd (7.7, 1) |
| 17 | 3.60 d (2) | | 5.08 br s | 5.11 br s | 5.00 dd (7.7, 2) |
| 18 | 1.72 ddd (12.5, 10, 8) | 2.01 ddd (13, 11, 7.5) | 1.67 m | 1.72 m | 1.29 m |
| | 1.83 ddd (12.5, 10, 2) | 2.09 ddd (13, 11, 2.5) | 1.92 m | 1.72 m | 1.89 dddd (13, 11, 7.5, 1) |
| 19 | 1.23 ddd (12.5, 10, 2) | 1.44 ddd (13, 11, 2.5) | 1.02 td (11, 2) | 0.87 m | 1.12 ddt (13, 11, 2) |
| | 1.65 ddd (12.5, 10, 8) | 1.64 m | 1.92 m | 1.85 m | 2.11 ddd (13, 11, 7.5) |
| 21 | 2.77 d (2) | 3.56 s | 2.69 s | 2.95 s | 2.73 s |
| $CO_2Me$ | 3.82 s | 3.83 s | 3.83 s | 3.85 s | |
| 16-OH | 8.22 br s | | | | |
| NH | 3.41 br s | 4.11 br s | 4.34 br s | 4.26 br s | 3.82 br s |
| $OCH_2O$ | 5.83 d (1.5) | 5.86 d (1.5) | | | |
| | 5.91 d (1.5) | 5.93 d (1.5) | | | |
| $CO_2\underline{C}H_2CH_3$ | | | | | 4.25 q (7) |
| $CO_2CH_2\underline{C}H_3$ | | | | | 1.31 t (7) |

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

where the methylenedioxy function is absent in the spectrum of **2** and only three aromatic hydrogen signals are seen at $\delta$ 6.65 (d, $J$ = 7.8 Hz; H-9), 7.04 (t, $J$ = 7.8 Hz; H-10), and 6.86 (d, $J$ = 7.8 Hz; H-11). This, and the observed aromatic carbon resonances, indicated substitution at C-12, which was confirmed by NOE difference experiments. Irradiation of the doublet at $\delta$ 6.65 caused enhancement of the H-21 signal ($\delta$ 2.91), which allowed the assignment of this signal to H-9 and indicated the aromatic substitution to be at C-12. Since no additional methoxy signals were observed in the NMR spectra, it was concluded that the C-12 substituent was an OH function.

Kopsimaline C (**3**) was isolated as a colorless oil, $[\alpha]_D = -27$ ($CHCl_3$, *c* 0.09). The EI-mass spectrum showed a molecular ion at *m/z* 488, and HREIMS measurements gave the molecular formula, $C_{24}H_{28}N_2O_9$, differing from **2** by 30 mass units and suggesting that it differed from **2** in having an additional methoxy group. Comparison of the $^1$H NMR spectra (Table 1) indicated a general similarity of **2** and **3**, except for some notable differences in the aromatic region. The aromatic region of **3** showed only two hydrogens, which appeared as a pair of AB doublets centered at $\delta$ 6.56 and 6.63 ($J$ = 8 Hz), indicating an *ortho* arrangement. The two possible arrangements of the aromatic substituents are 11-OH,

**Table 3.** $^{13}C$ NMR Data ($\delta$) for **1**–**10** (100 MHz, $CDCl_3$)[a]

| C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 75.8 | 76.7 | 77.5 | 75.5 | 76.7 | 71.3 | 71.0 | 71.2 | 70.7 | 67.4 |
| 3 | 48.4 | 48.3 | 48.4 | 48.3 | 48.5 | 50.1 | 47.7 | 49.8 | 48.0 | 49.6 |
| 5 | 48.1 | 48.1 | 48.2 | 48.8 | 48.5 | 48.9 | 49.4 | 49.3 | 50.4 | 49.6 |
| 6 | 39.8 | 39.5 | 39.7 | 39.2 | 39.9 | 39.8 | 36.5 | 37.0 | 35.5 | 37.0 |
| 7 | 55.6 | 56.2 | 55.5 | 55.6 | 56.5 | 55.1 | 56.8 | 54.8 | 56.2 | 55.9 |
| 8 | 135.5 | 142.9 | 135.4 | 136.0 | 143.8 | 136.5 | 135.6 | 138.7 | 139.7 | 139.1 |
| 9 | 113.1 | 118.5 | 111.0 | 113.2 | 113.3 | 112.9 | 114.2 | 121.0 | 121.2 | 121.3 |
| 10 | 103.2 | 126.5 | 107.9 | 103.5 | 125.1 | 99.5 | 100.6 | 119.1 | 119.6 | 119.3 |
| 11 | 148.7 | 112.1 | 153.5 | 149.0 | 112.0 | 147.7 | 147.5 | 127.0 | 126.8 | 127.0 |
| 12 | 136.5 | 146.5 | 136.7 | 135.5 | 149.3 | 133.2 | 132.2 | 111.0 | 110.7 | 111.1 |
| 13 | 122.0 | 125.5 | 126.1 | 121.4 | 128.3 | 131.1 | 130.4 | 149.4 | 149.1 | 149.2 |
| 14 | 53.6 | 53.7 | 53.5 | 53.4 | 53.6 | 128.8 | 16.6 | 128.4 | 18.5 | 129.3 |
| 15 | 57.0 | 56.6 | 56.8 | 54.6 | 57.1 | 131.3 | 30.3 | 131.3 | 31.4 | 130.6 |
| 16 | 79.7 | 81.0 | 80.9 | 79.3 | 80.0 | 81.4 | 79.2 | 76.8 | 76.5 | 53.9 |
| 17 | 81.9 | 82.8 | 82.6 | 77.2 | 81.9 | 84.0 | 209.6 | 71.9 | 67.9 | 73.3 |
| 18 | 26.2 | 27.4 | 27.3 | 37.4 | 26.7 | 27.7 | 24.8 | 26.7 | 26.6 | 34.4 |
| 19 | 23.5 | 23.7 | 23.7 | 66.6 | 23.4 | 26.6 | 31.1 | 23.6 | 26.7 | 23.5 |
| 20 | 38.2 | 37.8 | 37.9 | 42.6 | 38.4 | 39.5 | 45.1 | 40.8 | 37.3 | 40.8 |
| 21 | 62.8 | 62.6 | 62.8 | 61.8 | 62.7 | 69.0 | 70.1 | 66.5 | 67.8 | 65.9 |
| 11-OMe | | | 56.5 | | | | | | | |
| 12-OMe | | | | | 56.0 | | | | | |
| $CO_2\underline{Me}$ | 52.0 | 52.0 | 52.0 | 52.2 | 52.0 | 51.9 | 53.0 | 52.8 | 53.0 | |
| $\underline{C}O_2Me$ | 171.6 | 171.7 | 171.7 | 170.7 | 171.9 | 170.5 | 170.8 | 174.0 | 174.6 | |
| $NCO_2\underline{Me}$ | 53.2 | 53.5 | 53.8 | 53.4 | 53.1 | | | | | |
| $N\underline{C}O_2Me$ | 154.5 | 153.5 | 153.5 | 154.6 | 155.8 | | | | | |
| $OCH_2O$ | 100.7 | | | 100.8 | | 100.7 | 100.8 | | | |
| $CO_2CH_2\underline{C}H_3$ | | | | | | | | | | 14.4 |
| $CO_2\underline{C}H_2CH_3$ | | | | | | | | | | 60.9 |
| $\underline{C}O_2CH_2CH_3$ | | | | | | | | | | 172.7 |

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

12-OMe and 11-OMe, 12-OH. Examination of the carbon chemical shifts (Table 3) showed that the values were more consistent with the 11-OMe, 12-OH aromatic substitution pattern.[25] This was confirmed by NOE experiments where irradiation of the aromatic OMe signal resulted in enhancement of the aromatic H-10 signal, thus confirming the position of aromatic methoxy substitution at C-11. Compound **3** is therefore the 11-methoxy derivative of **2**.

Kopsimaline D (**4**) was obtained as a colorless oil, $[\alpha]_D = +83$ ($CHCl_3$, *c* 0.36). The UV spectrum ($\lambda_{max}$ 225, 245, 256, and 286 nm) was similar to that of **1**, showing absorption maxima characteristic of a dihydroindole chromophore, while the IR spectrum showed bands due to OH (3350 and 3481 $cm^{-1}$), ester (1742 $cm^{-1}$), and carbamate (1677 $cm^{-1}$) functions. The mass spectrum gave a molecular ion at *m/z* 502, and HREIMS measurements (*m/z* 502.1561) established the formula $C_{24}H_{26}N_2O_{10}$ (calcd 502.1588). The $^{13}C$ NMR spectrum (Table 3) accounted for a total of 24 carbon resonances (two methyls, five methylenes, seven methines, and 10 quaternary carbons) and showed a general similarity to that of **1**. The molecular formula of **4** showed that it differed from **1** ($C_{24}H_{26}N_2O_9$) by an additional oxygen atom. This was supported by the NMR data, which confirmed the presence of an OH function as shown by the H and C resonances due to an oxymethine ($\delta_H$ 3.76, $\delta_C$ 66.6). Analysis of the COSY and HMQC data indicated that the OH group could be at either C-5 or C-6, or alternatively, at C-18 or C-19. On chemical shift grounds, however, the NMR data were more consistent with placement of the OH group at C-18 or C-19.[27,28] Confirmation of the position of the OH was provided by HMBC, which showed $^3J$ correlations from H-15 and H-21 to C-19. Irradiation of the H-19 signal resulted in a NOE enhancement of the H-21 resonance, and *vice versa*, indicating that the orientation of the OH substituent was $\beta$. Furthermore, irradiation of H-17 caused enhancement of the H-15 signal, providing support for $\alpha$-orientation of the epoxide function. The orientation of the 17-OH function was determined to be $\beta$ from the *W* coupling observed between H-17$\alpha$ and H-21.

Kopsimaline E (**5**) was first reported from leaves of *K. teoi* and was then identified as 14,15-$\beta$-epoxykopsingine.[25] The same compound has been obtained in the present study. The IR, UV, MS, and NMR data were similar to those of the previously reported compound.[25] In the previous report the orientation of the epoxide function was deduced to be $\beta$ on the basis of the apparent NOE observed between H-21 and H-14 ($^1H$ NMR at 270 MHz). In the present study, extensive NOE/NOESY experiments were repeated at 400 MHz, and the results indicated that a revision was necessary. In the present instance, irradiation of H-21 resulted only in enhancement of H-9 and *vice versa*, while irradiation of H-14 resulted in enhancement of H-3 and H-15. Irradiation of H-17 on the other hand caused enhancement of H-15 and *vice versa*. On the basis of these results the orientation of the 14,15-epoxide function was clearly shown to be $\alpha$.

Kopsinicine (**6**) was isolated as a colorless oil, $[\alpha]_D = +22$ ($CHCl_3$, *c* 0.38). The UV spectrum showed absorption maxima at 219, 246, and 279 nm characteristic of a dihydroindole chromophore. The IR spectrum showed bands at 3474, 3339, and 1745 $cm^{-1}$, indicating the presence of OH, NH, and ester groups, respectively. The mass spectrum revealed a molecular ion at *m/z* 412, corresponding to the molecular formula $C_{22}H_{24}N_2O_6$. The fragment at *m/z* 384 was attributed to loss of ethene and originated from a retro Diels–Alder fragmentation, characteristic of alkaloids possessing an aspidofractinine skeleton. Other major fragments were observed at *m/z* 368 ($M - CO_2$), 353 ($M - CO_2Me$), 122, and 107. The $^{13}C$ NMR spectrum (Table 3) is in agreement with the molecular formula deduced from the mass spectrum, accounting for all 22 carbons. The $^1H$ NMR spectrum (Table 2) showed signals due to adjacent aromatic hydrogens (AB doublets at $\delta$ 6.57, 6.32), a methylenedioxy function ($\delta$ 5.91, 5.83), two olefinic hydrogens ($\delta$ 5.97, 5.67), a singlet due to an ester methoxy group ($\delta$ 3.82), and two broad singlets at $\delta$ 8.22 and 3.41 due to 16-OH and indolic *N*H, respectively. The COSY and HMQC experiments showed the presence of two isolated methines, corresponding to H-17 and H-21, a $CH_2-CH{=}CH$ unit, corresponding to the C(3)−C(14)−C(15) fragment, and two other $CH_2-CH_2$ units, which were assigned to the C(5)−C(6) and C(18)−C(19) fragments of an aspidofractinine-type molecule. The NMR data therefore indicated that **6** was 11,12-methylenedioxykopsinol. The orientation of the 17-OH was determined to be $\beta$ from the *W* coupling ($J_{17-21} = 2$ Hz) observed

between H-17α (δ 3.60) and H-21 (δ 2.77). Further confirmation of this was provided by NOE experiments. Irradiation of the H-17 signal resulted in enhancement of H-15, while irradiation of the 17-OH signal resulted in enhancement of H-6β. These interactions are possible only if the orientation of H-17 is α and C(17)-OH is β.

Kopsofinone (**7**) was isolated in amorphous form, $[\alpha]_D = +37$ ($CHCl_3$, *c* 0.27). The UV spectrum ($\lambda_{max}$ 218, 244, and 284 nm) was similar to that of **6**, while the IR spectrum showed absorption bands at 3444 (NH or OH) and 1745 $cm^{-1}$ (ketone or ester). The EI-mass spectrum showed a molecular ion at *m/z* 412, and HREIMS measurements indicated the molecular formula $C_{22}H_{24}N_2O_6$. The $^1H$ and $^{13}C$ NMR data of **7** (Tables 2 and 3) were similar to those of **6** except for some notable differences. The signals due to the 14,15-double bond, H-17, and 17-OH were absent in both the $^1H$ and $^{13}C$ NMR spectra of compound **7**. The signals for the 14,15-olefinic hydrogens had been replaced by methylenes, which were shifted upfield, while signals corresponding to H-17 and 17-OH were absent. The presence of a ketone function at C-17 was indicated by the carbon resonance at δ 209.6 in the $^{13}C$ NMR spectrum.

The kopsiloscines A−G are a group of aspidofractinine compounds all having an α-oriented OH group at C-17.[29] In the present study an additional three new compounds of this group were obtained.

Kopsiloscine H (**8**) was isolated in minute amounts from the bark extract of *K. singapurensis*. The UV spectrum exhibited absorptions at 203, 243, and 293 nm, indicating the presence of a dihydroindole chromophore. The EIMS established its mass to be 368, corresponding to the molecular formula $C_{21}H_{24}N_2O_4$, indicating 11 degrees of unsaturation in the molecule. Major fragments observed at *m/z* 340, 122, and 107 are characteristic of aspidofractinine-type compounds. The $^1H$ NMR spectrum (Table 2) showed signals due to four hydrogens in the aromatic region. The signals at δ 7.05 (d, $J = 7.5$ Hz) and 6.68 (d, $J = 7.5$ Hz) were assigned to H-9 and H-12, respectively, while H-10 and H-11 appeared as triplets at δ 6.74 and 7.03, respectively. Two signals in the olefinic region at δ 5.85 (ddd, $J = 10$, 4, 2 Hz) and 5.64 (dt, $J = 10$, 2 Hz) were assigned to H-14 and H-15, respectively. The corresponding C-14 and C-15 resonances were found at δ 128.4 and 131.3, respectively. A broad singlet at δ 4.34 was assigned to the dihydroindole NH. The two H-3 signals ($\delta_H$ 3.38 and 3.50) showed cross-peaks to both olefinic hydrogens in the COSY spectrum, indicating the partial structure $CH_2CH{=}CH$, corresponding to the C(3)−C(14)−C(15) fragment. The COSY experiment also showed the presence of two $CH_2{-}CH_2$ fragments, which were assigned to the C(5)−C(6) and C(18)−C(19) fragments of an aspidofractinine-type molecule. The NMR data were quite similar to those of kopsinol[30] except for the H-17 resonance, which was shifted to lower field and which showed *W* coupling ($J = 2$ Hz) to H-19. This observation indicated that the orientation of H-17 is β and not α, as in kopsinol.

Kopsiloscine I (**9**) was obtained as a colorless oil, $[\alpha]_D = -29$ ($CHCl_3$, *c* 0.09). Its molecular formula was determined to be $C_{21}H_{26}N_2O_4$ from EIMS, differing from kopsiloscine H (**8**) by addition of two hydrogen atoms. The UV spectrum (205, 242, and 293 nm) was similar to that of **8**. This was supported by the $^1H$ and $^{13}C$ NMR data (Tables 2 and 3, respectively), which were also similar to those of **8**, except for the signals of the 14,15-double bond, which were absent. The 14,15-olefinic hydrogen signals were replaced by methylenes, which were found upfield. The same behavior was also shown by the C-14 and C-15 resonances, which also indicated that compound **9** is a 14,15-dihydro derivative of **8**.

Kopsiloscine J (**10**) was obtained as a colorless oil, $[\alpha]_D = +22$ ($CHCl_3$, *c* 0.23). The UV spectrum showed absorption maxima at 207, 241, and 289 nm, indicating the presence of an unsubstituted dihydroindole chromophore. The EIMS showed a molecular ion at *m/z* 366 ($C_{22}H_{26}N_2O_3$) with a fragment at *m/z* 337 ($M - CH_2CH_3$). The $^1H$ and $^{13}C$ NMR data (Tables 2 and 3, respectively) indicated an aspidofractinine derivative resembling 17-α-hydroxy-$\Delta^{14,15}$-kopsinine (**11**),[26,31] except that the C-16 methyl ester group was absent, having been replaced by a $CO_2CH_2CH_3$ function instead. This conclusion was supported by the $^1H$ NMR spectrum, which showed a triplet at δ 1.31 and a quartet at δ 4.25 due to an ethyl ester function. The orientation of the 17-OH in kopsiloscine J (**10**) was deduced to be α from the *W* coupling observed between H-17β and H-19α.

None of the compounds tested showed any appreciable cytotoxicity toward drug-sensitive or vincristine-resistant KB (KB/VJ300) cells ($IC_{50} > 25$ μg/mL).[37] Kopsimalines A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) and kopsiloscine J (**10**), however, were found to reverse multidrug-resistance in vincristine-resistant KB cells, with **1** showing the highest potency ($IC_{50}$ 3.9 μg/mL (8 μM) in the presence of 0.1 μg/mL (0.121 μM) vincristine). The $IC_{50}$ values for **2**, **3**, **4**, **5**, and **10** are 13.0, 18.2, 9.2, 18.0, and 15.0 μg/mL, respectively, in the presence of 0.1 μg/mL vincristine.

## Experimental Section

**General Experimental Procedures.** Optical rotations were determined 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. ESIMS were obtained on a Perkin-Elmer API 100 instrument. Mass spectra 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 and partitioning of the concentrated EtOH extracts with dilute acid were carried out as described in detail elsewhere.[32] 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 $Et_2O$, $CHCl_3$, EtOAc, $Et_2O$/hexane, $Et_2O$/MeOH, EtOAc/MeOH, $CHCl_3/NH_3$-saturated, and $CHCl_3$/MeOH. The yields (g $kg^{-1}$) of the alkaloids from the leaf extract[33] were as follows: **1** (0.003), **2** (0.011), **3** (0.002), **4** (0.012), **5** (0.003), **6** (0.001), **7** (0.006), **10** (0.006), **11** (0.001), mersilongine (0.008),[12] mersirachine (0.001),[34] mersinaline (0.003),[34] kopsiloscine G (0.001), *N*-decarbomethoxykopsamine (0.002), rhazinilam (0.002), 5,21-dihydrorhazinilam (0.003), 16-*epi*-akuammiline (0.003), 16-*epi*-deacetylakuammiline (0.006), and akuammidine (0.005). The yields (g $kg^{-1}$) of the alkaloids from the stem-bark extract were as follows: **8** (0.011), **9** (0.015), mersicarpine (0.002),[11] rhazinilam (0.001), 5,21-dihydrorhazinilam (0.064), leuconolam (0.006), leuconoxine (0.001), kopsinine (0.006), 16-*epi*-akuammiline (0.001), deacetylakuammiline (0.002), 16-*epi*-deacetylakuammiline (0.003), aspidodasicarpine (0.008), lonicerine (0.003), burnamine (0.002), 16-hydroxymethylpleiocarpamine (0.001), picramicine (0.001), mossambine (0.002), 14α-hydroxycondylocarpine (0.001), akuammidine (0.003), and tetrahydroalstonine (0.001).

**Kopsimaline A (1):** colorless oil; $[\alpha]_D$ +159 (*c* 0.13, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 225 (4.43), 255 (3.97), 286 (3.24) nm; IR (dry film) $\nu_{max}$ 3376, 1742, 1679 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 1 and 3, respectively; EIMS *m/z* 486 $[M]^+$ (100), 472 (23), 427 (18), 399 (35), 398 (56), 370 (45), 355 (22), 300 (29), 271 (15), 174 (10), 113 (10); HREIMS *m/z* 486.1640 (calcd for $C_{24}H_{26}N_2O_9$, 486.1638).

**Kopsimaline B (2):** colorless oil; $[\alpha]_D$ –5 (*c* 0.53, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log ε) 217 (4.38), 244 (3.97), 284 (3.59) nm; IR (dry film) $\nu_{max}$ 3551, 3426, 1742, 1666 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 1 and 3, respectively; EIMS *m/z* 458 $[M]^+$ (100), 400 (28), 342

(29), 341 (80), 297 (18), 283 (16), 205 (27), 187 (15), 172 (14), 152 (9); HREIMS *m/z* 458.1711 (calcd for $C_{23}H_{26}N_2O_8$, 458.1689).

**Kopsimaline C (3):** colorless oil; $[\alpha]_D$ –27 (*c* 0.09, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 220 (4.53), 252 (3.89), 283 (3.42) nm; IR (dry film) $\nu_{max}$ 3536, 3406, 1742, 1666 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 1 and 3, respectively; EIMS *m/z* 488 $[M]^+$ (79), 431 (100), 371 (96), 329 (50), 218 (42), 141 (31), 116 (27); HREIMS *m/z* 488.1786 (calcd for $C_{24}H_{28}N_2O_9$, 488.1795).

**Kopsimaline D (4):** colorless oil; $[\alpha]_D$ +83 (*c* 0.36, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 225 (4.15), 245 (3.72), 256 (3.62), 286 (3.07) nm; IR (dry film) $\nu_{max}$ 3350, 3481, 1742, 1677 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 1 and 3, respectively; EIMS *m/z* 502 $[M]^+$ (100), 469 (12), 443 (16), 415 (24), 414 (49), 400 (12), 397 (21), 386 (26), 385 (52), 314 (12), 287 (12), 260 (13); HREIMS *m/z* 502.1561 (calcd for $C_{24}H_{26}N_2O_{10}$, 502.1588).

**Kopsimaline E (5):** colorless oil; $[\alpha]_D$ +11 (*c* 0.12, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$ 217 (4.48), 254 (3.92), 287 (2.82) nm; IR (dry film) $\nu_{max}$ 3393, 1742, 1671 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 1 and 3, respectively; ESIMS *m/z* 473 $[M + H]^+$.

**Kopsinicine (6):** colorless oil; $[\alpha]_D$ +22 (*c* 0.38, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 219 (4.27), 246 (3.69), 279 (3.07) nm; IR (dry film) $\nu_{max}$ 3474, 3339, 1745 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 2 and 3, respectively; EIMS *m/z* 412 $[M]^+$ (100), 384 (32), 368 (23), 324 (66), 295 (84), 266 (41), 239 (27), 205 (96), 188 (40), 158 (19), 122 (11), 107 (28); HREIMS *m/z* 412.1659 (calcd for $C_{22}H_{24}N_2O_6$, 412.1634).

**Kopsofinone (7):** colorless, amorphous solid; $[\alpha]_D$ +37 (*c* 0.27, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 218 (4.23), 244 (3.73), 284 (3.01) nm; IR (dry film) $\nu_{max}$ 3444, 1745 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 2 and 3, respectively; EIMS *m/z* 412 $[M]^+$ (100), 367 (39), 337 (33), 324 (66), 295 (26), 253 (15), 213 (15), 168 (39), 140 (20), 109 (31); HREIMS *m/z* 412.1630 (calcd for $C_{22}H_{24}N_2O_6$, 412.1634).

**Kopsiloscine H (8):** yellowish oil; $[\alpha]_D$ –13 (*c* 0.17, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 203 (3.59), 243 (3.01), 293 (2.62) nm; $^1H$ NMR and $^{13}C$ NMR data, Tables 2 and 3, respectively; EIMS *m/z* 368 $[M]^+$ (100), 340 (14), 308 (16), 280 (15), 249 (13), 232 (13), 173 (12), 144 (12), 123 (13), 108 (11); HREIMS *m/z* 368.1739 (calcd for $C_{21}H_{24}N_2O_4$, 368.1736).

**Kopsiloscine I (9):** colorless oil; $[\alpha]_D$ –29 (*c* 0.09, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 205 (3.90), 242 (3.34), 293 (2.96) nm; $^1H$ NMR and $^{13}C$ NMR data, Tables 2 and 3, respectively; EIMS *m/z* 370 $[M]^+$ (100), 342 (16), 321 (14), 281 (17), 240 (20), 221 (10), 207 (26), 182 (15), 140 (11), 124 (16), 119 (57); HREIMS *m/z* 370.1895 (calcd for $C_{21}H_{26}N_2O_4$, 370.1893).

**Kopsiloscine J (10):** colorless oil; $[\alpha]_D$ +22 (*c* 0.23, $CHCl_3$); UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 207 (4.41), 241 (3.96), 289 (3.58) nm; IR (dry film) $\nu_{max}$ 3433, 3352, 1725 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR data, Tables 2 and 3, respectively; EIMS *m/z* 366 $[M]^+$ (100), 337 (18), 265 (27), 248 (37), 216 (56), 183 (31), 167 (36), 156 (60), 135 (76), 123 (57); HREIMS *m/z* 366.1956 (calcd for $C_{22}H_{26}N_2O_3$, 366.1943).

**Cytotoxicity Assays.** Cytotoxicity assays were carried out following procedures described in detail previously.[35,36]

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