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(+)-KURARAMINE, A POSSIBLE METABOLITE OF (−)-*N*-METHYLCYTISINE IN FLOWERS OF *SOPHORA FLAVESCENS*

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Key Word Index—*Sophora flavescens*; Leguminosae; alkaloid; (+)-kuraramine; (+)-mamanine; (−)-*N*-methylcytisine; (−)-anagyrine; metabolism; biosynthesis; variation of alkaloid content.

Abstract—From the flowers of *Sophora flavescens*, a new dipiperidine-type alkaloid, (+)-kuraramine, has been isolated together with (+)-mamanine and the other 15 known lupin alkaloids. Its structure has been characterized as *N*-methyl-3-hydroxymethyl-5-(2′-pyridon-6′-yl)-piperidine or its enantiomer by spectrometric data. The concentrations of (+)-kuraramine and (+)-mamanine in the budding flowers were very low, but increased rapidly during flower growth, unlike the contents of (−)-*N*-methylcytisine and (−)-anagyrine. From these results, (+)-kuraramine and (+)-mamanine are assumed to be metabolites of (−)-*N*-methylcytisine and (−)-anagyrine, respectively.

INTRODUCTION

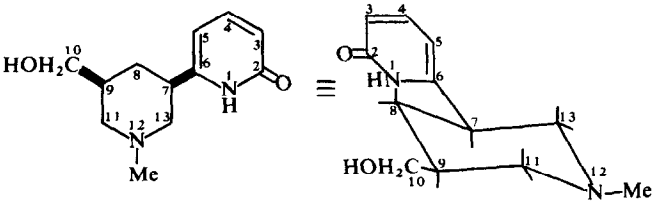
Previously, Murakoshi and Ohmiya *et al.* [1–14] have investigated the structure of the lupin alkaloids from a variety of Japanese leguminous plants of the genera *Sophora, Euchresta, Thermopsis, Echinosophora* and *Lupinus*, during which time the enzymes for the formation of (−)-(*trans*-4′-hydroxycinnamoyl) lupinine [15] from (−)-lupinine, and of (−)-*N*-methylcytisine [16] and (−)-*N*-acetylcytisine [17] from (−)-cytisine have also been found in the leguminous plants.

As part of this series, we have further investigated the basic constituents in the flowers of *S. flavescens* (Japanese name: kurara) which is native to the warm district of Japan. The roots are occasionally used as an indigenous crude drug in folk stomachics, diuretics, antipyretics, analgesics and as an insecticide. This paper describes the structure elucidation of a new dipiperidine-type alkaloid isolated from the flowers of this plant and variations in the alkaloid content at various stages of flower growth.

RESULTS AND DISCUSSION

From the *n*-hexane-insoluble fraction of the total crude alkaloid, obtained from the 75% EtOH extracts of the freshly harvested flowers at the last stages, a novel dipiperidine-type alkaloid, (+)-kuraramine (**1**), was isolated along with (+)-mamanine (**2**) and the other known lupin alkaloids.

(+)-Kuraramine (**1**) was obtained in a yield of 0.0021% of the fr. wt as a colourless, amorphous solid, $[\alpha]_D^{29}$ + 8.4° (EtOH), having a molecular formula of $C_{12}H_{18}N_2O_2$ (M^+, *m/z* 222.137, calc. 222.137). The IR spectrum of **1** showed bands at 1645, 1610 and 1550 cm^{-1} and the UV spectrum revealed the absorption maximum at 226



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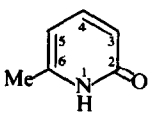

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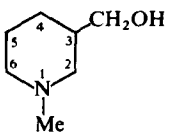

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($\log \varepsilon = 3.59$) and 304 ($\log \varepsilon = 3.64$) nm, both suggesting the presence of 2-pyridone moiety in the molecule [4, 9, 18]. The ^{1}H NMR spectrum ($CDCl_3$) of **1** showed a broad signal at δ 13.03 (1 H, NH) and three olefinic signals at 7.36 (1 H, *dd*, J = 9 and 7 Hz, 4-H), 6.42 (1 H, *dd*, J = 9 and 1 Hz, 3-H), 6.05 (1 H, *dd*, J = 7 and 1 Hz, 5-H) which were in accord with those of 6-methyl-2-pyridone (**3**). This indicates that **1** has the α-pyridone moiety substituted by an alkyl group at the C-6 position.

The high-field signals in the ^{1}H NMR spectrum of **1** were similar to those of *N*-methyl-3-hydroxymethylpiperidine (**4**). Thus, the signals at δ 3.49 (1 H, *s*) and 3.56 (2 H, *d*, J = 5.5 Hz) of **1** were assigned to the hydroxymethyl group, the three-proton singlet at 2.34 to the *N*-methyl group, the two-proton multiplet (apparent doublet) at 3.06 (J = 10 Hz) to the two equatorial protons on both C-11 and C-13 methylene carbons and the one-proton multiplet (apparent quartet) at 1.27 (J = 12 Hz) to the axial proton on the C-8 methylene carbon. In addition, the spectrum of **1** revealed the multiplet (apparent broad triplet, 1 H, J = 12 Hz) at δ 2.90 which could be assigned to the proton on the methine carbon bearing the 2-pyridone ring. This signal collapsed to an apparent broad doublet (J = 12 Hz) on irradiation at δ 1.27 due to 8-H_{ax}, indicating that the 2-pyridone moiety was linked at C-5 of **4** in the equatorial position.

From the above results, it can therefore be presumed that the structure of (+)-kuraramine is *N*-methyl-3-hydroxymethyl-5-(2′-pyridon-6′-yl)-piperidine (**1**) or its enantiomer which has the *cis*-configuration at the C-3 and C-5 positions.

The further identity of kuraramine as **1** was confirmed by the ^{13}C NMR spectrum (see Fig. 1), whose signal assignments were carried out by the aid of selective ^{1}H-decoupled and off-resonance ^{13}C NMR measurements.

(+)-Kuraramine (**1**) bears a marked structural resemblance to (+)-mamanine (**2**), one of the novel lupin alkaloids in the same flowers, which was isolated first by

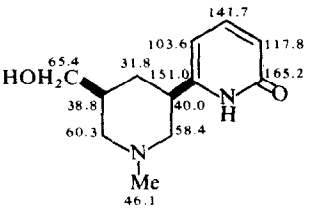

Fig. 1. ^{13}C NMR spectrum of (+)-kuraramine (**1**) in $CDCl_3$.

Kadooka *et al.* [19] from the bark of the endemic Hawaiian tree *Sophora chrysophylla*.

The structure of (+)-kuraramine (**1**) corresponds to an oxidative product derived from the N_1–C_{10} bond cleavage of (−)-*N*-methylcytisine (**5**) coexisting in the same flowers (Fig. 2). The structure of (+)-mamanine (**2**) could also be expected to be related to an oxidative product of (−)-anagyrine (**6**) at the same N_1–C_{10} position.

In fact, the concentrations of (+)-kuraramine (**1**) and (+)-mamanine (**2**), unlike those of (−)-*N*-methylcytisine (**5**) and (−)-anagyrine (**6**) in the budding flowers of *S. flavescens*, were low, but their concentrations increased rapidly during the flower's growth and became minor components again in the first stages of the seed's development (Fig. 3).

These facts strongly suggest that (+)-kuraramine (**1**) and (+)-mamanine (**2**) might be the oxidative metabolites of (−)-*N*-methylcytisine (**5**) and (−)-anagyrine (**6**), respectively. Detectable amounts of (+)-kuraramine (**1**) and (+)-mamanine (**2**) in the *Sophora* flower have been found only in the mature ovary and in the earlier stages of the capsule. In addition, a C-9 isomer of (+)-kuraramine has also been isolated from the same flowers but the available amount preclude exact characterization of the structure.

Further investigation on the absolute configuration and the biosynthesis of (+)-kuraramine (**1**) and (+)-

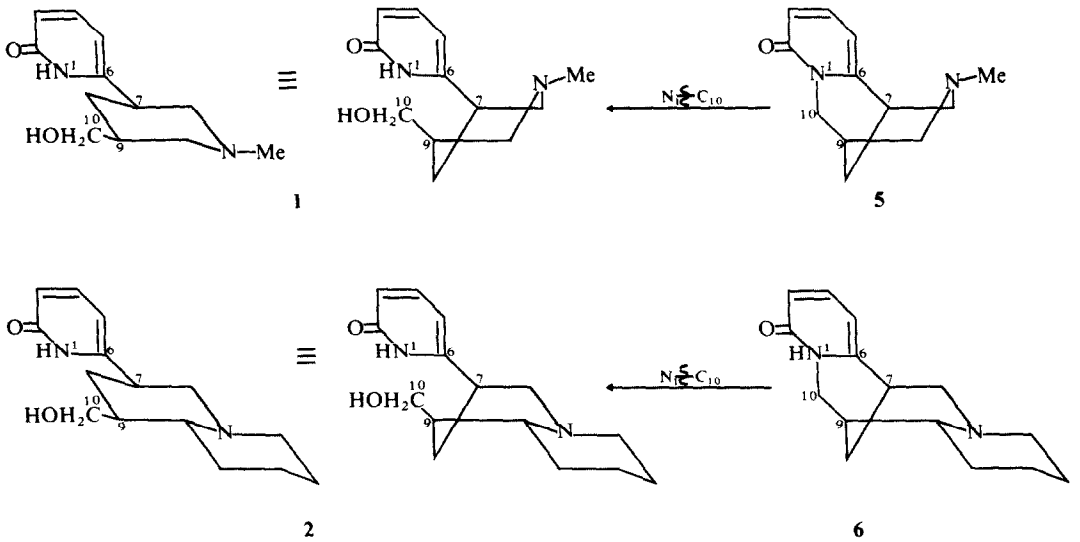

Fig. 2. Structural correlations of (+)-kuraramine (**1**) and (+)-mamanine (**2**) with (−)-*N*-methylcytisine (**5**) and (−)-anagyrine (**6**), respectively, co-existing in the flowers of *S. flavescens*.

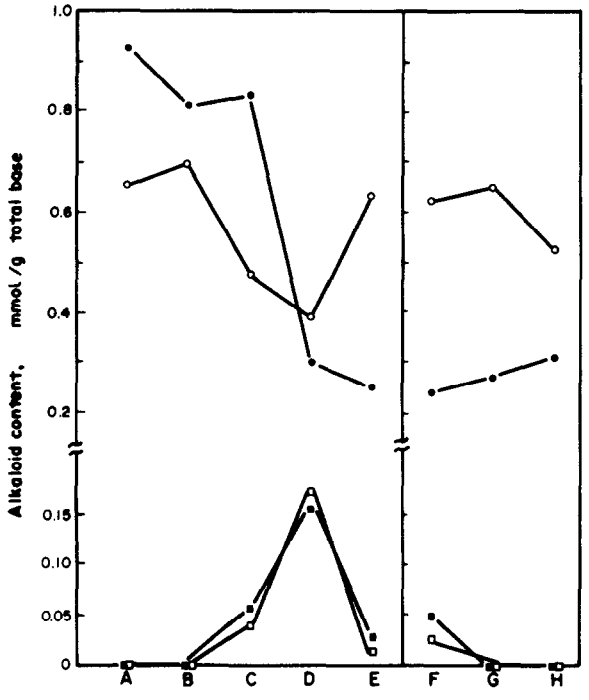

Fig. 3. Relative variations in contents of (+)-kuraramine (**1**) (■——■) and (+)-mamanine (**2**) (□——□) to those of (−)-*N*-methylcytisine (**5**) (●——●) and (−)-anagyrine (**6**) (○——○) in the flowers of *S. flavescens* during the flower development. A, Budding; B, growing; C, full; D, end; E, immature capsules and seeds; F, immature capsules; G, immature seeds; H, mature seeds.

mamanine (**2**) is currently being undertaken in our laboratories.

EXPERIMENTAL

The high and low resolution MS were measured at 70 eV. The ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) were recorded using TMS as int. standard. TLC were performed on Si gel plates with (1) CH_2Cl_2–MeOH–28% NH_4OH (90:9:1) and (2) CH_2Cl_2–MeOH–28% NH_4OH(43:6:1). Analytical HPLC were carried out with (3) 15% MeOH in Et_2O–2.5% NH_4OH (50:1) (4) 15% MeOH in Et_2O–H_2O–25% NH_4OH (500:10:3) and (5) 10% MeOH in CH_2Cl_2–25% NH_4OH (50:1) using a LiChrosorb SI 100 (Merck, particle size 10 μm, 0.3 × 50 cm) column employing a monitoring flow system (310 nm) coupled to recorder at a flow rate of 1 ml/min.

Isolation of (+)-kuraramine (**1**). The alkaloid fraction (20 g) obtained from the 75% EtOH extracts of fresh flowers (2.80 kg) of *S. flavescens* was separated to *n*-hexane-soluble (6.1 g) and -insoluble (13.9 g) portions. The insoluble fraction was subjected to chromatography on a Si gel column (Merck, type 60, 230–400 mesh, 200 g, 3.4 × 50 cm) using gradient elution with 4% MeOH in CH_2Cl_2–28% NH_4OH (1000:1) to 40% MeOH in CH_2Cl_2–28% NH_4OH (50:1). The **1**-rich fraction (0.3 g) obtained from the 40% MeOH in CH_2Cl_2–28% NH_4OH (50:1) eluate was purified by prep. HPLC (column; LiChrosorb SI 100, particle size 10 μm, 0.5 × 50 cm) monitoring UV detector (310 nm) with 10% MeOH in CH_2Cl_2–25% NH_4OH (50:1). **1** was obtained as a colourless, amorphous solid (58 mg, 0.0021%/fr. wt), $[\alpha]_D^{29}$ +8.4° (EtOH, c = 0.52). MS (probe) m/z (rel. int.): 222.137 (M^+, calc. for $C_{12}H_{18}N_2O_2$ 222.137, 91), 204 ($M^+ - H_2O$, 10), 191 ($M^+ - CH_2OH$, 35), 133 (34), 122 (50), 121 (81), 101 (44), 58 ($Me_2\overset{+}{N}=CH_2$, 100). IR ν_{max}^{KBr} cm^{-1}; 3350, 3150 (NH and OH), 2790 (>NMe), 1645, 1610, 1550 (α-pyridone). UV λ_{max}^{EtOH} nm (log ε): 226 (3.59), 304 (3.64). ^{1}H NMR ($CDCl_3$): δ 1.27 (1 H, *m* (apparent *q*), J = 12 Hz, 8-H_{ax}), 1.6–2.2 (4 H, *m*), 2.34 (3 H, *s*, NMe), 2.90 (1 H, *m* (apparent *br. t*, J = 12 Hz, 7-H), 3.06 (2 H, *m* (apparent *d*), J = 10 Hz, 11- and 13-H_{eq}), 3.49 (1 H, *s*, OH), 3.56 (2 H, *d*, J = 5.5 Hz, 10-H_2), 6.05 (1 H, *dd*, J = 7 and 1 Hz, 5-H), 6.42 (1 H, *dd*, J = 9 and 1 Hz, 3-H), 7.36 (1 H, *dd*, J = 9 and 7 Hz, 4-H), 13.03 (1 H, *br.*, NH). ^{13}C NMR ($CDCl_3$): δ 165.2 (*s*, 2-C), 117.8 (*d*, 3-C), 141.7 (*d*, 4-C), 103.6 (*d*, 5-C), 151.0 (*s*, 6-C), 40.0 (*d*, 7-C), 31.8 (*t*, 8-C), 38.8 (*d*, 9-C), 65.4 (*t*, 10-C), 60.3 (*t*, 11-C)*, 58.4 (*t*, 13-C)*, 46.1 (*q*, NMe). (*, These assignments could be reversed.)

The R_f values of **1** on Si gel TLC using solvents 1 and 2 were 0.13 and 0.20, respectively. The R_t (min) values of **1** on analytical HPLC using solvents 3, 4 and 5 were 56.7, 43.7 and 22.5, respectively.

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