Lupin Alkaloids from Chinese *Maackia Hupehensis*

Yong-Hong WANG,[a] Jia-Shi LI,[b] Hajime KUBO,[a] Kimio HIGASHIYAMA,[a] Hideaki KOMIYA[a] and Shigeru OHMIYA*,[a]

Institute of Medicinal Chemistry, Hoshi University,[a] Ebara 2–4–41, Shinagawa-ku, Tokyo 142–8501, Japan and Beijing University of Traditional Chinese Medicine,[b] 11, Beisan Huan Dong Ave, Beijing 100029, China.
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Two new lupin alkaloids, (−)-*N*-(2-oxopyrrolidinomethyl)cytisine and (−)-*N*-(*N*-acetylaminomethyl)cytisine, were isolated together with 9 known alkaloids from Chinese *Maackia hupehensis*, which grows in the south of China. The alkaloidal constituents of *M. hupehensis* were shown to be comparable to those of the southern species of Japanese *Maackia* plants.

Key words *Maackia hupehensis*; Leguminosae; lupin alkaloid; (−)-*N*-(2-oxopyrrolidinomethyl)cytisine; (−)-*N*-(*N*-acetylaminomethyl)cytisine; (−)-lusitanine

Maackia plants produce unusual types of lupin alkaloids, such as (−)-camoensidine,[1] (+)-tashiromine[2] and (+)-maackiamine,[3] which contain a pyrrolizidine or an indolizidine ring, together with common lupin alkaloids which consist of a piperidine or a quinolizidine ring. Furthermore, the structural types of lupin alkaloids which occur in Japanese *Maackia* plants (four species, *M. amurensis*, *M. tashiroi*, *M. floribunda* and *M. floribunda f. pubescens*) are related to the geographical distribution of the plants.[4] The plant of *M. amurensis* grows in the north of Japan. It accumulates sparteine-type lupin alkaloids such as (+)-sparteine and (−)-lupanine but no lupinine-type alkaloid.[3,4] The other *Maackia* plants, which grow in the south of Japan, accumulate mainly lupinine-type alkaloids such as (+)-epilupinine and (+)-tashiromine but no sparteine-type is found.[2,4] The cytisine- and anagyrine-type alkaloids are constituents common to plants of the above two groups. These phenomena are interesting from the viewpoints of chemotaxonomy of leguminous plants and biosynthesis of lupin alkaloids. In the course of our investigations on lupin alkaloids in *Maackia* species, we have recently reported the isolation of the possible non-basic metabolite of (−)-cytisine, (+)-hupeol, from one *Maackia* species native to China, *M. hupehensis*.[5] This report describes the isolation and structural determination of eleven alkaloids, in which (−)-*N*-(2-oxopyrrolidinomethyl)-cytisine (**1**) and (−)-*N*-(*N*-acetylaminomethyl)cytisine (**2**) were new alkaloids, and also describes a comparison of alkaloidal constituents of *M. hupehensis* with those of Japanese *Maackia* plants.

Results and Discussion

The alkaloid mixture (5.4 g) obtained from 75% methanol extracts of the air dried stems (1.2 kg) of *M. hupehensis*, collected in Jiang Xi province, China, in May, was repeatedly chromatographed on a silica gel column to give eleven lupin alkaloids, **1**, **2**, (−)-cytisine (**3**, 25%), (−)-*N*-methylcytisine (**4**, 5%), (−)-*N*-formylcytisine (3%), (−)-epibaptifoline (21%), (−)-lusitanine (12%), (+)-epilupinine (3%), (−)-*N*-(3-oxobutyl)cytisine (trace), (−)-rhombifoline (trace) and (+)-hupeol (trace), in which **1** and **2** were new alkaloids (Chart 1). The known alkaloids were identified by comparing directly with authentic samples in all measurable respects (MS, ^{1}H-NMR, IR, $[\alpha]_D$, co-TLC, HPLC) as described in our previous paper.[6]

The total base (1.3 g) obtained from the air dried leaves (750 g) was similarly treated to give seven known alkaloids, **3** (16%), **4** (5%), (−)-*N*-formylcytisine (2%), (−)-epibaptifoline (15%), (−)-lusitanine (11%), (+)-epilupinine (4%) and (−)-lupinine (1%). This is the first example of the coexistence of (+)-epilupinine and (−)-lupinine in plants of the genus *Maackia*.

Alkaloid **1** gave colorless crystals from $CHCl_3$, mp 169—170 °C, and alkaloid **2** was isolated as an oily compound. The molecular formulae of **1** and **2** were determined to be $C_{16}H_{21}N_3O_2$ and $C_{14}H_{19}N_3O_2$, respectively, by high resolution mass spectra. The MS spectra of both **1** and **2** revealed prominent fragment ions at *m/z* 203, 189, 160 and 146 which are characteristic of *N*-alkylcytisine like (−)-*N*-(3-oxobutyl)cytisine.[6,7] The ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra of **1** and **2**, which were assigned by analysis of ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{1}H–^{13}C COSY spectra, also resembled that of **4**, as shown in Tables 1 and 2. These results suggested that new alkaloids **1** and **2** might be an *N*-substituted cytisine.

The presence of an isolated methylene group in the structure of **1** was presumed from two doublets, which were coupled only with each other, at δ 3.91 (1H, d, J=12.2 Hz) and δ 3.77 (1H, d, J=12.2 Hz) in the ^{1}H-NMR spectrum of **1**. The signals at δ 175.9 (s), 31.2 (t), 18.0 (t) and 46.7 (t) in the ^{13}C-NMR spectrum of **1** were assigned to a lactam carbonyl and three methylene carbons of a 2-pyrrolidone moiety. Therefore, the structure of **1** was presumed to be *N*-(2-oxopyrrolidinomethyl)cytisine, which was determined by comparison with a synthetic sample obtained by refluxing a solution of **3**, formaldehyde and 2-pyrrolidone in EtOH.

The presence of a –CO–NH–CH_2–N< moiety in the structure of **2** was proposed from the ^{1}H-NMR signals at δ 5.73 (1H, broad) due to the amide NH, and at δ 4.03 (1H, dd, J=12.2, 5.7 Hz) and δ 3.84 (1H, dd, J=12.2, 5.7 Hz) assigned to the isolated methylene. The singlet at δ 1.98 (3H) was assigned to a methyl group adjacent to a carbonyl group, which was also confirmed by the signal at δ 23.4 (q) in the ^{13}C-NMR spectrum. The structure of **2** was presumed to be *N*-(*N*-acetylaminomethyl)cytisine, and identified by comparison with a synthetic sample, which was synthesized by refluxing a solution of **3**, formaldehyde and acetamide in EtOH.

The new alkaloids **1** and **2** have a methylene group inter-

* To whom correspondence should be addressed.

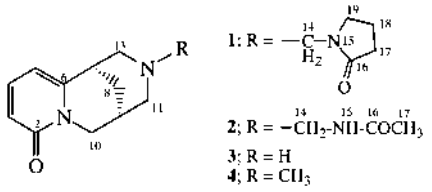


Chart 1

Table 1. ^{13}C-NMR Data of Alkaloids, **1**, **2**, **4** (δ ppm) in $CDCl_3$

C	1	2	4
2	163.3	163.7	163.3
3	116.6	116.8	116.6
4	138.6	138.7	138.4
5	104.5	104.7	104.4
6	151.2	151.2	151.3
7	35.1	35.1	35.3
8	25.7	26.6	25.8
9	27.8	27.6	27.9
10	50.0	49.9	49.8
11	57.7	51.7	62.4
13	58.0	51.0	62.1
14	63.8	61.3	46.1
16	175.9	170.7	—
17	31.2	23.4	—
18	18.0	—	—
19	46.7	—	—

Table 2. ^{1}H-NMR Data of Alkaloids, **1**, **2**, **4** (δ ppm, J=Hz) in $CDCl_3$

H	1	2	4
2	—	—	—
3	6.43 dd, J=9.0, 1.5	6.44 dd, J=9.1, 1.2	6.43 dd, J=9.0, 1.5
4	7.26 dd, J=9.0, 6.6	7.28 dd, J=9.1, 6.8	7.26 dd, J=9.0, 7.0
5	5.98 dd, J=6.6, 1.5	5.98 dd, J=6.8, 1.2	5.98 dd, J=7.0, 1.5
6	—	—	—
7	2.98 m	2.96 m	2.98 m
8	1.92 m	1.88 dd, J=12.8, 2.8	1.92 m
	1.75 m	1.75 dd, J=12.8, 1.6	1.75 m
9	2.47 m	2.46 m	2.47 m
10	4.06 d, J=15.2	4.01 d, J=14.8	4.06 d, J=15.5
	3.89 dd, J=15.2, 6.7	3.89 dd, J=14.8, 6.7	3.89 dd, J=15.0, 7.5
11	2.89 d, J=12.5	2.87 dd, J=11.0, 1.9	2.89 d, J=12.0
	2.55 d, J=12.5	2.55 dd, J=11.0, 2.1	2.55 d, J=12.0
13	2.80 dd, J=12.5, 1.8	2.89 d, J=11.0	2.87 dd, J=12.0, 2.4
	2.52 dd, J=12.5, 1.8	2.52 d, J=11.0	2.52 dd, J=12.0, 2.4
14	3.91 d, J=12.2	4.03 dd, J=12.2, 5.7	2.12 s
	3.77 d, J=12.2	3.84 dd, J=12.5, 5.7	
15	—	5.73 m (NH)	—
16	—	—	—
17	2.33 m	1.98 s (CH_3)	—
18	1.92 m	—	—
	1.80 m		
19	3.17 m	—	—
	3.00 m		

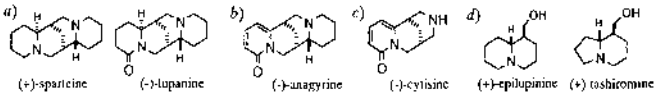
posed between the amino and the amide nitrogen in the structures. Lupin alkaloids having such a methylene group (>N-CH_2-N<) have not been found as far, except for (−)-12,12′-methylenedicytisine, which is isolated from *M. amurensis*[8] and *M. floribunda f. pubescens*.[9] It is interesting that only *Maackia* plants accumulate lupin alkaloids having the >N-CH_2-N<group.

M. hupehensis, which is native to the south of China, accumulates lupinine-type alkaloids, (−)-lupinine and (+)-epilupinine, together with cytisine- and anagyrine-type alkaloids, but no sparteine-type alkaloids were found. The components of *M. hupehensis* are the same as the south species of Japanese *Maackia* (Table 3). Thus, in analogy with the Japanese *Maackia* plants, the relationship between the geographical distribution of the plant and the structural-type of alkaloids which the plant accumulates, was also observed in the Chinese *Maackia* species.

Table 3. Comparison of Components of Chinese *M. Hupehensis* with Those of Japanese *Maackia* Species

Plants	Sparteine-type[a]	Anagyrine-type[b]	Cytisine-type[c]	Lupinine-type[d]
M. tashiroi		+	+++	++
M. floribunda		+	++	+
M. floribunda f. pubescens		+	+++	+++
M. amurensis	+++	+	+++	
M. hupehensis		++	+++	++

(+++; <30%, ++; <20%, +; <10%)



It is also interesting that the new alkaloid **1** has a pyrrolidine ring in the form of an amide, though unusual lupin alkaloids such as camoensidine and tashiromine, which are characteristic of *Maackia* plants, were not isolated from *M. hupehensis*. Further investigation on lupin alkaloids in the other *Maackia* plants native to China are being undertaken in our laboratories.

Experimental

Melting points were determined on Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 polarimeter. IR spectra were measured with a JASCO FT/IR-200 fourier transform IR spectrometer. The high and low resolution MS were measured at 70 eV using a direct inlet system. The ^{1}H-NMR (270 or 500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded using tetramethylsilane (TMS) as an internal standard. TLC was conducted on precoated silica gel plates (Merck 60 F_{254}).

Plant Material *M. hupehensis* was collected in May, 1995, at Jiang Xi province in the south of China, and identified by Prof. Jia-Shi Li, Department of Pharmacognosy, Beijing University of Traditional Chinese Medicine, and Director Ce-ming Tian, Jiang Xi Jiou Jiang Forest and Plant Research. A voucher specimen (No. 74568) is deposited at the Herbarium Institute of Botany Chinese Academy of Sciences, Xiangshan.

Isolation of Alkaloids The plant material collected was divided into leaves and stems. The air-dried stems (1.2 kg) were cut into small pieces and were extracted with 75% MeOH three times at room temperature. The combined extracts were concentrated, acidified with 10% HCl to pH 2 and then extracted with Et_2O three times. The aqueous layer was made alkaline with 25% NH_4OH to pH 11 and extracted with CH_2Cl_2 three times. The basic fraction was saturated with K_2CO_3 and extracted with CH_2Cl_2 repeatedly until it became negative to Dragendorff's reagent. The CH_2Cl_2 extracts were combined and dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The crude alkaloid (5.4 g) was obtained as a pale brown oil in a 0.45% yield of the dry stems. The dry leaves (750 g) were treated with a similar procedure as described for the stems to give the crude alkaloid (1.3 g, 0.17%). The crude alkaloid (5.4 g) from the stems was subjected to column chromatography on silica gel (230—400 mesh, 410 g) with CH_2Cl_2–MeOH–25%NH_4OH (43 : 6 : 1), 250-ml fractions being collected, monitoring with TLC, to give 17 fractions. Separation of fractions 1—2 (0.2 g) by silica gel column with Et_2O–MeOH–25%NH_4OH (50 : 15 : 1) yielded (−)-*N*-(3-oxobutyl)cytisine {mp 117—119 °C, $[\alpha]_D^{21}$ −210° (c=0.32, EtOH), 46 mg} and (−)-rhombifoline {oil, $[\alpha]_D^{21}$ −214° (c=0.49, EtOH), 25 mg}. Fraction 3 (50 mg) was

separated by silica gel column chromatography with CH_2Cl_2–AcOEt–MeOH (4 : 4 : 1) to yield (−)-*N*-(2-oxopyrrolidinomethyl)cytisine (**1**, 9 mg). Fraction 4 (25 mg) was chromatographed on silica gel column with CH_2Cl_2–AcOEt–MeOH (5 : 5 : 1) to yield (+)-hupeol (8 mg).[5] Fraction 5 (40 mg) was applied to column chromatography on silica gel using CH_2Cl_2–AcOEt–MeOH–25%NH_4OH (8 : 8 : 1 : 0.1) to give (−)-*N*-(*N*-acetylaminomethyl)cytisine (**2**, 5 mg). Fractions 6—7 (0.6 g) yielded (−)-*N*-methylcytisine {mp 135 °C, $[\alpha]_D^{20}$ −215° (*c*=0.45, EtOH), 0.27 g) and (−)-*N*-formylcytisine {mp 170 °C, $[\alpha]_D^{22}$ −232° (*c*=0.41, EtOH), 0.16 g}. Fractions 8—12 (3.2 g) were subjected to silica gel column chromatography with CH_2Cl_2–MeOH (4 : 1) to give **3** {mp 214 °C, $[\alpha]_D^{20}$ −110° (*c*=0.47, EtOH), 1.35 g} and (−)-epibatifoline {mp 154 °C, $[\alpha]_D^{22}$ −136° (*c*=0.26, EtOH), 1.13 g}. From fractions 14—15 (0.9 g), (−)-lusitanine {mp 184—186 °C, $[\alpha]_D^{22}$ −5.7° (*c*=0.20, EtOH), 0.6g} was obtained by silica gel column chromatography with CH_2Cl_2–MeOH–25%NH_4OH (50 : 2.5 : 0.5). (+)-Epilupinine {mp 75—77 °C, $[\alpha]_D^{22}$ +15.8° (*c*=0.16, EtOH), 0.2 g} was obtained from fractions 16—17 (0.3 g) by silica gel column chromatography with CH_2Cl_2–MeOH (10 : 1).

The crude alkaloid (1.3 g) from leaves was separated in a similar manner as described above to give **3** (230 mg), **4** (64 mg), (−)-*N*-formylcytisine (25 mg), (−)-epibaptifoline (195 mg), (−)-lusitanine (145 mg), (+)-epilupinine (40 mg) and (−)-lupinine (13 mg).

(−)-*N*-(2-Oxopyrrolidinomethyl)cytisine (**1**): Colorless crystals from $CHCl_3$, mp 169—170 °C, $[\alpha]_D^{23}$ −167° (*c*=0.36, EtOH), IR (KBr) cm^{-1}: 1650 (C=O). MS *m/z* (rel. int. %): 287.1627 (M^+, Calcd for $C_{16}H_{21}N_3O_2$: 287.1625) (13), 203 (6), 189 (27), 160 (10), 146 (13), 98 (100), 70 (58), 58 (10).

(−)-*N*-(*N*-Acetylaminomethyl)cytisine (**2**): Colorless oil, $[\alpha]_D^{23}$ −91° (*c*=0.48 , EtOH), IR (KBr) cm^{-1}: 3450 (NH), 1650 (C=O). MS *m/z* (rel. int. %): 261.1473 (M^+, Calcd for $C_{14}H_{19}N_3O_2$: 261.1478) (17), 218 (M^+-CH_3CO, 2), 203 (M^+-CH_3CONH, 24), 190 (85), 189 (60), 160 (23), 147 (100), 146 (76), 58 (93).

Synthesis of 1 A solution of **3** (38 mg, 0.2 mmol), 35% formalin 0.017 ml (0.2 mmol HCHO) and 2-pyrrolidone 17 mg (0.2 mmol) in EtOH (2 ml) was refluxed for 1 h. The solvent was removed and then the residue was chromatographed on silica gel column with CH_2Cl_2–AcOEt–MeOH (6 : 6 : 1) to give **1** (53 mg, 93%), which was identical with the natural **1** (MS, IR, ^{1}H-NMR, $[\alpha]_D$).

Synthesis of 2 Alkaloid **2** was obtained in the same way as the synthesis of **1**, by refluxing a solution of **3** (0.1 mmol), 35% formalin (0.1 mmol) and acetamide (0.1 mmol) in EtOH (1.0 ml). The reaction mixture was separated on column chromatography on silica gel with CH_2Cl_2–AcOEt–MeOH–25%NH_4OH (8 : 8 : 1 : 0.1) to yield **2** (22 mg, 86%), which was identical with the natural **2** (MS, IR, ^{1}H-NMR, $[\alpha]_D$).

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