(+)-Hupeol, a Possible Non-basic Metabolite of the Lupine Alkaloid (−)-Cytisine in Chinese *Maackia hupehensis*†

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A novel product, named (+)-hupeol (**1**), which is regarded as an intermediate in the metabolism of the lupine alkaloids to non-basic constituents, has been isolated from Chinese *Maackia hupehensis*.

Japanese *Maackia* species (Leguminosae) are a group of plants which are interesting from the viewpoints of chemotaxonomy and biosynthesis because they accumulate unusual lupine alkaloids containing a pyrrolizidine or indolizidine ring such as maackiamine[1], tashiromine[2] and camoensidine[3] together with common lupine alkaloids having a piperidine or quinolizidine ring. In the course of our studies on lupine alkaloids in *Maackia* plants, we isolated a novel constituent from *M. hupehensis* native to China. In this paper, we report the chemical characterization of the new constituent, named (+)-hupeol (**1**), and its biogenetic relationship with the typical lupine alkaloid (−)-cytisine (**2**), which is a main alkaloid (25% of the total base) of this plant.

The basic fraction (5.4 g) obtained from a 75% MeOH extract of the dry branches (1.2 kg) of *M. hupehensis*, collected in Jiang Xi province, China, in May, was subjected to repeated column chromatography on silica gel to yield (+)-hupeol (**1**; 8 mg), together with eight known lupine alkaloids, (−)-cytisine (**2**), (−)-*N*-methylcytisine, (−)-*N*-formylcytisine, (−)-epibaptifoline, (−)-lusitanine, epilupinine, *N*-3-oxobutylcytisine and rhombifoline.

(+)-Hupeol (**1**) was obtained as colourless needles from CH_2Cl_2–MeOH, $[\alpha]_D$ +32.3 (*c* 0.263, EtOH). The molecular formula, $C_{11}H_{13}NO_3$ [Found (EIMS): *m/z*, 207.0882. $C_{11}H_{13}NO_3$ requires M_r, 207.0894], contains one nitrogen and one hydrogen less and two oxygens more than that of the typical C_{11} lupine alkaloid (−)-cytisine ($C_{11}H_{14}N_2O$). The IR spectrum of **1** showed an absorption band at 3300 cm^{-1} (OH). The mass spectrum of **1** revealed prominent fragment ions at *m/z* 160 (43%) and 146 (97) which are characteristic of lupine alkaloids having a 2-pyridone ring such as in **2**.[4] The ^1H NMR spectrum (CD_3OD) of **1** exhibited two sets of signals in a 3:1 ratio, indicating that **1** was a 3:1 mixture of two structurally related compounds **1a** and **1b**, respectively, although **1** showed a single spot on TLC in several solvent systems.

The ^1H and ^{13}C NMR spectra of **1a** and **1b** (Table 1) were assigned by analysis of the ^1H–^1H COSY and ^1H–^{13}C COSY spectra. The similarity of the spectra of **1a** and **1b** with those of (−)-cytisine (**2**) (Table 1) suggested that both **1a** and **1b** had structures very similar to that of **2**. The hemiacetal structures for **1a** and **1b** were presumed from downfield shifts of the ^1H and ^{13}C signals at the 11 and 13 positions compared with those of **2**. The C-9 signals of **1a** and **1b** were shifted downfield by 4–5 ppm, compared with those of **2**, while the C-7 signals were only slightly shifted, indicating that the hydroxy groups of **1a** and **1b** were both situated at the 11 position.

The stereochemistry of the hydroxy group of **1a** and **1b** was concluded to be axial (α) and equatorial (β), respectively, by comparison of the ^{13}C signals at the 8, 10 and 13 positions of **1a** with those of **1b**. The ^{13}C signals of C-8 and C-13 of **1a** were at a higher field than those of **1b**, and the signal of C-10 of **1b** was at a higher field than that of **1a**, which could be explained by a γ effect of the hydroxy group (Scheme 1). Therefore, it was concluded that (+)-hupeol (**1**) was an inseparable equilibrium mixture (3:1) of hemiacetals **1a** and **1b**.

Table 1 ^1H and ^{13}C NMR data for (+)-hupeol (**1a** and **1b**) (CD_3OD) and (−)-cytisine (**2**) ($CDCl_3$)[a]

	1a		**1b**		**2**	
Carbon No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	165.7		165.7		166.6	
3	116.9	6.45 (dd, *J* 9.0, 1.2)	116.9	6.45 (dd, *J* 9.0, 1.3)	117.8	6.45 (dd, *J* 8.8, 1.4)
4	141.5	7.49 (dd, *J* 9.0, 6.5)	141.5	7.49 (dd, *J* 9.0, 6.5)	142.1	7.29 (dd, *J* 8.8, 6.7)
5	107.8	6.28 (dd, *J* 6.5, 1.2)	107.8	6.28 (dd, *J* 6.5, 1.2)	108.9	5.98 (dd, *J* 6.8, 1.4)
6	152.8	—	152.8	—	153.4	—
7	36.0	2.94 (m)	36.8	2.94 (m)	36.9	2.90 (m)
8 H_β	19.9	2.52 (d, *J* 12.8)	25.5	2.09 (dd, *J* 11.9, 3.0)	27.3	1.96 (m)
H_α		1.81 (dd, *J* 12.8, 3.1)		2.11 (d, *J* 11.9)		1.96 (m)
9	34.6	2.35 (m)	33.6	2.35 (m)	29.7	2.32 (m)
10 H_β	49.6	4.11 (d, *J* 15.8)	44.1	4.47 (d, *J* 16.0)	51.8	4.13 (d, *J* 15.3)
H_α		3.85 (dd, *J* 15.8, 6.8)		3.66 (dd, *J* 16.0, 6.8)		3.89 (dd, *J* 15.3, 6.7)
11 H_β	97.2	5.12 (s)	97.9	—	53.6	3.11 (m)
H_α		—		4.91 (d, *J* 3.0)		3.07 (m)
13 H_β	66.8	3.43 (dd, *J* 10.8, 1.7)	73.3	3.82 (dd, *J* 11.3, 1.9)	54.6	3.03 (m)
H_α		4.37 (dd, *J* 10.8, 1.8)		3.93 (dd, *J* 11.3, 1.8)		3.09 (m)

[a]*J* Values in Hz.

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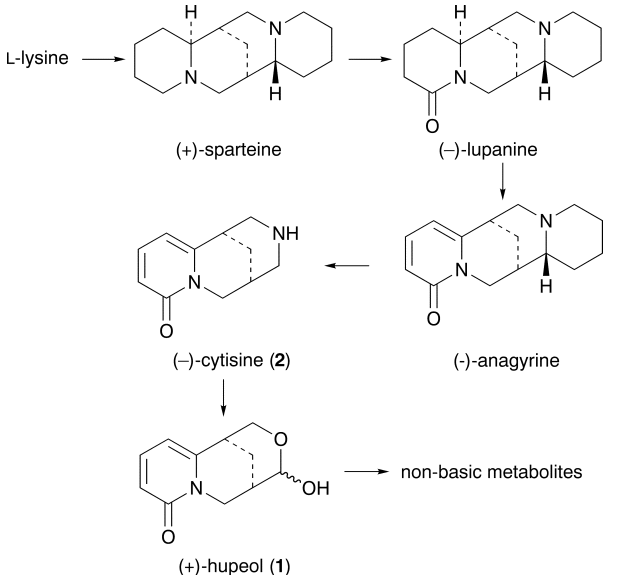


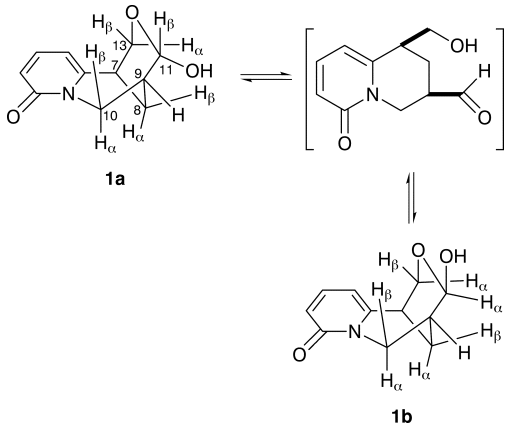
Fig. 1 A possible biosynthetic pathway for (+)-hupeol (**1**)

It is generally accepted in the biosynthesis of lupine alkaloids that tetracyclic sparteine-type alkaloids are first produced from three units of L-lysine and then metabolized oxidatively to tricyclic cytisine-type alkaloids *via* tetracyclic anagyrine-type alkaloids (Fig. 1).[4] (–)-Cytisine (**2**) is considered to be the ultimate metabolite in the biosynthetic pathway of the lupine alkaloids. (+)-Hupeol (**1**) does not have a basic amino group, which is one of the important characteristics of the other alkaloids, but has a structure closely related to that of **2**. Thus, (+)-hupeol (**1**) could be regarded as an intermediate in the metabolism of lupine alkaloids to non-basic compounds. To the best of our knowledge, this is the first example of such an intermediate in the biosynthesis of lupine alkaloids.

Investigation of the absolute stereochemistry of (+)-hupeol (**1**) is currently being undertaken in our laboratories.

Experimental

Mps are not corrected. High- and low-resolution mass spectra were measured at 70 eV using a direct-inlet system. ^{1}H NMR (270 or 500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using TMS as an internal standard.



Scheme 1

Isolation of (+)-*Hupeol* (**1**).—The crude alkaloid fraction (5.5 g) obtained from the 75% MeOH extracts was subjected to chromatography on a silica gel column (Merck, type 60, 230–400 mesh; 410 g) with CH_2Cl_2–MeOH–25% NH_4OH (43:6:1), monotoring with TLC, to give 17 fractions. The fourth fraction (25 mg), the **1**-rich fraction, was separated by silica gel column chromatography with CH_2Cl_2–AcOEt–MeOH (5:5:1) to yield (+)-*hupeol* (**1**; 8 mg), colourless needles from CH_2Cl_2–MeOH, $[\alpha]^{23}_D$+32.3 (c = 0.263, EtOH); m/z (EI) 207.0882 (M^+, $C_{11}H_{13}NO_3$ requires 207.0894, 57%), 190.0886 (M^+–OH, $C_{11}H_{12}NO_2$ requires 190.0868, 4), 189.0801 (M^+–H_2O, $C_{11}H_{11}NO_2$ requires 189.0790, 17), 178.0859 (M^+–CHO, $C_{10}H_{12}NO_2$ requires 178.0866, 20), 160.0761 ($C_{10}H_{10}NO$ requires 160.0761, 43), 149.0842 ($C_9H_{11}NO$ requires 149.0841, 68), 148.0771 ($C_9H_{10}NO$ requires 148.0761, 100), 1246.0613 (C_9H_8NO requires 146.0607, 97), 138 (38), 117 (36), 93 (35); ν_{max} (KBr)/cm^{-1} 3300 (OH), 1650 (C=O).

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