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21-OXOGELSEVIRINE, A NEW ALKALOID FROM *GELSEMIUM RANKINII*

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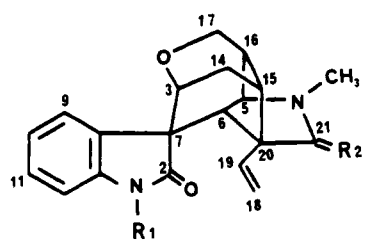
ABSTRACT.—A new oxindole alkaloid, 21-oxogelsevirine (**1**), has been isolated from the MeOH extract of the stem of *Gelsemium rankinii* and its structure elucidated. The ^{1}H- and ^{13}C-nmr spectra were assigned by comparison with the other three known analogs, gelsemine (**4**), 21-oxogelsemine (**2**), and gelsevirine (**3**).

Gelsemium rankinii Small, native to the southeastern United States, is one of the three *Gelsemium* species belonging to the family Loganiaceae. Although the other two species, *G. elegans* and *G. sempervirens*, have been extensively studied, *G. rankinii* has hitherto not been explored. As part of our interest in the anticancer activity of extracts of plants of this genus, we have studied the alkaloids of *G. rankinii*. We report here the isolation and structure elucidation of a new oxindole alkaloid, namely 21-oxogelsevirine (**1**).

Compound **1** displayed a molecular ion peak at *m/z* 366, 30 amu more than that of 21-oxogelsemine (**2**), and a base peak at *m/z* 122 characteristic of the fragmentation of 21-oxogelsemine (**2**), a minor alkaloid isolated from *G. sempervirens* (1). The observation in the ^{1}H-nmr spectrum of a three proton singlet at 3.96 ppm revealed the isolate to possess a methoxy group. The general similarity of all its spectra with those of compound **2** except for the chemical shifts of the aromatic protons, which were nearly identical with those of gelsevirine (**3**), pointed to compound **1** being 21-oxogelsevirine (Table 1).

Instead of gelsemine (**4**), the major alkaloid of *G. sempervirens*, gelsevirine (**3**) was the predominant alkaloid of *G. rankinii*. Previously, we have been able to assign unambiguously the proton and carbon spectra of gelsemine (**4**) (2). Using the same method, we found it also necessary to revise the previously assigned proton and carbon spectra of gelsevirine (3). Thus, the previous assignments for H-16, H-15, H-14a, H-14e, and H-6 for gelsevirine should be H-15, H-14a, H-16, H-6, and H-14e, respectively, from the evidence of a homonuclear 2-D COSY experiment. From the heteronuclear 2-D cor-



	R_1	R_2	
1	OCH_3	=O	21-oxogelsevirine
2	H	=O	21-oxogelsemine
3	OCH_3	H_2	gelsevirine
4	H	H_2	gelsemine

TABLE 1. ^{1}H-nmr Spectral Data of Gelsemine and Its Analogs[a]

Proton	Gelsemine[b] (4)	21-Oxogelsemine (2)	Gelsevirine (3)	21-Oxogelsevirine (1)
NH	8.71	7.89	—	—
H-9	7.40, d, J=7.6	7.39, d, J=7.6	7.46, d, J=7.6	7.44, d, J=7.6
H-11	7.17, dt, J=7.2, 7.2, 1.0	7.26, dt, J=7.6, 7.6, 0.8	7.29, dt, J=7.6, 7.6, 0.9	7.35, dt, J=7.6,7.6,0.9
H-10	6.99, dt, J=7.2, 7.2, 1.0	7.05, dt, J=7.6, 7.6, 0.8	7.06, dt, J=7.6, 7.6, 0.9	7.11, dt, J=7.6, 7.6, 0.9
H-12	6.74, d, J=7.6	6.85, d, J=7.6	6.96, d, J=7.6	7.00, d, J=7.6
H-19	6.26, dd, J=17.8, 11.0	6.06, dd, J=17.8, 11.1	6.23, dd, J=18.2, 11.0	6.07, dd, J=17.7, 11.0
H-18t	5.09, dd, J=11.0, 1.1	5.50, dd, J=11.1, 0.9	5.14, dd, J=11.0, 1.1	5.55, dd, J=11.0, 0.9
H-18c	4.95, dd, J=17.8, 1.1	5.21, dd, J=17.8, 0.9	4.97, dd, J=18.2, 1.1	5.23, dd, J=17.7, 0.9
H-17e	4.11, dd, J=11.0, 2.0	4.15, dd, J=11.4, 1.9	4.10, dd, J=11.1, 2.1	4.15, dd, J=11.6, 2.1
N-OCH$_3$	—	—	3.97, s	3.96, s
H-17a	3.92, dd, J=11.0, 2.0	4.01, dd, J=11.4, 1.9	3.91, dd, J=11.1, 2.1	4.00, dd, J=11.6, 2.1
H-3	3.81, m	3.86, m	3.81, m	3.86, m
H-5	3.46, s	3.88, d, J=1.2	3.42, s	3.83, d, J=1.4
H-14a	2.83, dd, J=14.2, 2.8	2.97, dd, J=14.7, 3.0	2.84, dd, J=14.4, 2.8	2.98, dd, J=14.7, 2.6
H-21exo	2.78, d, J=10.4	—	2.77, d, J=10.5	—
H-16	2.43, d, J=8.3	2.23, d, J=7.8	2.45, d, J=8.0	2.24, d, J=7.5
H-21endo	2.32, d, J=10.4	—	2.36, d, J=10.5	—
H-15	2.30, hidden	2.50, m	2.31, hidden	2.51, m
N-CH$_3$	2.25, s	2.79, s	2.26, s	2.79, s
H-14e	2.00, ddd, J=14.2, 6.1, 3.2	2.17, ddd, J=14.7, 6.3, 3.0	2.02, ddd, J=14.4, 5.6, 2.8	2.20, ddd, J=14.7, 5.7, 2.6
H-6	1.98, s	2.07, s	1.96, s	2.04, s

[a]Chemical shifts are given in ppm; δ_{TMS}=0 ppm; solvent $CDCl_3$; J=Hz.
[b]Data are from Yeh and Cordell (2).

relation spectrum, the assignments for C-16, C-15, C-6, and N-CH$_3$ should be revised to C-15, C-16, N-CH$_3$, and C-6, respectively.

Given the limited sample of 21-oxogelsemine isolated from the stem of *G. sempervirens*, it was not possible to employ a heteronuclear 2-D experiment for establishment of those nmr assignments. A simple and sensitive one-dimensional nmr technique for the correlation of proton and carbon chemical shifts (CSCM 1D) (4), however, made it possible to assign unambiguously the ^{13}C spectrum of 21-oxogelsemine (**2**) (Figure 1). For example, spectrum (C) was obtained by transfer from the downfield ^{13}C satellite of H-9 (920 Hz+80 Hz). Although the chemical shifts of C-9 and C-11 were close, their ^{1}H chemical shift difference (47 Hz) allowed selective irradiation to afford transfer only of C-9. In spectrum (D), the close ^{1}H chemical shifts of H-3 and H-5 led to the observation of both carbons. Each spectrum (B)-(H) is the result of only 400 scans.

21-Oxo substitution of gelsemine produced a significant downfield shift in C-18, C-21, C-20, C-6, and C-16, and an upfield shift for C-19, C-5, N-CH$_3$, and C-15 (Table 2). The close chemical shifts of C-6 and C-7 were distinguishable from an APT experiment, while C-17 and C-20 were confirmed by CSCM 1D for C-17. One of the carbonyl groups, C-21, was confirmed by the method of pulsed polarization transfer via long-range ^{1}H-^{13}C couplings (5), i.e., irradiating the proton chemical shift of N-CH$_3$ at 2.79 ppm permitted observation of the signal of C-21 at 176.84 ppm. Subsequently,

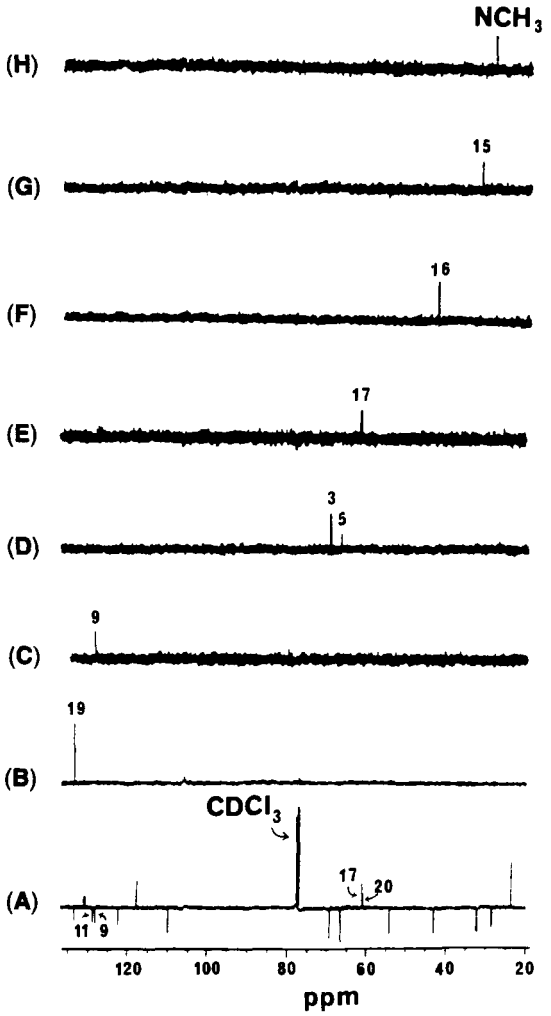


FIGURE 1. (A) ^{13}C APT spectrum of 21-oxogelsemine (**1**) in the range 20-135 ppm. (B)-(H) CSCM 1-D spectra from irradiating the satellites of H19, H9, H3, H17, H16, H15, and NCH_3, respectively.

the ^{13}C spectrum of the new isolate (**1**) was assigned by comparison with the three analogs, gelsemine, 21-oxogelsemine, and gelsevirine (Table 2).

The ^1H-nmr spectra of 21-oxogelsemine and 21-oxogelsevirine were also assigned by a 2-D COSY experiment. The absence of H-21, the downfield shifts of H-18, H-5, H-14, H-15, and N-CH_3 and upfield shifts of H-19 and H-16, supported the presence of the 21-oxo group.

The biological activity of these isolates will be reported subsequently.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The melting point was determined by means of a Kofler hotplate and is uncorrected. The uv spectrum was obtained with a Beckman model DU-7 spectrophotometer. The ir spectrum was determined on a Nicolet MX-1 interferometer. The ^1H-nmr spectrum was recorded on a Nicolet NMC 360 (360 MHz) using $CDCl_3$ as a solvent and TMS as an internal standard. The ^{13}C-nmr spectrum was obtained on the Nicolet NMC 360 instrument operating at 90.8 MHz. The mass

TABLE 2. ^{13}C-nmr Spectral Data of Gelsemine and Its Analogs[a]

Carbon	Gelsemine[b] (4)	21-Oxogelsemine (2)	Gelsevirine (3)	21-Oxogelsevirine (1)
C-2	179.3	177.3	172.5	171.8
C-13	140.5	140.1	138.8	139.5
C-19	138.5	133.1	137.8	132.7
C-8	131.7	131.8	127.6	126.4
C-9	128.0	127.8	127.8	127.6
C-11	127.7	128.4	127.8	128.7
C-10	121.4	121.9	122.3	122.8
C-18	111.9	117.3	112.5	117.9
C-12	108.8	109.4	106.7	107.5
C-5	71.8	66.1	71.8	66.2
C-3	69.2	68.9	69.0	68.8
C-21	65.9	176.8	65.8	176.7
N-OCH_3	—	—	62.8	63.3
C17	61.3	60.6	61.1	60.6
C-7	54.0	53.2	51.8	51.4
C-20	53.8	60.4	53.7	60.4
C-6	50.5	53.7	50.6	54.0
N-CH_3	40.4	27.9	40.2	27.9
C-16	37.9	42.6	37.6	42.5
C-15	35.5	31.6	35.5	31.7
C-14	22.7	23.1	22.7	23.3

[a]Chemical shifts are given in ppm; $\delta_{TMS}=0$ ppm; solvent $CDCl_3$.
[b]Data are from Yeh and Cordell (2).

spectrum was obtained with a Varian MAT 112S double focusing mass spectrometer operating at 70 eV. The 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, DE.

PLANT MATERIAL.—Dried stem material of *G. rankinii* was collected in the spring of 1984 and identified by one of us (M.G.). Specimens were deposited in the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND PURIFICATION.—Chopped stems of *G. rankinii* (300 g) were percolated five times with MeOH at room temperature for 2 days. The combined MeOH extracts were concentrated in vacuo at 30° to afford a thick dark syrup (ca. 35 g), which was dissolved in 2% citric acid and partitioned against Et_2O. After removal of the Et_2O extract, the acidic layer was basified with aqueous NH_3 to pH 8.0 and extracted extensively with EtOAc until a Dragendorff test was negative. The process was completed within the same day to avoid decomposition. The total alkaloid extract (2.5 g) was subjected to silica gel column chromatography eluting with mixtures of petroleum ether, EtOAc, and MeOH of increasing polarity. 21-Oxogelsemine (**2**), could not be found in the alkaloid extract of the stems of *G. rankinii*; gelsemine (**4**) was present in trace quantities.

ISOLATION OF 21-OXOGELSEVIRINE (**1**).—The residue (140 mg) from the pet. ether-EtOAc-MeOH (45:5:1) eluent was subjected to repeated preparative tlc using petroleum ether-C_6H_6-EtOAc-diethyl amine (25:10:10:4) as a solvent, and a purple band under uv light at Rf 0.3 was eluted with Me_2CO to afford white needles of **1** (2 mg), mp 226-228°; $[\alpha]^{25}D$ −67° (c 0.1, MeOH); uv λ max (MeOH) 210 (log ε 4.38) and 256 nm (3.77); ir ν max (AgCl) 1725, 1718, 1711, 1696, 1685, 1464, 1215, 1014, 780 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; ms *m/z* (rel. int.) 366 (M^+, 52), 335 (86), 253 (50), 149 (36), 122 (100).

ISOLATION OF GELSEVIRINE (**3**).—The major alkaloid of this plant was obtained by column chromatography using the same eluent as compound **1**. Using the same solvent system for preparative tlc, compound **3** was afforded at Rf 0.5 as a pale yellow oil (200 mg); $[\alpha]^{25}D$ −10° (c 0.3, MeOH); uv λ max (MeOH) 210 (log ε 4.26) and 256 nm (3.67); ir ν max (AgCl) 1731, 1721, 1616, 1464, 1317, 1100, 1081, 749 cm^{-1}; ^{1}H nmr, see Table 1; ^{13}C nmr, see Table 2; ms *m/z* (rel. int.) 352 (M^+, 24), 321 (75), 309 (21), 108 (100).

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