Isolation of Gelsedine-Type Indole Alkaloids from *Gelsemium elegans* and Evaluation of the Cytotoxic Activity of *Gelsemium* Alkaloids for A431 Epidermoid Carcinoma Cells

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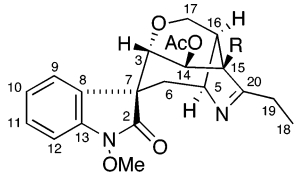
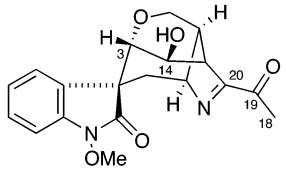
Received January 12, 2006

Four new gelsedine-type indole alkaloids (**1**−**4**) were isolated from the leaves of *Gelsemium elegans*, together with 11 known alkaloids. The structures were determined as 14-acetoxygelsenicine (**1**), 14-acetoxy-15-hydroxygelsenicine (**2**), 14-hydroxy-19-oxogelsenicine (**3**), and 14-acetoxygelselegine (**4**), respectively, by spectroscopic analysis. The cytotoxic effects of 14 *Gelsemium* alkaloids including two new compounds (**1**, **2**) were evaluated using the A431 human epidermoid carcinoma cell line. Of these, the gelsedine-type alkaloids 14-acetoxygelsenicine (**1**), 14,15-dihydroxygelsenicine (**5**), gelsedine (**7**), and gelsemicine (**8**) showed potent cytotoxic effects.

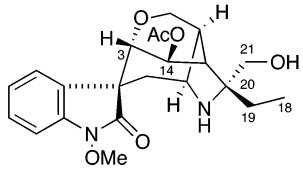
Gelsemium elegans Benth. (Loganiaceae), which is widely distributed in Southeast Asia, is known as a toxic plant and has been used in traditional Chinese medicine.[1] Our recent study has proved that the origin of "Yakatsu", one of the ancient medicines stored in the Shosoin repository in Japan, is *G. elegans*.[2] As this plant was used in traditional Chinese medicine as a remedy for certain kinds of skin ulcers, it is presumed to have been used as an external medication for dermatitis more than 1250 years ago in Japan. In addition, some pharmacological effects, such as analgesic, anti-inflammatory, and antitumor activities, of the *G. elegans* alkaloids have been reported.[3−5] In continuation of our study on *Gelsemium* alkaloids, we report herein the isolation of four new gelsedine-type alkaloids, namely, 14-acetoxygelsenicine (**1**), 14-acetoxy-15-hydroxygelsenicine (**2**), 14-hydroxy-19-oxogelsenicine (**3**), and 14-acetoxygelselegine (**4**), together with 11 known alkaloids, 14-hydroxygelsenicine,[6,7] 14,15-dihydroxygelsenicine (**5**),[8] gelsenicine (**6**),[9] gelsemoxonine,[8] 19(*Z*)-akuammidine,[7] humantenirine,[10] 11-methoxyhumantenine,[10] gelsemine,[11,12] gelsemine *N*-oxide,[7] 21-oxogelsemine,[12,13] and koumine.[14,15] The activity of 14 *Gelsemium* alkaloids including the new alkaloids (**1**, **2**) on the A431 human epidermoid carcinoma cell line was evaluated.



1 R=H 14-Acetoxygelsenicine
2 R=OH 14-Acetoxy-15-hydroxygelsenicine



3 14-Hydroxy-19-oxogelsenicine



4 14-Acetoxygelselegine

The HRFABMS of the new alkaloid **1** gave a protonated molecular ion peak at *m*/*z* 385.1768 ([MH]$^+$) that corresponded to the molecular formula $C_{21}H_{25}N_2O_5$ (*m*/*z* 385.1763). The UV absorptions at 258.0 and 213.5 nm revealed an oxindole nucleus.[16] The ^{1}H NMR spectrum showed signals assignable to four aromatic protons of the A ring of the oxindole system, an N_a-methoxy group at δ 3.95 (3H, s), an oxymethine proton at δ 3.81 (dd, H-3), a methine group bearing an imine nitrogen at δ 4.46 (m, H-5), oxymethylene protons at δ 4.43 (dd) and 4.33 (dd) (H_2-17), and an ethyl group [δ 1.31 (3H, dd, H_3-18), δ 2.93 and 2.54 (each 1H, dddd, H_2-19)], which were similar to those of 14-hydroxygelsenicine (**5**).[6,7] Furthermore, the characteristic signals of an acetyl methyl group at δ 2.08 (3H, s) and a low-field methine proton at δ 5.50 (d, H-14) were observed. The ^{13}C NMR spectrum revealed the presence of an acetyl carbonyl carbon at δ 170.1 together with a carbonyl carbon of the oxindole ring at δ 170.7 (C-2) and an imine carbon at δ 180.5 (C-20). In the ^{1}H−^{1}H COSY NMR spectrum, coupling was observed between the oxymethine proton at δ 3.81 due to H-3 and the low-field methine proton at δ 5.50. HMBC NMR spectroscopic correlations between the proton at δ 5.50 and the acetyl methyl protons and the carbon at δ 170.1 indicated that an acetoxy group was attached to C-14. The configuration of the acetoxy group at C-14 was inferred to be β from the H-14 (d, $J_{3,14}$ = 2.4 Hz) signal, which showed coupling with only H-3 and not H-15; the dihedral angle between H-14 and H-15 was ca. 90°. The above data indicated that **1** is a 14-*O*-acetyl derivative of 14-hydroxygelsenicine. When 14-hydroxygelsenicine was treated with Ac_2O in the presence of DMAP in CH_2Cl_2, 14-acetoxygelsenicine was obtained in 80% yield, thereby establishing the structure (**1**) of this new alkaloid.

The molecular formula of the alkaloid **2** was established as $C_{21}H_{24}N_2O_6$ from the HRFABMS (*m*/*z* 401.1707 [MH]$^+$), which indicated that **2** has an extra oxygen atom compared to 14-acetoxygelsenicine (**1**). The UV and NMR spectra revealed the existence of an oxindole nucleus. The ^{1}H NMR spectrum was very similar to that of **1** except for the lack of a H-15 signal. Signals corresponding to acetyl methyl protons and a low-field methine

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10.1021/np060016o CCC: $33.50
Published on Web 03/10/2006

Table 1. ^{1}H (500 MHz, *J* in Hz) and ^{13}C (125 MHz) NMR Data for Compounds **1**−**4** in $CDCl_3$

	1		**2**		**3**		**4**	
position	δ_H (mult., Hz)	δ_C	δ_H (mult., Hz)	δ_C	δ_H (mult., Hz)	δ_C	δ_H (mult., Hz)	δ_C
2		170.7		170.5		170.8		170.5
3	3.81 (dd, 2.4, 1.5)	76.1	3.87 (d, 2.4)	74.9	3.74 (dd, 2.4, 1.5)	78.9	3.50 (overlapped)	77.6
5	4.46 (m)	72.0	4.52 (m)	69.8	4.75 (ddd, 7.5, 4.9, 2.4)	74.1	3.64 (m)	58.3
6	2.44 (dd, 15.6, 4.9) 2.32 (dd, 15.6, 2.4)	37.4	2.45 (dd, 15.6, 4.6) 2.34 (dd, 15.6, 2.6)	36.3	2.62 (dd, 15.6, 4.9) 2.35 (dd, 15.6, 2.4)	37.8	2.20 (dd, 16.1, 3.8) 2.05 (overlapped)	33.8
7		53.9		53.8		54.2		55.1
8		131.4		131.2		131.2		130.5
9	7.53 (dd, 7.6, 0.6)	124.6	7.51 (d, 7.6)	124.6	7.55 (d, 7.7)	124.5	7.34 (d, 7.7)	124.9
10	7.07 (ddd, 7.6, 7.6, 0.6)	123.5	7.08 (dd, 7.6, 7.6)	123.6	7.10 (dd, 7.7, 7.7)	123.6	7.12 (dd, 7.7, 7.7)	123.7
11	7.27 (ddd, 7.6, 7.6, 0.6)	128.4	7.28 (dd, 7.6, 7.6)	128.6	7.29 (dd, 7.7, 7.7)	128.5	7.31 (dd, 7.7, 7.7)	128.6
12	6.88 (dd, 7.6, 0.6)	106.8	6.89 (d, 7.6)	106.9	6.89 (d, 7.7)	106.9	6.97 (d, 7.7)	107.4
13		138.1		138.1		137.9		138.4
14	5.50 (d, 2.4)	68.7	5.57 (d, 2.4)	69.0	4.46 (brs)	66.4	5.71 (s)	68.0
15	2.85 (dd, 8.5, 1.5)	49.7		79.2	3.42 (dd, 8.8, 1.5)	48.7	2.08 (overlapped)	43.7
16	2.59 (brdd, 8.5, 8.5)	38.6	2.44 (overlapped)	46.7	2.62 (overlapped)	38.1	2.82 (m)	38.1
17	4.43 (dd, 11.0, 3.2) 4.33 (dd, 11.0, 1.1)	61.7	4.39 (dd, 11.0, 3.0) 4.28 (d, 11.0)	60.6	4.52 (dd, 11.0, 3.3) 4.34 (brd, 11.0)	61.3	4.41 (dd, 11.0, 4.3) 4.32 (d, 11.0)	63.2
18	1.31 (3H, dd, 7.3, 7.3)	9.9	1.35 (3H, dd, 7.3, 7.3)	9.5	2.65 (3H, s)	26.0	0.88 (3H, dd, 7.5, 7.5)	9.0
19	2.93 (dddd, 17.4, 7.3, 7.3, 7.3) 2.54 (dddd, 17.4, 7.3, 7.3, 7.3)	26.1	2.73 (dddd, 17.8, 7.3, 7.3, 7.3) 2.56 (dddd, 17.8, 7.3, 7.3, 7.3)	21.8		197.3	2.00 (2H, overlapped)	23.2
20		180.5		182.2		174.7		68.6
21							3.48 (d, 10.1) 3.20 (brd, 10.1)	62.9
N_a-OMe	3.95 (3H, s)	63.4	3.95 (3H, s)	63.5	3.93 (3H, s)	63.4	4.08 (3H, s)	64.0
*C*OMe		170.1		170.9				174.0
CO*Me*	2.08 (3H, s)	21.2	2.16 (3H, s)	21.0			2.00 (3H, s)	21.0

proton of H-14 were observed at δ 2.16 (3H, s) and 5.57 (d), respectively. The ^{13}C NMR spectrum was also very similar to that of 14,15-dihydroxygelsenicine (**5**), which was recently isolated from the title plant,[8] except for the signals due to one acetyl group. The signal of an oxygenated quaternary carbon at C-15 was observed at δ 79.2. The positions of the acetoxy and hydroxyl groups were supported by the HMBC correlations between H-14 and an acetyl carbonyl carbon at δ 170.9, C-16, and C-20, and between H-3, H-14, and H-16 and the carbon at δ 79.2. The coupling constant ($J_{3,14}$ = 2.4 Hz) of the proton at C-14 indicated that the acetoxy group at C-14 is β-oriented. The hydroxyl group at C-15 was assigned as being β-oriented as in the case of 14,15-dihydroxygelsenicine (**5**) because of the formation of a ring consisting of the N_b, C-5, C-16, C-15, and C-20 atoms. From the above data, compound **2** was deduced to be 14-acetoxy-15-hydroxygelsenicine.

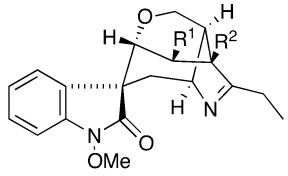
The alkaloid **3** was shown to have the molecular formula $C_{19}H_{20}N_2O_5$ from its HRFABMS (*m*/*z* 357.1420 $[MH]^+$). The UV absorptions at 256.5 and 213.0 nm revealed an oxindole nucleus. The ^{1}H and ^{13}C NMR spectra were very similar to those of GS-1[17] except for the signals in the aromatic region and the lack of a signal due to a methoxy group on ring A. In the ^{1}H NMR spectrum, signals assignable to four aromatic protons of the A ring of the oxindole system were observed at δ 7.55 (d, H-9), 7.29 (dd, H-11), 7.10 (dd, H-10), and 6.89 (d, H-12). The ^{13}C NMR spectrum revealed the presence of a ketone carbon at δ 197.3 (C-19), an imine carbon at δ 174.7 (C-20), and a methine carbon bearing an imine nitrogen at δ 74.1 (C-5), together with a carbonyl carbon of the oxindole ring at δ 170.8 (C-2). The HMBC correlations between H_3-18 and the carbons at δ 197.3 and 174.7 as well as other spectroscopic data obtained indicated that **3** is 14-hydroxy-19-oxogelsenicine. The configuration of the hydroxyl group at C-14 was shown to be β by the coupling constant between H-3 and H-14 (2.4 Hz), as in the case of other alkaloids of this type having a hydroxyl or an acetoxy group at C-14.

The molecular formula of alkaloid **4** was established as $C_{22}H_{28}N_2O_6$ from the HRFABMS (*m*/*z* 417.2012 $[MH]^+$). The UV absorptions at 258.0 and 209.5 nm revealed an oxindole nucleus. The ^{1}H NMR spectrum showed characteristic signals of a hydroxymethyl group at δ 3.48 (d) and 3.20 (brd) (H_2-21), a low-field methine proton at δ 5.71 (s, H-14), and an acetyl methyl group at δ 2.00 (3H, s), together with four aromatic protons, an N_a-methoxy group at δ 4.08 (3H, s), oxymethylene protons at δ 4.41 (dd) and 4.32 (d) (H_2-17), and an ethyl group. In the ^{13}C NMR spectrum, the hydroxymethyl carbon at δ 62.9, the quaternary carbon bearing a nitrogen atom at δ 68.6, and two carbonyl carbons due to C-2 in the oxindole ring and the acetoxy group at δ 170.5 and 174.0, respectively, were observed. From the above data and the HMBC correlations between the hydroxymethyl protons and C-19 (δ 23.2), and between the low-field methine proton at δ 5.71 and the carbons at δ 174.0, 68.6 (C-20), and 43.7 (C-15), **4** was deduced to be 14-acetoxygelselegine. The proton at C-14 was observed as a singlet in the ^{1}H NMR spectrum, suggesting that H-14 was not coupled with H-15, and therefore the acetoxy group at C-14 is β-oriented.

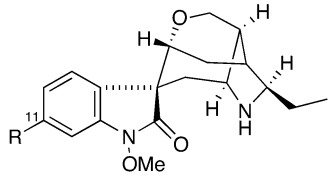
The cytotoxic effects were evaluated on a total of 14 *Gelsemium* alkaloids against the A431 human epidermoid carcinoma cell line. The *Gelsemium* alkaloids were classified into five types based on their chemical structures, i.e., sarpagine, koumine, humantenine, gelsedine, and gelsemine.[1] Among the 14 alkaloids examined, including gelsemicine (**8**)[17] and GS-2[18] from *Gelsemium sempervirens*, gelsedine-type alkaloids 14-acetoxygelsenicine (**1**), 14,15-dihydoxygelsenicine (**5**), gelsedine (**7**),[19] and gelsemicine (**8**) showed relatively strong cytotoxic effects. Their cytotoxic potency was compared to that of a positive control, cisplatin, which is used for the treatment of skin cancer. In particular, the new compound 14-acetoxygelsenicine (**1**) was found to show a potent effect (EC_{50} = 250 nM) (Table 2).

Experimental Section

General Experimental Procedures. Optical rotations were determined with a JASCO P-1020. UV spectra were recorded on a JASCO V-560. CD spectra were measured by a JASCO J-720WI. IR spectra



5 R^1=R^2=OH 14,15-Dihydroxygelsenicine
6 R^1=R^2=H Gelsenicine



7 R=H Gelsedine
8 R=OMe Gelsemicine

Table 2. Cytotoxicity (EC_{50}) of Selected *Gelsemium* Alkaloids for A431 Cells[a]

compound	EC_{50} (μM)
1, 14-acetoxygelsenicine	0.25
2, 14-acetoxy-15-hydroxygelsenicine	36
5, 14,15-dihydroxygelsenicine	1.3
6, gelsenicine	37
7, gelsedine	0.35
8, gelsemicine	0.75
cisplatin[b]	3.5

[a] 19(*Z*)-Akuammidine, 14-hydroxygelsenicine, gelsemoxonine, GS-2, humantenine, 11-methoxyhumantenine, gelsemine, and koumine were all inactive (EC_{50} > 100 μM). [b] Positive control.

were recorded using a JASCO FT/IR-230. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM A-500 at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR), respectively. EIMS were obtained on a JEOL GC Mate. FABMS were obtained on a JEOL JMS-AX500. HRFABMS were obtained on a JEOL JMS-HX110. TLC was done on precoated silica gel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography was carried out over silica gel 60 (Merck, 70−230 mesh). Medium-pressure liquid chromatography (MPLC) was carried out with C.I.G. prepacked column CPS-HS-221-05 (2.2 × 10 cm, Kusano Kagakukikai, silica gel).

Plant Material. *Gelsemium elegans* Benth. was collected from the Atagawa Tropical and Alligator Garden in Izu, Japan, in April 2002. A voucher specimen (no. 20011201) was deposited at the Faculty of Pharmaceutical Sciences, Chiba University.

Extraction and Isolation. The leaves of *G. elegans* (1.48 kg fresh weight) were extracted with MeOH (once at room temperature and five times under reflux) to give a MeOH extract (203.6 g). A portion of the MeOH extract (100 g) was dissolved in 1 N HCl (2 L). After extraction with ethyl acetate (3.4 L), the acidic layer was basified with Na_2CO_3 at 0 °C (pH 9) and extracted with 5% MeOH/$CHCl_3$ (4 L) to give a crude alkaloidal fraction (2.47 g). The crude alkaloids were separated by silica gel open column chromatography (4 × 15.2 cm) with a $CHCl_3$/MeOH gradient, to give 11 fractions: A, $CHCl_3$ (160 mL); B, 2% MeOH/$CHCl_3$ (240 mL); C, 2% (240 mL); D, 5% (320 mL); E, 5% (320 mL); F, 10% (320 mL); G, 10% (640 mL); H, 15% (320 mL) and 20% (320 mL); I, 30% (320 mL); J, 30% (160 mL), 50% MeOH/$CHCl_3$ (320 mL), and MeOH (200 mL); and K, MeOH (200 mL) and saturated NH_3 in 50% MeOH/$CHCl_3$ (200 mL). Fraction C (744.8 mg) was purified by silica gel open column chromatography (50% AcOEt/*n*-hexane−AcOEt−AcOEt/MeOH gradient). The fraction eluted with 5−10% MeOH/AcOEt was separated by MPLC (MeOH/$CHCl_3$ gradient) to give 14-acetoxygelsenicine (**1**, 93.7 mg) and 14-acetoxy-15-hydroxygelsenicine (**2**, 7.3 mg). The fraction eluted with 10−30% MeOH/AcOEt was purified by MPLC (MeOH/AcOEt gradient) to afford a further quantity of 14-acetoxygelsenicine (**1**, 18.2 mg). The fraction that was eluted with 2−5% MeOH/AcOEt on the silica gel open column chromatography of fraction C was further separated by MPLC (1% MeOH/AcOEt and 1% MeOH/$CHCl_3$) to give 14-hydroxy-19-oxogelsenicine (**3**, 3.6 mg). Fraction D (760.9 mg) was purified by silica gel open column chromatography (5−20% AcOEt/MeOH gradient). The fraction that eluted with 5−10% MeOH/AcOEt was purified by MPLC (3% MeOH/$CHCl_3$) to afford additional 14-acetoxy-15-hydroxygelsenicine (**2**, 3.2 mg). Fraction G (157 mg) was purified by silica gel open column chromatography (MeOH/$CHCl_3$/NH_4OH = 2:98:1 → 5:95:1 → 10:90:1 gradient), MPLC (10−30% MeOH/AcOEt gradient), and silica gel open column chromatography (NH_4OH-saturated $CHCl_3$) to give 14-acetoxygelselegine (**4**, 1.2 mg). Eleven known alkaloids were isolated. Gelsenicine (**6**, 75.9 mg), gelsemoxonine (72.9 mg), 21-oxogelsemine (2.0 mg), and 11-methoxyhumantenine (1.8 mg) were isolated from fraction C. 14-Hydroxygelsenicine (236.7 mg), 14,15-dihydroxygelsenicine (**5**, 61.1 mg), and 19(*Z*)-akuammidine (4.3 mg) were isolated from fraction D. Humantenirine (6.6 mg) was isolated from fraction E (189.4 mg). Koumine (1.7 mg) and gelsemine (91.0 mg) were isolated from fraction F (230.2 mg). Gelsemine (52.5 mg) was also obtained from fractions E and G. Gelsemine *N*-oxide (3.5 mg) was isolated from fraction J (55.2 mg). The structures of the known compounds were identified by comparing their spectroscopic data with the literature values or with those of authentic samples.

14-Acetoxygelsenicine (1): amorphous; $[\alpha]^{19}_D$ −102.6 (*c* 1.49, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 258.0 (3.77), 213.5 (4.27) nm; CD (*c* 0.330 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (nm) 0 (302), −6.30 (263), 0 (250), +12.91 (233), 0 (219), −12.82 (210); IR ($CHCl_3$) ν_{max} 2941, 1721, 1211 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 384 (M^+, 100), 353 (60), 311 (80); HRFABMS (NBA/PEG) *m*/*z* 385.1768 (MH^+, calcd for $C_{21}H_{25}N_2O_5$, 385.1763).

14-Acetoxy-15-hydroxygelsenicine (2): amorphous; $[\alpha]^{19}_D$ −66.3 (*c* 0.086, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 258.0 (3.73), 212.5 (4.26) nm; CD (*c* 0.315 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (nm) 0 (305), −5.67 (263), 0 (251), +13.52 (234), 0 (218), −10.30 (209); IR ($CHCl_3$) ν_{max} 2941, 1720, 1232 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 400 (M^+, 30), 340 (44), 285 (94), 254 (47), 215 (100); HRFABMS (NBA/PEG) *m*/*z* 401.1707 (MH^+, calcd for $C_{21}H_{25}N_2O_6$, 401.1713).

14-Hydroxy-19-oxogelsenicine (3): amorphous; $[\alpha]^{19}_D$ −146.1 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 256.5 (3.73), 213.0 (4.28) nm; CD (*c* 0.307 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (nm) 0 (325), +1.21 (279), 0 (273), −6.98 (258), 0 (244), +2.80 (237), 0 (231), −20.63 (213); IR ($CHCl_3$) ν_{max} 2927, 1715, 1219 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 356 (M^+, 100), 313 (49); HRFABMS (NBA/PEG) *m*/*z* 357.1420 (MH^+, calcd for $C_{19}H_{21}N_2O_5$, 357.1450).

14-Acetoxygelselegine (4): amorphous; $[\alpha]^{20}_D$ −62.1 (*c* 0.097, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 258.0 (3.60), 209.5 (4.23) nm; CD (*c* 0.327 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (nm) 0 (306), −4.48 (261), 0 (248), +9.48 (231), 0 (219), −11.80 (209); IR ($CHCl_3$) ν_{max} 2927, 1727, 1262 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS (NBA) *m*/*z* 417 $[M + H]^+$; HRFABMS (NBA/PEG) *m*/*z* 417.2012 (MH^+, calcd for $C_{22}H_{29}N_2O_6$, 417.2026).

Acetylation of 14-Hydroxygelsenicine. A solution of 14-hydroxygelsenicine (5.1 mg, 0.015 mmol) in dry CH_2Cl_2 (0.6 mL) was added to a solution of Ac_2O (1.5 μL, 0.016 mmol) and DMAP (1.5 mg, 0.012 mmol) in dry CH_2Cl_2 (0.1 mL) at room temperature under Ar gas, and the mixture was stirred at the same temperature for 1 h. Saturated aqueous NH_4Cl was added to the reaction mixture, and extraction was carried out with $CHCl_3$. The combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give an acetyl derivative (**1**, 4.6 mg, yield 80%). All of the spectroscopic data (^{1}H, ^{13}C NMR and CD) were identical with those of natural **1**.

Evaluation of Cytotoxic Activity. *Gelsemium* Alkaloids. 14-Acetoxygelsenicine (**1**), 14-acetoxy-15-hydroxygelsenicine (**2**), 14-hydroxygelsenicine, 14,15-dihydroxygelsenicine (**5**), gelsenicine (**6**), gelsedine (**7**), gelsemoxonine, 19(*Z*)-akuammidine, 11-methoxyhumantenine, humantenine,[7] gelsemine, and koumine were isolated from *G. elegans*. Gelsemicine (**8**) and GS-2 were isolated from *G. sempervirens*.

Cell Culture. Human epidermoid carcinoma A431 cells (purchased from RIKEN Cell Bank, Japan) were grown on 100 mm ϕ dishes until semiconfluent in a CO_2 incubator (5% CO_2, 37 °C). Then, cells were stripped with trypsin-EDTA (500 μL) for 3 min and were subcultured on the 12-well multiwell plates (1 × 10^5 cells/well). Biological assays were performed with the cells at semiconfluent state (approximately 5 × 10^5 cells/well) as follows.

Cell Counting. Each sample and the positive control substance (cisplatin) was dissolved in DMSO (100 mM). Each solution was added to the cells and diluted with serum-free RPMI-1640 cell culture medium (Kojin Bio, Osaka, Japan) so that the final concentration of each

compound was 10 nM to 100 μM. The concentration of DMSO in each sample solution was adjusted to less than 0.1% in order to prevent any cytotoxic effects. Cells were grown in these solutions for 48 h. Calculation of the number of cells was carried out using the Bürker-Turk's blood cell counting chamber by dyeing with trypan blue. Data were obtained in three independent experiments, and each was calculated as a survival ratio (%) compared with normal control and expressed as the mean ± SEM. The EC_{50} was calculated with GraphPad Prism version 4.0 software (GraphPad Software, Inc., San Diego, CA).

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research (C) (No. 16510156) from the Japan Society for the Promotion of Science.

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NP060016O