(−)-Camoensidine *N*-Oxide; A New Alkaloid from *Maackia tashiroi*[1)]

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A new alkaloid (3) was isolated from the stems of *Maackia tashiroi* (Leguminosae), together with (−)-camoensidine, tashiromine, ammodendrine and six known lupin (quinolizidine) alkaloids. The structure of 3 was characterized as the N_{15}-oxide of (−)-camoensidine, possessing an indolizidine-quinolizidine ring system, by a combination of spectroscopic and chemical methods.

Keywords *Maackia tashiroi*; Leguminosae; lupin alkaloid; quinolizidine alkaloid; quinolizidine-indolizidine alkaloid; (−)-camoensidine; (−)-camoensidine *N*-oxide; (−)-cytisine; (+)-epilupinine; tashiromine

In the course of our studies on lupin alkaloids in leguminous plants native to Japan, we have recently reported the isolation and structural determination of the new alkaloid named tashiromine from the stems of *Maackia tashiroi* (Leguminosae).[2)] Tashiromine **1** possesses an indolizidine ring system as a structural unit and is similar to the typical lupin (quinolizidine) alkaloid epilupinine (**2**) coexisting in the same plant. Further examination of alkaloid constituents in the plant led to the isolation of a new alkaloid (**3**), together with (−)-camoensidine (**4**), tashiromine (**1**), ammodendrine and six known lupin alkaloids. In this paper we wish to report the structural elucidation of **3** as the N_{15}-oxide of an indolizidine-quinolizidine alkaloid, (−)-camoensidine (**4**).

The basic fraction (5.9 g) obtained from the 75% MeOH extract of the dry stems (1.2 kg) of *M. tashiroi*, collected in Kumamoto prefecture in August, was subjected to repeated column chromatography on silica gel to yield the new alkaloid (**3**), together with the known alkaloids, (−)-camoensidine (**4**), (−)-cytisine, (−)-*N*-methylcytisine, (−)-rhombifoline, (−)-*N*-formylcytisine, (−)-lusitanine, (+)-epilupinine, tashiromine and ammodendrine, which were identified by direct comparison with authentic samples except for **4**.

The new alkaloid (**3**) was obtained as hygroscopic colorless crystals, $[\alpha]_D^{27}$ −57° (c=0.79, EtOH). The molecular formula, $C_{14}H_{22}N_2O_2$, was determined from the M^+ ion peak at m/z 250 in the in-beam mass spectrum (MS), the highest mass peak at m/z 234.1729 ($C_{14}H_{22}N_2O$) in the high-resolution electron impact (HR-EI) MS, and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I), showing the presence of 14 carbons and 22 hydrogens. The EI-MS of **3** showed fragment peaks at m/z 234 (M^+ −O), 233 (M^+ −OH), and 232 (M^+ −H_2O), characteristic of aliphatic amine *N*-oxides.[3)] Catalytic hydrogenation of **3** with Pd–C in MeOH gave the free base, which was identical with (−)-camoensidine (**4**), an oil, $[\alpha]_D^{27}$ −73° (c=1.13, EtOH), isolated from the same source. Treatment of **4** with H_2O_2 regenerated **3**. Accordingly, the

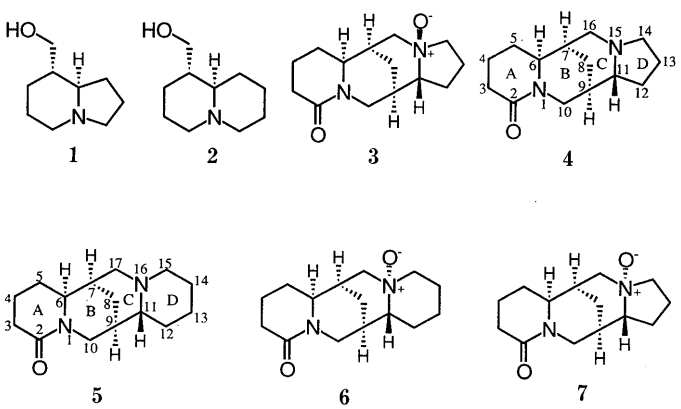


Chart 1

TABLE I. ^{13}C-NMR Data for **3**—**7** (δ ppm)

C	3	4	5[6)]	6[6)]	7
2	170.1	171.0	171.3	172.1	172.1
3	32.8	32.9	33.1	33.0	33.0
4	20.0	19.8	19.7	19.4	19.3
5	27.6	28.4	26.8	27.7	27.7
6	58.6	59.8	60.9	61.8	61.7
7	32.6	33.1	35.0	33.6	31.7
8	25.1	27.3	27.4	22.7	26.5
9	30.8	30.9	32.5	31.7	30.9
10	45.4	47.4	46.8	47.0	47.4
11	78.9	64.2	64.0	71.4	72.7
12	27.6	27.4	33.6	27.7	26.4
13	20.9	20.8	24.5	25.7	20.3
(14)[a)]			25.3	20.3	
14 (15)[a)]	72.2	54.2	55.4	69.6	69.6
16 (17)[a)]	61.7	49.1	52.9	65.2	61.7

Spectra were run in $CDCl_3$. *a*) Numbers in brackets refer to the extra carbon in the D-ring of **5** and **6**.

TABLE II. ^{1}H-NMR Data for **3**, **4** and **7** (δ ppm, J=Hz)

H	3	4	7
6	3.54, ddd, J=11.5, 3.2, 3.2	3.39, ddd, J=10.3, 4.9, 2.5	3.43, ddd, J=12.0, 4.8, 2.0
8α	1.78, m	1.46, dm, J=12.4	1.38, dm, J=12.5
8β	2.02, m	2.08, dddd, J=12.4, 3.9, 3.9, 2.4	3.90, dddd, J=12.5, 2.5, 2.5, 3.0
10α	2.85, dd, J=14.0, 3.7	2.67, dd, J=13.2, 2.7	2.60. dm, J=13.0
10β	4.87, d like, J=14.0	4.63, ddd, J=13.2, 2.4, 2.4	4.54, ddd, J=13.0, 3.0, 3.0
11	3.87, dd, J=9.3, 9.3	2.47, m	2.65, m
14α	3.66, m	2.40, dm, J=9.1	3.63, ddm, J=9.0, 9.0
14β	3.66, m	2.85, ddm, J=9.1, 9.1	3.14, ddd, J=9.0, 9.0, 9.0
16α	3.43, dd, J=13.4, 4.6	2.80, dd, J=11.9, 7.0	3.90, dd, J=12.5, 10.5
16β	3.78, dm, J=13.4	2.56, dm, J=11.9	2.98, dd, J=12.5, 2.5

Spectra were run in $CDCl_3$.

new base **3** was presumed to be an *N*-oxide of **4**.

The molecular formula of **4**, $C_{14}H_{22}N_2O$, determined from the HR-EI-MS, is one methylene unit less than that of a typical lupin alkaloid lupanine (**5**). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **4** showed signals at δ 4.63 (1H, ddd, J=13.2, 2.4 and 2.4 Hz, 10β-H), 2.67 (1H, dd, J=13.2 and 2.7 Hz, 10α-H) and 3.39 (1H, ddd, J=10.3, 4.9 and 2.5 Hz, 6-H), which correspond to the signals due to the C-10 methylene and the C-6 methine protons in the 2-quinolizidone moiety of lupanine-type lupin alkaloids.[4-6] The ^{13}C-NMR spectrum of **4** resembles that of **5**, especially the signals due to 2-C through 11-C (Table I). The base peak at m/z 122 and significant peaks at m/z 135 and 136 in the EI-MS of **4** were 14 mass units less than those (m/z 136, 149 and 150, respectively) of **5** which arise from the C/D ring.[7] These results indicate that 4 might be a homolog of lupanine (**5**), the D-ring of which is a pyrrolidine ring. The new alkaloid (**3**) was, therefore, presumed to be an N_{15}-oxide of **4**.

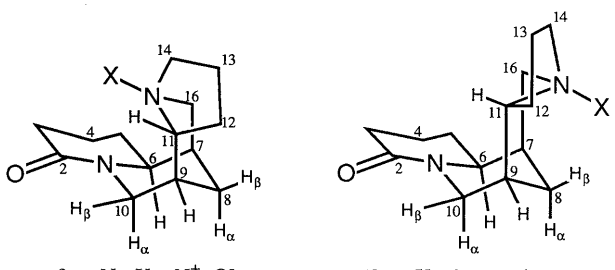
The relative stereochemistry at C-6, C-7, C-9 and C-11 of **4**, which is the same as that of **3**, was confirmed by measurements of the difference nuclear Overhauser effect (NOE) spectra of **3** and **4**. The ^{1}H- and ^{13}C-NMR spectra of **3** and **4** were assigned by means of ^{1}H–^{1}H and ^{1}H–^{13}C correlation spectroscopy (COSY), taking into account the assignments of lupanine (**5**)[5] and its *N*-oxide (**6**)[6] (Tables I and II). In the NOE spectrum of **4**, irradiation of the signal due to 6-H (δ 3.39) resulted in enhancements of the signals of 8α-H (δ 1.46) and 10α-H (δ 2.67), indicating a *trans* ring junction of the A/B ring (2-quinolizidone moiety) system, namely the α-configuration of 6-H, 7-H and 9-H of **4** (and **3**), as illustrated in **4a** or **4b** (and **3a** or **7a**) (Chart 2). The β-configuration of 11-H in **3** (and **4**) was revealed by the NOE experiment on **3**. Irradiation of the signal due to 10β-H (δ 4.87) induced enhancements of the signals corresponding to 11-H (δ 3.87) and 10α-H (δ 2.85) (Chart 2). The above results indicate that **4** has the same relative stereochemistry as that of lupanine (**5**) (Chart 1) and hence **4** was characterized as (−)-camoensidine,[8] isolated from *Camoensia maxima*[9] and *C. brevicalyx*[10] (Leguminosae). Therefore, it was concluded that the new alkaloid (**3**) was an N_{15}-oxide of (−)-camoensidine (**4**).

Two diastereomers (**3** and **7**) are possible for camoensidine *N*-oxide, taking into account the configuration of the *N*-oxide nitrogen: one (**3a**) involving a *cis*-indolizidine (C/D ring) with a chair piperidine ring (C-ring) and the other (**7a**) a *trans* indolizidine with a boat piperidine ring.

In fact, the two *N*-oxides (**3** and **7**) were obtained in 60% and 26% yields, respectively, on oxidation of **4** with *m*-chloroperbenzoic acid (MCPBA) in CH_2Cl_2, though oxidation of **4** with H_2O_2 gave only the natural *N*-oxide (**3**), as described above. The major product was identical with the natural *N*-oxide (**3**). The minor one (**7**), mp 111—113 °C, $[\alpha]_D^{27}$ −76°, was assigned as the other *N*-oxide of **4** from the M^+ peak at m/z 250.1683 and the similarity of the EI-MS of **7** to that of **3**.

The structural assignment of the two *N*-oxides **3** and **7** was confirmed by comparison of chemical shift differences between 8α-H and 8β-H in the ^{1}H-NMR spectra. The ^{1}H-NMR assignment of **7** was determined by analysis of the ^{1}H–^{1}H-COSY and ^{1}H–^{13}C-COSY spectra (Table II). The difference (2.52 ppm) in **7** was much larger than that (*ca.* 0.24 ppm) in **3**, which was attributable to the unusual down-field shift of 8β-H (δ 3.90) in **7**. This could be explained by a through-space deshielding effect of the *N*-oxide oxygen. Accordingly, the structure of the synthetic *N*-oxide (**7**) could be assigned as **7a** in which the 8β-H is very close to the *N*-oxide oxygen. Consequently, the structure of the new alkaloid **3** was determined as **3a**, in which the 8-H_2 is very far from the *N*-oxide oxygen.

Further investigation on the absolute stereochemistry of (−)-camoensidine (**4**) is being undertaken in our laboratories.



3a : N - X = N^+ - O^-
4a : X = lone pair

4b : X = lone pair
7a : N - X = N^+ - O^-

Chart 2

Experimental

Melting points were determined on a Yanagimoto micro melting apparatus and are uncorrected. The following equipments were used to obtain physical data: infrared (IR) spectra, Hitachi 215 grating infrared spectrometer; ^{1}H- and ^{13}C-NMR spectra, JEOL JNM-GX 400 (^{1}H, 399.78 MHz; ^{13}C, 100.43 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard; optical rotations, Jasco DIP-181 polarimeter; EI-MS and HR-EI-MS, JEOL D-300 spectrometer, in-beam MS; Hitachi RMU 7H spectrometer. Column chromatography was carried out with Kiesel gel 60 (70—230 mesh or 230—400 mesh, Merck). Thin-layer chromatography (TLC) was performed on 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 high-performance liquid chromatography (HPLC) was carried out as described previously.[11]

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

Extraction and Isolation of Alkaloids The dried stems (1.2 kg) of *M. tashiroi* were extracted three times with 75% MeOH at room temperature. The combined extracts were concentrated *in vacuo*, acidified with dilute HCl and filtered. The acid filtrate was extracted 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 (0.49%/dry material) of a crude alkaloid fraction. The alkaloid fraction was applied to a silica gel column (500 g) and eluted successively with CH_2Cl_2, 1%, 2%, 3%, 4%, 5%, 10%, 15% and 20% MeOH in $CHCl_3$, and MeOH, monitoring with TLC, to give seven fractions. Fraction 2 (170 mg) was purified on a silica gel column with C_6H_6–MeOH (20 : 1) to yield (−)-rhombifoline (61 mg, a colorless oil, $[\alpha]_D^{25}$ −175° (c=0.41, EtOH)). Fraction 3 (255 mg) consisted of (−)-anagyrine and (−)-*N*-methylcytisine, which were identified by gas liquid chromatography (GLC)-EI-MS and HPLC. Fraction 4 (2.15 g) was rechromatographed on a silica gel column using CH_2Cl_2–MeOH–28% NH_4OH (90 : 9 : 1) to give (−)-*N*-formylcytisine (72 mg, mp 172 °C, $[\alpha]_D^{25}$ −230° (c=0.30, EtOH)) and (−)-cytisine (1.95 g, mp 155 °C, $[\alpha]_D^{25}$ −110° (c=0.26, EtOH)). Fraction 5 (2.4 g) was fractionated through a silica gel column with CH_2Cl_2–MeOH–28% NH_4OH (95 : 5 : 0.5) to yield (+)-epilupinine (1.53 g, mp 77–78 °C, $[\alpha]_D^{25}$ +33° (c=0.50, EtOH), (−)-cytisine (0.75 g), ammodendrine (10 mg, a colorless oil), baptifoline (6 mg, mp 210 °C) and (−)-lusitanine (120 mg, mp 185—187 °C, $[\alpha]_D^{25}$ −7.7° (c=0.31, EtOH). Fraction 6 (0.6 g) was subjected to column chromatography on silica gel using CH_2Cl_2–MeOH–28% NH_4OH (90 : 9 : 1). The tashiromine (**1**)-rich fraction obtained was purified by preparative TLC with

Et_2O–MeOH–28%NH_4OH (17:2:1) to give **1**.[2] Fraction 7 (130 mg) was rechromatographed over a silica gel column with Et_2O–MeOH–25% NH_4OH (40:2:1) to give (−)-camoensidine (**4**, 7 mg) and (−)-camoensidine *N*-oxide (**3**, 15 mg). **4**: A colorless oil, $[\alpha]_D^{27}$ −73° (c=1.13, EtOH). EI-MS (70 eV) *m/z*: 234.1735 (M^+, 234.1733 for $C_{14}H_{22}N_2O$, 71), 233 (62), 136 (82), 135 (47), 122 (100), 84 (53). **3**: Hygroscopic colorless crystals, $[\alpha]_D^{27}$ −57° (c=0.79, EtOH). In-beam MS (70 eV) *m/z*: 250 (M^+, 25), 234 (M^+ −O, 61), 233 (M^+ −OH, 100), 232 (M^+ $-H_2O$, 24), 84 (69). EI-MS (70 eV) *m/z*: 234.1729 (M^+ −O, 234.1733 for $C_{14}H_{22}N_2O$, 50), 233 (M^+ −OH, 53), 232 (M^+ $-H_2O$, 88), 136 (50), 135 (55), 134 (60), 122 (82), 120 (100). All the known alkaloids except for **4** were identified by direct comparison with authentic compounds by co-TLC, co-HPLC, MS, IR, and ^{1}H-NMR.

Oxidation of (−)-Camoensidine (4) with H_2O_2 A solution of **4** (11.7 mg, 0.05 mmol) in 30% H_2O_2 (0.5 ml) was stirred for 15 h at ambient temperature. After decomposition of the excess H_2O_2 with a catalytic amount of MnO_2, the reaction mixture was made strongly alkaline with K_2CO_3 and extracted with CH_2Cl_2 several times. The combined extracts were dried over anhydrous K_2CO_3 and concentrated to dryness *in vacuo*. The residue was purified on a silica gel column using CH_2Cl_2–MeOH–28% NH_4OH (90:9:1) to give **3** as a colorless oily product (11.9 mg, 95% yield), which was identical with the natural *N*-oxide (**3**) (co-TLC, co-HPLC, MS and ^{1}H-NMR).

Oxidation of (−)-Camoensidine (4) with MCPBA MCPBA (85%, 22.3 mg, 0.11 mmol) was added to a solution of **4** (26.4 mg, 0.11 mmol) in 1 ml of CH_2Cl_2. After being stirred for 3 h at ambient temperature, the reaction mixture was washed with a saturated solution of K_2CO_3 (1 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel with CH_2Cl_2–CH_3OH–28% NH_4OH (90:9:1) to give the *N*-oxides **7** (7.2 mg, 26% yield) and **3** (16.9 mg, 60% yield), in that order of elution. **7**: Colorless crystals from C_6H_6–EtOH, mp 111—113 °C, $[\alpha]_D^{27}$ −76° (c=0.41, EtOH). EI-MS (70 eV) *m/z*: 250.1683 (M^+, 250.1682 for $C_{14}H_{22}N_2O_2$, 32), 234 (M^+ −O, 25), 233 (M^+ −OH, 38), 232 (M^+ $-H_2O$, 37), 134 (100), 122 (52), 120 (53).

Hydrogenation of (−)-Camoensidine *N*-Oxide (3) A solution of **3** (0.9 mg) in MeOH (1 ml) was hydrogenated over 10%Pd–C at atmospheric pressure and ambient temperature. The catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel with CH_2Cl_2–MeOH–28%NH_4OH (90:9:1) to give an oily product (0.7 mg), which was identical with (−)-camoensidine (**4**) (co-TLC, co-HPLC, co-GLC, MS).

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