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ISOKURARAMINE AND (−)-7,11-DEHYDROMATRINE, LUPIN ALKALOIDS FROM FLOWERS OF *SOPHORA FLAVESCENS**

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Key Word Index—*Sophora flavescens*; Leguminosae; alkaloids; (+)-kuraramine; (+)-mamanine; isokuraramine; (−)-7,11-dehydromatrine; lupanine; methylimidazoles; variation of alkaloid content.

Abstract—Two new lupin alkaloids, isokuraramine and (−)-7, 11-dihydromatrine, were isolated from the fresh flowers of *Sophora flavescens* along with 16 known lupin alkaloids, (+)-matrine, (−)-sophocarpine, (−)-sophoramine, (+)-sophoranol, (+)-5α, 9α-dihydroxymatrine, (−)-9α-hydroxysophoramine, (+)-matrine *N*-oxide, (+)-sophocarpine *N*-oxide, (+)-sophoranol *N*-oxide, lupanine, (−)-anagyrine, (−)-baptifoline, (−)-*N*-methycytisine, (−)-rhombifoline, (+)-mamanine and (+)-kuraramine. The structures of isokuraramine and (−)-7, 11-dehydromatrine are assumed to be diastereoisomers of (+)-kuraramine and (−)-7, 11-dehydromatrine, respectively, from their spectroscopic data. Two imidazoles were also isolated from the same source. However, these imidazole derivatives are presumed to be artifacts formed by the reaction of reducing sugars with ammonia during extraction. The variations in alkaloid content at various stages of flower and seed development of this plant were also examined.

INTRODUCTION

Sophora flavescens is a perennial herb belonging to the Leguminosae. Its dry roots have been used for stomachics, diuretics, antipyretic analgesics and insecticides as the Chinese drug 'Kusin'. Previous studies on the alkaloid constituents in the roots of *S. flavescens* have shown the presence of (+)-matrine, (+)-matrine *N*-oxide, (+)-allomatrine, (+)-isomatrine, (+)-sophoranol, (+)-sophoranol *N*-oxide, (−)-sophocarpin, (+)-sophocarpine *N*-oxide, (−)-sophoramine, (−)-*N*-methylcytisine, (−)-anagyrine and (−)-baptifoline [1–4]. From the aerial parts of this plant, Ueno *et al.* have recently isolated the seven new lupin alkaloids, (−)-13, 14-dehydrosophoridine, (+)-9α-hydroxymatrine, (−)-9α-hydroxysophocarpine, (−)-9α-hydroxysophocarpine *N*-oxide, (−)-7, 8-dehydrosophoramine, (−)-9α-hydroxysophoramine and the dimer of *N*-methylcytisine, in addition to the 13 known alkaloids including (−)-sophoridine and (+)-lehmannine which have not been found in the roots [4–6].

During the course of our studies of lupin alkaloids in leguminous plants which are mainly growing in Japan [7–13, 16, 17, 23], we have recently reported the isolation and the characterization of a novel dipiperidine-type alkaloid, (+)-kuraramine (**1**) which is assumed to be a new possible metabolite of (−)-*N*-methylcytisine, from the fresh flowers of *S. flavescens* [13]. Further examination of the alkaloidal constituents in the fresh flowers of this plant has resulted in the isolation of two more new lupin alkaloids, isokuraramine (**2**) corresponding to a diastereomer of (+)-kuraramine (**1**) and (−)-7, 11-dehydromatrine (**3**), along with 16 known lupin alkaloids. In addition to the lupin alkaloids, two imidazole derivatives, 4-methylimidazole (**5**) and 2, 4-dimethylimidazole (**6**), were also isolated from the same source. However, **5** and **6** were presumed to be artificial products arising from reducing sugars in the nectar and ammonia added to the concentrated ethanol extract in order to make it basic. This paper describes the structure elucidation of the two new lupin alkaloids isolated from the flowers of *S. flavescens* and the variations in alkaloid content at various stages of flower and seed development.

RESULTS AND DISCUSSION

The alkaloid fraction obtained from the 75% ethanol extract of the freshly harvested flowers of *S. flavescens*, collected at Chiba, was subjected to repeated Si gel CC followed by prep. HPLC to give the two new lupin alkaloids, isokuraramine (**2**) and (−)-7,11-dehydromatrine (**3**), along with (+)-kuraramine (**1**) and the other 15 known lupin alkaloids as shown in Table 1. The four known alkaloids, (+)-5α, 9α-dihydroxymatrine, lupanine, (−)-rhombifoline and (+)-mamanine so far have not been

*This work was partly presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, 28 August 1979 (*Meeting Abstract* p. 197).

Table 1. Physical properties and chromatographic behaviours of the alkaloids isolated from the flowers of *Sophora flavescens*

Alkaloids	mp°	$[\alpha]_D^{\circ}$	R_f on TLC*					R_t (min) on HPLC*		
			A	B	C	D	F	H	I	J
(+)-Matrine (4)	77	+39.1[b]	0.70	—	0.51	0.46	0.75	6.0	—	—
(−)-Sophocarpine	81–82	−29.4[a]	0.71	—	0.43	0.52	0.74	5.7	—	—
(−)-7, 11-Dehydromatrine (3)	107–108	−137.2[a]	0.65	—	0.24	0.42	0.69	12.0	—	—
(−)-Sophoramine	164–165	−98[a]	0.67	—	0.60	0.36	0.68	7.0	—	—
(+)-Sophoranol	171	+66[b]	0.55	—	0.45	0.23	0.56	7.9	—	—
(+)-5α, 9α-Dihydroxymatrine	192–193	+40.6[a]	0.23	0.48	0.42	0.05	—	14.0	13.0	—
(−)-9α-Hydroxysophoramine	230	−129[a]	0.38	0.58	0.52	0.10	—	11.0	9.6	—
(+)-Matrine *N*-oxide	162–163	+47.7[a]	0.27	0.44	0.11	—	0.30	—	—	20.3
(+)-Sophocarpine *N*-oxide	208–210	+37[a]	0.24	0.40	0.11	—	0.26	—	—	25.0
(+)-Sophoranol *N*-oxide	259–261	+38.1[a]	0.16	0.24	0.11	—	0.16	—	—	35.5
Lupanine	oil	†	0.67	—	0.26	0.41	0.71	15.0	10.5	—
(−)-Anagyrine	oil	−165.3[a]	0.65	—	0.57	0.34	0.70	8.4	5.6	—
(−)-Baptifoline	210	−137.2[a]	0.30	0.45	0.27	—	0.24	24.3	17.0	6.3
(−)-*N*-methylcytisine	137	−223.4[a]	0.61	—	0.52	0.26	0.64	10.5	7.5	—
(−)-Rhombifoline	oil	−232.4[a]	0.67	—	0.76	0.49	0.73	4.5	—	—
(+)-Mamanine	171–172	+31.7[a]	0.20	0.35	0.17	—	—	22.0	16.5	—
(+)-Kuraramine (1)	amorphous	+8.4[a]	0.13	0.20	—	—	—	56.7	43.7	8.8
Isokuraramine (2)	amorphous	†	0.35	—	—	—	—	13.0	—	—
4-Methylimidazole (5)	55–56	—	0.27	0.46	—	0.28	—	—	—	—
2,4-Dimethylimidazole (6)	91–92	—	0.28	0.48	—	0.28	—	—	—	—

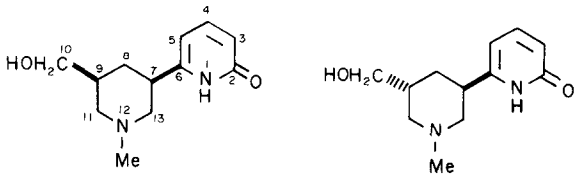
Solvents for $[\alpha]_D$ measurements: a, EtOH; b, H_2O.

*Solvents A–J for TLC and HPLC are described in the Experimental.

†Optical rotation has not been determined due to shortage of material.

found in either the aerial or underground parts of *S. flavescens*.

Isokuraramine (**2**) was a very minor component and was obtained as a colourless amorphous solid. The mass spectrum of **2** was in good agreement with that of (+)-kuraramine (**1**)[13]. The ^{13}C NMR signals of **2** (in $CDCl_3$) were also very similar to those of **1** except that the signals due to C-7 and C-9 of **2** were shifted up-field by δ 4.6 and 3.5, respectively compared to those of **1**, (Table 2). Furthermore, the ^1H NMR spectrum of **2** (in $CDCl_3$) showed signals at δ 12.64 (1H, *br*, NH), 7.36 (1H, *dd*, $J = 9$ and 7 Hz, H-4), 6.41 (1H, *dd*, $J = 9$ and 1 Hz, H-3) and 6.02 (1H, *dd*, $J = 7$ and 1 Hz, H-5) due to a 6-substituted 2-pyridone moiety and the three proton singlet at δ 2.31 assigned to an *N*-methyl group, all of which were characteristic of the spectrum of **1**. The above spectroscopic data suggest that **2** should be a diastereomer of (+)-kuraramine (**1**) (Scheme 1).



Scheme 1.

The ^1H NMR spectrum of **2** exhibited the AB part of an ABX system at δ 3.78 (1H, *dd*, $J = 11$ and 6.5 Hz, H-10) and 3.51 (1H, *dd*, $J = 11$ and 5 Hz, H'-10) assigned to the methylene protons of a hydroxymethyl group. The magnetic non-equivalence of the methylene protons suggests that the hydroxymethyl group is axially oriented and its hydroxy group forms an intramolecular hydrogen bond with the lone pair on N-12 by analogy with that of the hydroxymethyl group in (−)-lupinine[14]. Furthermore, the foregoing up-field shifts of the ^{13}C NMR signals due to C-7 and C-9 of **2** can be reasonably explained by the axial orientation of the hydroxymethyl group. Thus, it is suggested that the hydroxymethyl group of **2** adopts an axial orientation unlike the equatorial orientation of that in **1**. From the above results, it can therefore be presumed that the structure of **2** is a diastereomer of (+)-kuraramine (**1**), *N*-methyl-3-hydroxymethyl-5-(2'-pyridone-6'-yl)-piperidine which has the *trans*-configuration at the C-3 and C-5 positions.

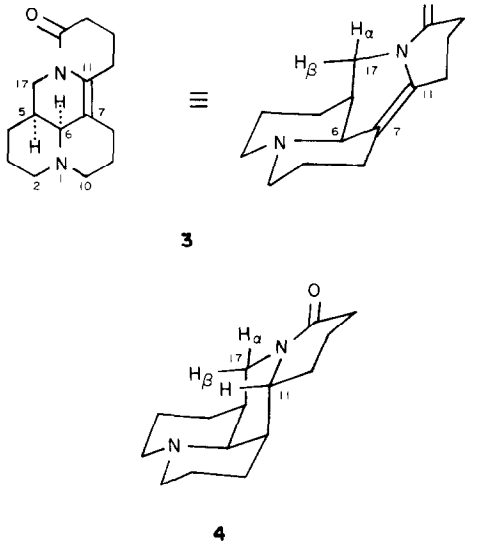
Table 2. ^{13}C NMR data for (+)-kuraramine (**1**) and isokuraramine (**2**) in $CDCl_3$*

Carbon No.	1	2
2	165.2 (*s*)	164.6 (*s*)
3	117.8 (*d*)	117.4 (*d*)
4	141.7 (*d*)	141.6 (*d*)
5	103.6 (*d*)	103.9 (*d*)
6	151.0 (*s*)	151.0 (*s*)
7	40.0 (*d*)	35.4 (*d*)[b]
8	31.8 (*t*)	32.5 (*t*)
9	38.8 (*d*)	35.3 (*d*)[b]
10	65.4 (*t*)	64.5 (*t*)
11	60.3 (*t*)[a]	58.1 (*t*)[c]
13	58.4 (*t*)[a]	57.9 (*t*)[c]
NMe	46.1 (*q*)	46.4 (*q*)

*TMS was used as int. standard and chemical shifts are given in δ values relative to the int. standard.

Values with the same superscript could be interchanged.

The second new base (**3**), $C_{15}H_{22}N_2O$ (M^+, *m/z* 246.171, calc. 246.170) was extracted as colourless prisms, mp 107–108° (ether), $[\alpha]_D^{24}$ −137.2° (EtOH). Its IR spectrum showed the presence of a lactam group (1640 cm^{-1}) and a *trans*-quinolizidine moiety (2770 and 2750 cm^{-1}). The UV spectrum of **3** exhibited an absorption band at 242 nm ($\log \epsilon = 4.15$, EtOH), indicative of the presence of a double bond conjugating with a nitrogen lone pair in the molecule[15, 16]. The ^1H NMR spectrum ($CDCl_3$) showed the low-field signals at δ 4.33 (1H, *dd*, $J = 12$ and 4 Hz, H-17α) and 3.22 (1H, *t*, $J = 12$ Hz, H-17β) separated from the other signals at higher field. Both the signals correspond to the characteristic signals due to H-17α and H-17β in the spectrum of matrine (**4**), which resonate at the unusually low chemical shifts of δ 4.49 (*dd*, $J = 12.5$ and 4 Hz) and 3.13 (*t*, $J = 12.5$ Hz) by the deshielding effects of the lactam carbonyl and the N-1 lone pair, respectively, and hence are clearly distinguishable from the other high field signals[17, 18]. These spectroscopic data suggest that **3** should be a derivative of matrine, which has a double bond adjacent to a nitrogen and has a conformation similar to that of matrine (**4**) (Scheme 2).



Scheme 2.

The position of the double bond in **3** is concluded to be situated in the C-7 and C-11 position from the following spectroscopic results: (1) the ^1H NMR spectrum of **3** did not show a signal due to an olefinic proton but the ^{13}C NMR spectrum exhibited the sig-

nals at δ 128.1 (*s*) and 115.3 (*s*) due to two olefinic quaternary carbons. (2) The IR spectrum showed the *trans*-quinolizidine band, indicating that the double bond should be located neither at the C-5 and C-6 nor the C-6 and C-7 positions [9, 17, 19]. (3) There was the presence of the ^{1}H NMR signal corresponding to H-11 which must resonate at a low field of *ca* δ 3.8 by the deshielding effect of the N-1 lone pair [17, 18]. Therefore, the second new base (**3**) is assumed to be (−)-7, 11-dehydromatrine, which is considered to be the same compound as that of leontalbinine, mp 107–108°, $[\alpha]_D$ −135.5°, isolated from *Leontice albertii* Rgl. (Berberidaceae) by Iskandarov *et al.* [20].

The residual alkaline aqueous layer after the extraction of the lupin alkaloids was further saturated with potassium carbonate and then extracted with chloroform. From the chloroform extract, two imidazole derivatives, 4-methylimidazole (**5**) and 2, 4-dimethylimidazole (**6**), were isolated by the application of Si gel CC and prep. HPLC. It is known that the methylimidazoles are formed by the reaction of reducing sugars such as glucose and rhamnose with ammonia in the presence of metal ions [21, 22], as shown in Scheme 3. Accordingly, the methylimidazoles isolated from the fresh flowers of *S. flavescens* are presumed to be artifacts arising from rhamnose or glucose in the nectar and ammonia added to the concentrated ethanol extract in order to make it basic.

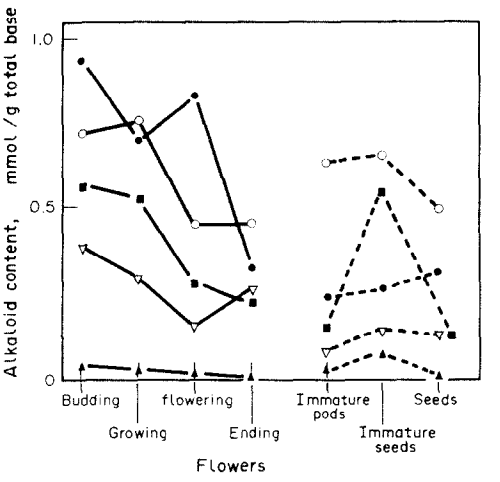
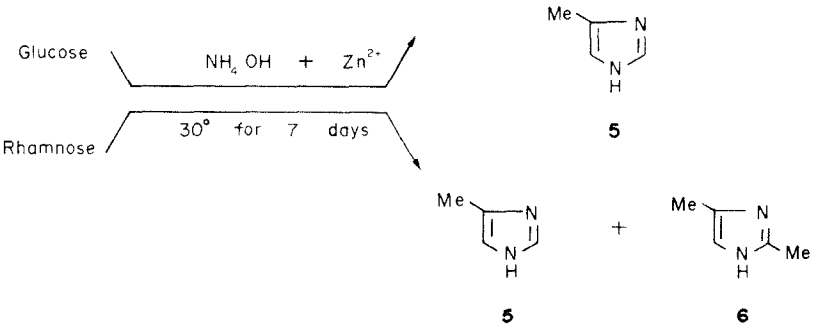


Fig. 1. Variations of alkaloid content at various stages of the flower and seed growth of *Sophora flavescens*. (■) (+)-matrine; (▽) (+)-sophoranol; (▲) (−)-sophocarpine; (●) (−)-*N*-methylcytisine; (○) (−)-anagyrine.



Scheme 3.

Variations in the alkaloid content at the various stages of the flower growth and the seed development of *S. flavescens* are shown in Figs. 1–3. This finding, analogous to that of (+)-kuraramine (**1**) and (+)-mamanine [13], (−)-epilamprolobine and its *N*-oxide [12], and of the cage-type lupin alkaloids, (−)-tsukushinamines [10, 11, 23], is interesting from the viewpoint of the metabolism and role of the lupin alkaloids in plants.

EXPERIMENTAL

General methods. Mps are uncorr. MS were recorded at 70 eV using a direct inlet system. ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) spectra were measured using TMS as int. standard. TLC was performed on Si gel G (Merck) in the following solvent systems: (A) CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); (B) CH_2Cl_2–MeOH–28% NH_4OH (43:6:1); (C) CH_2Cl_2–MeOH (4:1); (D) Et_2O–MeOH–28% NH_4OH (40:2:1); (E) Et_2O–MeOH–28% NH_4OH (70:30:1), and on Al_2O_3 plates (Merck) in: (F) C_6H_6–MeOH–Me_2CO (34:3:3).

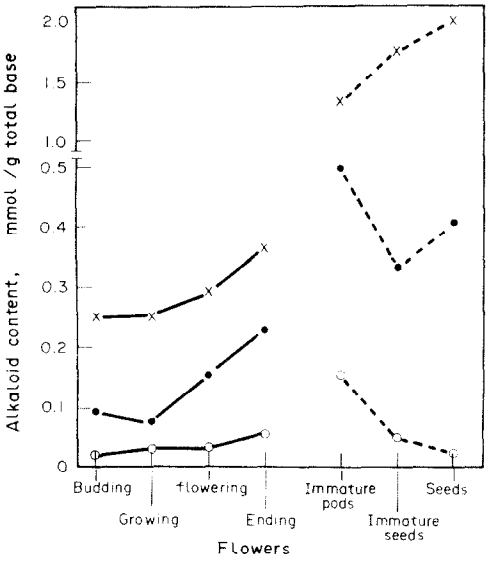


Fig. 2. Variations of alkaloid content at various stages of the flower and seed growth of *Sophora flavescens*. (×) (+)-matrine *N*-oxide; (●) (+)-sophocarpine *N*-oxide; (○) (+)-sophoranol *N*-oxide.

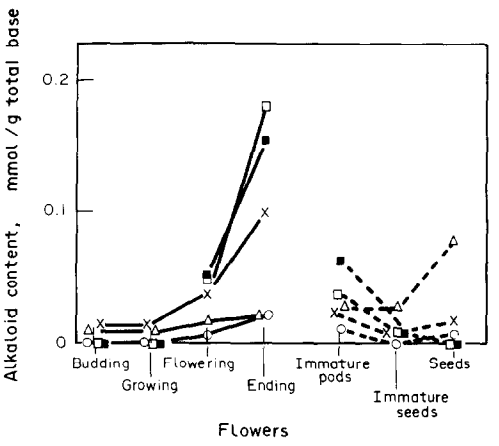


Fig. 3. Variations of alkaloid content at various stages of the flower and seed growth of *Sophora flavescens*. (□) (+)-mamanine; (▲) (+)-kuraramine; (×) (+)-5α,9α-dihydroxymatrine; (○) (−)-9α-hydroxymatrine; (△) (−)-baptifoline.

All spots were visualized by exposure to I_2 vapour or by spraying with Dragendorff's reagent and iodoplatinate reagent. PCAF (pentacyanoammonioferrate) reagent was also used for visualization of imidazoles. Analytical and prep. HPLC were carried out with solvents: (H) 15% MeOH in Et_2O–2.5% NH_4OH (50:1); (I) 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3); (J) 25% MeOH in Et_2O–H_2O–25% NH_4OH (100:4:3); (K) 10% MeOH in Et_2O–H_2O–2.5% NH_4OH (500:5:1); (L) 3% MeOH in CH_2Cl_2–25% NH_4OH (500:1), using a LiChrosorb SI 100 (10 μm, 0.3 × 50 cm for analytical and 0.5 × 50 cm for prep.) column connected with an UV detector. Flow rate for analytical HPLC was 1 ml/min.

Isolation and characterization of alkaloids. The 75% EtOH extract obtained from the fresh flowers (3.39 kg) of *S. flavescenes* Aiton, collected in June at Chiba-prefecture, Japan, was concd *in vacuo* below 40°. The aq. concentrate was acidified with 4% HCl to pH 1 and the resulting ppt was filtered off. The filtrate was extracted × 3 with $CHCl_3$, neutralized with 28% NH_4OH and adjusted to pH 10.5 with K_2CO_3 at 0°. The alkaline soln was extracted several times with $CHCl_3$. The aq. layer was made basic by further addition of dry K_2CO_3 at 0° and extd with $CHCl_3$ several times. All the $CHCl_3$ extracts from the alkaline aq. soln were combined, dried (K_2CO_3) and concd to dryness *in vacuo*. The crude alkaloid (23.7 g) was obtained in a yield of *ca* 0.7%/fr. wt (crude base I). In addition, the alkaline aq. layer was further satd with K_2CO_3 and extd with $CHCl_3$ several times. The combined $CHCl_3$ extracts were dried (Na_2SO_4) and concd to dryness *in vacuo* to give 1.7 g of a brown syrup (crude base II) which contained the imidazole derivatives. The crude base I (10 g) was chromatographed on a Si gel column (Merck, 70–230 mesh, 600 g, 4.3 × 102 cm) developing successively with the following solvent systems: Et_2O–MeOH–28% NH_4OH mixtures (40:2:0.5, fraction I, 1.36 g), (40:2:1, fraction II, 4.2 g), (40:4:1, fraction III 0.12 g), (40:6:1, fraction IV, 0.11 g), (40:8:1, fraction V, 0.08 g), and MeOH (fraction VI, 3.8 g). Fraction I (1.36 g) was rechromatographed on a Si gel column (Merck, 230–400 mesh, 80 g) using solvent H to give (−)-rhombifoline (7 mg), (−)-sophocarpine (80 mg), (+)-matrine (**4**, 0.93 g), (−)-7, 11-dehydromatrine (**3**, 12 mg) and lupanine (3 mg) in order of elution. Fraction II (2.20 g) was subjected to Si gel (Merck, 230–400 mesh, 200 g) CC with 15% MeOH in CH_2Cl_2–28% NH_4OH (1000:1), from which (−)-sophoramine (15 mg), (−)-anagyrine (0.68 g), (+)-sophoranol (0.27 g) and (−)-*N*-methylcytisine (0.95 g) were eluted in this order. Fraction III (0.12 g) was separated repeatedly by prep. HPLC using solvents H, K and L. (−)-9α-Hydroxysophoramine (13 mg), isokuraramine (**2**, 5 mg) and (+)-5α, 9α-dihydroxymatrine (68 mg), along with remaining (−)-*N*-methylcytisine and (+)-sophoranol, were obtained. Fraction IV (0.11 g) was rechromatographed on a Si gel (Merck, 230–400 mesh, 10 g) column with solvent C to give (−)-baptifoline (15 mg) and (+)-mamanine (64 mg). The main component of fraction V (80 mg) was purified by prep. HPLC using solvent H to give (+)-kuraramine (**1**, 54 mg). Fraction VI (0.38 g) was subjected to prep. HPLC using solvent J to give (+)-matrine *N*-oxide (0.29 g), (+)-sophocarpine *N*-oxide (35 mg) and (+)-sophoranol *N*-oxide (6 mg). The crude base II (1.5 g) was chromatographed on a Si gel (Merck, 70–230 mesh, 60 g) column with Et_2O–MeOH–28% NH_4OH (80:17:3). The first eluate (0.4 g) was subjected to Si gel (Merck, 230–400 mesh, 30 g) CC with 2% MeOH in CH_2Cl_2–28% NH_4OH (500:1). The first eluate (0.1 g) contained mainly imidazoles, which were further separated by prep. HPLC using solvent L to yield 4-methylimidazole (80 mg) and 2, 4-dimethylimidazole (9 mg).

Identification of known alkaloids and imidazoles. Some physical data and chromatographic behaviours of the alkaloids and imidazoles from *S. flavescens* are listed in Table 1. All alkaloids and imidazoles were identified by mp, colour reaction, TLC, HPLC and by comparison of the IR, MS and NMR with those of authentic samples, as described in our previous papers[7–13, 16, 17, 23].

Isokuraramine (**2**). The $[\alpha]_D$ of **2** has not been measured yet because of the limited amount of the material. Colourless amorphous solid. MS *m/z* (rel. int.): 222.138 $[M]^+$, calc. for $C_{12}H_{18}N_2O_2$, 222.137 (90), 204 $[M-H_2O]^+$ (5), 191 $[M-CH_2OH]^+$ (19), 133 (18), 122 (28), 121 (52), 101 (33) 58 $[Me_2\overset{+}{N}=CH_2]$, (100). ^1H NMR (in $CDCl_3$) δ 12.64 (1H, *br*, NH), 7.36 (1H, *dd*, *J* = 9 and 7 Hz, H-4), 6.41 (1H, *dd*, *J* = 9 and 1 Hz, H-3), 6.02 (1H, *dd*, *J* = 7 and 1 Hz, H-5), 3.78 (1H, *dd*, *J* = 11 and 6.5 Hz, H-10), 3.51 (1H, *dd*, *J* = 11 and 5 Hz, H'-10), 3.39 (1H, *s*, OH), 3.00 (1H, *m*, H-7), 2.7–2.6 (3H, *c*), 2.31 (3H, *s*, NMe), 2.2–1.5 (4H, *c*). The ^{13}C NMR data of **1** and **2** are shown in Table 2.

(−)-7, 11-*Dehydromatrine* (**3**). Colourless prisms from Et_2O mp 107–108°, $[\alpha]_D^{24}$ − 137.2° (EtOH: *c* 0.18). MS *m/z* (rel. int.): 246.171 (M^+, calc. for $C_{15}H_{22}N_2O$, 246.170, 65), 245 (100), 218 (56), 204 (22), 190 (35), 176 (15), 175 (14). IR ν_{max}^{KBr} cm^{-1}: 2770, 2750 (*trans*-quinolizidine band), 1640 (lactam C=O). UV λ_{max}^{EtOH} nm (log ε): 242 (4.15). ^1H NMR (in $CDCl_3$) δ 4.33 (1H, *dd*, *J* = 12 and 4 Hz, H-17α), 3.22 (1H, *t*, *J* = 12 Hz, H-17β). ^{13}C NMR (in $CDCl_3$): 168.5 (*s*, C-15), 128.1 (*s*, C-11), 115.3 (*s*, C-7), 61.8 (*d*, C-6), 57.3 (*t*, C-10), 55.6 (*t*, C-2), 40.8 (*t*, C-17), 32.8 (*t*, C-14), 32.1 (*d*, C-5), 29.2 (*t*, C-12), 27.1 (*t*, C-4), 27.0 (*t*, C-8), 24.7 (*t*, C-4), 21.7 (*t*, C-3), 19.6 (*t*, C-13).

Estimation of alkaloid content. Samples of flowers and seeds of *S. flavescens* were collected during the periods of flower-budding, flower-growth, flowering, flower-ending (immediately after the petals fell off), seed-growth, which was divided into the seeds and pods, and maturity of seeds. Individual crude alkaloids were extracted in a similar manner to that described above. The contents of individual alkaloids were estimated by HPLC using solvents H and J.

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