Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 3638–3642
Tetrahedron Letters

New humantenine-type indole alkaloids with iridoid unit from *Gelsemium* species

Noriyuki Kogure [a], Hiromi Kobayashi [a], Naoko Ishii [a], Mariko Kitajima [a], Sumphan Wongseripipatana [b], Hiromitsu Takayama [a,*]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[b] *Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10500, Thailand*

Received 5 March 2008; revised 25 March 2008; accepted 31 March 2008
Available online 8 April 2008

Abstract

Two new oxindole alkaloids, rankiniridine (**1**) and humanteniridine (**2**), having a nitrogen–carbon linkage between a humantenine-type monoterpenoid indole alkaloid and a monoterpene unit with an iridoid skeleton, were isolated from *Gelsemium rankinii* and *Gelsemium elegans*, respectively.

Keywords: Indole alkaloid; *Gelsemium*; Iridoid; Structure elucidation

The genus *Gelsemium*, which belongs to Loganiaceae, comprises three species: *Gelsemium elegans* Benth., *Gelsemium rankinii* Small, and *Gelsemium sempervirens* Ait. Among them, *G. elegans*, which is widely distributed in Southeast Asia, is known as a toxic plant and has been used in traditional Chinese medicine. Our previous study has proved that the origin of 'Yakatsu', one of the ancient medicines stored in the Shosoin repository in Japan, is *G. elegans*.[1] Presently, more than seventy *Gelsemium* alkaloids are known and they are classified into six types on the basis of their chemical structures, that is, sarpagine, koumine, humantenine, gelsedine, gelsemine, and yohimbane.[2,3] Recently, our research revealed that among the structurally diverse *Gelsemium* alkaloids, some gelsedine-type alkaloids showed potent cytotoxic effects against A431 epidermoid carcinoma cells.[4] In our continuing chemical studies on *Gelsemium* alkaloids,[5] two new oxindole alkaloids that are composed of a humantenine-type monoterpenoid indole alkaloid and an iridoid were isolated. In this Letter, we describe the structure elucidation of these novel alkaloids, named rankiniridine (**1**) and humanteniridine (**2**) (Fig. 1).

The leaves and stems of *G. rankinii*[6] (1144 g, dry weight) were extracted with MeOH to give the crude extract (232.6 g), which was dissolved in H_2O containing a small amount of MeOH and then extracted successively with *n*-hexane, AcOEt, 5% MeOH–$CHCl_3$, and *n*-BuOH. The 5% MeOH–$CHCl_3$ extract (8.92 g) was separated by SiO_2 flash column chromatography to afford rankiniridine (**1**,

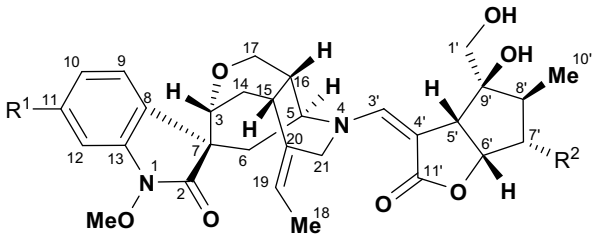


Fig. 1. Structures of new alkaloids (**1** and **2**).

* Corresponding author. Tel./fax: +81 43 290 2901.
E-mail address: htakayam@p.chiba-u.ac.jp (H. Takayama).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.03.148

Table 1
^{1}H and ^{13}C NMR data of **1**–**3** and **5**

Position	Rankiniridine (**1**)[a]		Rankinidine (**3**)[b]		Humanteniridine (**2**)[b]		Humantenirine (**5**)[b]	
	δ_H (600 MHz)[c]	δ_C (150 MHz)[d]	δ_H (500 MHz)[d]	δ_C (125 MHz)[d]	δ_H (600 MHz)[c]	δ_C (150 MHz)[c]	δ_H (500 MHz)[d]	δ_C (125 MHz)[d]
2		173.8		174.1		173.3		174.6
3	3.71 (d, 6.3)	71.9	3.55 (d, 8.5)	73.8	3.65 (d, 6.6)	72.2	3.52 (d, 8.2)	74.1
5	3.39 (overlapped)	64.1	3.72 (m)	54.4	3.98 (m)	64.6	3.69 (m)	56.4
6	2.47 (dd, 15.5, 4.4)	33.2	2.45 (dd, 15.9, 3.4)	34.1	2.44 (dd, 10.0, 4.8)	33.1	2.29 (dd, 15.7, 5.7)	34.3
	1.81 (dd, 15.5, 7.4)		2.19 (dd, 15.9, 4.3)		1.78 (overlapped)		2.18 (dd, 15.7, 3.7)	
7		54.5		56.9		54.2		54.4
8		127.0		131.3		118.9		123.0
9	7.30 (d, 7.6)	125.9	7.43 (d, 7.6)	125.2	7.17 (d, 8.2)	126.8	7.31 (d, 8.2)	126.0
10	7.18 (dd, 7.6, 7.6)	123.1	7.14 (dd, 7.6, 7.6)	123.5	6.63 (dd, 8.2, 2.2)	107.7	6.63 (dd, 8.2, 2.4)	108.0
11	7.40 (dd, 7.6, 7.6)	128.3	7.31 (dd, 7.6, 7.6)	128.1		160.3		160.2
12	7.06 (d, 7.6)	107.3	6.98 (d, 7.6)	107.2	6.61 (d, 2.2)	95.0	6.56 (d, 2.4)	94.6
13		138.3		138.3		140.0		140.2
14	2.45 (2H, overlapped)	30.1	2.45 (dd, 15.2, 7.6)	30.0	2.53 (ddd, 15.1, 11.8, 6.6)	30.3	2.42 (dd, 15.2, 7.6)	30.0
			2.31 (m)		2.39 (dd, 15.1, 5.8)		2.31 (m)	
15	2.91 (m)	31.5	2.62 (m)	34.2	2.86 (m)	31.8	2.60 (ddd, 11.5, 7.6, 3.8)	34.3
16	2.59 (m)	37.0	2.24 (m)	34.6	2.34 (m)	37.6	2.21 (m)	34.8
17	4.20 (d, 11.2)	65.2	4.33 (d, 10.8)	67.1	4.15 (d, 11.3)	65.9	4.29 (d, 10.4)	67.1
	4.08 (dd, 11.2, 3.8)		4.05 (dd, 10.8, 4.6)		4.05 (dd, 11.3, 4.1)		4.03 (dd, 10.4, 4.6)	
18	1.76 (3H, d, 6.8)	12.7	1.61 (3H, d, 6.8)	12.6	1.74 (3H, d, 6.9)	13.7	1.59 (3H, d, 6.8)	12.6
19	5.68 (q, 6.8)	122.9	5.24 (q, 6.8)	117.4	5.60 (m)	123.4	5.23 (q, 6.8)	117.5
20		134.9		140.3		134.6		139.4
21	4.63 (d, 16.1)	42.2	3.89 (d, 16.8)	41.3	4.64 (d, 16.4)	42.0	3.88 (d, 16.7)	41.1
	4.31 (d, 16.1)		3.32 (d, 16.8)		4.24 (d, 16.4)		3.32 (d, 16.7)	
N_a-OMe	3.99 (3H, s)	63.5	4.00 (3H, s)	63.4	3.96 (3H, s)	63.8	3.98 (3H, s)	63.4
11-OMe					3.85 (3H, s)	55.9	3.83 (3H, s)	55.6
1′	3.39 (2H, overlapped)	66.0			3.46 (2H, m)	67.5		
3′	7.23 (s)	146.0			7.21 (s)	145.7		
4′		88.0				89.8		
5′	3.63 (d, 6.0)	51.3			3.71 (d, 6.3)	54.6		
6′	4.73 (dd, 6.0, 4.2)	82.0			4.82 (br dd, 6.3, 4.7)	82.1		
7′	3.87 (dd, 11.5, 4.2)	76.7			2.13 (br dd, 14.0, 6.8)	38.1		
					1.78 (overlapped)			
8′	1.70 (m)	43.5			2.01 (m)	39.4		
9′		80.5				82.9		
10′	1.10 (3H, d, 6.9)	9.9			1.04 (3H, d, 6.8)	13.5		
11′		176.1				175.8		

[a] In $CDCl_3/CD_3OD = 19:1$.
[b] In $CDCl_3$.
[c] Measured at −20 °C.
[d] Measured at rt.

7.0 mg). Humanteniridine (**2**, 5.7 mg) was isolated from the leaves of *G. elegans*[7] using the same extraction and isolation procedure as above.

The molecular formula of rankiniridine (**1**)[8] was established as $C_{30}H_{36}N_2O_8$ from HR-FAB-MS [*m/z* 553.2556 (MH^+)]. Compound **1** possesses 10 carbons more than common *Gelsemium* alkaloids.[5d,9] The ^{1}H NMR spectrum (−20 °C) showed signals that were readily assignable to the alkaloid moiety and the iridoid moiety. Signals assigned to four aromatic protons [δ 7.40 (dd, H-11), δ 7.30 (d, H-9), δ 7.18 (dd, H-10), δ 7.06 (d, H-12)], an N_a-methoxy group [δ 3.99 (3H, s)], oxymethylene protons [δ 4.20 and 4.08 (H_2-17)], and ethylidene protons [δ 5.68 (q, H-19), δ 1.76 (3H, d, H_3-18)] are characteristic of humantenine-type alkaloids having an oxindole skeleton derived from sarpagine-type indole alkaloid.[3] The ^{1}H and ^{13}C NMR signals of the oxindole moiety in **1** are very similar (Table 1) to those of rankinidine (**3**),[10] a known humantenine-type *Gelsemium* alkaloid, except for the chemical shift of the carbons around the nitrogen atom (N-4). On the other hand, signals assigned to methyl protons [δ 1.10 (3H, d, H_3-10′)], oxymethylene protons [δ 3.39 (2H, br s, H_2-1′)], and a low-field methine proton attached to ester oxygen [δ 4.73 (dd, H-6′)] are characteristic of an iridoid moiety. Further, the signals at δ_H 7.23 (H-3′), δ_H 4.73 (H-6′), δ_C 146.0 (C-3′), δ_C 88.0 (C-4′), δ_C 176.1 (C-11′), and δ_C 82.0 (C-6′) indicate the existence of an α, β-unsaturated lactone with a hetero atom (N or O) at the β position in the iridoid moiety. The UV spectrum supports the presence of this function (296.0 nm). The gross structure of the iridoid moiety was assigned on the basis of ^{1}H–^{1}H COSY and HMBC correlations (Fig. 2), particularly from H-3′ to C-5′ and C-11′, and from H-6′ and H_3-10′ to C-9′. Finally, HMBC correlations from H-3′ to C-5 and C-21 indicate that the monoterpene unit is attached to N_b group at the C-3′ position.

The geometry of the ethylidene group at C-19–C-20 and the double bond at C-3′–C-4′ was elucidated by NOE experiments, as shown in Figure 2. The relative stereochemistry of sequential chiral centers (C-5′–C-8′) in the iridoid moiety was deduced from NOE correlations among H-5′, 6′, 7′, and 10′. In addition, the stereochemistry of C-9′ was determined from the NOE correlation from H-3′ to H-1′.

From a biogenetic point of view, **1** would originate in rankinidine (**3**) and gelsemide (**4**),[11] a coexisting iridoid in this plant, via the Michael-type addition, followed by ring opening of the dihydropyran moiety (Scheme 1). Based on this idea, the condensation of rankinidine (**3**) and gelsemide (**4**) was attempted. A mixture of rankinidine (**3**) and gelsemide (**4**) in THF was heated at 100–150 °C in a sealed tube for 26 h to give **1** in 19% yield. All the spectroscopic data including CD spectrum of semi-synthetic **1** were identical with those of natural **1** and therefore, the structure including the absolute configuration of rankiniridine (**1**) was established. Rankiniridine (**1**) is a new type of oxindole alkaloid having a nitrogen–carbon linkage between a humantenine-type monoterpenoid indole alkaloid and a monoterpene unit having an iridoid skeleton.

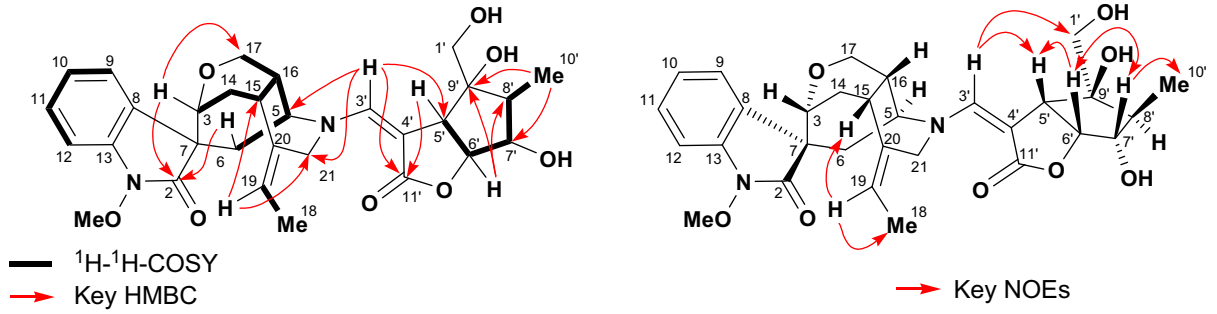
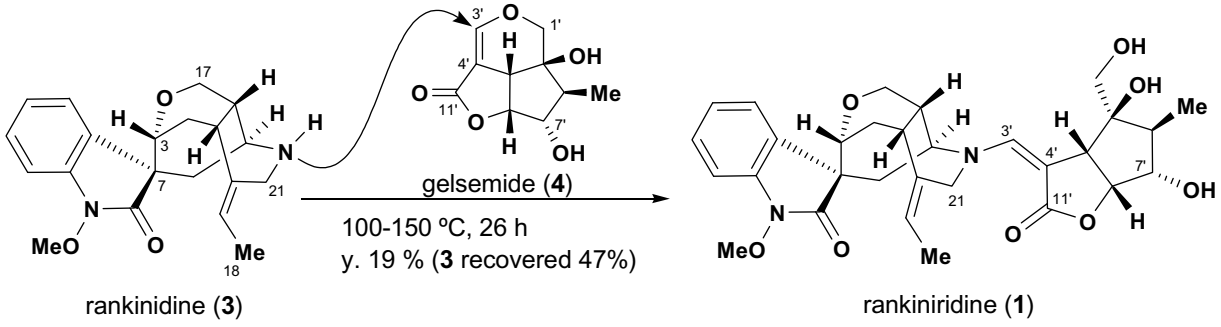


Fig. 2. Rankiniridine (**1**).



Scheme 1. Condensation of rankinidine (**3**) and gelsemide (**4**).

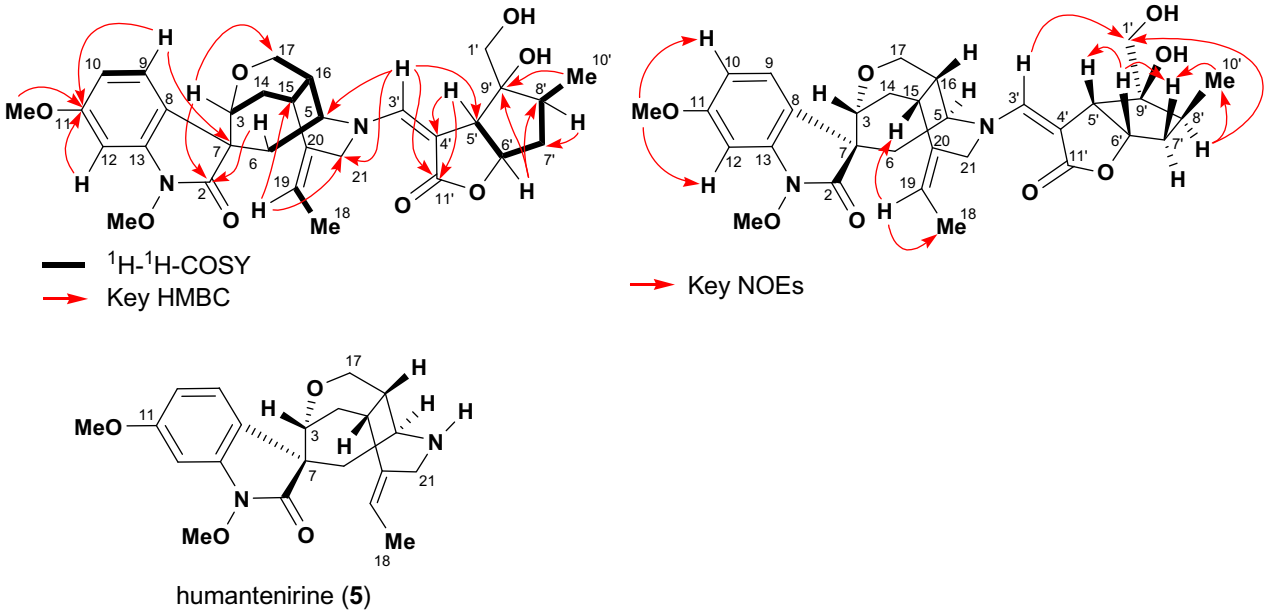


Fig. 3. 2D NMR and NOE of humanteniridine (**2**) and structure of humantenirine (**5**).

The HR-FAB-MS spectrum of humanteniridine (**2**)[12] gave a protonated molecular ion peak at *m/z* 567.2722 (MH^+) that corresponded to the molecular formula $C_{31}H_{39}N_2O_8$ (*m/z* 567.2706). The UV spectrum as well as the ^{1}H and ^{13}C NMR (−20 °C) spectra is very similar to those of **1** (Table 1). However, signals in the aromatic region indicate that **2** has an ABX system [δ 7.17 (d, H-9), δ 6.63 (dd, H-10), δ 6.61 (d, H-12)] and a methoxy group [δ 3.85 (3H, s)] on the benzene ring, suggesting that **2** has a methoxy group on C-11 position of humantenine-type alkaloid. This was supported by comparison of the NMR data of humanteniridine (**2**) with those of humantenirine (**5**)[13] (Table 1) and NOE experiments, as shown in Figure 3. Taking the molecular formula into consideration at this stage, **2** should have one less oxygen than **1** in the iridoid moiety. Comparison of the ^{1}H and ^{13}C NMR (−20 °C) data of the iridoid moiety with those of **1** (Table 1), particularly of the chemical shift at C-7′, suggested that **2** is the 7′-dehydroxy derivative of **1**. ^{1}H–^{1}H COSY and HMQC analyses revealed the presence of a five sp^3 carbon chain (–CHCHCH$_2$CHCH$_3$, C-5′, 6′, 7′, 8′, 10′) in the iridoid moiety (Fig. 3).

Both the geometry of the ethylidene group at C-19–C-20 and the double bond at C-3′–C-4′ were deduced to have the *Z*-configuration from NOE experiments (Fig. 3). In addition, the relative stereochemistry of the iridoid moiety was analyzed by performing NOE experiments (Fig. 3). The stereochemistry of the quaternary carbon (C-9′) was elucidated from the NOE correlation from H-8′ to H-1′. From these spectroscopic analyses, **2** was deduced to be an 7′-dehydroxy-11-methoxy derivative of **1**. Biogenetically, **2** was considered to originate in humantenirine (**5**) and 7-deoxygelsemide.[14]

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, the Research Foundation for Pharmaceutical Sciences, the Astellas Foundation for Research on Medicinal Resources, and the Takeda Science Foundation.

References and notes

1. Kitajima, M.; Arai, Y.; Takayama, H.; Aimi, N. *Proc. Jpn. Acad., Ser. B* **1998**, *74*, 159–163.
2. Kitajima, M. *J. Nat. Med.* **2007**, *61*, 14–23.
3. Takayama, H.; Sakai, S.. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 1997; Vol. 49, Chapter 1 and references cited therein.
4. Kitajima, M.; Nakamura, T.; Kogure, N.; Ogawa, M.; Mitsuno, Y.; Ono, K.; Yano, S.; Aimi, N.; Takayama, H. *J. Nat. Prod.* **2006**, *69*, 715–718; and *J. Nat. Prod.* **2007**, *70*, 142.
5. (a) Kitajima, M.; Kogure, N.; Yamaguchi, K.; Takayama, H.; Aimi, N. *Org. Lett.* **2003**, *5*, 2075–2078; (b) Kogure, N.; Nishiya, C.; Kitajima, M.; Takayama, H. *Tetrahedron Lett.* **2005**, *46*, 5857–5861; (c) Kitajima, M.; Urano, A.; Kogure, N.; Takayama, H.; Aimi, N. *Chem. Pharm. Bull.* **2003**, *51*, 1211–1214; (d) Kogure, N.; Ishii, N.; Kitajima, M.; Wongseripipatana, S.; Takayama, H. *Org. Lett.* **2006**, *8*, 3085–3088; (e) Kogure, N.; Someya, A.; Urano, A.; Kitajima, M.; Takayama, H. *J. Nat. Med.* **2007**, *61*, 208–212.
6. *Gelsemium rankinii* Small was harvested from the medicinal plant garden of Chiba University, Japan, which was identified by Dr. F. Ikegami, and the voucher specimen (No. 20051201) was deposited at the Faculty of Pharmaceutical Sciences, Chiba University, Japan.
7. *Gelsemium elegans* Benth. was collected in Phu Laung, Loei Province, Thailand, which was identified by Dr. Sumphan Wongseripipatana, and the voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.
8. Rankiniridine (**1**), white amorphous powder, FAB-MS (NBA): *m/z* 553 (MH^+), HR-FAB-MS (NBA/PEG): *m/z* 553.2556 (MH^+, calcd

for $C_{30}H_{37}N_2O_8$, 553.2550), UV (MeOH): λ_{max} nm ($\log\varepsilon$): 296.0 (4.25), 208.5 (4.18), $[\alpha]_D^{21}$ −270.5 (*c* 0.14, MeOH), CD ($c = 0.335$ mmol/L, MeOH, 24 °C) $\Delta\varepsilon$ (nm): 0 (326), −12.9 (287), −1.23 (257), −1.64 (251), 0 (241), +7.45 (226), 0 (219), −16.9 (209).

9. (a) Ponglux, D.; Wongseripipatana, S.; Takayama, H.; Ogata, K.; Aimi, N.; Sakai, S. *Tetrahedron Lett.* **1988**, *29*, 5395–5396; (b) Lin, L. Z.; Cordell, G. A.; Ni, C. Z.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 3199–3202; (c) Xu, Y.-K.; Yang, S.-P.; Liao, S.-G.; Zhang, H.; Lin, L.-P.; Ding, J.; Yue, J.-M. *J. Nat. Prod.* **2006**, *69*, 1347–1350.
10. Yeh, S.; Cordell, G. A. *J. Nat. Prod.* **1986**, *49*, 806–808.
11. Jensen, S. R.; Kirk, O.; Nielsen, B. J.; Norrestam, R. *Phytochemistry* **1987**, *26*, 1725–1731.
12. Humanteniridine (**2**), yellowish amorphous powder, FAB-MS (NBA): *m*/*z* 567 (MH^+), HR-FAB-MS (NBA/PEG): *m*/*z* 567.2722 (MH^+: calcd for $C_{31}H_{39}N_2O_8$, 567.2706), UV (MeOH): λ_{max} nm ($\log\varepsilon$): 293.0 (4.35), 215.0 (4.51), $[\alpha]_D^{16}$ −409.4 (*c* 0.26, MeOH), CD ($c = 0.131$ mmol/L, MeOH, 16 °C) $\Delta\varepsilon$ (nm): 0 (325), −14.6 (294), 0 (246), +13.1 (233), 0 (224), −22.5 (213) 0 (203).
13. Yang, J. S.; Chen, Y. W. *Acta Pharm. Sinica* **1984**, *19*, 686.
14. Takayama, H.; Morohoshi, Y.; Kitajima, M.; Aimi, N.; Wongseripipatana, S.; Ponglux, D.; Sakai, S. *Nat. Prod. Lett.* **1994**, *5*, 15–20.