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14β-HYDROXYGELSEDINE, A NEW OXINDOLE ALKALOID FROM *GELSEMIUM SEMPERVIRENS*

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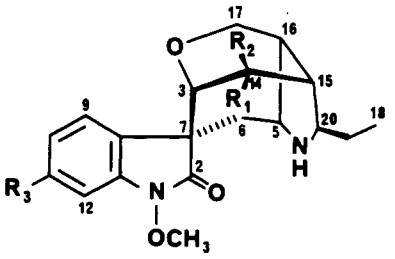
ABSTRACT.—An extract of the stem of *Gelsemium sempervirens* has afforded a new oxindole alkaloid, 14β-hydroxygelsedine, whose structure and stereochemistry were deduced by spectral methods, principally high resolution ^{1}H nmr, 2-D COSY, and decoupling experiments.

The genus *Gelsemium*, belonging to the family Loganiaceae, is represented by one species in southeastern Asia, *Gelsemium elegans* (Gardn. & Champ.) Benth., a second species in the southeastern United States, *Gelsemium rankinii* Small, and a third species *Gelsemium sempervirens* (L.) Jaume St.-Hilaire, native to the southeastern United States, the highlands of southern Mexico, and Guatemala (1). It has been known for more than a century that these plants cause death in both humans and livestock (2), and gelsemicine was the first toxic principle of *G. sempervirens* isolated (3).

The genus is known to contain indole alkaloids of a unique skeletal type (4). *G. sempervirens* is the best studied of these plants and has yielded gelsemine (5), gelsevirine (1-methoxygelsemine), gelsedine (6,7), gelsemicine (8) (11-methoxygelsedine), 14-hydroxygelsemicine, 21-oxogelsemine (9-11), and sempervirine (12).

As part of our program on the isolation of plant anticancer principles, we have studied the alkaloids of *G. sempervirens*[1] and wish to report here the isolation and structure elucidation of a new oxindole alkaloid 14β-hydroxygelsedine (**1**).

The molecular ion of **1** at *m/z* 344 was 30 amu less than that of 14-hydroxygelsemicine (**2**), while both compounds displayed the same intense fragment ions at *m/z* 168 and 84. The 360 MHz ^{1}H-nmr spectrum of **1** was nearly identical with that reported at 220 MHz for 14-hydroxygelsemicine (10), except for the presence of an unsubstiuted aromatic region, i.e., the absence of an aromatic methoxy group. The higher field, however, did permit enhanced resolution in the region between 2.3 and 1.8 ppm (Table 1). The ^{1}H-nmr spectrum δ (ppm) of **1** revealed exceptional similarity of its aromatic region, H-9, 7.39 (d, J=7.7 Hz); H-10, 7.13 (ddd, J=7.7, 7.7, 1.1 Hz); H-



	R_1	R_2	R_3	
1	H	OH	H	14β-hydroxygelsedine
2	H	OH	OCH_3	14-hydroxygelsemicine
3	H	H	H	gelsedine
4	H	H	OCH_3	gelsemicine

[1]Details of the anticancer activity of *G. sempervirens* will be reported subsequently.

TABLE 1. ^{1}H-nmr Spectra of 14-Hydroxygelsemicine (**2**) and 14β-Hydroxygelsedine (**1**)

	14-Hydroxygelsemicine (**2**)[a] δ ppm	14β-Hydroxygelsedine (**1**)[b] δ ppm
H-3	3.38 s	3.42 s
H-5	3.56 dt J=9, 3 Hz	3.61 dt J=9.3, 3.0 Hz
H-6	1.95, 2.11 J_{AB}=16, J_{AX}=3.5, J_{BX}=3 Hz	H_{6a} 2.16 H_{6e} 1.98 $JH_{6a,6e}$=16.3 Hz, $JH_{6a,5}$=3.3, Hz $JH_{6e,5}$=2.4 Hz
H-9	7.28 d J=8 Hz	7.39 d J=7.7 Hz
H-10	6.63 dd J=8, 2 Hz	7.13 ddd J=7.7, 7.7, 1.1 Hz
H-11	—	7.29 ddd J=7.7, 7.7, 1.1 Hz
H-12	6.53 d J=2 Hz	6.95 d J=7.7 Hz
H-14	4.28 s	4.42 bd s
H-15	2.00 (obscured)	2.05 t J=4.5 Hz
H-16	2.45 dt J=9, 4 Hz	2.49 dt J=9.0, 4.8 Hz
H-17	4.27, 4.41 J_{AB}=11 Hz, J_{AX}=4 Hz	4.31 H_{17a}, 4.44 H_{17e} $JH_{17a,17e}$=11.0 Hz $JH_{17e,16e}$=4.5 Hz
H-18	1.07 t J=7.2 Hz	1.09 t J=7.5 Hz
H-19	1.87 quinet J=7 Hz	1.89 quintet J=7.6 Hz
H-20	2.95 td J=7, 3.5 Hz	2.98 td J=7.4, 3.9 Hz
N-OCH_3	3.97 s	3.97 s
Ar-OCH_3	3.80 s	—

[a]Data from reference 10.
[b]Recorded at 360 MHz in $CDCl_3$.

11, 7.29 (ddd, J=7.7, 7.7, 1.1 Hz); and H-12, 6.95 (d, J=7.7 Hz) with that of gelsedine (**3**) (7), and the presence of an *N*-methoxy group at δ 3.97 was also apparent.

These data supported the structure 14-hydroxygelsedine for the isolate. The stereochemistry of the hydroxy group at C-14 was determined through decoupling experiments. Irradiation of H-3 at 3.42 ppm sharpened H-14 to a doublet (J=1.0 Hz), and irradiation of H-15 collapsed both H-3 and H-14 to doublets (J=0.7 Hz). The small coupling constants between H-3 and H-14 (J=0.7 Hz), and H-14 and H-15 (J=1.0 Hz) indicated that the angles between them are approximately 70° and 105°, respectively. The 14-hydroxy group therefore has the axial configuration.

A Dreiding model showed H-16 to be in the equatorial position, and the angles between H-16 and H-17e, and H-17a were 15° and 90°, respectively. These data supported the evidence that the resonance at 4.45 ppm (dd, J=11.0, 4.5 Hz) should be assigned to H-17e, and that at 4.31 ppm (d, J=11.0 Hz) to H-17a. The axial and equatorial protons at C-6 were assigned on the basis of a larger $J_{5,6a}$=3.3 Hz than $J_{5,6e}$=2.4 Hz, because H-6a was closer to H-5 (40°) than H-6e (55°). Consequently, the downfield peak at 2.16 ppm could be assigned to H-6a, while the signal at 1.98 ppm was attributed to H-6e. This could be further confirmed by a 2-D COSY experiment, since H-6e had a W coupling with H-3 rather than H-6a. The remaining proton assignments were also confirmed by the 2-D COSY experiment (Figure 1).

The ^{13}C-nmr spectra of 14β-hydroxygelsedine (**1**) and gelsedine (**3**) (7) (Table 2), were nearly identical except for C-14, which was shifted downfield 43.1 ppm due to the substitution by a hydroxyl group. Small shifts were also noted for C-3 (+6.8 ppm), C-15 (+4.5 ppm), and C-16 (+5.2 ppm). The previously ambiguous assignments of C-15 and C-16, and C-5 and C-20 (7) were clarified through irradiation of H-16, H-5, and H-20 during low power single frequency off-resonance (sford) decoupling experi-

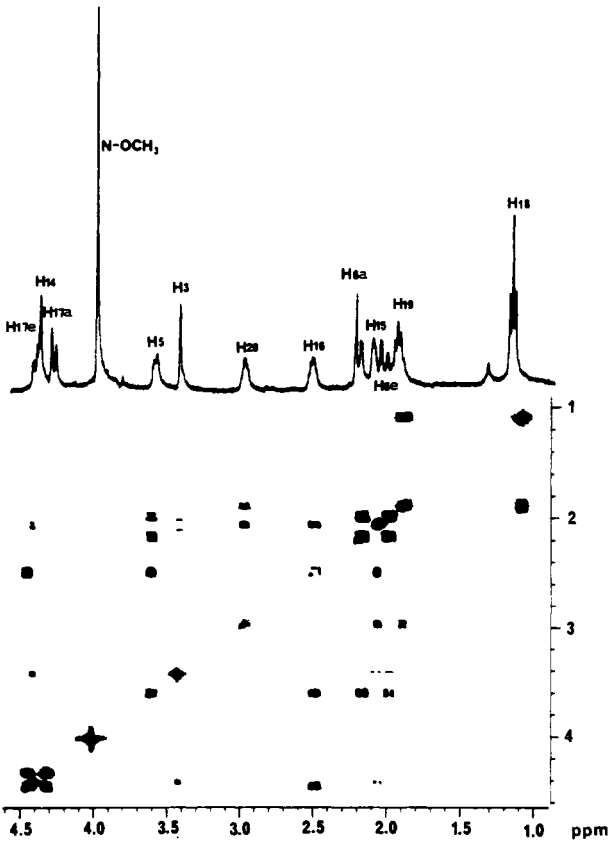


FIGURE 1. Two dimensional correlation spectrum of 14β-hydroxygelsedine (**1**) in the range 1.0-4.5 ppm.

ments. The close chemical shifts of C-20, C-14, and N-OCH_3 were also clarified by this method.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting point was determined by means of a Kofler hotplate and is uncorrected. The uv spectrum was obtained with a Beckman model DB-G grating spectrometer. The ir spectrum was determined on a Nicolet MX-1 interferometer. ^{1}H-nmr spectra were recorded on a Nicolet NMC 360 (360 MHz) using $CDCl_3$ as solvent and TMS as an internal standard. The ^{13}C-nmr spectrum was obtained on a Nicolet NMC 360 instrument operating at 90.8 MHz. The mass spectrum was obtained with a Varian MAT 112S double focusing spectrometer operating at 70 eV. Optical rotation was measured with a Perkin-Elmer, Model 241 polarimeter. Silica gel for chromatography was purchased from E. Merck, Darmstadt, W. Germany and preparative tlc plates were from Analtech, Newark, Delaware.

PLANT MATERIAL.—Dried stem material of *G. sempervirens* was collected in Texas and identified by Dr. Clifford W. Morden during the spring of 1983. Specimens were deposited in the S.M. Tracy Herbarium, Texas A&M University, College Station, Texas, and in the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND PURIFICATION.—Chopped stems of *G. sempervirens* (3 kg) were percolated with MeOH at room temperature for 2 days. This process was repeated five times, and the combined MeOH extracts were concentrated in vacuo at 30° to afford a thick dark syrup (ca. 520 g). The MeOH extract was dissolved in 2% citric acid, the acidic solution was basified with aqueous NH_3 to pH 8.0 and extracted extensively with EtOAc until a Dragendorff test was negative. The process was finished within the same day to avoid decomposition. The total alkaloid extract (35 g) was subjected to silica gel column chromatography eluting with mixtures of petroleum ether, EtOAc, and MeOH by increasing polarity.

TABLE 2. Carbon-13 nmr Spectra of Gelsedine (**3**) and 14β-Hydroxygelsedine (**1**)

	Gelsedine (**3**)[a] δ ppm	14β-hydroxygelsedine (**1**)[b] δ ppm
C-2	174.4	173.72
C-3	74.3	81.06
C-5	59.4[c]	58.27
C-6	33.7	34.26
C-7	52.7	54.61
C-8	131.7	130.77
C-9	125.2	125.09
C-10	123.4	123.78
C-11	127.8	128.30
C-12	106.9	107.16
C-13	138.0	137.61
C-14	21.2	63.87
C-15	41.7[d]	46.15
C-16	34.5[d]	39.66
C-17	63.6	63.39
C-18	11.7	11.80
C-19	21.2	21.16
C-20	65.3[c]	64.29
OCH_3	63.1	63.44

[a]Data are from Wenkert *et al.* (7).

[b]Recorded at 90.8 MHz in $CDCl_3$.

[c,d] Assignment may need to be interchanged in Wenkert *et al.* (7).

ISOLATION OF COMPOUND **1**.—The residue from the most polar eluant (MeOH) was further subjected to repeated preparative tlc using petroleum ether-C_6H_6-EtOAc-diethyl amine (25:10:10:4) as a solvent and a bluish purple band (under uv) at Rf 0.17 was eluted with Me_2CO to afford white needle-like crystals of **1** (15 mg), mp 216-218°; $[\alpha]^{25}D$ −113° (c 0.3, MeOH); uv λ max (MeOH) 216 and 260 nm (log ε 4.26 and 3.68); ir υ max (KBr) 2855 (broad, hydrogen bonded NH, and OH), 1694 (CO), 1619, 1467, 1245, and 755 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; ms *m/z* (rel. int.) 344 (M^+, 35), 314 (8), 313 (36), 295 (36), 168 (60), and 84 (100).

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