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(+)-12α-Hydroxysophocarpine, a new quinolizidine alkaloid and related anti-HBV alkaloids from *Sophora flavescens*

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Abstract—(+)-12α-Hydroxysophocarpine (**8**), a new quinolizidine alkaloid was isolated from the roots of *Sophora flavescens*, together with 10 known quinolizidine alkaloids, (+)-oxymatrine (**1**), (+)-matrine (**2**), (+)-9α-hydroxymatrine (**3**), (+)-allomatrine (**4**), (+)-oxysophocarpine (**5**), (−)-sophocarpine (**6**), (−)-9α-hydroxysophocarpine (**7**), (+)-lehmannine (**9**), (−)-13,14-dehydrosophoridine (**10**), and (−)-anagyrine (**11**). Their structures were elucidated by spectroscopic methods, and the stereochemistry of **8** was confirmed by X-ray analysis. These alkaloids were tested for anti-hepatitis B virus (HBV) activity in vitro, compounds **5**, **6**, **9**, and **10** showed significant anti-HBV activity with inhibitory potency against HBsAg secretion at 48.3–79.3% and that against HBeAg secretion at 24.6–34.6%.

Sophora species (Leguminosae) are important sources of Chinese herbal drugs. They accumulate quinolizidine alkaloids as principal constituents with potentially useful pharmacological effects such as analgesic, antipyretic, anti-inflammatory, anti-tumor, and notable antiviral activities.[1,2] The major quinolizidine alkaloids oxymatrine and matrine were reported to exhibit anti-hepatitis B virus (HBV) activity, oxymatrine could downregulate HBV gene expression and decrease HBsAg and HBeAg content in HBV transgenic mice,[3] and protect mice from fulminant hepatitis induced by GalN (galactosamine)/LPS (lipopolysaccharide) and block hepatocyte apoptosis as well,[4] and matrine could protect the D-GalN-treated mice from the development of fatal hepatitis induced by LPS.[5]

The roots of *Sophora flavescens* Ait., a species widely distributed throughout China, are commonly used as the traditional Chinese medicine 'Kushen' for the treatment of skin diseases and gynecological diseases, such as eczema, dermatitis, and colpitis.[6] Pharmacological studies showed that the alkaloids of *S. flavescens* inhibited CBV_3 (coxsackie B_3 virus) replication and possessed protective effect on infected myocardial cells,[7] and its components anagyrine, oxymatrine, and sophoranol also exhibited potent antiviral activities against RSV (respiratory syncytial virus).[8] During the course of our screening for anti-HBV agents from *Sophora* plants, a phytochemical investigation on the alkaloid constituents of *S. flavescens* was carried out and resulted in the isolation of a new quinolizidine alkaloid, (+)-12α-hydroxysophocarpine (**8**), together with 10 known quinolizidine alkaloids (**1**–**7**, **9**–**11**) (Fig. 1). This paper reports the isolation and structure elucidation of the new alkaloid, as well as the in vitro anti-HBV activity of these isolated alkaloids.

The roots of *S. flavescens* Ait.[9] (14 kg) were extracted with aqueous 1% (v/v) H_2SO_4, followed by partition with $CHCl_3$ after being basified with Na_2CO_3 to give the crude alkaloids, which were subjected to repeated silica gel column chromatography and prep. TLC to give a new quinolizidine alkaloid, (+)-12α-hydroxysophocarpine (**8**), as well as 10 known ones (**1**–**7**, **9**–**11**).[10] These known alkaloids were identified as (+)-oxymatrine (**1**),[11] (+)-matrine (**2**),[11] (+)-9α-hydroxymatrine (**3**),[12] (+)-allomatrine (**4**),[13] (+)-oxysophocarpine (**5**),[14] (−)-sophocarpine (**6**),[15,16] (−)-9α-hydroxysophocarpine (**7**),[15] (+)-lehmannine

Keywords: *Sophora flavescens*; Quinolizidine alkaloids; (+)-12α-Hydroxysophocarpine; Anti-HBV.
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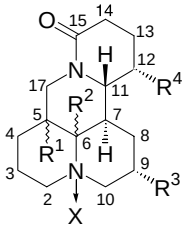

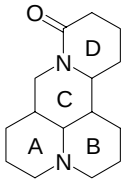

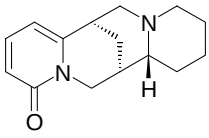

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	X	R^1	R^2	R^3	R^4	C-12–C-13	C-13–C-14
1	O	α-H	α-H	H	H	single bond	single bond
2	lone pair	α-H	α-H	H	H	single bond	single bond
3	lone pair	α-H	α-H	OH	H	single bond	single bond
4	lone pair	α-H	β-H	H	H	single bond	single bond
5	O	α-H	α-H	H	H	single bond	double bond
6	lone pair	α-H	α-H	H	H	single bond	double bond
7	lone pair	α-H	α-H	OH	H	single bond	double bond
8	lone pair	α-H	α-H	H	OH	single bond	double bond
9	lone pair	α-H	α-H	H	H	double bond	single bond
10	lone pair	β-H	α-H	H	H	single bond	double bond

Figure 1. Chemical structures of compounds **1**–**11**.

(**9**),[17,18] (−)-13,14-dehydrosophoridine (**10**),[13] and (−)-anagyrine (**11**),[19] respectively, by comparison of their $[\alpha]_D$, IR, EIMS, ^{1}H NMR, and ^{13}C NMR spectroscopic data with those reported.

Compound **8**[20] was obtained as white plates, mp 90–92 °C, $[\alpha]_D^{24}$ +137.3° (*c* 0.10, MeOH). The quasi-molecular ion $[M+H]^+$ was detected by HRESIMS at *m*/*z* 263.1761, consistent with the formula of $C_{15}H_{22}N_2O_2$. Its IR spectrum showed absorption bands characteristic of a hydroxyl group (3311 cm^{-1}), an α,β-unsaturated lactam system (1665 and 1598 cm^{-1}), and *trans*-quinolizidine functionalities (2811 and 2783 cm^{-1}). The EI mass spectrum of **8** showed a base peak at *m*/*z* 245 (100), corresponding to $[M-OH]^+$, and fragmentations similar to those of (−)-sophocarpine (**6**) and (−)-12β-hydroxysophocarpine,[15] indicating it might be a hydroxyl derivative of **6**. The ^{1}H NMR spectrum of **8** agreed well with that of **6**, except that there was an additional isolated signal at δ_H 4.17 (1H, m) in the spectrum of **8** which could be assigned to a methine proton bearing a hydroxyl group because of its low chemical shift, and that the signals due to the olefinic protons H-13 and H-14 simplified in splitting pattern from octets and sextets in **6** to quartets and doublets in **8**, and shifted to downfield for 0.29 and 0.10 ppm, respectively (Table 1). These differences were very similar to those of (−)-12β-hydroxysophocarpine as compared with **6**.[15] The hydroxyl group was thus deduced to be located on C-12, which was further supported by the HMBC correlations of the methine proton at δ_H 4.17 (H-12) bearing a hydroxyl group with C-13 (δ_C 138.7) and C-14 (δ_C 126.6), and of the olefinic protons at δ_H 6.74 (H-13) and δ_H 5.99 (H-14) with the carbon at δ_C 60.9 which correlated with the methine proton at δ_H 4.17 in the HMQC spectrum. In the ^{13}C NMR spectrum of **8**, the signals corresponding to C-2–C-6, C-8–C-10, C-15, and C-17 were consistent with those of **6** with $\Delta\delta_C \leqslant 1.2$ ppm, and the olefinic carbons C-13 and C-14 shifted to downfield for 1.3 and 1.9 ppm,

Table 1. Selected ^{1}H NMR data of compounds **6** and **8** (in $CDCl_3$, δ_H in ppm, *J* in Hz)

Compound	11β	12β	13	14	17α	17β
6[a]	3.98 (dd, 16.9, 9.6)	—[c]	6.45 (ddd, 9.8, 5.0, 3.8)	5.89 (dt, 9.8, 2.2)	4.14 (dd, 12.9, 4.7)	3.17 (t, 12.9)
8[b]	3.73 (br d, 10.4)	4.17 (m)	6.74 (dd, 9.6, 6.0)	5.99 (d, 9.6)	3.98 (dd, 13.2, 4.6)	3.22 (t, 13.2)

[a] 400 MHz.
[b] 500 MHz.
[c] Not assigned.

Table 2. ^{13}C NMR data of compounds **6** and **8** (in $CDCl_3$, δ_C in ppm)

Compound	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17
6[a]	57.3	21.1	27.8	34.7	63.6	41.6	26.6	20.8	57.3	51.5	27.4	137.4	124.7	165.7	42.1
8[b]	57.4	21.2	27.7	33.5	62.7	34.7	26.3	21.0	57.3	54.7	60.9	138.7	126.6	165.8	41.0
Δδ (**8**–**6**)	+0.1	+0.1	−0.1	−1.2	−0.9	−6.9	−0.3	+0.2	0	+3.2	+33.5	+1.3	+1.9	+0.1	−1.1

[a] 100 MHz.
[b] 125 MHz.