A Monoterpene Alkaloid from *Incarvillea sinensis*

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A novel monoterpene alkaloid, named incarvillateine E, possessing three moles of incarvilline moieties, has been obtained from the aerial parts of *Incarvillea sinensis* LAM. (Bignoniaceae). On the basis of spectroscopic evidence, the structure of incarvillateine E has been characterized.

Key words *Incarvillea sinensis*; Bignoniaceae; monoterpene alkaloid; incarvillateine E

Incarvillea sinensis LAM., a well-known traditional Chinese crude drug, has been used to treat rheumatism and to relieve pain. In the studies on its pharmacological active substances, seventeen novel alkaloids including incarvilline,[1,2] incarvine A (**2**),[3] incarvine C (**3**)[4] and incarvillateine (**4**)[5] were isolated and their structures were characterized.[1—8] Incarvillateine (**4**) was found to show a more potent antinociceptive effect than morphine in the formalin test, and the mechanism of antinociception was regarded to be different from that of morphine.[9] In this paper, we describe the isolation and structure elucidation of a new monoterpene alkaloid, incarvillateine E (**1**).

The aerial parts (3.0 kg) of *I. sinensis* were extracted with EtOH, and the extract was subsequently treated with weak acid and alkali in order to attain a facile separation of alkaloids, followed by Al_2O_3 and silica gel column chromatography to yield compound **1** (14.9 mg).

Incarvillateine E (**1**) showed peaks at *m/z* 888 $[M+H]^+$, 887 $[M]^+$, 529 [incarvine A+H]$^+$, 182 [incarvilline−H]$^-$ and 166 [incarvilline−OH]$^+$ in the positive FAB-MS. Its molecular formula was determined to be $C_{53}H_{82}N_3O_8$ from high resolution (HR)-FAB-MS. Alkaline hydrolysis of **1** yielded a monoterpene alkaloid identical to incarvilline, whose structure and absolute configuration were previously determined by X-ray analysis.[1,2] The signals at δ 0.71, 0.81, 0.84 (each 3H, d, J=6.6, 6.9, 6.9 Hz), 0.82, 0.93, 0.94 (each 3H, s, J=7.3, 7.3, 7.3 Hz), 2.19, 2.21, 2.23 (each 3H, s), 4.85 (1H, m) and 5.26 (2H, m) in the ^{1}H-NMR spectrum could be assigned to 4,4′,4″-Me, 8,8′,8″-Me, *N*,*N*′,*N*″-Me, 7″-H and 7,7′-H of three sets of incarvilline, indicating the presence of three monoterpene alkaloid moieties. The ^{13}C-NMR spectrum showed characteristic signals putting those of incarvine A (**2**)[3] and incarvine C (**3**)[4] together, except for the disappearance of the signals due to double bonds and the new occurrence of the signals at δ 39.2, 42.9, 50.3 and 50.9 in **1** as listed in Table 1. Twelve carbon signals at δ 75.4, 76.3, 76.9,

Table 1. ^{13}C-NMR Data for Incarvillateine E (**1**), Incarvine A (**2**),[3] Incarvine C (**3**)[4] and Incarvillateine (**4**)[6]

Incarvilline moiety					C_6–C_3 unit				Hildbrandt's acid moiety[3]		
C	**1**	**2**	**3**	**4**	C	**1**	**3**	**4**	C	**1**	**2**
1,1′,1″-C	57.4	57.1[a]	57.1	57.2	7′,7″,7‴-C	42.9	149.9	40.3	1″,1‴-C	174.6	167.3
	57.7[a]			57.3				41.7	2″,2‴-C	19.2	12.2
3,3′,3″-C	57.9[a]	57.3[a]	57.3	57.5	8′,8″,8‴-C	50.3	115.3	47.2	3″,3‴-C	50.9	128.7
	58.3			57.6				47.8	4″,4‴-C	39.2	139.9
4,4′,4″-C	30.5, 30.7	30.2[a]	30.1	30.2[a]	9′,9″,9‴-C	172.7	167.1	171.7	5″,5‴-C	25.2	26.4
	30.8							171.9	6″,6‴-C	39.1	39.3
5,5′,5″-C	37.5	37.3	37.5	37.3[a]	3′,3″,3‴-OMe	55.8	55.8	55.6	7″,7‴-C	18.9	18.6
	37.7[a]	37.4						55.7	8″,8‴-C	159.0	157.9
6,6′,6″-C	29.1, 29.9	29.6	29.9	29.2	1′,1″,1‴-C	130.9	126.4	130.2	9″,9‴-C	115.9	116.2
	30.4	29.7		29.7				130.4	10″,10‴-C	166.6	166.2
7,7′,7″-C	75.4, 76.3	75.1	75.8	76.4	2′,2″,2‴-C	110.2	109.7	110.8			
	76.9	75.9		76.6				110.9			
8,8′,8″-C	40.6, 40.7	40.6	41.0	40.3	3′,3″,3‴-C	145.6	147.5	145.3			
	40.8	40.7		40.4				145.5			
9,9′,9″-C	46.1, 46.2	45.7[a]	45.6	45.8	4′,4″,4‴-C	146.5	148.9	146.8			
	46.3			45.9				146.9			
N,*N*′,*N*″-Me	46.3	45.9[a]	45.8	46.1	5′,5″,5‴	114.4	115.2	114.7[a]			
	46.4[a]			47.4	6′,6″,6‴-C	120.1	123.8	119.8			
4,4′,4″-Me	17.0, 17.3	17.3[a]	17.3	16.9				120.3			
	17.4			17.1							
8,8′,8″-Me	14.9, 15.0	14.6[a]	14.7	14.4							
	15.1			14.8							

a) Overlapped signals.

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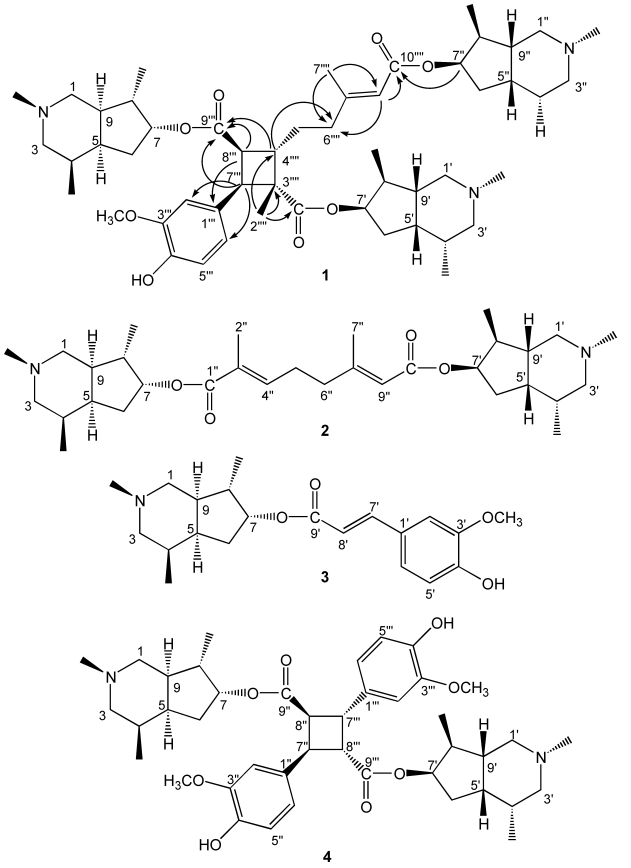

Fig. 1. Structures of Compounds **1**—**4** and Key HMBC Correlations of Compound **1**

46.3, 46.4×2, 17.0, 17.3, 17.4, 14.9, 15.0 and 15.1 could be assigned, respectively, to 7,7′,7″-C, *N*,*N*′,*N*″-Me, 4,4′,4″-Me and 8,8′,8″-Me of three sets of incarvilline by comparison with those of incarvine A (**2**)[3] and incarvine C (**3**),[4] and by the aid of ^{1}H-detected heteronuclear correlation through multiple quantum coherence (HMQC). The remaining signals at δ 130.9 (s), 110.2 (d), 145.6 (s), 146.6 (s), 120.1 (d) and 55.8 (q) suggested the presence of a 3-methoxy-4-hydroxylphenyl moiety by comparison with those of incarvine C (**3**)[4] and incarvillateine (**4**)[6] and by the aid of ^{1}H-detected heteronuclear multiple-bond correlation (HMBC). The methine proton signals at δ 3.32, 3.66 and 3.74 showed correlations with the carbon signals at δ 39.2, 42.9 and 50.3, respectively, in the HMQC spectrum. The correlations of δ 3.66/δ 3.74/δ 3.32 assignable to H-7‴–H-8‴–H-4‴ in the ^{1}H–^{1}H shift correlated spectroscopy (COSY) and the HMBC correlations as shown in Fig. 1, implied newly occurring carbon signals at δ 39.2 (d), 42.9 (d), 50.3 (d) and 50.9 (s) forming a cyclobutane ring by comparison with incarvillateine (**4**), the structure of **4** was also previously determined by X-ray analysis.[5] The relative configuration of the cyclobutane ring was established by nuclear Overhauser effect correlation spectroscopy (NOESY), especially NOEs between 7‴-H and 8‴-H, and 2⁗-H and 4⁗-H were observed. The absolute configuration of **1** could be assumed to be analogous with that of incarvillateine (**4**). The structure of **1** was consequently established as shown in Fig. 1 and named incarvillateine E (**1**), which was presumably derived from [2π+2π] cycloaddition between the 3⁗,4⁗-double bond of incarvine A (**2**) and 7‴,8‴-double bond of incarvine C (**3**).

Experimental

^{1}H- and ^{13}C-NMR: JEOL JNM-GX 500 NMR, int. standard (TMS); FAB-MS and HR-FAB-MS: JEOL DX-303HF mass spectrometer; TLC: precoated Kieselgel 60 F_{254} plate (0.2 mm, Merck), detection by spraying Dragendorff and 10% aq. H_2SO_4; CC: Kieselgel 60 (70—230 and 230—400 mesh, Merck), Aluminium oxide 90 aktiv (70—230 mesh, Merck).

Extraction and Separation *Incarvillea sinensis* (Bignoniaceae) was collected in the Hebei province, China, in August 1998. A voucher sample has been preserved in the Department of Natural Medicine, Kumamoto University. The aerial parts were then exhaustively extracted with EtOH and the EtOH extract was concentrated under reduced pressure to a syrup, which was dissolved in 1% HCl and filtered. The filtrate was adjusted to pH 10—11 by adding NH_4OH, and the alkaloid was extracted with $CHCl_3$. After removal of the solvent *in vacuo* to dryness to give a residue, the residue was repeatedly subjected to Al_2O_3 column chromatography with $CHCl_3$–MeOH–H_2O (10 : 1 : 0→6 : 4 : 1) as eluant as well as silica gel column chromatography with cyclohexane–EtOH–Et_2NH (30 : 1 : 1→5 : 1 : 1) to afford compound **1** (14.9 mg).

Alkaline Hydrolysis of 1 After a mixture of incarvillateine E (**1**, 10 mg) and 3% KOH–MeOH was heated at 60 °C for 1 h, the reaction mixture was acidified and partitioned between EtOAc and H_2O. The aqueous layer was neutralized and evaporated to give a residue which was subjected to Amberlite XAD-2 column chromatography and elution with aqueous MeOH affording a monoterpene alkaloid identical to incarvilline ([α]$_D$, ^{1}H- and ^{13}C-NMR).

Incarvillateine E (**1**): A white powder, $[\alpha]_D^{23}$ −6.4° (c=0.32, $CHCl_3$). Positive FAB-MS *m*/*z* (rel. int): 888 $[M+H]^+$ (20), 887 $[M]^+$ (12), 529 (22), 182 (52), 167 (15), 166 (100), 164 (49), 162 (14), 154 (26). HR-FAB-MS *m*/*z*: 888.6093 $[M+H]^+$ (Calcd for $C_{53}H_{82}N_3O_8$, 888.6105). ^{1}H-NMR ($CDCl_3$) δ: 0.71, 0.81, 0.84 (each 3H, d, J=6.6, 6.9, 6.9 Hz, 4,4′,4″-Me), 0.82, 0.93, 0.94 (each 3H, d, J=7.3, 7.3, 7.3 Hz, 8,8′,8″-Me), 1.46 (3H, m, 6,6′,6″-H*a*), 1.50 (3H, s, 2⁗-H), 1.63—1.78 (8H, m, 1,1′,1″-H*a*, 3,3′,3″-H*a*, 5⁗-H), 1.81—2.06 (6H, m, 4,4′,4″-H, 8,8′,8″-H), 1.88—2.10 (6H, m, 6,6′,6″-H*b*, 9,9′,9″-H), 2.11 (2H, m, 6⁗-H), 2.16 (3H, s, 7⁗-H), 2.19, 2.21, 2.23 (each 3H, s, *N*,*N*′,*N*″-Me), 2.28—2.39 (3H, m, 5,5′,5″-H), 2.53 (3H, m, 3,3′,3″-H*b*), 2.67 (3H, m, 1,1′,1″-H*b*), 3.32 (1H, m, 4⁗-H), 3.66 (1H, m, 7⁗-H), 3.74 (1H, m, 8‴-H), 3.86 (3H, s, O-Me), 4.85 (1H, m, 7″-H), 5.26 (2H, m, 7,7′-H), 5.65 (1H, s, 9⁗-H), 6.66 (2H, d, J=8.6 Hz, 2‴,6‴-H), 6.78 (1H, d, J=8.6 Hz, 5‴-H).

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