A LUPIN ALKALOID, (–)-TENUAMINE (NORLUSITANINE), FROM *MAACKIA TENUIFOLIA*

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Abstract– A lupin alkaloid, (–)-tenuamine, was isolated from the stem parts of *Maackia tenuifolia* together with eight known alkaloids. The structure of (–)-tenuamine was determined to be (–)-acetylaminomethylideneindolizidine by comparison of its chemical and spectroscopic data with those of (–)-lusitanine, (+)-tashiromine and indolizidine.

In the course of our phytochemical study on *Maackia* (Leguminosae) plants, we have reported maackiamine,[1] tashiromine,[2] and camoensidine[3] which all contain a pyrrolizidine or indolizidine ring. It is interesting from the perspectives of both chemotaxonomy and biosynthesis that *Maackia* species accumulate unusual lupin alkaloids containing a pyrrolizidine or indolizidine ring together with common lupin alkaloids with a piperidine or quinolizidine ring (Figure 1). In the present paper, we report the isolation and structure determination of (–)-tenuamine (**1**), a new lupin alkaloid with an indolizidine ring, from the stems of *Maackia tenuifolia*, together with (–)-lusitanine[4] which contains a quinolizidine ring, and seven other known alkaloids.

From the air-dried stems of *Maackia tenuifolia*, a new alkaloid (**1**) was isolated as colorless needles, mp 136~138 ℃, $[\alpha]_D^{23}$ –4.5° (*c* 0.12, EtOH) in a yield of 1.2% of the total base by repeated column chromatography. We also isolated eight known lupin alkaloids, (–)-anagyrine, (–)-*N*-methylcytisine, (–)-*N*-formylcytisine, (–)-cytisine (main base), (–)-epibatifoline, (–)-lusitanine, (+)-epilupinine and (–)-12,12'-methylenedicytisine. The known alkaloids were identified by direct comparison with authentic samples (mp, $[\alpha]_D$, TLC, IR, NMR and MS).[5]

The chromatographic behavior of alkaloid (**1**) on a silica gel column was very similar to that of lusitanine (**2**). The IR spectrum of **1** also showed a similar pattern to that of **2**, with an amide carbonyl band at 1660 cm^{-1} and a N-H band at 3330 cm^{-1}. The molecular formula of **1** was determined to be $C_{11}H_{18}N_2O$ by the ^{13}C NMR spectrum (Table 1) and HREIMS spectrometry at *m/z* 194.1409 (M^+, calcd for 194.1419). In the

EIMS spectrum of **1**. M^+ at *m/z* (rel. int.) 194 (55) and fragment ions at *m/z* 151 ($M^+ - COCH_3$, 40), 136 ($M^+ - NHCOCH_3$, 55), 122 (100) and 96 (38) were one methylene less than the corresponding ions of **2** at *m/z* 208 (M^+, $C_{12}H_{20}N_2O$, 70), 166 (90), 165 (35), 150 (30), 136 (100) and 110 (82), respectively. These suggested that **1** was a congener of lusitanine. The assignments in the ^1H NMR and ^{13}C NMR spectra were based on an analysis of $^{13}C - ^1H$ and $^1H - ^1H$ COSY. The ^1H NMR spectrum of **1** also suggested the presence of an acetylaminomethylidene group based on signals of an olefinic methine proton at δ 6.62 (1H, d, *J*=10.4 Hz), which was coupled with 11-NH at δ 7.65 (1H, d, *J*=10.4 Hz), and also on the signal of the methyl of an acetylamine group at δ 2.04 (3H, s). In the ^{13}C NMR spectrum, the signals of C-2 (δ 52.6, t), C-9 (δ 54.4, t) and C-6 (δ 65.8, d) adjacent to the nitrogen (N-1) coincided with those of indolizidine (**5**) and tashiromine (**3**), but were different from those of quinolizidine (**6**) and epilupinine (**4**). The substituent effects of the acetylaminomethylidene group in the ^{13}C NMR signals at C-4 (+1.0), C-6 (+1.7) and C-7 (–4.0) of **1**, compared to those of an indolizidine ring, were also similar to those of **2** (Table 1).

Table 1. ^{13}C NMR data of **1**, **2**, **3**, **4**, indolizidine (**5**) and quinolizidine (**6**) in $CDCl_3$

C	**1**	**3**	**5**	**1–5**	C	**2**	**4**	**6**	**2–6**
2	52.6	52.7	52.7	–0.1	2	57.0	57.0	56.4	+0.6
3	25.5	25.2	25.1	+0.4	3	24.2	24.9	25.6	–1.4
4	25.2	29.2	24.2	+1.0	4	27.8	29.5	24.4	+3.4
5	120.2	44.7	30.7	+89.5	5	121.6	43.8	33.2	+88.4
6	65.8	66.4	64.1	+1.7	6	64.2	64.4	62.9	+1.3
7	26.1	27.6	30.1	–4.0	7	26.4	28.3	33.2	–6.8
8	20.3	20.3	20.3	0.0	8	25.1	24.6	24.4	+0.7
					9	25.4	25.5	25.6	–0.2
9	54.4	54.2	53.9	+0.5	10	56.4	56.6	56.4	0.0
10	114.7	65.9			11	116.2	64.1		
12	167.7				13	167.9			
13	23.2				14	23.3			

The above data suggested the presence of an indolizidine ring in the structure of **1**, instead of a quinolizidine ring in **2**, and an acetylaminomethylidene group at the 5-position. Accordingly, the structure of **1** was presumed to be 5-acetylaminomethylideneindolizidine. As far as we know, this is the first isolation of the compound which has an indolizidine ring in its structure, corresponding to lusitanine (**2**) with a quinolizidine ring. Thus, we propose **1** to be named tenuamine.

It is interesting that *M. tenuifolia* accumulates the two alkaloids, tenuamine and lusitanine, which contain an indolizidine ring and a quinolizidine ring, respectively. The unusual lupin alkaloids that contain a pyrrolizidine or indolizidine ring have so far been isolated from *Maackia* plants are shown in Figure 1, together with common lupin alkaloids with a piperidine or quinolizidine ring. It can be speculated that

Maackia species can utilize ornithine instead of lysine as a precursor amino acid for alkaloids or can transform the piperidine moiety to the corresponding pyrrolidine group.

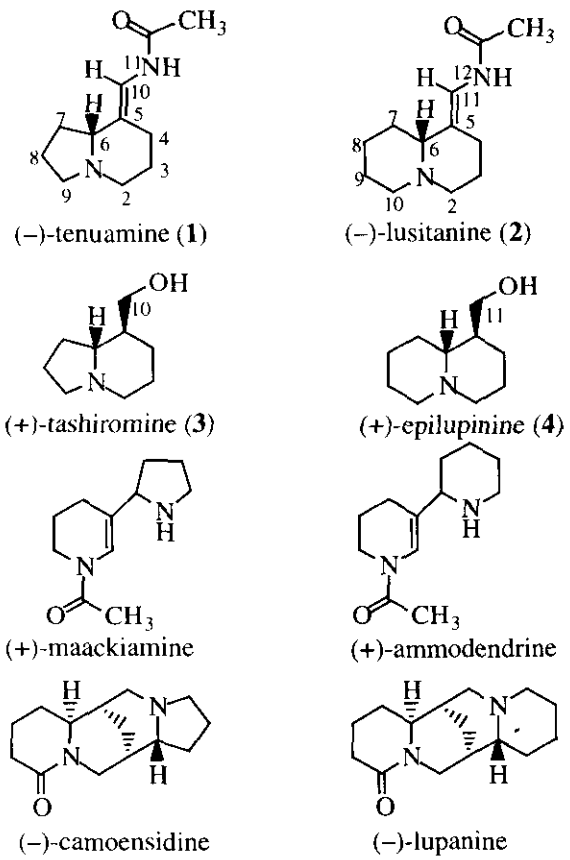

Figure 1. Two typical lupin alkaloids that coexist in the *Maackia* plants

EXPERIMENTAL

General Experimental Procedures. 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 Infrared Spectrophotometer. The high and low resolution MS were measured at 70 eV using direct inlet system with a JEOL JMS-600W instruments. NMR spectra were recorded with a 500 MHz JEOL NMR instruments using TMS as an internal standard and $CDCl_3$ as solvent. TLC was conducted on precoated silica gel plates (Merck 60 F_{254}).

Extraction and Isolation. The stems of *M. tenuifolia* were collected in Zhejiang Province, China, September, 1998, and identified by Director of Jiangxi Jioujiang Forest and Plant Research, Ce-ming Tan. A voucher specimen (No. 981013) is deposited in the Herbarium of the same Forest and Plant Research. The air-dried stem parts (1.6 kg) were extracted with 75% MeOH (x 3) at room temperature for 24 h. The combined extracts were concentrated and acidified with 10% HCl to pH 2. The acid phase was washed with

Et_2O (x 3), basified with 25% NH_4OH to pH 11 and extracted with CH_2Cl_2. The aqueous phase was saturated with K_2CO_3 and extracted with CH_2Cl_2 repeatedly until it became negative to Dragendorff's reagent. The all CH_2Cl_2 extracts were combined, dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The crude alkaloid was obtained as a pale brown oil in a 0.28% yield (4.5 g) of the dry stem parts and subjected to silica gel column chromatography (230-400 mesh, 400 g) with Et_2O-CH_2Cl_2-MeOH-25%NH_4OH (2.5:2.5:1:0.1), monitoring with TLC to give 11 fractions. Separation of frs 2, 3 (0.5 g) by silica gel column with Et_2O-CH_2Cl_2-MeOH-25%NH_4OH (10:10:1:0.1) yield (–)anagyrine {$[\alpha]_D^{23}$ –163° (*c* 0.28, EtOH), 0.4 g} and (–)-*N*-methylcytisine (80 mg). Fr 4 (0.4 g) yielded (–)-*N*-methylcytisine {mp 135~136℃, $[\alpha]_D^{23}$ –216° (*c* 0.3, EtOH), 0.15 g} and (–)-*N*-formylcytisine {mp 169~170℃, $[\alpha]_D^{23}$ –226° (*c* 0.16, EtOH), 0.2 g}. Frs 5~7 (1.5 g) were subjected to silica gel column chromatography with CH_2Cl_2-MeOH (4:1) to give (–)-epibatifoline {mp 207~208 ℃, $[\alpha]_D^{23}$ –132° (*c* 0.35, EtOH), 0.4 g}, (–)-cytisine {mp 153~155 ℃, $[\alpha]_D^{23}$ –110° (*c* 0.52, EtOH), about 1 g} and **2** (30 mg). In those frs. the dimer of cytisine, 12,12'-methylenedicytisine, was confirmed by ^{1}H NMR spectrum, but decomposed into cytisine on the isolation process. Fr 8 (0.6 g) contains (–)-cytisine and **2**. From frs 9,10 (0.5 g), **2** {mp 186~187 ℃, $[\alpha]_D^{23}$ –6.0° (*c* 0.3, EtOH), 0.35 g} and (+)-epilupinine {mp 75~76 ℃, $[\alpha]_D^{23}$ +16.8° (*c* 0.13, EtOH), 55 mg} were separated. Fr 11 (0.2 g) was purified by silica gel column chromatography (20 g) using Et_2O-CH_2Cl_2-MeOH-25%NH_4OH (3:1:1:0.1) to yield **1** (57 mg) and **2** (40 mg).

Tenuamine (1). Colorless needles, mp 136~138℃ ($CHCl_3$), $[\alpha]_D^{23}$ –4.5° (*c* 0.12, EtOH), IR (KBr): 3330, 1660 cm^{-1}(-CONH-). HRMS *m/z* 194.1409 $[M]^+$ (calcd for $C_{11}H_{18}N_2O$, 194.1419). MS *m/z* (% rel. int.): 194 [M^+] (55), 179 (10), 159 (60), 151 (40), 136 (55), 122 (100), 96 (38). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.65 (1H, d, *J*=10.4 Hz, 11-NH), 6.62 (1H, d, *J*=10.4 Hz, 10-H), 3.14 (2H, m, 2, 9-Heq), 2.56 (1H, dd, *J*=14.9, 4.2 Hz, 4-H), 2.43 (1H, m, 6-H), 2.22 (1H, dd, *J*=11.6, 8.5 Hz, 9-Hax), 2.16 (1H, dt, *J*=11.7, 2.8 Hz, 2-Hax), 2.04 (3H, s, 13-CH_3). ^{13}C NMR data: see Table 1.

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