Online Structural Elucidation of Alkaloids and Other Constituents in Crude Extracts and Cultured Cells of *Nandina domestica* by Combination of LC-MS/MS, LC-NMR, and LC-CD Analyses

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Received March 10, 2008

The combination of NMR, MS, and CD data permitted the structural elucidation including the absolute configuration of the known alkaloids and unknown components in the extract matrix solution of *Nandina domestica* without isolation and sample purification prior to the coupling experiments. Unstable natural stereoisomers were identified by LC-NMR and LC-MS. Five known alkaloids, (*S*)-isoboldine, (*S*)-domesticine, (*S*)-nantenine, sinoacutine, and menispermine, were identified from *N. domestica*. *O*-Methylpallidine and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-terrestribisamide were also characterized for the first time from this plant. Known jatrorrhizine, palmatine, and berberine and unknown (*R*)-carnegine and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-terrestribisamide were identified in the callus of *N. domestica*.

Nandina domestica Thumb. grows wild in Japan and China.[1] In Japan, the fruits of this plant have long been used to treat asthma, whooping cough, pharyngeal tumors, and uterine bleeding.[2] In China, other parts of the plant, e.g., stems and leaves, have also been used as medicines.[3] Shoji et al.[2] reported that the alkaloid nantenine is a serotonergic receptor antagonist. The fruits, seeds, roots, and stem bark contain aporphine-, promorphine-, protoberberine-, and protopine-type alkaloids.[4−18] Twelve protoberberines and magnoflorine have been identified from tissue cultures of *N. domestica*.[19−22]

Application of LC-NMR combined with LC-MS to drug metabolism, identification of natural products in crude plant extracts and the characterization of isomeric mixtures prepared by chemical reactions have been summarized.[23−25] The coupling of HPLC to a CD detector is one of the most powerful hyphenated techniques for stereochemical investigation.[26] The combination of three online coupling methods, LC-NMR, LC-MS, and LC-CD, permitted determination of the structures including absolute configuration of the natural products in crude plant extracts without the necessity of isolation and purification.[27,28]

In this report, we describe the characterization of components, particularly alkaloids, in crude extracts of intact plants and tissue cultures of *N. domestica* using LC-MS-MS, LC-NMR, and LC-CD analyses.

Results and Discussion

The ground parts of *N. domestica* were extracted to give Plant Fr. E and Plant Fr. C (see Experimental Section), which were subjected to LC-NMR, LC-MS, and LC-CD analyses. In the LC-MS chromatogram of Plant Fr. E (Figure 1), peaks a_1, b_1, c_1, and d_1 showed protonated molecular ions $[M + 1]^+$ at *m*/*z* 328 (a_1 and b_1), 326 (c_1), and 340 (d_1), respectively. The stopped-flow ^{1}H NMR spectrum of peak a_1 showed three aromatic proton singlets at δ 8.02, 6.83, and 6.77, two methoxy groups at δ 3.85 and 3.82, and an *N*-methyl group at δ 2.99. From these data, the compound associated with peak a_1 was identified as isoboldine (Figure 2, A). NOESY spectroscopic data (Figure 2) also supported this structure. Similarly, the compounds associated with peaks b_1 and c_1 were identified as sinoacutine and domesticine, respectively (Figure 2, B and C), on the basis of their stopped-flow ^{1}H NMR [peak b_1 displayed two aromatic proton doublets ($J = 8.0$ Hz) at δ 6.89 and 6.71, two olefinic proton singlets at δ 7.78 and 6.46, two methoxy groups at δ 3.76 and 3.66, an *N*-methyl group at δ 2.70; c_1 showed three aromatic proton singlets at δ 7.85, 6.84, and 6.76, a methylenedioxy group at δ 5.93, a methoxy group at δ 3.81, and an *N*-methyl group at δ 2.95] and NOESY data (Figure 2). The stopped-flow ^{1}H NMR spectrum of peak d_1 displayed three aromatic proton singlets at δ 7.77, 6.86, and 6.81, a methylenedioxy group at δ 5.96, two methoxy groups at δ 3.81 and 3.58, and an *N*-methyl group at δ 2.87. The compound corresponding to peak d_1 was identified as nantenine by comparison of its LC-NMR spectrum (Figure 2, D) with that of domesticine (Figure 2, C). Isoboldine, sinoacutine, domesticine, and nantenine have been isolated previously from *N. domestica*.[4−8,10−18]

The absolute configuration at C-6a of the isolated aporphine alkaloids (isoboldine, domesticine, and nantenine) were determined from LC-CD analysis. Initially, CD spectra of commercially available (*S*)-boldine and (*S*)-isocorydine in the stopped-flow mode were recorded at 220−420 nm to find a suitable wavelength for the measurement of LC-CD spectra. (*S*)-Boldine and (*S*)-isocorydine showed a positive CD sign near 240 nm (Figure 3). A wavelength of 236 nm was used in subsequent LC-CD measurements. In an LC-CD spectrum of Plant Fr. E measured under the same conditions, peaks a_1, c_1, and d_1 corresponding to isoboldine, domesticine, and nantenine, respectively, had a positive CD sign (Figure 4). Therefore, the absolute configuration at C-6a of these alkaloids was identified as *S*. The proaporphine-type alkaloid sinoacutine (peak b_1) showed a negative CD sign.

LC peaks a_2−e_2 of Plant Fr. C (Figure 5) exhibited protonated molecular ions $[M + 1]^+$ or molecular ions $[M]^+$ of *m*/*z* 356 (a_2), *m*/*z* 342 (b_2), *m*/*z* 328 (c_2), *m*/*z* 441 (d_2), and *m*/*z* 336 (e_2). The stopped-flow ^{1}H NMR spectrum of peak a_2 contained a singlet aromatic proton at δ 7.02, two aromatic proton doublets ($J = 8.0$ Hz) at δ 7.04 and 6.99, three methoxy groups at δ 3.86, 3.80, and 3.60, and two *N*-methyl groups at δ 3.28 and 2.89 (Figure 6, A). The compound associated with peak a_2 was identified as menispermine by comparison of LC-NMR data with those of magnoflorine. NOESY data (Figure 6) supported this structure.

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10.1021/np8001496 CCC: $40.75
Published on Web 08/01/2008

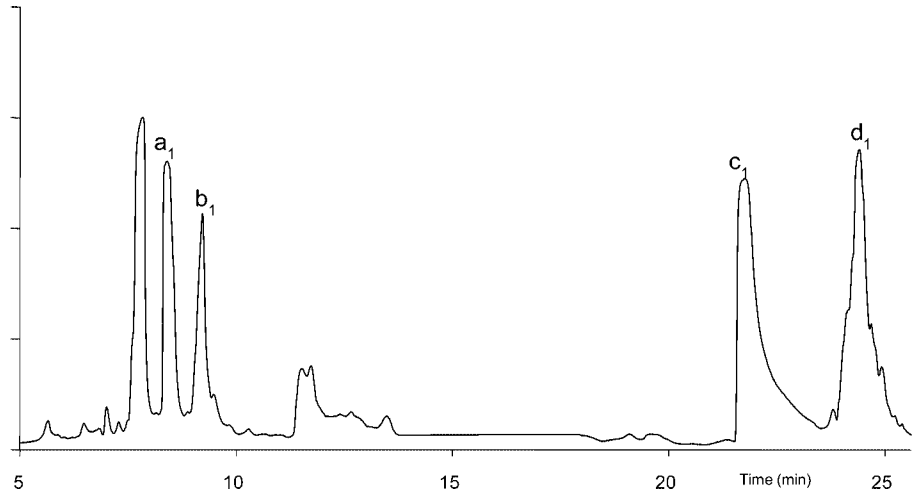


Figure 1. LC data of the alkaloid fraction (Plant Fr. E) obtained from *N. domestica*. Column: Cosmosil 5C -AR-II (4.6 × 150 mm). Eluent: A: 0.1 M NH_4OAc/D_2O (0.05% TFA); B: MeCN (0.05% TFA). Gradient A/B: initial 90/10, 5 min 75/25, 15 min 75/25, 30 min 0/100. Flow rate: 1.0 mL/min. UV detector: 280 nm.

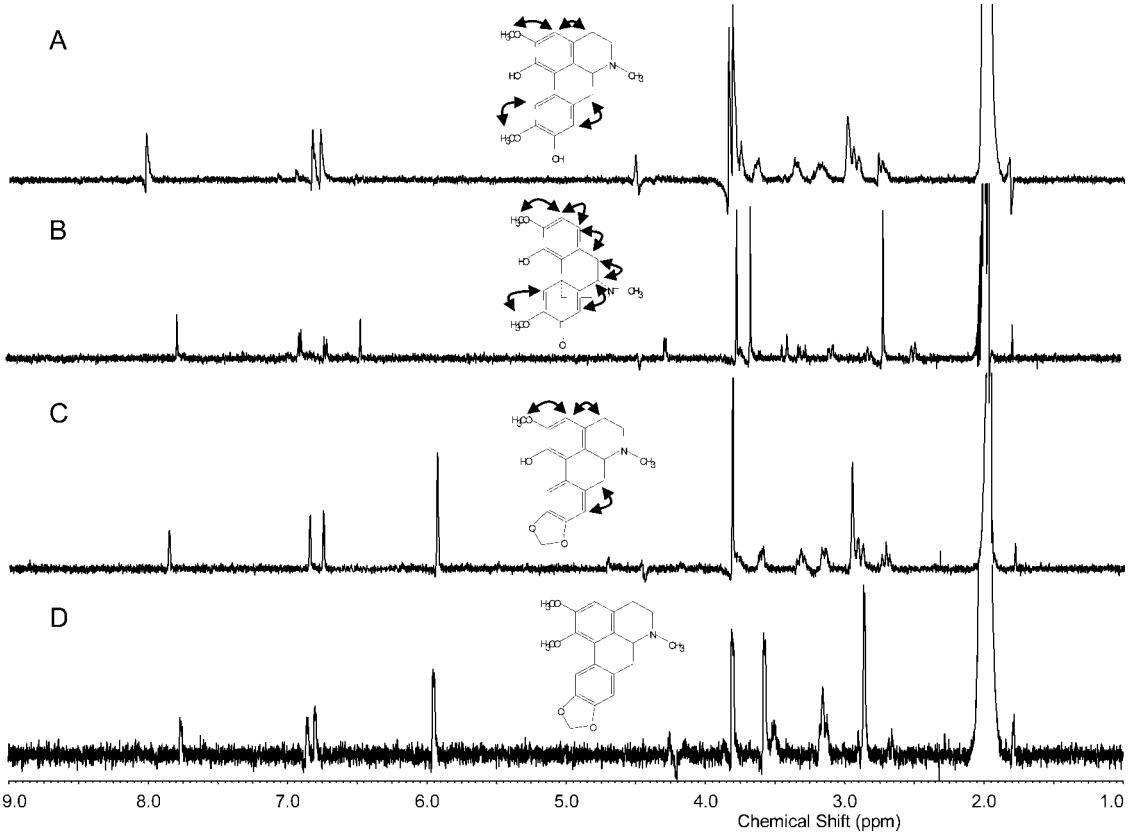


Figure 2. Stopped-flow LC-^{1}H NMR spectra and NOESY data of major components in the alkaloid fraction (Plant Fr. E) obtained from *N. domestica*. (A) ^{1}H NMR spectrum of peak a_1 (isoboldine). (B) ^{1}H NMR spectrum of peak b_1 (sinoacutine). (C) ^{1}H NMR spectrum of peak c_1 (domesticine). (D) ^{1}H NMR spectrum of peak d_1 (nantenine).

The LC-NMR spectrum of peak b_2 exhibited two aromatic proton singlets at δ 7.07 and 6.83, two singlet olefinic protons at δ 6.93 and 6.50, three methoxy groups at δ 3.80, 3.79, and 3.67, and one *N*-methyl group at δ 2.74 (Figure 6, C). The compound associated with peak b_2 was recognized as *O*-methylpallidine by comparison of LC-NMR data with those of sinoacutine. NOESY data (Figure 6) supported this structure.

Peak d_2 displayed a protonated molecular ion at *m*/*z* 441 and product ion at *m*/*z* 177. The stopped-flow ^{1}H NMR spectrum of peak d_2 showed an aromatic proton singlet at δ 7.12, two aromatic proton doublets (J = 8.0 Hz) at δ 7.04 and 6.82, two olefinic proton doublets (J = 16.0 Hz) at δ 7.35 and 6.41, a methoxy group at δ 3.80, and two methylene proton singlets at δ 3.23 and 1.53 (Figure 7, Table 1). After preparative HPLC of Plant Fr. C, HR-SIMS analysis of the compound corresponding to peak d_2 gave a molecular formula of $C_{24}H_{28}N_2O_6$ with a molecular ion at *m*/*z* 440 and fragment ions at *m*/*z* 265 and 177. ^{13}C NMR and DEPT spectra showed only 12 carbons: a methyl at δ 56.36, two methylenes at δ 40.14 and 27.92, five methines at δ 142.02, 123.15, 118.73, 116.46, and 111.53, three quaternary carbons at δ 149.82, 149.27, and 128.25, and a carbonyl at δ 169.23 (Figure 8 and Table 2). Thus, on the basis of HR-SIMS and ^{13}C NMR data, this compound is

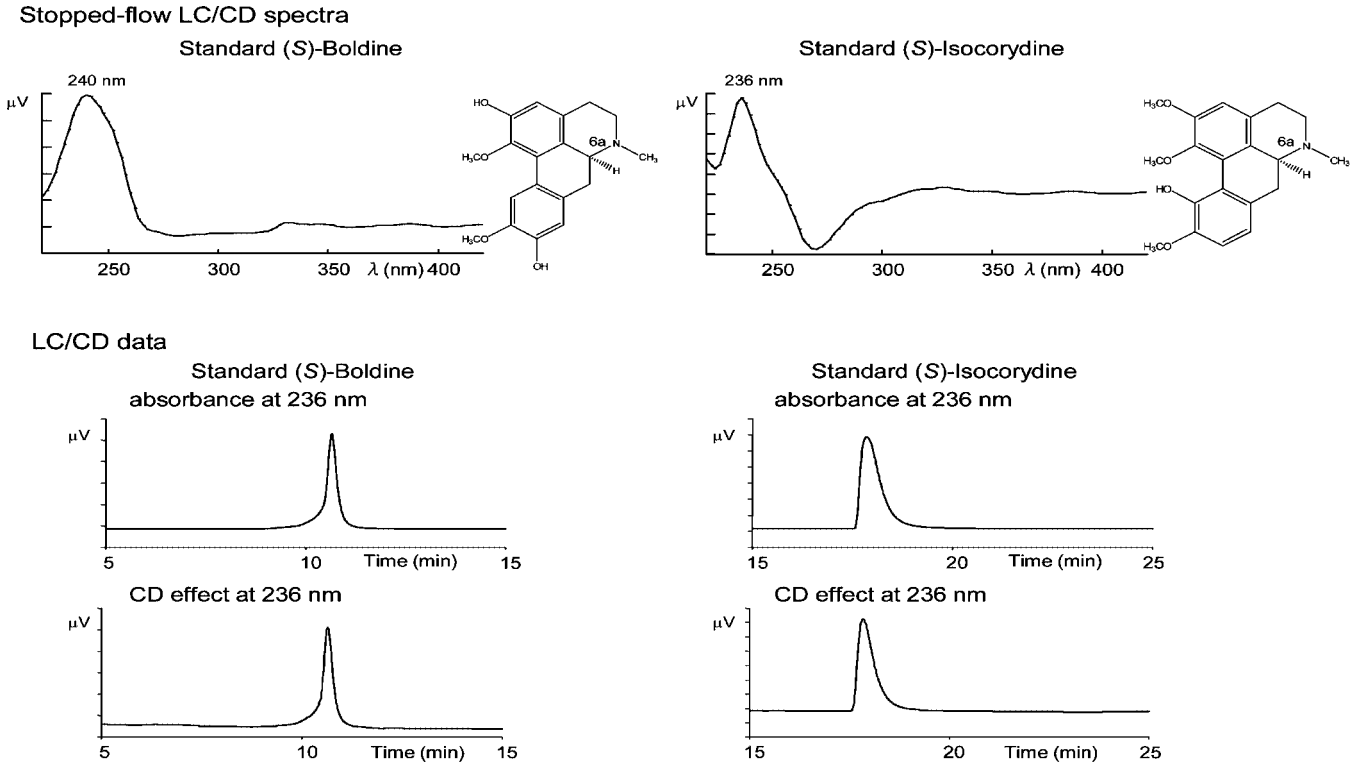


Figure 3. Stopped-flow LC-CD spectra and LC-CD data of (*S*)-boldine and (*S*)-isocorydine. Column: Cosmosil $5C_{18}$-AR-II (6.0 × 150 mm). Gradient: A: 0.1 M NH_4OAc (0.05% TFA); B: MeCN (0.05% TFA). A/B: initial 100/0, 40 min 0/100. Flow rate: 2.0 mL/min. Temp: 40 °C.

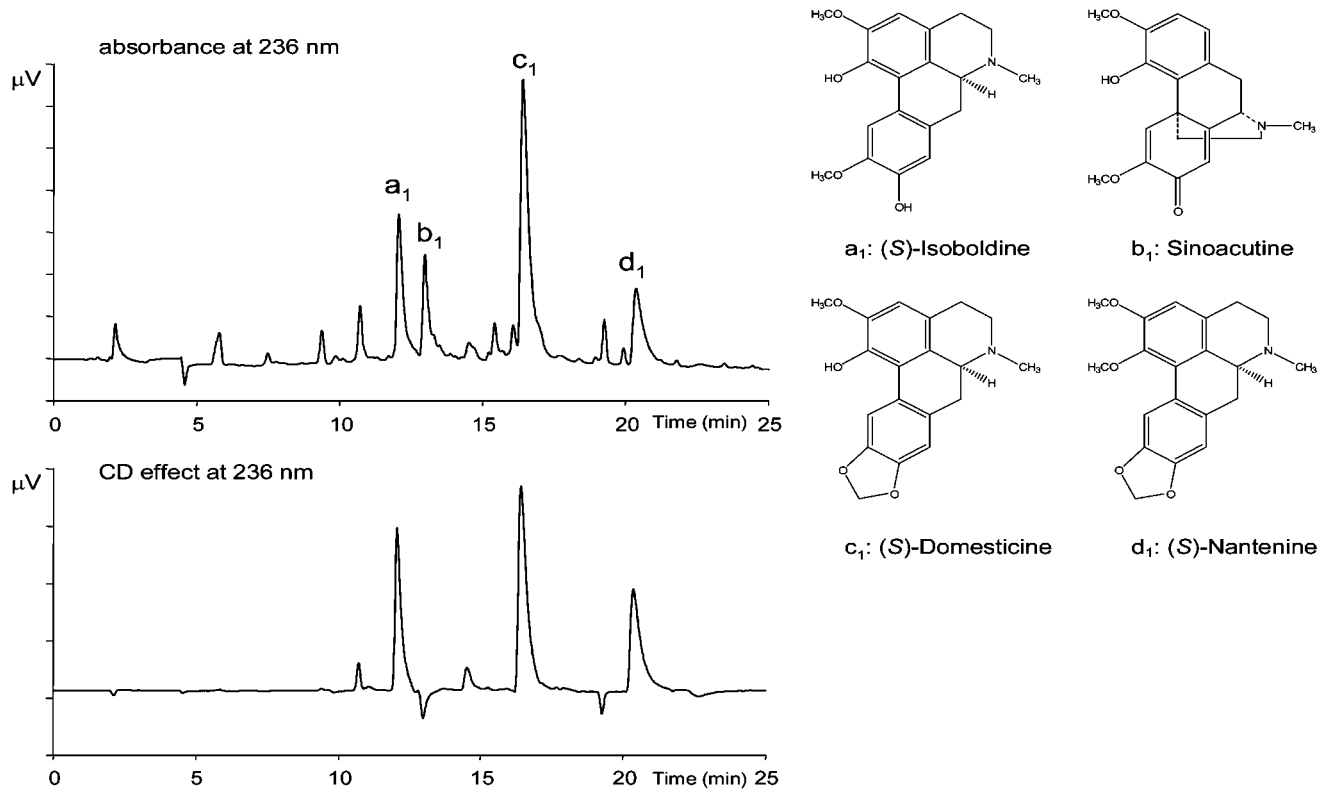


Figure 4. LC-CD data of the alkaloid fraction (Plant Fr. E) obtained from *N. domestica*. Column: Cosmosil $5C_{18}$-AR-II (6.0 × 150 mm). Gradient: A: 0.1 M NH_4OAc (0.05% TFA); B: MeCN (0.05% TFA). A/B: initial 100/0, 40 min 0/100. Flow rate: 2.0 mL/min. Temp: 40 °C. UV detector: 236 nm.

dimeric. The structure corresponding to peak d_2 was identified as terrestribisamide. Compositions of $C_{14}H_{21}N_2O_3$ and $C_{10}H_9O_3$ for HR-SIMS fragment ions *m*/*z* 265 and 177, respectively, supported this structure (Scheme 1). The coupling constant (16 Hz) of the

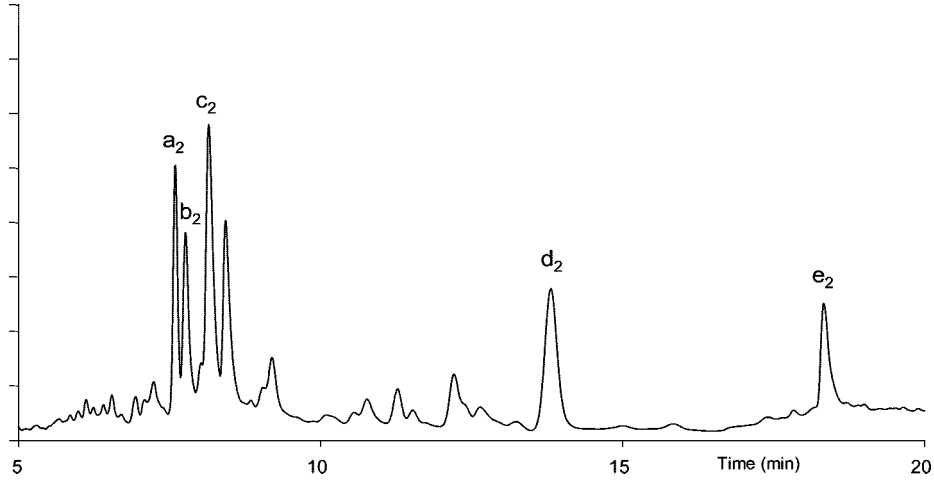


Figure 5. LC data of the alkaloid fraction (Plant Fr. C) obtained from *N. domestica*. Column: Cosmosil 5C_{18}-AR-II (4.6 × 150 mm). Eluent: A: 0.1 M NH_4OAc/D_2O (0.05% TFA); B: MeCN (0.05% TFA). Gradient A/B: initial 90/10, 5 min 75/25, 15 min 75/25, 30 min 0/100. Flow rate: 1.0 mL/min. UV detector: 280 nm.

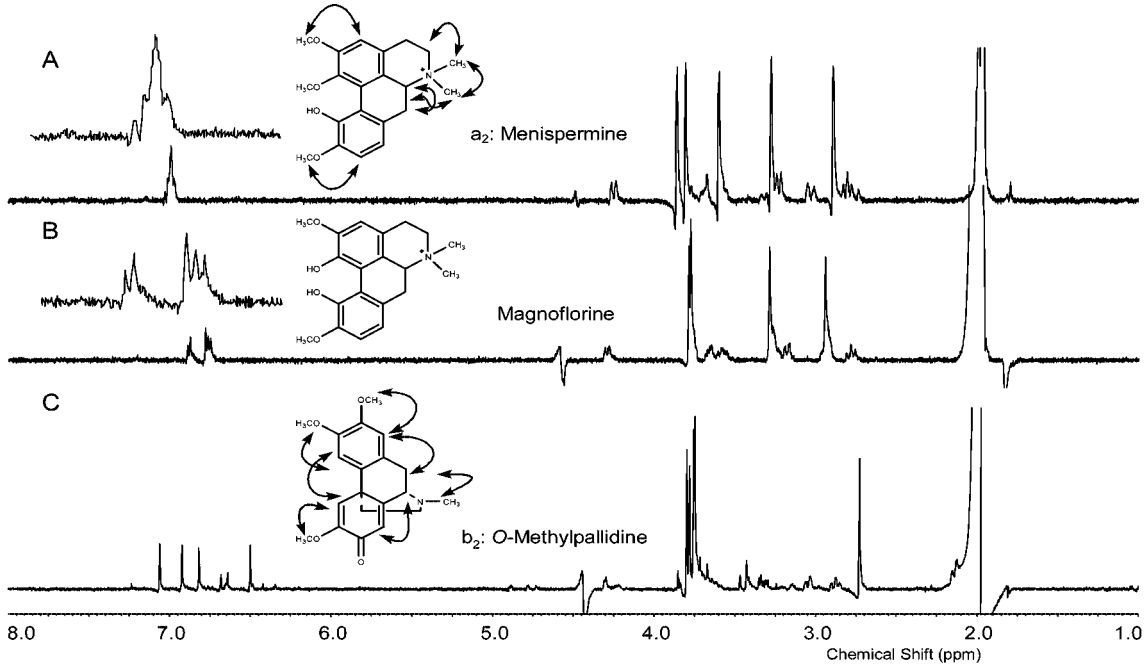


Figure 6. Stopped-flow LC-^{1}H NMR spectra of peaks a_2 and b_2 in LC of the alkaloid fraction (Plant Fr. C) obtained from *N. domestica* and magnoflorine. (A) ^{1}H NMR spectra of peak a_2 (menispermine). (B) ^{1}H NMR spectra of standard magnoflorine. (C) ^{1}H NMR spectra of peak b_2 (*O*-methylpallidine).

olefinic protons indicated that both double bonds have an *E* configuration. HMBC data (Table 2) were also consistent with identification of this compound (peak d_2) as (*E*,*E*)-terrestribisamide.

The LC-NMR spectrum of peak e_2 exhibited four aromatic proton singlets at δ 9.60, 8.55, 7.54, and 6.94, two aromatic proton doublets (J = 9.0 Hz) at δ 8.03 and 7.95, a methylendioxy group at δ 6.06, and two methoxy groups at δ 4.06 and 4.04. The compound associated with peak e_2 was determined to be berberine (identical with the ^{1}H NMR spectrum of C in Figure 15) by comparison of LC-NMR data with those of an authentic sample.

By using LC-NMR, LC-MS/MS, and LC-CD techniques, we have identified four known alkaloids, (*S*)-isoboldine, (*S*)-domesticine, (*S*)-nantenine, sinoacutine, and menispermine, from *N. domestica*. *O*-Methylpallidine and (*E*,*E*)-, (*E*,*Z*)-, and (*Z*,*Z*)-terrestribisamide were also characterized for the first time from this plant.

Calli of *N. domestica* were derived from the stems of wild plants grown in Kobe (Japan) and cultured in the dark on Murashige and Skoog's medium. Methanol extracts of callus cultured for 5−6 weeks were extracted to give Callus Frs. E and C (see Experimental Section), which were subjected to LC-NMR, LC-MS, and LC-CD analyses. In the LC of Callus Fr. E (Figure 9), peaks a_3, b_3, c_3, and d_3 exhibited protonated molecular ions at m/z 222 (a_3) and 441 (b_3, c_3, and d_3). The stopped-flow ^{1}H NMR spectrum of peak a_3 showed the presence of two aromatic proton singlets at δ 6.82 and 6.76, two methoxy groups at δ 3.77 (6H, s), and one methyl doublet (J = 6.0 Hz) at δ 1.56. The compound associated with peak a_3 was determined to be carnegine by comparison of its LC-NMR data with those of an authentic sample (Figure 10). The absolute configuration at C-1 of carnegine was predicted from LC-CD analysis. (*R*)- and (*S*)-Carnegine were resolved with L-(+)- and D-(−)-tartaric acid and showed negative and positive CD signs, respectively, in LC-CD spectra on Chiralcel OD-RH (Figure 11). An LC-CD spectrum of Callus Fr. E was measured under the same conditions. The peak corresponding to carnegine had a negative CD sign (Figure 11); therefore, the absolute configuration at C-1 of the isolated alkaloid was determined to be *R*.

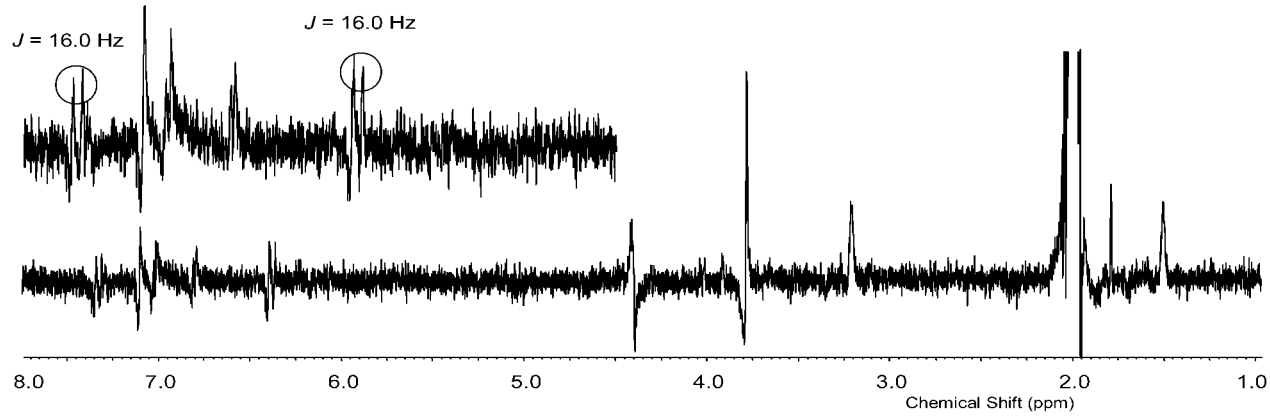


Figure 7. Stopped-flow LC-^{1}H NMR spectrum of peak d_2 in LC of the alkaloid fraction (Plant Fr. C) obtained from *N. domestica*.

Table 1. Stopped-Flow ^{1}H NMR and HMBC Data of (*E,E*)- and (*Z,Z*)-Terrestribisamide

protons	(*E,E*)-terrestribisamide	(*Z,Z*)-terrestribisamide
2-H	7.12	7.08
5-H	6.82[a]	6.75[c]
6-H	7.04[a]	6.83[c]
7-H	7.35[b]	6.63[d]
8-H	6.41[b]	5.83[d]
10-H	3.23	3.07
11-H	1.53	1.28
3-OCH3	3.80	3.73

[a] d J = 8.0 Hz. [b] d J = 16.0 Hz. [c] d J = 7.5 Hz. [d] d J = 12.0 Hz.

The compounds associated with peaks b_3−d_3 had the same protonated molecular ion (*m/z* 441) and, thus, were isomers of terrestribisamide with different double-bond configurations. The compound corresponding to peak d_3 had identical data to those described above for (*E,E*)-terrestribisamide. The stopped-flow ^{1}H NMR spectrum of peak b_3 showed an aromatic proton singlet at δ 7.08, two aromatic proton doublets (J = 7.5 Hz) at δ 6.83 and 6.75, two olefinic proton doublets (J = 12.0 Hz) at δ 6.63 and 5.83, a methoxy group at δ 3.73, and two methylene proton singlets at δ 3.07 and 1.28 (Figure 12). The coupling constant (12 Hz) of the olefinic protons indicated that the configuration of both double bonds was *Z*. Thus, the compound responsible for peak b_3 was identified as (*Z,Z*)-terrestribisamide. The stopped-flow ^{1}H NMR spectrum of peak c_3 displayed olefinic signals for both *E* and *Z* double bonds (Figure 13). Therefore, peak c_3 was identified as (*E,Z*)-terrestribisamide.

Similarly, in the LC-MS/MS of Callus Fr. C, peaks a_4, b_4, and d_4 (Figure 14) showed the same protonated molecular ion at *m/z* 441 and product ion at *m/z* 177. Peaks a_4, b_4, and d_4 were identified as (*Z,Z*)-, (*E,Z*)-, and (*E,E*)-terrestribisamide, respectively. Peaks c_4, e_4, and f_4 in Figure 14 displayed molecular ions at *m/z* 338, 352, and 336 and product ions at *m/z* 322, 336, and 320, respectively, in the LC-MS/MS. The stopped-flow ^{1}H NMR spectrum of peak c_4 showed four aromatic proton singlets at δ 9.57, 8.61, 7.55, and 6.88, two aromatic proton doublets (J = 9.0 Hz) at δ 8.02 and 7.94, and three methoxy groups at δ 4.05, 4.03, and 3.93 (Figure 15, A). The compound associated with peak c_4 was determined to be jatrorrhizine by comparison of LC-NMR data with those of an authentic sample. The LC-NMR spectrum of peak e_4 exhibited four aromatic proton singlets at δ 9.60, 8.64, 7.55, and 7.03, two aromatic proton doublets (J = 9.5 Hz) at δ 8.03 and 7.95, and four methoxy groups at δ 4.06, 4.03, 3.92, and 3.87 (Figure 15, B). The compound related to peak e_4 was confirmed to be palmatine by comparison of LC-NMR data with those of an authentic sample. The compound corresponding to peak f_4 was

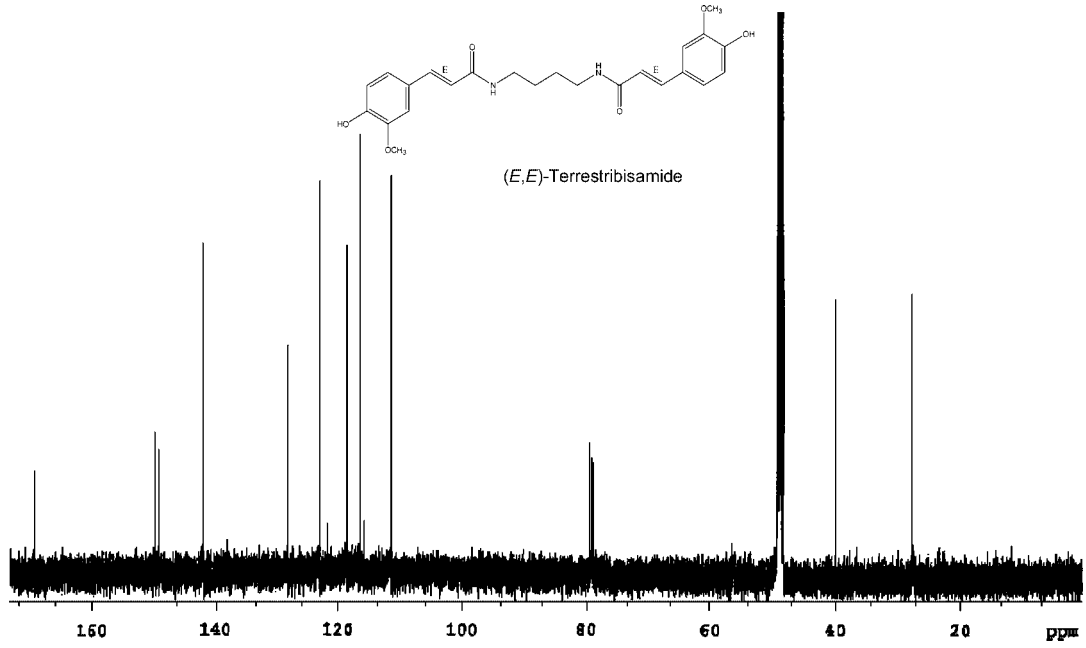
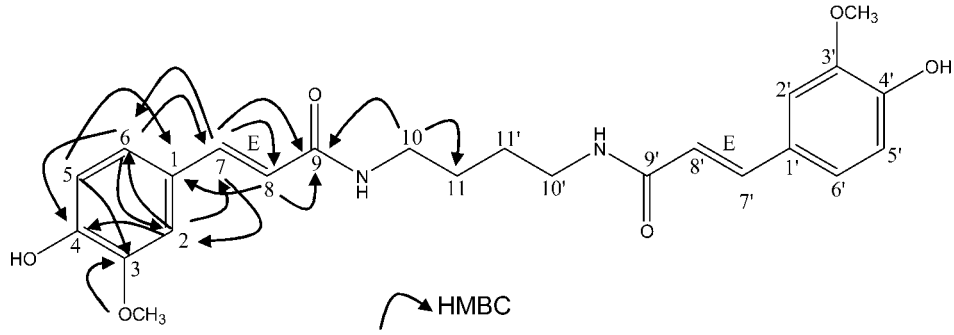


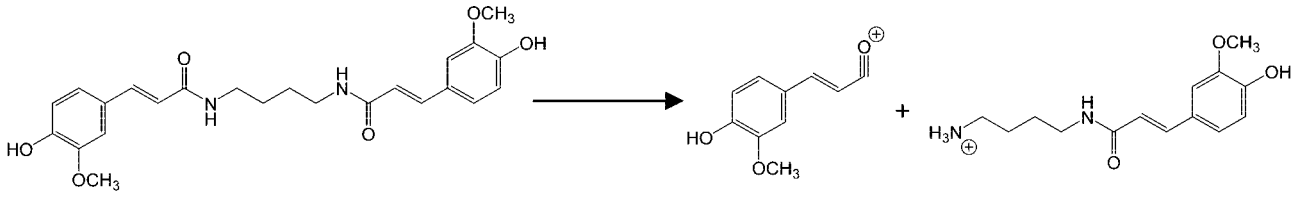
Figure 8. ^{13}C NMR spectrum of (*E,E*)-terrestribisamide.

Table 2. ^{13}C NMR HMBC Data of (*E,E*)-Terrestribisamide



C-1	C	128.25	C-7	CH	142.02
C-2	CH	111.53	C-8	CH	118.73
C-3	C	149.27	C-9	C=O	169.23
C-4	C	149.82	C-10	CH_2	40.14
C-5	CH	116.46	C-11	CH_2	27.92
C-6	CH	123.15	C(3)-OCH_3	CH_3	56.36

Scheme 1



$C_{24}H_{28}N_2O_6$ *m/z* = 440 $C_{10}H_9O_3^+$ *m/z* = 177 $C_{14}H_{21}N_2O_3^+$ *m/z* = 265

identical with berberine in LC-NMR (Figure 15, C), also recognized in Plant Fr. C.

Accordingly, jatrorrhizine, palmatine, berberine, (*R*)-carnegine, and (*E,E*)-, (*E,Z*)-, and (*Z,Z*)-terrestribisamide have been identified in the callus of *N. domestica*. (*R*)-Carnegine and (*E,E*)-, (*E,Z*)-, and (*Z,Z*)-terrestribisamide were characterized herein for the first time. Identification of unstable (*E,Z*)- and (*Z,Z*)-terrestribisamide formed by *trans*−*cis* isomerization of (*E,E*)-terrestribisamide indicates the usefulness of LC-NMR for structural analysis.

Experimental Section

General Experimental Procedures. Conventional ^1H NMR and NOESY spectra were obtained on a Varian VXR-500 spectrometer (500 MHz) in CD_3OD as solvent. ^{13}C NMR and DEPT spectra were measured on a Varian VXR-500 spectrometer (125 MHz). Mass spectra were determined on a Hitachi M 80 instrument at 75 eV.

Materials. In 2003, calli of *N. domestica* were derived from the stems of wild plants grown in Kobe (Japan) on Murashige and Skoog's medium containing 2,4-dichlorophenoxyacetic acid (1 mg/L), kinetin (0.1 mg/L), yeast extract (0.1%), and agar (1%). The callus tissues were subcultured every four or five weeks on fresh medium at 25 °C in the dark.

Extraction of *N. domestica*. Ground parts of *N. domestica* (217 g) were cut into pieces, extracted several times with MeOH under reflux, and concentrated after addition of H_2O. Methanol extracts were extracted with 2% HCl several times. The extracts were washed with Et_2O, made basic with NH_4OH, and extracted with Et_2O and then $CHCl_3$ to give Plant Frs. E (11.0 mg) and C (10.2 mg), which were subjected to LC-NMR, LC-MS, and LC-CD measurements.

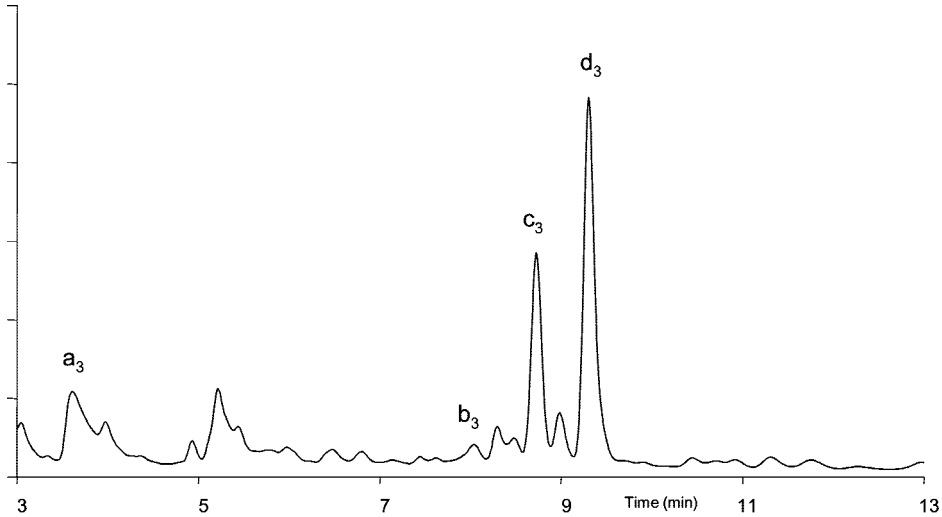


Figure 9. LC data of the alkaloid fraction (Plant Fr. E) obtained from *N. domestica*. Column: Cosmosil $5C_{18}$-AR-II (4.6 × 150 mm). Eluent: A: 0.1 M NH_4OAc/D_2O (0.05% TFA); B: MeCN (0.05% TFA). Gradient A/B: initial 80/20, 10 min 60/40, 20 min 60/40, 30 min 0/100. Flow rate: 1.0 mL/min. UV detector: 280 nm.

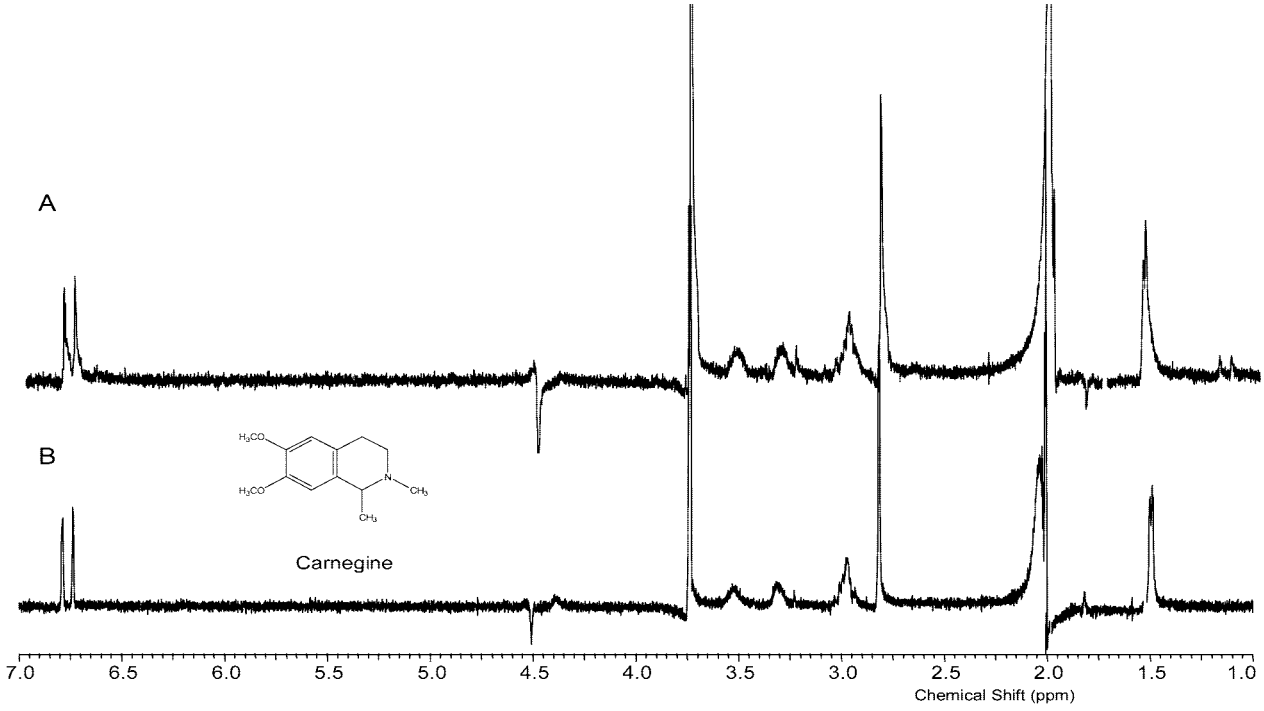


Figure 10. Stopped-flow LC-^{1}H NMR spectrum of peak a_3 in LC of the alkaloid fraction (Callus Fr. E) obtained from *N. domestica* and carnegine. (A) ^{1}H NMR spectrum of peak a_3 (carnegine). (B) ^{1}H NMR spectrum of standard carnegine.

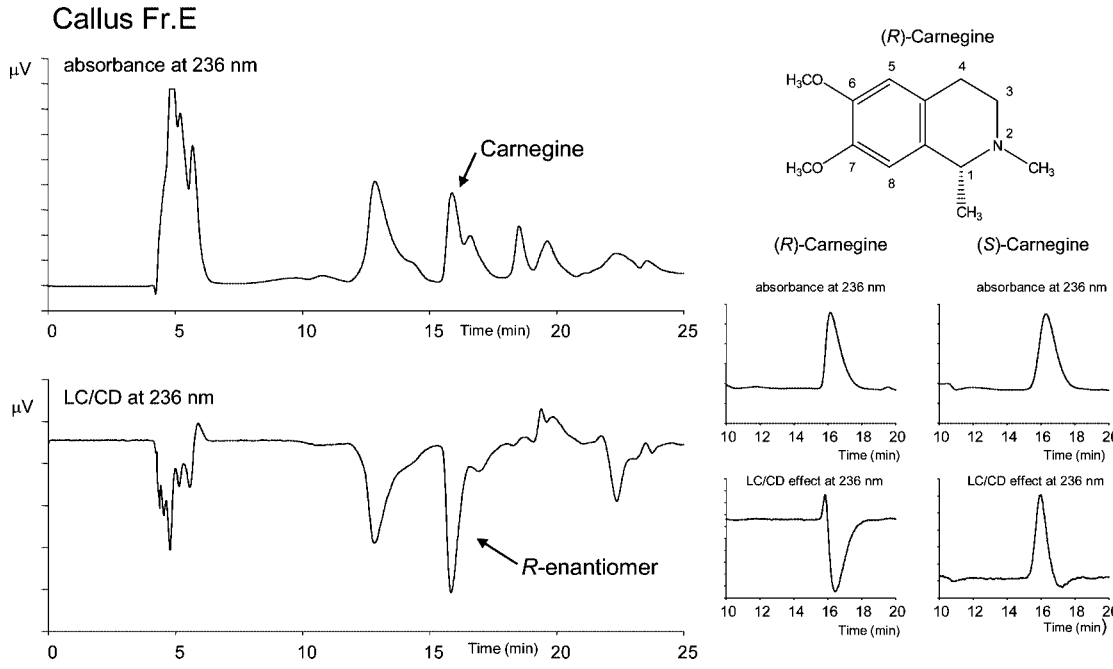


Figure 11. LC-CD data of the alkaloid fraction (Callus Fr. E) obtained from *N. domestica* and (*R*) or (*S*)-carnegine. Column: Chiralcel OD-RH (4.6 × 150 mm). Eluent: A: 0.1 M NH_4OAc (0.05% TFA); B: MeCN (0.05% TFA). Gradient A/B: initial 100/0, 5 min 95/5, 15 min 75/25, 25 min 75/25, 35 min 0/100. Flow rate: 0.5 mL/min. Temp: 40 °C. UV detector: 236 nm.

Extraction of Callus of *N. domestica*. After being subcultured for 5−6 weeks, calli (441 g) and medium were freeze-dried. Water was separated, and callus and medium were extracted several times with hot MeOH. H_2O and MeOH extracts were concentrated. The extracts were washed with Et_2O after acidification, made basic with NH_4OH, and extracted with Et_2O and then $CHCl_3$ to give Callus Frs. E (15.2 mg) and C (20.6 mg), which were subjected to LC-NMR, LC-MS, and LC-CD measurements.

Peak d_4 (*E*,*E*-terrestribisamide) in LC of Callus Fr. C (Figure 14) was separated by preparative HPLC [Cosmosil 5 C_{18}-AR (20 × 250 mm), (A) 0.1 M NH_4OAc (0.05% TFA) and (B) CH_3CN (0.05% TFA): A/B initial 80/20, 75/25 (5 min), 75/25 (15 min), 0/100 (30); 6 mL/min; 280 nm].

Optical Resolution of (±)-Carnegine. A solution of the free base obtained from (±)-carnegine hydrochloride (4.12 g)[29] in H_2O (10 mL) was added to a solution of L-(+)-tartaric acid (2 g) in H_2O (5 mL). The mixture was left in the refrigerator overnight. The resulting white crystals were filtered and washed with cold water to provide the salt [(+)-carnegine·(+)-tartaric acid] (2.72 g, *R*/*S*: 70/30). This salt (910 mg) was dissolved in H_2O (5 mL) and left in the refrigerator for 3 days. The resulting crystals were filtered, and the collected salt (216 mg, *R*/*S*: 93/7) was dissolved in H_2O, basified with aqueous NH_3, and

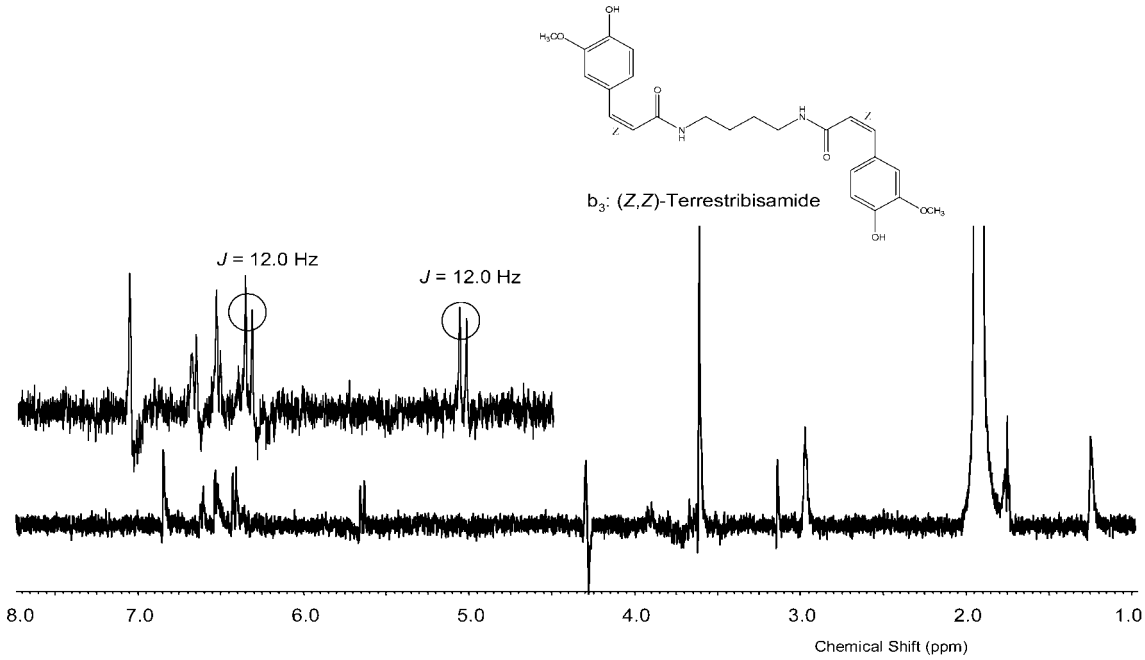


Figure 12. Stopped-flow LC-^{1}H NMR spectrum of peak b_3 in LC of the alkaloid fraction (Callus Fr. E) obtained from *N. domestica*.

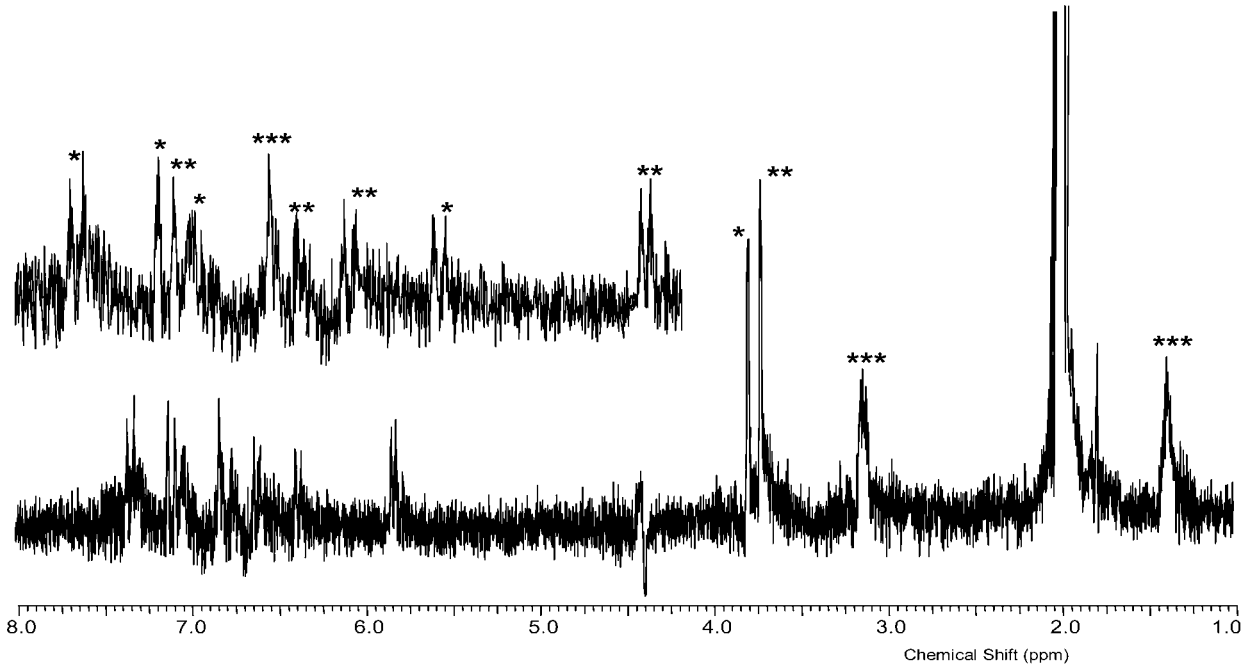


Figure 13. Stopped-flow LC-^{1}H NMR spectrum of peak c_3 in LC of the alkaloid fraction (Callus Fr. E) obtained from *N. domestica*. *Signals attributed to (*E*,*E*)-terrestribisamide. **Signals attributed to (*Z*,*Z*)-terrestribisamide. ***Signals attributed to (*E*,*E*)-terrestribisamide and (*Z*,*Z*)-terrestribisamide.

extracted with Et_2O. The ether extract was dried and evaporated to give (+)-carnegine as an oil (118 mg). Similarly, a solution of the free base obtained from (±)-carnegine hydrochloride (7.50 g) in H_2O (10 mL) was added to a solution of D-(−)-tartaric acid (2.7 g) in H_2O (14 mL). The mixture was left in the refrigerator for 4 days. The resulting white crystals were filtered and washed with cold H_2O to give the salt [(−)-carnegine•(−)-tartaric acid] (3.34 g, *R*/*S*: 40/60). This salt was dissolved in H_2O (5 mL) and left in the refrigerator for 2 days. The resulting crystals were filtered, and the resulting salt (1.01 g, *R*/*S*: 4/96) was converted to the free base [(−)-carnegine].

LC-APCI-MS Method. LC-APCI-MS (/MS) was measured on an Applied Biosystems API 3000 triple quadrupole mass spectrometer (MS/MS) with a heated nebulizer interface as described in a previous paper.[30]

LC-CD Method. LC-CD analyses were carried out on a chiral phase column at 236 nm. Chromatographic separations were performed using a Jasco PU-2080Plus intelligent pump with a column oven (Jasco 860-CO), Jasco Browin NT, HSS-2000 data processor, and Jasco CD-2095Plus CD chiral detector (Hg−Xe lamp), simultaneously monitoring the CD and UV signals at one specific wavelength (range 220−420 nm).

LC-NMR Method. LC-NMR data were acquired using a Varian UNITY-INOVA-500 spectrometer (^{1}H: 499.83 MHz) equipped with a 60 μL triple-resonance microflow NMR probe. 1D ^{1}H NMR spectra were obtained in stopped-flow mode as described in the previous paper.[30]

LC-^{1}H NMR: isoboldine δ 2.74 (1H, t, J = 13.5 Hz, H-7), 2.94 (1H, m, H-4), 2.99 (3H, s, NCH_3), 3.18 (1H, m, H-7), 3.22 (1H, m, H-4), 3.36 (1H, m, H-5), 3.65 (1H, m, H-5), 3.82 (3H, s, OCH_3-10), 3.85 (3H, s, OCH_3-2), 4.12 (1H, m, H-6a), 6.77 (1H, s, H-3), 6.83 (1H, s, H-8), 8.02 (1H, s, H-11); sinoacutine δ 1.95 (overlap with H_2O,

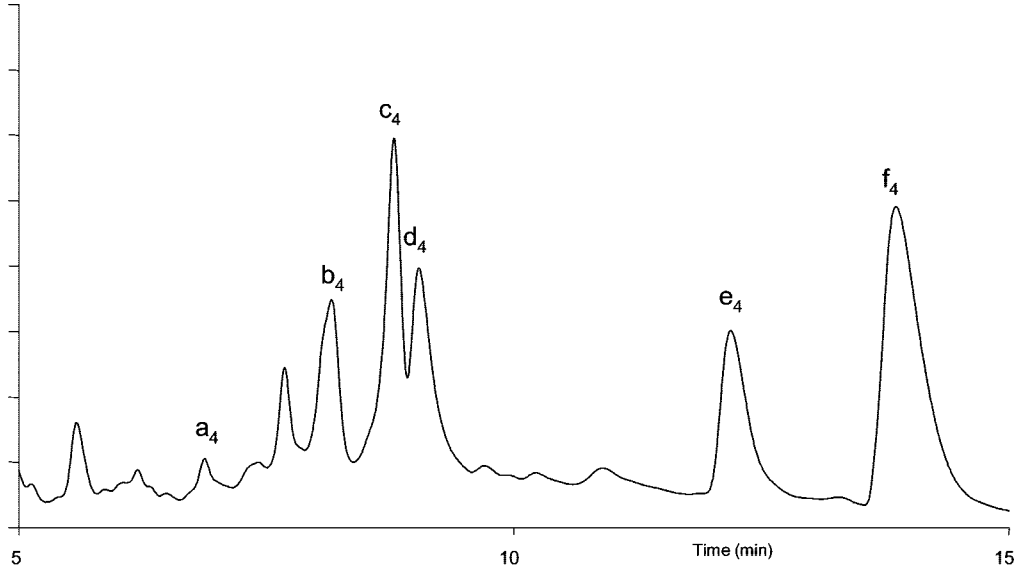


Figure 14. LC data of the alkaloid fraction (Callus Fr. C) obtain from *N. domestica*. Column: Cosmosil $5C_{18}$-AR-II (4.6 × 150 mm). Gradient: A: 0.1 M NH_4OAc/D_2O (0.05% TFA), B: MeCN (0.05% TFA).A/B: initial 80/20, 5 min 70/30, 15 min 70/30, 25 min 0/100.Flow rate: 1.0 mL/min.UV detector: 280 nm.

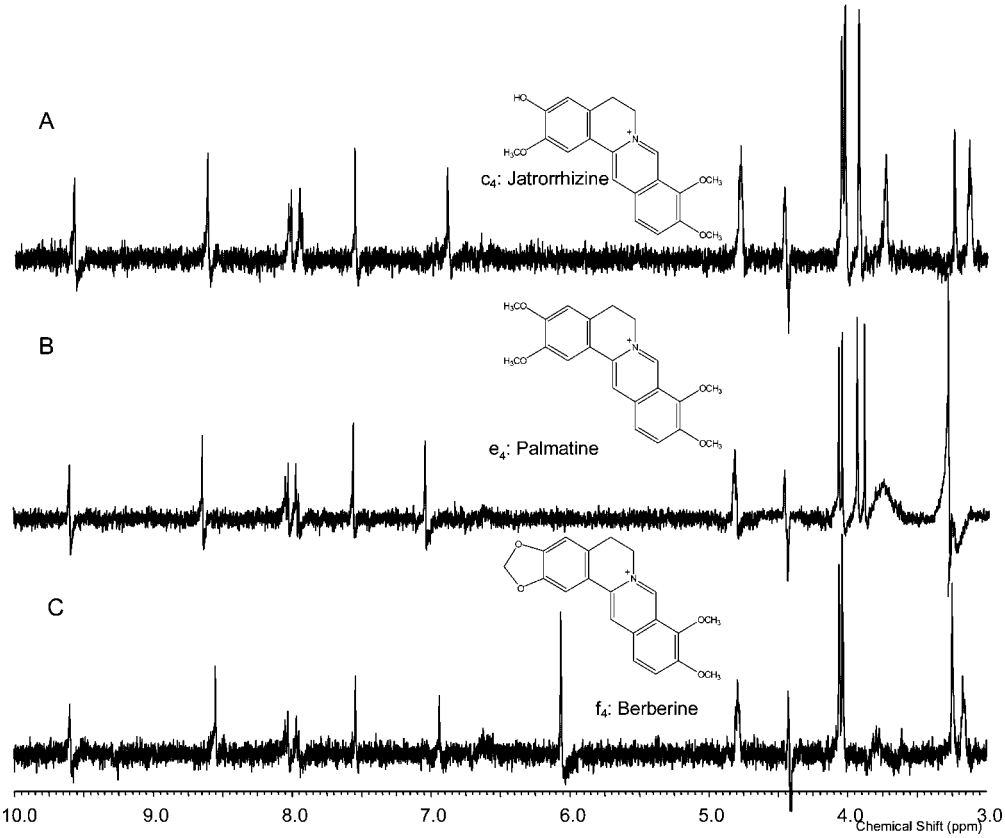


Figure 15. Stopped-flow LC-^{1}H NMR spectra of protoberberine-type alkaloids in the alkaloid fraction (Callus Fr. C) obtained from *N. domestica*. (A) ^{1}H NMR spectrum of peak c_4 (jatrorrhizine). (B) ^{1}H NMR spectrum of peak e_4 (palmatine). (C) ^{1}H NMR spectrum of peak f_4 (berberine).

H-15), 2.49 (1H, m, H-15), 2.70 (3H, s, NCH_3), 2.81 (1H, m, H-16), 3.07 (1H, m, H-16), 3.29 (1H, dd, J = 19.0, 5.5 Hz, H-10), 3.42 (1H, d, J = 19.0 Hz, H-10), 3.67 (3H, s, OCH_3-6), 3.77 (3H, s, OCH_3-3), 4.26 (1H, d, J = 5.5 Hz, H-9), 6.47 (1H, s, H-8), 6.72 (1H, d, J = 8.0 Hz, H-1), 6.90 (1H, d, J = 8.0 Hz, H-2), 7.78 (1H, s, H-5); domesticine δ 2.73 (1H, *t*, J = 14.0 Hz, H-4), 2.91 (1H, m, H-7), 2.97 (3H, s, NCH_3), 3.16 (1H, m, H-7), 3.20 (1H, m, H-4), 3.33 (1H, m, H-5), 3.62 (1H, m, H-5), 3.83 (3H, s, OCH_3), 4.09 (1H, s, H-6a), 5.94 (2H, s, OCH_2O), 6.76 (1H, s, H-3), 6.86 (1H, s, H-8), 7.87 (1H, s, H-11); nantenine δ 2.66 (1H, t, J = 14.0 Hz, H-4), 2.87 (3H, s, NCH_3), 2.89 (1H, m, H-7), 3.13 (1H, m, H-7), 3.16 (1H, m, H-4), 3.18 (1H, m, H-5), 3.50 (1H, br s, H-5), 3.58 (3H, s, OCH_3-1), 3.81 (3H, s, OCH_3-2), 5.96 (2H, s, OCH_2O), 6.81 (1H, s, H-3), 6.86 (1H, s, H-8), 7.77 (1H, s, H-11); menispermine δ 2.83 (1H, t, J = 13.0 Hz, H-7), 2.92 (3H, s, NCH_3), 3.06 (1H, m, H-4), 3.25 (1H, *m*, H-7), 3.30 (3H, s, NCH_3), 3.34 (1H, m, H-4), 3.59 (1H, m, H-5), 3.62 (3H, s, OCH_3-1), 3.71 (1H, m, H-5), 3.83 (3H, *s*, OCH_3-10), 3.88 (3H, s, OCH_3-2), 4.27 (1H, d, J = 13.0 Hz, H-6a), 7.01 (1H, d, J = 8.5 Hz, H-8), 7.02 (1H, s, H-3), 7.04 (1H, d, J = 8.5 Hz, H-9); *O*-methylpallidine δ 1.95 (overlap with H_2O, H-15), 2.15 (1H, m, H-15), 2.74 (3H, s, NCH_3),

2.88 (1H, m, H-16), 3.06 (1H, m, H-16), 3.33 (1H, dd, $J = 19.0, 5.5$ Hz, H-10), 3.45 (1H, d, $J = 19.0$ Hz, H-10), 3.75 (3H, s, OCH_3-6), 3.76 (3H, s, OCH_3-2), 3.80 (3H, s, OCH_3-3), 4.31 (1H, d, $J = 5.5$ Hz, H-9), 6.50 (1H, s, H-8), 6.83 (1H, s, H-1), 6.93 (1H, s, H-5). 7.07 (1H, s, H-4).

HPLC Parameters for LC-NMR, LC-MS, and LC-CD. Chromatographic preparations were performed using a Cosmosil 5 C_{18}-AR (4.6 i.d. × 150 mm) reversed-phase column. The mobile phases [(A) 0.1 M NH_4OAc (0.05% TFA, D_2O for LC-NMR) and (B) CH_3CN (0.05% TFA) or CH_3OH (0.05% TFA)] were used by nonlinear gradient elution. Chiral analytical separation was carried out on a chiral OJ-RH column (4.6 i.d. × 150 mm, Daicel Chemical Ltd.) at 40 °C for LC-CD.

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NP8001496