(−)-14β-Acetoxymatrine and (+)-14α-Acetoxymatrine, Two New Matrine-Type Lupin Alkaloids from the Leaves of *Sophora tonkinensis*

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Two new lupin alkaloids, which are the first ester derivatives of matrine-type lupin alkaloids, (−)-14β-acetoxymatrine and (+)-14α-acetoxymatrine, were isolated from the leaves of *Sophora tonkinensis* along with fourteen known lupin alkaloids: 17-oxo-α-isosparteine, (−)-sophocarpine, (+)-matrine, (−)-14β-hydroxymatrine, (+)-14α-hydroxymatrine, 13,14-dehydrosophoranol (5α-hydroxysophocarpine), (+)-sophoranol, (+)-9α-hydroxymatrine, lamprolobine, (+)-5α,9α-dihydroxymatrine, (−)-baptifoline, (+)-matrine *N*-oxide, (+)-sophocarpine *N*-oxide, and (+)-sophoranol *N*-oxide.

Key words *Sophora tonkinensis*; Leguminosae; lupin alkaloid; (−)-14β-acetoxymatrine; (+)-14α-acetoxymatrine

In the course of our research on the relationship between medicinal application and alkaloid constituents of Chinese drugs, we previously reported the presence of twelve known lupin alkaloids in the roots of *Sophora tonkinensis*,[1] which have been used as the Chinese drug Shan-Dou-Gen[2] to treat fever, throat inflammation, pain, hemorrhoids, tumors, *etc*., and reported that a major lupin alkaloid of this plant, (+)-matrine, and its stereoisomer (+)-allomatrine possesses significant antinociceptive activities which are mediated mainly by κ-opioid receptors.

As a continuation of these studies, we have examined the constituents of the fresh leaves of this plant and characterized two new alkaloids, (−)-14β-acetoxymatrine (**1**) and (+)-14α-acetoxymatrine (**2**), together with fourteen known lupin alkaloids (**3**—**16**) (Chart 1). The new alkaloids are the first examples of matrine-type alkaloids having an ester group. This paper describes the structural elucidation of the two new lupin alkaloids and the variations in alkaloid content in the roots and the leaves of *S. tonkinensis*.

The leaves of *S. tonkinensis*, which were collected in Guang-Xi province of China in July, 1993, gave an alkaloid mixture in a yield of 2% (fresh weight). The total base was subjected to repeated silica gel column chromatography to yield two new lupin alkaloids, (−)-14β-acetoxymatrine (**1**, 0.06%/total base) and (+)-14α-acetoxymatrine (**2**, 0.05%), together with seven known alkaloids, 17-oxo-α-isosparteine (**14**, trace), (+)-14α-hydroxymatrine (**4**, trace), (+)-9α-hydroxymatrine (**7**, 0.3%), lamprolobine (**15**, trace), (+)-5α,9α-dihydroxymatrine (**6**, 1%), (−)-baptifoline (**16**, trace) and (+)-sophoranol *N*-oxide (**10**, 3%), which have not been isolated previously from this plant, and the previously reported (−)-sophocarpine (**11**, 1.3%), (+)-matrine (**8**, 24%), (−)-14β-hydroxymatrine (**3**, trace), 13,14-dehydrosophoranol, (**12**, trace), (+)-sophoranol (**5**, 25%), (+)-matrine *N*-oxide (**9**,

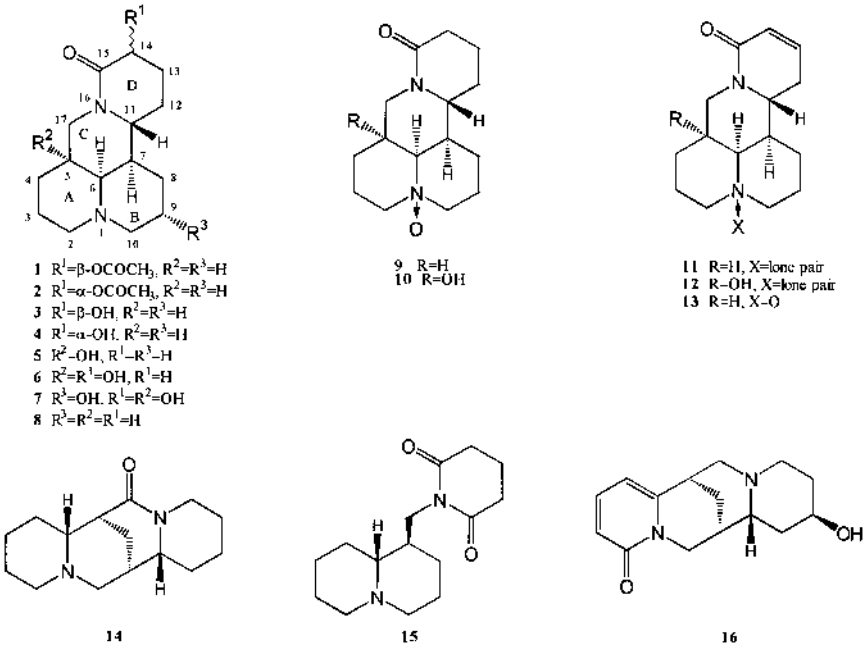

Chart 1

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of **1**—**3** in $CDCl_3$, δ ppm, J (Hz)

Carbon	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	57.2	2.80, dm, J=12.8 (β-H)	57.3	2.82, dm, J=11.6 (β-H)	57.1	2.80, dm, J=13.6 (β-H)
3	21.1		21.2		21.1	
4	27.7		27.7		27.7	
5	35.1		35.7		35.2	
6	63.4		63.9		63.3	
7	43.7		41.5		43.9	
8	26.0		26.7		26.0	
9	20.7		20.7		20.7	
10	57.1	2.80, dm, J=12.8 (β-H)	57.3	2.82, dm, J=11.6 (β-H)	57.1	2.80, dm, J=13.6 (β-H)
11	53.3	3.82, ddd, J=10.4, 10.4, 5.5	53.2	3.90, ddd, J=10.4, 4.9, 4.9	53.9	*ca*. 3.89, m
12	25.1		21.1		25.1	
13	25.4		24.0		27.3	
14	69.1	5.20, dd, J=11.9, 5.5 (α-H)	69.7		67.9	*ca*. 3.89, m, (α-H)
				5.21, dd, J=5.5, 5.5 (β-H)		
15	170.4		170.0		171.8	
17	41.6	4.32, dd, J=12.8, 4.9 (α-H)	42.6	4.30, dd, J=12.8, 4.9 (α-H)	41.9	4.25, dd, J=12.8, 4.3 (α-H)
		3.09, dd, J=12.8, 12.8 (β-H)		3.13, dd, J=12.8,12.8 (β-H)		3.15, dd, J=12.8, 12.8 (β-H)
$\underline{C}OCH_3$	166.5		165.6			
$CO\underline{C}H_3$	21.0	2.12, s	21.0	2.11, s		

37%), and (+)-sophocarpine *N*-oxide (**13**, 0.3%). The structures of the known alkaloids were identified by comparison with authentic samples (co-TLC, co-HPLC, $[\alpha]_D$, MS, IR and ^{1}H-, ^{13}C-NMR spectral data).[3)]

The molecular formula of the new alkaloid (**1**, colorless crystals, mp 113 °C, $[\alpha]_D^{25}$ −26.7°) was determined to be $C_{17}H_{26}N_2O_3$ from the high resolution-electron impact (HR-EI)-MS spectrum. Its IR spectrum ($CHCl_3$) showed absorptions due to an ester group (1100, 1740 cm^{-1}) and a tertiary amide group (1650 cm^{-1}). The EI-MS spectrum of **1** ($[M]^+$ *m*/*z* 306 (65)) showed peaks at *m*/*z* 246 (100), corresponding to $[M-CH_3COOH]^{+\cdot}$, indicating the presence of an acetoxyl group in the molecule. This was confirmed by the presence of a signal (s, 3H) at δ 2.12 which was assigned to an acetyl methyl in the ^{1}H-NMR spectrum of **1** ($CDCl_3$). The fragment ions at *m*/*z* 277 and *m*/*z* 263 in the EI-MS spectra were 58 mass units ($C_2H_2O_2$) larger than those (*m*/*z* 219, 205) of (+)-matrine (**8**) which were made up of the A/C/D rings or B/C/D rings.[3)] The other main fragment ions at *m*/*z* 177 (12), 150 (19), 148 (12), 136 (13) and 96 (24) were very similar to those of (+)-matrine which were made up of the A/B/C rings. From these results, the new alkaloid **1** would be assumed to be a matrine-type alkaloid possessing an acetoxyl group on the D ring. The ^{1}H-NMR spectrum of **1** ($CDCl_3$) showed signals corresponding to H-11, H-17β and H-17α which were all similar to those of (−)-14β-hydroxymatrine (**3**) (Table 1). The spectrum exhibited a signal due to a methine proton bearing an acetoxyl group at δ 5.20 (1H, dd, J=11.9, 5.5 Hz). In the ^{13}C-NMR spectrum of **1**, the signals corresponding to C-2—C-11 and C-17 on the A, B and C rings were consistent with those of (−)-14β-hydroxymatrine (**3**) (Table 1). The remaining signals at δ 69.1 (d), 25.4 (t), and 25.1 (t) were reasonably assigned to C-14, C-13, and C-12, respectively, by considering the substituent effects of an acetoxyl group[4)] on the basis of the ^{13}C-NMR assignment of **3**. The above results indicated that the acetoxyl group was situated at C-14 and oriented equatorially. Therefore, the new alkaloid **1** was assigned to be (−)-14β-acetoxymatrine.

Table 2. Distribution of Lupin Alkaloids in the Roots and Leaves of *Sophora tonkinensis* (%/Total Base)

Alkaloid	Roots	Leaves
(−)-14β-Acetoxymatrine (**1**)	—	Trace
(+)-14α-Acetoxymatrine (**2**)	—	Trace
(−)-14β-Hydroxymatrine (**3**)	0.09	Trace
(+)-14α-Hydroxymatrine (**4**)	—	Trace
(+)-Sophoranol (**5**)	2	25
(+)-5α,9α-Dihydroxymatrine (**6**)	—	1
(+)-9α-Hydroxymatrine (**7**)	—	0.3
(+)-Matrine (**8**)	65	24
(+)-Matrine *N*-oxide (**9**)	12	37
(+)-Sophoranol *N*-oxide (**10**)	—	3
(−)-Sophocarpine (**11**)	1.5	1.3
13,14-Dehydrosophoranol (**12**)[a)]	4	Trace
(+)-Sophocarpine *N*-oxide (**13**)	0.8	0.3
17-Oxo-α-isosparteine (**14**)[a)]	—	Trace
Lamprolobine (**15**)[a)]	—	Trace
(−)-Baptifoline (**16**)	—	Trace

a) Optical rotation has not been measured due to the shortage of material.

The second new alkaloid **2** (colorless crystals, mp 96 °C, $[\alpha]_D^{25}$ +33.0°) had the same molecular formula ($C_{17}H_{26}N_2O_3$) as that of **1**. Its IR spectrum ($CHCl_3$) also showed the absorption due to an ester (1100, 1740 cm^{-1}) and a tertiary amide (1650 cm^{-1}) group. The EI-MS spectrum of **2** resembled that of **1**. The ^{13}C-NMR spectrum ($CDCl_3$) of **2** was also similar to that of **1** (Table 1), except for the up-field shift of the signal (δ 21.1) of C-12 by 4.0 ppm compared with that (δ 25.1) of **1**. This difference in the chemical shifts can be explained by an γ-effect of the axial acetoxyl group in **2**. The axial orientation of the acetoxyl group in **2** was confirmed by the coupling characteristics of the signals due to the acetoxymethine proton (H-14, δ 5.12, dd, J=5.5, 5.5 Hz) of **2**. Thus, the structure of **2** was presumed to be (+)-14α-acetoxymatrine.

Lupin alkaloids are classified into three main structural types, namely bicyclic lupinine-type, tetracyclic sparteine-type and tetracyclic matrine-type. It is generally accepted in the biosynthesis of lupin alkaloids that they are first pro-

duced in the intact form of lupinine, sparteine and matrine from L-lysine and then oxidatively metabolized to their hydroxyl and/or unsaturated derivatives. Some of the hydroxyl derivatives of lupinine- and sparteine-type alkaloids have frequently been found in the form of ester, but those of matrine-type alkaloids have never been isolated in ester forms.[3] This is the first case of isolation in the form of ester of matrine-type lupin alkaloids.

Variations in the alkaloid contents at the roots and the leaves of *S. tonkinensis* are shown in Table 2. Compared with the alkaloids found in the roots, the leaves contained the sparteine- and anagyrine-types (**14**, **16**) and lupinine type (**15**). The main alkaloid in the roots was (+)-matrine (**8**, 65%/total base), and in leaves (+)-matrine *N*-oxide (**9**, 37%) and (+)-matrine (**8**, 25%) were the main alkaloids.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus without correction. Optical rotations were measured on a JASCO DIP-181 polarimeter at 25 °C with EtOH as a solvent. IR spectra were measured with a JASCO FT/IR-200 spectrometer. High and low-resolution MS were measured at 70 eV using a direct inlet system on a JEOL D300 spectrometer. ^{1}H-NMR (270, 500 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on Kieselgel (Merck, 70—230 mesh, or 230—400 mesh), Wako gel C-300 and Lichrospher Si 60 (40—63 μm, Merck).

Plant Material The leaves of *Sophora tonkinensis* were identified by Prof. Jia-Shi Li and Yu-Ning Yan, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine.

Extraction and Isolation of Alkaloids Dry leaves (4.9 kg) of *Sophora tonkinensis* were extracted with 75% aq. MeOH three times at room temperature. The concentrate was acidified to pH 4 with 10% HCl and extracted three times with Et_2O. Then the aqueous layer was made alkaline with K_2CO_3 to pH 11 and extracted with CH_2Cl_2 three times. The CH_2Cl_2 extracts were dried over K_2CO_3, and concentrated *in vacuo* to give 98.7 g crude base in a yield of 2%. The crude base (15.4 g) was chromatographed on silica gel column (7.5×17 cm) with solvent systems with increasing concentrations of MeOH and 25% NH_4OH in CH_2Cl_2. Seven fractions were obtained. Fraction 2 (32 mg) was further chromatographed on Lichroprep Si 60 (ϕ1×25 cm) with solvent MeOH : CH_2Cl_2 : 2.5% NH_4OH (15 : 85 : 2) to give (−)-14β-acetoxymatrine (**1**, mp 113 °C, $[\alpha]_D^{25}$ −26.7°, 9 mg), (+)-14α-acetoxymatrine (**2**, mp 96 °C, $[\alpha]_D^{25}$ +33.0°, 7 mg) and 17-oxo-α-isospartein (**14**, 5 mg). Fraction 3 (4.2 g) was further separated to five alkaloids with solvent MeOH : CH_2Cl_2 : 25% NH_4OH (1 : 240 : 1) on silica gel column chromatography (ϕ5×27 cm) to yield (−)-sophocarpine (**11**, mp 81—82 °C, $[\alpha]_D^{25}$ −29.4°, 0.2 g), (+)-matrine (**8**, mp 77 °C,$[\alpha]_D^{25}$ +39.1°, 3.7 g), (−)-14β-hydroxymatrine (**3**, mp 69—70 °C, $[\alpha]_D^{25}$ −76.5°, 7 mg), (+)-14α-hydroxymatrine (**4**, mp 120 °C, $[\alpha]_D^{25}$ +30.4°, 8 mg), and 13,14-dehydrosophoranol, (**12**, 4 mg). Fraction 4 (4.0 g) contained (+)-sophoranol (**5**, mp 171 °C, $[\alpha]_D^{25}$ +66.0°, 3.9 g). Fraction 5 (300 mg) was further chromatographed on silica gel (30 g) column and solvent CH_2Cl_2 : MeOH : 25% NH_4OH (90 : 9 : 1) to yield four alkaloids, lamprolobine (**15**, oil, 3 mg), (+)-5α,9α-dihydroxymatrine (**6**, mp 192—193 °C, $[\alpha]_D^{25}$ +40.6°, 154 mg), (+)-9α-hydroxymatrine (**7**, mp 155 °C, $[\alpha]_D^{25}$ +32.0°, 46 mg) and (−)-baptifoline (**16**, mp 210 °C, $[\alpha]_D^{25}$ −137.2°, 10 mg). Fraction 6 (6.4 g) contained (+)-matrine *N*-oxide (**9**, mp 162—163 °C, $[\alpha]_D^{25}$ +47.7°, 5.7 g), (+)-sophocarpine *N*-oxide (**13**, mp 208—210 °C, $[\alpha]_D^{25}$ +37.0°, 46 mg) and (+)-sophoranol *N*-oxide (**10**, mp 259—261 °C, $[\alpha]_D^{25}$ +38.1°, 462 mg) which were separated with solvent CH_2Cl_2 : MeOH : 25% NH_4OH (90 : 9 : 1) on silica gel column chromatography (ϕ4.5×45 cm).

The known alkaloids were identified by direct comparison with the authentic samples (mp, TLC, HPLC, GC, IR, MS, and NMR spectral data).

(−)-14β-Acetoxymatrine (**1**): Colorless crystals from dichloromethane–*n*-hexane, mp 113 °C, $[\alpha]_D^{25}$ −26.7° (*c*=1, EtOH). HR-MS *m*/*z*: 306.1942 (Calcd for $C_{17}H_{26}N_2O_3$: 306.1943). EI-MS *m*/*z* (rel. int. %): 306 (M^+, 65), 305 ($[M-H]^+$, 33), 277 (3), 263 (28), 247 ($[M-OCOCH_3]^+$, 27), 246 ($[M-CH_3COOH]^+$, 100), 218 (65), 177 (12), 150 (19), 148 (12), 136 (13), 96 (24). ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1.

(+)-14α-Acetoxymatrine (**2**): Colorless crystals from dichloromethane–*n*-hexane, mp 96 °C, $[\alpha]_D^{25}$ +33.0° (*c*=1, EtOH). HR-MS: *m*/*z*: 306.1937 (Calcd for $C_{17}H_{26}N_2O_3$: 306.1943). EI-MS *m*/*z* (rel. int. %): 306 ($[M]^+$, 100), 305 ($[M-H]^+$, 87), 277 (6), 263 (77), 247 ($[M-OCOCH_3]^+$, 9), 246 ($[M-CH_3COOH]^+$, 25), 218 (45), 177 (12), 150 (23), 148 (11), 136 (12), 96 (27). ^{1}H- and ^{13}C-NMR ($CDCl_3$): Table 1.

Acknowledgement We thank Iwaki Scholarship Foundation for supporting this research.

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