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Structure Reinvestigation of Gelsemoxonine, a Constituent of *Gelsemium elegans*, Reveals a Novel, Azetidine-Containing Indole Alkaloid

Mariko Kitajima,[†] Noriyuki Kogure,[†] Kentaro Yamaguchi,[‡] Hiromitsu Takayama,[†] and Norio Aimi*[,†]

Graduate School of Pharmaceutical Sciences and Chemical Analysis Center, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

aimi@p.chiba-u.ac.jp

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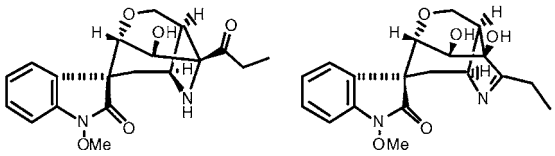
ABSTRACT



Gelsemoxonine (**2**, revised) 14,15-Dihydroxygelsenicine (**3**)

The structure of gelsemoxonine, isolated from *Gelsemium elegans* Benth., was revised to be a novel oxindole alkaloid having an azetidine unit. A new alkaloid, 14,15-dihydroxygelsenicine, which was presumed to be a biosynthetic precursor of gelsemoxonine, was also isolated.

The *Gelsemium* alkaloids[1] have attracted the attention of many synthetic organic chemists as challenging target molecules due to their markedly diverse and complex architectures. To date, the total syntheses of gelsemine,[2] gelsedine,[3] and koumine and their related sarpagine-type alkaloids,[4] as well as the biomimetic transformation of simple sarpagine-type alkaloids into humantenines, gelsedines, and koumine,[5] have been achieved. Our recent study proved that the original plant of "Yakatsu", one of the ancient medicines stored more than one and a quarter millennia ago in Shosoin repository in Japan, was *Gelsemium elegans* Benth.[6] Presently, more than fifty *Gelsemium* alkaloids are known, and they are classified into five types on the basis of their chemical structures, i.e., sarpagine, koumine, humantenine, gelsedine, and gelsemine types. Among them, gelsemoxonine, a representative of the gelsedine type, was first isolated by Lin et al. from *G. elegans* in 1991.[7] On the basis of a spectroscopic analysis, its chemical structure was proposed

[†] Graduate School of Pharmaceutical Sciences.
[‡] Chemical Analysis Center.

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Table 1. ^{1}H and ^{13}C NMR Data for Gelsemoxonine (**2**) and 14,15-Dihydroxygelsenicine (**3**)

	2				**3**	
	in pyridine-d_5, at room temperature		in pyridine-d_5, at −30 °C		in $CDCl_3$, at room temperature	
	δ_H (500 MHz)	δ_C (125 MHz)	δ_H (600 MHz)	δ_C (150 MHz)	δ_H (500 MHz)	δ_C (125 MHz)
2		174.5		174.3		170.6
3	4.21 (d, 2.4)	79.8	4.25 (s)	79.5	3.82 (d, 2.1)	77.2
5	3.84 (m)	56.1	3.76 (m)	55.8	4.44 (m)	69.2
6	2.43 (d, 15.6)	35.1	2.34 (d, 15.7)	34.6	2.38 (dd, 15.6, 4.6)	36.1
	2.19 (dd, 15.6, 4.6)		2.12 (br d, 15.7)		2.28 (dd, 15.6, 2.4)	
7		55.1		54.8		53.6
8		131.8		131.6		131.3
9	7.68 (d, 7.6)	126.0	7.65 (br d, 7.7)	125.9	7.49 (d, 7.6)	124.6
10	7.17 (t, 7.6)	124.0	7.15 (br t, 7.7)	123.9	7.08 (td, 7.6, 0.9)	123.6
11	7.33 (t, 7.6)	128.8	7.31 (br t, 7.7)	128.8	7.28 (td, 7.6, 0.9)	128.5
12	7.04 (d, 7.6)	107.6	7.01 (br d, 7.7)	107.5	6.88 (d, 7.6)	106.9
13		138.7		138.5		138.0
14	5.00 (br d, 2.4)	69.4	5.01 (s)	69.1	4.31 (d, 2.1)	66.0
15		67.8		67.6		78.8
16	3.52 (m)	34.2	3.48 (m)	33.7	2.38 (overlapped)	46.3
17	4.45 (dd, 11.3, 3.7)	61.8	4.39 (br d, 11.4)	61.6	4.31 (dd, 11.0, 3.5)	60.5
	4.16 (d, 11.3)		4.10 (br d, 11.4)		4.22 (br d,11.0)	
18	1.13 (3H, t, 7.3)	7.6	1.03 (3H, br t, 6.9)	7.5	1.28 (3H, t, 7.3)	9.6
19	2.91 (dq, 18.0, 7.3)	28.7	2.87 (m)	28.4	2.54 (2H, m)	22.0
	2.66 (dq, 18.0, 7.3)		2.59 (m)			184.0
20		210.5		210.9	3.91 (3H, s)	63.4
N_a-OMe	3.94 (3H, s)	63.5	3.87 (3H, s)	63.4		
N_b-H	4.57 (br s)		4.64 (s)			

to be an unusual *N*4/C20 seco-oxindole alkaloid (**1**). However, during the reinvestigation of the chemical constituents of *G. elegans* Benth., we found that gelsemoxonine had an unusual azetidine unit, which was the first example among monoterpenoid indole alkaloids. In this paper, we describe the structure reinvestigation of gelsemoxonine and the isolation of a new oxindole alkaloid (**3**), which appears to be biogenetically related to gelsemoxonine.

The leaves of *G. elegans* Benth. (1483.6 g fresh weight)[8] were extracted with MeOH to give the extract (203.6 g). The crude alkaloidal fraction (2.47 g) was obtained by the usual procedure from a portion of the MeOH extract (100.0 g) and purified by SiO_2 column chromatography to afford gelsemoxonine (**2**, 74.4 mg) and a new alkaloid **3** (61.6 mg) together with known alkaloids, gelsemine, gelsenicine (**4**), and 14-hydroxygelsenicine (**5**).

Compound **2**[9] was obtained as colorless prisms (mp 171−172 °C), and the molecular formula was established to be $C_{19}H_{22}N_2O_5$ from the HRFABMS spectrum [*m*/*z* 359.1611 ([M + H]$^+$)]. The ^{1}H and ^{13}C NMR data of **2** were completely identical with the published data[7] for gelsemoxonine. However, acetylation of **2** under ordinary conditions (Ac_2O, Py) gave an unexpected diacetylated derivative (two acetyl methyl signals at δ_H 2.02 and δ_H 1.91 and two acetyl carbonyl carbons at δ_C 169.5 and δ_C 169.3). This finding raised doubts regarding the reported structure (**1**) of gelsemoxonine. Thus, we reinvestigated the chemical structure of this compound carefully. The UV and NMR spectra of gelsemoxonine exhibited the characteristic N_a-methoxyoxindole chromophore. The ^{1}H NMR spectrum in pyridine-d_5 (Table 1) showed an N_b-H at δ 4.57 together with two oxymethine protons at δ 4.21 (d, J = 2.4 Hz, H-3) and δ 5.00 (br-*d*, J = 2.4 Hz, H-14). The ^{13}C NMR spectra revealed the presence of five sp^3 carbons (one quaternary, three methines, and one methylene) bearing an oxygen or a nitrogen atom at δ 67.8 (C-15), 56.1 (C-5), 79.8 (C-3), 69.4 (C-14), and 61.8 (C-17), together with an isolated propanoyl residue. The HMBC spectrum was measured at −30 °C in pyridine-d_5 by utilizing the phenomenon that the signal of N_b-H in **2** becomes sharp and well defined at low temperatures. As a result, clear correlations between N_b-H and the two carbons at C-14 and C-20 (δ 210.9) were observed. Formula **1** cannot explain these long-range couplings. Taken together with the results of the acetylation experiment above, these spectroscopic data enabled us to construct an azetidine ring consisting of the N_b, C-15, C-16, and C-5 positions.

At this stage, X-ray crystallographic analysis[10] of **2** was carried out, confirming that gelsemoxonine was an N_a-methoxyoxindole alkaloid having a novel azetidine unit in the molecule. To the best of our knowledge, this is the first

(8) *Gelsemium elegans* Benth. was collected from the Atagawa Tropical and Alligator Garden in Izu in Japan in April 2002.

(9) **Gelsemoxonine (2):** colorless prisms; mp 171−172 °C (benzene); $[\alpha]^{22}_D$ −41.3° (*c* 0.988, MeOH); UV (MeOH) λ_{max} nm (log ϵ) 257.5 (3.64), 209.0 (4.31); IR (KBr) 3410, 3250, 2936, 1698, 1616 cm^{-1}; EIMS *m*/*z* (%) 358 (M$^+$, 100), 301 (67), 270 (47); HRFABMS *m*/*z* 359.1611 (MH$^+$; calcd for $C_{19}H_{22}N_2O_5$, 359.1607); CD (*c* = 0.335 mmol/L, MeOH, 23 °C) $\Delta\epsilon$ (nm) 0 (302), −4.98 (262), 0 (250), +11.84 (234), 0 (222), −15.78 (211).

example of an azetidine-containing monoterpenoid indole alkaloid, although natural products having an azetidine unit have been reported.[11]

The HRFABMS spectrum of the new alkaloid **3**[12] gave a protonated molecular ion peak at *m/z* 359.1611 ([M + H]$^+$) corresponding to the molecular formula $C_{19}H_{23}N_2O_5$ (*m/z* 359.1607), which possessed one oxygen more than that of a known alkaloid, 14-hydroxygelsenicine (**5**).[13] The UV absorptions of **3** at 212 and 258 nm revealed an oxindole nucleus. The ^{1}H NMR spectrum showed four aromatic protons due to the A ring of the oxindole system, an N_a-methoxy group at δ 3.91 (3H, s), a methine group bearing imine nitrogen at δ 4.44 (m, H-5), an oxymethine proton at δ 3.82 (d, H-3), a methine group bearing a hydroxyl group at δ 4.31 (d, H-14), oxymethylene protons at δ 4.31 (dd) and δ 4.22 (br-d) (H_2-17), and an ethyl group [δ 1.28 (3H, t, H_3-18), δ 2.54 (2H, m, H_2-19)], which were similar to those of 14-hydroxygelsenicine. The ^{13}C NMR spectrum showed 19 carbons, including an imine carbon (δ 184.0, C-20), two oxygenated methines (δ 77.2, C-3, and δ 66.0, C-14), and a methylene (δ 60.5, C-17) bearing an oxygen atom. Furthermore, an additional quaternary carbon bearing a hydroxyl group was observed at δ 78.8. The HMBC cross-peaks between protons, H-3, H-5, and H-17, and the carbon at δ 78.8 demonstrated that C-15 had a hydroxyl group. From the above data, compound **3** was deduced to be 14,15-dihydroxygelsenicine. Treatment of **3** with 2,2-dimethoxypropane and pyridinium *p*-toluenesulfonate (PPTS) in dry acetone gave an acetonide derivative in 23% yield (50% recovery from the starting material), revealing that the diol at C-14 and C-15 had a cis orientation. Therefore, the stereochemistry of the hydroxyl group at C-14 was suggested to possess the β configuration.

The CD spectra of gelsemoxonine (**2**) and 14,15-dihydroxygelsenicine (**3**) were similar to that of gelsenicine (**4**). The absolute configuration of **4** was established by chemical correlation with gardnerine,[14] indicating that **2** and **3** possessed the absolute configuration depicted in Figure 1.

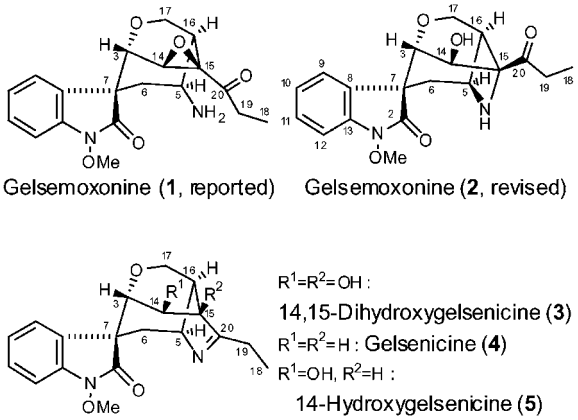

Figure 1. Structure of gelsemoxonine and gelsenicines.

From a biogenetic point of view, gelsemoxonine (**2**) would be derived from a sarpagine-type alkaloid, N_a-methoxy-(19*Z*)-anhydrovobasinediol (**6**),[15] via the sequence shown in Figure 4: (i) oxidative rearrangement from **6** to the humantenine-type oxindole alkaloid, rankinidine (**7**);[16] (ii) loss of the C-21 carbon in **7** to form a gelsedine-type alkaloid, gelsenicine (**4**);[17] (iii) introduction of two hydroxyl groups at positions C-14 and C-15 to give the new alkaloid **3**; (iv) hydrolytic cleavage of the imine part in **3** to form an intermediate having

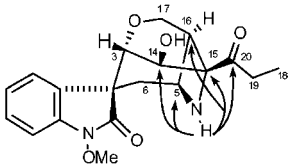

Figure 2. Selected HMBC correlations for gelsemoxonine (**2**).

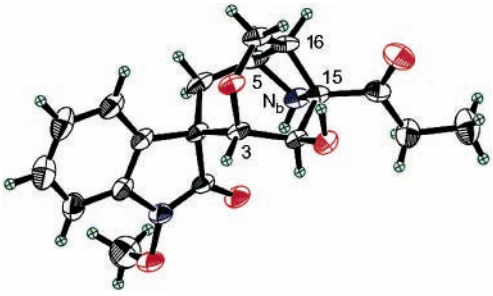

Figure 3. X-ray structure of gelsemoxonine (**2**).

(10) **X-ray Crystallographic Analysis of 2.** All measurements were made on a Bruker SMART 1000 CCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71069 Å). Crystal data: orthorhombic, $C_{19}H_{22}N_2O_5 \cdot 1/2C_6H_6$ (M_r = 397.45), space group $P2_12_12$ with a = 12.642(4) Å, b = 26.156(9) Å, c = 6.193(2) Å, V = 2047(1) Å^3, Z = 4, and D_{calcd} =1.289 g/cm^3. The structure was solved by direct methods (SHELXS-97) and expanded using Fourier techniques (DIRDIF94). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2224 reflections ($I > 0\sigma(I)$, $2\theta < 57.28°$) and 263 variable parameters and converged with unweighted and weighted agreement factors of R = 0.078 and R_w = 0.044.

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(12) **Compound 3:** colorless amorphous; UV (MeOH) λ_{max} nm (log ϵ) 258.0 (3.76), 212.0 (4.29); IR ($CHCl_3$) 3472, 3011, 2941, 1719, 1220 cm^{-1}; EIMS *m/z* (%) 358 (M$^+$, 100), 327 (75); HRFABMS *m/z* 359.1611 (MH$^+$; calcd for $C_{19}H_{22}N_2O_5$, 359.1607); CD (c = 0.307 mmol/L, MeOH, 22 °C) $\Delta\epsilon$ (nm) 0 (300), −5.9 (263), 0 (250), +11.0 (235), 0 (221), −17.3 (210).

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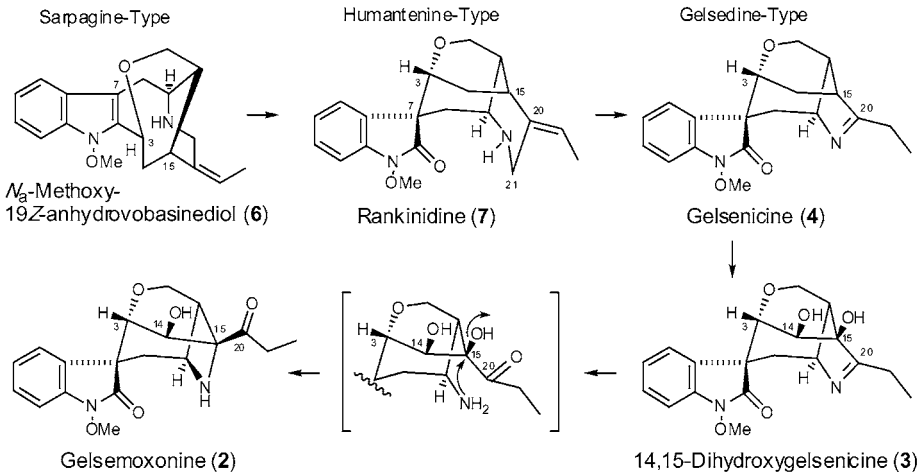

Figure 4. Hypothetical biogenetic route of gelsemoxonine (**2**).

a primary amine and a carbonyl functionality, which corresponded to the first reported structure of gelsemoxonine; and (v) ring closure between the N_b and C-15 positions to form the azetidine ring (Figure 4).

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Supporting Information Available: ^{1}H and ^{13}C NMR of gelsemoxonine (**2**) and 14,15-dihydroxygelsenicine (**3**) and experiment procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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