Synthesis and Pharmacological Activity of Alkaloids from Embryo of Lotus, Nelumbo nucifera

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Bisbenzylisoquinoline alkaloid, nelumboferine which was recently isolated from the embryo of Nelumbo nucifera, and stereoisomers of neferine, which is a major alkaloid of the embryo of N. nucifera, were stereoselectively synthesized. Pharmacological activity of nelumboferine, stereoisomers of neferine, liensinine, isoliensinine, and O-methylneferine were evaluated.

Key words nelumboferine; neferine; liensinine; isoliensinine; O-methylneferine

Lotus (Nelumbo nucifera Gaertner, Nelumboaceae) has a wide distribution, such as in Japan, China, India, and Australia. All parts of this plant have been used from ancient times in traditional medicine.1–3 Recently in Japan, lotus seeds have been recorded in the Japanese Pharmacopoeia. Chemical constituents of the embryo of N. nucifera were studied in the 1960s to isolate neferine,2 liensinine,3–5 and isoliensinine6 as major alkaloids (Fig. 1).

Biological studies on the lotus embryo reported biological activities such as antihypertensive activity7 and anti-pulmonary fibrosis8; however, most of these activities were examined by using a crude extract of the lotus embryo, and there have been few experiments using pure constituents.9 Recently, we investigated the chemical constituents of the lotus embryo to isolate and structurally elucidate a new alkaloid, nelumboferine, as a minor component (Fig. 1).10 We also found that neferine decreases locomotor activities in mice.11,12 These results prompted us to synthesize nelumboferine and to evaluate its pharmacological activity.

Neferine, a major alkaloid, has two chiral centers in the molecule, and there are structural differences among neferine, liensinine, and isoliensinine as to whether is O-methylated. From the viewpoint of the structure–activity relationship, we synthesized three stereoisomers of neferine and O-methylneferine,5,13,14 and examined these pharmacological activities.

Results and Discussion

Neferine, liensinine, and isoliensinine were isolated from the embryo of N. nucifera. O-Methylneferine was prepared by methylation of neferine with trimethylsilyldiazomethane.10

Synthesis of Nelumboferine Our synthesis plan is shown in Chart 1. Because nelumboferine is a dimer of benzylisoquinoline, it is synthesized by Ullmann coupling15,16 of two chiral isoquinoline monomers (R)-5a (top half) and (R)-5b (bottom half). Chiral 5a and 5b are synthesized by Gawley’s asymmetric alkylation of lithiated isoquinoline (1)-6-Li, which has an oxazolidine derived from (1)-valine as a chiral auxiliary, with substituted benzyl chlorides 7a and 7b, respectively.17

Tetrahydroisoquinoline (1)-6, which is a substrate for asymmetric alkylation, was prepared according to the reported procedure.17,18 Asymmetric alkylation was carried out by lithiation of (1)-6 with n-butyllithium in tetrahydrofuran (THF) at −78°C, followed by treating with benzyl chloride 7a at −98°C to give benzylisoquinoline (1)-(R)-8a in 59% yield (Chart 2). Chiral auxiliary in 8a was removed by treating with hydrazine and toluenesulfonic acid. The resulting amino group was reductively methylated with formalin and sodium borohydride in methanol to give N-methylated isoquinoline (R)-9a in 70% yield. Chiral stationary phase HPLC analysis of (R)-9a showed enantiomeric excess of >99%. Removal of the benzyl protective group by catalytic hydrogenation gave benzyltetrahydroisoquinoline (R)-5a, the top half of nelumboferine, in quantitative yield. The bottom half of nelumboferine ((R)-5b) was synthesized using the same procedure (Chart 3); asymmetric alkylation of (1)-6 with benzyl chloride 7b gave isoquinoline (1)-(R)-8b in 57% yield. Removal of the chiral auxiliary, followed by reductive N-methylation, gave the bottom half (R)-5b in 70% yield with >99% optical purity.

According to the reported procedure for Ullmann coupling of tetrahydroisoquinoline,21 we attempted to couple the top and bottom halves. Reaction of phenol (R)-5a with bromide (R)-5b in the presence of copper(II) oxide, potassium carbonate and potassium iodide in refluxing pyridine gave the desired isoquinoline dimer 10 only in 10% yield (Chart 4). The yield was improved by treating (R)-5a and (R)-5b with copper(I) bromide-dimethyl sulfide complex22 and cesium carbonate when refluxing pyridine for 20 h to give bisbenzylisoquinoline 10 in 34% yield along with 42% recovery of unchanged (R)-5a. Deprotection of both benzyl and isopropyl groups with boron trichloride in dichloromethane at −15°C proceeded smoothly.
to give nelumboferine in 70% yield. Spectroscopic and analytical data of synthetic nelumboferine were agreed with those of natural product.

Synthesis of Stereoisomers of Neferine

Three stereoisomers of neferine (1-epi-neferine, 1′-epi-neferine and ent-neferine) were synthesized by the same methodology used for the synthesis of nelumboferine. Thus, top and bottom halves were prepared by asymmetric alkylation, and two isoquinoline units were connected by Ullmann coupling. Reaction of (l)-6 with butyllithium followed by 4-methoxybenzyl chloride in THF at −98°C gave benzylisoquinoline (l)-(R)-8c in 54% yield (Chart 5). Removal of the chiral auxiliary with hydrazine, followed by reductive N-methylation with formalin and sodium borohydride gave (R)-9c in 60% yield. Deprotection of the benzyl group by catalytic hydrogenation gave the top half (R)-5c in quantitative yield. Enantiomer of the top half ((S)-5c) was also synthesized by asymmetric alkylation of (o)-6, which has a chiral auxiliary derived from o-valine. Both enantiomers of the bottom half ((R)- and (S)-13) were also synthesized through asymmetric alkylation of (l)- or (o)-11, removal of the chiral auxiliary, and reductive N-methylation (Chart 6).

Ullmann coupling of (S)-5c and (R)-13 with copper(I) bromide-dimethyl sulfide and cesium carbonate in refluxing pyridine gave isoquinoline dimer 14 in 35% yield (Chart 7). Deprotection of the benzyl group with boron trichloride in dichloromethane at −15°C gave 1-epi-neferine in 68% yield. Ullmann coupling of (R)-5c and (S)-13, followed by deprotection gave 1′-epi-neferine. In the same way, Ullmann coupling of (S)-5c and (S)-13, followed by deprotection, gave ent-neferine.

Pharmacological Activity

Previously we demonstrated that neferine reduces locomotor activity in mice dose dependently, showing that neferine has sedative effects; however, it is not yet clear whether liensinine and isoliensinine, and nelumboferine isolated from the embryo of N. nucifera, have sedative effects or not. In the present study, therefore, we examined the effects of these alkaloids, including a newly synthesized nelumboferine, on locomotor activity in mice. The effects of O-methylneferine were also studied.

Effects of neferine, liensinine, isoliensinne, nelumboferine and O-methylneferine on locomotor activity are shown in Fig. 2. As shown in Results, all compounds decreased locomotor activity in mice. These results suggest that the bisbenzylisoquinoline alkaloids examined in the present study possess sedative effects. This is the first study demonstrating that alkaloids from the embryo of N. nucifera have sedative effects similar to neferine. Neferine and O-methylneferine at 25 mg/kg had no effect but had significant effects at a dosage of 50 mg/kg. In contrast, liensinine, isoliensinine, and nelumboferine elicited...
apparent effects on locomotor activity at 25 and 50 mg/kg. Thus, the efficacy of liensinine, isoliensinine and nelumboferine is more potent than neferine and O-methylneferine. Liensinine, isoliensinine, and nelumboferine have a hydroxy group at C-12 and/or C-7' respectively, different from neferine and O-methylneferine. O-methylation of the hydroxy group at C-12' in neferine could not increase pharmacological activity and rather shortened the sedative effects, since the effects of O-methylneferine disappeared 50 min after treatment. These results suggest that the presence of a hydroxy group at C-12 or C-7', in place of a methoxy group, may contribute to enhancing the sedative effects of bisbenzylisoquinolines.

From the viewpoint of the structure–activity relationship, three stereoisomers of neferine were synthesized and the effects of stereochemistry on locomotor activity were examined (Fig. 3). All compounds decreased locomotor activity in mice. Neferine and ent-neferine elicited significant sedative effects at 50 mg/kg, while at doses of 10 and 25 mg/kg they did not alter locomotor activity. 1-epi-Neferine and 1'-epi-neferine decreased locomotor activity above 25 and 10 mg/kg, respectively. 1-epi-Neferine elicited the most potent sedative effects among stereoisomers and 1'-epi-neferine at 50 mg/kg elicited strong sedative effects, with the result that 30% of mice died during experiments. These results reveal that the activity of the enantiomer was not improved; however, 1-epimer and 1'-epimer both had greater activity than neferine.

The detailed mechanisms of the sedative effects of bisbenzylisoquinoline alkaloids from the embryo of *Nelumbo nucifera* are not clear at present; however, we reported that neferine has antidepressant effects in mice mediated by the serotonin 5-HT_{1A} receptor\(^{12}\); therefore, the sedative effects of these compounds may be related to serotonergic transmission.

**Conclusions**

We synthesized nelumboferine, an alkaloid of the embryo of *Nelumbo nucifera* by using asymmetric alkylation and Ullmann coupling as key reactions. This is the first synthesis of nelumboferine. We also synthesized stereoisomers of neferine, a major alkaloid of the embryo of *N. nucifera* and its derivative O-methylneferine. Alkaloids from the embryo of *N. nucifera*, liensinine, isoliensinine, and nelumboferine, have sedative effects similar to neferine. Synthesized nelumboferine showed a stronger effect than neferine. Synthesized nelumboferine also elicited significant sedative effects. From the structure–activity relationship, the presence of a hydroxy group at C-12 or C-7' may contribute to enhancing sedative activity. Among stereoisomers of neferine, activities of 1-epi- and 1'-epi-neferine were higher than those of neferine. These interesting findings implied that the configuration change at C-1 or C-1' might enhance the activity of other alkaloids such as liensinine.

**Experimental**

All melting points were recorded on Yanagimoto hot plate melting points apparatus and are uncorrected. IR spectra were taken by Shimadzu FTIR-8200 spectrophotometer. NMR
spectra were taken by Varian Mercury 300 spectrometer at 300 MHz for 1H- and 75 MHz for 13C-NMR or by Varian VXR-500 spectrometer at 500 MHz for 1H- and 125 MHz for 13C-NMR. MS and HRMS spectra were taken by Hitachi M-4000 spectrometer.

7-(Benzyloxy)-2-[(4S)-4,5-dihydro-4-(1-methyl ethyl)-2-oxazolyl]-6-methoxy-1,2,3,4-tetrahydroisoquinoline ((l)-6)

To a solution of 7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline (l)-6) (7.30 g, 27 mmol) in benzene (100 mL) were added (S)-2-ethoxy-4-isopropyl-4,5-dihydrooxazole 17) (4.30 g, 27 mmol) and p-toluenesulfonic acid (92 mg, 0.54 mmol), and the mixture was refluxed for 2 h. After being cooled to room temperature (rt), the solution was washed with saturated sodium bicarbonate, dried over sodium sulfate, filtered, and evaporated. Silica gel column chromatography (ethyl acetate/ethanol = 4/1) gave (l)-6 (9.0 g, 88%) as white solid of mp 72–74°C. MS (electron ionization (EI)) m/z: 380 (M⁺), 337, 289, 91. HR-MS (EI) m/z: 380.2113 (Calcd for C23H28N2O3 (M⁺): 380.2100). IR (Nujol) cm⁻¹: 1654, 1609. [α]D²⁸ −29.8 (c=1.00, benzene). ¹H-NMR (300 MHz, CDCl₃) δ: 0.84 (3H, d, J=6.9 Hz), 0.95 (3H, d, J=6.9 Hz), 1.69 (1H, separt of d, J=6.9, 6.6 Hz), 2.78 (2H, dd, J=5.8, 5.8 Hz), 3.56 (1H, dt, J=12.6, 5.8 Hz), 3.63 (1H, dt, J=12.6, 5.8 Hz), 3.81 (1H, ddd, J=8.8, 6.8, 6.6 Hz), 3.86 (3H, s), 4.00 (1H, dd, J=8.0, 6.8 Hz), 4.26 (1H, dd, J=8.8, 8.0 Hz), 4.39 (1H, d, J=17.3 Hz), 4.45 (1H, d, J=17.3 Hz), 5.11 (2H, s), 6.60 (1H, s), 6.63 (1H, s), 7.28–7.44 (5H, m). ¹³C-NMR (75 MHz, CDCl₃) δ: 17.5, 18.8, 28.0, 33.1, 42.8, 46.8, 55.9, 70.0, 70.4, 71.0, 111.8, 111.9, 125.0, 126.7, 127.0, 127.6, 128.4, 137.0, 146.6, 148.1, 160.7.

(1R)-7-Benzyl-2-[[4S]-4,5-dihydro-4-(1-methyl ethyl)-2-oxazolyl]-1-(4-isopropylbenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline ((l)-(R)-8a)

To a solution of isoquinoline (1)-6 (3.82 g, 10 mmol) in THF (80 mL) was added n-butyl-lithium (1.22 M in hexane, 8.6 mL, 11 mmol) at −80°C, and the mixture was stirred for 10 min. A solution of the benzyl chloride 7a (2.27 g, 12 mmol) in THF (20 mL) was added at −98°C, and the mixture was stirred for 2 h from −98 to 0°C.
Saturated ammonium chloride solution was added, and the whole was extracted with ethyl acetate. Organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated. Silica gel column chromatography (ethyl acetate/ethanol=9/1) gave (1)-(R)-8a (3.10 g, 59%) as an amorphous solid of mp 85–88°C. MS (EI) m/z: 529 (MH⁺), 379, 91. HR-MS (EI) m/z: 529.3056 (Calcd for C₃₃H₄₁N₂O₄ (MH⁺): 529.3066). IR (Nujol) cm⁻¹: 1644. [α]D₂⁷ −135.4 (c=1.00, benzene). ¹H-NMR (300 MHz, CDCl₃) δ: 0.81 (3H, d, J=6.6 Hz), 0.89 (3H, d, J=6.6 Hz), 1.30 (6H, d, J=6.6 Hz), 1.66 (1H, m), 2.48 (1H, ddd, J=15.9, 4.4, 4.0 Hz), 2.78 (1H, m), 2.82 (1H, ddd, J=15.9, 9.8, 6.3 Hz), 2.88 (1H, dd, J=13.5, 6.5 Hz), 2.99 (1H, dd, J=13.5, 6.5 Hz), 3.33 (1H, ddd, J=13.6, 9.8, 4.4 Hz), 3.80 (1H, m), 3.85 (3H, s), 3.95 (1H, dd, J=8.5, 8.5 Hz), 4.08 (1H, dd, J=8.5, 8.5 Hz), 4.48 (1H, septet, J=6.1 Hz), 4.89 (1H, d, J=12.4 Hz), 4.95 (1H, d, J=12.4 Hz), 5.01 (1H, t, J=6.5 Hz), 6.30 (1H, s), 6.58 (1H, s), 6.75 (2H, d, J=8.5 Hz), 6.93 (2H, d, J=8.5 Hz), 7.27–7.37 (5H, m). ¹³C-NMR (75 MHz, CDCl₃) δ: 17.6, 18.4, 21.7, 21.8, 27.7, 33.1, 39.5, 41.1, 55.6, 57.1, 69.4, 69.6, 70.1, 70.6, 111.4, 113.0, 115.2, 126.6, 126.9, 127.5, 128.1, 128.2,

Fig. 2. Effects of Benzylisoquinoline Alkaloids on Locomotor Activity in Mice
Results are shown as the means±S.E.M. of 5–9 mice. (A) Neferine; (B) liensinine; (C) isoliensinine; (D) nelumboferine; (E) O-methylneferine. * p<0.05, ** p<0.01, significantly different from the respective saline-treated group.
To a solution of (R)-(1)-8a (1.00 g, 1.89 mmol) in ethanol (19 mL) were added hydrazine hydrate (1.8 mL, 27.8 mmol) and p-toluenesulfonic acid monohydrate (718 mg, 3.78 mmol), and the mixture was refluxed for 2 h. Water was added, and the whole was extracted with ethyl acetate. Organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated to give crude amine. To a solution of this amine in methanol (29 mL) were successively added formalin (37%, 1.4 mL, 18.9 mmol) and sodium borohydride (472 mg, 12.6 mmol) at 0°C, and the mixture was stirred for 1 h at rt. Ten percent acetic acid and 10% ammonia solution were successively added to the reaction mixture at 0°C, and the whole was extracted with ether. Organic layers were washed with brine, dried over potassium carbonate, filtered, and evaporated. Silica gel column chromatography (ethyl acetate/ethanol = 4/1) gave (R)-9a (570 mg, 70%) as an amorphous solid of mp 76–78°C. MS (EI) m/z: 432 (MH+), 282, 191. HR-MS (EI) m/z: 432.2536 (Calcd for C28H34NO3 (MH+): 432.2539). IR (Nujol) cm⁻¹: 1606. [α]D 28: −8.0 (c = 1.00, benzene). 1H-NMR (300 MHz, CDCl3) δ: 1.29 (3H, d, J = 6.1 Hz), 1.30 (3H, d, J = 6.1 Hz), 2.50 (3H, s), 2.54–2.86 (3H, m), 2.69 (1H, dd, J = 13.5, 7.7 Hz), 3.06 (1H, dd, J = 13.5, 5.0 Hz), 3.15 (1H, m), 3.60 (1H, dd, J = 7.7, 5.0 Hz), 3.83 (3H, s), 4.48 (1H, septet, J = 6.1 Hz), 4.75 (1H, d, J = 12.1 Hz), 4.82 (1H, d, J = 12.1 Hz), 6.08 (1H, s), 6.57 (1H, s), 6.78 (2H, d, J = 8.5 Hz), 6.94 (2H, d, J = 8.5 Hz), 7.24–7.33 (5H, m). 13C-NMR (75 MHz, CDCl3) δ: 22.0, 25.6, 40.0, 42.6, 46.9, 55.8, 64.7, 69.6, 70.6, 111.6, 113.7, 115.4, 126.4, 127.2, 127.5, 128.3, 129.2, 130.6, 131.7, 137.2, 145.3, 147.7, 156.1. HPLC: Daicel Chiralpak IB, hexane–2-propanol–ethylenediamine = 80:20:0.1, 1.0 mL/min, UV 270 nm, R: 5.2 min (dl-9a: 4.7, 5.3 min).

To a solution of (R)-9a (757 mg, 1.75 mmol) in methanol (150 mL) were added 10% palladium on carbon (208 mg) and acetic acid (0.42 mL), and the mixture was stirred under hydrogen atmosphere for 7 h at rt. Reaction mixture was filtered, and the filtrate was evaporated. The residue was dissolved in dichloromethane, and the solution was washed with saturated sodium bicarbonate and brine, dried over sodium sulfate, filtered, and evaporated. Silica gel column chromatography (chloroform/methanol = 9/1) gave (R)-5a (583 mg, 99%) as a colorless oil. MS (EI) m/z: 342 (MH+), 192. HR-MS (EI) m/z: 342.2072 (C21H28NO3 (MH+): 342.2069). IR (neat) cm⁻¹: 3543, 3408, 1610. [α]D 27: +27.6 (c = 1.00, benzene). 1H-NMR (300 MHz, CDCl3) δ:
1.32 (6H, d, J=6.0 Hz), 2.45 (3H, s), 2.53 (1H, m), 2.67–2.77 (2H, m), 2.82 (1H, dd, J=14.0, 6.0 Hz), 3.02 (1H, dd, J=14.0, 6.0 Hz), 3.16 (1H, m), 3.65 (1H, t, J=6.0 Hz), 3.84 (3H, s), 4.50 (1H, septet, J=6.0 Hz), 3.69 (1H, s), 3.9 (1H, s), 3.72 (3H, m), 3.77 (2H, d, J=8.5 Hz), 6.02 (2H, d, 5.5 Hz), 2.90 (1H, dd, J=14.6, 5.5 Hz), 2.97 (1H, dd, J=13.7, 5.2 Hz), 3.05–3.15 (2H, m), 3.51–3.64 (2H, m), 3.75 (3H, s), 3.80 (3H, s), 4.38 (1H, septet, J=6.0 Hz), 4.84 (2H, s), 5.03 (2H, s), 6.18 (1H, s), 6.39 (1H, s), 6.51 (1H, s), 6.61 (1H, s), 6.62 (2H, d, J=8.5 Hz), 6.65 (1H, dd, J=8.3, 2.2 Hz), 6.80 (1H, d, J=8.3 Hz), 6.87 (2H, d, J=8.5 Hz), 7.22–7.37 (11H, m).

13C-NMR (75 MHz, CDCl3) δ: 22.0, 22.0, 25.0, 40.5, 42.4, 46.8, 55.6, 64.5, 69.7, 110.5, 113.9, 115.5, 125.1, 130.0, 130.3, 131.8, 143.5, 145.2, 156.0.

(1R)-7-(Benzylxoy)-1-(4-(benzoyloxy)-3-((R)-1-(4-isopropoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline-7yloxyl)-benzoyloxy)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (10)

To a solution of (R)-Sa (191 mg, 0.56 mmol) and (R)-Sb (310 mg, 0.56 mmol) in pyridine (3.9 mL) were added copper(I) bromide–dimethyl sulfide complex (230 mg, 1.12 mmol) and cesium carbonate (1.09 g, 3.36 mmol), and the mixture was refluxed for 20 h. Reaction mixture was filtered, and the filtrate was evaporated. Silica gel column chromatography (chloroform/methanol=9/1) gave 10 (156 mg, 34%) as a brown oil. MS (EI m/z): 819 (MH⁺), 282, 192. HR-MS (EI m/z): 819.4368 (Calcd for C₃₉H₄₃NO₅ (MH⁺): 819.4371). (IR (neat) cm⁻¹: 1508. [α]D²⁰ +76.8 (c=1.00, MeOH).

1H-NMR (300 MHz, CDCl₃) δ: 1.23 (3H, d, J=6.0 Hz), 1.25 (3H, d, J=6.0 Hz), 2.43 (3H, s), 2.44 (3H, s), 2.55 (1H, m), 2.6 (1H, dd, J=13.7, 5.8 Hz) 2.64–2.83 (5H, m), 2.75 (1H, dd, J=14.6, 5.5 Hz), 2.90 (1H, dd, J=14.6, 5.8 Hz), 2.97 (1H, dd, J=13.7, 5.2 Hz), 3.05–3.15 (2H, m), 3.51–3.64 (2H, m), 3.75 (3H, s), 3.80 (3H, s), 4.38 (1H, septet, J=6.0 Hz), 4.84 (2H, s), 5.03 (2H, s), 6.18 (1H, s), 6.39 (1H, s), 6.51 (1H, s), 6.61 (1H, s), 6.62 (2H, d, J=8.5 Hz), 6.65 (1H, dd, J=8.3, 2.2 Hz), 6.80 (1H, d, J=8.3 Hz), 6.87 (2H, d, J=8.5 Hz), 7.22–7.37 (11H, m).

13C-NMR (75 MHz, CDCl₃) δ: 22.0, 22.0, 25.0, 40.5, 42.4, 46.8, 55.6, 64.5, 69.7, 110.5, 113.9, 115.5, 125.1, 130.0, 130.3, 131.8, 143.5, 145.2, 156.0.

(1R)-7-(Benzylxoy)-1-(4-(benzoyloxy)-3-((R)-1-(4-isopropoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline-7yloxyl)-benzoyloxy)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (10) To a solution of (R)-5a (191 mg, 0.56 mmol) and (R)-5b (310 mg, 0.56 mmol) in pyridine (3.9 mL) were added copper(I) bromide–dimethyl sulfide complex (230 mg, 1.12 mmol) and cesium carbonate (1.09 g, 3.36 mmol), and the mixture was refluxed for 20 h. Reaction mixture was filtered, and the filtrate was evaporated. Silica gel column chromatography (chloroform/methanol=9/1) gave 10 (156 mg, 34%) as a brown oil. MS (EI m/z): 819 (MH⁺), 282, 192. HR-MS (EI m/z): 819.4368 (Calcd for C₃₉H₄₃NO₅ (MH⁺): 819.4371). (IR (neat) cm⁻¹: 1508. [α]D²⁰ +76.8 (c=1.00, MeOH).

1H-NMR (300 MHz, CDCl₃) δ: 1.23 (3H, d, J=6.0 Hz), 1.25 (3H, d, J=6.0 Hz), 2.43 (3H, s), 2.44 (3H, s), 2.55 (1H, m), 2.6 (1H, dd, J=13.7, 5.8 Hz) 2.64–2.83 (5H, m), 2.75 (1H, dd, J=14.6, 5.5 Hz), 2.90 (1H, dd, J=14.6, 5.8 Hz), 2.97 (1H, dd, J=13.7, 5.2 Hz), 3.05–3.15 (2H, m), 3.51–3.64 (2H, m), 3.75 (3H, s), 3.80 (3H, s), 4.38 (1H, septet, J=6.0 Hz), 4.84 (2H, s), 5.03 (2H, s), 6.18 (1H, s), 6.39 (1H, s), 6.51 (1H, s), 6.61 (1H, s), 6.62 (2H, d, J=8.5 Hz), 6.65 (1H, dd, J=8.3, 2.2 Hz), 6.80 (1H, d, J=8.3 Hz), 6.87 (2H, d, J=8.5 Hz), 7.22–7.37 (11H, m).

13C-NMR (75 MHz, CDCl₃) δ: 22.0, 22.0, 25.0, 40.5, 42.4, 46.8, 55.6, 64.5, 69.7, 110.5, 113.9, 115.5, 125.1, 130.0, 130.3, 131.8, 143.5, 145.2, 156.0.
1270, 1276, 1283, 1304, 1305, 1307, 1370, 1457, 1480, 1579, 1602.

(o)-5c. [α]_D^{24} +80.4 (c=1.00, benzene).

(2R,7S)-{2-(Aminomethyl)-3,4-dihydroxy-1-methyl-2-oxazolyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (2R,7S)-11 This compound was prepared from (o)-5c as described above in 5% yield. MS (EI) m/z: 797 (MH⁺), 398 (MH⁺), 382 (MH⁺), 265 (MH⁺). IR (neat) cm⁻¹: 3370, 1650. [α]_D^{23} 47.1 (c=1.00, benzene).

(2R,7S)-{2-(Aminomethyl)-3,4-dihydroxy-1-methyl-2-oxazolyl}-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (2R,7S)-11 This compound was prepared from (o)-5c and 7b as described above in 12% yield. MS (EI) m/z: 579 (MH⁺), 303. IR (KBr) cm⁻¹: 3422, 1611, 1510. [α]_D^{23} −278 (c=1.00, CHCl₃).

1-epi-Neferine This compound was prepared from 1c as described above in 68% yield. mp 69−72°C. UV λ_max (methanol) nm (log ε): 227 (4.48), 284 (3.94). MS (EI) m/z: 625 (MH⁺), 503, 206. HR-MS (EI) m/z: 625.3279 (Calcd for C_{23}H_{25}NO_{5} (MH⁺): 625.3278). IR (KBr) cm⁻¹: 3422, 1611, 1510. [α]_D^{23} −278 (c=1.00, CHCl₃).
8.0, 6.0 Hz, H-4), 3.02 (1H, dd, J=14.0, 5.5 Hz, H-a), 3.08 (1H, dd, J=14.0, 5.0 Hz, H-α'). 3.11–3.17 (2H, m, H-3 or 3'). 3.55 (3H, s, OCH3-6) 3.61 (1H, dd, J=8.0, 5.0 Hz, H-1') 3.63 (1H, dd, J=6.5, 5.5 Hz, H-I) 3.72 (3H, s, OCH3-12), 3.80 (3H, s, OCH3-6), 3.82 (3H, s, OCH3-7'), 6.01 (1H, s, H-8), 6.39 (1H, s, H-8), 6.53 (1H, s, H-5'), 6.58 (1H, d, J=2.0 Hz, H-10'), 6.64 (1H, s, H-5), 6.69 (2H, d, J=8.5 Hz, H-11, 13), 6.70 (1H, dd, J=8.0, 2.0 Hz, H-14'), 6.85 (1H, d, J=8.0 Hz, H-13'), 6.90 (2H, d, J=8.5 Hz, H-10, 14). 13C-NMR (125 MHz, CDCl3) δ: 25.2, 26.0, 39.9, 40.6, 42.5, 42.7, 46.7, 47.0, 50.1, 51.5, 55.5, 55.7, 55.8, 64.4, 64.8, 110.9, 111.1, 112.3, 113.4, 115.9, 119.1, 120.0, 125.3, 125.6, 129.0, 130.4, 130.5, 131.0, 131.4, 131.9, 142.8, 144.6, 145.3, 146.3, 147.2, 149.8, 157.8. Hydrochloride: mp 181–183°C. Anal. Caled for C16H12Cl2N2O2.HCl: C, 54.93; H, 3.87. Found: C, 54.61; H, 3.81.

1-epi-Neferine This compound was prepared from 15 in 84% yield. mp 77–80°C. [α]D 24 +24.4 (c=1.00, CHCl3). Hydrochloride: mp 185–189°C. Anal. Caled for C16H12Cl2N2O2.HCl: C, 62.98; H, 6.82; N, 3.87. Found: C, 63.32; H, 6.93; N, 3.92.

Results

Determination of Locomotor Activity

Experiments were undertaken in accordance with the Guiding Principles for Care and Use of Laboratory Animals as approved by The Japanese Pharmaceutical Society. The study protocol was approved by the Ethics Committee of the Yokohama College of Pharmacy (Yokohama, Japan). Male ICR mice (age, 5 weeks) were purchased from SLC Japan Inc. (Shizuoka, Japan). Mice were housed in groups of five under a controlled 12-h-12-h light–dark cycle (light from 7 a.m. to 7 p.m.) with a room temperature of 23±1°C and humidity of 55±5%. Mice had free access to food and water. Each mouse was only used once. All drugs were injected via the intraperitoneal (i.p.) route. Mice in the control group received saline. The locomotor activity of mice was measured using a digital counter with an infrared sensor (NS-AS01, Neuroscience Inc., Tokyo, Japan) following the method described by previous reports. 12 An infrared sensor was set on a zero-length clear polycarbonate cage (22.5×33.8×14.0 cm) into which each mouse was placed. Locomotor activity was determined over 60 min. The apparatus was used to detect and record a digital count of the horizontal movements of animals. Results are shown as means±S.E.M. of 5–9 mice in behavioral studies. Results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test post-hoc test.

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