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

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Experimental

General

Melting point: Yamato MP-21 (uncorrected). ^1H - and ^{13}C -NMR spectra: JEOL JNM ECP-600 or a JEOL JNM A-500 at 600 or 500 MHz (^1H -NMR) and at 150 or 125 MHz (^{13}C -NMR), respectively. UV: JASCO V-560. IR: JASCO FT/IR-230. EI-MS: JEOL GC mate. HR-FAB-MS: JEOL JMS-HX110. Optical rotation: JASCO P-1020. CD: JASCO J-720WI. TLC: precoated Silica gel 60 F₂₅₄ plates (Merck, 0.25 mm thick). Column chromatography: Silica gel 60 (Merck, 70-230 mesh). Medium pressure liquid chromatography (MPLC): C. I. G. prepacked column CPS-HS-221-05 (Kusano Kagakukikai, SiO_2).

Extraction and Isolation of Gelsemoxonine (2) and 14,15-Dihydroxygelsenicine (3).

The leaves of *G. elegans* Benth. (1483.6 g fresh weight) were extracted with MeOH (One time at room temperature and five times under reflux) to give the extract (203.6 g). A portion of the MeOH extract (100.0 g) was dissolved in 1N 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 the crude alkaloidal fraction (2.47 g). The crude alkaloid was separated by SiO_2 open column chromatography eluted with CHCl_3 /MeOH gradient to give 11 fractions; fr. A CHCl_3 , fr. B and C 2%, fr. D and E 5%, fr. F and G 10%, fr. H 15-20%, fr. I 30%, fr. J 30-50% MeOH/ CHCl_3 , MeOH, fr. K MeOH, sat. NH_3 in 50% MeOH/ CHCl_3 . Fr. C (744.8 mg) was purified by SiO_2 open column chromatography (50% AcOEt/*n*-Hex – AcOEt/MeOH gradient – 5% Et_2NH /MeOH) and then by MPLC (1% MeOH/AcOEt or 3% MeOH/ CHCl_3) to afford gelsemoxonine (**2**, 61.0 mg). Separation of Fr. D (760.9 mg) by SiO_2 open column chromatography (5-20% MeOH/AcOEt) and subsequent MPLC (2% MeOH/AcOEt or 3% MeOH/ CHCl_3) gave **2** (13.4 mg) and 14,15-dihydroxygelsenicine (**3**, 61.1 mg). **3** (4.3 mg) was also obtained from fr. E (189.4 mg).

NMR Data (in CDCl_3) for Gelsemoxonine (2).

^1H -NMR (500 MHz, CDCl_3) δ 7.47 (1H, dd, $J=7.6$, 0.6 Hz, H-9), 7.36 (1H, ddd, $J=7.6$, 7.6, 0.9 Hz, H-11), 7.18 (1H, ddd, $J=7.6$, 7.6, 0.9 Hz, H-10), 7.02 (1H, dd, $J=7.6$, 0.6 Hz, H-12), 4.51 (1H, br-d, $J=6.1$, H-14), 4.26 (1H, dd, $J=12.0$, 4.1 Hz, H-17), 4.17 (1H, d, $J=12.0$ Hz, H-17), 4.05 (3H, s, Na-OMe), 3.89 (1H, br-ddd, $J=8.2$, 4.6, 1.5 Hz, H-5), 3.80 (1H, d, $J=2.4$ Hz, H-3), 3.34 (1H, br-dd, $J=8.2$, 4.0 Hz, H-16), 2.82 (1H, dq, $J=18.3$, 7.3 Hz, H-19), 2.52 (1H, dq, $J=18.3$, 7.3 Hz, H-19), 2.39 (1H, dd, $J=16.2$, 1.5 Hz, H-6), 2.29 (1H, dd, $J=16.2$, 4.6 Hz, H-6), 1.11 (3H, t, $J=7.3$ Hz, H_3 -18); ^{13}C -NMR (125 MHz, CDCl_3) δ 211.8 (C-20), 173.4 (C-2), 138.0 (C-13), 130.3 (C-8), 128.8 (C-11), 125.2 (C-9), 124.1 (C-10), 107.5 (C-12), 78.6 (C-3), 68.7 (C-14), 67.2 (C-15), 63.7 (Na-OCH₃), 61.8 (C-17), 55.6 (C-5), 53.9 (C-7), 34.7 (C-6), 33.6 (C-16), 28.9 (C-19), 7.0 (C-18).

Acetylation of Gelsemoxonine (2).

To a solution of **2** (6 mg, 0.017 mmol) in dry pyridine (1.5 ml) was added dry acetic anhydride (1.0 ml) at 0 °C under Ar and the mixture was stirred at room temperature for 2.5 hrs. After concentration of the solvent, the residue was dissolved in CHCl_3 . 5% aqueous NaHCO_3 solution was added and the whole mixture was extracted with CHCl_3 . The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by SiO_2 open column chromatographies (MeOH- CHCl_3 gradient and 5% MeOH-Benzene) to give diacetylated derivative (7 mg, y. 94 %). **Diacetylated derivative of 2**. UV (MeOH) λ_{max} nm 257, 207; IR (CHCl_3) cm^{-1} 3005, 2940, 1730, 1658; EIMS m/z (%) 442 (M^+ , 100), 411

(35), 173 (53); $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ 7.38 (1H, d, $J=7.7$ Hz, H-9), 7.32 (1H, ddd, $J=7.7, 7.7, 1.1$ Hz, H-11), 7.11 (1H, ddd, $J=7.7, 7.7, 1.1$ Hz, H-10), 6.98 (1H, d, $J=7.7$ Hz, H-12), 6.08 (1H, dd, $J=2.5, 0.8$ Hz, H-14), 4.56 (1H, ddd, $J=8.8, 4.4, 1.4$ Hz, H-5), 4.14 (2H, dd, $J=3.0, 1.9$ Hz, H₂-14), 4.05 (3H, s, $N_a\text{-OCH}_3$), 3.94 (1H, d, $J=2.5$ Hz, H-3), 3.74 (1H, br-d, $J=8.5$ Hz, H-16), 3.18 (1H, dddd, $J=17.6, 7.1, 7.1, 7.1$ Hz, H-19), 2.50 (1H, dd, $J=15.9, 4.4$ Hz, H-6), 2.44 (1H, dddd, $J=17.6, 7.1, 7.1, 7.1$ Hz, H-19), 2.35 (1H, dd, $J=15.9, 1.4$ Hz, H-6), 2.02 (3H, s, COCH_3), 1.92 (3H, s, COCH_3), 1.12 (3H, dd, $J=7.1, 7.1$ Hz, H₃-18); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ 205.9 (C-20), 171.6 (C-2), 169.5 and 169.3 (2 x COCH_3), 138.8 (C-13), 129.6 (C-8), 129.0 (C-11), 125.2 (C-9), 123.5 (C-10), 107.4 (C-12), 75.3 (C-3), 69.2 (C-15), 68.2 (C-14), 64.1 ($N_a\text{-OCH}_3$), 61.2 (C-5), 60.7 (C-17), 53.5 (C-7), 34.3 (C-19), 32.8 (C-6), 32.7 (C-16), 21.1 and 20.6 (2 x COCH_3), 8.5 (C-18).

Preparation of Acetonide of 14,15-Dihydroxygelsenicine (3).

To a solution of **3** (5.0 mg, 0.014 mmol) in dry acetone (0.6 ml) were added 2,2-dimethoxypropane (8.5 μl , 0.069 mmol) and PPTS (4.0 mg, 0.016 mmol) at 0 °C under Ar and the mixture was stirred at room temperature for 2 hrs. 2,2-Dimethoxypropane (17 μl , 0.138 mmol) was further added, and the reaction mixture was stirred at room temperature for 7.5 hrs. 2,2-Dimethoxypropane (17 μl , 0.138 mmol) was added again, and the mixture was stirred at room temperature for 8.5 hrs and under reflux for 8 hrs. Saturated aq. NaHCO_3 was added to the reaction mixture and the whole was extracted with CHCl_3 . The organic layer was washed with brine, dried over MgSO_4 and evaporated. The residue was purified by medium pressure liquid chromatographies (SiO_2 , 20% AcOEt-n-Hex. and 1-2% MeOH-AcOEt) to give acetonide derivative (1.3 mg, y. 23 %) and starting material (**3**, 2.5 mg). **Acetonide derivative of 3**. UV (MeOH) λ_{max} nm 258, 210.5; IR (CHCl_3) cm^{-1} 2940, 1717, 1618; EIMS m/z (%) 398 (M^+ , 39), 312 (57), 309 (100), 281(37); $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 7.57 (1H, d, $J=7.6$ Hz, H-9), 7.28 (1H, dd, $J=7.6, 7.6$ Hz, H-11), 7.10 (1H, dd, $J=7.6, 7.6$ Hz, H-10), 6.89 (1H, d, $J=7.6$ Hz, H-12), 4.80 (1H, d, $J=2.8$ Hz, H-3), 4.44 (1H, dd, $J=11.0, 3.0$ Hz, H-17), 4.42 (1H, m, H-5), 4.22 (1H, d, $J=11.0$ Hz, H-17), 4.02 (1H, d, $J=2.8$ Hz, H-14), 3.95 (3H, s, $N_a\text{-OCH}_3$), 2.64 (2H, m, H₂-19), 2.50 (1H, dd, $J=15.6, 4.7$ Hz, H-6), 2.36 (1H, br-d, $J=7.9$ Hz, H-16), 2.31 (1H, dd, $J=15.6, 2.1$ Hz, H-6), 1.62 and 1.52 (each 3H, s, 2 x CH_3), 1.33 (3H, dd, $J=7.3, 7.3$ Hz, H₃-18); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ 181.7 (C-20), 170.9 (C-2), 137.9 (C-13), 131.4 (C-8), 128.5 (C-11), 124.7 (C-9), 123.7 (C-10), 111.1 ($\text{C}(\text{CH}_3)_2$), 106.8 (C-12), 87.3 (C-15), 74.2 (C-3), 72.7 (C-14), 67.9 (C-5), 63.5 ($N_a\text{-OCH}_3$), 60.2 (C-17), 53.9 (C-7), 45.9 (C-16), 36.9 (C-6), 28.0 and 26.3 (2 x CH_3), 22.8 (C-19), 9.5 (C-18).

Gelsemoxonine

DFILE
 COMNT
 DATIM
 OBNUC
 EXMOD
 OBFRQ
 OBSET
 OBFIN
 POINT
 FREQU
 SCANS
 ACQTM
 PD
 FWI
 IRNUC
 CTEMP
 SLVNT
 EXREF
 BF
 RGAIN

Gelsemoxonine
 1H
 SINGL-NON LQNR 8A
 500.00 MHz
 160.00 KHz
 2410.0 Hz
 32768
 10000.0 Hz
 16
 3.277 sec
 3.000 sec
 6.1 us
 1H
 -204.8 c
 C5D5N
 7.55 ppm
 0.01 Hz
 18

¹H-NMR of Gelsemoxonine (2)
(500 MHz, in Py- d₅, at rt)

Gelsemoxonine-BCM

DFILE Gelsemoxonine-BCM
 COMNT
 DATIM
 OBNUC 13C
 EXMOD SINGL-BCM LONOR Å@
 OBFRQ 125.65 MHz
 OBSET 120.00 KHz
 OBFIN 7782.6 Hz
 POINT 65536
 FREQU 30030.0 Hz
 SCANS 600
 ACQTM 2.182 sec
 PD 1.500 sec
 PW1 3.9 us
 IRNUC 1H
 CTEMP -204.8 c
 SIVNT C5D5N
 EXREF 123.50 ppm
 BF 0.01 Hz
 RGAIN 23

¹³C-NMR of Gelsemoxonine (**2**)
 (125 MHz, in Py-*d*₅, at rt)

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Sample ID = SKKQJRE-C7D1-PY
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Revision Date = 28-FEB-2003 18:58:45
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Spec Type = DELTA_RMR
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Din Units = ppm
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Actual_end_time = 3-DEC-2002 18:44:13
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Relaxation_delay = 4[s]
Scans = 8
Sweep = PYRIDINE-D5
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Spin_lock_attn = 11.5[db]
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Tri_noise = WALTZ
Tri_noise = 10[us]
Qua90 = 10[us]
Qua_noise = WAUGH
Qua_noise = 1[us]
Qua_width = 1[us]

¹H-NMR of Gelsemoxonine (2)
(600 MHz, in Py-d₅, at -30°C)

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File Name = SEKOGRE-C7D1-PY-13C
Author = JEOL LTD. C7D1-PY
Contact ID = C7D1 13C VT-30
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Revision Date = 11-DEC-2002 17:35:01
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Dim Units = Hz
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End time = 11-DEC-2002 19:47:16
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F1_offset2 = 0.000000 [Hz]
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Filter width = 18.8641788 [kHz]
Irr_code = 194
Irr_domain = 1H
Irr_freq = 600.17530548 [MHz]
Irr_gate = 1000000000 [Hz]
Irr_offset = 5.0 [ppm]
Irr_width = 60 [us]
Iterations = 1
Local time = 11-DEC-2002 17:11:24
Obs_noise = 1.5 [us]
Obs_width = 2592 [us]
Probe = 1H/13C
Recvr_gain = 23
Relaxation_delay = 1.5 [s]
Scans = 2312
Solvent = PYRIDINE-D5
Spin_get = 12 [Hz]
Spin_lock_90 = 45 [us]
Spin_lock_attn = 11.5 [dB]
Temp_get = -30 [deg]
X90 = 13 [us]
X_acq_duration = 1.736704 [s]
X_angle = 10 [deg]
X_center = 130.930327 [MHz]
X_freq = 110 [ppm]
X_offset = 65536
X_points = 4
X_prescans = 4
X_pulse = 333333 [us]
X_pulse2 = 0.578121 [Hz]
X_ref = 37.73584906 [kHz]
X_sweep = 10 [us]
Tri90 = WALTZ
Tri_noise = 1 [us]
Tri_width = 10 [us]
Qn90 = 10 [us]
Obs_noise = 1.5 [us]
Qn90_noise = 1 [us]

14,15-Dihydroxygelsenicine

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN

14,15-Dihydroxygelsenicine

1H
SINGL-NON LQOR @A

500.00 MHz

160.00 KHz

2410.0 Hz

32768

10000.0 Hz

16

3.277 sec

3.000 sec

6.1 us

1H

-204.8 c

CDCL3

0.00 ppm

0.01 Hz

23

¹H-NMR of 14,15-Dihydroxygelsenicine (**3**)
(500 MHz, in CDCl₃, at rt)

14,15-Dihydroxygelsenicine BCM

DFILE
COMNT
DATIM
OBNUC 13C
SINGL-BCM LQOR Å
EXMOD 125.65 MHz
OBPRO 120.00 KHz
OBSET 8520.4 Hz
OBFIN 65536
POINT 30030.0 Hz
FREQU 4000
SCANS 2.182 sec
ACQTM 1.500 sec
PD 3.9 us
FW1 1H
IRNUC -204.8 c
CTEMP CDCL3
SLVNT 0.00 ppm
EXREF 2.00 Hz
BF 23
RGAIN

¹³C-NMR of 14,15-Dihydroxygelsenicine (**3**)
(125 MHz, in CDCl₃, at rt)