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New oxindole and indole alkaloids from *Gelsemium rankinii*

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ABSTRACT

Six new humantenine-type (**1**–**6**) and two new gelsemine-type (**7**, **8**) oxindole alkaloids and one new indole alkaloid (**9**) were isolated from the leaves and branches of *Gelsemium rankinii*. The structures of the new alkaloids were determined by spectroscopic analyses. Among them, 6-hydroxyhumantenine (**5**) is the first example of a *Gelsemium* alkaloid with an oxygen function at C-6 position, and is a plausible biogenetic precursor of gelsemine-type alkaloids.

1. Introduction

The genus *Gelsemium* comprises three species: *G. elegans*, which is widely distributed in Southeast Asia, and *G. sempervirens* and *G. rankinii*, which are distributed in North America. All of them are known to be rich sources of indole alkaloids. To date, more than seventy alkaloids have been isolated and classified into six types on the basis of their chemical structures.[1–3] Recently, we have found a new type of oxindole alkaloid from *G. rankinii* called rankiniridine, which has a nitrogen–carbon linkage between a humantenine-type alkaloid and an iridoid unit.[4] In our continuing chemical studies on the *Gelsemium* alkaloids,[5] we isolated six new humantenine-type (**1**–**6**), two new gelsemine-type (**7**, **8**), and one new sarpagine-type (**9**) alkaloids from *G. rankinii* (Fig. 1). In this paper, we report the structure elucidation of these new alkaloids.

2. Results and discussion

New alkaloid **1** was found to have the molecular formula $C_{20}H_{22}N_2O_3$ from HRFABMS [*m*/*z* 339.1737 (MH^+)]. It possesses two hydrogens less than rankinidine (**10**),[6,7] the main alkaloid of this plant. The UV spectrum exhibited a characteristic oxindole chromophore. ^1H and ^{13}C NMR spectra (Table 1) revealed some readily assignable signals due to the rankinidine skeleton, including signals assigned to an oxindole system with a non-substituted A ring [δ_H 7.48 (d, H-9), δ_H 7.32 (ddd, H-11), δ_H 7.14 (ddd, H-10), δ_H 6.98 (d, H-12); δ_C 171.4 (C-2)], an ethylidene group [δ_H 5.41 (m, H-19), δ_H 1.67 (3H, d, H_3-18)], an N_a-methoxy group [δ_H 3.98 (3H, s)], an oxymethylene group [δ_H 4.61 (d), δ_H 4.16 (dd); δ_C 64.9 (C-17)], an oxymethine group [δ_H 3.59 (d); δ_C 75.2 (C-3)] and a methylene group bearing a nitrogen atom [δ_H 4.91 (d), δ_H 3.76 (br d) (H_2-21)]. Comparison of the ^1H NMR data of **1** with those of rankinidine (**10**) indicated the lack of a nitrogen-bearing methine proton due to H-5 and the downfield shift of the signals due to H_2-6, H-16 and H_2-21 in **1**. Furthermore, a signal at δ 173.2 corresponding to an imine carbon was observed in the ^{13}C NMR spectrum of **1**. The above data implied the existence of an imine residue between N_4 and C-5 in **1**. The W-coupling (J=3.0 Hz) between H-6 and H-16 in the ^1H NMR spectrum and the HMBC correlations between the protons of H-17 and H-21 and the imine carbon at δ 173.2 supported the existence of an N_4–C-5 imine residue (Fig. 2). The anisotropy effect of this imine group might have caused the ^1H NMR signals of H-6 and H-21 to shift to the lower field. The *Z* configuration of the ethylidene group at C-19–C-20 was confirmed by the NOE correlation of H-19 to H-15. Therefore, compound **1** was deduced to be 4,5-dehydrorankinidine. This is the first example of a *Gelsemium* alkaloid with an imine moiety between N_4 and C-5 position.

New alkaloid **2** was shown to have the molecular formula $C_{20}H_{24}N_2O_4$ from HRFABMS [*m*/*z* 357.1844 (MH^+)], which indicated that **2** has an extra oxygen atom compared to rankinidine (**10**). The ^1H NMR spectrum was very similar to that of rankinidine (**10**) and included signals assignable to an N_a-methoxy oxindole system with a non-substituted A ring, an ethylidine group, an oxymethylene group (H_2-17), an oxymethine group (H-3), and methylene (H_2-21) and methine (H-5) groups bearing a nitrogen atom. However, the H-14 signal was observed as a methine proton in the lower field [δ 4.64 (d, H-14)] relative to that of **10**. In addition, an oxygenated methine

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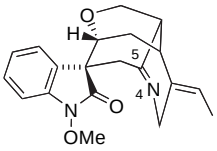
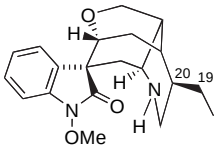
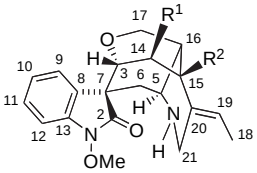


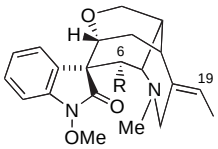
4,5-Dehydrorankinidine (**1**)



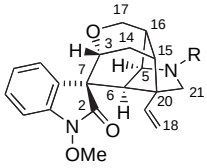
19,20-Dihydrorankinidine (**4**)



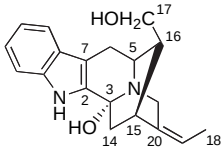
R^1=OH, R^2=H :
14-Hydroxyrankinidine (**2**)
R^1=H, R^2=OH :
15-Hydroxyrankinidine (**3**)
R^1=H, R^2=H : Rankinidine (**10**)



R=OH :
6-Hydroxyhumantenine (**5**)
R=H, 19(*E*) :
19(*E*)-Humantenine (**6**)
R=H : Humantenine (**11**)



R=H :
N_b-Demethylgelsevirine (**7**)
R=Me, N_b-oxide :
Gelsevirine *N*-oxide (**8**)
R=Me : Gelsevirine (**12**)



3-Hydroxykoumidine (**9**)

Figure 1. Structures of new (**1**–**9**) and known (**10**–**12**) alkaloids.

carbon signal was observed at δ_C 71.2 besides C-3 and C-17 oxygenated carbon signals in the ^{13}C NMR spectrum, suggesting the existence of an additional hydroxyl group. HMBC correlation of the proton at δ 3.51 due to H-3 to the carbon at δ 71.2 and that of the proton at

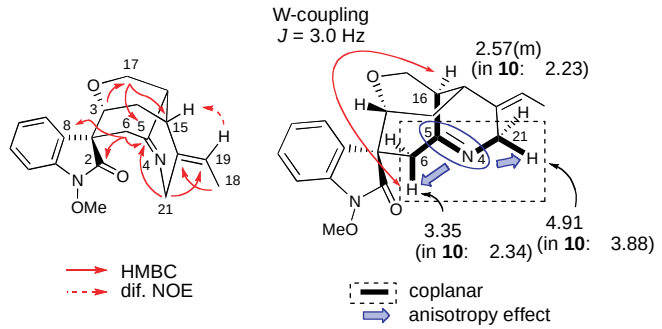


Figure 2. Selected HMBC and NOE correlations and NMR analysis of 4,5-dehydrorankinidine (**1**).

δ 4.64 to the carbon at δ 137.5 (C-20) indicated that the hydroxyl group was attached to C-14 (Fig. 3). The configuration of the hydroxyl group at C-14 was shown to be β on the basis of the coupling constant of H-14 ($J_{14,15}$=5.8 Hz) that shows coupling only with H-15 and not with H-3; the dihedral angle between H-14 and H-3 is ca. 90 degrees. The *Z* configuration of the ethylidene group at C-19–C-20 was confirmed by the NOE correlation of H-19 to H-15. From these data, compound **2** was deduced to be 14-hydroxyrankinidine.

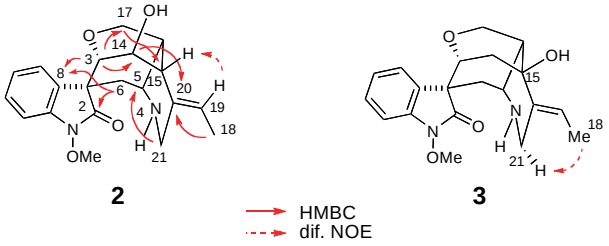


Figure 3. Selected HMBC and NOE correlations of 14-hydroxyrankinidine (**2**) and 15-hydroxyrankinidine (**3**).

New alkaloid **3** was found to have the molecular formula $C_{20}H_{24}N_2O_4$ from HRFABMS [*m*/*z* 357.1822 (MH^+)], which is the same as that of compound **2**. The ^1H NMR spectrum was very similar to that of rankinidine (**10**) except for the lack of a signal due

Table 1
^1H (500 MHz) and ^{13}C (125 MHz) NMR data for **1**–**4** in $CDCl_3$

Position	**1**		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2		171.4		173.7		173.9		174.8
3	3.59 (d, 8.9)	75.2	3.51 (s)	81.8	3.65 (d, 8.5)	73.2	3.68 (d, 8.3)	72.8
5		173.2	3.67 (m)	53.0	3.76 (m)	53.9	3.60 (m)	54.8
6	3.35 (dd, 13.5, 3.0)	41.7	2.40 (dd, 16.0, 5.3)	34.4	2.43 (dd, 16.0, 5.8)	34.5	2.54 (dd, 15.8, 7.6)	31.4
	2.89 (d, 13.5)		2.15 (dd, 16.0, 2.7)		2.17 (dd, 16.0, 4.0)		1.86 (dd, 15.8, 9.5)	
7		50.2		54.8		55.9		55.7
8		129.8		130.7		131.0		129.8
9	7.48 (d, 7.7)	125.2	7.42 (d, 7.6)	125.1	7.45 (d, 7.6)	125.3	7.42 (d, 7.7)	125.7
10	7.14 (ddd, 7.7, 7.7, 1.1)	123.3	7.15 (ddd, 7.6, 7.6, 1.1)	123.9	7.15 (dd, 7.6, 7.6)	123.7	7.11 (ddd, 7.7, 7.7, 1.1)	123.1
11	7.32 (ddd, 7.7, 7.7, 1.1)	128.4	7.32 (ddd, 7.6, 7.6, 1.1)	128.5	7.32 (dd, 7.6, 7.6)	128.3	7.31 (ddd, 7.7, 7.7, 1.1)	128.1
12	6.98 (d, 7.7)	107.1	6.99 (d, 7.6)	107.4	6.98 (d, 7.6)	107.3	7.00 (d, 7.7)	107.3
13		138.8		138.2		138.3		138.9
14	2.30 (dd, 14.9, 7.6)	30.2	4.64 (d, 5.8)	71.2	2.97 (d, 16.2)	38.3	2.35 (dd, 14.8, 8.1)	21.9
	2.18 (ddd, 14.9, 8.9, 8.9)				2.15 (overlapped)		1.98 (ddd, 14.8, 10.7, 8.3)	
15	2.75 (m)	33.6	2.38 (overlapped)	46.1		68.4	2.16 (m)	28.8
16	2.57 (m)	38.6	2.29 (m)	32.7	2.19 (overlapped)	41.4	2.11 (m)	39.7
17	4.61 (d, 10.9)	64.9	4.35 (d, 10.7)	66.8	4.56 (dd, 10.4, 4.6)	62.5	4.20 (d, 11.0)	67.6
	4.16 (dd, 10.9, 4.2)		4.14 (dd, 10.7, 4.9)		4.23 (d, 10.4)		4.02 (dd, 11.0, 5.5)	
18	1.67 (3H, d, 7.0)	13.2	1.64 (3H, d, 6.7)	12.8	1.65 (3H, d, 7.0)	12.6	0.95 (3H, dd, 7.4, 7.4)	11.4
19	5.41 (m)	119.0	5.46 (br q, 6.7)	119.1	5.86 (br q, 7.0)	116.0	1.36 (2H, dq, 7.4, 7.4)	23.1
20		137.3		137.5		144.2	1.70 (m)	41.9
21	4.91 (d, 17.6)	49.6	3.88 (d, 17.0)	41.4	3.90 (d, 16.7)	41.6	3.11 (dd, 13.6, 11.4)	40.6
	3.76 (br d, 17.6)		3.32 (d, 17.0)		3.45 (d, 16.7)		2.77 (dd, 13.3, 5.0)	
N_a-OMe	3.98 (3H, s)	63.2	4.01 (3H, s)	63.6	3.98 (3H, s)	63.5	4.00 (3H, s)	63.4

to H-15. In the ^{13}C NMR spectrum, a low-field quaternary carbon signal was observed at δ 68.4, which was assigned to C-15. The *Z* configuration of the ethylidene group at C-19–C-20 was confirmed by the NOE correlation of H_3-18 to H-21. Therefore, compound **3** was deduced to be 15-hydroxyrankinidine.

New alkaloid **4** was revealed to have the molecular formula $C_{20}H_{26}N_2O_3$ from HRFABMS [*m*/*z* 343.2007 (MH^+)]. It possesses two hydrogens more than rankinidine (**10**). ^1H and ^{13}C NMR spectra showed signals due to an N_a-methoxy oxindole system with a non-substituted A ring, an oxymethylene group (H_2-17), an oxymethine group (H-3), and methylene (H_2-21) and methine (H-5) groups bearing a nitrogen atom. In addition, signals due to an ethyl group [δ_H 1.36 (2H, dq, H_2-19), δ_H 0.95 (3H, dd, H_3-18); δ_C 23.1 (C-19), δ_C 11.4 (C-18)] were observed instead of those of an ethylidene group in **10**. By analyzing HMBC spectra (Fig. 4) together with the above data, compound **4** was deduced to be 19,20-dihydrorankinidine. The NOE correlation of H-20 to H-16 as well as the high-field shift of C-14 (δ_C 21.9) caused by γ-*gauche* effect of the ethyl side chain suggested that the configuration at C-20 position is as depicted in Figure 4.

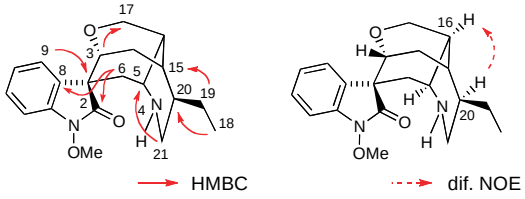


Figure 4. Selected HMBC and NOE correlations of 19,20-dihydrorankinidine (**4**).

New alkaloid **5** was found to have the molecular formula $C_{21}H_{26}N_2O_4$ from HRFABMS [*m*/*z* 371.1978 (MH^+)]. It possesses one oxygen more than humantenine (**11**).[7,8] The ^1H and ^{13}C NMR spectra (Table 2) revealed characteristic signals assigned to an N_a-methoxy oxindole system with a non-substituted A ring [δ_H 7.27 (d, H-9), δ_H 7.33 (ddd, H-11), δ_H 7.12 (ddd, H-10), δ_H 7.01 (dd, H-12), δ_H 3.98 (3H, s); δ_C 172.5 (C-2)], an ethylidene group [δ_H 5.59 (br q, H-19), δ_H 1.72 (3H, ddd, H_3-18)] and an N_b-methyl group [δ_H 2.46 (3H, s)], together with an oxymethylene group [δ_H 4.17 (dd), δ_H 4.13 (dd); δ_C 66.6 (C-17)], an oxymethine group [δ_H 3.66 (m); δ_C 70.3 (C-3)] and a methylene group bearing a nitrogen atom [δ_H 3.82 (d), δ_H 2.84 (overlapped) (H_2-21)], suggesting that **5** possessed the humantenine (**11**) skeleton. Furthermore, the H-6 signal was observed as a methine proton in a lower field [δ_H 4.35 (d)] than that of humantenine (**11**). In the ^{13}C NMR spectrum, an oxygenated methine carbon signal was observed at δ_C 73.0 in addition to the oxygenated carbon signals due to C-3 (δ_C 70.3) and C-17 (δ_C 66.6), suggesting the existence of an additional hydroxyl group. HMBC correlations of H-6 to both C-2 and C-8 and that of H-3 to C-6 indicated that the hydroxyl group was attached to C-6 (Fig. 5). By γ-effect of the hydroxy group on C-6, the C-16 signal was observed in the higher field (δ 30.7) relative to that of **11** in the ^{13}C NMR spectrum. The configuration of the hydroxyl group at C-6 was determined on the basis of the NOE correlations of H-6 to both H-14 and H-21, as shown in Figure 5. The *Z* configuration of the ethylidene group at C-19–C-20 was confirmed from the NOE correlation of H-19 to H-15. From the above data, compound **5** was deduced to be 6-hydroxyhumantenine. The oxygenation at C-6 position is unprecedented among *Gelsemium* alkaloids. From a biogenetic point of view, 6-hydroxyhumantenine (**5**) seems to be a plausible biogenetic precursor of gelsemine-type alkaloids, such as gelsevirine (**12**), which is characterized by the ring closure between C-6 and C-20, as shown in Figure 6.

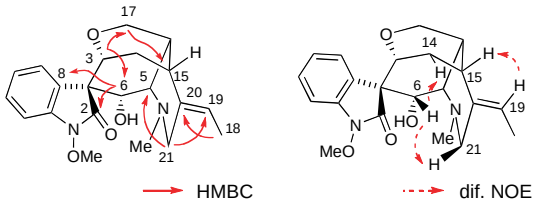


Figure 5. Selected HMBC and NOE correlations of 6-hydroxyhumantenine (**5**).

Table 2
^1H and ^{13}C NMR data for **5** and **6** in $CDCl_3$

Position	**5**		**6**	
	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[b]
2		172.5		174.4
3	3.66 (m)	70.3	3.67 (br d, 6.6)	72.0
5	2.82 (overlapped)	70.1	3.44 (br ddd, 8.5, 8.5, 3.1)	61.6
6	4.35 (d, 9.2)	73.0	2.55 (dd, 15.4, 8.5)	24.9
			1.67 (overlapped)	
7		59.9		55.2
8		123.7		129.1
9	7.27 (d, 7.6)	127.0	7.41 (d, 7.6)	125.9
10	7.12 (ddd, 7.6, 7.6, 1.2)	122.7	7.12 (ddd, 7.6, 7.6, 1.0)	123.0
11	7.33 (ddd, 7.6, 7.6, 1.2)	128.4	7.33 (ddd, 7.6, 7.6, 1.0)	128.2
12	7.01 (dd, 7.6, 1.2)	107.3	7.01 (dd, 7.6, 1.0)	107.4
13		140.3		139.0[d]
14	2.49 (2H, overlapped)	30.2	2.29 (2H, overlapped)	26.7
15	2.69 (m)	32.1	3.00 (m)	27.6
16	2.29 (m)	30.7	2.24 (overlapped)	37.7
17	4.17 (dd, 11.0, 1.5)	66.6	4.23 (d, 11.0)	67.1
	4.13 (dd, 11.0, 3.7)		4.10 (dd, 11.0, 5.4)	
18	1.72 (3H, ddd, 7.0, 1.8, 1.8)	13.5	1.66 (3H, dd, 6.9, 1.6)	12.7
19	5.59 (br q, 7.0)	120.8	5.41 (br q, 6.9)	119.5
20		138.6		136.8[d]
21	3.82 (d, 13.4)	49.2	3.63 (br d, 15.1)	52.2
	2.84 (overlapped)		3.04 (d, 15.1)	
N_a-OMe	3.98 (3H, s)	63.2	4.00 (3H, s)	63.4
N_b-Me	2.46 (3H, s)	45.0	2.32 (3H, s)	42.2

[a] At 500 MHz.
[b] At 125 MHz.
[c] At 400 MHz.
[d] Interchangeable.

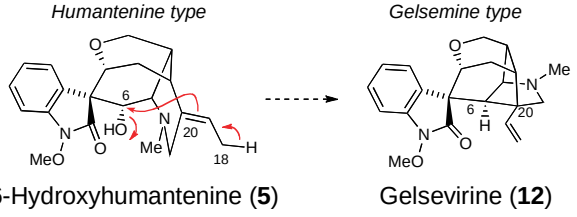


Figure 6. Possible biogenesis from humantenine-type to gelsemine-type alkaloid.

New alkaloid **6** was found to have the same molecular formula $C_{21}H_{26}N_2O_3$ as humantenine (**11**) from HRFABMS [*m*/*z* 355.2006 (MH^+)]. Its ^1H and ^{13}C NMR spectra were similar to those of humantenine (**11**) except for the chemical shifts of H-15, C-15 and C-21; H-15 and C-21 were observed in the lower field and C-15 was observed in the higher field compared to those of **11**. In the NOE experiments (Fig. 7), the correlations of H_3-18 to H-15 and of H-19 to H-21 were observed, suggesting the existence of an *E*-ethylidene group in **6**. Therefore, compound **6** was deduced to be 19(*E*)-humantenine.

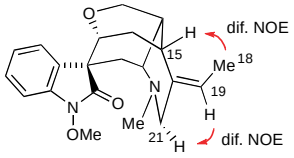


Figure 7. Selected NOE correlations of 19(*E*)-humantenine (**6**).

New alkaloid **7** was revealed to have the molecular formula $C_{20}H_{22}N_2O_3$ from HRFABMS [*m*/*z* 339.1702 (MH^+)]. The ^1H NMR spectrum (Table 3) showed characteristic signals of a vinyl group [δ_H 6.23 (dd, *J*=17.8, 11.0 Hz, H-19), δ_H 5.18 (dd, *J*=11.0, 1.2 Hz, H-18), δ_H 5.00 (dd, *J*=17.8, 1.2 Hz, H-18)], an N_a-methoxy oxindole system with a non-substituted A ring, an oxymethylene group (H_2-17), an oxymethine group (H-3), and methylene (H_2-21) and methine (H-5) groups bearing a nitrogen atom, suggesting that **7** was a gelsemine-type compound. The ^{13}C NMR spectrum revealed 20 carbons, including a carbonyl carbon of oxindole (δ 172.6) and carbons due to a vinyl group (δ 137.7, δ 113.3). These NMR data were similar to those of gelsevirine (**12**)[9] except for the lack of an N_b-methyl group and the chemical shift of protons (H-5, H_2-21, N_b-Me) and carbons (C-5, C-21, N_b-Me) around the N_b nitrogen atom. From the above data, compound **7** was deduced to be N_b-demethylgelsevirine.

Table 3
^1H and ^{13}C NMR data for **7** and **8** in $CDCl_3$

Position	**7**		**8**	
	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[b]
2		172.6		171.2
3	3.81 (m)	69.4	3.84 (m)	69.0
5	3.79 (br s)	65.8	4.02 (br s)	85.1
6	1.73 (s)	54.6	2.25 (br s)	51.0
7		52.8[d]		52.1[d]
8		127.9		126.1
9	7.51 (d, 7.7)	128.2	7.41 (d, 7.7)	128.1
10	7.08 (ddd, 7.7, 7.7, 1.2)	122.9	7.13 (ddd, 7.7, 7.7, 1.2)	123.5
11	7.31 (ddd, 7.7, 7.7, 1.2)	128.4	7.37 (ddd, 7.7, 7.7, 1.2)	129.1
12	6.97 (d, 7.7)	107.2	7.01 (d, 7.7)	107.6
13		139.5		139.3
14	2.87 (dd, 14.6, 3.2)	23.0	2.86 (dd, 14.7, 3.2)	22.6
	2.04 (ddd, 14.6, 5.8, 3.2)		2.14 (ddd, 14.7, 6.2, 2.8)	
15	2.43 (br dd, 8.2, 5.8)	35.9	2.64 (br dd, 7.7, 6.2)	34.6
16	2.32 (br d, 8.2)	43.6	4.28 (br d, 7.7)	34.3
17	4.04 (dd, 11.2, 2.1)	61.3	4.21 (dd, 11.5, 2.6)	61.3
	3.96 (dd, 11.2, 1.8)		4.06 (dd, 11.5, 2.0)	
18	5.18 (dd, 11.0, 1.2)	113.3	5.26 (d, 11.2)	115.8
	5.00 (dd, 17.8, 1.2)		5.04 (d, 17.8)	
19	6.23 (dd, 17.8, 11.0)	137.7	6.18 (dd, 17.8, 11.0)	134.3
20		52.4[d]		53.1[d]
21	3.00 (d, 11.3)	57.3	3.51 (d, 12.6)	80.1
	2.71 (d, 11.3)		3.31 (d, 12.6)	
N_a-OMe	3.97 (3H, s)	63.2	3.98 (3H, s)	63.2
N_b-Me			3.26 (3H, s)	59.3

[a] At 500 MHz.
[b] At 125 MHz.
[c] At 400 MHz.
[d] Interchangeable.

New alkaloid **8** was shown to have the molecular formula $C_{21}H_{24}N_2O_4$ from HRFABMS [*m*/*z* 369.1823 (MH^+)]. It possesses one oxygen more than gelsevirine (**12**). Its ^1H and ^{13}C NMR spectra were similar to those of gelsevirine (**12**) except for the chemical shift of the protons and carbons around the N_b nitrogen atom, the signals of which were observed in the lower field, suggesting that **8** is an N_b-oxide derivative of gelsevirine (**12**). The configuration at the N_b position was deduced to be *S* from the NOE correlations of N_b-CH_3 to H-6 (Fig. 8). This was supported by the lower-field-shifted H-16 interpreted from the anisotropy effect of the oxygen atom on N_b and the higher-field-shifted C-16 interpreted from the γ-*gauche* effect of the same oxygen atom, compared to those of **12** in the NMR spectra. As expected, **8** was obtained by the *m*-CPBA oxidation of gelsevirine (**12**). All the spectroscopic data, including the CD data of synthetic **8**, were identical with those of the natural one. Therefore, the structure of **8** was decided to be gelsevirine *N*-oxide.

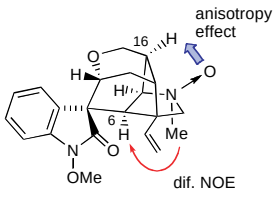


Figure 8. Selected NOE correlation of gelsevirine *N*-oxide (**8**).

The configuration at the C-7 spiro center of all the new oxindole compounds (**1**–**8**) was deduced to be *S* from the negative and positive Cotton effects that appeared, respectively, around 260 and 230 nm in the CD spectrum, and was the same as the absolute configuration of the other *Gelsemium* alkaloids.

New sarpagine-type alkaloid **9** was revealed to have the molecular formula $C_{19}H_{22}N_2O_2$ from HRFABMS [*m*/*z* 311.1744 (MH^+)]. The UV spectrum exhibited a typical indole chromophore. Its ^1H and ^{13}C NMR spectra showed characteristic signals due to a non-substituted A ring of the indole system, an ethylidene group [δ_H 5.25 (br qdd, H-19), δ_H 1.50 (3H, br ddd, H_3-18)], an oxymethylene group [δ_H 3.29 (dd), δ_H 2.87 (dd); δ_C 61.2 (C-17)] and a methylene [δ_H 4.04 (br d), δ_H 3.34 (br d) (H_2-21)] and a methine [δ_H 3.63 (dd, H-5)] group bearing a nitrogen atom. Furthermore, a hemiaminal carbon was observed at δ 82.5 in the ^{13}C NMR spectrum, which was correlated with proton signals due to H-5, H-14, H-15 and H-21 in the HMBC spectra (Fig. 9), suggesting that the hemiaminal carbon was assigned to C-3. The stereochemistry at C-16 and C-19 positions was confirmed from the NOE correlations of H-16 to H-5 and of H-19 to H-15, respectively. The CD spectrum demonstrated a similar curve to those of 16-*epi*-voacarpine[10] and 19*Z*-16-*epi*-voacarpine,[11] revealing that these compounds have the same absolute configuration. From the above data, compound **9** was deduced to be 3-hydroxykoumidine.

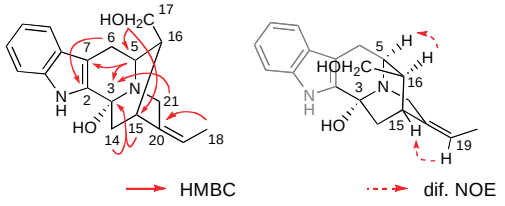


Figure 9. Selected HMBC and NOE correlations of 3-hydroxykoumidine (**9**).

In conclusion, six new humantenine-type (**1**–**6**), two new gelsedine-type (**7**, **8**) and one new sarpagine-type (**9**) alkaloids were isolated from the leaves and branches of *G. rankinii*. Among them, 6-hydroxyhumantenine (**5**) is the first example of a *Gelsemium* alkaloid with an oxygen function at C-6 position, and is a plausible biogenetic precursor of gelsemine-type alkaloids.

3. Experimental

3.1. General

^1H and ^{13}C NMR spectra: JEOL JNM ECP-400 at 400 MHz (^1H) and JEOL JNM A-500 at 500 MHz (^1H) or 125 MHz (^{13}C), respectively. UV: JASCO V-560. EIMS: JEOL GC mate. FABMS: JEOL JMS-AX500. HRFABMS: JEOL JMS-HX110. CD: JASCO J-720WI. TLC: Precoated silica gel 60 F_{254} plates (Merck, 0.25 mm thick), Precoated amino-silica gel plates (Fuji Silysia Chemical), Aluminium oxide F_{254} (Merck, Type-E). Column chromatography: Silica gel 60 (Merck, 70–230 mesh), Silica gel 60N [Kanto Chemical, 40–50 mm (for flash chromatography)], Chromatorex NH [Fuji Silysia Chemical, 100–200 mesh (for amino-silica gel column chromatography)], Aluminium oxide 60 (Merck, 70–230 mesh), Sephadex LH-20 (GE Healthcare). Medium pressure liquid chromatography (MPLC): C.I.G. prepacked column CPS-HS-221–05 (Kusano Kagakukikai, SiO_2), Ultra Pack NH-40A (Yamazen, amino-silica gel).

3.2. Plant material

G. rankinii small was harvested from the medicinal plant garden of Chiba University, Japan. It was identified by Dr. F. Ikegami and a voucher specimen (No. 20051201) was deposited at the Faculty of Pharmaceutical Sciences, Chiba University, Japan.

3.3. Isolation

5% MeOH/$CHCl_3$ (8.92 g) and *n*-BuOH (40.01 g) extracts were obtained from the leaves and stems of *G. rankinii* (1144 g, dry weight).[4,12] The 5% MeOH/$CHCl_3$ extract (8.92 g) was separated by SiO_2 open column chromatography with a $CHCl_3$/MeOH gradient to afford eight fractions: fr. CA 0–1% MeOH/$CHCl_3$ (1500 mL, 131 mg); fr. CB 1–3% MeOH/$CHCl_3$ (1000 mL, 59 mg); fr. CC 3% MeOH/$CHCl_3$ (500 mL, 728 mg); fr. CD 5% MeOH/$CHCl_3$ (500 mL, 459 mg); fr. CE 5–10% MeOH/$CHCl_3$ (2500 mL, 3813 mg); fr. CF 20% MeOH/$CHCl_3$ (750 mL, 317 mg); fr. CG 20–50% MeOH/$CHCl_3$ (1000 mL, 489 mg); and fr. CH 50–100% MeOH/$CHCl_3$ (750 mL, 376 mg). Fraction CE (3813 mg) was separated by SiO_2 flash column chromatography with MeOH/$CHCl_3$ gradient. The fraction eluted with 2–5% MeOH/$CHCl_3$ was successively purified by SiO_2 flash column chromatography (MeOH/$CHCl_3$ gradient), MPLC (SiO_2, AcOEt/*n*-hexane gradient), and amino-silica gel open column chromatography ($CHCl_3$) to give 4,5-dehydrorankinidine (**1**, 1.7 mg). Fraction CF (317 mg) was separated by SiO_2 flash column chromatography with MeOH/AcOEt gradient. The fraction eluted with 30% MeOH/AcOEt and then MeOH was purified by SiO_2 flash column chromatography (MeOH/AcOEt gradient) and silica gel open column chromatography (NH_4OH saturated $CHCl_3$) to give 6-hydroxyhumantenine (**5**, 1.4 mg). The fraction that was eluted with MeOH on SiO_2 flash column chromatography of fraction CF was purified by SiO_2 flash column chromatography (MeOH/NH_4OH saturated $CHCl_3$ gradient) and amino-silica gel open column chromatography (AcOEt/*n*-hexane gradient) to afford 19,20-dihydrorankinidine (**4**, 2.7 mg). Fraction CG (489 mg) was separated by SiO_2 flash column chromatography with MeOH/AcOEt–MeOH–20% MeOH/NH_4OH saturated $CHCl_3$ gradient. The fraction that was eluted with MeOH and 20% MeOH/NH_4OH saturated $CHCl_3$ was purified by amino-silica gel open column chromatography to give four fractions. The fraction that was eluted with AcOEt was purified by MPLC (amino-silica gel, AcOEt/*n*-hexane–MeOH/AcOEt gradient) to afford 19(*E*)-humantenine (**6**, 1.3 mg). Further purification of the fraction eluted with MeOH gave gelsevirine *N*-oxide (**8**, 0.4 mg). The *n*-BuOH extract (22.50 g) was separated by Sephadex LH-20 column chromatography with MeOH/H_2O gradient to give eight fractions (frs. BA-BH). Fraction BA that was eluted with H_2O (7749 mg) was purified by SiO_2 flash column chromatography with MeOH/$CHCl_3$ gradient. The fraction that was eluted with 10–15% MeOH/$CHCl_3$ (983.3 mg) was separated by SiO_2 flash column chromatography (AcOEt/*n*-hexane–MeOH/AcOEt–5% concn NH_4OH/MeOH gradient and then MeOH/$CHCl_3$ gradient). The fractions that were eluted with 7–10% MeOH/$CHCl_3$ and 10% MeOH/$CHCl_3$ were purified by repeated chromatography to afford N_b-demethylgelsevirine (**7**, 1.6 mg from the former fraction), 14-hydroxyrankinidine (**2**, 0.8 mg) and 15-hydroxyrankinidine (**3**, 1.4 mg). The fractions BB (1914 mg) that was eluted with 10–20% MeOH/H_2O was purified by SiO_2 flash column chromatography. The fraction that was eluted with 15–30% MeOH/$CHCl_3$ (176.1 mg) was separated by repeated chromatography including MPLC (amino-silica gel) and Al_2O_3 open column chromatography to give 3-hydroxykoumidine (**9**, 3.9 mg). Additional 6-hydroxyhumantenine (**5**, 2.2 mg) was obtained from the *n*-BuOH extract. Twelve known alkaloids, rankinidine (**10**), humantenine (**11**), humantenirine, 20-hydroxydihydrorankinidine, humantenine *N*-oxide, gelsemamide, gelsevirine, 21-oxogelsevirine, 19(*S*)-hydroxydihydrogelsevirine, gelsenicine, 14-hydroxygelsenicine and 4,20-dehydrogelsemicine were isolated from the MeOH extract.

3.4. Characteristics of each alkaloid

*3.4.1. 4,5-Dehydrorankinidine (**1**).* ^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 255.0 (3.65), 209.0 (4.19); FABMS (NBA) *m*/*z* 339 (MH^+); HRFABMS (NBA/PEG) 339.1737 (MH^+, calcd for $C_{20}H_{23}N_2O_3$, 339.1709); CD (*c*=0.379 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (318), −2.09 (267), 0 (250), +5.45 (232), 0 (222), −13.10 (211).

*3.4.2. 14-Hydroxyrankinidine (**2**).* ^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 257.5 (3.55), 210.5 (4.20); EIMS *m*/*z* (%) 356 (M^+, 100), 325 (75), 180 (49), 108 (100); HRFABMS (NBA/PEG) 357.1844 (MH^+, calcd for $C_{20}H_{25}N_2O_4$, 357.1814); CD (*c*=0.227 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (300), −1.54 (261), 0 (245), +2.21 (230), 0 (222), −8.32 (211).

*3.4.3. 15-Hydroxyrankinidine (**3**).* ^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 256.0 (3.58), 211.5 (4.07); EIMS *m*/*z* (%) 356 (M^+, 98), 325 (23), 307 (9), 124 (100); HRFABMS (NBA/PEG) 357.1822 (MH^+, calcd for $C_{20}H_{25}N_2O_4$, 357.1814); CD (*c*=0.365 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (298), −2.20 (262), 0 (249), +9.61 (230), 0 (220), −16.72 (210).

*3.4.4. 19,20-Dihydrorankinidine (**4**).* ^1H and ^{13}C NMR data, see Table 1; UV (MeOH) λ_{max} nm (log ε) 256.0 (3.66), 208.0 (4.27); FABMS (NBA) *m*/*z* 343 (MH^+); HRFABMS (NBA/PEG) 343.2007 (MH^+, calcd for $C_{20}H_{27}N_2O_3$, 343.2022); CD (*c*=0.380 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (301), −2.76 (290), −5.68 (259), 0 (245), +16.11 (228), 0 (220), −35.84 (210).

*3.4.5. 6-Hydroxyhumantenine (**5**).* ^1H and ^{13}C NMR data, see Table 2; UV (MeOH) λ_{max} nm (log ε) 255.0 (3.68), 208.0 (4.44); EIMS *m*/*z* (%) 370 (M^+, 52), 339 (63), 122 (100); HRFABMS (NBA/PEG) 371.1978 (MH^+, calcd for $C_{21}H_{27}N_2O_4$, 371.1971); CD (*c*=0.216 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (327), −3.37 (257), 0 (241), +5.06 (228), 0 (221), −17.14 (211).

*3.4.6. 19(E)-Humantenine (**6**).* ^1H and ^{13}C NMR data, see Table 2; UV (MeOH) λ_{max} nm (log ε) 256.0 (3.82), 208.0 (4.43); EIMS *m*/*z* (%) 354 (M^+, 76), 323 (100); HRFABMS (NBA/PEG) 355.2006 (MH^+, calcd for $C_{21}H_{27}N_2O_3$, 355.2022); CD (*c*=0.277 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (325), −1.68 (266), 0 (245), +4.64 (230), 0 (220), −11.47 (211).

*3.4.7. N_b-Demethylgelsevirine (**7**).* ^1H and ^{13}C NMR data, see Table 3; UV (MeOH) λ_{max} nm (log ε) 256.5 (3.58), 210.5 (4.19); EIMS *m*/*z* (%) 338 (M^+, 81), 307 (100), 278 (32); HRFABMS (NBA/PEG) 339.1702 (MH^+, calcd for $C_{20}H_{23}N_2O_3$, 339.1709); CD (*c*=0.367 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (306), +0.64 (291), 0 (276), −3.87 (259), 0 (248), +6.39 (235), 0 (223), −8.47 (214), 0 (205).

*3.4.8. Gelsevirine N-oxide (**8**).* ^1H and ^{13}C NMR data, see Table 3; UV (MeOH) λ_{max} nm (log ε) 256.0 (3.61), 208.0 (4.23); EIMS *m*/*z* (%) 368 (M^+, 14), 352 (62), 321 (77), 108 (100); HRFABMS (NBA/PEG) 369.1823 (MH^+, calcd for $C_{21}H_{25}N_2O_4$, 369.1814); CD (*c*=0.236 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (313), +0.99 (291), 0 (275), −3.16 (259), 0 (248), +5.17 (236), 0 (223), −8.29 (214).

*3.4.9. 3-Hydroxykoumidine (**9**).* ^1H NMR (500 MHz, CD_3OD) δ 7.29 (1H, br d, *J*=7.8 Hz, H-9), 7.22 (1H, br d, *J*=8.1 Hz, H-12), 6.97 (1H, ddd, *J*=8.1, 7.0, 1.0 Hz, H-11), 6.87 (1H, ddd, *J*=7.8, 7.0, 1.0 Hz, H-10), 5.25 (1H, br qdd, *J*=6.8, 2.6, 2.6 Hz, H-19), 4.04 (1H, br d, *J*=17.6 Hz, H-21), 3.63 (1H, dd, *J*=10.5, 4.1 Hz, H-5), 3.34 (1H, br d, *J*=17.6 Hz, H-21), 3.29 (1H, dd, *J*=11.0, 6.1 Hz, H-17), 2.87 (1H, dd, *J*=11.0, 9.2 Hz, H-17), 2.87 (1H, overlapped, H-6), 2.80 (1H, d, *J*=15.9 Hz, H-6), 2.43 (1H, m, H-15), 2.18 (1H, dd, *J*=13.8, 3.8 Hz, H-14), 2.06 (1H, m, H-16), 1.69 (1H, d, *J*=13.8 Hz, H-14), 1.50 (3H, br ddd, *J*=6.8, 1.1, 1.1 Hz, H_3-18); ^{13}C NMR (125 MHz, CD_3OD) δ 142.6 (C-20), 139.1

(C-2), 138.6 (C-13), 127.2 (C-8), 122.6 (C-11), 119.9 (C-10), 119.2 (C-9), 114.5 (C-19), 112.3 (C-12), 108.2 (C-7), 82.5 (C-3), 61.2 (C-17), 58.0 (C-5), 47.8 (C-21), 43.7 (C-16), 38.3 (C-15), 37.4 (C-14), 23.0 (C-6), 12.5 (C-18); UV (MeOH) λ_{max} nm (log ε) 290.5 (3.71), 282.5 (3.79), 224.5 (4.48); EIMS *m*/*z* (%) 310 (M^+, 51), 279 (18), 184 (100); HRFABMS (NBA/PEG) 311.1744 (MH^+, calcd for $C_{19}H_{23}N_2O_2$, 311.1760); CD (*c*=0.387 mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (λ nm) 0 (304), +0.66 (270), 0 (247), −10.91 (229), 0 (213).

*3.4.10. m-CPBA oxidation of gelsevirine (**12**).* To a solution of gelsevirine (**12**, 10.3 mg, 0.029 mmol) in CH_2Cl_2 (0.5 mL) was added *m*-CPBA (11.4 mg, 0.066 mmol) at 0 °C under Ar atmosphere and the mixture was stirred for 2 h at rt. 5% NH_4OH aq 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 Al_2O_3 open column chromatography ($CHCl_3$) and then SiO_2 open column chromatography (NH_4OH saturated $CHCl_3$) to afford gelsevirine *N*-oxide (**8**, 2.5 mg, 24%). All the spectroscopic data of synthetic **8** were identical with those of natural one.

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