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TWO ALKALOIDS FROM *INCARVILLEA SINENSIS*

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Key Word Index—*Incarvillea sinensis*; Bignoniaceae; alkaloids; incarvines B and C; anti-rheumatism agent.

Abstract—Two novel ester alkaloids, incarvines B and C, were isolated from the aerial parts of *Incarvillea sinensis*. On the basis of chemical and spectroscopic evidence, incarvine B was characterized as the ester of the monoterpene alkaloid, incarvilline, and the monoterpene acid, Hildebrandt's acid, whilst the structure of incarvine C was established as incarvilline 7-*O*-ferulate.

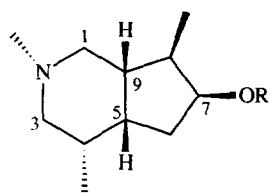
INTRODUCTION

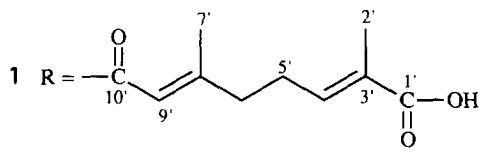
Incarvillea sinensis is a well-known Chinese crude drug which has been used to treat rheumatism and to relieve pain. We have previously investigated the pharmacologically-active substances of this species and reported the isolation and structural elucidation of three new alkaloids, incarvilline, incarvillateine and incarvine A [1–3]. The present paper describes further work leading to the isolation and structural elucidation of two new ester alkaloids, incarvines B and C.

RESULTS AND DISCUSSION

The aerial parts of *I. sinensis* were extracted with EtOH and the resulting extract treated with weak acid and alkali, followed by silica gel column chromatography, to yield incarvine B (**1**) and incarvine C (**2**).

Incarvine B (**1**) was obtained as a powder, $[\alpha]_D$ + 14.0° ($CHCl_3$), in a yield of 0.029%. The EI mass spectrum showed peaks at *m/z* 363 $[M]^+$ (14), 183 [incarvilline]$^+$ (15), 182 [incarvilline – H]$^+$ (100), 166 [incarvilline – OH]$^+$ (38). Its ^{13}C NMR signals (Table 1) suggested the presence of the monoterpene alkaloid moiety, incarvilline, which was previously determined by X-ray analysis [1]. The remaining carbon signals disclosed the occurrence of a monoterpene derivative composed of two carboxylic carbons, two methyl groups, two tri-substituted double bonds and two methylene groups. Moreover, the proton signals revealed the presence of one *N*-methyl, two secondary methyls, three methylenes, four methines and one oxygen methine on the incarvilline moiety, and two olefinic methyls, two olefinic protons and two methylenes on the monoterpene residue. The structure of the monoterpene moiety was identified as Hildebrandt's acid, which was obtained as a metabolite of monoterpenes in animals [4]. The connection between incarvilline and Hildebrandt's acid was elucidated by a ^1H-^{13}C long-range NMR spectrum and comparison of the chemical shifts with those of incarvine A. Long-range COSY was observed between C-10′ of Hildebrandt's acid and H-7 of incarvilline. Moreover, in comparing the chemical shifts of the ^{13}C NMR of **1** with those of incarvine A, downfield shifts (+ 7.0 and + 4.5) at C-1′ and C-3′ and an upfield shift (– 4.7) at C-4′ on the Hildebrandt's acid moiety in incarvine B, suggested that the C-10′ carboxyl group was





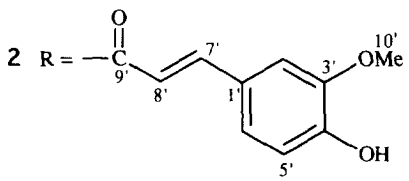


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Table 1. ^{13}C NMR data for compounds **1** and **2**

C	1	2
	Monoterpene alkaloid	
1	56.3	57.1
3	56.6	57.4
4	29.6	30.1
5	37.2	37.5
6	29.6	29.9
7	74.9	75.8
8	40.6	41.0
9	45.2	45.6
N-Me	45.2	45.8
4-Me	17.0	17.3
8-Me	14.6	14.8
	Hildebrandt's acid	Ferulic acid
1′	174.3	126.4
2′	13.2	109.7
3′	133.2	147.5
4′	135.2	148.9
5′	39.8	115.3
6′	26.5	123.8
7′	18.9	144.9
8′	159.0	115.2
9′	115.7	167.1
10′	166.4	55.8

Table 2. ^{1}H NMR data for compounds **1** and **2**

H	1	2
	Monoterpene alkaloid	
1a	1.73 *t* J = 11.9	1.75 *t* J = 11.6
1b	2.82 *dd* J = 11.4, 5.5	2.73 *dd* J = 11.6, 5.5
3a	2.67 *m*	2.57 *m*
3b, 6a, 4, 8, 9	1.85–2.18 *m*	1.93–2.14 *m*
5	2.41 *m*	2.45 *m*
6b	1.59 *m*	1.66 *t* J = 11.6
7	5.27 *m*	5.38 *m*
N-Me	2.33 *s*	2.27 *s*
4-Me	0.87 *d* J = 7.0	0.88 *d* J = 6.7
8-Me	0.94 *d* J = 7.3	0.99 *d* J = 7.3
	Hildebrandt's acid	Ferulic acid
2′	1.83 *s*	7.03 *d* J = 1.8
4′	6.51 *br.t* J = 7.3	—
5′	2.25–2.35 *m*	6.86 *d* J = 7.9
6′	2.25–2.35 *m*	7.05 *br.d* J = 7.9
7′	2.17 *s*	7.60 *d* J = 15.9
8′	—	6.27 *d* J = 15.9
9′	5.69 *s*	—
10′	—	3.91 *s*

esterified. The chemical shift of the signal at δ 5.27 (1H, *m*) assignable to H-7 in the ^{1}H NMR spectrum (Table 2) also showed that the 7-hydroxyl group was acylated. Alkaline treatment of **1** liberated an alkaloid, which was identical to incarvilline in all respects, and the monoterpene derivative. The latter was subsequently methylated with ethereal diazomethane and the methyl ester identified as Hildebrandt's acid dimethyl ester. Consequently, the structure of incarvine B (**1**) has been established as shown in the formulae.

Incarvine C (**2**) was obtained as a powder, $[\alpha]_D$ − 20.8° ($CHCl_3$), in a yield of 0.0025%. The EI mass spectrum showed peaks at *m/z* 359 $[M]^+$ (20), 182 [incarvilline − H]$^+$ (100), 177 [ferulic acid − OH]$^+$ (12), 166 [incarvilline − OH]$^+$ (15). The ^{13}C NMR signals (Table 1) also suggested the presence of incarvilline. The remaining signals suggested the occurrence of ferulic acid [5] whose ^{1}H NMR spectrum showed signals due to two olefinic protons, three aromatic protons and one methoxy group. The position of the methoxy group was confirmed by NOE experiments; irradiation of the signal at H_3-10′ (–OMe) increased the intensity of H-2′, but the H-5′ signal intensity remained unchanged. The chemical shift of signals at δ 5.38 (1H, *m*) assignable to H-7 in the ^{1}H NMR spectrum (Table 2) suggested that the 7-hydroxyl group was acylated. Consequently, the structure of incarvine C (**2**) has been established as shown in the formulae.

The isolated compounds might be one of the possible substances responsible for relieving pain. The Hildebrandt's acid moiety in incarvine B (**1**) is reported for the first time from a plant source. Moreover, incarvine C (**2**) is considered to be the biosynthetic precursor of incarvillateine [2].

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR were measured on a JEOL JNM–GX 400 NMR spectrometer and chemical shifts are given in δ with TMS as an int. standard. TLC was then performed on precoated Kieselgel 60 F_{254} plates (Merck) and detection was achieved by spraying with Dragendorff's reagent and 10% aq. H_2SO_4. CC was carried out on Kieselgel 60 (70–230 and 230–400 mesh, Merck).

Extraction and separation. Aerial parts of *I. sinensis* Lam collected in Hebei province, China, were exhaustively extracted with EtOH. The EtOH extract was concd under red. pres. to a syrup. The residue was than extracted with $CHCl_3$ after weak acid and alkaline treatment. After the solvent was evapd *in vacuo* to dryness, the residue was repeatedly chromatographed on a silica gel column with cyclohexane–MeOH–Et_2NH (30:1:1–5:1:1) to afford compounds **1** and **2**.

Incarvine B (**1**). Powder $[\alpha]_D$ + 14.0° ($CHCl_3$; *c* 0.45). EIMS *m/z* (rel. int.): 363 $[M]^+$ (14), 183 (15), 182 (100), 166 (38). ^{1}H and ^{13}CNMR in Tables 1 and 2.

Alkaline hydrolysis of **1**. After a mixt. of incarvine B (**1**, 50 mg) and 3% KOH-MeOH was heated at 60° for 1 hr, the reaction mixt. was acidified and partitioned between EtOAc and H_2O. The organic layer was methylated with CH_2N_2–Et_2O and successively purified over silica gel to

give a compound identical with Hildebrandt's acid Me ester syrup . ^{1}H NMR ($CDCl_3$) : δ1.85 (3H, *s*), 2.18 (3H, *s*), 2.20–2.38 (4H, *m*), 3.69 (3H, *s*, OMe), 3.74 (3H, *s*, OMe), 5.69 (1H, *s*), 6.70 (1H, *t*). Meanwhile, the aq. layer was neutralized and evapd to dryness and the residue, which was subjected to Amberlite XAD–2 CC with aq. MeOH, afforded a monoterpene alkaloid identical to incarvilline ($[\alpha]_D$ ^{1}H NMR and ^{13}C NMR).

Incarvine C (**2**). Powder. $[\alpha]_D$ − 20.8° ($CHCl_3$; *c* 0.46). EIMS *m/z* (rel. int.): 359 $[M]^+$ (20), 183 (16), 182 (100), 177 (12), 166 (15), 58 (30), 44 (11). ^{1}H and ^{13}C NMR in Tables 1 and 2.

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