TASHIROMINE; A NEW ALKALOID FROM MAACKIA TASHIROI [1]

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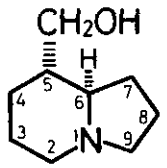
Abstract - A new alkaloid (**1**) containing an indolizidine ring, named tashiromine, was isolated from the stems of Maackia tashiroi (Leguminosae) together with seven lupin alkaloids and ammodendrine. The structure of **1** was determined as (5S,6R)-5-hydroxymethyl-trans-indolizidine or its enantiomer by comparison of the spectral data with those of the synthetic diastereomers of 5-hydoxymethyl-trans-indolizidine.

In the course of our studies on lupin alkaloids in leguminous plants, we have recently reported that Maackia amurensis accumulates alkaloids containing pyrrolidine or indolizidine ring such as maackiamine and camoensidine together with the corresponding piperidine or quinolizidine alkaloids such as ammodendrine and lupanine.[2] Examination of alkaloid constituents in another Maackia species, M. tashiroi, has resulted in the isolation of a new alkaloid (**1**), named tashiromine, possessing indolizidine ring together with seven lupin (quinolizidine) alkaloids and ammodendrine. M. tashiroi is a deciduous shrub distributed widely in subtropical Asia. This paper describes the structural determination of **1**.

Tashiromine[3] (**1**, 2 mg) was isolated as a colorless oil from the basic fraction (5.7 g) obtained from the 75% MeOH extracts of the dry stems (1.2 Kg) of M. tashiroi (collected in Kumamoto prefecture in August) together with (-)-cytisine, (-)-lusitanine, (-)-N-methylcytisine, (-)-anagyrine, (-)-N-formylcytisine, (-)-rhombifoline, (+)-epilupinine and ammodendrine.

The molecular formula of **1**, $C_9H_{17}NO$, which was determined by the hrms spectrum

(M^+, m/z 155.1307), is one CH_2 unit (14 mass unit) less than that of epilupinine (2), coexisting in the same plant. The significant fragment ions of the eims spectrum of 1 were also 14 mass unit less than those (designated in parentheses) of 2; m/z 154 (168), 138 (152), 124 (138), 110 (124), 97 (111), 96 (110), 84 (98), 83 (97), 82 (96), 69 (83). The ^{13}C-nmr spectrum of 1 showed nine signals due to a hydroxymethyl carbon, two methylene and one methine carbon adjacent to a nitrogen, one methine carbon and four methylene carbons (Table I). The above results suggested that 1 might be an indolizidine derivative having a hydroxymethyl group.



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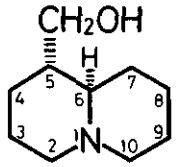

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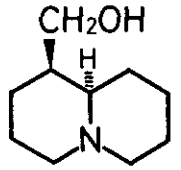

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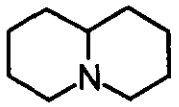

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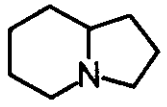

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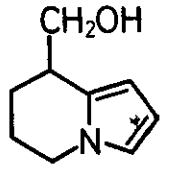

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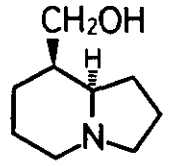

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Table I. ^{13}C-Nmr data of 1 - 5 and 7 in $CDCl_3$

carbon No.	2	3	4	$\Delta\delta_{2-4}$	$\Delta\delta_{3-4}$	1	7	5	$\Delta\delta_{1-5}$	$\Delta\delta_{7-5}$
2	57.0	56.9	56.4	0.6	0.5	52.7	54.4	52.7	0.0	1.7
3	24.9	22.7	25.6	-0.7	-2.9	25.2	23.2	25.1	0.1	-1.9
4	29.5	30.8	24.4	5.1	6.4	29.2	30.5	24.2	5.0	6.3
5	43.8	38.5	33.2	10.6	5.3	44.7	35.4	30.7	14.0	4.7
6	64.4	65.0	62.9	1.5	2.1	66.4	66.8	64.1	2.3	2.7
7	28.3	29.5	33.2	-4.9	-3.7	27.6	25.8	30.1	-2.5	-4.3
8	24.6	24.6	24.4	0.2	0.2	20.3	20.8	20.3	0.0	0.5
9	25.5	25.5	25.6	-0.1	-0.1	54.2	53.5	53.9	0.3	-0.4
10	56.6	56.9	56.4	0.2	0.5	65.9	65.6	-	-	-
11	64.1	64.7	-	-	-	-	-	-	-	-

The ^{13}C-nmr signals of **1** were assigned on the basis of the data of **2**,[4] lupinine (**3**),[4] quinolizidine (**4**)[4] and indolizidine (**5**).[5] The most reasonable assignment was that to 5-hydroxymethylindolizidine. Epilupinine (**2**) and lupinine (**3**) are a pair of diastereomers with an equatorial and an axial hydroxymethyl group, respectively, at the C-5 position of trans-quinolizidine.[6] The substituent effects of the hydroxymethyl group in the ^{13}C-nmr spectrum of **1** were similar to those of **2** rather than those of **3** (Table I). Accordingly, the structure of **1** was presumed to be 5-hydroxymethyl-trans-indolizidine, in which the hydroxymethyl group was oriented equatorially.

The structure of **1** was confirmed by comparison of the spectral data with those of the diastereomers of 5-hydroxymethylindolizidine obtained synthetically: 5-hydroxymethyl-1-azabicyclo[4.3.0]nona-6,8-diene (**6**) was synthesized according to Tanis's method.[7] Hydrogenation of **6** in AcOH with PtO_2 gave the diastereomers (**1** and **7**) of 5-hydroxymethylindolizidine in 45% and 31% yields, respectively. One of the diastereomers was completely identical with **1** in all measurable respects (tlc, hplc, ms, ^{1}H-nmr and ^{13}C-nmr). The ^{13}C-nmr spectrum of the other diastereomer (**7**) was reasonably assigned as shown in Table I. The differences in the chemical shifts of the ^{13}C-nmr spectra between **5** and **7** were in fair agreement with those between **4** and **3** (Table I). In addition, the characteristic ^{1}H-nmr signals correponding to the methylene protons of the hydroxymethyl group of **1** and **7** showed good coincident with those of **2** and **3**, respectively, as shown in Table II. These spectral results indicated that **1** and **7** were a pair of diastereomers of 5-hydroxymethyl-trans-indolizidine and their stereochemistries corresponded to those of **2** and **3**, respectively.

Consequently, the structure of the new alkaloid, tashiromine, was determined to be (5S,6R)-5-hydroxymethyl-trans-indolizidine (**1**) or its enantiomer.[8]

Table II. ^{1}H-Nmr signals due to methylene protons of the hydroxymethyl group in **1**, **2**, **3** and **7** in $CDCl_3$

compounds	H_1	H_2
1	3.64, dd, J = 10.0 and 4.0	3.48, dd, J = 10.0 and 6.0
7	4.18, ddd, J = 9.2, 4.3 and 1.2	3.73, dd, J = 9.2 and 1.1
2	3.66, dd, J = 11.0 and 4.5	3.60, dd, J = 11.0 and 3.4
3	4.17, ddd, J = 10.5, 4.3 and 1.8	3.70, d, J = 10.5

It is well known that quinolizidine alkaloids are biosynthesized from lysine.[10,11] In pyrrolidine and indolizidine alkaloids such as tabacco, tropane, Elaeocarpus and phenanthroindolizidine alkaloids, the pyrrolidine ring is shown to be derived from ornithine.[10,12] M. tashiroi accumulates the alkaloid containing a indolizidine ring, tashiromine, together with quinolizidine (lupin) alkaloids in analogy with the case of M. amurensis.[2] This suggests that Maackia species have the biosynthetic ability of utilizing ornithine as well as lysine as amino acid precursors. Thus, Maackia species are of interest from biochemical and chemotaxonomical stand points.

Further investigations on the absolute stereochemistry of **1** and on alkaloid constituents in M. tashiroi are being undertaken in our labolatories.

EXPERIMENTAL

The following equipments were used: ^{1}H- (400 MHz) and ^{13}C-nmr (100 MHz) spectra, JEOL JNM-GX 400 spectrometer with tetramethylsilane (TMS) as an internal standard; ms spectra, JEOL JMS D-300 mass spectrometer. Column chromatography was carried out with kieselgel 60. Thin-layer chromatography (tlc) was performed 0.25 mm precoated silica gel (60 F_{254}, Merck), and spots were detected by exposure to I_2 vapor or spraying with Dragendorff's reagent. Preparative tlc was conducted with 1.0 mm precoated silica gel (Merck). Analytical hplc was carried out with solvent, 15% MeOH in ether-H_2O-25% NH_4OH (500:10:3), on a LiChrosorb SI-100 (5 μm, 0.4 x 25 cm, Merck) column.

Plant material.

M. tashiroi was collected early in August in Kumamoto prefecture, Japan.

Extraction and isolation.

The air-dried stems (1.2 kg) of M. tashiroi were extracted three times with 75% MeOH (10 l) at room temperature for 9 days. The combined extracts were concentrated *in vacuo*, acidified with dil. HCl and filtered. The acid filtrate was washed twice with ether, made strongly alkaline with K_2CO_3 under ice-cooling, and then extracted with CH_2Cl_2 several times. The CH_2Cl_2 extracts were combined, dried over anhydrous K_2CO_3 and evaporated to dryness to give 5.7 g of a crude

alkaloid fraction. The alkaloid fraction was applied to a silica gel (500 g) column and eluted successively with $CHCl_3$, 1%, 2%, 3%, 4%, 5%, 10%, 15% and 20% MeOH in $CHCl_3$ to give nine fractions. The 20% MeOH in $CHCl_3$ eluent (0.61 g) was rechromatographed on silica gel column using CH_2Cl_2-MeOH-25% NH_4OH(90:9:1). The **1**-rich fraction was purified by preparative tlc with Et_2O-MeOH-25% NH_4OH(17:2:1) to give **1** (2 mg); colorless oil, eims and hreims m/z (%): 155.1307 (M^+, calcd for $C_9H_{17}NO$ 155.1309, 63), 154 (65), 138 (M^+-OH, 97), 124 (60), 110 (20), 97 (68), 96 (100), 84 (43), 83 (46), 82 (18), 70 (31), 69 (43). ^1H- and ^{13}C-nmr data are given in Tables II and I, respectively.

Hydrogenation of 5-hydroxymethyl-1-azabicyclo[4.3.0]nona-6,8-diene (6).

The compound **6** was synthesized according to the method reported by Tanis and Ragon.[7] To a solution of **6** (76.7 mg, 0.51 mmol) in AcOH (16 ml) was added PtO_2 (13 mg). The mixture was hydrogenated under H_2 (6 Kg/cm^2) at room temperature for 8 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was chromatographed on a silica gel column (15 g) using CH_2Cl_2-MeOH-25% NH_4OH (90:9:1) to give **1** (35.5 mg, 45%) and **7** (24.4 mg, 31%). The synthetic **1** was consistent with the natural **1** on tlc, hplc, ms, ^1H-nmr and ^{13}C-nmr spectra. **7**; colorless oil, eims m/z (%): 155 (M^+, 55), 154 (51), 138 (M^+-OH, 70), 124 (50), 110 (27), 97 (51), 96 (100), 84 (63), 83 (62), 82 (27), 70 (37), 69 (42). ^1H- and ^{13}C-nmr data are given in Tables II and I, respectively.

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