New Iridoids from *Gelsemium* Species

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Four new iridoids structurally related to gelsemide (5) were isolated from two Loganiaceous plants, *Gelsemium elegans* and *G. rankinii*. Among them, GEIR-1 (1) has a novel tetracyclic caged structure.

Key words *Gelsemium*; iridoid; gelsemide

The genus *Gelsemium*, which belongs to Loganiaceae, comprises three species: *G. elegans* BENTH., *G. rankinii* SMALL, and *G. sempervirens* AIT., from which more than seventy indole alkaloids have been isolated.[1,2] We have proved that the origin of "Yakatsu," one of the ancient medicines stored in the Shosoin repository in Japan, is *G. elegans*.[3] Recently, we found that among the structurally diverse *Gelsemium* alkaloids, some gelsedine-type alkaloids showed cytotoxicity against A431 epidermoid carcinoma cells.[4] In our continuing chemical studies on *Gelsemium* plants,[5—9] we found new iridoids (**1**—**4**) in the leaves of *G. elegans* and *G. rankinii*. In this paper, we describe the structure elucidation of these compounds (Fig. 1).

Results and Discussion

The leaves of *G. elegans* (3575 g dry weight) were extracted with hot MeOH to yield the extract (941.8 g). The MeOH extract was dissolved in H_2O containing a small amount of MeOH and extracted successively with *n*-hexane, AcOEt, 5% MeOH/$CHCl_3$, and *n*-BuOH. The 5% MeOH/$CHCl_3$ extract (6.91 g) was separated by SiO_2 flash column chromatography to afford two new iridoids, GEIR-1 (**1**, 41.2 mg) and GEIR-2 (**3**, 3.6 mg), along with known alkaloids, gelsenicine, 14,15-dihydroxygelsenicine,[5] gelsemoxonine,[5] gelsedilam,[8] 14-acetoxygelsedilam,[8] gelseiridone,[8] and gelsefuranidine.[8] Further, the third new iridoid, GEIR-3 (**4**, 119.7 mg), was isolated from the *n*-BuOH fraction. Using a similar procedure, the fourth new iridoid, GRIR-1 (**2**, 24.4 mg), was isolated from the *n*-BuOH fraction of *G. rankinii* (see Experimental).

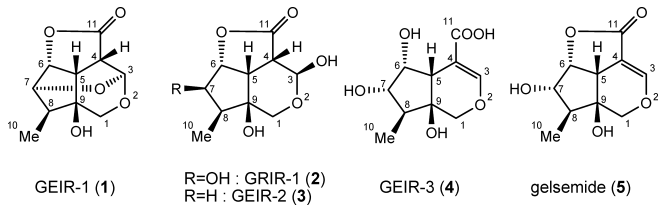

Fig. 1. Structures of New Iridoids (**1**—**4**) and Gelsemide (**5**)

GEIR-1 (**1**) was obtained as colorless prisms (mp 119—120 °C). The HR-FAB-MS spectrum gave a protonated molecular ion peak at *m/z* 213.0763 ($M+H^+$) that corresponded to the molecular formula $C_{10}H_{13}O_5$ (*m/z* 213.0768). The IR spectrum suggested that **1** has a hydroxyl group (3558 cm^{-1}) and a lactone moiety (1766 cm^{-1}). The ^{1}H-NMR spectrum showed characteristic signals for iridoids, such as methyl protons [δ 1.02 (d, H_3-10)] and low-field oxygenated protons [δ 5.23 (dd, H-6), δ 5.16 (br s, H-3)] (Table 1). The ^{13}C-NMR spectrum showed 10 carbons including one carbonyl carbon [δ 176.2 (C-11)] and one acetal carbon [δ 94.1 (C-3)] (Table 1). ^{1}H–^{1}H COSY and HMQC analyses (Fig. 2a) indicated the presence of a seven sp^3 carbon chain (–CHCHCHCHCHCH$_3$, C-3, 4, 5, 6, 7, 8, 10), the terminal methine carbon of which was estimated to be an acetal residue from the chemical shift (δ_C 94.1). HMBC crosspeaks of two protons [δ 5.23 (H-6), δ 2.98 (H-4)] and a carbonyl carbon (δ 176.2) indicated the presence of a γ-lactone (Fig. 2a). In addition, the presence of a quaternary carbon (δ 74.9) and an oxymethylene carbon (δ 64.0) was suggested. HMBC

Table 1. ^{1}H- and ^{13}C-NMR Data of **1**—**4**

	GEIR-1 (**1**)		GRIR-1 (**2**)		GEIR-2 (**3**)		GEIR-3 (**4**)	
	δ_H (400 MHz)	δ_C (100 MHz)	δ_H (500 MHz)	δ_C (125 MHz)	δ_H (600 MHz)	δ_C (150 MHz)	δ_H (400 MHz)	δ_C (125 MHz)
1	3.61 (d, 9.4)	64.0	3.80 (d, 11.9)	58.9	4.01 (d, 11.7)	59.1	3.94 (dd, 11.0, 2.0)	74.1
	3.57 (d, 9.4)		3.29 (d, 11.9)		3.47 (d, 11.7)		3.75 (d, 11.0)	
3	5.16 (br s)	94.1	5.27 (s)	91.0	5.49 (s)	89.7	7.73 (s)	157.0
4	2.98 (overlapped)	44.8	2.90 (d, 11.2)	44.8	2.99 (overlapped)	44.2		107.1
5	2.98 (overlapped)	49.3	3.12 (dd, 11.2, 6.4)	47.2	2.95 (overlapped)	46.7	2.49 (br s)	46.9
6	5.23 (dd, 7.0, 3.6)	83.0	4.70 (br d, 6.4)	87.3	5.02 (dd, 4.4, 4.4)	83.3	4.24 (dd, 4.0, 4.0)	80.3
7	3.88 (m)	79.5	3.95 (br d, 3.7)	79.0	2.08 (2H, m)	38.2	3.70 (dd, 9.3, 4.0)	73.1
8	2.67 (q, 7.9)	49.8	1.82 (qd, 7.0, 3.7)	40.1	1.85 (m)	35.9	1.62 (m)	44.4
9		74.9		76.0		73.3		74.4
10	1.02 (3H, d, 7.9)	12.7	0.96 (3H, d, 7.0)	6.0	0.99 (3H, d, 6.4)	10.1	1.08 (3H, d, 7.1)	12.9
11		176.2		177.3		175.7		174.0

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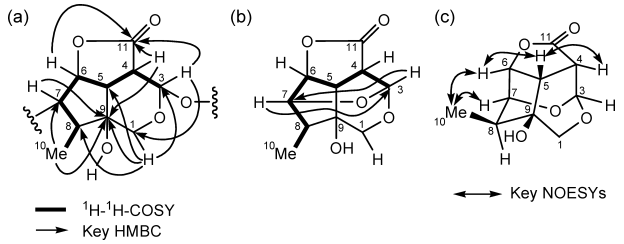

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC and NOESY Correlations of GEIR-1 (**1**)

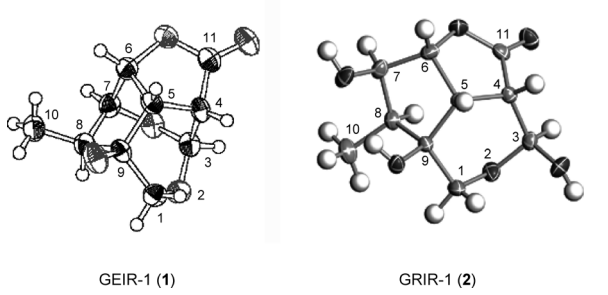

Fig. 3. X-Ray Structures of **1** and **2**

correlations between oxymethylene protons (δ 3.61, 3.57, H_2-1) and an acetal carbon (δ 94.1) indicated that the oxymethylene carbon was attached to one of the acetal oxygens. HMBC correlations between terminal methyl protons (δ 1.02, H_3-10) and H-7 and an oxygenated quaternary carbon (δ 74.9) suggested that the quaternary carbon was attached to C-8. Other cross peaks of three protons (H_2-1, H-4) and the quaternary carbon (δ 74.9) suggested the existence of a 3-oxo-bicyclo[4,3,0]nonane ring (Fig. 2a). HMBC cross-peaks between the protons at δ 3.88 (H-7) and the acetal carbon at δ 94.1 (C-3), and between the protons at δ 5.16 (H-3) and δ 79.5 (C-7) revealed that C-3 and C-7 were connected by an oxygen atom (Fig. 2b). From these analyses, structure **1** having a unique tetracyclic caged structure constructed by an intramolecular acetal function was proposed. NOESY observations illustrated in Fig. 2c supported the stereostructure of **1**.

The structure inferred by spectroscopic analysis above was confirmed by X-ray analysis (Fig. 3).[10] This iridoid was considered to be derived from gelsemide (**5**)[11] by Michael attack from 7-OH to C-3 of α,β-unsaturated lactone. When compound **5** was treated with PTSA in dioxane, iridoid **1** was formed in 12% yield (Chart 1).

GRIR-1 (**2**) isolated from *G. rankinii* was obtained as colorless prisms (mp 165—169 °C). Its ^{1}H- and ^{13}C-NMR spectra were similar to those of GEIR-1 (**1**), of which molecular formula $C_{10}H_{14}O_6$ had one H_2O molecule more than **1**. Coupling constants of the protons at C-6, C-7, and C-8 ($J_{H6–H7}$=*ca.* 0 Hz, $J_{H7–H8}$=3.7 Hz) and NOE between H-7 and H-8 suggested β-orientation of the oxygen function on C-7 (Fig. 4). Taking this finding into account, the molecular formula, and HMBC correlations depicted in Fig. 4, GRIR-1 (**2**) was considered to be an epimer at C-7 of the dissociated form of the intramolecular acetal function of **1**. The structure, including the stereochemistry at C-3 of the hemiacetal function, was finally determined by X-ray crystallographic analysis (Fig. 3).[12]

The molecular formula of new iridoid GEIR-2 (**3**) was es-

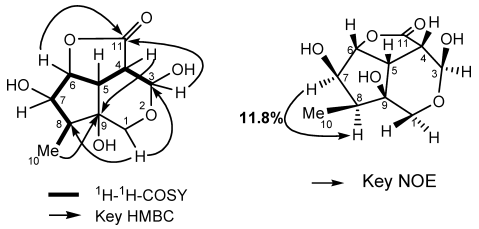

Fig. 4. Key ^{1}H–^{1}H COSY, HMBC and NOE Correlations of GRIR-1 (**2**)

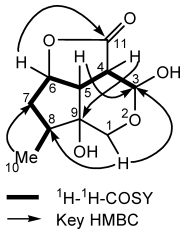

Fig. 5. Key ^{1}H–^{1}H COSY and HMBC Correlations of GEIR-2 (**3**)

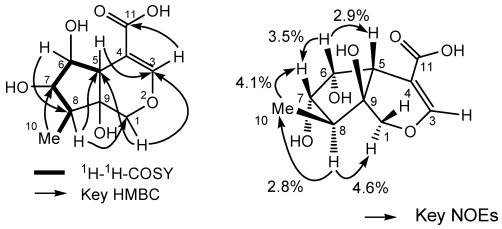

Fig. 6. Key ^{1}H–^{1}H COSY, HMBC and NOE Correlations of GEIR-3 (**4**)

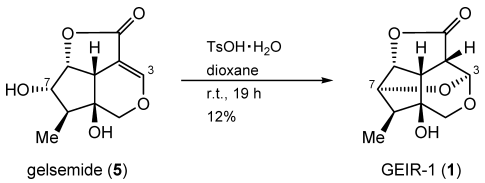

Chart 1. Chemical Conversion of Gelsemide (**5**) to GEIR-1 (**1**)

tablished to be $C_{10}H_{14}O_5$ from HR-FAB-MS (*m/z* 237.0729 $[M+Na]^+$), which possessed one oxygen less than **2**. The ^{1}H-NMR spectrum showed characteristic signals of iridoids, such as methyl protons [δ 0.99 (d, H_3-10)], acetal proton [δ 5.49 (s, H-3)], and low-field oxymethine proton [δ 5.02 (dd, H-6)]. Comparison of the ^{13}C-NMR data of **3** with those of **2** (Table 1) particularly of the chemical shift at C-7, indicated that **3** is the 7-deoxyderivative of **2** (Fig. 5). GEIR-2 (**3**) was previously prepared by the enzymatic hydrolysis of 9-hydroxysemperoside (**6**).[11]

The HR-EI-MS spectrum of GEIR-3 (**4**) gave a protonated molecular ion peak at *m/z* 230.0780 (M^+) that corresponded to the molecular formula $C_{10}H_{14}O_6$ (*m/z* 230.0790). UV spectrum and NMR signals at δ_H 7.73 (H-3), δ_C 157.0 (C-3), δ_C 107.1 (C-4), and δ_C 174.0 (C-11) revealed the existence of a β-alkoxyacrylate residue. The ^{13}C-NMR spectrum showing one carbonyl carbon [δ 174.0 (C-11)] and four oxygenated carbons [δ 80.3 (C-6), δ 74.4 (C-9), δ 74.1 (C-1), δ 73.1 (C-7)], together with HMBC correlations from H-1 to C-3 and C-5, from H-8 to C-1 and C-5, and from H-3 to C-11, suggested that GEIR-3 (**4**) has a gelsemide skeleton (Fig. 6).

Comparing the chemical shift of the proton at C-6 (δ_H 4.24) and the molecular formula of **4** with those of gelsemide (**5**), it was revealed that compound **4** was a hydrolysis derivative of the lactone moiety of **5**. The relative stereochemistry was established by NOE experiments, as shown in Fig. 6.

Experimental

General Procedure ^{1}H- and ^{13}C-NMR spectra: JEOL JNM ECP-600, JEOL JNM A-500 or JNM A-400 at 600, 500 or 400 MHz (^{1}H-NMR) and at 150, 125 or 100 MHz (^{13}C-NMR), respectively. UV: JASCO V-560. IR: JASCO FT/IR-230. FAB-MS: JEOL JMS-AX500 or AX-505. HR-FAB-MS: JEOL JMS-HX110. EI-MS: JEOL GC-mate. Optical rotation: JASCO P-1020. CD: JASCO J-720WI. TLC: Precoated silica gel 60 F_{254} plates (Merck, 0.25 mm thick). Column chromatography: Silica gel 60 (Merck, 70—230 mesh). Flash column chromatography: Silica gel 60N (Kanto Chemical, 40—50 μm). Medium pressure liquid chromatography (MPLC): C. I. G. prepacked column CPS-HS-221-05 (Kusano Kagakukikai, SiO_2). X-ray crystallography: Rigaku AFC-7 and Bruker APEX II.

Plant Material *Gelsemium elegans* Benth. was collected in Phu Laung, Loei Province, Thailand. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. *Gelsemium rankinii* Small was harvested from the medicinal plant garden of Chiba University, Japan. A voucher specimen (No. 20051201) was deposited at the Faculty of Pharmaceutical Sciences, Chiba University, Japan.

Extraction and Isolation of 1—5 The leaves of *G. elegans* Benth. (3575 g dry weight) were extracted with MeOH (20.5 l, once at room temperature and four times under reflux) to give the extract (941.8 g). The MeOH extract (940.8 g) was dissolved in H_2O (3 l×2) containing a small amount of MeOH and extracted with *n*-hexane (1.7 l×3) to give the *n*-hexane extract (95.91 g). The aqueous layer was successively extracted with AcOEt (1.7 l×3), 5% MeOH/$CHCl_3$ (1.7 l×4), and *n*-BuOH (1.7 l×4) to give the AcOEt extract (76.91 g), the 5% MeOH/$CHCl_3$ extract (7.04 g), and the *n*-BuOH extract (278.31 g), respectively. The 5% MeOH–$CHCl_3$ extract (6.91 g) was separated by SiO_2 flash column chromatography with $CHCl_3$/MeOH gradient to give 6 fractions: fr. A 0—3% MeOH/$CHCl_3$ (48.4 mg); fr. B 3—5% MeOH/$CHCl_3$ (180.4 mg); fr. C 5—10% MeOH/$CHCl_3$ (1648 mg); fr. D 10—20% MeOH/$CHCl_3$ (2037 mg); fr. E 20—50% MeOH/$CHCl_3$ (1059 mg); and fr. F 50% MeOH/$CHCl_3$ and MeOH (1572 mg). Fr. C was purified successively by SiO_2 flash column chromatography (MeOH/$CHCl_3$ gradient or MeOH/AcOEt/$CHCl_3$ gradient), MPLC (3% MeOH/$CHCl_3$), and MPLC (50% AcOEt/$CHCl_3$) to afford GEIR-1 (**1**, 41.2 mg). Fr. D was separated by SiO_2 flash column chromatography with MeOH/$CHCl_3$ gradient to give 5 fractions: fr. DA 5% MeOH/$CHCl_3$ (174.6 mg); fr. DB 5% MeOH/$CHCl_3$ (740.9 mg); fr. DC 5% MeOH/$CHCl_3$ (618.9 mg); fr. DD 5—10% MeOH/$CHCl_3$ (439.3 mg); and fr. DE MeOH (91.8 mg). Fr. DC was further purified by SiO_2 flash column chromatography (MeOH/AcOEt gradient), SiO_2 flash column chromatography (MeOH/$CHCl_3$ gradient), and MPLC (5% MeOH/$CHCl_3$ and 50% AcOEt/hexane) to afford GEIR-2 (**3**, 3.6 mg). The *n*-BuOH extract (18.93 g) was separated on a Sephadex LH-20 column with H_2O/MeOH gradient to give 23 fractions. The fraction that was eluted with MeOH (6993 mg) was subjected to SiO_2 flash column chromatography (MeOH/AcOEt gradient or MeOH/$CHCl_3$ gradient) and purified several times to afford GEIR-3 (**4**, 119.7 mg).

The aerial part of *G. rankinii* Small (1144 g dry weight) was extracted with MeOH (1.8 l, twice at room temperature and four times under reflux) to give the extract (232.7 g). The MeOH extract was dissolved in H_2O (0.5 l×2) containing a small amount of MeOH and extracted with *n*-hexane (0.4 l×3) to give the *n*-hexane extract (29.08 g). The aqueous layer was successively extracted with AcOEt (0.6 l, 0.5 l×2), 5% MeOH/$CHCl_3$ (0.6 l, 0.5 l×2), and *n*-BuOH (0.6 l, 0.5 l×2) to give the AcOEt extract (14.41 g), the 5% MeOH/$CHCl_3$ extract (8.92 g), and the *n*-BuOH extract (40.01 g), respectively. The *n*-BuOH extract (40.01 g) was separated on a Sephadex LH-20 column with H_2O/MeOH gradient to give 8 fractions. The fraction that was eluted with H_2O (7749 mg) was purified by SiO_2 flash column chromatography (MeOH/$CHCl_3$ and AcOEt/*n*-hexane gradient) to afford GRIR-1 (**2**, 24.4 mg).

GEIR-1 (**1**): Colorless prisms, mp 119—120 °C ($CHCl_3$). FAB-MS (NBA) *m/z*: 213 ($M+H^+$). HR-FAB-MS (NBA/PEG) *m/z*: 213.0763 ($M+H^+$, Calcd for $C_{10}H_{13}O_5$: 213.0768). ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3558, 3426, 1766. $[\alpha]_D^{24}$ +41.9° (c=1.02, MeOH). CD (c=0.450 mmol/l, MeOH, 24 °C) $\Delta\varepsilon$ (nm): 0 (248), −0.34 (215). *Anal.* Calcd for $C_{10}H_{12}O_5$: C, 56.6; H, 5.7; O, 37.7. Found: C, 56.8; H, 5.8; O, 37.5.

GRIR-1 (**2**): Colorless prisms, mp 165—169 °C (AcOEt). EI-MS *m/z*: 212 ($M-H_2O^+$). HR-EI-MS *m/z*: 212.0668 ($M-H_2O^+$, Calcd for $C_{10}H_{12}O_5$: 212.0684). ^{1}H- and ^{13}C-NMR: see Table 1. $[\alpha]_D^{24}$ +24.7° (c=0.017, MeOH).

GEIR-2 (**3**): White amorphous powder, FAB-MS (NBA+NaCl) *m/z*: 237 ($M+Na^+$), HR-FAB-MS (Gly+NaCl+H_2O/PEG) *m/z*: 237.0729 ($M+Na^+$, Calcd for $C_{10}H_{14}O_5Na$: 237.0739). ^{1}H- and ^{13}C-NMR: see Table 1. $[\alpha]_D^{24}$ +5.7° (c=0.16, MeOH).

GEIR-3 (**4**): Yellowish amorphous powder, EI-MS *m/z*: 230 (M^+), 194, 166, 153. HR-EI-MS *m/z*: 231.0780 (M^+, Calcd for $C_{10}H_{14}O_6$: 230.0790). UV λ_{max} (MeOH) nm (log ε): 236.5 (4.03). ^{1}H- and ^{13}C-NMR: see Table 1. $[\alpha]_D^{21}$ −100.1° (c=1.21, MeOH). CD (c=0.202 mmol/l, MeOH, 16 °C) $\Delta\varepsilon$ (nm): 0 (265), −7.91 (231), 0 (207), +1.37 (203).

Chemical Conversion of Gelsemide (5) to GEIR-1 (1) To a stirred solution of gelsemide (**5**, 5.0 mg, 0.024 mmol) in 1,4-dioxane (0.6 ml), *p*-toluenesulfonic acid monohydrate (50.0 mg, 0.263 mmol) was added and the mixture was stirred at room temperature under Ar. After 19 h, the reaction mixture was quenched with 5% aq. $NaHCO_3$ and extracted with 10% MeOH/$CHCl_3$. Then, the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by SiO_2 column chromatography (2% MeOH/$CHCl_3$) to afford **1** (0.6 mg, 12%), which was identical with the natural product in all respects.

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10) X-ray crystallographic analysis of **1**. All measurements were carried out on a Rigaku AFC7S diffractometer with graphite monochromated Cu$K\alpha$ radiation. Crystal data: orthorhombic, $C_{10}H_{12}O_5$ (Mw: 212.2), space group $P2_12_12_1$ with a=9.231(1) Å, b=11.200(2) Å, c=8.910(2) Å, V=921.3(3) Å^3, Z=4, and D_{calc}=1.53 g/cm^3. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 979 reflections ($I>3.00\sigma(I)$, $2\theta<137.88$) and 138 variable parameters and converged with unweighted and weighted agreement factors of R=0.055, R_w=0.092.
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12) X-ray crystallographic analysis of **2**. All measurements were carried out on a Bruker APEX II with graphite monochromated Mo$K\alpha$ radiation. Crystal data: orthorhombic, $C_{10}H_{14}O_6$ (Mw: 230.2), space group $P2_12_12_1$ with a=6.3717(4) Å, b=10.9746(7) Å, c=14.6919(9) Å, V=1027.36(11) Å^3, Z=4, and D_{calc}=1.488 g/cm^3. The structure was solved by direct methods (SHELEX97) and expanded using Fourier techniques (DIRDIF94). Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 2366 reflections and 150 variable parameters and converged with unweighted and weighted agreement factors of R=0.028, R_w=0.069.