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Lupin alkaloids from seeds of *Sophora viciifolia*

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Abstract

Three new lupin alkaloids, (−)-14β-hydroxysophoridine, (−)-12β-hydroxysophocarpine and (−)-9α-hydroxysophocarpine, were isolated from the seeds of *Sophora viciifolia* together with 10 known lupin alkaloids, (+)-9α-hydroxymatrine, (−)-14β-hydroxymatrine, (+)-lupanine, (−)-5,6-dehydrolupanine, (−)-cytisine, (+)-matrine, (+)-matrine *N*-oxide, (−)-sophocarpine, (+)-sophocarpine *N*-oxide and (−)-sophoridine.

Keywords: *Sophora viciifolia*; Leguminosae; Seeds; Bai-Ci-Hua, lupin alkaloid; (−)-14β-Hydroxysophoridine; (−)-12β-Hydroxysophocarpine; (−)-9α-Hydroxysophocarpine

1. Introduction

Plants of the genus *Sophora* are important sources of Chinese drugs. They accumulate lupin alkaloids, particularly matrine-type alkaloids, as main constituents. During our research on the relationship between the medicinal application and alkaloid constituents of Chinese drugs, we previously reported the alkaloid constituents in the roots of *S. tonkinensis* (Xiao et al., 1996). The roots of this species have been used as the Chinese drug Shan-Dou-Gen to treat fever, throat inflammation, hemorrhoids, tumours, etc. (Xiao, 1993a). We have also studied the pharmacological effects of the main alkaloid and found that (+)-matrine had an antinociceptive effect identical to that of pentazocine (Kamei et al., 1997). In the present study, we have examined the alkaloid constituents in the seeds of *S. viciifolia*.

Sophora viciifolia is a bush that grows widely throughout south west China. Its roots have been used as the Chinese drug BAI-CI-HUA to treat fever, cystitis, haematuria, oedema, etc. (Xiao, 1993b). A previous study on the alkaloid constituents of this species showed the presence of (+)-matrine, (+)-matrine *N*-oxide, (−)-sophocarpine, (+)-sophocarpine *N*-oxide, (−)-sophoridine and (+)-sophoramine as the main alkaloids (Wang, Li, Wei, & Ohmiya, 1995), which were contained equally in the seeds and the aerial and ground parts of the plant (Dou et al., 1988). By further examination of the seeds of this plant, we have isolated three new lupin alkaloids, (−)-14β-hydroxysophoridine (**1**), (−)-12β-hydroxysophocarpine (**2**) and (−)-9α-hydroxysophocarpine (**3**), together with the 10 known lupin alkaloids (**4**–**13**). We describe here the structural elucidation of the three new compounds.

2. Results and discussion

Seeds of *S. viciifolia*, which were collected in Yun-Nan province of China, in June, 1993, gave an alkaloid mixture in a yield of 2.8% (fr. wt). The total based were subjected to repeated silica gel column chromatography, to give three new lupin alkaloids, (−)-14β-hydroxysophoridine (**1**, 0.2%/total base), (−)-12β-hydroxysophocarpine (**2**, 0.2%) and (−)-9α-hydroxysophocarpine (**3**, 0.3%), together with five known alkaloids, (+)-9α-hydroxymatrine (**10**, trace), (−)-14β-hydroxymatrine (**9**, trace), (+)-lupanine (**11**, trace), (−)-5,6-dehydrolupanine (**12**, trace) and (−)-cytisine (**13**, trace), which have not been isolated

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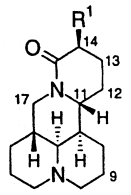
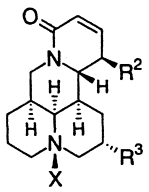
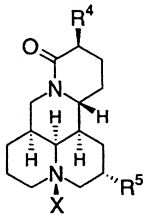


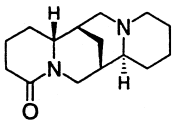
1. R^1=OH, (-)-14βhydroxy sophoridine
4. R^1=H, (-)-sophoridine



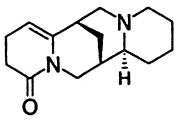
2. R^2=OH, R^3=H,X=lone pair, (-)-12βhydroxysophocarpine
3. R^2=H, R^3=OH, X=lone pair, (-)-9αhydroxysophocarpine
5. R^2=H, R^3=H, X=lone pair, (-)-sophocarpine
6. R^2=H, R^3=H, X=O, (+)-sophocarpine *N*-oxide



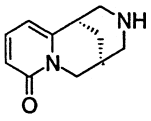
7. R^4=H, R^5=H, X=lone pair, (+)-matrine
8. R^4=H, R^5=H, X=O, (+)-matrine *N*-oxide
9. R^4=OH, R^5=H, X=lone pair, (-)-14β-hydroxymatrine
10. R^4=H, R^5=OH, X=lone pair, (+)-9o-hydroxymatrine



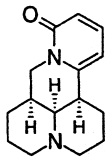
11. (+)-lupanine



12. (-)-5,6-dehydro-lupanine



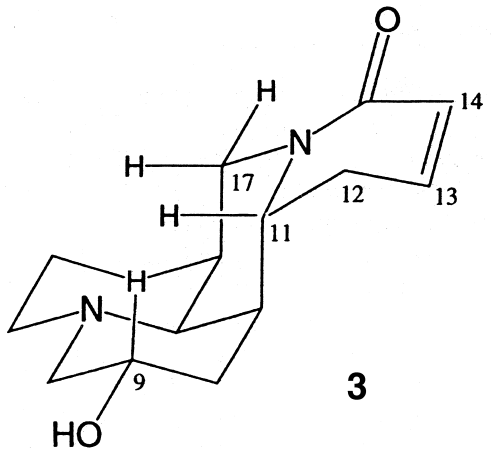
13. (-)-cytisine



14. (-)-sophoramine

previously from this species, and the previously reported (+)-matrine (**7**, 5%), (+)-matrine *N*-oxide (**8**, 25%), (−)-sophocarpine (**5**, 12%), (+)-sophocarpine *N*-oxide (**6**, 35%) and (−)-sophoridine (**4**, 3%). The structures of the known alkaloids were identified by comparison with authentic samples (co-TLC, co-HPLC and $[\alpha]_D$, mass spectrometry, IR and ^{1}H NMR, ^{13}C NMR spectral data (Ohmiya, Saito, & Murakoshi, 1995; Aslanov, Kushmuradov, & Sadykov, 1987).

The molecular formula of the new alkaloid, **1** was determined to be $C_{15}H_{24}N_2O_2$. Its IR spectrum ($CHCl_3$) showed absorption bands due to hydroxyl (ν_{max} 3400 cm^{-1}) and a lactam C=O group (ν_{max} 1635 cm^{-1}). The EI mass spectrum was similar to that of (−)-14β-hydroxymatrine (**9**). These results suggest that **1** was a matrine-type alkaloid possessing a hydroxyl group on the D ring. The ^{1}H NMR spectrum



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of **1** ($CDCl_3$) exhibited signals due to H-17β, H-17α and H-11 at δ 3.58, 3.10 and 3.42, respectively, which were shifted up-field in comparison with those of **9** (Table 1). The spectrum also showed a signal due to a methine proton bearing a hydroxyl group at δ 3.95 (1H, dd, J = 12.2 and 4.9 Hz), which was assigned to the axial proton (α) at C(14) adjacent to the lactam carbonyl based on its coupling characteristics. These results indicated that the new alkaloid **1** was a stereoisomer of **9**. 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 (−)-sophoridine (**4**) (Table 2). The remaining signals at δ 67.9 (d), 27.4 (t) and 26.7 (t) were reasonably assigned to C(14), C(13) and C(12), respectively, by considering the substituent effect of an equatorial hydroxyl group at C(14) Xiao et al., 1951 (Table 2). Therefore, the new alkaloid **1** was presumed to be (−)-14β-hydroxysophoridine.

The second new alkaloid, **2** had a molecular formula of $C_{15}H_{22}N_2O_2$. Its IR spectrum ($CHCl_3$) showed absorptions of hydroxyl (ν_{max} 3360 cm^{-1}) and an α, β-unsaturated lactam system (ν_{max} 1600 cm^{-1} for C=C and ν_{max} 1660 cm^{-1} for C=O). The EI mass spectrum of **2** (m/z 262 $[M]^+$, 18) showed a base peak at m/z 244 (100), corresponding to $[M-H_2O]^+$ and fragment ions very similar to those of (−)-sophoramine (**14**), indicating the presence of a hydroxyl group on the D ring. The ^{1}H NMR spectrum of **2** (in $CDCl_3$) was very similar to that of (−)-sophocarpine (**5**) (Table 1). The spectrum of **2** had one additional isolated signal at δ 4.27 (1H, dd, J = 11.3 and 5.5 Hz) which was assigned

Table 1. ^{1}HNMR data of **1**, **2** and **3** compared with **9** and **5** in $CDCl_3$, δ, J(Hz)

H	1	9	2	5	3
9β	–	–	–	–	ca. 3.83
11	3.42 m	ca. 3.89	4.33 dd (11.3, 11.3)	4.01 dm (11.6)	ca. 3.83
12	–	–	4.27 dd (11.3, 5.5)	–	–
13	–	–	6.60 dd (9.8, 5.5)	6.46 ddd (17.1, 17.1, 7.9)	6.47 dm (9.8)
14	3.95 dd (12.2, 4.9)	ca. 3.89	6.01 d (9.8)	5.89 d (9.9)	5.90 d (9.8)
17α	3.10 dd (12.2, 12.2)	4.25 dd (12.8, 4.3)	4.22 dd (12.5, 4.3)	4.14 dd (12.8, 4.9)	4.14 dd (13.0, 4.3)
17β	3.58 dd (12.2, 4.3)	3.15 dd (12.8, 12.8)	3.20 dd (12.5,2.5)	3.09 dd (12.8, 12.8)	3.10 dd (13.0, 12.9)

to a methine proton bearing a hydroxyl group because of its low chemical shift. Assignment of the ^{13}C NMR spectrum of **2** (in $CDCl_3$) was determined by analysis of H–H COSY and C–H COSY spectra. The signals corresponding to C(2)–C(10) on rings A and B were consistent with those of **5** to within 1.2 ppm (Table 2). The remaining signals at δ 62.1 (d) and 60.5 (d) were assigned to C(12) and C(11) by considering the substituent effects of a hydroxyl group based on the ^{13}C NMR assignment of **5**. Thus, a hydroxyl group was located at the C(12) position. An equatorial hydroxyl group on C12 is supported by the coupling constants between H-12 and H-13 (5.5 Hz) and between H-11 and H-12 (11.3 Hz) in the ^{1}H NMR spectrum of **2**. From the above results, **2** was expected to be (−)-12β-hydroxysophocarpine.

The third new alkaloid **3** had a molecular formula of $C_{15}H_{22}N_2O_2$. Its IR spectrum ($CHCl_3$) also showed absorption of a hydroxyl group (ν_{max} 3360 cm^{-1}) and an α, β-unsaturated lactam system (ν_{max} 1600 cm^{-1} for C=C and ν_{max} 1660 cm^{-1} for C=O). The EI mass spectrum of **3** was also similar to that of (−)-sophocarpine (**5**). The fragment ions at m/z 193 (18), 166 (45) and 96 (74), which are made up of the A/B/C, A/B and A or B rings, respectively, were 16 m.u. larger than those of **5** (m/z 177, 150 and 80) (Dou et al.), 1988. These results indicated that the new alkaloid **3** was a derivative of **5** with a hydroxyl group on the A or B ring. The ^{1}H NMR spectrum of **3** showed signals corresponding to H-14, H-13, H-11, H-17β and H-17α, which were all similar to those of **5** (Table 1). The presence of an equatorial (β) hydroxyl group could be presumed from the signal width (21.1 Hz) of the methine proton bearing the hydroxyl group in the ^{1}H NMR spectrum (C_5D_5N). Assignment of the ^{13}C NMR spectrum of **3** (in $CDCl_3$) was determined by analysis of H–H COSY and C–H COSY spectra. Two possible structures for **3** were considered, one with an equatorial hydroxyl group at C(3), in which case **3** would be 3α-hydroxysophocarpine (**3′**) and another in which the equatorial hydroxyl group is at C(9), in which case **3** would be 9α-hydroxysophocarpine (**3**) (Table 2). To clarify this point, difference nuclear overhauser effect (NOE) spectroscopy (in C_5D_5N) of **3** was used. H-11 was enhanced (7.5%) when the methine proton bearing a hydroxyl group was saturated. This indicated that the equatorial hydroxyl group was at C9. Thus, the new alkaloid **3** was concluded to be (−)-9α-hydroxysophocarpine.

Contents of the main alkaloids (**5**–**8**) in the roots, aerial parts and seeds of *S. viciifolia* were also examined and the results obtained are shown in Table 3.

(+)-Matrine was the main alkaloid in *S. tonkinensis* and comprised ca. 65% of the total bases. In contrast, *S. viciifolia* contained only 5% (+)-matrine and,

Table 2. ^{13}CNMR data of **1**, **2**, **3** and **3′** compared with **4** and **5** in $CDCl_3$, δ

	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-17
1	55.8	23.9	26.7	31.2	63.6	42.3	21.0	20.4	50.5	56.1	29.7	27.4	67.9	172.0	47.7
4	55.8	23.7	28.1	30.7	63.3	40.9	21.8	21.5	50.2	55.7	30.2	18.9	32.5	171.8	47.5
$\Delta\delta_{1-4}$	±0	+ 0.2	+ 1.6	+ 0.5	+ 0.3	+ 1.4	− 0.8	− 1.1	+ 0.3	+ 0.4	− 3.5	+ 8.5	+ 35.4	+ 2.2	+ 0.2
2	57.2	20.9	27.4	35.9	64.3	40.5	26.8	20.5	57.3	60.5	62.1	137.2	126.1	162.5	43.6
5	57.3	21.1	27.8	34.7	63.5	41.6	26.6	20.8	57.3	51.5	27.4	137.3	124.5	165.4	41.9
$\Delta\delta_{2-5}$	− 0.1	− 0.2	− 0.4	+ 1.2	+ 0.8	− 1.1	+ 0.2	− 0.3	±0	+ 9.0	+ 34.7	− 0.1	+ 1.6	− 2.9	+ 1.7
3	57.1	20.7	27.6	34.1	62.5	42.3	35.7	63.1	64.5	52.1	27.3	137.2	124.6	165.4	42.0
$\Delta\delta_{3-5}$	− 0.1	− 0.4	− 0.2	− 0.6	− 0.1	+ 0.7	+ 9.1	+ 42.3	+ 7.3	+ 0.6	− 0.1	− 0.1	+ 0.1	±0	+ 0.1
3′	64.5	63.1	35.7	34.1	62.5	42.3	27.6	20.7	57.1	52.1	27.3	137.2	124.6	165.4	42.0
$\Delta\delta_{3'-5}$	+ 7.3	+ 42.0	+ 7.9	− 0.6	− 0.1	+ 0.7	+ 1.0	− 0.1	− 0.1	+ 0.6	− 0.1	− 0.1	+ 0.1	±0	+ 0.1

Table 3. Alkaloid contents[a] in roots, aerial parts and seeds of *Sophora viciifolia*

Alkaloids	Roots	Aerial parts	Seeds
Total base[b]	0.81	0.42	2.8
(+)-Matrine (**7**)[c]	40	19	5
(+)-Matrine *N*-oxide (**8**)[c]	15	28	25
(−)-Sophocarpine (**5**)[c]	14	9	12
(+)-Sophocarpine *N*-oxide (**6**)[c]	9	23	35

[a]Alkaloid contents were estimated by HPLC.
[b]%/fr. wt.
[c]%/total base.

instead, primarily contained (+)-sophocarpine *N*-oxide (35%) and (−)-sophocarpine (12%).

Pharmacological studies of (+)-sophocarpine *N*-oxide and (−)-sophocarpine are currently under way.

3. Experimental

3.1. General

M.p.'s uncorr. Optical rotations: 25°, 10 cm cell, EtOH. High and low resolution MS were measured at 70 eV using a direct inlet system. ^{1}H NMR (270 and 500 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using TMS as int. standard. Analytical HPLC was carried out with, 5% MeOH in Et_2O–H_2O–25% NH_4OH (500:5:1) for **5** and **7** and 25% MeOH in Et_2O–H_2O–25% NH_4OH (500:20:15) for **6** and **8**, using a LiChrospher Si 60 (5 μm, 0.4 × 25 cm) column.

3.2. Plant material

Seeds of *S. viciifolia* were collected in the Yun-Nan province of China in August 1993. The species was identified by Professor Jia-Shi Li and Yu-Ning Yan, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine. A voucher specimen (No. 1054) is deposited in the Herbarium of Beijing University of Traditional Chinese Medicine.

3.3. Extraction and isolation

Viable seeds (3.75 kg) were extracted ×3 with 75% aq. MeOH at room temp. After evapn of MeOH, the aq. concentrate was acidified to pH 4 with dil. HCl and extracted ×3 with Et_2O. Then, the aq. layer was made alkaline with K_2CO_3 and extracted ×3 with CH_2Cl_2. The CH_2Cl_2 extracts were dried (K_2CO_3) and concd in vacuo to give crude base (105 g) in a yield of 2.8%. This (15.1 g) was chromatographed on a silica gel column with solvent systems containing increasing conc. ns of MeOH and 28% NH_4OH in CH_2Cl_2 and (−)-sophocarpine (1.8 g), (+)-matrine (750 mg), (−)-14β-hydroxymatrine (5.8 mg), (+)-lupanine (19 mg), (−)-sophoridine (0.4 g), (−)-5,6-dehydrolupanine (8.6 mg), (−)-9α-hydroxysophocarpine (**3**, 44.5 mg), (+)-9α-hydroxymatrine (13.1 mg), (−)-12β-hydroxysophocarpine (**2**, 24.5 mg), (−)-14β-hydroxysophoridine (**1**, 30.7 mg), (−)-cytisine (13.4 mg), (+)-matrine *N*-oxide (3.8 g) and (+)-sophocarpine *N*-oxide (5.3 g) were eluted consecutively.

3.4. Identification of known alkaloids

Known alkaloids were identified by direct comparison with the authentic samples (m.p., TLC, HPLC, GC, IR, MS and NMR).

3.4.1. (−)-14β-Hydroxylsophoridine (**1**)

Colourless needles from CH_2Cl_2–*n*-hexane, m.p. 90°. $[\alpha]_D^{25}$ −94.8°(EtOH, *c* 0.47). HR-MS *m*/*z* 264.1835 ($C_{15}H_{24}N_2O_2$ requires: 264.1839). EI-MS *m*/*z* (rel. int.): 264 $[M]^+$ (86), 263 $[M–H]^+$ (100), 247 $[M–OH]^+$ (4), 235 (6), 222 (19), 221 (30), 218 (15), 205 (8), 193 (25), 192 (18), 177 (34), 150 (29), 136 (17), 96 (54). ^{1}H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2.

3.4.2. (−)-12β-Hydroxylsophocarpine (**2**)

Colourless crystals from benzene, m.p. 146°. $[\alpha]_D^{25}$ −215.1°(EtOH, *c* 0.22). HR-MS: *m*/*z* 262.1679 ($C_{15}H_{22}N_2O_2$ requires: 262.1683). EI-MS *m*/*z* (rel. int.): 262 $[M]^+$ (18), 261 $[M–H]^+$ (16), 244 $[M–H_2O]^+$ (100), 243 (78), 215 (17), 160 (7), 149 (36), 136 (82), 122 (12), 96 (30). ^{1}H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2.

3.4.3. (−)-9α-Hydroxylsophocarpine (**3**)

Colourless crystals from benzene, m.p. 120°. $[\alpha]_D^{25}$ −44.2°(EtOH, *c* 0.36). HR-MS: *m*/*z* 262.1677 ($C_{15}H_{22}N_2O_2$ requires: 262.1683). EI-MS *m*/*z* (rel. int.): 262 $[M]^+$ (72), 261 $[M–H]^+$ (82), 245 $[M–OH]^+$ (13), 233 (5), 219 (6), 217 (12), 203 (23), 193 (18), 166 (45), 154 (27), 136 (29), 110 (34), 96 (74). ^{1}H NMR (500 MHz, C_5D_5N): δ 6.32 (1H, dm, J = 9.8 Hz, H-13), 6.05 (1H, d, J = 9.8 Hz, H-14), 4.35 (1H, dd, J = 13.0 and 4.3 Hz, H-17α), 4.08 (1H, m, H-9β), 3.85 (1H, m, H-11), 3.19 (1H, dd, J = 13.0 and 12.9 Hz, H-17β). Difference NOE: H-9 [H-11 (7.5), H-10β (3.3), H-8β (3.9)], H-11 [H-9 (11.1), H-17β (3.3), H-12 (3.8)]. ^{1}H NMR ($CDCl_3$): Table 1. ^{13}C NMR ($CDCl_3$): Table 2.

Acknowledgements

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