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, Nelumbonaceae) 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)] 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 isoliensinine[6)] as major alkaloids (Fig. 1).

Biological studies on the lotus embryo reported biological activities such as antihypertensive activity[7)] and anti-pulmonary fibrosis[8)]; 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 isoliensine 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.

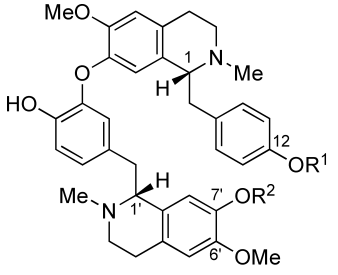
Results and Discussion

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

Synthesis of Nelumboferine Our synthesis plan is shown in Chart 1. Because nelumboferine is a dimer of benzylisoquinoline, it is synthesized by Ullmann coupling[15,16)] 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 (L)-**6**-Li, which has an oxazolidine derived from (L)-valine as a chiral auxiliary, with substituted benzyl chlorides **7a** and **7b**, respectively.[17)]

Tetrahydroisoquionoline (L)-**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 (L)-**6** with *n*-butyllithium in tetrahydrofuran (THF) at −78°C, followed by treating with benzyl chloride **7a** at −98°C to give benzylisoquinoline (L)-(*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 (L)-**6** with benzyl chloride **7b** gave isoquinoline (L)-(*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,[2)] 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 complex[15)] 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



$R^1 = R^2 =$ Me: neferine (**1**)
$R^1 =$ H, $R^2=$ Me: liensinine (**2**)
$R^1 =$ Me, $R^2 =$ H: isoliensinine (**3**)
$R^1 = R^2 =$ H: nelumboferine (**4**)

Fig. 1. Structures of Alkaloids of the Embryo of *Nelumbo nucifera*

The authors declare no conflict of interest.

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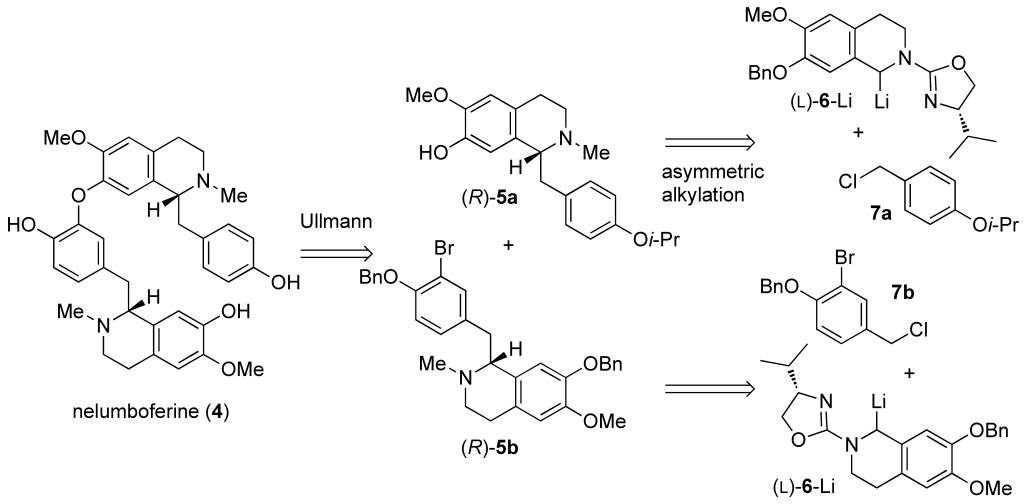
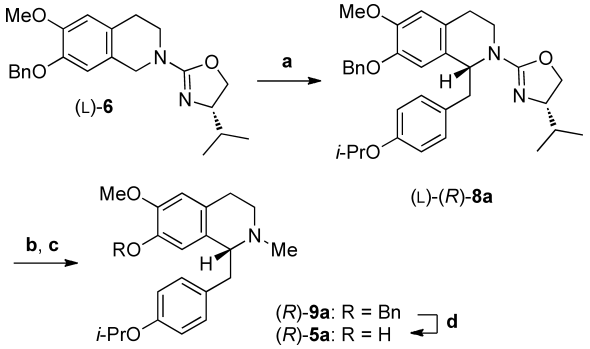
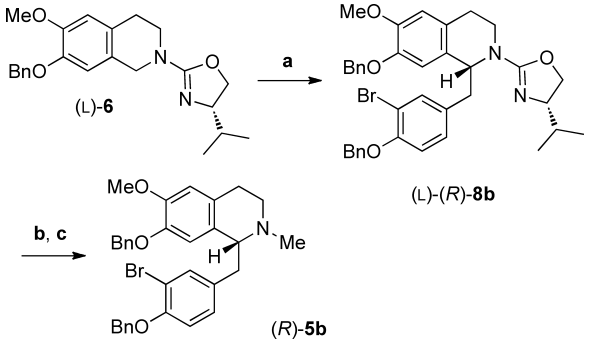


Chart 1. Retrosynthesis of Nelumboferine



Reagents and conditions: (a) *n*-BuLi, **7a**, THF, −98 to 0°C, 2 h, 59%; (b) hydrazine, *p*-TsOH, EtOH, reflux, 2 h; (c) formalin, $NaBH_4$, MeOH, rt, 1 h, 70%; (d) H_2, Pd/C, MeOH, rt, 7 h, 99%.

Chart 2. Asymmetric Alkylation of Top Half



Reagents and conditions: (a) *n*-BuLi, **7b**, THF, −98 to 0°C, 2 h, 57%; (b) hydrazine, *p*-TsOH, EtOH, reflux, 2 h; (c) formalin, $NaBH_4$, MeOH, rt, 1 h, 70%.

Chart 3. Asymmetric Alkylation of Bottom Half

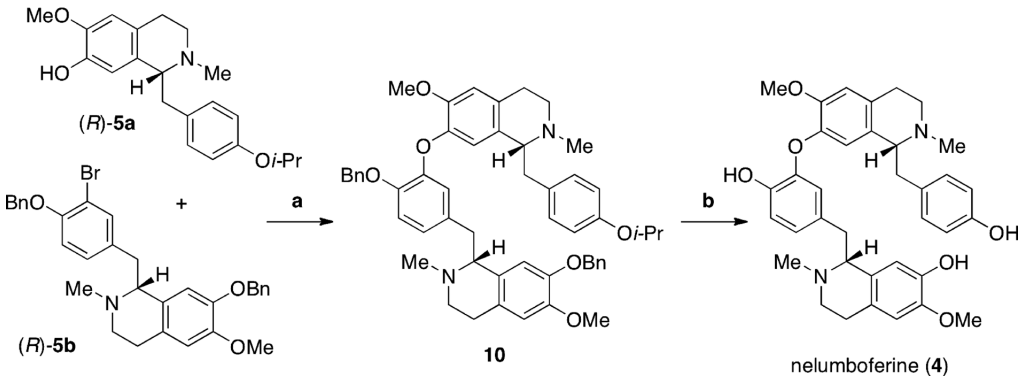
to give nelumboferine in 70% yield. Spectroscopic and analytical data of synthetic nelumboferine were agreed with those of natural product.

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 (D)-**6**, which has a chiral auxiliary derived from D-valine. Both enantiomers of the bottom half ((*R*)- and (*S*)-**13**) were also synthesized through asymmetric alkylation of (L)- or (D)-**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.

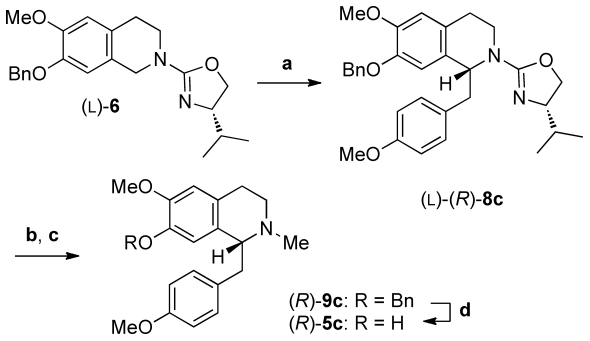
Pharmacological Activity Previously we demonstrated that neferine reduces locomotor activity in mice dose dependently, showing that neferine has sedative effects;[11] however, it is not yet clear whether liensinine and isoliensinine, and nelumboferine isolated from the embryo of *N. nucifera*, have sedative 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* liensinine, isoliensinine, and nelumboferine 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



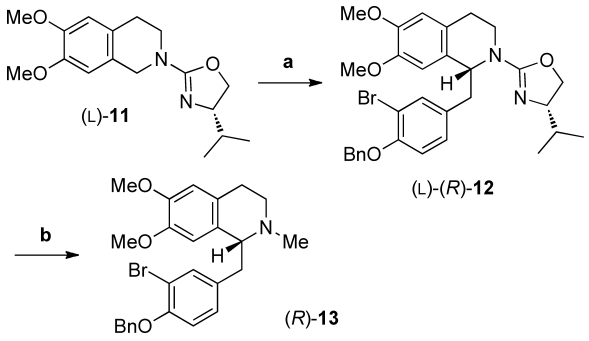
Reagents and conditions: (a) CuBr·SMe_2, Cs_2CO_3, pyridine, reflux, 20 h, 34%; (b) BCl_3, CH_2Cl_2, −15°C, 1 h, 70%.

Chart 4. Synthesis of Nelumboferine



Reagents and conditions: (a) *n*-BuLi, *p*-methoxybenzyl chloride, THF, −98 to 0°C, 2 h, 54%; (b) hydrazine, *p*-TsOH, EtOH, reflux, 2 h; (c) formalin, $NaBH_4$, MeOH, rt, 1 h, 60%; (d) H_2, Pd/C, MeOH, rt, 16 h, 99%.

Chart 5. Asymmetric Alkylation of Top Half



Reagents and conditions: (a) *n*-BuLi, **7b**, THF, −98 to 0°C, 2 h, 75%; (b) hydrazine, *p*-TsOH, EtOH, reflux, 2 h; (c) formalin, $NaBH_4$, MeOH, rt, 1 h, 64%.

Chart 6. Asymmetric Alkylation of Bottom Half

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.

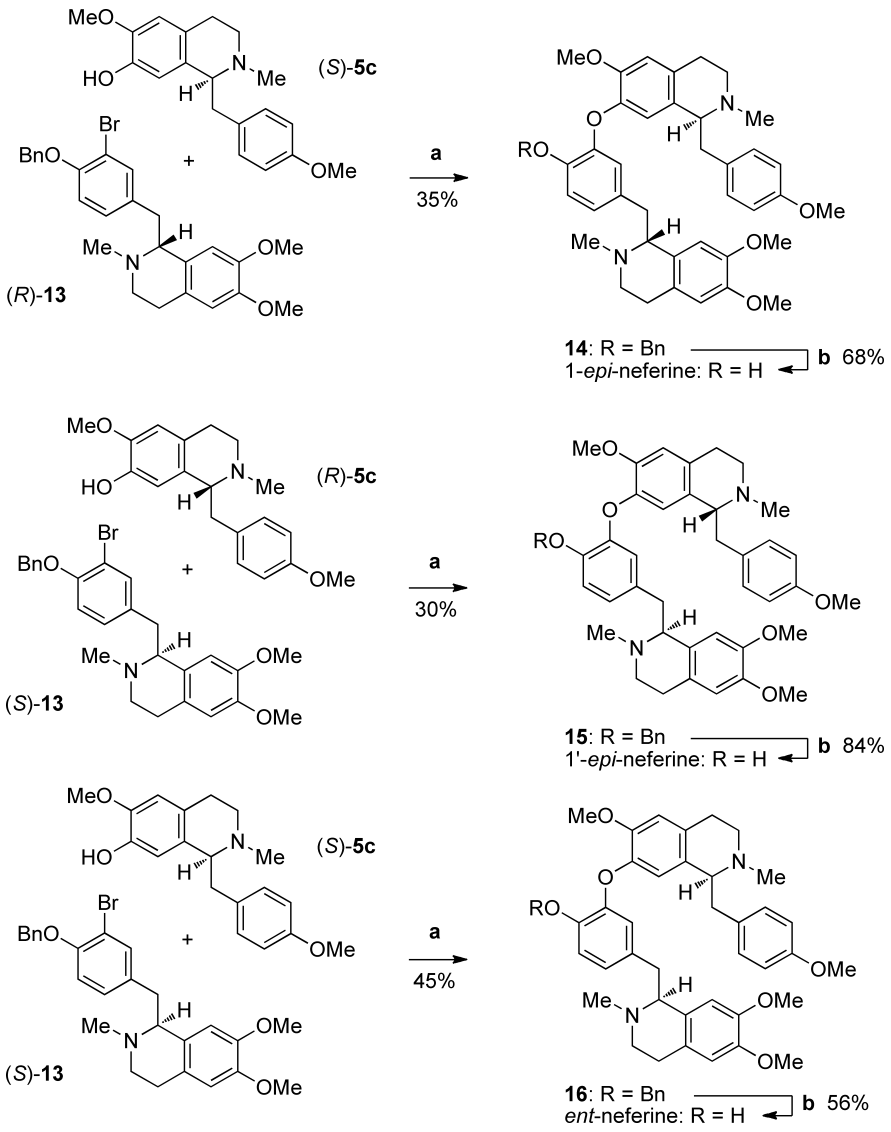
Conclusions

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. The newly synthesized *O*-methylneferine also elicited significant sedative effects. From the structure–activity relationship, the presence of a hydroxyl 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



Reagents and conditions: (a) $CuBr \cdot SMe_2$, Cs_2CO_3, pyridine, reflux, 20 h; (b) BCl_3, CH_2Cl_2, −15°C, 1 h.

Chart 7. Synthesis of 1-*epi*- and 1′-*epi*- and *ent*-Neferine

spectra were taken by Varian Mercury 300 spectrometer at 300 MHz for ^{1}H- and 75 MHz for ^{13}C-NMR or by Varian VXR-500 spectrometer at 500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR. MS and HRMS spectra were taken by Hitachi M-4000 spectrometer.

7-(Benzyloxy)-2-[(4*S*)-4,5-dihydro-4-(1-methylethyl)-2-oxazolyl]-6-methoxy-1,2,3,4-tetrahydroisoquinoline ((L)-6) To a solution of 7-(benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline[18] (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 $C_{23}H_{28}N_2O_3$ (M^+): 380.2100). IR (Nujol) cm^{-1}: 1654, 1609. $[\alpha]_D^{28}$ −29.8 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.84 (3H, d, *J*=6.9 Hz), 0.95 (3H, d, *J*=6.9 Hz), 1.69 (1H, septet 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). ^{13}C-NMR (75 MHz, $CDCl_3$) 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.

(D)-**6**. mp 76–77°C. $[\alpha]_D^{23}$ +25.0 (*c*=1.00, benzene).

(1*R*)-7-Benzyloxy-2-[(4*S*)-4,5-dihydro-4-(1-methylethyl)-2-oxazolyl]-1-(4-isopropylbenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline ((L)-(*R*)-8a) To a solution of isoquinoline (L)-**6** (3.82 g, 10 mmol) in THF (80 mL) was added *n*-butyllithium (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.

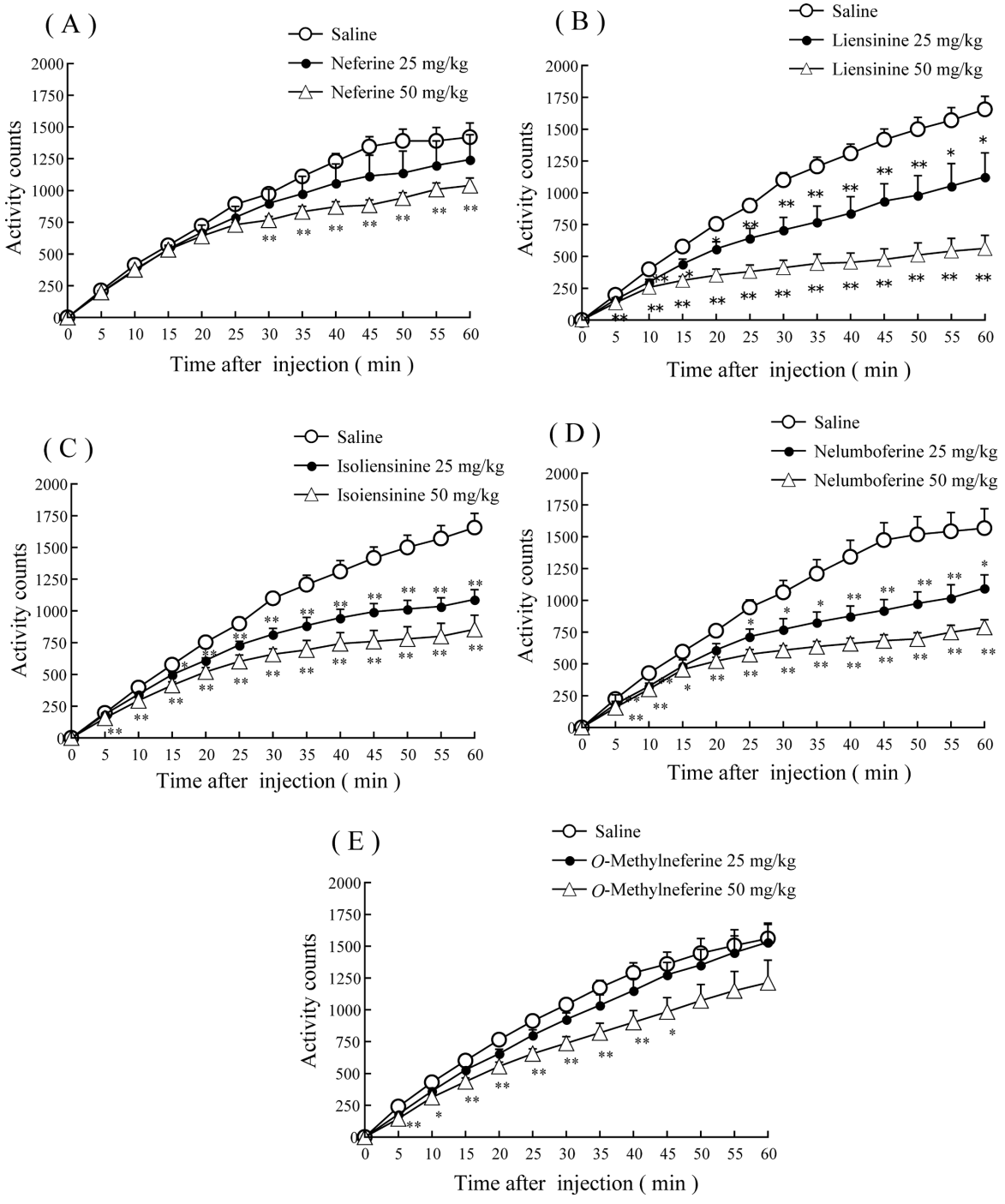


Fig. 2. Effects of Benzylisoquinoline Alkaloids on Locomotor Activity in Mice

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.

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 (L)-(*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 $C_{33}H_{41}N_2O_4$ (MH^+): 529.3066). IR (Nujol) cm^{-1}: 1644. $[\alpha]_D^{27}$ −135.4 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, d, *J*=6.6 Hz), 0.89 (3H, d, *J*=6.6 Hz), 1.30 (6H, d, *J*=6.1 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). ^{13}C-NMR (75 MHz, $CDCl_3$) 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,

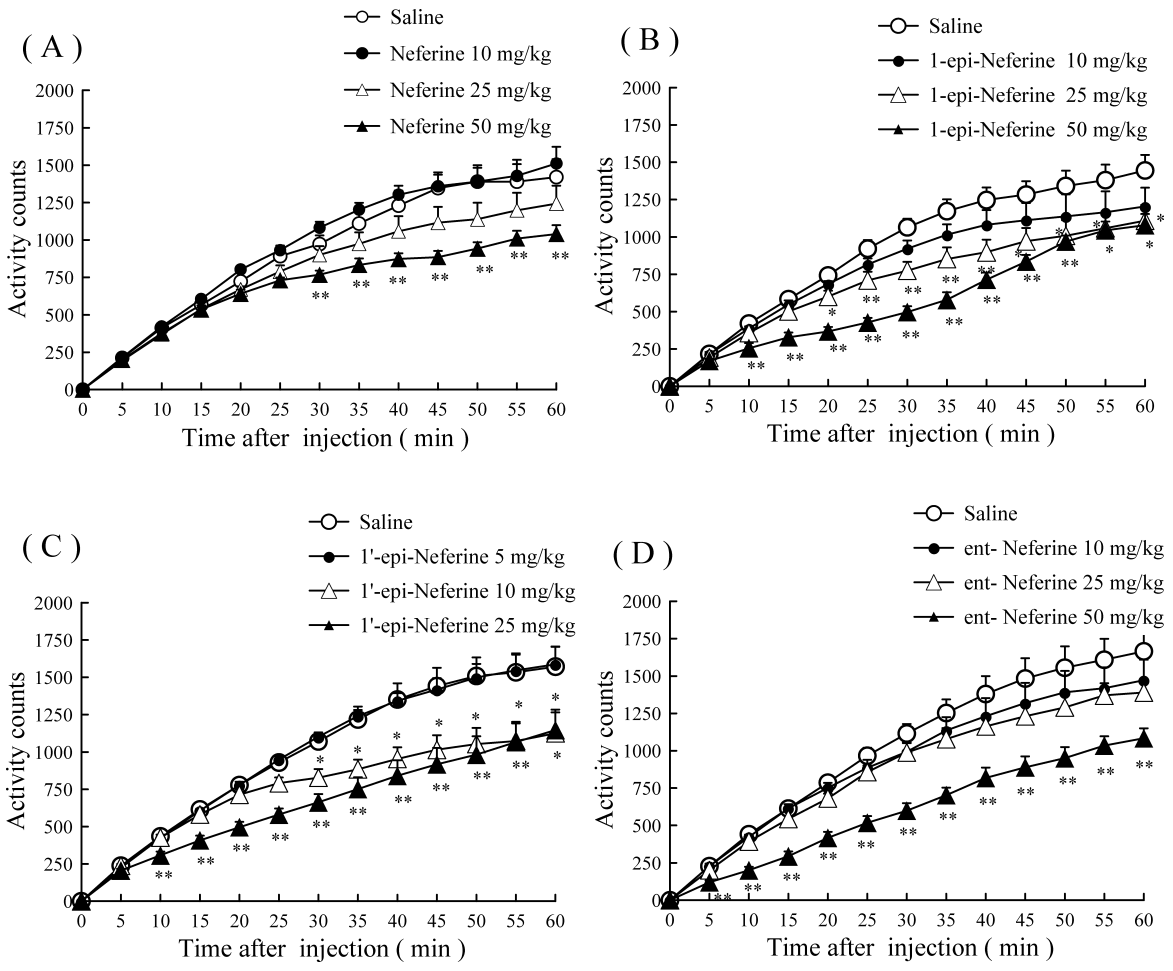


Fig. 3. Effects of Neferine and Its Stereoisomers on Locomotor Activity in Mice

Results are shown as the means±S.E.M. of 5–9 mice. (A) Neferine; (B) 1-*epi*-neferine; (C) 1′-*epi*-neferine; (D) *ent*-neferine; *$p<0.05$, **$p<0.01$, significantly different from the respective saline-treated group.

130.2, 130.6, 136.9, 145.6, 147.9, 156.1, 160.1.

(*R*)-7-(Benzyloxy)-1-(4-isopropoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-9a) To a solution of (L)-(*R*)-**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 $C_{28}H_{34}NO_3$ (MH^+): 432.2539). IR (Nujol) cm^{-1}: 1606. $[\alpha]_D^{28}$ −8.0 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) 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). ^{13}C-NMR (75 MHz, $CDCl_3$) 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).

(*R*)-1-(4-Isopropoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((*R*)-5a) 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 (Calcd for $C_{21}H_{28}NO_3$ (MH^+): 342.2069). IR (neat) cm^{-1}: 3543, 3408, 1610. $[\alpha]_D^{27}$ +27.6 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) 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), 6.39 (1H, s), 6.52 (1H, s), 6.77 (2H, d, J=8.5 Hz), 7.02 (2H, d, J=8.5 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) 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, 143.4, 145.2, 156.0.

(1*R*)-7-(Benzyloxy)-1-(4-(benzyloxy)-3-bromobenzyl)-2-[(4*S*)-4,5-dihydro-4-(1-methylethyl)-2-oxazolyl]-6-methoxy-1,2,3,4-tetrahydroisoquinoline ((L)-(*R*)-8b) This compound was prepared from the isoquinoline (L)-**6** and the benzyl chloride **7b** as described above in 57% yield. MS (EI) m/z: 654 (M^+), 379, 91. HR-MS (EI) m/z: 654.2103 (Calcd for $C_{37}H_{39}BrN_2O_4$ (M^+): 654.2093). IR (neat) cm^{-1}: 1647. $[\alpha]_D^{24}$ −107.6 (c=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.80 (3H, d, J=6.6 Hz), 0.88 (3H, d, J=6.6 Hz), 1.62 (1H, m), 2.46 (1H, ddd, J=15.9, 4.4, 4.4 Hz), 2.73–2.84 (2H, m), 2.85 (1H, dd, J=13.5, 6.6 Hz), 2.94 (1H, dd, J=13.5, 6.6 Hz), 3.34 (1H, ddd, J=9.6, 4.4, 4.4 Hz), 3.76 (1H, m), 3.85 (3H, s), 3.93 (1H, dd, J=8.0, 6.0 Hz), 4.05 (1H, dd, J=8.5, 8.0 Hz), 4.91 (1H, d, J=12.4 Hz), 4.98 (1H, d, J=12.4 Hz), 5.00 (1H, t, J=6.6 Hz), 5.11 (2H, s), 6.32 (1H, s), 6.58 (1H, s), 6.79 (1H, d, J=8.4 Hz), 6.90 (1H, dd, J=8.4, 2.0 Hz), 7.27–7.46 (11H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.7, 18.6, 27.8, 33.2, 39.7, 41.1, 55.9, 57.1, 69.8, 70.3, 70.7, 71.0, 111.7, 111.8, 113.1, 113.4, 126.8, 126.9, 127.1, 127.7, 127.8, 127.9, 128.4, 129.7, 132.6, 134.4, 136.5, 137.0, 145.9, 148.2, 153.4, 160.2.

(*R*)-7-(Benzyloxy)-1-(4-(benzyloxy)-3-bromobenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-5b) This compound was prepared from (L)-(*R*)-**8b** as described above in 58% yield. MS (EI) m/z: 557 (M^+), 282. HR-MS (EI) m/z: 557.1580 (Calcd for $C_{32}H_{32}BrNO_3$ (M^+): 557.1566). IR (neat) cm^{-1}: 1607. $[\alpha]_D^{25}$ +24.4 (c=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.48 (3H, s), 2.49 (1H, m), 2.73–2.82 (3H, m,), 2.97 (1H, dd, J=13.7, 5.0 Hz), 3.11 (1H, m), 3.57 (1H, t, J=5.0 Hz), 3.84 (3H, s), 4.81 (1H, d, J=12.3 Hz), 4.89 (1H, d, J=12.3 Hz), 5.10 (2H, s), 6.14 (1H, s), 6.56 (1H, s), 6.78 (1H, d, J=8.5 Hz), 6.84 (1H, dd, J=8.5, 1.9 Hz), 7.23–7.46 (11H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.6, 39.7, 42.6, 47.1, 55.8, 64.5, 70.7, 70.8, 111.7, 111.8, 113.3, 113.6, 126.8, 127.1, 127.6, 127.7, 128.3, 128.4, 128.8, 129.6, 133.9, 134.3, 136.5, 137.2, 145.6, 147.9, 153.1. HPLC: Daicel Chiralpak IB, hexane–2-propanol–ethylenediamine=80 : 20 : 0.1, 1.0 mL/min, UV 270 nm, R: 8.3 min (DL-**5b**: 7.2, 8.4 min).

(*R*)-7-(Benzyloxy)-1-[(4-(benzyloxy)-3-[(*R*)-1-(4-isopropoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yloxy]benzyl)-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 $C_{53}H_{59}N_2O_6$ (MH^+): 819.4371). IR (neat) cm^{-1}: 1508. $[\alpha]_D^{26}$ −76.8 (c=1.00, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) 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), 2.62 (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). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 22.0, 25.5, 25.8, 40.2, 40.4, 42.6, 42.6, 46.9, 47.3, 55.8, 55.8, 64.3, 64.5, 69.6, 70.8, 70.9, 111.6, 112.3, 113.6, 114.8, 115.4, 117.9, 120.3, 120.6, 124.7, 126.6, 127.0, 127.3, 127.4, 127.6, 128.2, 128.3, 129.1, 129.5, 130.3, 131.5, 133.3, 137.2, 137.3, 144.0, 145.6, 146.4, 147.6, 147.8, 148.6, 155.9.

Nelumboferine (4) To a solution of **10** (410 mg, 0.5 mmol) in dichloromethane (35 mL) was added a solution of boron trichloride (1 M in dichloromethane, 2.5 mL, 2.5 mmol) at −15°C, and the mixture was stirred for 1 h. Water and 27% ammonia solution were added, and the whole was extracted with dichloromethane. Organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporated. Silica gel column chromatography (chloroform/methanol/water=80/20/1) gave nelumboferine (210 mg, 70%) as a pale yellow amorphous solid of mp 117–120°C. UV λ_{max} (methanol) nm (log ε): 225 (4.51), 284 (4.02). MS (EI) m/z: 596 (M^+), 489, 192. HR-MS (EI) m/z: 596.2876 (Calcd for $C_{36}H_{40}N_2O_6$ (M^+): 596.2886). IR (KBr) cm^{-1}: 3385, 1612, 1510. $[\alpha]_D^{26}$ −66.0 (c=1.00, MeOH). Lit.[10] $[\alpha]_D$ −64.0 (c=1.0, MeOH). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.52 (3H, s,), 2.54 (3H, s), 2.62 (1H, m), 2.66 (1H, dd, J=13.5, 10.0 Hz) 2.69 (1H, dd, J=13.5, 10.5 Hz), 2.75 (1H, ddd, J=12.0, 6.0, 6.0 Hz), 2.79–2.87 (2H, m), 2.91 (1H, m), 2.97 (1H, m), 3.10 (1H, dd, J=13.5, 3.0 Hz), 3.20 (1H, dd, J=13.5, 2.5 Hz), 3.21 (1H, m), 3.42 (1H, m) 3.51 (1H, dd, J=10.5, 2.5 Hz) 3.67 (1H, dd, J=10.0, 3.0 Hz) 3.85 (3H, s), 3.90 (3H, s), 5.97 (1H, s), 6.34 (1H, s), 6.46 (1H, dd, J=8.0, 2.0 Hz), 6.56 (1H, s), 6.68 (1H, s), 6.75 (1H, d, J=8.0 Hz), 6.75 (1H, d, J=2.0 Hz), 6.76 (2H, d, J=8.5 Hz), 6.94 (2H, d, J=8.5 Hz). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 22.1, 26.3, 39.8, 40.5, 42.3, 42.6, 44.5, 47.6, 55.8, 56.0, 64.2, 65.3, 110.8, 112.2, 114.4, 115.5, 116.6, 118.2, 121.3, 123.2, 127.0, 127.8, 129.5, 130.5, 130.6, 130.9, 143.2, 143.3, 143.9, 145.6, 146.7, 148.3, 155.6. Hydrochloride: To a solution of **4** (230 mg, 0.39 mmol) in methanol (5 mL) was added hydrochloric acid (12 M, 0.2 mL) at 0°C. Evaporation of the solvent gave hydrochloride (250 mg). mp 207–210°C. *Anal.* Calcd for $C_{36}H_{42}Cl_2N_2O_6 \cdot 2H_2O$: C, 61.27; H, 6.57; N, 3.97. Found: C, 61.26; H, 6.63; N, 3.89.

(1*R*)-7-(Benzyloxy)-2-[(4*S*)-4,5-dihydro-4-(1-methylethyl)-2-oxazolyl]-6-methoxy-1-(4-methoxy-benzyl)-1,2,3,4-tetrahydroisoquinoline ((L)-(*R*)-8c) This compound was prepared from (L)-**6** and *p*-methoxybenzyl chloride as described above in 54% yield. MS (EI) m/z: 501 (MH^+), 379. HR-MS (EI) m/z: 501.2741 (Calcd for $C_{31}H_{37}N_2O_4$ (MH^+): 501.2753). IR (neat) cm^{-1}: 1644. $[\alpha]_D^{24}$ −67.8 (c=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, d, J=6.6 Hz), 0.89 (3H, d, J=6.6 Hz), 1.64 (1H, m), 2.46 (1H, ddd, J=15.9, 4.4, 4.4 Hz), 2.71 (1H, m), 2.79 (1H, ddd, J=15.9, 9.9, 5.8 Hz), 2.90 (1H, dd, J=13.5, 6.6 Hz), 3.00 (1H, dd, J=13.5, 6.3 Hz), 3.32 (1H, ddd, J=12.9, 9.9, 4.4 Hz), 3.75 (1H, m), 3.76 (3H, s), 3.84 (3H, s), 3.95 (1H, dd, J=8.0, 6.0 Hz,), 4.09 (1H, dd, J=8.5, 8.0 Hz), 4.89 (1H, d, J=12.6 Hz), 4.95 (1H, d, J=12.6 Hz), 5.02 (1H, dd, J=6.6, 6.3 Hz), 6.30 (1H, s), 6.58 (1H, s), 6.77 (2H, d, J=8.8 Hz), 6.94 (2H, d, J=8.8 Hz), 7.24–7.37 (5H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.6, 18.5, 27.8, 33.2, 39.7, 41.2, 55.0, 55.8, 57.1, 69.7, 70.2, 70.8, 111.5, 113.1, 113.3, 126.8,

127.0, 127.6, 128.3, 130.4, 130.5, 130.7, 137.0, 145.7, 148.0, 157.9, 160.2.

(D)-(*S*)-**8c**. $[\alpha]_D^{24}$ +80.4 (*c*=1.00, benzene).

(*R*)-7-(Benzyloxy)-6-methoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-9c) This compound was prepared from (L)-(*R*)-**8c** as described above in 60% yield. MS (EI) *m/z*: 404 (MH^+), 282. HR-MS (EI) *m/z*: 404.2201 (Calcd for $C_{26}H_{30}NO_3$ (MH^+): 404.2226). IR (neat) cm^{-1}: 1610, 1511. $[\alpha]_D^{27}$ +15.2 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.50 (3H, s), 2.57 (1H, m), 2.69–2.85 (2H, m), 2.71 (1H, dd, *J*=13.7, 7.4 Hz), 3.06 (1H, dd, *J*=13.7, 5.2 Hz), 3.16 (1H, m), 3.60 (1H, dd, *J*=7.4, 5.2 Hz) 3.77 (3H, s), 3.84 (3H, s), 4.77 (1H, d, *J*=12.4 Hz), 4.84 (1H, d, *J*=12.4 Hz), 6.01 (1H, s), 6.57 (1H, s), 6.79 (2H, d, *J*=8.8 Hz), 6.95 (2H, d, *J*=8.8 Hz), 7.27–7.34 (5H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.6, 39.9, 42.6, 46.9, 55.0, 55.7, 64.7, 70.6, 111.5, 113.4, 113.7, 126.5, 127.0, 127.5, 128.2, 129.2, 130.6, 131.9, 137.2, 145.3, 147.7, 157.7. HPLC: Daicel Chiralpak IB, hexane–2-propanol–ethylenediamine=80 : 20 : 0.1, 1.0 mL/min, UV 270 nm, *R*: 6.8 min (DL-**9c**: 5.8, 6.8 min).

(*S*)-**9c**. $[\alpha]_D^{24}$ −11.2 (*c*=1.00, benzene).

(*R*)-6-Methoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-ol ((*R*)-5c) This compound was prepared from (*R*)-**9c** as described above in 99% yield. MS (EI) *m/z*: 314 (MH^+), 192. HR-MS (EI) *m/z*: 314.1751 (Calcd for $C_{19}H_{24}NO_3$ (MH^+): 314.1756). IR (neat) cm^{-1}: 1611, 1509. $[\alpha]_D^{27}$ +40.0 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.54 (3H, s), 2.55 (1H, m), 2.68–2.78 (2H, m), 2.84 (1H, dd, *J*=14.1, 6.0 Hz), 3.06 (1H, dd, *J*=14.1, 6.0 Hz), 3.17 (1H, m), 3.67 (1H, t, *J*=6.0 Hz) 3.78 (3H, s), 3.84 (3H, s), 6.39 (1H, s), 6.53 (1H, s), 6.79 (2H, d, *J*=8.5 Hz), 7.04 (2H, d, *J*=8.5 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 24.9, 40.4, 42.3, 46.8, 55.0, 55.6, 64.5, 110.5, 113.3, 113.8, 124.9, 129.6, 130.3, 131.6, 143.4, 145.3, 157.7.

(*S*)-**5c**. $[\alpha]_D^{24}$ −39.4 (*c*=1.00, benzene).

2-[(4*S*)-4,5-Dihydro-4-(1-methylethyl)-2-oxazolyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline ((L)-11) This compound was prepared from commercially available 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline and (*S*)-2-ethoxy-4-isopropyl-4,5-dihydrooxazole[17] as described above in 82% yield. mp 67–69°C. MS (EI) *m/z*: 304 (M^+), 261. HR-MS (EI) *m/z*: 304.1803 (Calcd for $C_{17}H_{24}N_2O_3$ (M^+): 304.1787). IR (Nujol) cm^{-1}: 1660, 1609. $[\alpha]_D^{27}$ −40.8 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.85 (3H, d, *J*=6.6 Hz), 0.95 (3H, d, *J*=6.6 Hz), 1.69 (1H, septet of d, *J*=6.6, 6.6 Hz), 2.79 (2H, t, *J*=6.0 Hz), 3.59 (1H, dt, *J*=11.5, 6.0 Hz), 3.65 (1H, dt, *J*=11.5, 6.0 Hz), 3.82 (1H, ddd, *J*=8.8, 6.6, 6.6 Hz), 3.84 (3H, s), 3.85 (3H, s), 4.01 (1H, dd, *J*=8.0, 6.6 Hz), 4.27 (1H, dd, *J*=8.8, 8.0 Hz), 4.47 (1H, d, *J*=17.0 Hz), 4.53 (1H, d, *J*=17.0 Hz), 6.56 (1H, s), 6.61 (1H, s). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.3, 18.5, 27.7, 32.9, 42.6, 46.7, 55.5, 69.7, 70.2, 108.7, 111.2, 124.8, 125.8, 147.2, 147.2, 160.6.

(D)-**11**. mp 64–65°C. $[\alpha]_D^{26}$ +37.8 (*c*=1.00, benzene).

(1*R*)-1-[4-(Benzyloxy)-3-bromobenzyl]-2-[(4*S*)-4,5-dihydro-4-(1-methylethyl)-2-oxazolyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline ((L)-(*R*)-12) This compound was prepared from (L)-**11** and **7b** as describe above in 75% yield. MS (EI) *m/z*: 579 (MH^+), 303. HR-MS (EI) *m/z*: 579.1853 (Calcd for $C_{31}H_{36}BrN_2O_4$ (MH^+): 579.1858). IR (neat) cm^{-1}: 1650. $[\alpha]_D^{22}$ −104.4 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.79 (3H, d, *J*=6.9 Hz), 0.88 (3H, d, *J*=6.9 Hz), 1.55–1.66 (1H, m), 2.52 (1H, ddd, *J*=15.9, 4.4, 4.4 Hz), 2.78–2.87 (2H, m), 2.92 (1H, dd, *J*=13.5, 6.6 Hz), 3.04 (1H, dd, *J*=13.5, 6.9 Hz), 3.40 (1H, ddd, *J*=12.9, 9.6, 4.4 Hz), 3.66 (3H, s), 3.72 (1H, ddd, *J*=8.8, 6.0, 6.0 Hz), 3.84 (3H, s), 3.93 (1H, dd, *J*=8.0, 6.0 Hz), 4.05 (1H, dd, *J*=8.8, 8.0 Hz), 5.09 (1H, dd, *J*=6.9, 6.6 Hz), 5.13 (2H, s), 6.29 (1H, s), 6.58 (1H, s), 6.81 (1H, d, *J*=8.3 Hz), 6.98 (1H, dd, *J*=8.3, 2.2 Hz), 7.28–7.48 (6H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 17.7, 18.5, 27.7, 33.2, 39.6, 41.2, 55.6, 55.7, 57.1, 69.8, 70.4, 70.7, 110.2, 111.2, 111.9, 113.4, 126.1, 126.8, 127.8, 128.1, 128.4, 129.6, 132.6, 134.4, 136.5, 146.8, 147.6, 153.3, 160.2.

(D)-(*S*)-**12**. $[\alpha]_D^{28}$ +91.0 (*c*=1.00, benzene).

(*R*)-1-(4-(Benzyloxy)-3-bromobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline ((*R*)-13) This compound was prepared from (L)-(*R*)-**12** as described above in 64% yield. MS (EI) *m/z*: 482 (MH^+), 206. HR-MS (EI) *m/z*: 482.1323 (Calcd for $C_{26}H_{29}BrNO_3$ (MH^+): 482.1331). IR (neat) cm^{-1}: 1607. $[\alpha]_D^{24}$ −73.0 (*c*=1.00, MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.50 (3H, s), 2.57 (1H, m), 2.72–2.86 (2H, m), 2.73 (1H, dd, *J*=13.7, 7.1 Hz), 3.07 (1H, dd, *J*=13.7, 5.5 Hz), 3.16 (1H, ddd, *J*=12.4, 8.8, 8.8 Hz), 3.58 (3H, s), 3.65 (1H, dd, *J*=7.1, 5.5 Hz), 3.84 (3H, s), 5.13 (2H, s), 6.08 (1H, s), 6.55 (1H, s), 6.81 (1H, d, *J*=8.2 Hz), 6.91 (1H, dd, *J*=8.2, 2.2 Hz), 7.27–7.48 (6H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.1, 39.7, 42.3, 46.6, 55.3, 55.4, 64.4, 70.4, 110.6, 111.0, 111.7, 113.2, 125.8, 126.6, 127.5, 128.2, 128.7, 129.3, 133.8, 134.1, 136.3, 146.2, 147.0, 152.9. HPLC: Daicel Chiralpak IB, hexane–2-propanol–ethylenediamine=80 : 20 : 0.1, 1.0 mL/min, UV 270 nm, *R*: 12.5 min (DL-**13**: 7.9, 12.1 min).

(*S*)-**13**. $[\alpha]_D^{26}$ +75.2 (*c*=1.00, MeOH).

(*R*)-1-[4-(Benzyloxy)-3-[(*S*)-6-methoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy]benzyl]-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (14) This compound was prepared from (*S*)-**5c** and (*R*)-**13** as described above in 35% yield. MS (EI) *m/z*: 715 (MH^+), 593, 296, 206. HR-MS (EI) *m/z*: 715.3723 (Calcd for $C_{45}H_{51}N_2O_6$ (MH^+): 715.3747). IR (Nujol) cm^{-1}: 1611, 1509. $[\alpha]_D^{23}$ −46.6 (*c*=1.00, benzene). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.46 (6H, s), 2.52–2.63 (2H, m), 2.66–2.85 (5H, m) 2.70 (1H, dd, *J*=13.7, 8.0 Hz), 2.94 (1H, dd, *J*=14.0, 5.8 Hz), 3.07 (1H, dd, *J*=13.7, 5.2 Hz), 3.12–3.15 (2H, m), 3.52 (3H, s), 3.57–3.64 (2H, m), 3.67 (3H, s), 3.78 (3H, s), 3.82 (3H, s), 5.06 (2H, s), 6.03 (1H, s), 6.33 (1H, s), 6.52 (1H, s), 6.62 (1H, s), 6.63 (2H, d, *J*=8.8 Hz), 6.65 (1H, dd, *J*=8.2, 1.9 Hz), 6.72 (1H, d, *J*=1.9 Hz), 6.82 (1H, d, *J*=8.2 Hz) 6.89 (2H, d, *J*=8.8 Hz), 7.22–7.32 (5H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.2, 25.8, 40.0, 40.4, 42.4, 42.6, 46.7, 47.2, 54.8, 55.3, 55.5, 55.7, 64.2, 64.5, 70.8, 110.7, 111.0, 112.1, 113.2, 114.8, 117.6, 120.6, 124.7, 125.9, 126.8, 127.4, 128.1, 129.0, 129.3, 129.8, 130.1, 131.4, 133.3, 137.2, 143.9, 146.2, 146.2, 147.0, 147.4, 148.4, 157.5.

1-*epi*-Neferine This compound was prepared from **14** 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 $C_{38}H_{45}N_2O_6$ (MH^+): 625.3278). IR (KBr) cm^{-1}: 3422, 1611, 1510. $[\alpha]_D^{22}$ −27.8 (*c*=1.00, $CHCl_3$). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.46 (3H, s, NCH_3-2′), 2.49 (3H, s, NCH_3-2), 2.55 (1H, ddd, *J*=16.0, 4.5, 4.5 Hz, H-4′), 2.62 (1H, ddd, *J*=16.5, 5.0, 5.0 Hz, H-4) 2.67 (1H, dd, *J*=14.0, 8.0 Hz, H-α'), 2.71–2.75 (2H, m, H-3 or 3′), 2.75 (1H, dd, *J*=14.0, 6.5 Hz, H-α), 2.81 (1H, ddd, *J*=16.0, 8.0, 6.0 Hz, H-4′), 2.83 (1H, ddd, *J*=16.5,

8.0, 6.0 Hz, H-4), 3.02 (1H, dd, J=14.0, 5.5 Hz, H-α), 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, OCH_3-6′) 3.61 (1H, dd, J=8.0, 5.0 Hz, H-1′) 3.63 (1H, dd, J=6.5, 5.5 Hz, H-1) 3.72 (3H, s, OCH_3-12), 3.80 (3H, s, OCH_3-6), 3.82 (3H, s, OCH_3-7′), 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), 6.68 (1H, dd, J=8.0, 2.0 Hz, H-14′), 6.70 (2H, d, J=8.5 Hz, H-11, 13), 6.85 (1H, d, J=8.0 Hz, H-13′), 6.93 (2H, d, J=8.5 Hz, H-10, 14). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 25.2, 26.0, 39.9, 40.6, 42.5, 42.7, 46.7, 47.0, 55.1, 55.5, 55.7, 55.8, 64.4, 64.8, 110.9, 111.1, 112.3, 113.4, 115.4, 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, 148.9, 157.8. Hydrochloride: mp 181–183°C. *Anal.* Calcd for $C_{38}H_{46}Cl_2N_2O_6 \cdot 1.5H_2O$: C, 62.98; H, 6.82; N, 3.87. Found: C, 63.51; H, 6.83; N, 3.97.

(*S*)-1-(4-(Benzyloxy)-3-((*R*)-6-methoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yloxy)benzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (15) This compound was prepared from (*R*)-**5c** and (*S*)-**13** as described above in 30% yield. $[\alpha]_D^{27}$ +43.4 (c=1.00, benzene).

1′-*epi*-Neferine This compound was prepared from **15** in 84% yield. mp 77–80°C. $[\alpha]_D^{25}$ +24.4 (c=1.00, $CHCl_3$). Hydrochloride: mp 185–189°C. *Anal.* Calcd for $C_{38}H_{46}Cl_2N_2O_6 \cdot 1.5H_2O$: C, 62.98; H, 6.82; N, 3.87. Found: C, 63.32; H, 6.93; N, 3.92.

(*S*)-1-(4-(Benzyloxy)-3-((*S*)-6-methoxy-1-(4-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yloxy)benzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (16) This compound was prepared from (*S*)-**5c** and (*S*)-**13** as described above in 45% yield. $[\alpha]_D^{24}$ −46.4 (c=1.00, benzene). MS (EI) m/z: 715 (MH^+), 593, 296, 206. HR-MS (EI) m/z: 715.3723 (Calcd for $C_{45}H_{51}N_2O_6$ (MH^+): 715.3725). IR (Nujol) cm^{-1}: 1611, 1509. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s), 2.46 (3H, s), 2.50–2.62 (2H, m), 2.66–2.84 (5H, m) 2.76 (1H, dd, J=14.3, 6.5 Hz), 2.92 (1H, dd, J=14.3, 5.8 Hz), 3.02–3.15 (3H, m), 3.54 (3H, s), 3.57–3.64 (2H, m), 3.68 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 5.06 (2H, s), 6.06 (1H, s), 6.34 (1H, s), 6.50 (1H, s), 6.62 (1H, s), 6.63 (2H, d, J=8.8 Hz), 6.66 (1H, dd, J=8.2, 2.2 Hz), 6.72 (1H, d, J=2.2 Hz), 6.81 (1H, d, J=8.2 Hz) 6.88 (2H, d, J=8.8 Hz), 7.22–7.32 (5H, m). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 25.2, 25.9, 39.9, 40.4, 42.5, 42.6, 46.7, 47.3, 54.9, 55.4, 55.5, 55.7, 64.3, 64.5, 70.8, 110.7, 111.0, 112.1, 113.2, 114.8, 117.6, 120.5, 124.8, 125.7, 126.8, 127.4, 128.1, 129.0, 129.5, 130.2, 131.4, 133.3, 137.2, 143.9, 146.2, 146.3, 147.0, 147.4, 148.4, 157.5.

***ent*-Neferine** This compound was prepared from **16** as described above in 56% yield. mp 70–73°C. UV λ_{max} (methanol) nm ($\log\varepsilon$): 225 (4.40), 282 (3.96). MS (EI) m/z: 625 (MH^+), 503, 206. HR-MS (EI) m/z: 625.3255 (Calcd for $C_{38}H_{45}N_2O_6$ (MH^+): 625.3278). IR (KBr) cm^{-1}: 3423, 1611, 1510. $[\alpha]_D^{22}$ +35.0 (c=1.00, $CHCl_3$). (Natural neferine $[\alpha]_D^{28}$ −37.8 (c=1.43, $CHCl_3$)[2]). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.47 (3H, s, NCH_3-2′), 2.50 (3H, s, NCH_3-2), 2.55 (1H, ddd, J=15.0, 5.5, 3.5 Hz, H-4′), 2.62 (1H, ddd, J=16.0, 5.0, 5.0 Hz, H-4) 2.67 (1H, dd, J=13.5, 7.5 Hz, H-α'), 2.71–2.76 (2H, m, H-3 or 3′), 2.77 (1H, dd, J=14.0, 7.0 Hz, H-α), 2.78–2.84 (2H, m, H-4, 4′), 3.01 (1H, dd, J=14.0, 5.5 Hz, H-α), 3.08 (1H, dd, J=13.5, 4.5 Hz, H-α'), 3.12–3.17 (2H, m, H-3 or 3′), 3.53 (3H, s, OCH_3-6′) 3.61 (1H, dd, J=7.5, 4.5 Hz, H-1′) 3.65 (1H, dd, J=7.0, 5.5 Hz, H-1) 3.73 (3H, s, OCH_3-12), 3.80 (3H, s, OCH_3-6), 3.81 (3H, s, OCH_3-7′), 5.99 (1H, s, H-8′), 6.38 (1H, s, H-8), 6.51 (1H, s, H-5′), 6.55 (1H, d, J=2.0 Hz, H-10′), 6.63 (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). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 25.2, 26.0, 39.9, 40.6, 42.5, 42.7, 46.7, 47.0, 55.1, 55.5, 55.7, 55.8, 64.4, 64.8, 110.9, 111.1, 112.3, 113.4, 115.4, 119.1, 120.0, 125.3, 130.4, 131.0, 131.3, 131.9, 142.8, 144.6, 145.4, 146.4, 147.3, 148.9, 157.8. Hydrochloride: mp 187–190°C. *Anal.* Calcd for $C_{38}H_{46}Cl_2N_2O_6 \cdot H_2O$: C, 63.77; H, 6.76; N, 3.91. Found: C, 63.92; H, 6.64; N, 3.94.

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 Pharmacological 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 over an open-top 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 horizonal 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 Dunnet's multiple comparison *post-hoc* test.

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