Inhibitory Effects of Diterpenoid Alkaloids on the Growth of A172 Human Malignant Cells

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The cytotoxicity against A172 human malignant glioma cells was examined for 14 alkaloids from the roots of *Aconitum yesoense* var. *macroyesoense* and of *Aconitum japonicum* and from the seeds of *Delphinium elatum* as well as for 25 semisynthetic derivatives. The major alkaloid constituents of *A. yesoense* var. *macroyesoense,* kobusine (**2**) and pseudokobusine (**3**), a minor alkaloid constituent of *A. japonicum*, aljesaconitine A (**5**), and six alkaloid derivatives, *N*-deethyldelcosine (**10**), *N*-deethyldelsoline (**11**), 12-benzoylluciculine (**18**), 12-anisoylluciculine (**19**), 6,11-dibenzoylpseudokobusine (**28**), and 6-veratroylpseudokobusine (**29**), had only very weak activity. Four acylated alkaloid derivatives, 12-acetylluciculine (**23**), 11-veratroylpseudokobusine (**30**), 11-(*m*-trifluoromethylbenzoyl)pseudokobusine (**32**), and 11-(*m*-trifluoromethylbenzoyl)kobusine (**39**), exhibited more potent activity, while pseudokobusine 11-cinnamoate (**31**), 11-anisoate (**33**), and 11-*p*-nitrobenzoate (**34**) were found to be the most potent cytotoxic agents.

A large number of diterpenoid alkaloids have been isolated from various species of *Aconitum* and *Delphinium* (Ranunculaceae) and are classified according to their chemical structure as C_{19}-norditerpenoid alkaloids, which consist of an aconitine or a lycoctonine skeleton, and C_{20}-diterpenoid alkaloids, consisting of an atisine or a veatchine skeleton (Chart S1, Supporting Information). The former group includes aconitine, mesaconitine, hypaconitine, and jesaconitine, all having extremely high toxicity, whereas the latter group, including lucidusculine, kobusine, pseudokobusine, and atisine, are far less toxic.[1] The roots of *Aconitum* plants have been used as "bushi", a herbal drug in some prescriptions of traditional Chinese medicine for the treatment of hypometabolism, dysuria, cardiac weakness, chills, neuralgia, gout, and certain rheumatic diseases.[1]

The pharmacological properties of the C_{19}-norditerpenoid alkaloids have been studied extensively and reviewed.[2] Aconitine is a representative toxin that exhibits activity both centrally and peripherally, with predominant effects on the cardiovascular and respiratory systems, by preventing the normal closing of sodium channels.[2] However, there is little information about the pharmacological properties of the C_{20}-diterpenoid alkaloids and their chemically transformed products. Two reports on the effects of the C_{19}-norditerpenoid alkaloids on cancer cells have appeared in recent years. Chodoeva and co-workers reported that 8-*O*-azeloyl-14-benzoylaconine, an aconitine-type C_{19}-norditerpenoid alkaloid, exhibits antiproliferative activity.[3] The cytotoxic effects of various C_{19}-norditerpenoid alkaloids against tumor cell lines have been reported by de Ines et al.[4]

Aconitum japonicum Thunb. is a plant that grows naturally in the Zenibako district of Hokkaido in Japan, and extracts from the roots were found to contain the C_{19}-norditerpenoid alkaloids jesaconitine, aconitine, and mesaconitine, as major alkaloids, together with 20 minor alkaloids.[5–9] Aconitine and mesaconitine have strong toxicity,[10] and mesaconitine has potent analgesic activity.[11] *Aconitum yesoense* var. *macroyesoense* (Nakai) Tamura is a plant that grows naturally in the Jozankei district of Hokkaido in Japan, and extracts from the roots were found to contain the C_{19}-norditerpenoid alkaloids delcosine and 14-acetyldelcosine (**1**) and the C_{20}-diterpenoid alkaloids kobusine (**2**), pseudokobusine (**3**), and lucidusculine, as major alkaloids, together with 27 minor alkaloids.[12–16] Administration of lucidusculine is known to result in peripheral and coronary vasodilation[17] and reduction of blood pressure.[18] We previously examined diterpenoid alkaloids for peripheral vasoactivity by continuously measuring blood flow in the hind feet of anesthetized mice using a Doppler-type laser blood flow-meter.[19–21] Kobusine (**2**) and pseudokobusine (**3**) and certain semisynthetic derivatives of these alkaloids induced marked increases in cutaneous blood flow in the hind feet of mice. The effects of kobusine 15-veratroate, pseudokobusine 15-anisoate, 15-veratroate (**4**), and 15-*p*-nitrobenzoate at 0.1 mg/kg were notable.[21]

In spite of the progress made using surgery, radiotherapy, and chemotherapeutic agents, the prognosis for patients with malignant glioma remains poor. Further improvements, especially novel drugs, are required urgently. In the present study, we examined the effects of various naturally occurring and semisynthetic diterpenoid alkaloids on growth of the A172 human malignant glioma cell line.

Results and Discussion

In order to obtain diterpenoid alkaloids for rapid testing of the structure–activity relationships among substituted diterpenoid alkaloids, the readily available kobusine (**2**), pseudokobusine (**3**), and luciculine were used as templates for functional group transformations. The natural alkaloids 14-acetyldelcosine (**1**), kobusine (**2**), pseudokobusine (**3**), 15-veratroylpseudokobusine (**4**), 14-acetylbrowniine (**14**), yesoxine (**16**), dehydrolucidusculine (**17**), and 12-acetyllucidusculine (**22**) were isolated and purified from the roots of *A. yesoense* var. *macroyesoense* according to procedures described in the literature.[12–16] Five alkaloids of *A. japonicum*, aljesaconitine A (**5**), deoxyjesaconitine (**6**), hokbusine A (**7**), hypaconitine (**8**), and deoxyaconitine (**9**), were purified from the roots as described previously.[5–9] Delpheline (**15**) was purified from the seeds of *Delphinium elatum* cv. Pacific Giant by a previously described procedure.[22] Thirteen acylated derivatives, 12-benzoylluciculine (**18**),[23] 1,12,15-triacetylluciculine (**21**),[13] 12-acetylluciculine (**23**),[19] 6-benzoylpseudokobusine (**27**),[13] 6,11-dibenzoylpseudokobusine (**28**),[13] 6-veratroylpseudokobusine (**29**),[21] 11-veratroylpseudokobusine (**30**),[21] 11-cinnamoylpseudokobusine (**31**),[20] 11-anisoylpseudokobusine (**33**),[21] 11-*p*-nitrobenzoylpseudokobusine (**34**),[21] 6-cinnamoylpseudokobusine (**35**),[20] 6-anisoylpseudokobusine (**37**),[21] and 6-*p*-nitrobenzoylpseudokobusine (**38**),[13] were prepared from the parent alkaloids, luciculine and pseudokobusine, by literature procedures. Eight semisynthetic alkaloids, 12-anisoylluciculine (**19**), 12-veratroylluciculine (**20**), *N*-benzyl-*N*,6-seco-6-dehydropseudokobusine (**24**), *N*,15-dibenzyl-*N*,6-seco-6-

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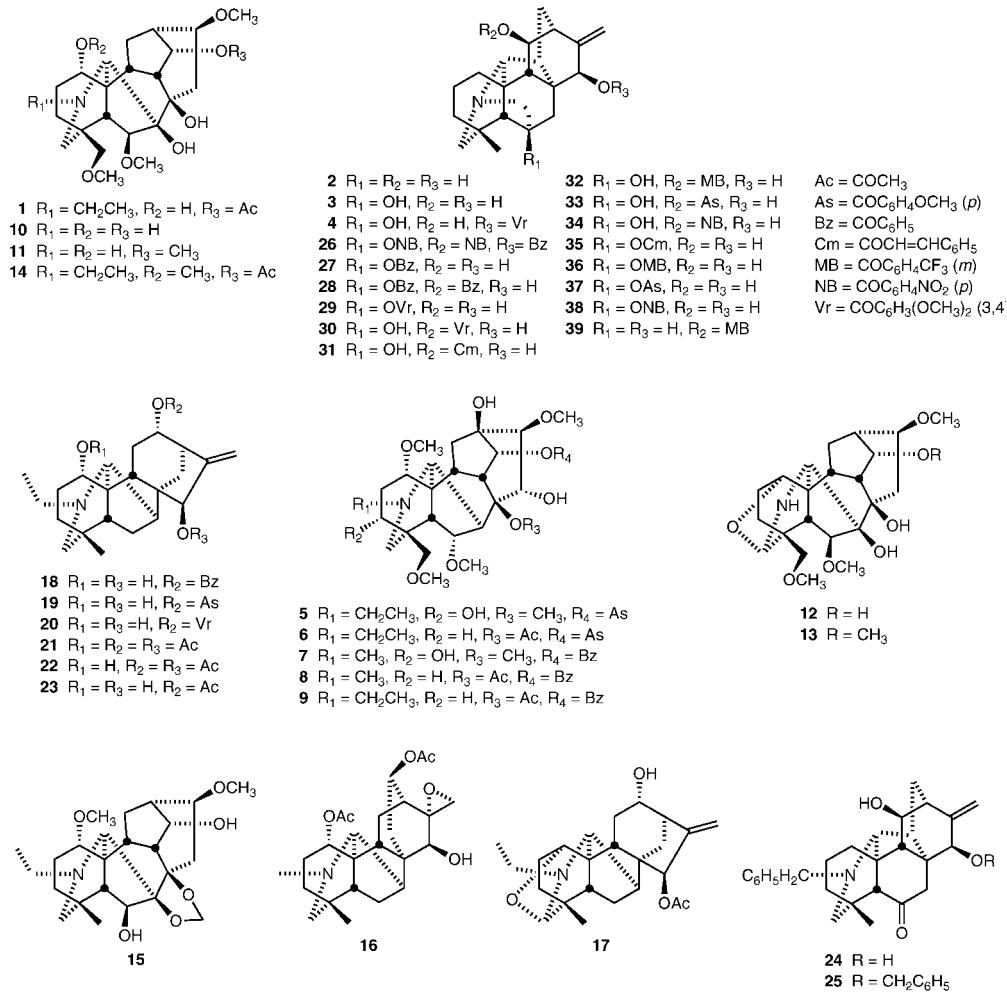

Table 1. Inhibitory Effects of C_{19}-Norditerpenoid Alkaloids on A172 Cell Growth

compound	mean ± SD	compound	mean ± SD
control	1.00		
1	1.11 ± 0.32	**10**	0.90 ± 0.25
5	0.93 ± 0.08	**11**	0.93 ± 0.22
6	0.97 ± 0.02	**12**	1.04 ± 0.07
7	1.03 ± 0.20	**13**	1.14 ± 0.13
8	1.08 ± 0.13	**14**	0.99 ± 0.18
9	0.99 ± 0.02	**15**	1.10 ± 0.05

Table 2. Inhibitory Effects of Veatchine-Type C_{20}-Diterpenoid Alkaloids on A172 Cell Growth

compound	mean ± SD	compound	mean ± SD
control	1.00		
16[b]	1.01 ± 0.22	**20**	0.97 ± 0.23
17[b]	1.18 ± 0.09	**21**[b]	1.02 ± 0.28
18	0.82 ± 0.06[a]	**22**	1.03 ± 0.22
19	0.84 ± 0.38	**23**[b]	0.66 ± 0.11[a]

[a] $p < 0.001$: significantly different from control value. [b] IC_{50} is the compound concentration required to inhibit tumor cell growth by 50%. Data are expressed as means ± SD from the dose–response curves of at least three independent experiments. IC_{50} (μg/mL): **16** > 5.0, **17** > 5.0, **21** > 5.0, **23** = 5.6 ± 0.15.

dehydropseudokobusine (**25**), 15-benzoyl-6,11-di-*p*-nitrobenzoylpseudokobusine (**26**), 11-(*m*-trifluoromethylbenzoyl)pseudokobusine (**32**), 6-(*m*-trifluoromethylbenzoyl)pseudokobusine (**36**), and 11-(*m*-trifluoromethylbenzoyl)kobusine (**39**), were prepared from the parent diterpenoid alkaloids, kobusine, pseudokobusine, 6,11-di-*p*-nitrobenzoylpseudokobusine, and luciculine. These semisynthetic alkaloids were synthesized at controlled reaction times and temperatures. Four semisynthetic C_{19}-norditerpenoid alkaloids, *N*-deethyldelcosine (**10**),[24] *N*-deethyldelsoline (**11**), *N*-deethylanhydrohydroxydelcosine (**12**),[24] and *N*-deethylanhydrohydroxydelsoline (**13**),[25] were prepared from delcosine.

Table 1 summarizes the inhibitory effects of several C_{19}-norditerpenoid alkaloids on growth of the A172 human malignant glioma cell line. In a test at a single concentration (1 μg/mL per well) against a human cell line, an alkaloid is considered active when it reduces the growth rate relative to that of untreated controls. The five aconitine-type alkaloids (**5**–**9**) were inactive. Aljesaconitine A (**5**) slightly influenced the growth of A172 cells, and the activity of **5** was slightly affected by a methyl group [acetyl, deoxyjesaconitine (**6**)] substituent at C-8 or by an anisoyl group [benzoyl, hokbusine A (**7**)] substituent at C-14 or an *N*-ethyl group [*N*-methyl, hokbusine A (**7**)]. Chodoeva and co-workers reported that 8-*O*-azeloyl-14-benzoylaconine, an aconitine-type C_{19}-norditerpenoid alkaloid, exhibits antiproliferative activity.[3] Replacement by an alkoxyl group at C-8 may contribute to the enhancement of activity of the parent alkaloids more than when an acetyloxy group is present at this position. Among the seven lycoctonine-type alkaloids tested (**1**, **10**–**15**), *N*-deethyldelcosine (**10**) and *N*-deethyldelsoline (**11**) showed slight growth inhibitory activities, and the growth inhibitory activities were affected by having a hydroxyl group [ether group, *N*-deethylanhydrohydroxydelcosine (**12**) and *N*-deethylanhydrohydroxydelsoline (**13**), or methoxy, 14-acetylbrowniine (**14**)] substituent at C-1 or by an *N*-H group [*N*-ethyl, 14-acetyldelcosine (**1**) and 14-acetylbrowniine (**14**)]. Delpheline (**15**) was inactive.

Table 2 shows the inhibitory effects of C_{20}-diterpenoid alkaloids on growth of the A172 human malignant glioma cell line. The alkaloid yesoxine (**16**), including an epoxy group, was inactive. Seven veatchine-type alkaloids (**17**–**23**) were also examined. Dehydrolucidusculine (**17**), a natural alkaloid, was inactive. Among

Table 3. Inhibitory Effects of Atisine-Type C_{20}-Diterpenoid Alkaloids on A172 Cell Growth

compound	mean ± SD	compound	mean ± SD
control	1.00	**30**[c]	0.47 ± 0.07[b]
2[c]	0.76 ± 0.28	**31**[c]	0.49 ± 0.18[b]
3[c]	0.84 ± 0.23	**32**	0.58 ± 0.16[b]
4[c]	0.97 ± 0.09	**33**[c]	0.23 ± 0.04[b]
24	1.01 ± 0.14	**34**[c]	0.22 ± 0.11[b]
25[c]	1.04 ± 0.10	**35**	0.97 ± 0.16
26	0.97 ± 0.09	**36**	1.04 ± 0.12
27	0.93 ± 0.11	**37**	0.99 ± 0.13
28[c]	0.76 ± 0.14[a]	**38**	0.96 ± 0.12
29[c]	0.84 ± 0.19	**39**	0.60 ± 0.09[b]

[a] $p < 0.01$, [b] $p < 0.001$: significantly different from control value. [c] IC_{50} is the compound concentration required to inhibit tumor cell growth by 50%. Data are expressed as means ± SD from the dose–response curves of at least three independent experiments. IC_{50} (μg/mL): **2** > 5.0, **3** > 5.0, **4** = 2.9 ± 0.09, **25** > 5.0, **28** = 1.3 ± 0.72, **29** = 3.7 ± 0.57, **30** = 1.2 ± 0.10, **31** = 0.89 ± 0.16, **33** = 1.3 ± 0.11, **34** = 1.5 ± 0.01.

the C-12 aryl ester derivatives (**18**–**20**), 12-benzoylluciculine (**18**) showed a modest inhibitory effect. Substitution on the phenyl ring had variable effects. A *p*-methoxy group [12-anisoylluciculine (**19**)] gave minimal effects relative to those of **18**. However, the inclusion of two methoxy substituents [12-veratroylluciculine (**20**)] on the phenyl ring led to a loss of any inhibitory effect. Among the acetyl derivatives (**21**–**23**) of luciculine, 1,12,15-triacetylluciculine (**21**) and 12,15-diacetylluciculine (**22**, 12-acetyllucidusculine) were inactive. In contrast, 12-acetylluciculine (**23**) had a more potent inhibitory effect. The contribution of the acetyl group to activity, as in **23**, was position-dependent. Considering substitution of the veatchine-type alkaloid at the C-1 and C-15 positions, a hydroxy group seems to be a factor related to A172 cell growth inhibition. Esterification of the hydroxyl group at C-12 may contribute to the enhancement of activity of the parent alkaloids more than that of the OH group at C-12.

The inhibitory effects of various atisine-type C_{20}-diterpenoid alkaloids (**2**–**4**, **24**–**39**) on growth of the A172 human malignant glioma cell line were also examined (Table 3). Alkaloids kobusine (**2**) and pseudokobusine (**3**) influenced the growth of A172 cells slightly, as did the veatchine-type alkaloids 12-benzoylluciculine (**18**) and 12-anisoylluciculine (**19**). Alkaloids **2** and **3** contain two and three hydroxy groups, respectively, in the common basic structure of the atisine skeleton, being devoid of any other substituents. In the molecules of **2** and **3**, the effects of substitution on the hydroxy groups and semisynthetic derivatives were examined. *N*-Benzyl-*N*,6-seco-6-dehydropseudokobusine (**24**) and *N*,15-dibenzyl-*N*,6-seco-6-dehydropseudokobusine (**25**) were inactive. Among the benzoyl derivatives (**26**–**28**) of **3**, 15-benzoyl-6,11-di-*p*-nitrobenzoylpseudokobusine (**26**) and 6-benzoylpseudokobusine (**27**) had moderate inhibitory effects on the growth of A172 cells. 6,11-Dibenzoylpseudokobusine (**28**) had a more potent inhibitory effect, which was affected by an aryl substituent at C-11 or by a hydroxy group at C-15. Among the veratroyl derivatives (**4**, **29**, **30**) of **3**, 15-veratroylpseudokobusine (**4**) and 6-veratroylpseudokobusine (**29**) displayed modest inhibitory effects. 11-Veratroylpseudokobusine (**30**) had a significantly more potent inhibitory effect, which was affected by the presence of an acyl substituent at C-11. In fact, 11-acyl derivatives (**31**–**34**), like compound **30**, exhibited potent inhibitory effects. The contribution of the acyl group to activity, as in **30**, was position-dependent. Placing an acyl group at C-6 (**35**–**38**) resulted in less active compounds. Substitution of the hydroxy at C-11 had variable effects. The inhibitory effect of 11-cinnamoylpseudokobusine (**31**) was equivalent to that of **30**. However, 11-*m*-trifluoromethylbenzoylpseudokobusine (**32**) showed minimal effects relative to those of **30**, while the effect of 11-(*m*-trifluoromethylbenzoyl)kobusine (**39**) was about the same as that of **32**. 11-Anisoylpseudokobusine (**33**) and 11-*p*-nitrobenzoylpseudokobusine (**34**) exhibited the most potent inhibitory effects. Compound **31** showed an IC_{50} value against the A172 cell line of 0.89 ± 0.16 μg/mL. The results of this study suggest that the hydroxyl groups of pseudokobusine at C-6 and C-15 are necessary for an inhibitory effect. Esterification of the hydroxyl group at C-11 thus may contribute to the enhancement of activity of the parent alkaloids more than that of the OH group at C-11. Anisoyl, cinnamoyl, *p*-nitrobenzoyl, and veratroyl substitutions were also effective. Current studies are focused on the use of semisynthetic analogues of diterpenoid alkaloids to further probe the mechanisms of the inhibitory effect on growth of the A172 human malignant glioma cell line.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. ^{1}H NMR spectra in $CDCl_3$ were recorded on JEOL GX-270 and AL-400 spectrometers using tetramethylsilane as an internal standard. Chemical shifts are given in ppm. Electron impact (EI) or secondary ion (SI) mass spectra were measured on a Hitachi M-2000 spectrometer. All products reported showed ^{1}H NMR spectra and mass spectra in agreement with the assigned structures. Reactions were carried out under an inert atmosphere of dry nitrogen or argon, unless otherwise described. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were monitored routinely by TLC on silica gel (precoated Merck F_{254} plates) and visualized with Dragendroff reagent. Chromatography was performed using silica gel and the indicated solvent system. All other chemicals used were of analytical grade.

Alkaloids. The following diterpenoid alkaloids were used after extraction from the roots of *A. yesoense* var. *macroyesoense*, followed by purification and identification by methods described previously:[12,13] 14-acetyldelcosine (**1**), kobusine (**2**), pseudokobusine (**3**), 15-veratroylpseudokobusine (**4**), 14-acetylbrowniine (**14**), yesoxine (**16**), dehydrolucidusculine (**17**), and 12-acetyllucidusculine (**22**). Five natural alkaloids of *A. japonicum*, aljesaconitine A (**5**), deoxyjesaconitine (**6**), hokbusine A (**7**), hypaconitine (**8**), and deoxyaconitine (**9**), were purified from the roots by previously described procedures.[8–10] Delpheline (**15**) was purified from the seeds of *Delphinium elatum* cv. Pacific Giant by a previously described procedure.[22] Thirteen acyl derivatives, 12-benzoylluciculine (**18**),[23] 1,12,15-triacetylluciculine (**21**),[13] 12-acetylluciculine (**23**),[19] 6-benzoylpseudokobusine (**27**),[13] 6,11-dibenzoylpseudokobusine (**28**),[13] 6-veratroylpseudokobusine (**29**),[21] 11-veratroylpseudokobusine (**30**),[21] 11-cinnamoylpseudokobusine (**31**),[20] 11-anisoylpseudokobusine (**33**),[21] 11-*p*-nitrobenzoylpseudokobusine (**34**),[21] 6-cinnamoylpseudokobusine (**35**),[20] 6-anisoylpseudokobusine (**37**),[21] and 6-*p*-nitrobenzoylpseudokobusine (**38**),[13] were prepared by methods described previously.

Synthesis of *N*-Deethyldelcosine (10) and *N*-Deethylanhydrohydroxydelcosine (12).[24] A solution of delcosine (0.06 g, 0.13 mmol) and mercury(II) acetate (0.42 g, 1.3 mmol) in 3% acetic acid (10 mL) was refluxed with stirring for 4 h. After cooling, the reaction mixture was filtered. The filtrate was extracted with chloroform after addition of aqueous NH_4OH. The organic layer was washed with 5% aqueous $NaHCO_3$ and water and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography eluting with NH_4OH-saturated $CHCl_3$ to give **10** (30 mg, 54%) and **12** (12 mg, 22%). *N*-Deethyldelcosine (**10**): colorless crystals (methanol−acetone); mp 238–241 °C (lit.[24] 240–241 °C); HREIMS *m*/*z* 425.2391 (calcd for $C_{22}H_{35}NO_7$, 425.2411). *N*-Deethylanhydrohydroxydelcosine (**12**): colorless crystals (acetone−hexane); mp 226–227 °C (lit.[24] 218–219 °C); HREIMS *m*/*z* 423.2254 (calcd for $C_{22}H_{33}NO_7$, 423.2255).

Synthesis of *N*-Deethyldelsoline (11) and *N*-Deethylanhydrohydroxydelsoline (13).[25] A solution of delsoline[26] (0.06 g, 0.13 mmol) and mercury(II) acetate (0.42 g, 1.3 mmol) in 3% acetic acid (10 mL) was refluxed with stirring for 2 h. After cooling, the reaction mixture was filtered. The filtrate was extracted with chloroform after addition of aqueous NH_4OH. The organic layer was washed with 5% aqueous $NaHCO_3$ and water and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography eluting with NH_4OH-

saturated $CHCl_3$ to give **11** (30 mg, 53%) and **13** (16 mg, 28%). *N*-Deethyldelsoline (**11**): colorless crystals (acetone−hexane); mp 207–209 °C; $[\alpha]^{22}_D$ +69.1 (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3432, 3326, 2938, 1464, 1106, 1083 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.49 and 1.64 (each 1H, m, H-3), 1.67 and 2.05 (each 1H, m, H-12), 1.74 (1H, m, H-15), 1.75 and 1.97 (each 1H, m, H-2), 1.92 (1H, d, J = 1.9 Hz, H-5), 1.96 (1H, dd, J = 6.8, 4.6 Hz, H-10), 2.40 and 2.78 (each 1H, d, J = 11.2 Hz, H-19), 2.39 (1H, s, H-13), 2.55 (1H, dd, J = 14.4, 8.5 Hz, H-15), 2.82 (1H, d, J = 2.1 Hz, H-17), 2.96 and 3.35 (each 1H, d, J = 8.7 Hz, H-18), 3.01 (1H, dd, J = 6.8, 5.1 Hz, H-9), 3.25 (1H, t. J = 8.3 Hz, H-16), 3.33 (3H, s, H-18′), 3.34 (3H, s, H-16′), 3.38 (3H, s, H-14′), 3.42 (3H, s, H-6′), 3.64 (1H, t, J = 4.1 Hz, H-14β), 3.73 (1H, s, H-1), 3.98 (1H, s, H-6); EIMS *m*/*z* 439 [M^+] (20), 424 (100), 422 (88), 406 (72); HREIMS *m*/*z* 439.2569 (calcd for $C_{23}H_{37}NO_7$, 439.2568). *N*-Deethylanhydrohydroxydelsoline (**13**): colorless crystals (acetone−hexane); mp 191–192 °C (lit.[25] 185–187 °C); HREIMS *m*/*z* 437.2394 (calcd for $C_{23}H_{35}NO_7$, 437.2411).

Synthesis of 12-Anisoylluciculine (19). A solution of luciculine (0.2 g, 0.56 mmol) and anisoyl chloride (0.59 g, 3.5 mmol) in pyridine (4 mL) was stirred for 7 h at ambient temperature. After adding water, the reaction mixture was extracted with chloroform. The organic layer was washed with 5% aqueous $NaHCO_3$ and brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography, eluting first with 33% and then 25%, 20%, and 10% hexane/NH_4OH-saturated $CHCl_3$ to give **19** (107 mg, 39%). 12-Anisoylluciculine (**19**): colorless crystals (acetone−hexane); mp 175–178 °C; $[\alpha]^{22}_D$ −12.5 (*c* 0.37, $CHCl_3$); IR (KBr) ν_{max} 3436, 2936, 1709, 1607, 1280, 1259, 1170, 891 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.78 (3H, s, H-18), 1.11 (3H, t, J = 7.0 Hz, N-$CH_2C\underline{H}_3$), 1.11 and 2.00 (each 1H, d, J = 12.2 Hz, H-14), 1.34 (1H, s, H-5), 1.35 and 2.56 (each 1H, m, H-6), 1.38 (1H, m, H-3), 1.56 (1H, dd, J = 13.1, 6.5 Hz, H-9), 1.64 (1H, dt, J = 11.2, 5.3 Hz, H-3), 1.87 and 2.04 (each 1H, m, H-2), 2.06 (2H, m, H-11), 2.14 (1H, d, J = 5.1 Hz, H-7), 2.27 and 2.52 (each 1H, d, J = 11.4 Hz, H-19), 2.56 (2H, m, N-$C\underline{H}_2CH_3$), 2.61 (1H, d, J = 3.9 Hz, H-13), 3.47 (1H, s, H-20), 3.86 (3H, s, Ar-OCH_3), 3.92 (1H, dd, J = 7.8, 6.1 Hz, H-1), 4.22 (1H, s, H-15), 4.73 (1H, dd, J = 10.0, 7.0 Hz, H-12), 5.27 and 5.43 (each 1H, s, H-17), 6.90 and 8.00 (each 2H, d, J = 9.0 Hz, H-Ar); EIMS *m*/*z* 493 [M^+] (100), 475 (22), 358 (8), 340 (65), 135 (36); HREIMS *m*/*z* 493.2851 (calcd for $C_{30}H_{39}NO_5$, 493.2826).

Synthesis of 12-Veratroylluciculine (20). A solution of luciculine (0.15 g, 0.42 mmol) and veratroyl chloride (0.59 g, 2.9 mmol) in pyridine (3 mL) was stirred for 3 h at ambient temperature. After adding water, the reaction mixture was extracted with chloroform. The organic layer was washed with 5% aqueous $NaHCO_3$ and brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography, eluting first with 33% hexane and then 1% MeOH/NH_4OH-saturated ether to give **20** (176 mg, 80%). 12-Veratroylluciculine (**20**): colorless crystals (acetone−hexane); mp 194–196 °C; $[\alpha]^{22}_D$ −12.4 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3420, 2938, 1707, 1603, 1272, 1224, 1178, 901 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.79 (3H, s, H-18), 1.10 (1H, dd, J = 12.2, 4.1 Hz, H-14), 1.15 (3H, t, J = 7.0 Hz, N-$CH_2C\underline{H}_3$), 1.33 (1H, s, H-5), 1.35 (1H, dd, J = 13.9, 5.3 Hz, H-3), 1.37 and 2.59 (each 1H, m, H-6), 1.61 (1H, d, J = 4.8 Hz, H-9), 1.63 (1H, dt, J = 13.6, 5.3 Hz, H-3), 1.93 and 2.11 (each 1H, m, H-2), 2.02 (1H, d, J = 12.4 Hz, H-14), 2.05 (2H, m, N-$C\underline{H}_2CH_3$), 2.05 and 2.20 (each 1H, m, H-11), 2.16 (1H, d, J = 5.1 Hz, H-7), 2.30 and 2.61 (each 1H, d, J = 11.4 Hz, H-19), 2.62 (1H, d, J = 3.9 Hz, H-13), 3.53 (1H, s, H-20), 3.90 (1H, m, H-1), 3.94 (6H, s, Ar-OCH_3), 4.24 (1H, s, H-15), 4.74 (1H, dd, J = 10.4, 7.0 Hz, H-12), 5.29 and 5.45 (each 1H, s, H-17), 6.87 (1H, d, J = 8.5 Hz, H-Ar), 7.54 (1H, d, J = 1.9 Hz, H-Ar), 7.70 (2H, dd, J = 8.5, 1.9 Hz, H-Ar); EIMS *m*/*z* 523 [M^+] (100), 505 (32), 358 (6), 340 (97), 160 (32); HREIMS *m*/*z* 523.2910 (calcd for $C_{31}H_{41}NO_5$, 523.2931).

Synthesis of *N*-Benzyl-*N*,6-seco-6-dehydropseudokobusine (24) and *N*,15-Dibenzyl-*N*,6-seco-6-dehydropseudokobusine (25). A solution of **3** (0.1 g, 0.30 mmol), sodium hydride (74 mg, 3.1 mmol), and benzyl chloride (0.35 mL, 0.30 mmol) in dioxane (5 mL) was stirred for 6 h at 60 °C and then for 14 h at ambient temperature. The reaction mixture was filtered, and then the solvent was evaporated under reduced pressure. The resulting residue was purified by alumina column chromatography, eluting with $CHCl_3$, to give **24** (55 mg, 44%) and **25** (27 mg, 17%). *N*-Benzyl-*N*,6-seco-6-dehydropseudokobusine (**24**): colorless crystals (acetone−hexane); mp 153–156 °C; $[\alpha]^{22}_D$ −57.9 (*c* 0.38, $CHCl_3$); IR (KBr) ν_{max} 3236, 1688, 1597, 903 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (1H, d, J = 13.1 Hz, H-13), 1.29 (1H, d, J = 3.4 Hz, H-1), 1.36 (1H, m, H-2), 1.40 (3H, s, H-18), 1.46 (1H, m, H-1), 1.48 (1H, m, H-3), 1.62 (1H, d, J = 12.2 Hz, H-2), 1.73 (1H, s, H-5), 1.81 (1H, d, J = 12.2 Hz, H-3), 1.88 (1H, s, H-9), 1.96 (1H, m, H-13), 2.12 and 2.78 (each 1H, d, J = 11.7 Hz, H-19), 2.33 (1H, d, J = 10.9 Hz, H-14), 2.38 (1H, s, H-20), 2.56 (1H, d, J = 3.6 Hz, H-12), 2.63 and 2.90 (each 1H, d, J = 17.5 Hz, H-7), 3.75 and 4.16 (each 1H, d, J = 14.4 Hz, CH_2-C_6H_5), 3.99 (1H, s, H-15), 4.16 (1H, d, J = 4.3 Hz, H-11), 5.16 and 5.30 (each 1H, s, H-17), 7.21 (2H, d, J = 7.0 Hz, H-Ar), 7.31 (1H, t, J = 6.8 Hz, H-Ar), 7.35 (2H, t, J = 6.8 Hz, H-Ar); EIMS *m*/*z* 419 [M^+] (100), 404 (26), 402 (20), 328 (11); HREIMS *m*/*z* 419.2452 (calcd for $C_{27}H_{33}NO_3$, 419.2458). *N*,15-Dibenzyl-*N*,6-seco-6-dehydropseudokobusine (**25**): colorless crystals (acetone−hexane); mp 134 °C (dec); $[\alpha]^{22}_D$ −20.0 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3332, 1715, 1601, 911 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.12 (1H, d, J = 13.9 Hz, H-13), 1.25 (2H, m, H-1), 1.36 (3H, s, H-18), 1.45 (1H, m, H-3), 1.47 (1H, m, H-2), 1.62 (1H, m, H-2), 1.66 (1H, s, H-5), 1.79 (1H, d, J = 9.5 Hz, H-3), 1.92 (1H, s, H-9), 1.94 (1H, m, H-13), 1.98 and 2.58 (each 1H, d, J = 11.7 Hz, H-19), 2.23 (1H, s, H-14), 2.26 (1H, s, H-20), 2.65 (1H, s, H-12), 2.72 and 2.89 (each 1H, d, J = 18.0 Hz, H-7), 3.42 and 4.16 (each 1H, d, J = 14.6 Hz, CH_2-C_6H_5), 3.69 (1H, s, H-15), 4.03 (1H, s, H-11), 4.42 and 4.83 (each 1H, d, J = 10.9 Hz, CH_2-C_6H_5), 5.16 and 5.26 (each 1H, s, H-17), 7.13–7.37 (10H, m, H-Ar); EIMS *m*/*z* 509 [M^+] (13), 418 (100), 91 (50); HREIMS *m*/*z* 509.2930 (calcd for $C_{34}H_{39}NO_3$, 509.2928).

Synthesis of 15-Benzoyl-6,11-di-*p*-nitrobenzoylpseudokobusine (26). A solution of 6,11-di-*p*-nitrobenzoylpseudokobusine[16] (22 mg, 0.035 mmol) and benzoyl chloride (0.04 mL, 0.34 mmol) in pyridine (0.6 mL) was stirred overnight at ambient temperature. After adding water, the reaction mixture was extracted with chloroform. The organic layer was washed with 5% aqueous $NaHCO_3$, water, and brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography eluting with 40% hexane/NH_4OH-saturated $CHCl_3$ to give **26** (23 mg, 91%). 15-Benzoyl-6,11-di-*p*-nitrobenzoylpseudokobusine (**26**): colorless crystals (acetone−hexane); mp 253–256 °C; $[\alpha]^{22}_D$ +9.7 (*c* 0.21, $CHCl_3$); IR (KBr) ν_{max} 1721, 1607, 1531, 1350, 1321, 1286, 1267, 874 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.99 (3H, s, H-18), 1.19 and 2.04 (each 1H, d, J = 13.9 Hz, H-13), 1.45 (1H, dd, J = 14.1, 4.4 Hz, H-3), 1.54 (1H, d, J = 13.4 Hz, H-2), 1.83 (1H, m, H-2), 1.85 and 2.08 (each 1H, m, H-1), 1.86 (1H, m, H-3), 1.94 and 2.94 (each 1H, d, J = 12.9 Hz, H-7), 2.30 (1H, s, H-9), 2.33 (1H, m, H-14), 2.67 and 3.21 (each 1H, d, J = 12.2 Hz, H-19), 2.68 (1H, s, H-5), 2.78 (2H, s, H-12 and H-20), 5.18 and 5.48 (each 1H, s, H-17), 5.49 (1H, d, J = 5.1 Hz, H-11), 5.82 (1H, s, H-15), 7.27 (2H, t, J = 8.0 Hz, H-Ar), 7.51 (1H, t, J = 7.5 Hz, H-Ar), 7.92 (4H, d, J = 9.0 Hz, H-Ar), 8.04 (2H, d, J = 8.7 Hz, H-Ar), 8.16 (2H, d, J = 9.0 Hz, H-Ar), 8.25 (2H, d, J = 9.0 Hz, H-Ar); EIMS *m*/*z* 731 [M^+] (22), 581 (28), 459 (4), 150 (32), 105 (100); HRSIMS *m*/*z* [M + H]$^+$ 732.2576 (calcd for $C_{41}H_{38}N_3O_{10}$, 732.2525).

Synthesis of 11- (32) and 6-(*m*-Trifluoromethylbenzoyl)pseudokobusine (36). A solution of **3** (0.1 g, 0.31 mmol) and *m*-trifluoromethylbenzoyl chloride (0.044 mL, 0.30 mmol) in pyridine (2 mL) was stirred for 15 min at ambient temperature. After adding 5% aqueous $NaHCO_3$, the reaction mixture was extracted with chloroform. The organic layer was washed with water and brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography eluting with NH_4OH-saturated $CHCl_3$ to give **32** (13 mg, 8%), **36** (19 mg, 12%), and **3** (46 mg). 11-(*m*-Trifluoromethylbenzoyl)pseudokobusine (**32**): colorless crystals (acetone−hexane); mp 218 °C (dec); $[\alpha]^{22}_D$ −0.6 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3078, 1717, 1620, 1338, 1261, 907 cm^{-1}; ^1H NMR δ 1.08 (1H, d, J = 14.1 Hz, H-13), 1.36 (1H, m, H-3), 1.39 (3H, s, H-18), 1.48 (2H, d, J = 12.6 Hz, H-2 and H-3), 1.57 (1H, s, H-5), 1.65 (1H, dd, J = 13.1, 3.6 Hz, H-1), 1.72 (1H, m, H-2), 1.87 (1H, m, H-7), 1.89 (1H, m, H-1), 1.90 (1H, s, H-9), 1.92 (1H, m, H-13), 2.00 (1H, d, J = 10.7 Hz, H-7), 2.38 and 3.13 (each 1H, d, J = 12.2 Hz, H-19), 2.50 (1H, d, J = 13.1 Hz, H-14), 2.59 (1H, s, H-20), 2.72 (1H, d, J = 4.1 Hz, H-12), 4.08 (1H, s, H-15), 5.16 and 5.33 (each 1H, s, H-17), 5.39 (1H, d, J = 4.8 Hz,

H-11), 7.59 (1H, t, $J = 7.8$ Hz, H-Ar), 7.81 (1H, d, $J = 7.8$ Hz, H-Ar), 8.13 (1H, d, $J = 7.8$ Hz, H-Ar), 8.26 (1H, s, H-Ar); EIMS *m/z* 501 [M^+] (100), 328 (35), 173 (19); HREIMS *m/z* 501.2111 (calcd for $C_{28}H_{30}F_3NO_4$, 501.2125). 6-(*m*-Trifluoromethylbenzoyl)pseudokobusine (**36**): colorless crystals (acetone−hexane); mp 219–220 °C; $[\alpha]^{22}_D$ +45.1 (*c* 0.14, $CHCl_3$); IR (KBr) ν_{max} 3434, 1729, 1620, 1338, 1263, 893 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (1H, d, $J = 13.4$ Hz, H-13), 0.92 (3H, s, H-18), 1.31 (1H, dd, $J = 9.6$, 4.3 Hz, H-3), 1.40 (1H, d, $J = 12.6$ Hz, H-2), 1.45 (1H, d, $J = 12.2$ Hz, H-1), 1.53 (1H, m, H-3), 1.62 (1H, m, H-2), 1.68 and 3.05 (each 1H, d, $J = 12.5$ Hz, H-7), 1.72 (1H, m, H-1), 1.78 (1H, dd, $J = 13.3$, 3.0 Hz, H-13), 1.85 (1H, s, H-14), 1.87 (1H, s, H-9), 2.44 (1H, s, H-12), 2.46 and 3.07 (each 1H, d, $J = 12.5$ Hz, H-19), 2.47 (1H, s, H-20), 2.48 (1H, s, H-5), 3.90 (1H, s, H-15), 4.02 (1H, d, $J = 4.6$ Hz, H-11), 5.08 and 5.18 (each 1H, s, H-17), 7.51 (1H, t, $J = 7.8$ Hz, H-Ar), 7.75 (1H, d, $J = 7.8$ Hz, H-Ar), 8.16 (1H, d, $J = 7.8$ Hz, H-Ar), 8.23 (1H, s, H-Ar); EIMS *m/z* 501 [M^+] (100), 424 (100), 328 (42), 173 (44); HREIMS *m/z* 501.2140 (calcd for $C_{28}H_{30}F_3NO_4$, 501.2125).

Synthesis of 11-(*m*-Trifluoromethylbenzoyl)kobusine (39). Compound **2** (0.1 g, 0.32 mmol) was mixed with *m*-trifluoromethylbenzoyl chloride (0.09 mL, 0.61 mmol) in pyridine (2 mL) and stirred at 0 °C (ice bath) for 5.5 h. The reaction mixture was extracted with chloroform after addition of 5% aqueous $NaHCO_3$. The organic layer was washed with water and brine and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography, eluting with 10% hexane/NH_4OH-saturated $CHCl_3$ to give **39** (73 mg, 47%). 11-(*m*-Trifluoromethylbenzoyl)kobusine (**39**): colorless crystals (acetone−hexane); mp 222–224 °C; $[\alpha]^{22}_D$ +46.7 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 3144, 1727, 1601, 1338, 1253, 1135, 907 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.99 (3H, s, H-18), 1.11 and 1.88 (each 1H, d, $J = 10.0$ Hz, H-13), 1.40 and 2.13 (each 1H, m, H-2), 1.33 (1H, dd, $J = 14.0$, 4.0 Hz, H-3), 1.47 (1H, d, $J = 10.2$ Hz, H-3), 1.54 (1H, s, H-5), 1.71 and 2.13 (1H, dd, $J = 13.5$, 2.4 Hz, H-7), 1.86 (1H, s, H-9), 1.88 and 1.99 (1H, d, $J = 9.5$ Hz, H-1), 1.90 (1H, s, H-14), 2.40 and 2.53 (1H, d, $J = 12.3$ Hz, H-19), 2.53 (1H, s, H-20), 2.71 (1H, d, $J = 3.2$ Hz, H-12), 3.26 (1H, s, H-6), 4.03 (1H, s, H-15), 5.09 and 5.26 (each 1H, s, H-17), 5.41 (1H, d, $J = 4.6$ Hz, H-11), 7.57 (1H, t, $J = 7.8$ Hz, H-Ar), 7.80 (1H, d, $J = 7.5$ Hz, H-Ar), 8.12 (1H, d, $J = 7.5$ Hz, H-Ar), 8.26 (1H, s, H-Ar); EIMS *m/z* 485 [M^+] (100), 312 (43), 295 (16), 173 (14); HREIMS *m/z* 485.2201 (calcd for $C_{28}H_{30}F_3NO_3$, 485.2176).

Inhibition of Growth of Human A172 Cells. All test alkaloids were dissolved in dimethyl sulfoxide (DMSO) at 1 mg/mL immediately before use and diluted in the medium before addition to the cells. Cells were cultured in a high-glucose DMEM medium supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotics [penicillin (100 UI/mL) and streptomycin (100 UI/mL)]. To determine the effects of the alkaloids on cell growth, exponentially growing A172 cells (5×10^3 cells/mL) were seeded in 24-well plates (Falcon, Becton-Dickinson Labware, Lincoln Park, NJ) with 1 mL of medium; 24 h later, each alkaloid (final concentration: 1 μg/ mL) was added to each plate. The total cell numbers were determined after 6 days using a model Z series particle counter (Coulter Electronics, Hialeah, FL). The results are expressed as inhibition values related to untreated controls and as IC_{50} values (concentration causing 50% inhibition relative to untreated controls). All experiments were repeated at least three times. Considering the possible antiproliferative effects of DMSO, control cultures were always performed using the maximum levels of DMSO employed for the administration of the alkaloids tested. The concentration of DMSO employed for the assays was never higher than 0.5% and did not affect growth of the cell lines employed.

Statistical Analysis. The data are expressed as means ± SD of three cultures of a group, and the differences from the values determined from the control culture were analyzed statistically by Student's paired *t*-test at a significance level of 0.001 or less.

Note Added after ASAP Publication: The structure chart in the Nov 29, 2007, version contained an error. The current version is correct.

Supporting Information Available: Chart S1, Table S1, and ^1H NMR data for synthesized compounds are available free of charge via the Internet at http://pubs.acs.org.

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