Nematicidal Activity of Matrine and Its Derivatives against Pine Wood Nematodes

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The nematicidal activity of matrine and its derivatives isolated from the epigeal part of *Sophora flavescens* was examined against the pine wood nematode (*Bursaphelenchus xylophilus*). The nematicidal activity of matrine, which is one of the major alkaloids in the root of the plant, was poor. However, sophocarpine, one of the unsaturated derivatives of matrine, had strong nematicidal activity against nematodes; another unsaturated derivative, sophoramine, had such activity, but it was less than that of sophocarpine. These results suggest that the degree of unsaturation in the δ-lactam ring of matrine-type alkaloids is important to nematicidal activity.

INTRODUCTION

The pine wilt disease is a very serious problem affecting pine trees (*Pinus densiflora* and *P. thunbergii*) in Japan. The disease is caused by the pine wood nematode (*Bursaphelenchus xylophilus*), spreading with the vector beetle (*Monochamus alternatus*). One method of controlling the disease is to kill or suppress the parasitic nematode, but no effective and practical controls by chemicals have so far been established.

A variety of lupin alkaloids have been isolated and their structures characterized (Figure 1). Some have biological activities against plants, microorganisms, and molluscs (Wink, 1983, 1984a,b). *N*-Methylcytisine (**5**) and anagyrine (**6**), isolated from the root of *Sophora flavescens*, are nematicidal toward the pine wood nematode (Matsuda et al., 1989). In that study, the crude fraction containing matrine (**1**) and sophocarpine (**2**) was found to be less active against the nematodes than that containing the cytisine-type alkaloids **5** and **6**. However, matrine shows biological activity against human parasitic nematodes (Terada et al., 1982), and therefore matrine and its derivatives may also have nematicidal activities against *B. xylophilus*. A few matrine-type alkaloids were consequently isolated and their nematicidal activity against pine wood nematodes was examined to investigate the structure–activity relationship with respect to the δ-lactam moiety of the alkaloids.

MATERIALS AND METHODS

Instruments. Ultraviolet (UV) absorption spectra were recorded on a Shimadzu UV-204 spectroscope. Infrared (IR) spectra were obtained on a Shimadzu IR-408 spectroscope. Mass (MS) spectrometry was carried out by a Hitachi M80-MS spectroscope. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM-GSX 500 (500-MHz) spectroscope. Samples were dissolved in chloroform-*d* ($CDCl_3$), and chemical shifts were expressed in parts per milltion (ppm) downfield from tetramethylsilane (TMS).

Chemicals. Sparteine for use in the nematicidal test was purchased from Sigma Chemical Co. Wako-gel C 300, used for silica gel column chromatography, was obtained from Wako Pure Chemical Industries, Ltd. For thin-layer chromatography (TLC), Merck silica gel plates (60 F_{254}, No. 5554) were used.

Isolation of Matrine and Its Derivatives. The epigeal part of *S. flavescens* (45 kg) was extracted with methanol (30 L × 3), and the solvent was evaporated below 40 °C. The residual aqueous solution was extracted with benzene (20 L), and the organic layer was extracted with the same volume of 1 N HCl solution. The acidic aqueous layer was made basic (pH 10) with

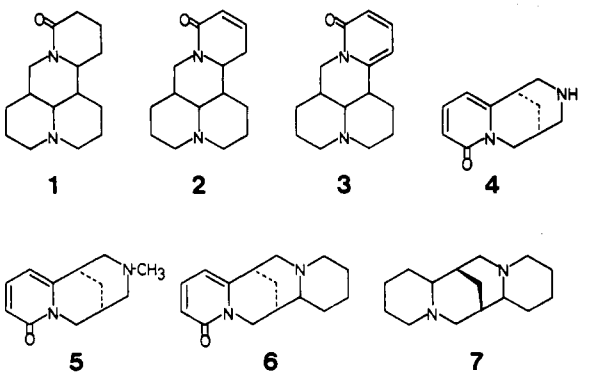

Figure 1. Chemical structures of lupin alkaloids. **1**, Matrine; **2**, sophocarpine; **3**, sophoramine; **4**, cytisine; **5**, *N*-methylcytisine; **6**, anagyrine; **7**, sparteine.

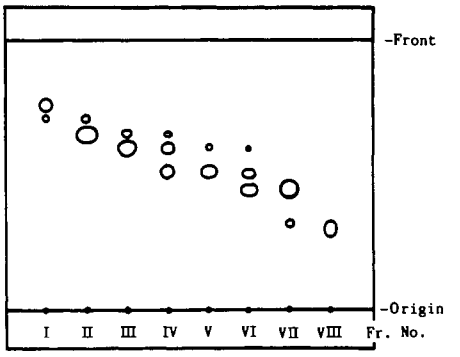

Figure 2. Thin-layer chromatogram of each fraction separated from the benzene-soluble basic fraction. Solvent system: Et_2O–MeOH–28% NH_4OH (40:4:1).

K_2CO_3 in an ice–water bath and extracted with benzene (23 L). The organic solvent was evaporated to give a benzene-soluble basic fraction (8.5 g). One gram of the basic fraction was put on a silica gel column (76 g, 2 × 63 cm) and eluted with ether–MeOH–28% NH_4OH (40:2:1). Fractions I–VIII were collected with reference to spot densities, and their R_f values on silica gel TLC were detected by UV absorption and I_2 vapor (TLCs of eight fractions are shown in Figure 2). On the basis of the TLC, sophocarpine (**2**), matrine (**1**), and sophoramine (**3**) were isolated from fractions II, III, and V, respectively, as described below.

Fraction II (120 mg) was separated on a silica gel column (17 g, 1.2 × 36 cm), by eluting with CH_2Cl_2–MeOH (10:1), and the eluate containing the most abundant component in the fraction was collected. The obtained crude solid was purified by use of a silica gel column (18 g, 1.2 × 37 cm) with CH_2Cl_2–MeOH (20:1) to give yellowish crystals of sophocarpine (23 mg). Recrystal-

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lization of the compound from petroleum ether yielded colorless fine prisms (4.2 mg). The physicochemical data of sophocarpine were as follows: mp 43–45 °C; $[\alpha]^{20}_D$ (c 0.1) = −31°; UV λ_{max} (EtOH) (log ϵ) 260 (3.40) nm; IR ν_{max} (KBr) 2830, 2780 (*trans*-quinolizidine), 1660, 1596 (α,β-unsaturated lactam C=O) cm^{-1}; EIMS m/z (relative abundance, %) 246.1709 (M^+, $C_{15}H_{22}ON_2$; calcd 246.1732, 90), 245 (100), 203 (12), 150 (30), 138 (18), 96 (38); ^{1}H NMR δ ($CDCl_3$) 6.45 (1 H, ddd, J = 9, 5, 4 Hz, C14-H), 5.89 (1 H, ddd, J = 10, 2, 2 Hz, C17-H), 4.14 (1 H, dd, J = 13, 5 Hz, C17-H), 3.98 (1 H, ddd, J = 9, 9, 7 Hz, C11-H), 3.16 (1 H, t, J = 13 Hz, C17-H), 2.86–2.76 (2 H, m, C2-H and C10-H), 2.60 (1 H, ddd, J = 18, 6, 6 Hz), 2.20 (1 H, ddt, J = 18, 9, 3 Hz), 2.10 (1 H, br s), 2.00–1.90 (2 H, m), 1.86–1.40 (10 H, m); ^{13}C NMR δ ($CDCl_3$) 165.7 (C15), 137.3 (C13), 124.7 (C14), 63.5 (C6), 57.3 (C2 and C10), 51.4 (C11), 42.0 (C17), 41.6 (C7), 34.6 (C5), 27.8 (C12), 27.4 (C4), 26.6 (C8), 21.1 (C9), 20.8 (C3). The analytical data shown above were in good agreement with those reported in the literature (Okuda et al., 1965; Bohlmann and Zeisberg, 1975).

Matrine, which was most abundant in fraction III, was isolated from the fraction by use of a silica gel column (16 g, 1.2 × 34 cm) and elution with ether–MeOH–28% NH_4OH (80:2:1). It was recrystallized from petroleum ether to give colorless needles (1.7 mg). The physicochemical properties shown below agreed with published data for the compound (Okuda et al., 1965; Bohlmann and Zeisberg, 1975; Morinaga et al., 1978): mp 75 °C; $[\alpha]^{20}_D$ (c 0.1, EtOH) = +37°; IR ν_{max} (KBr) 2860, 2800 (*trans*-quinolizidine), 1613 (δ-lactam C=O) cm^{-1}; EIMS 248.1863 (M^+, $C_{15}H_{24}ON_2$; calcd 248.1889, 81), 247 (60), 205 (58), 150 (55), 137 (47), 96 (100); ^{1}H NMR δ ($CDCl_3$) 4.40 (1 H, dd, J = 12.5, 4 Hz, C17-H), 3.82 (1 H, ddd, J = 10, 10, 6 Hz, C11-H), 3.05 (1 H, t, J = 12.5 Hz, C17-H), 2.87–2.76 (2 H, m, C2-H and C10-H), 2.43 (1 H, br d, J = 17 Hz), 2.28–2.19 (1 H, m), 2.12–2.05 (2 H, m), 2.00–1.87 (3 H, m), 1.84–1.75 (1 H, m), 1.76–1.35 (11 H, m); ^{13}C NMR δ ($CDCl_3$) 169.4 (C15), 63.8 (C6), 57.2 (C2 and C10), 53.2 (C11), 43.2 (C17), 41.5 (C7), 35.4 (C5), 32.9 (C14), 27.8 (C12), 27.2 (C4), 26.5 (C8), 21.2 (C9), 20.8 (C3), 19.0 (C13).

Recrystallization of the crude solid of fraction V from ether gave colorless needles of sophoramine (3, 30.4 mg) with mp 163–164 °C. The structure was confirmed by comparison of the following data with those already reported (Okuda et al., 1962): $[\alpha]^{20}_D$ (c 0.1, EtOH) = −96°; UV λ_{max} (EtOH) 310 (3.92), 235 (3.81) nm; IR ν_{max} (KBr) 2870, 2800 (*trans*-quinolizidine), 1650, 1578, 1547 (α-pyridone) cm^{-1}; EIMS 244.1572 (M^+, $C_{15}H_{20}ON_2$; calcd 244.1575, 100), 243 (54), 149 (30), 136 (69), 96 (18); ^{1}H NMR δ ($CDCl_3$) 7.29 (1 H, dd, J = 9, 7 Hz, C13-H), 6.45 (1 H, ddd, J = 9, 1.5, 1.5 Hz, C12-H), 6.23 (1 H, br d, J = 7 Hz, C12-H), 4.19 (1 H, dd, J = 15, 7 Hz, C17-H), 3.82 (1 H, dd, J = 15, 12 Hz, C17-H), 2.91 (1 H, br s, C6-H), 2.82–2.78 (2 H, m, C2-H and C10-H), 2.36 (1 H, br d, J = 14 Hz), 2.21 (1 H, t, J = 3 Hz), 2.12 (1 H, m), 2.04–1.91 (3 H, m), 1.78 (1 H, br d, J = 14 Hz), 1.73–1.55 (3 H, m), 1.55–1.46 (2 H, m); ^{13}C NMR δ ($CDCl_3$) 164.0 (C15), 148.0 (C11), 138.4 (C13), 116.5 (C14), 103.2 (C12), 60.5 (C6), 56.8 (C10), 56.6 (C2), 43.6 (C17), 38.5 (C7), 32.2 (C5), 28.1 (C4), 27.0 (C8), 21.2 (C9), 20.4 (C3).

Nematicidal Test. Substances were tested for nematicidal activity as reported elsewhere (Kawazu et al., 1980). Pine wood nematodes (*B. xylophilus*) were cultured on mycelia of *Botrytis cinerea* grown on Czapek-Dox agar plates at 26 °C and separated from the culture through double sheets of tissue paper (Wipers S-200, Kimbery-Clark Co.) by use of a Baermann funnel. A cotton ball (i.d. = 5 mm) containing a certain dose of the test substance was prepared by the injection of 100 μL of a methanol solution of the compound into the ball, which was dried at reduced pressure. A fungal mat of *B. cinerea* was made by culture of the fungi at 22 °C for 4 days on 3 mL of Czapek-Dox agar in a Petri dish (i.d. = 4 cm), and the cotton ball was placed in the center of the mat. Then 100 μL of an aqueous suspension of cultured nematodes (15 000 nematodes/mL) was pipetted into the cotton ball and the test culture was incubated at 26 °C. After 5 days, the living nematodes were separated from the culture through the tissue paper in the Baermann funnel and collected by centrifugation (650g, 3 min). The nematodes were killed by heating in boiling water, stained with 1% methylene blue, and counted. The dose–response relationship for the nematicidal effect of sophocarpine (2) is shown in Figure 3. The ID_{50} value in moles (the dose at which the propagation seen in the control was inhibited by 50%) was calculated by probit transformation for each compound (Finney, 1952). The log ($1/ID_{50}$) value was used as an index of the nematicidal activity of the compounds.

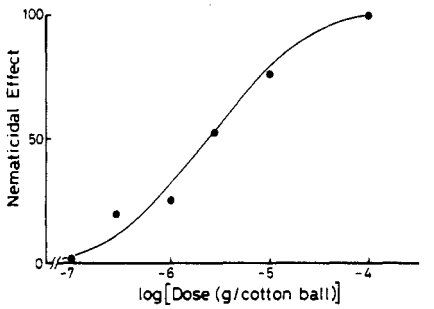

Figure 3. Dose–response relationship for the nematicidal effect of sophocarpine (2). The nematicidal effect was expressed as 100 × (1 − nematode number of test culture/nematode number of control culture).

Table I. Nematicidal Activity of Lupin Alkaloids

no.	compd	log ($1/ID_{50}$), mol/cotton ball
1	matrine	6.39
2	sophocarpine	7.78
3	sophoramine	6.68
4	cytisine	8.23[a]
5	*N*-methylcytisine	7.91[a]
6	anagyrine	7.54[a]
7	sparteine	7.96

[a] Data are quoted from Matsuda et al. (1989).

RESULTS AND DISCUSSION

The results of nematicidal tests of lupin alkaloids are given in Table I. This is the first report that sophocarpine is strongly nematicidal. The nematicidal activity varied with the number of olefinic bonds in the δ-lactam moiety of matrines (compounds 1–3). Since matrine-type alkaloids have a rigid skeleton constructed from rings, even a small structural change in a ring could result in a large conformational change of the molecule. An increase in the number of olefinic bonds in the δ-lactam moiety of matrine does alter slightly the hydrophobicity of the compound, but variations in the conformation of matrines might be more important as a cause of variations in their nematicidal activities.

Previously, we suggested that a cytisine-type structure contributes to nematicidal potency (Matsuda et al., 1989). The structure common to cytisines is an α-pyridone ring. Here, however, the nematicidal potency of sophoramine, which has an α-pyridone ring, was poor and that of sparteine (7), which does not have an α-pyridone ring, was almost as strong as that of *N*-methylcytisine (5). These results show that an α-pyridone ring is not necessarily favorable to nematicidal activity.

The mechanisms of action of matrine and cytisine in other animal species have been studied (Barlow and McLeod, 1969; Ishida and Shinozaki, 1984; Yamazaki and Arai, 1985). Matrine inhibits the response to glutamate at the crayfish neuromuscular junction, but *N*-methylcytisine (5) had no such effect (Ishida and Shinozaki, 1984). Cytisine acted as a nicotinic cholinergic drug in some physiological studies that used rat brain tissues (Schwartz and Kellar, 1985) and guinea pig (Beani et al., 1985), but matrine has not been found to have nicotinic neuroactivities. It is possible that sophocarpine had a different mechanism of nematicidal effects from that of cytisine-type alkaloids.

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