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Four novel gelsedine-type oxindole alkaloids from *Gelsemium elegans*

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ABSTRACT

Four new gelsedine-type oxindole alkaloids (**1**–**4**) were isolated from the leaves and branches of *Gelsemium elegans*, together with 10 known alkaloids. The structures of the new alkaloids were determined by spectroscopic analyses and partial synthesis from known compounds. Gelsecrotonidine (**1**), 14-hydroxygelsecrotonidine (**2**), and 11-methoxygelsecrotonidine (**3**) possess an additional C_2 unit with an acetic acid residue compared to gelsenicine-related monoterpenoid indole alkaloids. 14-Hydroxygelsedilam (**4**) is an 18,19-nor-type monoterpenoid indole alkaloid.

1. Introduction

Gelsemium elegans Benth. (Loganiaceae), a toxic plant widely distributed in Southeast Asia, was used in traditional Chinese medicine. Our previous study has proved that the origin of 'Yakatsu,' one of the ancient medicines stored in the Shosoin repository in Japan, is *G. elegans*.[1] This plant was used as a remedy for certain kinds of skin ulcers in traditional Chinese medicine and is presumed to have been used as an external medication for dermatitis more than 1250 years ago in Japan. The genus *Gelsemium* comprises three species, all of which are known to be rich sources of alkaloids. To date, more than 70 alkaloids have been isolated and classified into 6 types on the basis of their chemical structures.[2,3] Recently, we found that among the structurally diverse *Gelsemium* alkaloids, some gelsedine-type compounds showed potent cytotoxic effects on A431 epidermoid carcinoma cells.[4] In our continuing chemical studies on *Gelsemium* alkaloids,[5] 4 new gelsedine-type alkaloids, namely, gelsecrotonidine (**1**), 14-hydroxygelsecrotonidine (**2**), 11-methoxygelsecrotonidine (**3**), and 14-hydroxygelsedilam (**4**), were isolated from *G. elegans*, together with 10 known alkaloids (Fig. 1). In this paper, the structural elucidation of these new alkaloids by means of spectroscopic analyses and chemical conversion from known alkaloids is described.

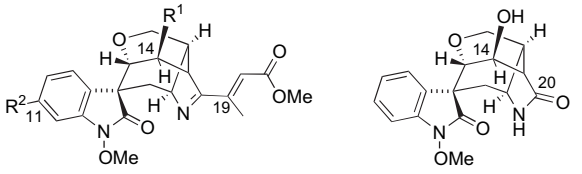

Figure 1. Structures of new alkaloids.

2. Results and discussion

The leaves and branches of *G. elegans* Benth. (711.1 g) were extracted with MeOH to afford the MeOH extract. The MeOH extract was dissolved in H_2O containing a small volume of MeOH and extracted successively with *n*-hexane, AcOEt, 5% MeOH/$CHCl_3$, and *n*-BuOH to give the *n*-hexane extract, the AcOEt extract, the 5% MeOH/$CHCl_3$ extract, and the *n*-BuOH extract, respectively. From the 5% MeOH/$CHCl_3$ extract, 4 new alkaloids, gelsecrotonidine (**1**, 0.6 mg, 0.00008%), 14-hydroxygelsecrotonidine (**2**, 0.8 mg, 0.00011%), 11-methoxygelsecrotonidine (**3**, 0.3 mg, 0.00004%), and 14-hydroxygelsedilam (**4**, 2.2 mg, 0.0003%), were isolated, together with 10 known alkaloids, gelsenicine (**5**),[6] 14-hydroxygelsenicine (**6**),[7,8] 14,15-dihydroxygelsenicine,[5a] 14-acetoxygelsenicine,[4]

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Table 1
^{1}H (400 MHz) and ^{13}C (125 MHz) NMR data for gelsecrotonidine (**1**), 14-hydroxygelsecrotonidine (**2**), and 11-methoxygelsecrotonidine (**3**) in $CDCl_3$

Position	**1**		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		171.0		170.6		171.5
3	3.74 (dd, 4.8, 2.1)	74.9	3.68 (br dd, 1.9, 1.9)	79.4	3.71 (dd, 4.3, 1.7)	75.2
5	4.67 (br ddd, 7.7, 5.0, 2.4)	73.7	4.67 (br ddd, 7.6, 5.0, 2.9)	73.1	4.65 (ddd, 7.9, 5.0, 2.4)	73.6
6	2.51 (dd, 15.5, 5.0), 2.35 (dd, 15.5, 2.4)	37.6	2.54 (dd, 15.8, 5.0), 2.38 (dd, 15.8)	37.4	2.47 (dd, 15.4, 5.0), 2.32 (dd, 15.4, 2.4)	37.8
7		55.9		53.8		55.6
8		131.8		131.3		123.7
9	7.54 (d, 7.6)	124.6	7.53 (d, 7.5)	124.6	7.41 (d, 8.3)	125.4
10	7.08 (ddd, 7.6, 7.6, 1.0)	123.4	7.10 (ddd, 7.5, 7.5, 1.1)	123.6	6.57 (dd, 8.3, 2.4)	107.8
11	7.27 (ddd, 7.6, 7.6, 1.0)	128.2	7.29 (ddd, 7.5, 7.5, 1.1)	128.5		160.2
12	6.89 (d, 7.6)	106.6	6.89 (d, 7.5)	106.9	6.47 (d, 2.4)	94.0
13		138.1		138.1		139.2
14	2.41 (dd, 14.9, 2.1), 2.24 (ddd, 14.9, 9.5, 4.8)	27.9	4.49 (d, 1.9)	67.0	2.38 (dd, 15.0, 1.7), 2.23 (ddd, 15.0, 9.5, 4.3)	27.8
15	3.33 (dd, 9.5, 9.5)	39.4	3.33 (br d, 8.5)	49.2	3.32 (dd, 9.5, 9.5)	39.4
16	2.64 (m)	39.8	2.67 (br ddd, 8.5, 8.5, 3.4)	38.3	2.63 (m)	39.8
17	4.34 (dd, 11.3, 2.8), 4.30 (dd, 11.3, 1.8)	61.9	4.50 (dd, 10.9, 3.4), 4.37 (d, 10.9)	61.6	4.33 (dd, 11.2, 3.0), 4.29 (dd, 11.2, 1.5)	61.9
18	2.59 (3H, br d, 1.3)	14.8	2.58 (3H, br d, 1.4)	14.8	2.58 (3H, d, 0.7)	14.8
19		149.0		148.6		149.0
20		179.7		176.5		179.7
21	6.20 (br d, 1.3)	122.7	6.41 (br d, 1.4)	123.2	6.20 (br d, 0.7)	122.6
22		167.0		167.0		167.0
23	3.77 (3H, s)	51.4	3.78 (3H, s)	51.4	3.77 (3H, s)	51.4
N_a-OMe	3.92 (3H, s)	63.3	3.92 (3H, s)	63.4	3.91 (3H, s)	63.4
11-OMe					3.81 (3H, s)	55.6

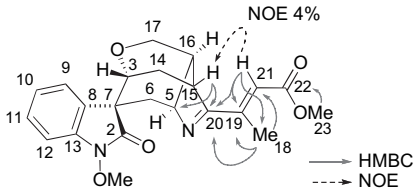


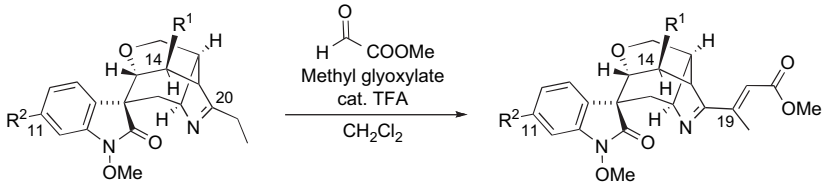
Figure 2. Selected HMBC and NOE correlations of gelsecrotonidine (**1**).

19-oxogelsenicine,[8] 14-hydroxy-19-oxogelsenicine,[8] gelsemoxonine,[5a] 19(*Z*)-akuammidine,[8] 11-methoxyhumantenine,[9] and gelsemine,[10,11] by a combination of column chromatographies.

New alkaloid **1**, named gelsecrotonidine, was found to have the molecular formula $C_{22}H_{24}N_2O_5$ from HREIMS [*m*/*z* 396.1677 (M^+)]. ^{1}H and ^{13}C NMR spectra (Table 1) revealed some readily assignable signals due to the gelsenicine (**5**) skeleton, including signals assigned to an oxindole system with a nonsubstituted A ring [δ_H 7.54 (d, H-9), δ_H 7.27 (ddd, H-11), δ_H 7.08 (ddd, H-10), δ_H 6.89 (d, H-12), δ_C 171.0 (C-2)], an N_a-methoxy group [δ_H 3.92 (3H, s)], an oxymethylene group [δ_H 4.34 (dd), δ_H 4.30 (dd), δ_C 61.9 (C-17)], and an oxymethine group [δ_H 3.74 (dd), δ_C 74.9 (C-3)]. Instead of signals due to H-19 methylene protons, signals assignable to a trisubstituted olefin group [δ_H 6.20 (br d, H-21), δ_C 149.0 (C-19), δ_C 122.7 (C-21)] and a methyl carboxylate group [δ_H 3.77 (3H, s, H_3-23), δ_C 167.0 (C-22), δ_C 51.4 (C-23)] were observed. By examining HMBC correlations of the olefinic proton signal at δ 6.20 (H-21) to the methyl carbon signal at δ 14.8 (C-18), the olefinic carbon signal at δ 149.0 (C-19), and the imine carbon signal at δ 179.7 (C-20) as well as the proton signal at δ 2.59 (H_3-18) to the olefinic carbon signal at δ 122.7 (C-21), a crotonic acid methyl ester residue comprising C-18 and C-19 was confirmed to be connected to the C-20 imine carbon, as shown in Figure 2. The geometry of the double bond between C-19 and C-21 was elucidated to be *E* based on the NOE correlation of H-21 (δ 6.20) to H-15 (δ 3.33). From these data, gelsecrotonidine (**1**) was proposed to be a gelsenicine (**5**) derivative having a methyl acetate residue on C-19 position.

To confirm the structure inferred by spectroscopic analyses above, the chemical transformation of gelsenicine (**5**) into **1** was attempted (Scheme 1). Gelsenicine (**5**) was treated with methyl glyoxylate[12] in the presence of a catalytic amount of TFA in CH_2Cl_2 to give **1** in 86% yield. All of the spectroscopic data including the CD spectrum of semi-synthetic **1** were identical with those of the natural product, thereby establishing the structure including the absolute configuration. To the best of our knowledge, gelsecrotonidine (**1**) is the first example of a monoterpenoid indole alkaloid having a C_2 unit with an acetic acid residue on the side chain.

New alkaloid **2** was shown to have the molecular formula $C_{22}H_{24}N_2O_6$ from HREIMS [*m*/*z* 412.1636 (M^+)], which indicated that **2** had an extra oxygen compared to gelsecrotonidine (**1**). ^{1}H and ^{13}C NMR spectral data revealed the same backbone structure as **1**, namely, four aromatic protons [δ 7.53 (d, H-9), δ 7.29 (ddd, H-11), δ 7.10 (ddd, H-10), δ 6.89 (d, H-12)], an N_a-methoxy group [δ 3.92 (3H, s)], oxymethylene protons [δ 4.50 (dd), δ 4.37 (d) (H_2-17)], an oxymethine proton [δ 3.68 (br-dd, H-3)], and a methyl crotonate residue [δ_H 6.41 (br d, H-21), δ_H 3.78 (3H, s, H_3-23), δ_H 2.58 (3H, br d,



R^1=H, R^2=H : Gelsenicine (**5**)
R^1=OH, R^2=H : 14-Hydroxygelsenicine (**6**)
R^1=H, R^2=OMe : 4,20-Dehydrogelsemicine (**7**)

R^1=H, R^2=H : Gelsecrotonidine (**1**)
R^1=OH, R^2=H : 14-Hydroxygelsecrotonidine (**2**)
R^1=H, R^2=OMe : 11-Methoxygelsecrotonidine (**3**)

Scheme 1.

H_3-18), δ_C 167.0 (C-22), δ_C 148.6 (C-19), δ_C 123.2 (C-21), δ_C 51.4 (C-23), δ_C 14.8 (C-18)]. In addition, a low-field methine proton signal at δ 4.49 (d, H-14) and an oxygenated methine carbon signal at δ_C 67.0 were observed, suggesting the existence of an additional hydroxyl group. HMBC correlations of the proton signal at δ 3.68 due to H-3 and that at δ 3.33 due to H-15 to the carbon signal at δ 67.0 indicated that the hydroxyl group was attached to C-14. The configuration of the hydroxyl group at C-14 was shown to be β on the basis of the coupling constant ($J_{3,14}$=1.9 Hz) of the proton at C-14, as in the case of other compounds having a hydroxyl or acetoxy group at C-14.[4,5b,d] The *E* configuration of the C-19–C-21 double bond was determined by NOE correlation of H-21 to H-15. From the above data, new alkaloid **2** was deduced to be a 14β-hydroxy derivative of gelsecrotonidine (**1**). To confirm the structure, 14-hydroxygelsenicine (**6**), a 14β-hydroxy derivative of gelsenicine (**5**), was treated with methyl glyoxylate according to the method for the partial synthesis of gelsecrotonidine (**1**) to give **2** in 82% yield. All of the spectroscopic data including the CD spectrum of semi-synthetic **2** were identical with those of the natural product, thereby establishing the structure including the absolute configuration.

New alkaloid **3** was found to have the molecular formula $C_{23}H_{26}N_2O_6$ from HREIMS [*m*/*z* 426.1791 (M^+)], which indicated that **3** had an extra CH_2O compared to gelsecrotonidine (**1**). ^{1}H and ^{13}C NMR spectral data revealed the same backbone structure as gelsecrotonidine (**1**), including a methyl crotonate residue [δ_H 6.20 (br d, H-21), δ_H 3.77 (3H, s, H_3-23), δ_H 2.58 (3H, d, H_3-18), δ_C 167.0 (C-22), δ_C 149.0 (C-19), δ_C 122.6 (C-21), δ_C 51.4 (C-23), δ_C 14.8 (C-18)], except for the signals in the aromatic region. NMR data suggested the presence of an N_a,6-dimethoxyoxindole system [δ_H 7.41 (d, H-9), δ_H 6.57 (dd, H-10), δ_H 6.47 (d, H-12), δ_H 3.91 (3H, s, N_a-OMe), δ_H 3.81 (3H, s, 11-OMe), δ_C 171.5 (C-2), δ_C 63.4 (N_a-OMe), δ_C 55.6 (11-OMe)]. UV spectra [293.0 (sh), 249.0 (sh), 218.5] also supported the presence of a 6-methoxyoxindole chromophore. HMBC crosspeaks of the methoxy proton signal at δ 3.81 (11-OMe) to the aromatic carbon signal at δ 160.2 (C-11) and of the proton signal at δ 6.57 (dd, H-10) to the quaternary sp^2 carbon signal at δ 123.7 (C-8) indicated that a methoxy group was attached to C-11 in the A ring of the oxindole. From the above data, new alkaloid **3** was deduced to be an 11-methoxy derivative of gelsecrotonidine (**1**). To reveal the structure of **3**, partial synthesis from 4,20-dehydrogelsemicine (**7**),[5b,13] an 11-methoxy derivative of gelsenicine (**5**), to afford **3** was accomplished in 42% yield. All of the spectroscopic data including the CD spectrum of semi-synthetic **3** were identical with those of the natural product, thereby establishing the structure including the absolute configuration.

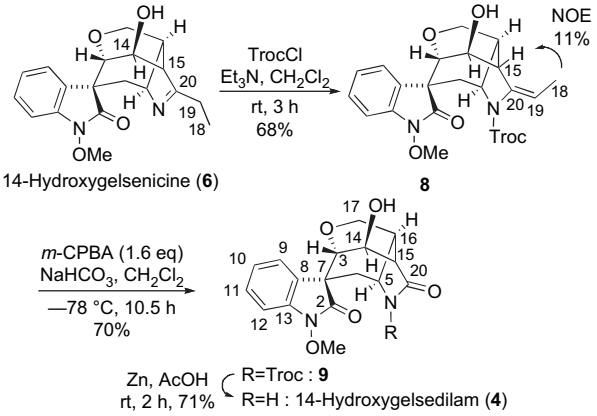
New alkaloid **4** was shown to have the molecular formula $C_{17}H_{18}N_2O_5$ from HREIMS [*m*/*z* 330.1213 (M^+)], which possessed two carbons less than common *Gelsemium* alkaloids. UV and NMR spectra exhibited the characteristic N_a-methoxyoxindole chromophore. ^{1}H and ^{13}C NMR data (Table 2) revealed the presence of a nonsubstituted A ring of the oxindole system, an N_a-methoxy group (δ_H 3.87, δ_C 64.0), two oxymethine groups (δ_H 4.41, δ_C 62.1, C-14, and δ_H 3.56, δ_C 80.5, C-3), a methine group bearing nitrogen (δ_H 3.99, δ_C 57.8, C-5), and an oxymethylene group (δ_H 4.27, 4.20 δ_C 67.5, C-17). In addition to the carbonyl carbon signal due to the oxindole nucleus at δ 172.9, a lactam carbonyl carbon signal at δ 179.6 was observed in the ^{13}C NMR spectra. These spectral data were very similar to those of gelsedilam,[5d] which had an 18,19-nor-gelsedine backbone structure, except for the existence of a low-field methine function (δ_H 4.41, δ_C 62.1), suggesting the addition of a hydroxyl group. ^{1}H–^{1}H COSY correlation between the H-3 oxymethine proton signal at δ 3.56 and the low-field methine proton signal at δ 4.41 indicated that a hydroxyl group was attached to C-14. The configuration of the hydroxyl group at C-14 was shown to be β on the basis of the coupling constant ($J_{3,14}$=2.2 Hz) of the proton at C-14.

Table 2
^{1}H (400 MHz) and ^{13}C (125 MHz) NMR data for 14-hydroxygelsedilam (**4**) in CD_3OD

Position	**4**	
	δ_H	δ_C
2		172.9
3	3.56 (br s)	80.5
5	3.99 (m)	57.8
6	2.39 (dd, 15.7, 3.8), 1.91 (br dd, 15.7, 2.5)	37.4
7		55.1
8		132.8
9	7.47 (d, 7.7)	125.8
10	7.04 (ddd, 7.7, 7.7, 1.1)	124.8
11	7.24 (ddd, 7.7, 7.7, 1.1)	129.6
12	6.90 (d, 7.7)	108.1
13		139.3
14	4.41 (br d, 2.2)	62.1
15	2.44 (d, 8.4)	47.7
16	2.79 (m)	37.0
17	4.27 (dd, 10.9, 3.0), 4.20 (d, 10.9)	67.5
20		179.6
N_a-OMe	3.87 (3H, s)	64.0

Therefore, **4** was deduced to be a 14β-hydroxy derivative of gelsedilam.

To confirm the structure of **4** proposed by spectroscopic analysis, partial synthesis using 14-hydroxygelsenicine (**6**) as starting material was performed (Scheme 2). 14-Hydroxygelsenicine (**6**) was treated with trichloroethoxycarbonyl (Troc) chloride in the presence of Et_3N in CH_2Cl_2 to give enamine-carbamate **8** in 68% yield. Treatment of **8** with *m*-CPBA afforded lactam **9** in 70% yield. Finally, removal of the N_b-Troc group (Zn, AcOH) gave 14-hydroxygelsedilam (**4**) in 71% yield. All of the spectroscopic data including the CD spectrum of synthetic **4** were identical with those of natural **4** and, therefore, the structure including the absolute configuration was established.



Scheme 2.

In conclusion, four new gelsedine-type oxindole alkaloids (**1**–**4**) were isolated from the leaves and branches of *G. elegans*. To the best of our knowledge, gelsecrotonidine (**1**), 14-hydroxygelsecrotonidine (**2**), and 11-methoxygelsecrotonidine (**3**) are the first examples of a monoterpenoid indole alkaloid possessing a C_2 unit with an acetic acid residue on the side chain.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra: JEOL JNM ECP-400 at 400 MHz (^{1}H) and JEOL JNM A-500 at 125 MHz (^{13}C), respectively. UV: JASCO V-560. EIMS and HREIMS: JEOL GC mate. CD: JASCO J-720WI. TLC:

precoated silica gel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Silica gel 60 (Merck, 70–230 mesh). Column chromatography: Silica gel 60N [Kanto Chemical, 40–50 mm (for flash chromatography)] and Chromatorex NH [Fuji Silysia Chemical, 100–200 mesh (for amino-silica gel column chromatography)]. Medium pressure liquid chromatography (MPLC): C.I.G. prepacked column CPS-HS-221–05 (Kusano Kagakukikai, SiO_2).

3.2. Plant material

G. elegans Benth. was collected from the Atagawa Tropical and Alligator Garden in Izu, Japan, in April 2002 (branches 529.7 g) and in March 2006 (branches 82.4 g, and leaves 99.0 g), and identified by Dr. F. Ikegami, Chiba University. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Chiba University.

3.3. Extraction and isolation

The leaves and branches of *G. elegans* Benth. (711.1 g, dry weight) were extracted with MeOH (once at room temperature and three times under reflux) to give the MeOH extract (88.36 g). The MeOH extract was dissolved in 8% MeOH/H_2O (1.0 L) and extracted with *n*-hexane (1.0 L, 0.4 L, 0.5 L) to give the *n*-hexane extract (12.30 g). The aqueous layer was successively extracted with AcOEt (1.0 L, 0.8 L, 0.8 L), 5% MeOH/$CHCl_3$ (1.0 L×3), and *n*-BuOH (0.8 L×3) to give the AcOEt extract (19.22 g), the 5% MeOH/$CHCl_3$ extract (1.28 g), and the *n*-BuOH extract (20.86 g), respectively. The 5% MeOH/$CHCl_3$ extract (1.28 g) was separated by SiO_2 flash column chromatography with a $CHCl_3$/MeOH gradient to afford eight fractions: fr. A 2% MeOH/$CHCl_3$ (160 mL, 21.9 mg); fr. B 2% MeOH/$CHCl_3$ (120 mL, 9.2 mg); fr. C 2% MeOH/$CHCl_3$ (200 mL, 457.0 mg); fr. D 5% MeOH/$CHCl_3$ (360 mL, 177.3 mg); fr. E 5% MeOH/$CHCl_3$ (80 mL, 16.0 mg); fr. F 5% MeOH/$CHCl_3$ (60 mL, 22.9 mg); fr. G MeOH (60 mL, 464.9 mg); and fr. H MeOH (110 mL, 7.0 mg). Fraction C (457.0 mg) was separated by SiO_2 flash column chromatography with MeOH/AcOEt gradient. The fraction eluted with 2% MeOH/AcOEt was purified by MPLC (7% MeOH/AcOEt and 1% MeOH/$CHCl_3$) to give gelsecrotonidine (**1**, 0.6 mg) and 11-methoxygelsecrotonidine (**3**, 0.3 mg). The fraction eluted with 2% MeOH/AcOEt was further separated by MPLC (7% MeOH/AcOEt and 7% MeOH/$CHCl_3$) to give 14-hydroxygelsecrotonidine (**2**, 0.8 mg). The AcOEt-soluble part of fraction G (439.0 mg) was separated by SiO_2 flash column chromatography with MeOH/$CHCl_3$ gradient. The fraction eluted with 10–20% MeOH/$CHCl_3$ was purified by MPLC (10% MeOH/$CHCl_3$) to give 14-hydroxygelsedilam (**4**, 2.2 mg). Ten known alkaloids were isolated. Gelsenicine (**5**, 2.7 mg), 14-acetoxygelsenicine (1.8 mg), 19-oxogelsenicine (0.4 mg), 14-hydroxy-19-oxogelsenicine (1.1 mg), gelsemoxonine (9.0 mg), and 11-methoxyhumantenine (20.1 mg) were isolated from fraction C. 11-Methoxyhumantenine (36.2 mg) was also isolated from fraction D and fraction G. 14,15-Dihydroxygelsenicine (15.5 mg) was isolated from fraction D. 14-Hydroxygelsenicine (**6**, 2.7 mg) was isolated from fraction F and fraction G. 19(*Z*)-Akuammidine (4.5 mg) was isolated from fraction G and gelsemine (7.0 mg) was isolated from fraction H (7.0 mg). The structures of the known compounds were identified by comparing their spectroscopic data with the literature values.

3.4. Characteristics of each alkaloid

3.4.1. Gelsecrotonidine (*1*)

^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 248.5 (4.03), 209.5 (4.30); EIMS *m*/*z* (%) 396 (M^+, 100), 365 (57); HREIMS 396.1677 (M^+, calcd for $C_{22}H_{24}N_2O_5$, 396.1685); CD (*c* 0.252 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (286), −3.47 (256), 0 (239), +0.75 (236), 0 (229), −14.12 (210).

3.4.2. 14-Hydroxygelsecrotonidine (*2*)

^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 248.5 (4.10), 210.5 (4.36); EIMS *m*/*z* (%) 412 (M^+, 100), 146 (52); HREIMS 412.1636 (M^+, calcd for $C_{22}H_{24}N_2O_6$, 412.1634); CD (*c* 0.291 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (346), +0.52 (307), 0 (292), −4.01 (251), −1.00 (237), −13.79 (213).

3.4.3. 11-Methoxygelsecrotonidine (*3*)

^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 293.0 (sh, 3.36), 249.0 (sh, 4.01), 218.5 (4.26); EIMS (%) *m*/*z* 426 (M^+, 100), 395 (72), 132 (81); HREIMS 426.1791 (M^+, calcd for $C_{23}H_{26}N_2O_6$, 426.1790); CD (*c* 0.244 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (nm) 0 (298), −2.17 (256), 0 (242), +1.02 (239), 0 (236), −17.27 (219), 0 (205), +4.36 (201).

3.4.4. 14-Hydroxygelsedilam (*4*)

^1H and ^{13}C NMR data, see Table 2; UV (MeOH) λ_{max} nm (log ε) 257.5 (3.52), 209.0 (4.19); EIMS *m*/*z* (%): 330 (M^+, 87), 144 (100); HREIMS 330.1213 (M^+, calcd for $C_{17}H_{18}N_2O_5$, 330.1215); CD (*c* 0.280 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (304), −3.11 (260), 0 (248), +4.33 (235), 0 (224), −12.65 (213).

3.5. Partial synthesis of gelsecrotonidine (1), 14-hydroxygelsecrotonidine (2), and 11-methoxygelsecrotonidine (3)

3.5.1. Conversion of gelsenicine (*5*) into gelsecrotonidine (*1*)

To a stirred solution of gelsenicine (**5**, 3.9 mg, 0.012 mmol) and TFA (5 μL) in CH_2Cl_2 (0.8 mL) was added methyl glyoxylate (0.25 mL, excess), and the mixture was stirred for 4.5 h at room temperature under Ar atmosphere. Saturated aqueous Na_2CO_3 was added to the reaction mixture under ice cooling and the entire mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was subjected to amino-silica gel open column chromatography ($CHCl_3$) to remove excess aldehyde. The fraction eluted with $CHCl_3$ was purified by SiO_2 open column chromatography ($CHCl_3$) to afford **1** (3.9 mg, 86%). All the spectroscopic data were identical with those of natural **1**.

3.5.2. Conversion of 14-hydroxygelsenicine (*6*) into 14-hydroxygelsecrotonidine (*2*)

To a stirred solution of 14-hydroxygelsenicine (**6**, 3.6 mg, 0.011 mmol) and TFA (5 μL) in CH_2Cl_2 (0.8 mL) was added methyl glyoxylate (0.25 mL, excess), and the mixture was stirred for 4.5 h at room temperature under Ar atmosphere. Saturated aqueous Na_2CO_3 was added under ice cooling and the entire mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was subjected to amino-silica gel open column chromatography ($CHCl_3$) to remove excess aldehyde. The fraction eluted with $CHCl_3$ was purified by MPLC (2.5% MeOH/$CHCl_3$) to afford **2** (3.4 mg, 82%). All the spectroscopic data were identical with those of natural **2**.

3.5.3. Conversion of 4,20-dehydrogelsemicine (*7*) into 11-methoxygelsecrotonidine (*3*)

To a stirred solution of 4,20-dehydrogelsemicine (**7**, 2.0 mg, 0.006 mmol) and TFA (0.2 μL) in CH_2Cl_2 (1.1 mL) was added methyl glyoxylate (0.1 mL, excess), and the mixture was stirred for 5 h at room temperature under Ar atmosphere. Saturated aqueous Na_2CO_3 was added under ice cooling and the entire mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was subjected to amino-silica open column chromatography ($CHCl_3$) to remove excess aldehyde. The fraction eluted with $CHCl_3$ was purified by MPLC

(0.5% MeOH/$CHCl_3$) to afford **3** (1.0 mg, 42%). All the spectroscopic data were identical with those of natural **3**.

3.6. Partial synthesis of 14-hydroxygelsedilam (4)

*3.6.1. Preparation of enamine-carbamate **8** from 14-hydroxygelsenicine (**6**)*

To a solution of 14-hydroxygelsenicine (**6**, 5.5 mg, 0.017 mmol) in CH_2Cl_2 (0.5 mL) were added Et_3N (4.3 μL, 0.034 mmol) and 2,2,2-trichloroethoxycarbonyl (Troc) chloride (2.7 μL, 0.019 mmol) at 0 °C under Ar atmosphere. After stirring at room temperature for 3 h, additional Troc chloride (1.3 μL, 0.009 mmol) was added and the mixture was stirred for 1 h. Saturated aqueous NH_4Cl was added to the reaction mixture and the entire mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by MPLC (50% AcOEt/*n*-hexane) to give enamine-carbamate **8** (5.6 mg, 68%).

*3.6.1.1. Enamine-carbamate **8**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (1H, d, J=7.6 Hz), 7.30 (1H, dd, J=7.6, 7.6 Hz), 7.10 (1H, dd, J=7.6, 7.6 Hz), 6.92 (1H, d, J=7.6 Hz), 6.38 (1H, ddd, J=7.1, 7.1, 7.1 Hz), 4.86 (1H, d, J=12.0 Hz), 4.69 (1H, d, J=12.0 Hz), 4.61 (1H, m), 4.40 (2H, overlapped), 4.34 (1H, d, J=11.2 Hz), 3.92 (3H, s), 3.68 (1H, br s), 3.24 (1H, br d, J=7.9 Hz), 2.63 (1H, br d, J=15.9 Hz), 2.57 (1H, m), 2.30 (1H, dd, J=15.9, 3.7 Hz), 1.86 (3H, d, J=7.1 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 150.2, 140.2, 138.3, 131.1, 128.5, 124.4, 123.5, 107.0, 103.8, 95.5, 79.4, 74.4, 70.2, 63.5, 61.7, 61.3, 53.2, 43.7, 34.8, 33.6, 13.9; UV (MeOH) λ_{max} nm (log ε) 253.5 (sh, 3.47), 209.0 (4.01); EIMS m/z (%) 516 (M^+, 100), 518 (M^++2, 97), 520 (M^++4, 33); HREIMS 516.0604 (M^+, calcd for $C_{22}H_{23}N_2O_6Cl_3$, 516.0621); CD (c 0.282 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (301), −1.18 (269), 0 (260), +4.67 (232), 0 (223), −30.61 (210).

*3.6.2. Oxidative cleavage of **8** with m-CPBA*

To a solution of enamine-carbamate **8** (5.6 mg, 0.011 mmol) in CH_2Cl_2 (0.2 mL) were added $NaHCO_3$ (1.0 mg, 0.011 mmol) and a solution of *m*-CPBA (2.2 mg, 0.009 mmol) in CH_2Cl_2 (0.22 mL) at −78 °C under Ar atmosphere. After stirring for 3.5 h, a solution of *m*-CPBA (2.2 mg, 0.009 mmol) in CH_2Cl_2 (0.22 mL) was added and the mixture was stirred for 7 h at −60 °C under Ar atmosphere. The reaction mixture was quenched by adding saturated aqueous $NaHSO_3$ and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated. The residue was purified by SiO_2 open column chromatography (50% AcOEt/*n*-hexane to 5% MeOH/AcOEt) to afford **9** (3.8 mg, 70%).

*3.6.2.1. Compound **9**.* ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (1H, d, J=7.6 Hz), 7.31 (1H, ddd, J=7.6, 7.6, 1.2 Hz), 7.12 (1H, ddd, J=7.6, 7.6, 1.1 Hz), 6.94 (1H, d, J=7.6 Hz), 4.98 (1H, d, J=12.0 Hz), 4.78 (1H, d, J=12.0 Hz), 4.70 (1H, m), 4.68 (1H, d, J=2.4 Hz), 4.42 (1H, dd, J=11.3, 3.3 Hz), 4.32 (1H, d, J=11.3 Hz), 3.94 (3H, s), 3.85 (1H, br s), 2.97 (1H, d, J=9.0 Hz), 2.86 (1H, m), 2.60 (1H, dd, J=16.1, 2.3 Hz), 2.45 (1H, dd, J=16.1, 3.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.0, 170.7, 149.1, 138.2, 130.4, 128.8, 124.2, 123.8, 107.2, 94.5, 78.7, 74.9, 66.4, 63.7, 60.8, 59.8, 53.4, 48.1, 33.4, 32.6; UV (MeOH) λ_{max} nm (log ε) 257.5 (3.36), 209.5 (4.07); EIMS m/z 504 (M^+, 18), 506 (M^++2, 16), 270 (83), 176 (100); HREIMS 504.0266 (M^+, calcd for $C_{20}H_{19}N_2O_7Cl_3$, 504.0257); CD (c 0.142 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (307), −7.26 (259), 0 (245), +8.57 (229), 0 (222), −26.21 (213).

*3.6.3. Preparation of 14-hydroxygelsedilam (**4**)*

To a solution of **9** (10.0 mg, 0.020 mmol) in AcOH (1.5 mL) was added zinc powder (100 mg, 1.529 mmol). The reaction mixture was stirred for 2 h at room temperature and the zinc powder was removed by filtration. The acidic layer was basified with cold aqueous NH_3 at 0 °C and extracted with 20% MeOH/$CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by MPLC (10% MeOH/AcOEt) to give 14-hydroxygelsedilam (**4**, 4.8 mg, 71%). All the spectroscopic data were identical with those of natural **4**.

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