Monoterpene Alkaloids from *Incarvillea sinensis*. VI.[1] Absolute Stereochemistry of Incarvilline and Structure of a New Alkaloid, Hydroxyincarvilline

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Received August 12, 1996; accepted November 18, 1996

The absolute configuration of incarvilline (1), an important core compound of a number of alkaloids isolated from *Incarvillea sinensis* LAM., has been determined by the application of Mosher's method and X-ray crystallographic analysis of incarvilline methiodide (1a). Furthermore, the structure of hydroxyincarvilline (2) was also determined by spectroscopic means.

Key words *Incarvillea sinensis*; Bignoniaceae; incarvilline; hydroxyincarvilline; absolute configuration

Incarvillea sinensis has been used to treat rheumatism and to relieve pain. We previously reported the isolation and structural elucidation of many new alkaloids,[2] among which the absolute configuration of incarvilline (**1**) has been remained to be solved. The present paper describes the absolute configuration of incarvilline (**1**), and the additional structural characterization of hydroxyincarvilline (**2**).

Incarvilline (**1**) was isolated from the aerial parts of *I. sinensis* and its relative structure was established previously by spectroscopic and X-ray analysis.[2a] However, its absolute stereochemistry remained to be determined. Thus, in order to elucidate the absolute configurations at C-4, C-5, C-7, C-8 and C-9, **1** was esterified, respectively, with (*R*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetic acid (MTPA) and (*S*)-(−)-MTPA. The absolute configurations of these positions were thus deduced from (*S*)-(−)-MTPA and (*R*)-(+)-MTPA esters of **1** by Mosher's method.[3,4] The $\Delta\delta$ ($\delta_S-\delta_R$) values for the MTPA esters of **1** shown in Fig. 1 allowed us to assign **1** as (−)-(4*R*,5*S*,7*R*,8*S*,9*S*)-incarvilline.

Furthermore, to confirm the absolute configuration of **1**, X-ray analysis of incarvilline methiodide (**1a**) was also undertaken.

Incarvilline methiodide (**1a**) was prepared as a colorless crystal, mp >300 °C, $[\alpha]_D$ −20.4° (MeOH), whose ^{1}H-NMR signals were assigned as follows: δ: 0.97 (3H, d, J=6.7 Hz, 4-Me), 1.04 (3H, d, J=7.3 Hz, 8-Me), 1.63 (1H, m, 6-H$_a$), 1.91 (2H, m, 6-H$_b$, 8-H), 2.21 (1H, m, 9-H), 2.45 (1H, m, 4-H), 2.57 (1H, m, 5-H), 2.86 (1H, t, J=13.1 Hz, 1-H$_a$), 3.00 (1H, t, J=12.8 Hz, 3-H$_a$), 3.05, 3.13 (each, 3H, s, N-Me×2), 3.18 (1H, m, 3-H$_b$), 3.32 (1H, m, 1-H$_b$), 4.29 (1H, m, 7-H). The structure was determined by a direct method using MULTAN82[5] from the data collected with an Enraf–Nonius CAD4 diffractometer, and was refined by a block-diagonal least squares method with anisotropic thermal parameters for all non-hydrogen atoms and isotropic ones for hydrogen atoms. A total of 1564 reflections were collected, and 1455 of them were observed after correction. All hydrogen atoms were located, and the R value at the conclusion of refinement was 4.00% for the observed reflections. The absolute stereochemistry was determined by an anomalous dispersion method.[6] The X-ray diffraction of crystals of **1a** established the stereochemistry at the asymmetric centers as 4*R*, 5*S*, 7*R*, 8*S*, 9*S*, as shown in **1a** and Fig. 2. Concerning the stereochemistry at *N*-Me in **1**, since the relative configuration was already determined by X-ray analysis,[2a] it was determined to be β-methyl bond. Incarvilline (**1**), therefore, has the absolute stereochemistry as that shown in **1**, whose result doesn't contradict that derived by Mosher's method.

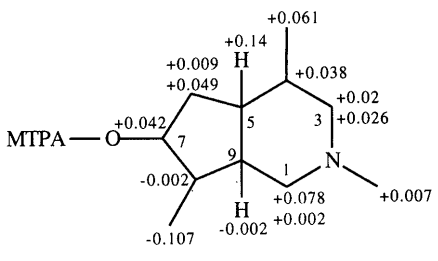

Fig. 1. $\Delta\delta$ Values for MTPA Esters of (−)-(4*R*,5*S*,7*R*,8*S*,9*S*)-Incarvilline (**1**)

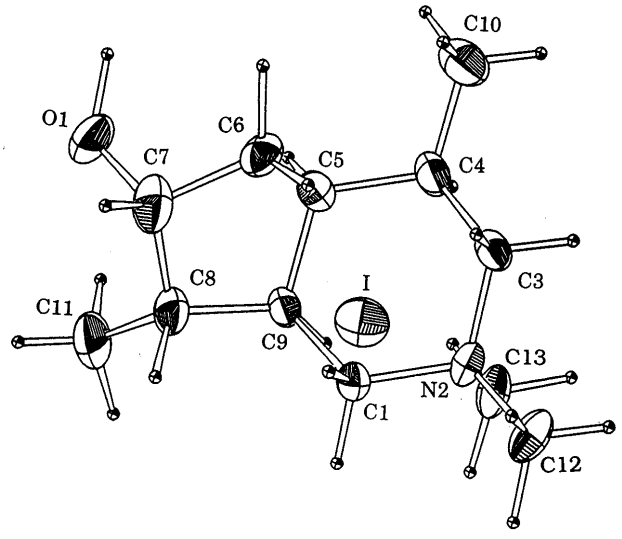

Fig. 2. Stereoplot of Incarvilline Methiodide (**1a**)

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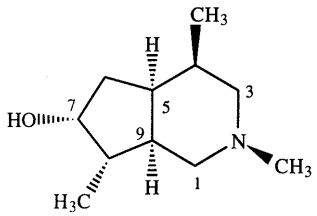

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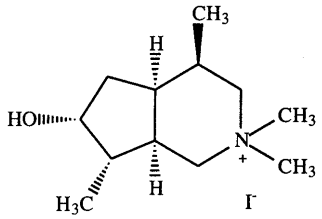


1a

Table 1. ^{1}H-NMR Spectral Data for Compounds **2** and **1** (500 MHz, $CDCl_3$, TMS as Standard)

	2	1
4-Me	0.94 (3H, d, J=6.7 Hz)	0.86 (3H, d, J=7.0 Hz)
8-Me	1.16 (3H, d, J=7.3 Hz)	1.02 (3H, d, J=7.3 Hz)
1-H_a	1.50 (1H, t, J=11.6 Hz)	1.54 (1H, t, J=11.7 Hz)
3-H_a	1.63 (1H, t, J=11.6 Hz)	1.65 (1H, t, J=11.5 Hz)
6-H_a	1.74 (1H, m)	1.45 (1H, m)
8-H	1.74 (1H, m)	1.80 (1H, m)
6-H_b	1.80 (1H, m)	1.80 (1H, m)
9-H	1.87 (1H, m)	1.90 (1H, m)
4-H	1.94 (1H, m)	2.06 (1H, m)
N-Me	2.21 (3H, s)	2.21 (3H, s)
5-H		2.41 (1H, m)
3-H_b	2.65 (1H, m)	2.49 (1H, m)
1-H_b	2.88 (1H, m)	2.65 (1H, m)
7-H	4.18 (1H, t, J=6.1 Hz)	4.29 (1H, m)

Table 2. ^{13}C-NMR Spectral Data for Compounds **2** and **1** (500 MHz, $CDCl_3$, TMS as Standard)

	2	1
Me-4	13.6 (q)	17.3 (q)
Me-8	14.9 (q)	14.2 (q)
C-4	39.1 (d)	30.4 (d)
C-6	38.9 (t)	32.4 (t)
C-5	83.5 (s)	37.3 (d)
C-8	43.4 (d)	42.2 (d)
C-9	54.5 (d)	45.8 (d)
N-Me	45.7 (q)	46.2 (q)
C-1	62.2 (t)	57.9 (t)
C-3	60.6 (t)	58.0 (t)
C-7	74.1 (d)	72.7 (d)

Hydroxyincarvilline (**2**) was obtained as a white powder, $[\alpha]_D$ −6.1° ($CHCl_3$). Its EI-MS showed a molecular ion peak at *m/z* 199 $[M]^+$ (100), and prominent fragment ion peaks at 182 $[M-OH]^+$ (98) and 166 $[M-OH\times2+H]^+$ (52) which corresponded to $[incarvilline-H]^+$ and $[incarvilline-OH]^+$ peaks, respectively. Comparing the ^{1}H-NMR spectrum (Table 1) of **2** with that of **1**, signals at δ 0.94 (3H, d, J=6.7 Hz), 1.16 (3H, d, J=7.3 Hz), 2.21 (3H, s), 2.65 (1H, m), 2.88 (1H, m), 4.18 (1H, t, J=6.1 Hz) could be attributed to 4-Me, 8-Me, *N*-Me, 3-H_b, 1-H_b, and 7-H, respectively. In the ^{13}C-NMR spectrum (Table 2) of **2**, downfield shifts (+8.7 ppm) at C-4, +6.5 ppm at C-6 and +8.7 ppm at C-9 were observed by comparison with those of **1**, suggesting that **2** was the hydroxy derivative of incarvilline (**1**) and that the hydroxyl group was located at C-5. The structure of **2** was confirmed by ^{1}H-detected heteronuclear multiple-bond correlation

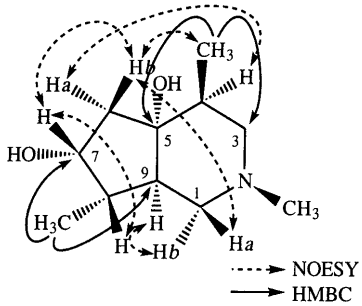
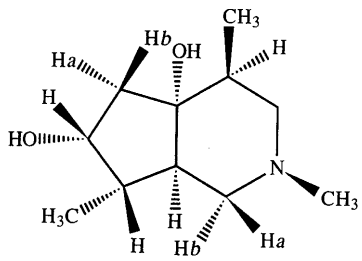


Fig. 3. NOESY and HMBC Correlations of **2**



2

(HMBC), and the relative configuration was established by nuclear Overhauser effect correlation spectroscopy (NOESY) (Fig. 3); especially, NOEs between 1-H_a and 6-H_b, and 4-H and 6-H_a were observed. It is suggested that the configurations of 5-OH and 9-H oriented to *cis*, and were anticoplanar to 1-H_a and 6-H_b bonds. The absolute configuration of **2** could be assumed to be the analogous with that of **1**.

Experimental

Preparation of MTPA Esters of 1 A solution of **1** (10.6 mg) and 4-dimethylaminopyridine (DMAP) (3.1 mg) in Et_2O (5 ml) was treated with (−)-MTPA (10.6 mg) and *N,N'*-dicyclohexylcarbodiimide (DCC) (16.6 mg) Et_2O solution (5 ml), and was stirred at room temperature for 12 h. After the solvent was evaporated under reduced pressure, the residue was subjected to silica gel column elution with $CHCl_3$: MeOH = 50 : 1—10 : 1 to give the (−)-MTPA ester of **1** (10.3 mg). The (+)-MTPA (4.8 mg) ester of **1** was obtained by the same method.

^{1}H-NMR ($CDCl_3$) of (−)-MTPA ester of **1**: δ 0.86 (6H, d, J=7.0 Hz, 4, 8-Me), 1.57 (1H, m, 6-H_a), 1.64 (1H, t, J=11.7 Hz, 1-H_a), 1.67 (1H, t, J=11.5 Hz, 3-H_a), 1.96 (1H, m, 6-H_b), 1.94 (1H, m, 9-H), 2.03 (1H, m, 8-H), 2.08 (1H, m, 4-H), 2.24 (3H, s, *N*-Me), 2.35 (1H, m, 5-H), 2.52 (1H, m, 3-H_b), 2.68 (1H, m, 1-H_b), 5.46 (1H, m, 7-H). (+)-MTPA ester of **1**: δ 0.80 (3H, d, J=7.0 Hz, 4-Me), 0.97 (3H, d, J=7.0 Hz, 8-Me), 1.56 (1H, m, 6-H_a), 1.56 (1H, t, J=11.7 Hz, 1-H_a), 1.65 (1H, t, J=11.5 Hz, 3-H_a), 1.91 (1H, m, 6-H_b), 1.94 (1H, m, 9-H), 2.04 (1H, m, 8-H), 2.04 (1H, m, 4-H), 2.23 (3H, s, *N*-Me), 2.21 (1H, m, 5-H), 2.50 (1H, m, 3-H_b), 2.68 (1H, m, 1-H_b), 5.42 (1H, m, 7-H).

Preparation of 1a To an acetone solution of **1** was added methyl iodide, filtered, and then recrystallized from dil. MeOH to give a crystal

Table 3. Experimental Data for X-Ray Diffraction Study of Incarvilline Methiodide (**1a**)

Formula	$C_{12}H_{24}INO$
Formula weight	325.24
System	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	9.240 (1)
b (Å)	17.260 (2)
c (Å)	8.808 (1)
Z	4
V (Å^3)	1404.7 (2)
D_x (mg/m^3)	1.537
F (000)	656
λ(Cu-K_α) (Å)	1.5418
μ(Cu-K_α) (mm^{-1})	17.919
R	0.040
R_w	0.048

Table 4. Positional and Thermal Parameters for Non-Hydrogen Atoms of **1a**

Atom	x	y	z	B_{eqv}
I	39073 (5)	86608 (3)	73888 (5)	2.99 (1)
O1	79863 (60)	83093 (36)	20679 (94)	4.69 (18)
N2	19936 (55)	88466 (32)	27135 (67)	2.16 (12)
C1	34274 (71)	92562 (37)	29364 (81)	2.00 (14)
C3	22327 (81)	79848 (40)	29407 (88)	2.47 (16)
C4	33255 (86)	76460 (42)	18406 (95)	2.71 (17)
C5	47839 (79)	80767 (38)	19153 (84)	2.22 (15)
C6	57332 (79)	79355 (44)	33197 (93)	2.58 (17)
C7	68922 (89)	85576 (52)	31437 (102)	3.45 (20)
C8	61061 (69)	92708 (42)	25127 (96)	2.88 (16)
C9	46203 (74)	89712 (39)	18883 (80)	1.94 (13)
C11	69682 (100)	97328 (54)	13355 (143)	4.53 (27)
C10	34913 (102)	67741 (46)	21588 (115)	3.87 (22)
C12	9362 (84)	91202 (53)	39312 (92)	3.16 (19)
C13	13681 (73)	90271 (51)	11892 (90)	2.78 (17)

Table 5. Positional Parameters for Hydrogen Atoms of **1a**

Atom	x	y	z	B
H(O1)1	820 (10)	768 (5)	201 (10)	3.9 (2.0)
H(C1)1	388 (11)	920 (6)	420 (11)	4.9 (2.4)
H(C1)2	323 (11)	986 (5)	278 (11)	4.7 (2.2)
H(C3)1	123 (10)	770 (5)	294 (11)	4.7 (2.3)
H(C3)2	264 (10)	789 (5)	424 (11)	4.1 (2.2)
H(C4)1	291 (10)	771 (5)	58 (10)	3.7 (2.1)
H(C5)1	525 (10)	795 (5)	54 (11)	3.5 (2.0)
H(C6)1	616 (10)	732 (6)	345 (12)	4.8 (2.4)
H(C6)2	505 (11)	799 (5)	451 (12)	4.6 (2.4)
H(C7)1	752 (11)	865 (6)	435 (11)	4.6 (2.3)
H(C8)1	604 (10)	971 (5)	356 (11)	4.4 (2.2)
H(C9)1	383 (10)	916 (6)	71 (11)	4.4 (2.2)
H(C11)1	632 (11)	1021 (5)	72 (12)	4.6 (2.4)
H(C11)2	794 (11)	994 (6)	149 (12)	5.0 (2.5)
H(C11)3	714 (11)	933 (6)	22 (11)	4.8 (2.4)
H(C10)1	419 (12)	653 (6)	114 (13)	5.7 (2.7)
H(C10)2	394 (11)	668 (6)	328 (12)	5.2 (2.4)
H(C10)3	251 (10)	650 (5)	202 (11)	4.3 (2.2)
H(C12)1	−3 (10)	886 (5)	394 (13)	4.7 (2.6)
H(C12)2	140 (9)	899 (5)	506 (10)	3.6 (1.9)
H(C12)3	75 (10)	975 (5)	402 (11)	4.2 (2.2)
H(C13)1	36 (12)	873 (5)	88 (12)	4.8 (2.4)
H(C13)2	126 (10)	966 (5)	95 (11)	4.0 (2.1)
H(C13)3	207 (11)	884 (5)	20 (11)	4.7 (2.4)

Table 6. Bond Distances and Bond Angles of **1a**

Bond distances (Å)			
O1–C7	1.450 (11)	N2–C1	1.514 (8)
N2–C3	1.517 (9)	N2–C12	1.526 (10)
N2–C13	1.494 (10)	C1–C9	1.519 (10)
C3–C4	1.517 (11)	C4–C5	1.540 (11)
C4–C10	1.538 (11)	C5–C6	1.536 (11)
C5–C9	1.551 (9)	C6–C7	1.524 (11)
C7–C8	1.533 (11)	C8–C9	1.567 (10)
C8–C11	1.531 (13)		
Bond angles (°)			
C1–N2–C3	108.3 (5)	C1–N2–C12	108.9 (5)
C1–N2–C13	110.9 (5)	C3–N2–C12	107.7 (6)
C3–N2–C13	112.3 (6)	C12–N2–C13	108.6 (5)
N2–C1–C9	113.9 (5)	N2–C3–C4	113.0 (6)
C3–C4–C5	111.7 (6)	C3–C4–C10	109.1 (7)
C5–C4–C10	112.2 (6)	C4–C5–C6	117.2 (6)
C4–C5–C9	113.2 (6)	C6–C5–C9	103.1 (6)
C5–C6–C7	102.0 (6)	O1–C7–C6	110.4 (7)
O1–C7–C8	109.3 (7)	C6–C7–C8	105.6 (6)
C7–C8–C9	106.1 (6)	C7–C8–C11	114.6 (6)
C9–C8–C11	113.0 (7)	C1–C9–C5	112.5 (6)
C1–C9–C8	108.4 (6)	C5–C9–C8	103.7 (5)

Table 7. Torsion Angles (°) of **1a**

C3–N2–C1–C9	57.3 (7)
C12–N2–C1–C9	174.1 (6)
C13–N2–C1–C9	−66.4 (7)
C1–N2–C3–C4	−59.6 (7)
C12–N2–C3–C4	−177.3 (6)
C13–N2–C3–C4	63.2 (8)
N2–C1–C9–C5	−50.9 (7)
N2–C1–C9–C8	−165.1 (5)
N2–C3–C4–C5	55.5 (8)
N2–C3–C4–C10	−179.9 (6)
C3–C4–C5–C6	73.0 (8)
C3–C4–C5–C9	−46.8 (8)
C10–C4–C5–C6	−49.8 (9)
C10–C4–C5–C9	−169.6 (6)
C4–C5–C6–C7	−169.5 (6)
C9–C5–C6–C7	−44.4 (7)
C4–C5–C9–C1	44.7 (8)
C4–C5–C9–C8	161.6 (6)
C6–C5–C9–C1	−83.0 (7)
C6–C5–C9–C8	34.0 (7)
C5–C6–C7–O1	−80.4 (8)
C5–C6–C7–C8	37.7 (8)
O1–C7–C8–C9	102.2 (7)
O1–C7–C8–C11	−23.1 (10)
C6–C7–C8–C9	−16.5 (8)
C6–C7–C8–C11	−141.9 (7)
C7–C8–C9–C1	108.9 (7)
C7–C8–C9–C5	−10.9 (7)
C11–C8–C9–C1	−124.7 (7)
C11–C8–C9–C5	115.5 (7)

(**1a**), mp >300 °C, $[\alpha]_D$ −20.4° (c=0.44, MeOH), which was suitable for X-ray diffraction. Crystal data are listed in Table 3. Positional parameters are given in Tables 4 and 5. Bond distances and bond angles are given in Table 6, and torsion angles are listed in Table 7.

Extraction and Separation of 2 The aerial parts of *Incarvillea sinensis* (Bignoniaceae) (3.5 kg) collected in Hebei province, China, were exhaustively extracted with EtOH. The EtOH extract was concentrated under reduced pressure to a syrup, which was dissolved in 2% HCl and filtered. The filtrate was then adjusted to pH 11 by adding NH_4OH, and the alkaloid was extracted with $CHCl_3$. After the solvent was evaporated *in vacuo* to dryness, the resulting residue was repeatedly chromatographed over an Al_2O_3 column with $CHCl_3$ and silica gel column with cyclohexane–EtOH–Et_2NH (20 : 1 : 1) to afford hydroxyincarvilline (**2**)

(21.8 mg).

Hydroxyincarvilline (2) A white powder, $[\alpha]_D$ −6.1° (c=1.46, $CHCl_3$). EI-MS m/z: 199 $[M]^+$ (100), 198 $[M-1]^+$ (68), 182 $[M-OH]^+$ (98), 181 (38), 166 (52), 84 (48), 74 (53), 58 (47).

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