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A NEW QUINOLIZIDINE ALKALOID FROM *Sophora flavescens*

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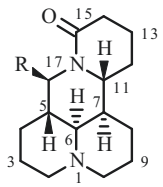
We studied the alkaloid constituents and bioactivity of Sophora flavescens *(Leguminosae). The constituents were isolated with silica gel column chromatography, semi-preparative HPLC, Sephadex LH-20, and MPLC packed with MCI gel, and their structures were elucidated on the basis of physical characteristics and spectral data. Compounds **1** and **5–8** were evaluated for their* in vitro *cytotoxicity against human tumor HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines. Ten alkaloids were obtained, and their structures were identified as 17*β*-hydroxysophoridine (**1**), matrine (**2**), sophocarpine (**3**), sophoridine (**4**), isomatrine (**5**), 7,11-dehydromatrine (**6**), mamanine (**7**), sophoranol (**8**), oxymatrine (**9**), and oxysophocarpine (**10**). Compound **1** is a new alkaloid, and compound 7 was isolated from the* Sophora flavescens *for the first time. None of the compounds were cytotoxic to five human cancer cell lines.*

Keywords: *Sophora flavescens*, Leguminosae, alkaloids, cytotoxicity.

Sophora flavescens Aiton is a perennial herbaceous plant widely distributed in China, Russia, and India [1, 2]. Recent studies showed that it has antibacterial, antipyrotic, antipyretic, antiarrhythmic, antiasthmatic, antiulcerative, anti-HBV, and antineoplastic effects, and it has been used to treat jaundice, leukorrhea, carbuncles, pyogenic infections of the skin, scabies, enteritis, and dysentery [1, 3]. Previous phytochemical investigations led to the isolation of alkaloids, flavonoids, coumarins, phenylpropanoids, quinones, and triterpenoid saponins from this species [4]. A phytochemical investigation on the constituents of *S. flavescens* was carried out and led to the isolation of a new quinolizidine alkaloid, 17*β*-hydroxysophoridine (**1**), along with nine known quinolizidine alkaloids, matrine (**2**) [5], sophocarpine (**3**) [5], sophoridine (**4**) [5], isomatrine (**5**) [6], 7,11-dehydromatrine (**6**) [7], mamanine (**7**) [8], sophoranol (**8**) [9], *N*-oxymatrine (**9**) [5], and *N*-oxysophocarpine (**10**) [5]. The current report describes the isolation, structure elucidation, and cytotoxic evaluation of these compounds.



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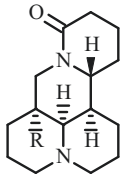

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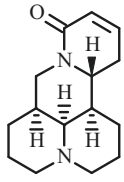

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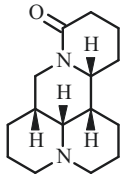

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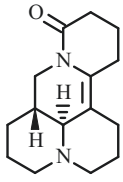

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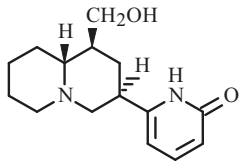

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1, 8: R = OH; **2, 4:** R = H

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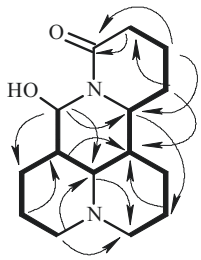
TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectra of **1** (CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2a	2.97 (1H, m)	55.4	10a	2.94 (1H, m)	48.8
2b	2.68 (1H, td, J = 12.9, 2.6)		10b	2.57 (1H, td, J = 11.2, 5.5)	
3a	1.79 (1H, m)	20.8	11	3.48 (1H, ddd, J = 9.3, 5.9, 3.2)	54.7
3b	1.52 (1H, m)		12a	1.91 (1H, m)	27.2
4a	2.26 (1H, m)	31.0	12b	1.78 (1H, m)	
4b	1.29 (1H, m)		13a	1.79 (1H, m)	18.2
5	1.84 (1H,m)	34.9	13b	1.70 (1H, m)	
6	2.44 (1H, dd, J = 12.6, 8.0)	58.6	14a	2.33 (1H, m)	33.4
7	1.94 (1H,m)	40.1	14b	2.27 (1H, m)	
8a	1.69 (1H, m)	24.1	15	–	173.2
8b	1.32 (1H, m)		17	5.40 (1H, d, J = 7.2)	81.1
9a	1.84 (1H, m)	23.7			
9b	1.59 (1H, m)				



1

Fig. 1. Key ^{1}H–^{1}H COSY and HMBC correlations of compound **1**.

Compound **1** was obtained as a yellow oil. Its molecular formula was determined as $C_{15}H_{24}N_2O_2$ by positive HR-ESI-MS at *m/z* 265.1914 $[M + H]^+$ (calcd for $C_{15}H_{25}N_2O_2$, 265.1916). Its IR spectrum exhibited absorption bands for the hydroxy (3431 cm^{-1}), carbonyl (1629 cm^{-1}), and quinolizidine moiety (2934, 2859, 2810, and 2749 cm^{-1}). The ^{1}H NMR data showed a characteristic signal at δ_H 5.40 (1H, d, J = 7.2 Hz, H-17) for one oxygenated methine. The ^{13}C and DEPT NMR spectra displayed 15 carbon resonances, including five methines (one oxygenated carbon), nine methylenes, and one carbonyl group. The 1D NMR data of **1** were similar to those of sophoridine (**4**), except for an additional hydroxy group at C-17 in **1**, as deduced from the HMBC correlations of H-17 to C-4 (δ_C 31.0), C-5 (δ_C 34.9), C-6 (δ_C 58.6), and C-11 (δ_C 54.7), and the correlations between H-17 and H-5 in the ^{1}H–^{1}H COSY experiment. The structure was further confirmed by the correlations in the HMBC experiment (Fig. 1).

The relative stereochemistry of **1** was established through an analysis of coupling constants and a ROESY experiment. The large coupling constant (J = 12.6, 8.0 Hz) [4] of H-6 with H-5 and H-7 suggested that these three protons, H-5, H-6, H-7, had β-, α-, and α-orientation, respectively, which was confirmed by the correlations of H-6 with H-4b, H-5 with H-4a, and H-11 with H-8b. In the ROESY spectrum of **1**, the correlations of H-5 with H-11 indicated that H-11 was β-oriented, while the correlations of H-6 and H-4b with H-17, suggested that OH-17 has β-orientation.

Compounds **1** and **5–8** were evaluated for their *in vitro* cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cancer cell lines using the reported MTT method [10]. However, none of them were active against the above five human cancer cell lines.

EXPERIMENTAL

General. UV spectra were recorded on a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained on a Tensor 27 spectrometer. 1D and 2D NMR spectra were performed on Bruker AM-400, DRX-500, or Avance III-600 spectrometers with TMS as internal standard. HR-ESI-MS were carried out using VG Auto Spec-3000 or API-Qstar-Pulsar instruments. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical

Co. Ltd., Qingdao, People′s Republic of China), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with Zorbax SB-C_{18} (5 μm, 9.4 × 250 mm). MPLC was performed on a Lisui EZ Purify III system, including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Lisui Chemical Engineering Co., Ltd., China), and on a column packed with MCI gel (CHP 20P, 75–150 μm; Mitsubishi Chemical Corporation, Japan). Fractions were monitored by TLC, and spots were visualized using Dragendorff′s reagent. Solvents were distilled prior to use.

Plant Material. The roots of *Sophora flavescens* were bought from the Kunming Chrysanthemum Village medicine market, Yunnan, China, in March 2011 and was identified by Prof. X. Cheng at Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20110320s01) has been deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried roots (20 kg) were extracted with MeOH for three times at room temperature successively to afford a black residue (3 kg) after evaporation *in vacuo*. The residue was dissolved in warm water, acidified with 0.5% HCl to pH 2, and extracted with ethyl acetate. The aqueous layer was neutralized with 10% ammonia/water to pH 9 and extracted with $CHCl_3$ once again and concentrated *in vacuo* to obtain the total alkaloids (300 g). It was subjected to MPLC (MCI gel) using a stepwise gradient (MeOH–H_2O, 40:60 to 100:0) to obtain three fractions, A–C. Fraction A (100 g) was subjected to silica gel CC (200–300 mesh) and eluted with a gradient of petroleum ether–acetone (9:1 to 6:4) to give four further subfractions, A1–A4. Subfraction A1 was further purified by column chromatography on silica gel with petroleum ether–EtOAc (9:1) to afford **2** (1 g) and **3** (900 mg). Subfraction A2 was subjected to semi-preparative HPLC (MeOH–H_2O, 11:89) to yield **4** (15 mg) and **5** (12 mg). Subfraction A3 was loaded on a column of silica gel and eluted with $CHCl_3$–acetone–diethylamine (8:2:0.05), and then further purified by Sephadex LH-20 (MeOH), yielding **6** (13 mg) and **7** (7 mg). Fraction B (30 g) was subjected to silica gel CC with $CHCl_3$–MeOH (9:1), followed by semi-preparative HPLC (MeOH–H_2O, 10:90) to afford **1** (70 mg) and **8** (600 mg). Fraction C (150 g) was re-separated by silica gel CC (100–200 mesh) using petroleum ether–acetone–diethylamine (5:3.5:0.5 to 5:4:1) to give **9** (57 g) and **10** (17 g).

Compound 1. Yellow oil. $[\alpha]_D^{17.8}$ –39.76° (*c* 0.218, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 421 (1.73), 204 (3.27). IR (KBr, ν_{max}, cm^{-1}): 3431, 2934, 2859, 2810, 2749, 1729, 1629, 1463, 1453, 1383, 1333, 1264, 1221, 1111, 1043. For ^1H and ^{13}C NMR spectral data, see Table 1. ESI-MS *m/z* 265 $[M + H]^+$; HR-ESI-MS at *m/z* 265.1914 $[M + H]^+$ (calcd for $C_{15}H_{25}N_2O_2$, 265.1916).

Matrine (2). White needles (petroleum ether). ESI-MS *m/z* 249 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 4.30 (1H, dd, J = 12.7, 4.1, H-17a), 3.81 (1H, m, H-11), 3.06 (1H, t, J = 12.7, H-17b), 2.85 (2H, m, H-2a, H-10a), 2.34–2.03 (7H, m), 1.95–1.45 (14H, m). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 172.9 (C-15), 63.0 (C-6), 58.3 (C-2), 58.2 (C-10), 54.7 (C-11), 44.5 (C-17), 42.8 (C-7), 36.8 (C-5), 33.4 (C-14), 28.7 (C-12), 28.0 (C-4), 27.2 (C-8), 22.1 (C-3), 21.6 (C-9), 19.6 (C-13).

Sophocarpine (3). White needles (petroleum ether). ESI-MS *m/z* 247 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.62 (1H, m, H-13), 5.80 (1H, dt, J = 9.8, 1.9, H-14), 4.05 (1H, dd, J = 12.9, 4.7, H-17a), 3.96 (1H, m, H-11), 3.14 (1H, t, J = 12.9, H-17b), 2.82 (2H, m, H-2a, H-10a), 2 60 (1H, m, H-6), 2.21–1.47 (14H, m). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 167.7 (C-15), 140.9 (C-13), 124.1 (C-14), 64.7 (C-6), 58.3 (C-10), 58.3 (C-2), 52.9 (C-11), 43.1 (C-17), 42.8 (C-7), 35.9 (C-5), 28.7 (C-4), 28.3 (C-12), 27.3 (C-8), 22.0 (C-3), 21.6 (C-9).

Sophoridine (4). White needles (MeOH). ESI-MS *m/z* 249 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 3.40–3.30 (3H, m, H-11, H_2-17), 2.87 (2H, m, H-2a, 10a), 2.30 (3H, m, H_2-14, H-6), 2.14 (2H, m, H-2b, 10b), 2.03 (2H, m, H-3a, 5), 1.86 (2H, m, H-4a, 12a), 1.60–0.90 (10H, m). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 172.5 (C-15), 63.5 (C-6), 57.5 (C-2), 56.6 (C-11), 50.6 (C-10), 49.6 (C-17), 41.6 (C-7), 32.9 (C-14), 31.3 (C-5), 30.5 (C-12), 28.6 (C-4), 23.5 (C-3), 22.8 (C-8), 22.7 (C-9), 19.4 (C-13).

Isomatrine (5). Colorless prisms (MeOH). ESI-MS *m/z* 249 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 3.92 (1H, m, H-11), 3.72 (1H, dd, J = 9.7, 3.9, H-17a), 3.53 (1H, d, J = 9.7, H-17b). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 174.0 (C-15), 62.8 (C-6), 56.1 (C-2), 52.9 (C-11), 52.1 (C-10), 43.0 (C-17), 38.3 (C-7), 33.4 (C-14), 29.9 (C-5), 26.6 (C-12), 25.3 (C-4), 20.3 (C-3), 19.7 (C-8), 19.2 (C-9), 17.6 (C-13).

7,11-Dehydromatrine (6). Colorless prisms (acetone). ESI-MS *m/z* 247 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm): 4.24 (1H, m, H-17a), 3.31 (1H, m, H-17b). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 171.6 (C-15), 136.4 (C-11), 109.1 (C-7), 62.7 (C-6), 58.0 (C-10), 56.2 (C-2), 41.9 (C-17), 33.5 (C-14), 33.2 (C-5), 29.6 (C-12), 27.6 (C-4), 27.6 (C-8), 25.4 (C-9), 22.3 (C-3), 20.5 (C-13).

Mamanine (7). Colorless oil. ESI-MS *m/z* 263 $[M + H]^+$. ^1H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 7.53 (1H, dd, J = 6.5, 9.0, H-4), 6.39 (1H, t, J = 8.7, H-3), 6.25 (1H, d, J = 6.8, H-5), 3.59 (2H, m, H_2-17). ^{13}C NMR (100 MHz, CD_3OD,

δ, ppm): 166.4 (C-2), 152.5 (C-6), 143.8 (C-4), 118.3 (C-3), 105.1 (C-5), 64.8 (C-10), 63.7 (C-17), 61.1 (C-16), 57.5 (C-14), 44.6 (C-9), 40.4 (C-7), 34.6 (C-11), 30.3 (C-8), 26.4 (C-13), 25.3 (C-12).

Sophoranol (8). White crystals (MeOH). ESI-MS *m/z* 265 $[M + H]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 4.48 (1H, d, J = 14.5, Ha-17), 3.77 (1H, m, H-11), 3.32 (1H, d, J = 14.5, H-17b), 3.16–3.02 (2H, m, H-2a, 10a). ^{13}C NMR (125 MHz, CD_3OD, δ, ppm): 173.5 (C-15), 68.5 (C-6), 67.0 (C-5), 57.0 (C-10), 56.3 (C-2), 53.4 (C-11), 46.0 (C-17), 36.7 (C-7), 35.8 (C-4), 33.4 (C-14), 28.0 (C-8), 24.5 (C-12), 21.1 (C-3), 21.1 (C-9), 19.5 (C-13).

Oxymatrine (9). Colorless prisms (acetone). ESI-MS *m/z* 265 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 4.87 (1H, dd, J = 11.7, H-11), 4.32 (1H, dd, J = 12.2, 4.5, H-17a), 4.02 (1H, t, J = 12.2, H-17b), 3.20 (5H, m), 2.55 (2H, m, H-3a, 9a), 2.38 (1H, m, H-14a), 2.25 (2H, m, H-14b, 12a), 2.10 (1H, m, H-8a), 1.58–1.87 (9H, m), 1.35 (1H, m, H-12b). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 172.8 (C-15), 69.7 (C-10), 69.3 (C-2), 67.9 (C-6), 54.9 (C-11), 43.5 (C-7), 43.2 (C-17), 35.6 (C-5), 33.6 (C-14), 29.3 (C-12), 26.7 (C-4), 25.1 (C-8), 19.6 (C-13), 18.2 (C-3), 18.1 (C-9).

Oxysophocarpine (10). Colorless prisms (acetone). ESI-MS *m/z* 263 $[M + H]^+$. ^{1}H NMR (400 MHz, CD_3OD, δ, ppm, J/Hz): 6.68 (1H, m, H-13), 5.84 (1H, dt, J = 12.1, 1.9, H-14), 4.77 (1H, m, H-11), 3.94 (2H, m, H_2-17), 3.31 (3H, m, H-6, 2a, 10a), 3.05 (2H, m, H-2b, 10b), 2.75 (1H, m, H-12a), 2.56 (2H, m, H-3a, 9a), 2.01 (2H, m, H-5, 12b), 1.97 (2H, m, H-7, 8a), 1.58–1.86 (5H, m). ^{13}C NMR (100 MHz, CD_3OD, δ, ppm): 168.8 (C-15), 141.2 (C-13), 124.7 (C-14), 69.5 (C-10), 69.2 (C-2), 67.3 (C-6), 53.4 (C 11), 43.7 (C-17), 41.6 (C-7), 34.0 (C-5), 30.7 (C-12), 26.8 (C-4), 25.2 (C-8), 18.2 (C-3), 18.1 (C-9).

Cytotoxicity Assay. The methyl thiazolyl tetrazolium assay (MTT assay) was used to determine cell viability. The following human tumor cell lines were used: HL-60 (human myeloid leukemia cell line), SMMC-7721 (human hepatocarcinoma cell line), A-549 (lung cancer cell line), MCF-7 (breast cancer cell line), and SW480 (human colon carcinoma). The cancer cells were all seeded in a 96-well plate at a density of 5000 to 10000 cells per well in 100 μL of medium. The test compounds and the positive control, cisplatin, were added to appropriate wells and the cells were incubated for 48 h (37°C, 5% CO_2). Then MTT (100 μg) solution was added into the assay plates. After shaking for 10 s, the plates were returned to the incubator and kept for 4 h, after which the cells were lysed with 100 μL 20% sodium dodecyl sulfate (SDS)-50% DMF after removal of 100 μL medium. The optical density of the lysate was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680, USA). The IC_{50} value of each compound was calculated by the Reed and Muench method [11].

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