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INCARVINE A, A MONOTERPENE ALKALOID FROM *INCARVILLEA SINENSIS*

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Key Word Index—*Incarvillea sinensis*; Bignoniaceae; incarvine A; monoterpene alkaloid; anti-rheumatism agent.

Abstract—A novel ester compound comprised of a monoterpene alkaloid and a monoterpene, named incarvine A, has been isolated from *Incarvillea sinensis*. Its chemical structure was characterized by chemical and spectroscopic means.

INTRODUCTION

Incarvillea sinensis has been used to treat rheumatism and relieve pain. An additional novel monoterpene alkaloid derivative designated as incarvine A (**1**) has now been characterized after the isolation and structural determination of incarvilline [1] and incarvillateine [2] from this crude drug.

RESULTS AND DISCUSSION

An ethanolic extract of the aerial parts of the title plant was subjected to weak acid treatment and silica gel column chromatography to provide incarvine A (**1**) as a white powder, $[\alpha]_D$ −0.9 ($CHCl_3$), in a yield of 0.23%. The EI mass spectrum showed peaks at *m/z* 529 ($[M + 1]^+$, 62%), 347 (12.7%), 319 (7.3%), 265 (20.5%) and 182 (100%). HR mass spectrometry suggested its molecule formula to be $C_{32}H_{52}O_4N_2$. ^{13}C NMR signals (Table 1) disclosed the presence of the two sets of the monoterpene alkaloid moiety, incarvilline, whose structure previously was determined by X-ray analysis [1]. The remaining signals suggested the occurrence of a monoterpenoic acid including two ester carboxylic carbons, two methyl groups, two tri-substituted double bonds and two methylene carbons. The proton signals were also attributed by ^1H–^1H COSY NMR (Table 1), indicating the presence of two moles of the incarvilline moiety and one monoterpene residue composed of two olefinic methyl groups, two olefinic protons and two methylene groups. This NMR evidence led to the identification of the monoterpene as Hildebrandt's acid [3], which has been obtained previously as a metabolite of monoterpenes in animals. The ^1H–^{13}C long-range NMR correlation between H-7, H-7′ on the alkaloid moieties and C-1″, C-10″ on the monoterpene residue revealed that the linkage was between C-7–OH and C-10″. Taking into account the chemical shift of signals at δ 5.28 (2H, *m*) assignable to H-7 and H-7′ in the ^1H NMR spectrum [4], it was apparent that the 7- and 7′-hydroxyl groups were acylated. Alkaline treatment of **1** liberated an alkaloid identical with incarvilline in all respects and the monoterpene, which was methylated with CH_2N_2 and identified as Hildebrandt's acid dimethyl ester. Consequently, the structure of incarvine A (**1**) has been established as shown in the formula. This compound might be one of the substances responsible for relieving pain.

EXPERIMENTAL

General. ^1H and ^{13}C NMR were measured with a JEOL JNM–GX 400 NMR spectrometer and chemical shifts are given in δ with TMS as int. standard. FAB–MS were recorded in a glycerol matrix containing NaI. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) and detection was achieved by spraying with Dragendorff's reagent. CC was carried out with 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 extracted with $CHCl_3$ after weak acid and alkaline treatment. After drying, the solvent was removed to give a residue, which was repeatedly chromatographed on silica gel and eluted with cyclohexane–MeOH–Et_2NH (30:1:1–5:1:1) to provide compound **1**.

Incarvine A (**1**). R_f value 0.77 benzene–Me_2CO–MeOH (7:2:1).

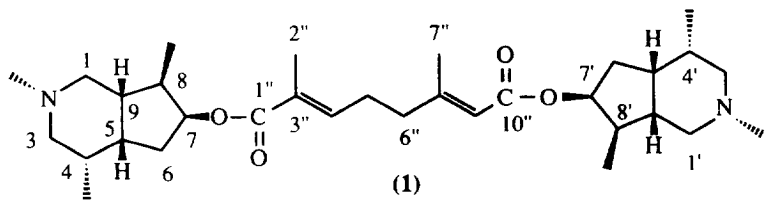
Alkaline hydrolysis of **1**. After a mixt. of incarvine A (**1**, 50 mg) and 3% KOH–MeOH was heated at 60° for 1 hr, the reaction mixt. was acidified and partitioned between EtOAc and H_2O. The organic layer was methylated with CH_2N_2 and successively purified by silica gel CC to give

Table 1. ^{1}H and ^{13}C NMR spectral assignments of compound **1**

Incarvilline moiety	1,1′	3,3′	4,4′	5,5′	6,6′	7,7′	8,8′	9,9′	N, N′-Me	4, 4′-Me	8, 8′-Me
^{1}H	2.72 (2H, *m*)	2.58 (2H, *m*)	1.85–2.18 (2H, *m*)	2.39 (2H, *m*)	1.57 (2H, *m*) 1.85–2.18 (2H, *m*)	5.28 (2H, *m*)	1.85–2.18 (2H, *m*)	1.85–2.18 (2H, *m*)	2.29 (6H, *s*)	0.87 (6H, *d* $J = 7.0$)	0.94, 0.96 (each, 3H, *d* $J = 7.3$)
^{13}C	57.1	57.3	30.2	37.3 37.4	29.6 29.7	75.1 75.9	40.6 40.7	45.7 *	45.9 *	17.1 *	14.6 *

Monoterpene moiety	1″	2″	3″	4″	5″	6″	7″	8″	9″	10″
^{1}H	—	1.84 (3H, *s*)	—	6.69 (1H, *t*)	2.25–2.45 (2H, *m*)	2.25–2.45 (2H, *m*)	2.17 (3H, *s*)	—	5.68 (1H, *s*)	—
^{13}C	167.3	12.2	128.7	139.9	26.4	39.3	18.6	157.9	116.1	166.2

*Signals overlapped in each column.



(1)

a compound identical with Hildebrandt's acid diMe ester, as a colourless 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*). The aq. layer was neutralized and evapd to give a residue which was subjected to Amberlite XAD-2 CC and elution with aq. MeOH affording a monoterpene alkaloid identical with incarvillin ($[\alpha]_D$, ^{1}H and ^{13}C NMR).

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