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Antinociceptive substances from *Incarvillea delavayi*

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Abstract

Antinociceptive activities of an *Incarvillea delavayi* extract, as well as its constituents, 8-epideoxyloganic acid and delavayine A, were evaluated in the acetic acid induced writhing test in mice. An oral administration of the *delavayi* extract weakly decreased the number of writhings and stretchings in this test, in a dose-dependent manner. Furthermore, orally administered 8-epideoxyloganic acid showed weak antinociceptive activity, whereas administration by subcutaneous injection did not. However, subcutaneous injection of delavayine A, a novel monoterpene alkaloid, showed a more significant level of antinociceptive activity.

Keywords: *Incarvillea delavayi*; Bignoniaceae; Monoterpene alkaloid; Iridoid; Antinociception

1. Introduction

We have previously reported various novel monoterpene alkaloid derivatives obtained from *Incarvillea sinensis* which display significant antinociception and anti-inflammatory effects (Chi, Hashimoto, Yan & Nohara, 1995a, 1995b; Chi, Hashimoto, Yan, Nohara, Yamashita & Marubayashi, 1997a; Chi, Hashimoto, Yan & Nohara, 1997b; Chi et al., 1997c; Nakamura et al., 1999). However, *Incarvillea delavayi*, which is used horticulturally in China, has not been chemically and pharmacologically investigated. As part of our continuing study of *Incarvillea* spp., we now report the structural elucidation and antinociceptive activity of a new monoterpene alkaloid and a known iridoid obtained from *I. delavayi*.

2. Results and discussion

The aerial parts of *I. delavayi* were extracted with MeOH, and the extract was partitioned with 80% MeOH and *n*-hexane. The 80% MeOH-soluble fraction was subjected to Diaion HP-20, Sephadex LH20 and silica gel column chromatography, respectively, to yield **1** as a major component and **2** as a minor component.

Compound **1** was identified as 8-epideoxyloganic acid by the aid of ^{1}H–^{1}H, ^{1}H–^{13}C COSY and HMBC spectra, and by comparison of its NMR spectral data with those of an authentic sample (Uesato, Miyauchi, Itoh & Inouye, 1986).

Compound **2**, delavayine A, showed a $[M]^{+}$ ion peak at m/z 302 in the positive FABMS, and its molecular formula was established as $C_{19}H_{28}NO_2$ by high resolution mass spectros copy. The ^{1}H-NMR spectrum of **2** contained signals for three methyl groups (δ 0.88, δ 3.50 and δ 3.83) and three methine groups (δ 8.25, δ 7.50 and δ 7.56) linked to the aromatic ring of benzoic acid. The ^{13}C-NMR spectrum of **2** revealed two quaternary carbons (δ 130.7, C-1′ and δ 166.6, C-7′) linked to benzoic acid and 12 signals belonging to a monoterpene alkaloid unit; suggesting that benzoic acid was attached to the hydroxy group at C-11 of the monoterpene alkaloid. This was confirmed by analysis of the ^{1}H–^{1}H COSY, HMBC and NOESY spectra (Table 1, Fig. 1). Therefore, the structure of compound **2** was established as shown in the formula, although

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Table 1
Spectral data for delavayine A (**2**) (in pyridine-d_5, 500.00 MHz for δ_H, 125.65 MHz for δ_C, TMS)

C/H	δ_C	δ_H	^1H–^1H COSY correlations	HMBC correlations (bond connectivity)
1 a		3.70 *m*	H-1b, H-9	
	60.8			
1 b		3.86 *m*	H-1a, H-9	
N–Me a	47.2	3.50 *s*		C-1 (3), C-3 (3), N–Me b (3)
N–Me b	56.2	3.83 *s*		C-1 (3), C-3 (3), N–Me a (3)
3 a		3.62 *m*	H-3b, H-4	
	62.5			
3 b		3.70 *m*	H-3a, H-4	
4	26.4	2.43 *m*	H-10, H-3a, H-3b	
5	38.7	2.10 *m*	H-6a, H-9	
6 a		1.53 *m*	H-5, H-6b, H-7a, H-7b	
	22.7			
6 b		2.10 *m*	H-6a, H-7a	
7 a		1.32 *m*	H-6a, H-6b, H-7b	
	26.0			
7 b		2.14 *m*	H-6a, H-7a, H-8	
8	41.5	2.20 *m*	H-7b, H-11	
9	37.6	2.56 *m*	H-1a, H-1b, H-5	
10	16.3	0.88 *d* (7.0)[a]	H-4	C-3 (3), C-4 (2), C-5 (3)
11	67.9	4.22 *m*	H-8	C-7 (3), C-9 (3), C-7′ (3)
1′	130.7			
2′, 6′	130.0	8.25 *d* (7.0)[a]	H-3′, 5′	C-4′ (3), C-7′ (3)
3′, 5′	129.1	7.50 *t* (7.0)[a]	H-2′, 6′, H-4′	C-1′ (3)
4′	133.4	7.56 *t* (7.0)[a]	H-3′, 5′	C-2′, 6′ (3)
7′	166.6			

[a] Values in parentheses are coupling constants (J) in Hz.

the absolute configurations at carbons 4, 5, 8, and 9 were not determined. This compound named delavayine A is a novel monoterpene alkaloid comprised of a benzoate ester.

The antinociceptive activities of 8-epideoxyloganic acid (**1**) and delavayine A (**2**), respectively, were evaluated by observing the writhing behavior of mice. A suspension of **1** or **2** was administered to mice, prior to intraperitoneal injection of 500 μl 1.0% acetic acid solution in saline, and the writhing behavior of their pain reaction was measured. Aminopyrine was used as a positive control treatment

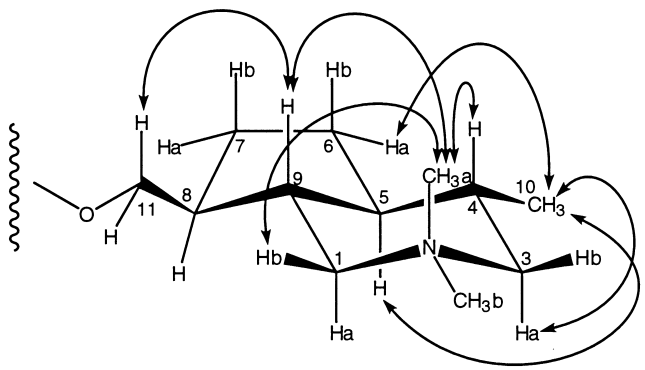


Fig. 1. NOESY correlations of delavayine A.

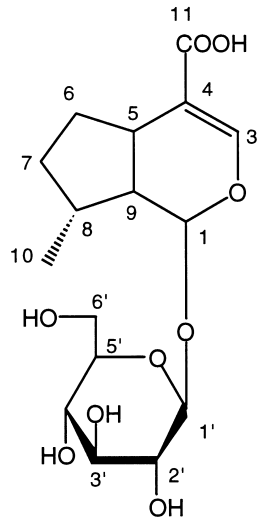
Oral administration of the *I. delavayi* extract attenuated writhing behavior in a dose-dependent manner. Further, the major component 8-epideoxyloganic acid (**1**) showed stronger activity at 100 mg/kg. Subcutaneous injection of 8-epideoxyloganic acid (**1**) did not, however, show any antinociceptive activity at any dose (Table 2). These results suggest the possibility that its action was due to metabolism of compound **1**. The antinociceptive effects of iridoid compounds including geniposidic acid have been previously reported (Okuyama, Fujimori, Yamazaki & Deyama, 1998), and the present study suggests that iridoids may become new lead compounds for antinociceptive drug development.

Subcutaneous injection of delavayine A (**2**) (50 mg/kg) significantly reduced writhing behavior (Table 2). We have already reported the potent antinociceptive and anti-inflammatory actions of similar monoterpene alkaloids, such as incarvillateine (Chi et al., 1997a, 1997b, 1997c; Nakamura et al., 1999). Therefore, monoterpene alkaloids seemed to be active as both antinociceptive and anti-inflammatory principles. Further investigation is required to elucidate the exact mechanisms underlying these effects.

Table 2
Inhibitory effect of *I. delavayi* extract, 8-epideoxyloganic acid and delavayine A on acetic acid-induced writhing in mice[a]

Drug	Dose (mg/kg)	Administration	No. of writhings (counts/20 min)	% Inhibition
2.5% Tween 80/saline	–	*p.o*	31.3 ± 4.3	–
	–	*s.c.*	32.0 ± 2.7	–
I. delavayi ext.	200	*p.o.*	31.0 ± 1.4	1
	400	*p.o.*	20.5 ± 2.9[b]	35
8-Epideoxyliganic acid (**1**)	50	*p.o.*	33.5 ± 7.2	−5
	100	*p.o.*	16.0 ± 2.0[b]	49
	50	*s.c.*	32.0 ± 3.2	0
	100	*s.c.*	30.0 ± 7.9	6
Delavayine A (**2**)	50	*s.c.*	17.6 ± 3.4[b]	45
Aminopyrine (positive control)	50	*p.o.*	4.0 ± 2.9[b]	87
	50	*s.c.*	1.8 ± 2.1[b]	94

[a] Each value is the mean of five mice with S.E.M. % inhibition produced by the *p.o.* of *I. delavayi* ext. 8-eoideoxyliganic acid and Aminopyrine was calculated with respect to *p.o.* of 2.5% Tween 80/saline, while *s.c.* of 8-eoideoxyliganic acid, delavayine A and aminopyrine with respect to *s.c.* of 2.5% Tween 80/saline.
[b] $p < 0.01$ compared with 2.5% Tween 80/saline group (Dunnett's test).



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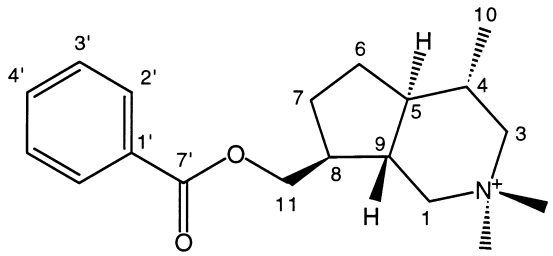

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(relative configuration)

3. Experimental

3.1. General

^{1}H- and ^{13}C-NMR, DEPT, ^{1}H–^{1}H COSY and HMBC spectra were recorded on a JEOL JNM-GX-400 and α-500 in C_5D_5N. TMS was used as international standard. FABMS (negative ion mode): 2–3 kV. HR-FABMS: JEOL DX-303HF. CC: silica gel 60 (spherical, 40–100 mesh, Kanto Chemicals), Chromatorex Chromatography Silica gel (DM-1020, 100–200 mesh, FUJI Silylia), Sephadex LH-20 (Pharmacia), Diaion HP-20 (Mitsubishi Chemical). TLC: silica gel 60 F_{254} (0.2 mm, Merck); visualized under UV light (254 and 366 nm). TLC solvent system: $CHCl_3$–MeOH–H_2O (7: 3: 0.5).

3.2. Plant material

The bulbs of *Incarvillea delavayi* (Bignoniaceae) were purchased from Heiwa-en, Japan, with the plants then grown in our garden (Botany section of Faculty of Pharmaceutical Sciences, Kumamoto University).

3.3. Extraction and separation

Dried aerial parts of *delavayi* (1.5 kg) were exhaustively extracted with MeOH. The MeOH extract was concentrated under reduced pressure to afford a syrup (yield 128 g), which was partitioned between 80% MeOH and *n*-hexane. The 80% MeOH-sol. fraction was subjected to various chromatographic steps, including Diaion HP-20 column chromatography, using H_2O–MeOH (10:0 → 0:10) as eluant, subjected to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (30:1:0 → 6: 4: 1) as eluant, chromatorex column chromatography with $CHCl_3$–MeOH–NH_3 (8:

2: 0.2 → 6: 4: 1) as eluant and Sephadex LH-20 column chromatography with MeOH as eluant to afford compounds **1** (18 mg) and **2** (11 mg), respectively.

3.4. 8-Epideoxyloganic acid (*1*)

Yellow powder, $[\alpha]_D^{23} -22.5^\circ$ ($c = 0.50$, C_5H_5N); Positive ion FABMS m/z: 361 $[M+H]^+$, Negative ion FABMS m/z : 359 $[M–H]^-$; ^{1}H-NMR (pyridine d_5); δ 1.10 (3H, *d*, $J = 7.0$ Hz, 10-H), 1.34 (1H, *m*, 7-H*a*), 1.65 (1H, *m*, 7-H*b*), 1.84 (1H, *m*, 6-H*a*), 2.08 (1H, *m*, 8-H), 2.20 (1H, *m*, 6-H*b*), 2.39 (1H, *dd*, $J = 7.0$, 8.0 Hz, 9-H), 3.24 (1H, *dd*, $J = 7.0$, 8.0 Hz, 5-H), 4.02 (1H, *brs*, glc 5′-H), 4.08 (1H, *t*, $J = 8.0$ Hz, glc 2′-H), 4.26 (1H, *m*, glc 4′-H), 4.28 (1H, *m*, glc 3′-H), 4.40 (1H, *m*, glc 6′-H*a*), 4.58 (1H, *m*, glc 6′-H*b*), 5.42 (1H, *d*, $J = 8.0$ Hz, glc 1′-H), 5.76 (1H, *d*, $J = 5.0$ Hz, 1-H), 7.92 (1H, *s*, 3-H); ^{13}C-NMR spectral data (pyridine-d_5); δ 16.5 (10′-Me), 31.8 (C-6), 32.6 (C-7), 34.0 (C-5), 36.5 (C-8), 43.6 (C-9), 62.8 (glc-6′), 71.6 (glc-4′), 74.9 (glc-2′), 78.5 (glc-3′), 78.8 (glc-5′), 95.5 (C-1), 100.5 (glc-1′), 113.5 (C-4), 151.3 (C-3), 169.6 (COO).

3.5. Delavayine A (*2*)

Yellow powder, $[\alpha]_D^{22} -5.1^\circ$ ($c = 0.90$, C_5H_5N); Positive FABMS m/z: 302$[M]^+$, Negative ion FABMS m/z: 372 $[M+2Cl]^-$. HR-FABMS m/z: 302.2119$[M]^+$ (calcd. for $C_{19}H_{28}NO_2$: 302.2120). ^{1}H-NMR and ^{13}C-NMR spectral data (pyridine-d_5); see Table 1.

3.6. Animals

Male ICR mice (Charles Liver) weighing 25–35 g were used in this study. Prior to experiments, animals were housed for at least one week in the laboratory animal room. Housing conditions were maintained at $23 \pm 1^\circ$C with 60% humidity, in 12: 12 h light–dark cycle. Food and water were given ad libitum.

3.7. Writhing test and treatments

A modified Whittle's method was used (Whittle, 1964). The tested drugs were prepared as suspensions with 2.5% Tween 80/saline. Oral administration; MeOH extract (200, 400 mg/kg), 8-Epideoxyloganic acid (**1**) (50, 100 mg/kg), aminopyrine (reference drug, 50 mg/kg) and 2.5% Tween 80/saline (vehicle) were administered 20 min prior to the injection of an inducer (1% acetic acid/saline, 500 ml, *i. p.*). Subcutaneous administration; 8-epideoxyloganic acid (**1**) (50, 100 mg/kg), Delavayine A (**2**) (50 mg/kg), aminopyrine (50 mg/kg) and 2.5% Tween 80/saline (vehicle) were administered 10 min prior to injection of inducer. The number of writhings and stretchings was counted over 20 min, beginning 5 min after introduction of the inducer by injection. The percentage of inhibition was determined for each experimental group of five mice as follows

$$\text{inhibition}\ (\%) = 100 \times (1 - \text{experimental/control})$$

3.8. Statistical analysis

The data are shown as mean $\pm$ SE ($n = 5$). The Dunnett's test was employed to determine the significance of difference between control and experimental samples.

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