Characterization of Isoquinoline Alkaloids from *Neolitsea sericea* var. *aurata* by HPLC-SPE-NMR

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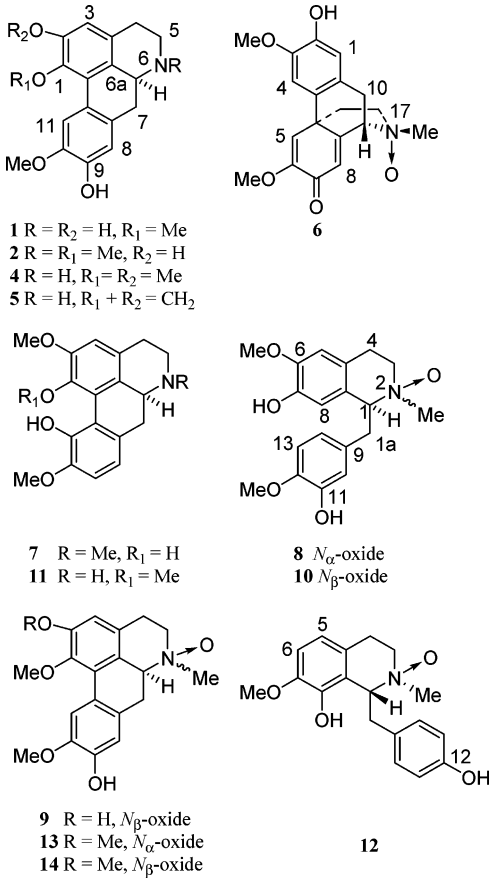
Application of the HPLC-SPE-NMR technique to identify the alkaloids in an EtOH extract of the leaves of *Neolitsea sericea* var. *aurata* led to the characterization of 14 alkaloids while consuming plant material equivalent to 1.1 g. Of these, seven are *N*-oxides, four of which are new, namely, 9*S*,17*S*-pallidine N_α-oxide (**6**), 1*S*,2*S*-reticuline N_α-oxide (**8**), 6*R*,6a*S*-boldine N_β-oxide (**9**), and 6*S*,6a*S*-*N*-methyllaurotetanine N_α-oxide (**13**). Their structures were also confirmed by partial synthesis.

Studies have revealed that Lauraceous plants, especially those of the *Cryptocarya*, *Dehaasia*, *Lindera*, *Litsea*, and *Neolitsea* genera, are good sources of isoquinoline alkaloids.[1,2] Despite intensive phytochemical studies on this plant family over the past two decades, more work is still required, including examining alkaloids of higher polarity and nonalkaloidal constituents. Advancements in isolation techniques and instrumentation have greatly facilitated studies of natural products. However, the isolation and characterization of compounds that are very minor, not well-resolved by LC, or unstable during workup are still a considerable challenge. In addition, typically it usually takes a considerable time and kilogram-scale isolation of plant materials to study the chemical constituents of a plant species. Moreover, most of the compounds isolated tend to be already known. Thus, the development of a highly efficient methodology for screening novel or desired compounds is essential to avoid the unnecessary consumption of time, materials, and manpower, and to accelerate research progress. It would also be beneficial if this methodology could address the problems associated with poorly resolvable or minor compounds. The HPLC-SPE (solid-phase extraction)-NMR technique (Figure 1) has been shown to be useful for identifying some natural constituents and drug metabolites.[3−5] This technique offers the following advantages over the more conventional HPLC-NMR: (i) multiple trapping by the SPE cartridge to increase the amount of sample and hence the sensitivity; (ii) drying of the cartridge using an inert gas (N_2) to reduce the effects of solvent/buffer on the NMR data and to prevent the decomposition of trapped compounds that are labile to oxygen; and (iii) simplifying the NMR spectra using a single solvent (*d*-solvent). Recently, we have used this powerful technique to identify lignans present in *Phyllanthus urinaria*.[6] Since we were interested in examining bioactive natural products from Lauraceous plants, we then used this technique to identify alkaloids in the leaves of *Neolitsea sericea* (Blume) Koidz. var. *aurata* Hayata, a plant native to Orchid Island, Taiwan,[7] whose alkaloidal constituents have not been reported previously. We report here the results of this investigation.



Results and Discussion

The total free bases (fraction I) of the EtOH extract of the leaves of *N. sericea* var. *aurata* were obtained in the usual manner. The phenolic portion was further divided into fractions that were soluble in chloroform (fraction II) and water (fraction III). TLC analysis revealed that the secondary amine laurolitsine (**1**) was the major base in fraction I. To identify other alkaloids in this fraction, N-formylation was performed to remove the major **1**, and after workup, two fractions (fractions Ia and Ib) were obtained. *N*-Formyllaurolitsine (**1a**) and *N*-formylactinodaphnine (**5a**) were identified from the fraction (Ia) containing neutral products. The fraction containing the nonreacted alkaloids (Ib) was analyzed by HPLC-SPE-NMR (600 MHz). Before this technique was used, we evaluated the abilities of six kinds of SPE cartridge [HySphere resin GP, C_{18}, C_{18} (HD, high density), C_8, C_8 (EC, end capped), and CN] to trap boldine (**2**), a common Lauraceous alkaloid. The results indicated that the C_8 (EC) cartridge was the best (Table S3, Supporting Information). However, due to the potential instability on the C_8 cartridge under long-term acidic conditions (0.1% trifluoroacetic acid, TFA), the GP-resin cartridge was chosen for

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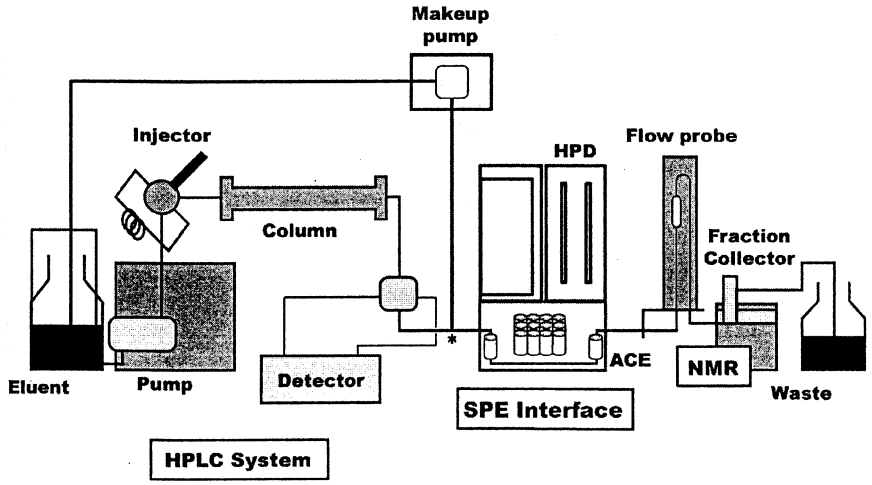


Figure 1. Schematic representation of the HPLC-DAD-SPE-NMR adopted from the website of Bruker Biospin GmbH with permission.

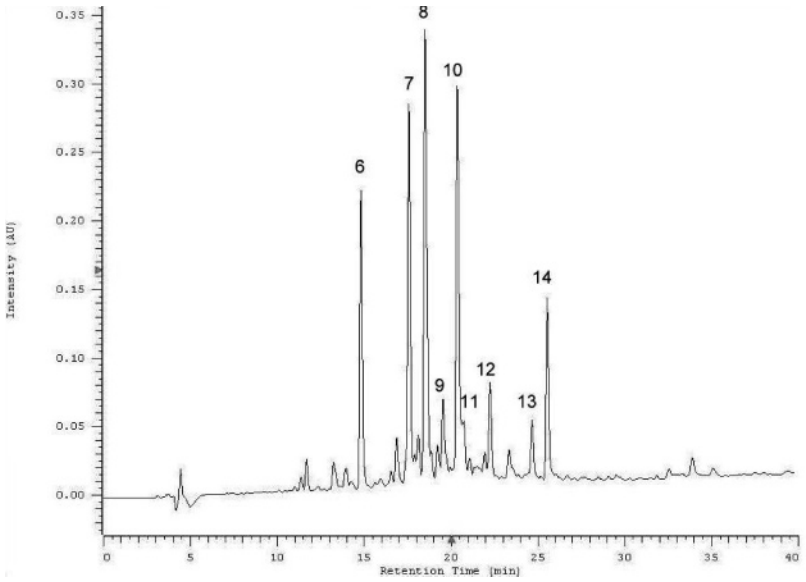


Figure 2. Separation of alkaloids **6**−**14** from fraction III by reversed-phase HPLC, monitored at UV 280 nm (for other HPLC conditions, see Experimental Section).

further work. The delivery conditions for HPLC to provide baseline separation for fraction Ib were found to be 0.1% TFA containing MeCN−H_2O with gradient elution (5:95 to 60:40) over 30 min. One injection of 112.5 μg of fraction Ib in 10 μL of MeOH was applied, and each pure alkaloid separated from an analytical C_{18} column (Figure S1, Supporting Information) was trapped with a GP-resin cartridge. Analysis of the on-line ^{1}H NMR spectra (Figure S2, Supporting Information) led to the identification of five known alkaloids, i.e., laurolitsine (**1**), boldine (**2**), reticuline (**3**), laurotetanine (**4**), and actinodaphnine (**5**).[8] Under such HPLC conditions, the alkaloids were separated as the TFA salts, whose ^{1}H NMR spectra revealed the downfield-shifted signals for protons vicinal to the protonated nitrogen (see Supporting Information). For instance, the signal of H-6a appeared at ca. 4.23 ppm for the TFA salt of the three secondary aporphines (**1**, **4**, and **5**) and at ca. 4.00 ppm for the TFA salt of the tertiary aporphine boldine (**2**), whereas it appears at ca. 3.00 ppm for the corresponding free bases.

HPLC analysis indicated great similarity of the alkaloids present in fractions Ib and II. Fraction III contained polar phenolic alkaloids, which were baseline separated by another LC delivery system: 0.1% TFA containing MeCN−H_2O with gradient elution (10:90 to 50:50 over 40 min). Application of these HPLC conditions, coupled with the SPE-NMR technique, led to the characterization of nine alkaloids (**6**−**14**) from fraction III (Figures 2 and 3). Alkaloids **7** and **11** were identified as (+)-corytuberine[8] and (+)-norisocorydine,[9] respectively, by comparison of their physical data (CD, MS, and NMR) with those reported in the literature. Alkaloids **6**, **8**−**10**, and **12**−**14** were identified as the corresponding *N*-oxides of pallidine (**6**), reticuline (**8** and **10**), boldine (**9**), juziphine (**12**), and *N*-methyllaurotetanine (**13** and **14**), based on ^{1}H NMR analysis, to be discussed below, and MS data, each showing a molecular ion that was 16 amu larger than the corresponding free base.

Alkaloids **10** and **14** were identified as the known (1*S*,*R*)-reticuline N_β-oxide[10]and (6*R*,6a*S*)-*N*-methyllaurotetanine N_β-oxide,[11] respectively. Accordingly, alkaloid **8**, the corresponding epimer of **10**, as evidenced by the identical MS data and great similarity of the ^{1}H NMR spectra, should be (1*S*,2*S*)-reticuline N_α-oxide. Likewise, alkaloid **13** should be (6*S*,6a*S*)-*N*-methyllaurotetanine N_α-oxide, the epimer of **14** at N^6. From the ^{1}H NMR spectroscopic analysis to be described below, alkaloid **9** as the TFA salt was designated as boldine *N*-oxide since the chemical shift of *N*-Me appeared at δ 3.65, similar to that in N_β-oxide **14**·TFA (δ 3.66) (Table 1). The absolute configuration of these *N*-oxides, i.e., 9*S* for **6**, 1*S* for **8** and **10**, and 6a*S* for **13** and **14**, was elucidated on the basis of the similarity of the shape of their circular dichroism (CD) spectra to those of the corresponding free bases, and the products were prepared from the partial synthesis described in the Experimental Section.

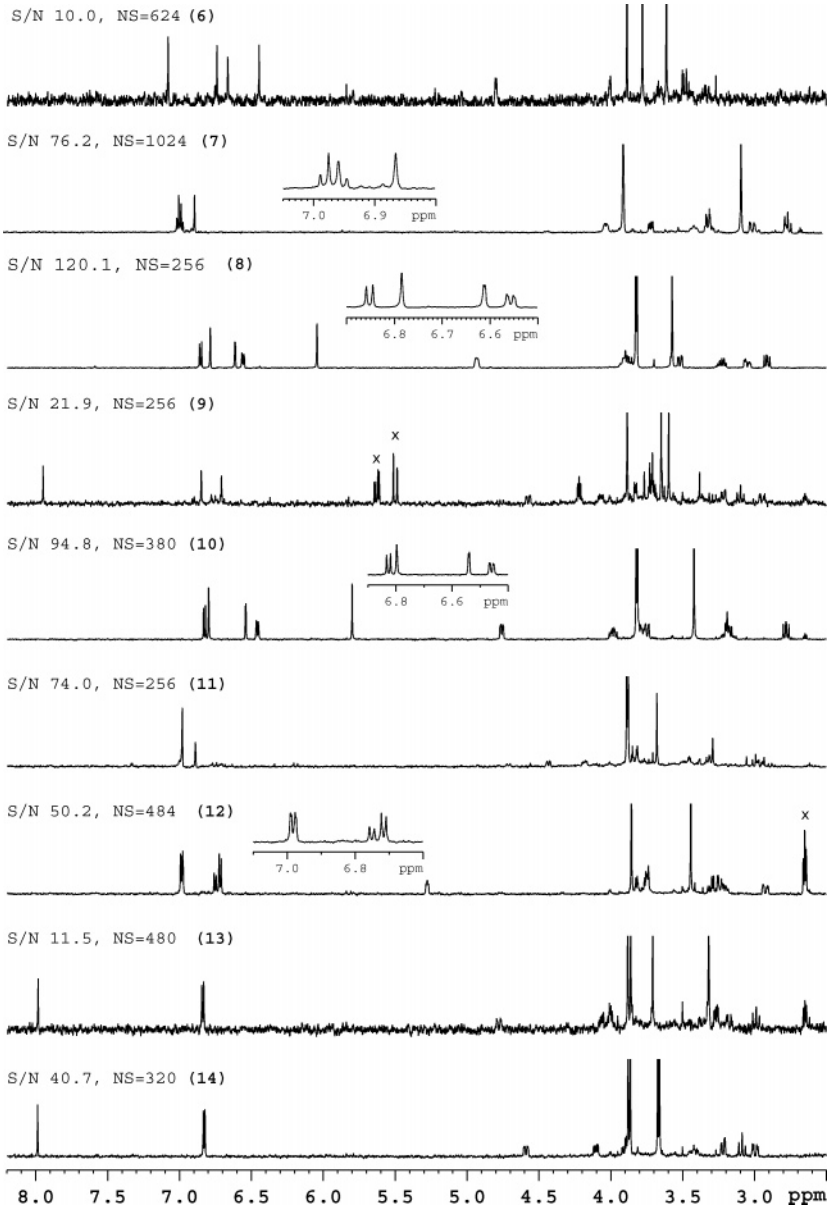


Figure 3. ^{1}H NMR spectra of alkaloids **6**−**14** (CD_3CN), separated from fraction III (HPLC chromatogram, see Figure 1), obtained from on-line HPLC-SPE-NMR (600 MHz).

Alkaloid **12** gave spectroscopic data similar to those reported for (1*R*)-juziphine *N*-oxide.[12] The CD spectrum of **12** showed two negative Cotton effects at 279 and 234 nm, suggesting a 1*R*-configuration.[13] The proton chemical shift of *N*-Me in **12**·TFA (Table S1, Supporting Information) is the same as that in the N_β-oxide **10**·TFA (δ 3.42), but different from that in the N_α-oxide **8**·TFA (δ 3.57) (Table 1), indicating **12** to be (1*R*,2*R*)-juziphine N_β-oxide.

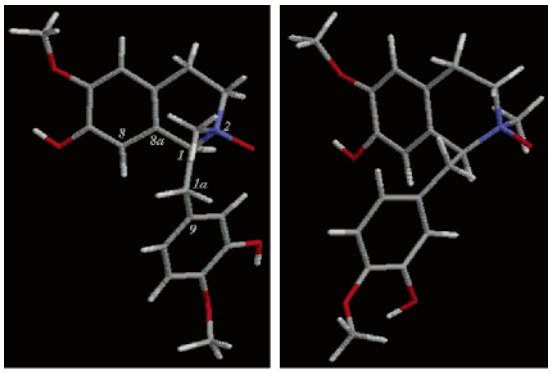
The ^{1}H NMR spectra of the *N*-oxide alkaloids (Figure 3) exhibited similar splitting patterns, but different chemical shifts for protons adjacent to the *N*-oxide moiety compared to those of the corresponding free bases (Figure S2, Supporting Information). For instance, the signals of Me-2 and H-1 of reticuline·TFA (**3**·TFA) (see Supporting Information) appeared at positions that were more upfield than those of (1*S*,2*R*)-reticuline N_β-oxide TFA salt (**10**·TFA): δ_{2-Me} 2.75 vs 3.42, and δ_{H-1} 4.42 vs 4.76 ppm (Table 1). The orientation of the *N*-oxide significantly affects the chemical shifts of the protons adjacent to the *N*-oxide function. The ^{1}H NMR spectra of the aporphine *N*-methyllaurotetanine *N*-oxides as the TFA salts showed the signals of *N*-Me and H-6a at δ 3.32 and 4.78 in the N_α-oxide **13** and at δ 3.66 (*N*-Me) and 4.58 (H-6a) in the N_β-oxide **14** (Table 1). This difference is attributable to an anisotropic effect (upfield-shifted *N*-Me_{ax} in **13**; downfield-shifted *N*-Me_{eq} in **14**) and an inductive effect of N_α-oxide *cis* to H-6a (**13**),[14] of which the latter was also observed for the signal of H-1 (δ 4.94 vs 4.76) in both epimers of the benzylisoquinoline reticuline *N*-oxides (**8**·TFA, N_α-oxide vs **10**·TFA, N_β-oxide). In contrast to the observations in **13**·TFA and **14**·TFA, axial-oriented *N*-Me (δ_{ax} 3.57, **8**·TFA) appeared downfield relative to the equatorial-oriented *N*-Me (δ_{eq} 3.42, **10**·TFA), ascribable to the flexible benzyl moiety, which also influenced the chemical shift of H-8 to some degree (δ 6.04 in **8**·TFA vs δ 5.80 in **10**·TFA) (Table 1).

To confirm the assigned structures, including the stereochemistry, chemical syntheses of five *N*-oxides, i.e., **6**, **8**/**10**, **13**/**14**, were undertaken, starting from the corresponding free bases. The preparations typically included three reaction steps, i.e., O-acetylation to protect the phenolic groups, *m*-CPBA oxidation to produce *N*-oxides, and O-deacetylation under mild alkaline conditions (NH_4OH−MeOH). This process yielded two epimers composed of the N_α- and N_β-oxides, which were separated by reversed-phase HPLC (C_{18} column) and were distinguished by NOESY or NOED experiments. For instance, in the aporphine series, while irradiation of the Me-6 singlet enhanced the signals of H-6a in the N_β-oxide epimer **14** (6*R*), this enhancement was not observed for

Table 1. ^{1}H NMR Data (δ_H) of 9*S*,17*S*-Pallidine N_α-oxide (**6**), Reticuline *N*-oxides, **8** (N_α) and **10** (N_β), Boldine N_β-oxide (**9**), and *N*-Methyllaurotetanine *N*-oxides, **13** (N_α) and **14** (N_β), (δ/ ppm)

position	**6**·TFA[a,d]	**6**[b]	**8**·TFA[a,d]	**8**[c]	**10**·TFA[a,d]	**10**[c]	**9**·TFA[a,d]	**13**·TFA[a,d]	**14**·TFA[a,d]	**14**[b]
H-1	6.66 s	6.62 s	4.94 dd	5.08 dd	4.76 brd	4.82 brd				
H-3							6.71 s	6.83 s	6.82 s	6.77 s
H-4	7.08 s	7.03 s								
H-5	6.74 s	6.69 s	6.78 s	6.77 s	6.80 s	6.79 s				
H-6a							4.57 dd	4.78 dd	4.58 dd	4.39 dd
H-8	6.45 s	6.29 s	6.04 s	6.05 s	5.80 s	5.75 s	6.85 s	6.84 s	6.83 s	6.82 s
H-9	4.80 d	4.33 s								
H-10			6.61 d	6.63 d	6.54 d	6.54 d				
H-11							7.95 s	7.98 s	7.98 s	7.96 s
H-13			6.85 d	6.85 d	6.83 d	6.82 d				
H-14			6.56 brd	6.56 brd	6.46 dd	6.48 brd				
OMe-1							3.59 s	3.71 s	3.67 s	3.63 s
OMe-2								3.88 s	3.87 s	3.85 s
OMe-3	3.89 s	3.87 s								
OMe-6	3.78 s	3.76 s	3.83 s	3.82 s	3.82 s	3.82 s				
OMe-10							3.88 s	3.86 s	3.86 s	3.83 s
OMe-12			3.82 s	3.81 s	3.81 s	3.81 s				
N-Me	3.61 s	3.32 s	3.57 s	3.59 s	3.42 s	3.46 s	3.65 s	3.32 s	3.66 s	3.52 s

[a] Data obtained from the analysis of on-line HPLC-SPE-NMR (MeCN-d_3, 600 MHz). [b]Data obtained from the analysis of off-line NMR measurement (MeCN-d_3, 400 MHz). [c] Data obtained from the analysis of off-line NMR measurement (CD_3OD, 400 MHz). [d]TFA: trifluoroacetic acid



Conformer A (**8**) Conformer B (**10**)

Figure 4. Conformer A for **8** with the lowest energy of 1213.5 kcal/mol and conformer B for **10** with the lowest energy of 1206.2 kcal/mol, obtained from an MD simulation (MeOH).

the N_α-oxide epimer **13** (6*S*). Similarly, in the benzylisoquinoline series, irradiation of the Me-2 singlet enhanced the signals of H-1 in the N_β-oxide epimer **10** (2*R*), but not in the N_α-oxide epimer **8** (2*S*). For the morphinan pallidine *N*-oxide (**6**), the NOESY spectrum showed the NOE relationships of *N*-Me to H-10α, H-16ax, and H-16eq (see Supporting Information), confirming *N*-Me to be oriented equatorially and *N*-oxide to be α-oriented.

The difference in chemical shift for H-8 in compounds **8** and **10**, caused by the orientation of the *N*-oxide, could be explained by a conformational analysis using a molecular dynamics simulation.[15] Conformer A for alkaloid **8** (Figure 4), which had the lowest energy of 1213.5 kcal/mol at 827 ps, showed the β-benzylic carbon (C_{1a}) and Me-2β to be pseudoequatorially and pseudoaxially oriented, respectively, with a dihedral angle of 62.9°, suggesting a *gauche* conformation for this part of the structure. Conformer B for compound **10** (Figure 4), which had the lowest energy of 1206.2 kcal/mol at 943 ps, showed that both β-benzylic carbon (C_{1a}) and the Me-2α are pseudoaxially oriented, with a dihedral angle of 170.0°, suggesting an *anti* conformation for this part of the structure. In addition, the dihedral angles formed by C-8a, C-1, C-1a, and C-9 in conformers A and B are 88.6° and 67.6°, respectively, indicating that conformer B is folded. The distance between adjacent protons in both conformers was measured (Table S2, Supporting Information), and the data integrated well with NOESY analysis (Figure 4). For instance, H-8 of alkaloid **8** showed NOE correlations to H_α-1a (3.685 Å), H_β-1a (2.369 Å), and H-1 (3.227 Å) in the NOESY spectrum. However, H-8 of alkaloid **10** showed NOE correlations only to H-1 (2.432 Å) and H_β-1a (3.431 Å) but not to H_α-1a (4.210 Å). In addition, the distance from H_β-3 to H_β-1a is 5.2189 and 2.276 Å in conformers A and B, respectively, and accordingly, a clear contour of these two signals was observed in the NOESY spectrum of **10** but not in that of **8**. The signal of H-8 in **10** appears relatively upfield compared to that in **8** (δ 5.75 vs 6.05), which is attributable to the larger shielding by the lower ring in **10**, and can be explained by the greater distance between H-8 and the lower ring in conformer A (**8**) compared to that in conformer B (**10**).

The elution order of these alkaloids was closely related to the charge/mass ratio for each molecule of similar structure under acidic RP-HPLC (C_{18}) conditions; that is, for the protonated salt, smaller molecules were eluted earlier than larger molecules, e.g., **1** > **2**. In the case of isomeric pairs, tertiary amines were eluted earlier than secondary amines, e.g., **2** > **4**; **7** > **11**. As with lignans,[6] if two compounds have an identical skeleton and differ only in two substitutions, i.e., a methylenedioxy group versus two methoxyl groups, the compound that contains the latter was eluted earlier, e.g., **4** > **5**. For the *N*-oxides, the α-*N*-oxide was eluted earlier than the corresponding β-*N*-oxide, e.g., **13** > **14**; **8** > **10**.

The presence of 0.1% TFA was found to increase the resolving power. We clarified whether the addition of trifluoroacetic acid to the HPLC delivery system will cause artifacts by direct ESIMS measurement of fraction III, dissolved in methanol. This mass spectrum (Figure S3, Supporting Information) showed the quasi-molecular ions, $[M + H]^+$, of alkaloids **6**−**14**, similar to those separated by the RP-HPLC conditions indicated above. This result demonstrated that these identified compounds are not artifacts produced by 0.1% TFA.

The present study has demonstrated that, in an analysis using the HPLC-SPE-NMR technique, 112.5 μg of a pretreated fraction (III), which was theoretically equivalent to ca. 1.10 g of dried leaves of *N. sericea* var. *aurata*, led to the identification of nine alkaloids (**6**−**14**). Seven of these, i.e., **6**, **8**−**10**, and **12**−**14**, contain *N*-oxide functions, which are relatively minor and highly polar and hence might be missed by conventional analysis. Four of these seven, i.e., 9*S*,17*S*-pallidine N_α-oxide (**6**), 1*S*,2*S*-reticuline N_α-oxide (**8**), 6*R*,6a*S*-boldine N_β-oxide (**9**), and 6*S*,6a*S*-*N*-methyllaurotetanine N_α-oxide (**13**), have not been reported previously. Additionally, the three known *N*-oxides, i.e., 1*S*,2*R*-reticuline N_β-oxide (**10**), 1*R*,2*R*-juziphine N_β-oxide (**12**), and 6*S*,6a*R*-*N*-methyllaurotetanine N_β-oxide (**14**), were found for the first time in plants of the *Neolitsea* genus. The ^{1}H NMR data (CD_3CN) of these separated alkaloids (as the TFA salts) obtained from on-line HPLC-SPE-NMR experiments are also useful for building a databank to facilitate the identification of alkaloids separated by this technique.

Experimental Section

General Experimental Procedures. The physical data of the alkaloids were obtained using the following instruments: CD (MeOH): JASCO J-710 spectropolarimeter. Off-line NMR spectra: Bruker Avance 400. HPLC-DAD (diode array detector)-SPE-NMR (600 MHz) carried out using an Agilent 1100 liquid chromatograph (Waldbronn, Germany) equipped with a photodiode array detector (Bruker DAD, Bruker, Rheinstetten, Germany), followed by a Prospekt 2 automated solid-phase extraction unit (Spark Holland, Emmen, Holland), containing 96 HySphere resin GP cartridges (10 × 2 mm, 10−12 μm), which was connected to a 30 μL inverse NMR probe equipped in a Bruker Avance 600 NMR spectrometer. ESIMS: Finnigan Mat TSQ 7000 and Bruker Daltonics Esquire 2000. (+)-*N*-Methyllaurotetanine, (−)-pallidine, and (+)-reticuline used for partial synthesis were previously isolated by our group.[8]

Plant Material. Leaves of *Neolitsea sericea* (Blume) Koidz. var. *aurata* were collected in July 1992 on Orchid Island, Taiwan, and were authenticated by Professor Ih-Sheng Chen, School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan. A voucher specimen (PHNTU920701) was stored at the herbarium library of the School of Pharmacy, National Taiwan University.

Extraction and Isolation. Powdered, dry leaves (6.80 kg) were percolated at room temperature with 95% EtOH (20 L × 5) to give, upon concentration under reduced pressure, an EtOH extract (1.33 kg). The EtOH extract was triturated with 0.1 N HCl (1.0 L × 3), and the acidic solution was extracted with $CHCl_3$ (0.7 L × 7) to remove neutral constituents (13.71 g), then adjusted to pH 9 with NH_4OH, and filtered to give a residue (42 g), in which **1** was found to be a major constituent. The filtrate was extracted with $CHCl_3$ (1.2 L × 3) to give the total bases (11.05 g, fraction I). The aqueous layer, after being adjusted to pH 3.0 with concentrated HCl in an ice bath, was passed through an Amberlite XAD-2 column, eluted with H_2O and MeOH, to give the MeOH eluate (222 g).

A small portion of the total bases (100 mg, fraction I) was reacted with ethyl formate (0.3 mL) in DMF (2 mL) at 90 °C under nitrogen for 1 day.[16] After cooling, the reaction mixture was partitioned between 1 N HCl (20 mL) and CH_2Cl_2 (20 mL × 3) to give a fraction that was soluble in CH_2Cl_2 (fraction Ia, 36.5 mg). The aqueous layer was adjusted to pH 9 with aqueous NH_4OH and then extracted with CH_2Cl_2 to give the tertiary base major fraction (fraction Ib, 17 mg).

A portion of the total bases (1.00 g) was partitioned between ethyl ether (50 mL × 3) and 1 N aqueous NaOH (50 mL) to give the nonphenolic alkaloids (138 mg). The aqueous layer was adjusted to pH 9 with NH_4Cl, then extracted with $CHCl_3$ (50 mL × 3) to give the phenolic bases (fraction II, 610 mg). The aqueous layer was passed through an Amberlite XAD-2 column and eluted with H_2O and MeOH. The MeOH eluate was further passed through a Sephadex LH-20 column (MeOH−$CHCl_3$, 1:1) to give an alkaloid-containing fraction (fraction III, 64 mg).

HPLC Conditions Used in HPLC-SPE-NMR (600 MHz) and Separation of Alkaloids 1−14. Delivery system: Eluent A acetonitrile containing 0.1% TFA-*d*, eluent B H_2O containing 0.1% TFA-*d*, eluent A/eluent B 5:95 to 60:40 within 30 min for fraction Ib and 10:90 to 50:50 within 40 min[17] for fraction III, both linear gradient elution; flow rate: 0.8 mL/min; HPLC column: C_{18} column (for fraction Ib, a Merck LiChroCART column, 250 mm × 4.6 mm, 5 μm; for fraction III, a Phenomenex Prodigy ODS3 100A column, 250 × 4.6 mm, 5 μm); amount injected: fraction Ib 183.3 μg/10 μL (MeOH), fraction III 112.5 μg/10 μL (MeOH); detection at 280 nm.

Fraction Ib was separated using the HPLC conditions described above to give alkaloids **1**−**5** with retention times of 13.20 (**1**), 13.65 (**2**), 14.40 (**3**), 17.00 (**4**), and 17.28 min (**5**) (Figure S1, Supporting Information). Fraction III gave alkaloids **6**−**14** with retention times of 12.73 (**6**), 15.35 (**7**), 16.03 (**8**), 17.16 (**9**), 17.97 (**10**), 18.27 (**11**), 19.32 (**12**), 22.55 (**13**), and 23.50 min (**14**) (Figure 2).

Comparison of Trapping Ability of Six Different SPE Cartridges to Boldine. The HPLC conditions, including column, eluents A and B, and detection, used in this study were the same as those used for analysis of fraction Ib. The delivery system was eluent A/B 1:4 and flow rate was 0.3 mL/min, and the makeup (H_2O) flow rate was 1 mL/min. Boldine (25 μg/5 μL, MeOH) was injected into the column, and each sample eluate was trapped by a SPE cartridge including CN, C_8, C_8 (EC) (8 μm), C_{18}, C_{18} (HD) (7 μm), and, resin GP (10−12 μm). Each loaded cartridge was then washed by acetonitrile (2 mL). To the washing was added acetonitrile to a final volume of 5 mL for UV quantitative analysis, measured at λ_{max} 281 nm.

SPE-NMR Procedure. A makeup flow of pure water with a flow rate of 2.4 mL/min and 2.0 mL/min for fractions I and II, respectively, was added to the postcolumn eluent, and the mixed sample volume of each peak was passed through a HySphere resin GP cartridge (10 × 2 mm). After the cartridges were dried by flushing with dry nitrogen, the compound trapped in each cartridge was eluted with deuterated acetonitrile into a 30 μL inverse NMR probe. The 1D ^1H NMR spectrum of each separated compound was recorded using a multiple solvent suppression pulse program for residual protons and water signals in the *d*-solvent. Shaped low-power rf pulse and CW decoupling on the F2 channel for the decoupling of the ^{13}C satellites were utilized. All spectra were measured at 300 K, and the ^1H chemical shift was referenced to a residual signal of CD_2HCN at δ 1.93. For each measurement 256−1024 scans were accumulated into 16k data points with a sweep width of 12 000 Hz. 2D NMR spectra were recorded by using standard pulse programs (COSY and NOESY), and the correlation maps consisted of 2048 × 256 data points per spectrum.

ESIMS and CD Procedure. Alkaloids **6**−**14**, corresponding to peaks 6−14 in Figure 2, were obtained by another HPLC run using the same conditions as in HPLC-SPE-NMR. These alkaloids were analyzed by ESIMS (Finnigan MAT TSQ 7000) and CD.

Effect of TFA on the Production of Artifacts. Fraction III at a concentration of 1 mg/10 mL (MeOH) was analyzed directly by ESIMS (Bruker Daltonics Esquire 2000) without HPLC separation, and the result is shown in Figure S3, Supporting Information.

9*S*,17*S*-Pallidine N_α-oxide (6): t_R 14.83 min (Figure 2); CD (MeOH) (Cotton effect, CE) 279 (−), 264 (−), 252 (−), 235 (−), 219 (+); ^1H NMR data, see Table 1; ESIMS *m*/*z* 344 $[M + H]^+$ (calcd for $C_{19}H_{21}NO_5$ + H).

1*S*,2*S*-Reticuline N_α-oxide (8): t_R 18.51 min (Figure 2); CD (MeOH) (CE) 300 (+), 291 (+), 236 (+), 227 (+); ^1H NMR data, see Table 1; ESIMS *m*/*z* 346 $[M + H]^+$ (calcd for $C_{19}H_{23}NO_5$ + H).

6*R*,6a*S*-Boldine N_β-oxide (9): t_R 19.55 min (Figure 2); ^1H NMR data, see Table 1; ESIMS *m*/*z* 344 $[M + H]^+$ (calcd for $C_{19}H_{21}NO_5$ + H).

1*S*,2*R*-Reticuline N_β-oxide (10): t_R 20.35 min (Figure 2); CD (MeOH) (CE) 291 (+), 237 (+), 226 (+); ^1H NMR data, see Table 1; ESIMS *m*/*z* 346 $[M + H]^+$ (calcd for $C_{19}H_{23}NO_5$ + H).

1*R*,2*R*-Juziphine N_β-oxide (12): t_R 22.24 min (Figure 2); CD (MeOH) (CE) 279 (−), 234 (−), 224 (−); ^1H NMR data, see Table S1, Supporting Information; ESIMS *m*/*z* 316 $[M + H]^+$ (calcd for $C_{18}H_{21}NO_4$ + H).

6*S*,6a*S*-*N*-Methyllaurotetanine N_α-oxide (13): t_R 24.67 min (Figure 2); CD (MeOH) (CE) 317 (−), 300 (−), 281 (−), 246 (+), 221 (−); ^1H NMR data, see Table 1; ESIMS *m*/*z* 358 $[M + H]^+$ (calcd for $C_{20}H_{23}NO_5$ + H).

6*R*,6a*S*-*N*-Methyllaurotetanine N_β-oxide (14): t_R 25.55 min (Figure 2); CD (MeOH) (CE) 316 (−), 291 (−), 280 (−1.35), 244 (+15.17), 220 (−8.07); ^1H NMR data, see Table 1; ESIMS *m*/*z* 358 $[M + H]^+$ (calcd for $C_{20}H_{23}NO_5$ + H).

Preparation of 6*S*,6a*S*- and 6*R*,6a*S*-*N*-Methyllaurotetanine *N*-Oxides (13 and 14), 1*S*,2*S*- and 1*S*,2*R*-Reticuline *N*-Oxide (8 and 10), and 9*S*,17*S*-Pallidine *N*-Oxide (6). (+)-*N*-Methyllaurotetanine (7.0 mg, 20.5 μmoL) was reacted with Ac_2O−py (2:1, 0.3 mL) in a sealed tube for 21 h at room temperature. Absolute EtOH (5 mL) was then added, and the solution was stirred for 2 h. Evaporation of the reaction mixture gave a residue that was partitioned between 2.5% aqueous NH_4OH (5 mL) and CH_2Cl_2 (5 mL × 2). The combined CH_2Cl_2 layers were dried (Na_2SO_4) and evaporated. To the residue dissolved in CH_2Cl_2 (2 mL) was added *m*-CPBA (2.7 mg, 16 μmoL), and the solution was stirred for 1 h.[11] $NaHCO_3$ powder (ca. 10 mg) was then added, and the mixture was filtered through cotton wool. The filtrate was evaporated to dryness. The residue was dissolved in MeOH (3 mL) and O-deacetylated by reaction with 25% aqueous NH_4OH (1 mL) at room temperature for 3 h. Evaporation of the reaction mixture gave a residue that was further purified via a Sephadex LH-20 column (MeOH) to give a mixture of **13** and **14** (4.1 mg, overall yield 55.9%) in a ratio of 10:1, based on HPLC analysis using the conditions for fraction III. Alkaloid **6** was obtained as the major product from pallidine (**6a**)[8] by the same procedure, in a yield of 40%. Alkaloids **8** and **10** were obtained in an approximately 1:1 ratio (1.6 mg and 1.3 mg, 67%) using reticuline

as a starting material following an approach similar to that for the preparation of **13** and **14**.

Molecular Dynamics Procedures. The software packages Linux, Amber 8, RasMol Version 2.7.1, and Gaussian were used. The NPT ensemble was applied to the molecular dynamics simulation. The temperature was set at 300 K and two alkaloids, 1*S*,2*S*-reticuline N_α-oxide (**8**) and 1*S*,2*R*-reticuline N_β-oxide (**10**), were simulated. The first step was a Gaussian single-point (SP) calculation, followed by solvation of the molecule in methanol and energy minimization. Next, an initial velocity was given to the molecule at low temperature and the system was heated slowly to 300 K. The time step was set to 2 fs, and many parameters, as well as coordinates, were recorded every 50 steps, such as temperature, density, and energy, including potential energy and kinetic energy. After the system reached an equilibrium state, the simulation was continued until 1000 ps.[15]

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Supporting Information Available: ^{1}H NMR data of the known alkaloids as the TFA salts (CD_3CN, 600 MHz), distance between selected atoms in conformers A (**8**) and B (**10**) obtained from MD simulation, trapping abilities of different cartridges to boldine, separation and ^{1}H NMR spectra of alkaloids **1**−**5** from fraciton Ib by HPLC-SPE-NMR, and ESIMS spectrum of fraction III are available free of charge via the Internet at http://pubs.acs.org.

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