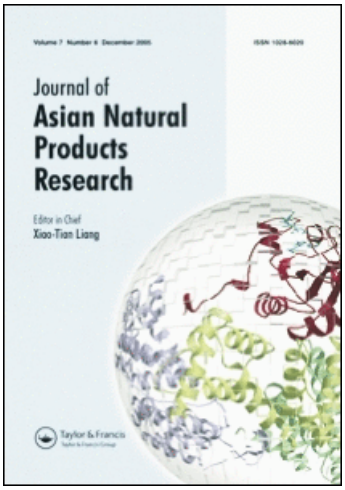

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A novel macrocyclic spermine alkaloid from *Incarvillea sinensis*

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A novel macrocyclic spermine alkaloid incasine C′ (1), along with a known compound incasine C (2), were isolated from the whole plants of *Incarvillea sinensis*, and their structures were elucidated on the basis of chemical and spectroscopic evidence.

Keywords: Incarvillea sinensis; Bignoniaceae; Spermine alkaloid; Incasine C′

1. Introduction

The whole plants of *Incarvillea sinensis* Lam. (Bignoniaceae) have been used to treat rheumatism and to relieve pain as a traditional Chinese crude drug designated as 'Tougucao'. In studies on its pharmacological active substances, five novel macrocyclic spermine alkaloids [1] and 12 novel monoterpene alkaloids [2–8] have been isolated and characterised. One of the monoterpene alkaloids, incarvillateine, was found to show more potent anti-nociceptive activity than morphine in the formalin test, and the mechanism of anti-nociception was regarded to be different from that of morphine [9]. In this paper, we describe the isolation and structural elucidation of a novel macrocyclic spermine alkaloid, incasine C′ (1).

2. Results and discussion

The whole plants of *I. sinensis* were extracted with EtOH, and the extract was subsequently treated with weak acid and alkali, followed by Al_2O_3 and silica gel column chromatographies

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and preparative HPLC separation to yield a novel compound incasine C′ (1), together with a known compound incasine C (2).

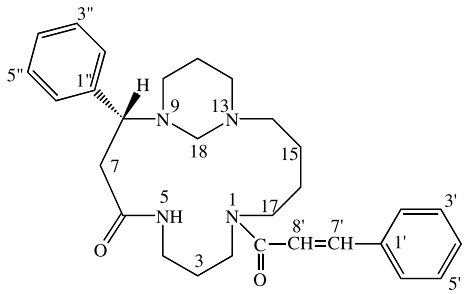
Compound 1 was obtained as an off-white amorphous powder. Its molecular formula $C_{29}H_{38}N_4O_2$ was provided by HREI-MS (at *m/z* 474.2987 $[M]^+$). The ^1H NMR and ^{13}C NMR spectra of 1 were quite similar to those of compound 2 except for the signals at δ 6.80, 6.81 (each 0.5H, d, $J = 15.4$ Hz) and 7.71, 7.73 (each 0.5H, d, $J = 15.4$ Hz) in the ^1H NMR spectrum, and at δ 142.0, 142.2 (d) and 117.1, 117.3 (d) in the ^{13}C NMR spectrum [1]. It was noted that a complexity, mostly with doublet signals for each carbon assignment in the ^{13}C NMR spectrum was due to the presence of *E*- and *Z*-isomers of the amide groups [10]. Its EI-MS exhibited the same molecular ion peak at *m/z* 474 and MS fragmentation as compound 2. A peak at *m/z* 139 was recognised as a characteristic fragment ion which was due to the initial cleavage of an unstable bond between C-8 and N-9, followed by a process of splitting bond and of hydrogen transfer between N-1 and C-17, suggesting a methylene group was attached to N-9 and N-13 on a 17-membered lactam ring [11]. In the ^1H NMR spectrum, signals at δ 6.80, 6.81 (each 0.5H, d, $J = 15.4$ Hz) and 7.71, 7.73 (each 0.5H, d, $J = 15.4$ Hz) were assigned as a pair of olefinic protons, suggesting the presence of an amidically bounded *trans*-cinnamoyl residue. In addition, catalytic hydrogenation of compounds 1 and 2 with 10% Pd/C under H_2 yielded one and the same saturated product 3. Thus, the structure of 1 was established as described in figure 1.

In order to confirm the absolute configuration involving a chirality at C-8 position, the CD spectrum of its saturated product 3 was compared with those of (*R*)-(+)- and (*S*)-(−)-α-phenylethylamines [12,13]. The CD spectrum of 3 was shown to be quite similar to that of (*R*)-(+)-α-phenylethylamine, thus the absolute configuration was determined to be *R*.

3. Experimental

3.1 General experimental procedures

^1H NMR and ^{13}C NMR spectra were obtained with a JEOL α-500 spectrometer, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. EI-MS were recorded on a JEOL DX-303 HF spectrometer. CD spectra were measured on a JASCO J-720 spectrometer. TLC: pre-coated 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). Preparative HPLC



1 7',8'-*trans*

2 7',8'-*cis*

3 7',8'-*dihydro*

Figure 1. Structures of compounds **1–3**.

separation was performed on a Hewlett–Packard (HP) Agilent 1100 series HPLC system (Agilent, Yokogawa Analytical Systems, Tokyo, Japan), with a diode array detector operating at 253 nm. The column used in this study was Hydrosphere C18 (5 μm, 250 × 20 mm i.d., YMC Co. Ltd., Japan).

3.2 Plant material

The dried whole plants of *I. sinensis* were collected in Qingdao of Shandong province, China in August 1999, and identified by the fifth author. A voucher specimen was deposited at the Herbarium of Beijing University of Traditional Chinese Medicine and Pharmacy.

3.3 Extraction and isolation

The whole plants (2.0 kg) were extracted twice with 95% EtOH for 2 h and the combined extracts were concentrated to a syrup at 60°C. The residue was then 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 (8.6 g), which was repeatedly subjected to Al_2O_3 column chromatography with $CHCl_3$/-MeOH/H_2O (10:1:0 → 6:4:1) as eluant and silica gel column chromatography with cyclohexane/EtOH/Et_2NH (40:1:1 → 5:1:1). The fractions were combined on the basis of their behaviour on TLC. A fraction seemed to be a pure substance according to its TLC behaviour; however, the multiplicity of ^{1}H NMR and ^{13}C NMR spectral data, and HPLC behaviour, showed it to be a mixture of *cis* and *trans* isomers. Further separation was performed by reverse-phase HPLC elution with CH_3CN/40 mM KH_2PO_4 (23:77) to give compounds 1 (13 mg) and 2 (18 mg).

3.3.1 Incasine C′ (1). An off-white amorphous powder, $[\alpha]_D^{24}$ + 6.4 ($CHCl_3$, *c* 0.08). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.26–1.87 (8H, m, H-3, 11, 15, 16), 2.30–3.78 (16H, m, H-2, 4, 7, 10, 12, 14, 17, 18), 3.98 (1H, m, H-8), 6.80, 6.81 (each 0.5H, d, J = 15.4 Hz, H-7′), 7.71, 7.73 (each 0.5H, d, J = 15.4 Hz, H-8′), 7.09, 7.29–7.42, 7.52 (10H, m, H-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 21.2, 21.3, 25.3, 25.6, 26.7, 26.9, 29.6, 29.7 (C-3, 11, 15, 16), 35.8, 36.4, 43.8, 44.1, 45.8, 47.2, 47.6, 50.8, 53.4, 53.7 (C-2, 4, 7, 10, 12, 14, 17, 18), 64.0 (C-8), 117.1, 117.3 (C-8′), 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 128.6, 128.7, 129.3, 129.4 (C-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″), 135.1, 135.2 (C-1′), 142.0, 142.2 (C-7′), 142.5, 142.6 (C-1″), 165.6, 165.7 (C-9′), 171.7, 171.8 (C-6). EI-MS (*m/z*): 474 ($[M]^+$, 21), 473 (28), 314 (35), 196 (17), 194 (18), 180 (22), 178 (15), 139 (17), 138 (18), 131 (23), 111 (31), 107 (27), 95 (24), 83 (35), 71 (39), 70 (36), 43 (100). HREI-MS (*m/z*): 474.2987 (calcd for $C_{29}H_{38}N_4O_2$, 474.2997).

3.3.2 7′,8′-dihydroincasine C (3). A white powder, $[\alpha]_D^{25}$ + 8.6 ($CHCl_3$, *c* 0.62). EI-MS (*m/z*): 476 ($[M]^+$, 90), 475 (100), 421 (19), 131 (18), 125 (12), 111 (13), 105 (28), 98 (17), 91 (38), 84 (28), 70 (12). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.47–1.83 (8H, m, H-3, 11, 15, 16), 2.37–3.90 (20H, m, H-2, 4, 7, 10, 12, 14, 17, 18, 7′, 8′), 3.98 (1H, m, H-8), 7.08, 7.20, 7.26–7.35 (10H, m, H-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″). CD (MeOH, c = 6.7 mg/2 ml, 220–300

nm): $[\theta]_{249}$ 0, $[\theta]_{250}$ −1010 (peak), $[\theta]_{252}$ +270 (trough), $[\theta]_{254}$ −1562 (peak), $[\theta]_{256}$ −511 (trough), $[\theta]_{260}$ −2267 (peak), $[\theta]_{264}$ −851 (trough), $[\theta]_{266}$ −2261 (peak), $[\theta]_{273}$ 0.

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