Three New Compounds from *Incarvillea delavayi*

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Phytochemical studies on the whole plant of *Incarvillea delavayi* resulted in the isolation of three new compounds, incarvilleaol (**1**), incarvillaldehyde (**2**) and 2-(1,4-dihydroxycyclohexyl)ethyl caffeate (**3**). Their structures were defined on the basis of UV, IR, 1D- and 2D-NMR (^{1}H,^{1}H-COSY, DEPT, HSQC, HMBC), as well as HR-ESI-MS analyses.

Introduction. – *Incarvillea delavayi*, a member of the genus *Incarvillea* (Bignoniaceae), is mainly distributed in Yunnan and Sichuan Provinces of P. R. China [1]. It is often treated as ornamental plant because of its beautiful flowers [2]. Recently, the *Incarvillea* species have been found to be a rich source of acridine-type alkaloids [3–6], and possess significant antinociceptive activity. In previous investigations of this plant, three monoterpene alkaloids and two iridoids [7–9] have been reported. In this work we describe the isolation and structural characterization of three new compounds, incarvilleaol (**1**), incarvillaldehyde (**2**) and 2-(1,4-dihydroxycyclohexyl)ethyl caffeate (**3**).

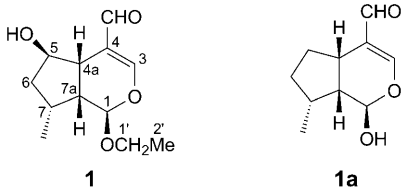
Results and Discussion. – Compound **1** was obtained as a colorless oil. The IR spectrum showed absorption bands due to a OH group (3312 cm^{-1}), Me groups (2958, 2874 cm^{-1}), and C=C bonds (1651 cm^{-1}). The molecular formula was deduced as $C_{12}H_{18}O_4$ by ESI-MS (positive-ion mode; *m/z* 249, $[M+Na]^+$) as well as by the ^{1}H- and ^{13}C-NMR data (*Table 1*), and further confirmed by HR-ESI-MS (positive-ion mode; $[M+Na]^+$ at *m/z* 249.1105).

The ^{1}H-NMR spectrum of **1** exhibited signals of an aldehyde H-atom at δ(H) 9.34 (*s*), of an olefinic H-atom at δ(H) 7.21 (*s*), of an O-bearing H-atom at δ(H) 5.18 (*d*, $J=3.0$) correlated with the C-atom at δ(C) 100.1 in the HSQC experiment, and of two Me groups at δ(H) 1.19 (*t*, $J=7.2$) and 1.05 (*d*, $J=7.2$). The ^{13}C-NMR spectrum showed resonances of twelve C-atoms, including two Me groups (δ(C) 15.1, 14.7), two CH_2 groups (δ(C) 64.8, 41.3), six CH groups (δ(C) 160.8, 100.1, 79.0, 43.6, 43.5, and 34.7), a CHO group (δ(C) 192.7), and a quaternary C-atom signal (δ(C) 123.7). The above evidences suggested that **1** possessed an iridoid skeleton [10].

Table 1. *^{1}H- and ^{13}C-NMR Spectral Data* (600 and 150 MHz, resp.; in $CDCl_3$) *of* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)	Key HMBC (H → C)	NOESY (H ↔ H)
H–C(1)	5.18 (*d*, *J* = 3.0)	100.1	C(3), C(4a), C(7a), C(1′)	H–C(7a), H_a–C(1′), H–C(2′)
H–C(3)	7.21 (*s*)	160.8	C(1), C(4), C(4a), C(9)	
C(4)		123.7		
H–C(4a)	2.64–2.69 (*m*)	43.5	C(1), C(3), C(4), C(5), C(6), C(7a), C(9), C(7a), C(9)	H–C(6), H–C(7a)
H–C(5)	4.17–4.22 (*m*)	79.0	C(4)	H–C(6)
CH_2(6)	1.81–1.87 (*m*)	41.3	C(4a), C(5), C(7), C(7a), C(8)	H–C(4a), H–C(7), H–C(5)
H–C(7)	2.64–2.69 (*m*, overlapped)	34.7	C(1), C(4a), C(5), C(6), C(7a), C(8)	H–C(7a)
H–C(7a)	2.25–2.29 (*m*)	43.6	C(5), C(6)	H–C(1), H–C(4a), H–C(7a)
Me–C(7)	1.05 (*d*, *J* = 7.2)	14.7	C(6), C(7), C(7a)	H–C(1), H–C(6), H–C(7)
CHO	9.34 (*s*)	192.7	C(3), C(4), C(4a)	
CH_2(1′)	3.53–3.58 (*m*, H_a)	64.8	C(1), C(2′)	H_b–C(1′)
	3.76–3.81 (*m*, H_b)		C(2′)	H_a–C(1′)
Me(2′)	1.19 (*t*, *J* = 7.2)	15.1	C(1′)	

The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of a known iridoid, **1a** (1,4a,5,6,7,7a-hexahydro-1-hydroxy-7-methylcyclopenta[*c*]pyran-4-carbaldehyde) [10] (see *Fig. 1*), except for the signals of C(4a), C(5), C(6), and C(1). Comparison of the ^{13}C-NMR spectra of **1** and **1a** showed that C(4a), C(5), and C(6) of compound **1** were shifted downfield by 11, 47.3, and 10.9 ppm, respectively, which implied that a OH group was attached to C(5) of **1**.



1 **1a**

Fig. 1. *Structures of compounds* **1** *and* **1a**

In the HMBC spectrum of **1**, the long-range correlations between the aldehyde H-atom with C(3) (δ(C) 160.8), C(4) (δ(C) 123.7), and C(4a) (δ(C) 43.5) suggested that the aldehyde was substituted at C(4). The HMBC cross-peak of H–C(1) with C(1′) (δ(C) 64.8) indicated that an EtO group was located at C(1). Moreover, the long-range correlations from *doublet* Me signal (δ(H) 1.05) to C(6) (δ(C) 41.3), C(7) (δ(C) 34.7), and C(7a) (δ(C) 43.6) located the Me group at C(7). Considering the downfield shift of H–C(5) at δ(H) 4.17–4.22, the OH group was positioned at C(5). The inference was confirmed by the long-range correlation between H–C(5) and C(4) in the HMBC spectrum.

Biogenetically, HO–C(1), H–C(4a), and H–C(7a) of the iridoid skeleton are in β-orientation [9][10]. The NOESY cross-peak between *Me*–C(7) and H–C(1) revealed that H–C(7) was in β-orientation. The configuration of HO–C(5) was determined to be β on the basis of γ-gauche effect (the γ-C-atom of **1** shifted downfield by 1.7 ppm). Therefore, the structure of compound **1** was established as shown in *Fig. 1*, and named incarvilleaol.

Compound **2** was obtained as an orange amorphous powder. The molecular formula was deduced as $C_{11}H_{12}O_2$ by HR-ESI-MS (negative-ion mode) ($[M-H]^-$ at m/z 175.0760). The IR spectrum of **2** showed absorption bands of a OH group (3276 cm^{-1}), a Me group (2954 cm^{-1}), a CHO group (2768, 2728, 1734 cm^{-1}), and a Ph moiety (1608, 1509 cm^{-1}).

The ^{1}H-NMR spectrum of **2** (see *Table 2*) exhibited a Me signal at δ(H) 1.18 (*d*, J = 8.4), a CHO H-atom at δ(H) 10.22 (*s*), and two aromatic H-atoms at δ(H) 7.55 (*d*, J = 1.8), 7.26 (*d*, J = 1.8). The ^{13}C-MNR spectrum of **2** displayed resonances of eleven C-atoms, including a CHO group at δ(C) 192.6, and six aromatic C-atom signals at δ(C) 158.2 (*s*), 152.7 (*s*), 136.8 (*s*), 133.4 (*s*), 117.6 (*d*), and 115.1 (*d*), which indicated the presence of a tetrasubstituted benzene ring, and that the CHO group was attached to the benzene ring.

Table 2. *^{1}H- and ^{13}C-NMR Spectral Data* (600 and 150 MHz, resp.; in C_5D_5N) *of* **2**. δ in ppm, J in Hz.

	δ(H)	δ(C)	^{1}H,^{1}H-COSY (H ↔ H)	Key HMBC (H → C)
H–C(1)	2.94–3.02 (*m*)	38.9	Me–C(1)	Me–C(1), C(2), C(3a), C(7a)
CH_2(2)	2.16–2.22 (*m*, H_a)	35.0	H_b–C(2), H–C(3)	C(3a), C(7a)
	1.46–1.52 (*m*, H_b)		H_a–C(2), H–C(3)	Me–C(1), C(1), C(3)
CH_2(3)	2.94–3.02 (*m*, H_a)	29.4	H–C(2), H_b–C(3)	C(2), C(3a), C(7a)
	3.23–3.28 (*m*, H_b)		H–C(2), H_a–C(3)	C(1), C(3a), C(7a)
C(3a)		136.8		
C(4)		133.4		
H–C(5)	7.55 (*d*, J = 1.8)	115.1		C(3a), C(6), C(7), CHO
C(6)		158.5		
H–C(7)	7.26 (*d*, J = 1.8)	117.6		C(1), C(3a), C(5), C(6)
C(7a)		152.7		
CHO	10.22 (*s*)	192.6		C(3a), C(4), C(5)
Me	1.18 (*d*, J = 8.4)	20.0	H–C(1)	C(1), C(2), C(7a)

In the ^{1}H,^{1}H-COSY spectrum, the cross-peaks of CH_2(2) (δ(H) 2.16–2.22, 1.46–1.52) with H–C(1) (δ(H) 2.94–3.02) and CH_2(3) (δ(H) 3.23–3.28, 2.94–3.02) indicated that there was a fragment of C(3)–C(2)–C(1). In the HMBC experiment, the long-rang correlations of H–C(1), CH_2(2), and CH_2(3) with δ(C) 136.8 (C(3a)) and 152.7 (C(7a)) implied that C(1) and C(3) were attached to the benzene ring at C(3a) and C(7a), respectively, and formed a five-membered ring (C(1), C(2), C(3), C(3a), C(7a)). In the HMBC spectrum of **2**, the cross-peaks of the Me group (δ(H) 1.18) with δ(C) 35.0 (C(2)), 38.9 (C(1)), and 152.7 (C(7a)) suggested that the Me group was attached to C(1), while the correlations from the CHO H-atom at δ(H) 10.22 to C(5), C(4), and C(3a) indicated that CHO was positioned at C(4). The HMBC cross-peaks of H–C(7) (δ(H) 7.26) with the aromatic C-atoms C(5) and C(3a) inferred the

presence of HO–C(6), which was further confirmed by the coupling constant of H–C(5) (δ(H) 7.55) with H–C(7) (δ(H) 7.26, $J = 1.8$).

The structure of **2** was then elucidated as shown in *Fig. 2*, and named incarvillaldehyde.

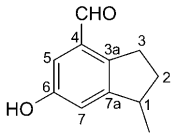


Fig. 2. *Structure of compound* **2**

Compound **3** was obtained as a yellow oil. The molecular formula was deduced as $C_{17}H_{22}O_6$ by HR-ESI-MS (negative-ion mode) ($[M-H]^-$ at m/z 321.1342) and by analysis of the NMR data. The IR spectrum exhibited absorption bands due to a OH group (3364 cm^{-1}), a Ph moiety (1607 and 1510 cm^{-1}), and an ester CO group (1745, 1250, and 1183 cm^{-1}).

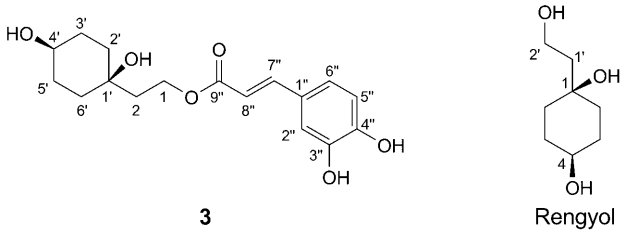
The caffeoyl moiety was determined by the ^{1}H- and ^{13}C-NMR signals (see *Table 3*) due to two olefinic H-atoms at δ(H) 7.52 (d, $J = 15.9$) and 6.24 (d, $J = 15.9$), three aromatic H-atoms at δ(H) 7.02 (d, $J = 1.8$), 6.93 (dd, $J = 1.8, 8.2$), and 6.77 (d, $J = 8.2$), one CO group at δ(C) 169.6, five CH groups at δ(C) 147.1, 123.2, 116.8, 115.6, and 115.4, and three quaternary C-atoms (δ(C) 149.9, 147.1, and 128.0).

Table 3. *^{1}H- and ^{13}C-NMR Spectral Data* (400 and 100 MHz, resp.; CD_3OD) *of* **3**. δ in ppm, J in Hz.

	δ(H)	δ(C)	Key HMBC (H → C)
CH_2(1)	4.32 (t, $J = 7.0$)	62.3	C(2), C(1′), C(9″)
CH_2(2)	1.83 (t, $J = 7.0$)	42.7	
C(1′)		70.5	
CH_2(2′)	1.42–1.49 (m, H_{ax})	36.4	C(4′)
	1.65–1.76 (m, H_{eq})		C(4′)
CH_2(3′)	1.65–1.76 (m, overlapped)	31.6	C(1′)
H–C(4′)	3.52–3.58 (m)	70.9	C(2′), C(6′)
CH_2(5′)	1.65–1.76 (m, overlapped)	31.6	C(1′)
CH_2(6′)	1.42–1.49 (m, H_{ax})	36.4	C(4′)
	1.65–1.76 (m, H_{eq})		C(4′)
C(1″)		128.0	
H–C(2″)	7.02 (d, $J = 1.8$)	115.4	C(4″), C (6″)
C(3″)		147.1	
C(4″)		149.9	
H–C(5″)	6.77 (d, $J = 8.2$)	116.8	C(1″), C(3″)
H–C(6″)	6.93 (dd, $J = 1.8, 8.2$)	123.2	C(4″)
H–C(7″)	7.52 (d, $J = 15.9$)	147.1	C(9″)
H–C(8″)	6.24 (d, $J = 15.9$)	115.6	C(1″)
C(9″)		169.6	

The other ^{1}H- and ^{13}C-NMR data of **3** was similar to those of rengyol [11] (see *Fig. 3*), except that the signal of C(1) was shifted downfield by 3.1 ppm, and the one of C(2) by 1.8 ppm, suggesting that **3** was a caffeate of rengyol. This was confirmed by the

cross-peaks from δ(H) 4.32 (CH_2(1)) to C(2), C(1′), and C(9″) in the HMBC spectrum. Thus, the structure of **3** was established as 2-(1,4-dihydroxycyclohexyl)ethyl caffeate.



3 Rengyol

Fig. 3. *Structures of compound* **3** *and Rengyol*

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Marine Chemical Factory*, Qingdao, P. R. China); *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA). TLC: SiO_2 plates, visualization by spraying with 10% H_2SO_4 in EtOH. Optical rotation: *Perkin-Elmer 343* polarimeter. UV Spectra: *SHIMADZU UV-2550* spectrophotometer; λ_{max} in nm. IR Spectra: *Bruker Vector-22* spectrophotometer; ν_{max} in cm^{-1}. NMR Spectra: *Bruker DRX-600* (600 MHz) and *DRX-400* spectrometer (400 MHz); δ in ppm, with Me_4Si as internal standard, *J* in Hz. MS: *Agilent MSD-Trap-XCT* (for ESI) and *Q-Tof* micro mass spectrometer (for HR-ESI), in *m*/*z*.

Plant Material. The whole plants of *I. delavayi* were collected in Eryuan County, Yunnan Province, P. R. China, in July 2006, and authenticated by Prof. *Li-Shan Xie* of Kunming Institute of Botany, the Chinese Academy of Sciences. A voucher specimen (No. 2006071003) is deposited with School of Pharmacy, Second Military Medical University.

Extraction and Isolation. The dried and powdered plants (17 kg) were refluxed with 80% EtOH 3 × 2 h. The EtOH extract was concentrated under reduced pressure to a syrup, which was dissolved in 2% HCl and filtered. The filtrate was adjusted to pH 9–10 by adding 10% NaOH, and then extracted with $CHCl_3$ to get a $CHCl_3$ fraction. The aq. soln. and the filter residue were collected together, were then adjusted to pH 7, then partitioned successively with petroleum ether (PE), AcOEt, and BuOH to yield a PE fraction, a AcOEt fraction, a BuOH fraction, and the H_2O layer, resp. The $CHCl_3$ fraction (350 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH gradient) to give *Frs. 1.1–1.8. Fr. 1.2* (4.1 g) was purified by repeated CC over SiO_2 ($CHCl_3$/MeOH 30:1 → 5:1) and *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) to provide **1** (5 mg) and **2** (8 mg). The AcOEt fraction (130 g) was subjected to CC (SiO_2; gradient $CHCl_3$/MeOH 10:0 → 0:10) to afford *Frs. 2.1–2.6. Fr. 2.4* was purified repeatedly by CC (SiO_2 and *Sephadex LH-20*; MeOH) to provide **3** (5 mg).

Incarvilleaol (=*(1*R*,4a*S*,5*R*,7*R*,7a*R*)-1-Ethoxy-1,4a,5,6,7,7a-hexahydro-5-hydroxy-7-methylcyclopenta[*c*]pyran-4-carbaldehyde*; **1**). Colorless oil. $[\alpha]_D^{20} = +83.5$ ($c = 0.165$, $CHCl_3$). UV (MeOH): 250, 208. IR (KBr): 3312, 2958, 2874, 1651. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Table 1*. ESI-MS: 249 ($[M+Na]^+$). HR-ESI-MS: 249.1105 ($[M+Na]^+$, $C_{12}H_{18}NaO_4^+$; calc. 249.1103).

Incarvillaldehyde (=*(+)-2,3-Dihydro-6-hydroxy-1-methyl-1*H*-indene-4-carbaldehyde*; **2**). Orange amorphous powder. $[\alpha]_D^{20} = +9$ ($c = 0.10$, MeOH). UV (MeOH): 225.0, 263.0, 335.2. IR (KBr): 3276, 2954, 2768, 2728, 1734, 1608, 1509. ^{1}H- and ^{13}C-NMR (C_5D_5N): *Table 2*. ESI-MS: 353 ($[2M+H]^+$), 175 ($[M-H]^-$). HR-ESI-MS: 175.0760 ($[M-H]^-$, $C_{11}H_{11}O_2^-$; calc. 175.0759).

2-(1,4-Dihydroxycyclohexyl)ethyl Caffeate (=*2-(*cis-*1,4-Dihydroxycyclohexyl)ethyl (2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoate*; **3**). Yellow oil. $[\alpha]_D^{20} = -2$ ($c = 0.285$, MeOH). UV (MeOH): 228.4, 283.8, 277.2. IR (KBr): 3364, 1745, 1607, 1510, 1250, 1183. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 3*. ESI-MS: 345 ($[M+Na]^+$), 321 ($[M-H]^-$). HR-ESI-MS: 321.1342 ($[M-H]^-$, $C_{17}H_{21}O_6^-$; calc. 321.1338).

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REFERENCES

[1] Editorial Board of Flora of China of Chinese Academy of Sciences, 'Flora of China', Science Press, Beijing, 1990, Vol. 69, p. 44.
[2] K. Xu, Y. L. Liu, H. Hu, *J. Yunnan Agric. Univ.* **2005**, *20*, 559.
[3] M. Nakamura, Y.-M. Chi, J. Kinjo, W.-M. Yan, T. Nohara, *Phytochemistry* **1999**, *51*, 595.
[4] Y.-M. Chi, F. Hashimoto, W.-M. Yan, T. Nohara, *Phytochemistry* **1997**, *46*, 763.
[5] Y. M. Chi, M. Nakamura, X. Y. Zhao, T. Yoshizawa, W. M. Yan, F. Hashimoto, T. Nohara, S. Sakurada, *Nat. Prod. Res. Dev.* (in Chinese) **2005**, *17*, 362.
[6] T. F. Ji, X. Z. Fen, *Chin. Tradit. Herb. Drugs* **2002**, *33*, 967.
[7] M. Nakamura, K. Kido, J. Kinjo, T. Nohara, *Phytochemistry* **2000**, *53*, 253.
[8] M. Nakamura, K. Kido, J. Kinjo, T. Nohara, *Chem. Pharm. Bull.* **2000**, *48*, 1826.
[9] T. Lu, W. D. Zhang, Y. H. Pei, C. Zhang, J. C. Li, Y. H. Shen, *Chin. Chem. Lett.* **2007**, *18*, 1512.
[10] S. E. Drewes, M. M. Horn, J. D. Connolly, B. Bredenkamp, *Phytochemistry* **1998**, *47*, 991.
[11] C. Kobler, F. Effenberger, *Tetrahedron* **2006**, *62*, 4823.

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