

(–)-14β-Hydroxymatrine, a New Lupine Alkaloid from the Roots of *Sophora tonkinensis*

Ping XIAO,^a Jiashi LI,^b Hajime KUBO,^a Kazuki SAITO,^c Isamu MURAKOSHI,^c and Shigeru OHMIYA^{*,a}

Faculty of Pharmaceutical Sciences, Hoshi University,^a 4–41, Ebara 2-chome, Shinagawa-ku, Tokyo 142, Japan, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine,^b Hepig Bei Kou, Beijing 100029, China, and Faculty of Pharmaceutical Sciences, Chiba University,^c 1–33 Yayoi-cho, Inage-ku, Chiba 260, Japan.

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A new lupine alkaloid, (–)-14β-hydroxymatrine (1**) was isolated from the dry roots of *Sophora tonkinensis* together with 11 known lupine alkaloids. The absolute structure (5*S*,6*S*,7*R*,11*R*,14*S*) of the new base was confirmed by comparison of the natural product with a synthetic sample derived from (+)-matrine.**

Key words lupine alkaloid; quinolizidine alkaloid; (–)-14β-hydroxymatrine; absolute structure; (+)-matrine; Leguminosae

Sophora tonkinensis is a deciduous shrub belonging to Leguminosae and its dry roots have been used to treat asthma, hemorrhoids, allergic dermatitis, etc. as the Chinese drug San-Zi-Gong.¹⁾ The previous study on the alkaloid constituents in the roots of *S. tonkinensis* demonstrated the presence of (+)-matrine, (+)-sophocarpine *N*-oxide, (+)-matrine *N*-oxide, (–)-cytisine, (+)-sophoramine and (+)-sophoranol.²⁾ Further examination on the alkaloidal constituents in the roots of this plant has resulted in isolation of a new alkaloid, (–)-14β-hydroxymatrine (**1**), and 7 known lupine alkaloids, (+)-allomatrine, (+)-lemannine, (+)-7,11-dehydromatrine, (–)-sophocarpine, (+)-13,14-dehydrosophoranol, (–)-*N*-methylcytisine and (–)-*N*-formylcytisine, which have not been isolated previously from this source, in addition to previously reported (+)-matrine, (+)-matrine *N*-oxide, (+)-sophocarpine *N*-oxide, and (+)-sophoranol. We now report the isolation of 12 lupine alkaloids from the roots of *S. tonkinensis* and the structural determination of the new alkaloid (**1**), including the absolute stereochemistry.

Results and Discussion

The dry roots of *Sophora tonkinensis*, which were collected in July, 1993 in Guang-Xi province in China, were treated as described previously³⁾ to give a crude alkaloid fraction in a yield of 2.1%. The alkaloid mixture obtained was separated by repeated column chromatography on silica gel and aluminum oxide to afford a new alkaloid, (–)-14β-hydroxymatrine (**1**, 0.09% of total base) together with 11 known alkaloids, (+)-matrine (**2**, 65%), (+)-matrine *N*-oxide (12%), (+)-allomatrine (1%), (+)-lemannine (trace), (+)-7,11-dehydromatrine (leontalbinine) (1.5%), (–)-sophocarpine (1.5%), (+)-sophocarpine *N*-oxide (0.8%), (+)-13,14-dehydrosophoranol ((–)-5α-hydroxysophocarpine) (4%), (+)-sophoranol (2%), (–)-*N*-methylcytisine (3.5%) and (–)-*N*-formylcytisine (1.5%), which were identified by direct comparison with authentic samples (co-TLC, co-HPLC, [α]_D, MS, IR and ¹H-NMR), except for (+)-lemannine, which was identified by comparison of the spectral data with the reported values.⁴⁾

The new alkaloid **1** formed colorless needles, [α]_D –8.3° (EtOH), mp 69–70 °C, from CH₂Cl₂–*n*-hexane. The

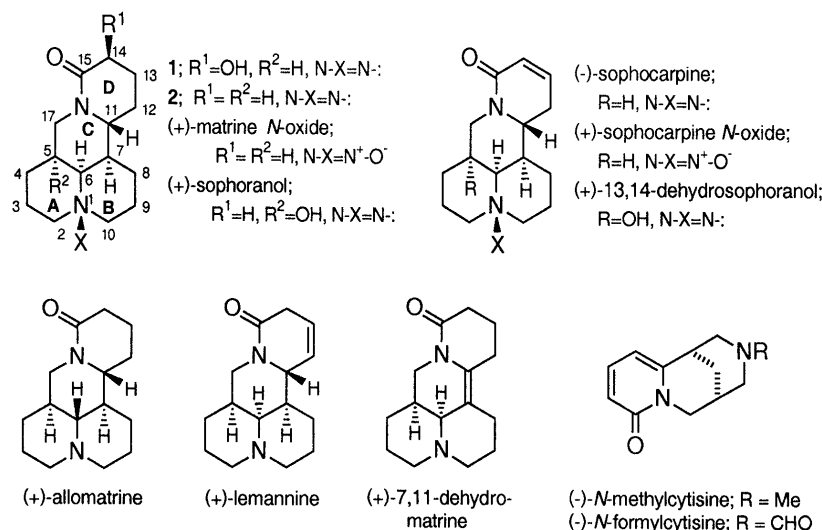


Chart 1

* To whom correspondence should be addressed.

molecular formula was determined by high-resolution mass spectrometry as $C_{15}H_{24}N_2O_2$ (M^+ m/z 264.1836; calcd for 264.1836). The electron impact-MS (EI-MS) of **1** (M^+ m/z 264, 100%) showed fragment ions at m/z 247 (22) and 246 (20) corresponding to $[M-OH]^+$ and $[M-H_2O]^+$, respectively, indicating the presence of a hydroxyl group in the molecule. The fragment ions at m/z 235 and m/z 221 were 16 mass units larger than those (m/z 219 and m/z 205) of matrine (**2**) which are made up of the A/C/D rings or the B/C/D rings.⁵⁾ The other main fragment ions at m/z 192 (35), 177 (21), 150 (57), 137 (31), 136 (31) and 96 (80) were very similar to those of (+)-matrine, and are characteristic fragment ions of **2** and its stereoisomers, arising from the A/B/C rings.⁵⁾ These results suggested that the new alkaloid **1** is a matrine-type alkaloid in which the hydroxyl group is on the D ring.

The IR spectrum ($CHCl_3$) of **1** shows absorptions attributable to a lactam carbonyl group (1626 cm^{-1}) and a hydroxyl group (3503 cm^{-1}). The $^1\text{H-NMR}$ spectrum ($CDCl_3$) of **1** also resembled that of (+)-matrine (**2**), as shown in Table 1. The spectrum exhibited one more signal at δ 3.89 in the low-field region which was assigned to a proton on a methine carbon bearing a hydroxyl group. These results suggested that **1** is a hydroxyl derivative of (+)-matrine (**2**) or its enantiomer. In the $^{13}\text{C-NMR}$ spectrum of **1**, the signals corresponding to C2—C11 and C17 on the A, B, and C rings were all coincident with those of **2** within 1 ppm (Table 2). The remaining signals at δ 67.9 (d), 27.3 (t), and 25.1 (t) were assigned to C14, C13, and C12, respectively, by considering the substituent effects⁶⁾ of an equatorial hydroxyl group on the basis of the $^{13}\text{C-NMR}$ assignment of **2**. This indicated that the hydroxyl group is situated at C14 and oriented equatorially. Thus, the new alkaloid was presumed to be 14 β -hydroxymatrine (**1**).

The structure of **1** including absolute stereochemistry was determined by comparison of the natural product with a synthetic sample obtained from hydroxylation of (+)-

matrine, whose absolute configuration (5*S*,6*S*,7*R*,11*R*)⁸⁾ is known (Chart 1). (+)-Matrine (**2**) was treated with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) at 0 °C followed by exposure to O_2 gas, and then reduction with $NaHSO_3$ to give a diastereomeric mixture of 14 β -hydroxymatrine (**1**) and 14 α -hydroxymatrine (**3**), which was difficult to separate. The mixture was acetylated and separated by silica gel column chromatography to give the two corresponding acetoxymatrine (**4** and **5**).

The $^1\text{H-NMR}$ spectra of the two acetoxymatrine, **4** and **5**, were very similar and only the coupling characteristics of the signals due to the acetoxymethine proton (C14-H) were different from each other. The methine proton signal of **4** (δ 5.20, dd) showed coupling constants (J) of 12.2 and 5.5 Hz, while that of **5** (δ 5.21, dd) showed $J=5.5$ and 5.5 Hz, indicating that the methine protons of **4** and **5** are oriented axially and equatorially, respectively. This stereochemical feature was confirmed by analysis of the $^{13}\text{C-NMR}$ spectra of **4** and **5** (Table 2). The $^{13}\text{C-NMR}$ signal due to C12 of **5** was shifted to higher field by 4.0 ppm than that of **4**. This can be explained by a γ -effect of the axial acetoxyl group in **5**. Thus, the structures of **4** and **5** were determined to be (–)-14 β -acetoxymatrine and (+)-14 α -acetoxymatrine, respectively. The former compound **4** was hydrolyzed by NH_4OH to give (–)-14 β -hydroxymatrine which was identical with natural (–)-14 β -hydroxymatrine in terms of $^1\text{H-NMR}$ and MS spectra, TLC and HPLC behavior, and $[\alpha]_D$.

From the above results, the structure of the new alkaloid

Table 2. $^{13}\text{C-NMR}$ Data for **1**–**5** [in $CDCl_3$, δ ppm]

R	2 ⁷⁾ H	1 β -OH	3 α -OH	4 β -OAc	5 α -OAc
2	57.4	57.1	57.3	57.2	57.3
3	21.3	21.1	20.7	21.1	21.2
4	27.9	27.7	27.7	27.7	27.7
5	35.5	35.2	36.1	35.1	35.7
6	64.0	63.3	64.3	63.4	63.9
7	43.4	43.9	40.4	43.7	41.5
8	26.6	26.0	26.9	26.0	26.7
9	20.9	20.7	20.4	20.7	20.7
10	57.4	57.1	57.3	57.1	57.3
11	53.3	53.9	53.7	53.3	53.2
12	27.2	25.1	21.2	25.1	21.1
13	19.1	27.3	24.6	25.4	24.0
14	32.9	67.9	68.2	69.1	69.7
15	169.6	171.8	171.0	170.4	170.0
17	41.5	41.9	43.5	41.6	42.6
$COCH_3$				166.5	165.6
$COCH_3$				21.0	21.0

Table 1. $^1\text{H-NMR}$ Data for **1** and **2** [in $CDCl_3$, δ ppm, J (Hz)]

	2	1
2 β -H	2.84, ^{a)} dm, $J=12.8$	2.83, ^{a)} dm, $J=13.6$
10 β -H	2.79, ^{a)} dm, $J=12.8$	2.78, ^{a)} dm, $J=13.6$
11 β -H	3.80, ddd, $J=9.2$	ca. 3.89
14 α -H	—	ca. 3.89
17 α -H	4.39, dd, $J=12.8, 4.3$	4.25, dd, $J=12.8, 4.3$
17 β -H	3.05, dd, $J=12.8, 12.8$	3.15, dd, $J=12.8, 12.8$

a) Assignments for these signals within each column may be reversed.

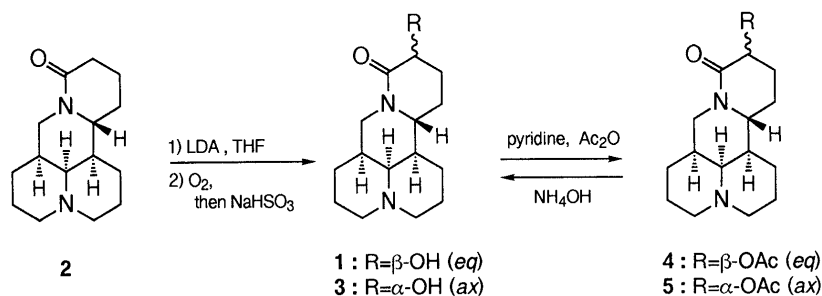


Chart 2

was determined to be (–)-14β-hydroxymatrine (**1**) (5S, 6S, 7R, 11R, 14S).

Experimental

General Procedures Melting points were determined on a Yanagimoto micro melting point apparatus without correction. High- and low- resolution MS were measured at 70 eV using a direct inlet system on a JEOL D300 spectrometer. ¹H-NMR and ¹³C-NMR spectra were recorded on a JEOL JNM-GSX270 (¹H; 270 MHz) or a JEOL JNM-GX400 (¹H; 400 MHz, ¹³C; 100 MHz), using tetramethylsilane (TMS) as an internal standard. Optical rotations were measured on a JASCO DIP-181 polarimeter. TLC was carried out on Silica gel 60 plates (0.25 mm). Analytical HPLC was performed on a LiChrosorb SI 60 (Merck, 5 mm, i.d. 4–250 mm) column, using a UV detector.

Plant Material *Sophora tonkinensis* was identified by Prof. Li, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine. Roots of *S. tonkinensis* were collected in Guang-Xi province in China in July 1993. Voucher specimens have been deposited in the Herbarium of Beijing University.

Extraction and Isolation of Alkaloids Dry plant materials were cut into small chips and extracted with 75% MeOH several times at room temperature. The aqueous concentrate was acidified with 10% HCl to pH 3 and the resulting precipitate was filtered off. The filtrate was extracted three times with CH₂Cl₂. The aqueous layer was made alkaline with NH₄OH to pH 11 and extracted with CH₂Cl₂ three times. The aqueous layer was saturated with K₂CO₃ and extracted three times with CH₂Cl₂. The CH₂Cl₂ extracts from the alkaline aqueous layers were combined, dried over Na₂SO₄, and concentrated *in vacuo* to give 14.6 g of a crude base. The crude alkaloid mixture (10.9 g) was chromatographed on a silica gel column (Wako C-300, 455 g) with solvent systems containing increasing concentrations of MeOH and 28% NH₄OH in CH₂Cl₂. The fractions obtained were further separated by silica gel column chromatography and then purified by aluminum oxide column chromatography or preparative HPLC to give 12 alkaloids, (+)-lemannine (6.5 mg), (–)-sophocarpine (170 mg), (+)-matrine (**2**, 7.1 g), (–)-14β-hydroxymatrine (**1**, 10 mg), (+)-13,14-dehydrosophoranol (452 mg), (+)-sophoranol (236 mg), (–)-N-methylcytisine (330 mg), (–)-N-formylcytisine (160 mg), (+)-7,11-dehydromatrine (160 mg), (+)-allomatrine (103 mg), (+)-matrine N-oxide (1.2 g), and (+)-sophocarpine N-oxide (88 mg). These alkaloids were identified by direct comparison with authentic samples, except for (+)-lemannine, the spectral data of which were identical with the reported values.⁴⁾

(+)-Lemannine (12,13-Dehydromatrine) Colorless needles, mp 93–94 °C, [α]_D²⁵ +37.0° (c=0.28, EtOH). EI-MS *m/z* (rel. int.): 246 (M⁺, 100), 245 (67), 217 (10), 203 (52), 159 (13), 150 (28), 136 (19), 122 (12), 121 (19), 96 (42). ¹H-NMR (CDCl₃) δ : 5.85 (1H, dm, *J*=10.5 Hz, C12-H), 5.76 (1H, dm, *J*=10.5 Hz, C13-H), 4.46–4.56 (2H, m, C11-H and C17 α -H), 3.11 (1H, dd, *J*=12.1, 12.1 Hz, C17 β -H), 2.93 (2H, m, C14-H₂). ¹³C-NMR (CDCl₃) δ : 166.0 (s, C(15)), 123.6 (d, C(13)), 122.6 (d, C(12)), 64.0 (d, C(6)), 57.2 (t, C(2)), 57.2 (t, C(10)), 54.7 (d, C(11)), 44.4 (d, C(7)), 41.6 (t, C(17)), 35.8 (d, C(5)), 29.7 (t, C(14)), 27.8 (t, C(4)), 26.7 (t, C(8)), 21.0 (t, C(3)), 21.0 (t, C(9)).

(–)-14β-Hydroxymatrine (1**)** Colorless needles from CH₂Cl₂-*n*-hexane, mp 69–70 °C, [α]_D²¹ –8.3° (c=0.28, EtOH). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{–1}: 3503 (OH), 1626 (C=O). EI-MS *m/z* (rel. int.): 264.1836 (M⁺, calcd for C₁₅H₂₄N₂O₂, 264.1836, 100), 263 (70), 247 ([M–OH]⁺, 6), 246 ([M–H₂O]⁺, 6), 235 (12), 222 (42), 221 (46), 218 (32), 193 (52), 192 (26), 178 (18), 177 (16), 150 (40), 148 (23), 136 (21), 98 (23), 96 (42).

Synthesis of 14-Acetoxymatrine **4 and **5**** A 1.6 M solution of *n*-BuLi in hexane (0.6 ml, 0.96 mmol) was added to a solution of diisopropylamine (33.6 μ l) in THF (3 ml) at 0 °C with stirring. After several minutes, a solution of **2** (51 mg, 0.12 mmol) in THF (2 ml) was added to the LDA solution at 0 °C and the whole was stirred for 10 min, then exposure to dry O₂ gas. An aqueous solution of NaHSO₃ was added, and the reaction mixture was stirred for 10 min, made alkaline with K₂CO₃, and extracted three times with CH₂Cl₂. The organic layers were combined and dried

over K₂CO₃. The solvent was evaporated *in vacuo*. The residue was subjected to silica gel column chromatography (Wako, C-300, 10 g) to give a mixture of **1** and **3**, which was acetylated with pyridine–Ac₂O at room temperature. The reaction mixture was separated by silica gel column chromatography to give **4** (15.9 mg, 18.2%) and **5** (16.1 mg, 18.4%).

14β-Acetoxymatrine (4**)** Colorless crystals from *n*-hexane, mp 113–114 °C. EI-MS *m/z* (rel. int.): 306.1942 (M⁺, calcd for C₁₇H₂₆N₂O₃, 306.1943, 18), 305 (10), 304 (22), 261 (35), 246 (100), 245 (55), 218 (56), 175 (20), 150 (36), 148 (24), 96 (57). ¹H-NMR (CDCl₃) δ : 5.20 (1H, dd, *J*=12.2, 5.5 Hz, C14-H), 4.31 (1H, dd, *J*=12.8, 4.9 Hz, C17 α -H), 3.83 (1H, ddd, *J*=10.4, 10.4, 5.4 Hz, C11-H), 3.09 (1H, dd, *J*=12.8, 12.8 Hz, C17 β -H), 2.80 (2H, dm, *J*=12.8 Hz, 2 β -H, 10 β -H), 2.12 (3H, s, –COCH₃).

14 α -Acetoxymatrine (5**)** Colorless crystals from *n*-hexane, mp 95–97 °C. EI-MS *m/z* (rel. int.): 306.1937 (M⁺, calcd for C₁₇H₂₆N₂O₃, 306.1943, 98), 305 (80), 264 (60), 263 (100), 246 (41), 245 (30), 218 (70), 150 (50), 96 (79). ¹H-NMR (CDCl₃) δ : 5.21 (1H, dd, *J*=5.5, 5.5 Hz, C14-H), 4.30 (1H, dd, *J*=12.8, 4.9 Hz, C17 α -H), 3.93 (1H, ddd, *J*=10.4, 4.9, 4.9 Hz, C11-H), 3.15 (1H, dd, *J*=12.8, 12.8 Hz, C17 β -H), 2.82 (2H, dm, *J*=11.6 Hz, C2 β -H, C10 β -H), 2.11 (3H, s, –COCH₃).

Hydrolysis of 14β-Acetoxymatrine (4**)** A solution of **4** (10.2 mg, 0.03 mmol) in ethanol (2 ml) was treated with 25% NH₄OH (0.5 ml) and the mixture was stirred at 50 °C for 4 h. The solvent was removed *in vacuo* and H₂O was added to the residue. The aqueous solution was made alkaline with K₂CO₃ and extracted with CH₂Cl₂ three times. The combined extract was dried over K₂CO₃ and evaporated *in vacuo*. The residue was subjected to silica gel column chromatography to give (–)-14β-hydroxymatrine, which was identical with the natural **1** (TLC, HPLC, ¹H-NMR, ¹³C-NMR, mp and [α]_D).

Hydrolysis of 14 α -acetoxymatrine (5**)** Compound **5** was hydrolyzed in the same manner as **4**, described above, to afford (+)-14 α -hydroxymatrine (**3**), colorless crystals from acetone, mp 120 °C, [α]_D²⁵ +30.4 °C (c=0.83, EtOH). EI-MS *m/z* (rel. int.): 264.1836 (M⁺, calcd for C₁₅H₂₄N₂O₂, 264.1838, 100), 263 (90), 247 (5), 235 (11), 222 (45), 221 (42), 218 (21), 193 (38), 192 (20), 178 (17), 177 (16), 150 (45), 148 (20), 136 (20), 96 (42). ¹H-NMR (CDCl₃) δ : 4.17 (1H, dd, *J*=12.8, 4.2 Hz, C17 α -H), 3.96–4.40 (2H, m, C11-H, C14-H), 3.17 (1H, dd, *J*=12.8, 12.8 Hz, C17 β -H), 2.78 (2H, dm, *J*=13.4 Hz, C2 β -H, C10 β -H).

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