Litebamine *N*-Homologues: Preparation and Anti-Acetylcholinesterase Activity

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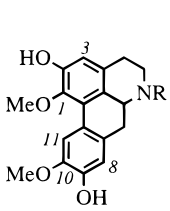
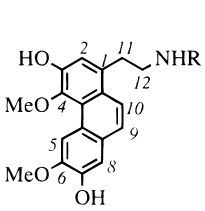
Litebamine *N*-homologues were easily prepared from laurolitsine, generally via three reaction steps (*N*-alkylation, solvolysis with 1 M NH_4OAc under reflux, and the Mannich reaction) in more than 80% overall yield. Among the prepared compounds, *N*-propyl-, *N*-isobutyl-, and *N*-isopropylnorlitebamines exhibited moderate antiacetylcholinesterase activity (IC_{50} ca. 7.0 μM), while the corresponding *N*-metho salt of *N*-propylnorlitebamine showed potent activity (IC_{50} 2.70 μM).

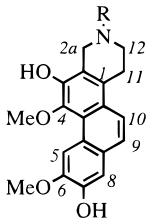
Litebamine (**11**) is a novel phenanthrene alkaloid isolated from the wood of *Litsea cubeba*.[1] It possesses antiplatelet aggregation activity through the inhibition of thromboxane B_2 formation induced by arachidonic acid in washed rabbit platelets (15 μM, 77% inhibition).[2] Recently, it was demonstrated to possess activity against acetylcholinesterase (AChE), with an IC_{50} value of 22.0 μM.[3] The latter biological activity stimulated our interest to study the potential of this type of isoquinoline alkaloid as an anti-AChE agent. The following describes our effort in the preparation of litebamine *N*-homologues (**13**−**19**) and their inhibitory effect on AChE.



1. R=Me
2. R=H
3. R=Et
4. R=^{n}Pr
5. R=^{n}Bu
6. R=^{i}Bu
7. R=allyl
8. R=Bn
9. R=^{i}Pr
10. R=Ac



1a. R=Me
2a. R=H
3a. R=Et
4a. R=^{n}Pr
5a. R=^{n}Bu
6a. R=^{i}Bu
7a. R=allyl
8a. R=Bn
9a. R=^{i}Pr
10a R=Ac



11. R=Me
12. R=H
13. R=Et
14. R=^{n}Pr
15. R=^{n}Bu
16.R=^{i}Bu
17. R=allyl
18. R=Bn
19. R=^{i}Pr
20. R=^{n}Pr, Me I^-; N^+

Results and Discussion

A three-step preparation of litebamine (**11**) from boldine (**1**) via a biogenetic approach has been developed in our laboratory.[4] The method for preparing the key intermediate secoboldine (**1a**) was modified recently to a facile one-pot reaction via solvolysis of **1** with 1 M NH_4OAc in EtOH−H_2O under reflux, which afforded **1a** in high yield (>90%).[5] This modification has increased the total yield of litebamine (**11**) up to 80% from boldine (**1**), a significant improvement compared with the 23% obtained after alkylation with iodoacetic acid, followed by a novel rearrangement.[6] Based on these surveys, we used the solvolysis method to prepare the key intermediates, the *N*-alkylsecolaurolitsines (**3a**−**10a**). These alkylsecoaporphines were prepared from laurolitsine (**2**), which is an abundant aporphine alkaloid in *Phoebe formosana* and was readily obtained as crude base (ca. 25% purity) upon alkalization of the acidic extract.[7]

N-Alkyllaurolitsines (**3**−**6**) and *N*-benzyllaurolitsine (**8**) were prepared from crude **2** via direct reaction with the corresponding halide under reflux in alkaline conditions.[8] *N*-Allyllaurolitsine (**7**), however, could be prepared easily only with suitable molar ratio (ca. 1: 0.72) of the reactant to reagent (allyl bromide). Because of the highly reactive property of allyl bromide, exhaustive *N*-allylation and subsequent Hoffman-like degradation reaction can occur if more reagent is added. These two successive reactions gave *N*,*N*-diallylsecolaurolitsine, which is almost TLC-inseparable from the desired product **7**. The diallylseco derivative displays a $[M]^+$ at *m*/*z* 393 and a base peak at *m*/*z* 110, corresponding to fragment ion **A** (Figure 1), with R = allyl and H replaced by another allyl group. The ^{1}H-NMR spectra of **3**−**8** showed *N*-alkyl, *N*-allyl, or *N*-benzyl signals in addition to that of laurolitsine, and their EIMS spectra displayed the common major peak at *m*/*z* 284 (fragment ion **B**, Figure 1), obtained probably from a *retro* Diels−Alder fragmentation process,[9] supporting their structures. Solvolysis of these 2-hydroxyaporphines with 1 M NH_4OAc in EtOH−H_2O (1:1) solution under a reflux condition overnight gave the corresponding *N*-alkylsecolaurolitsines (**3a**−**6a**), *N*-allylsecolaurolitsine, (**7a**) and *N*-benzylsecolaurolitsine (**8a**) in more than 90% yield at a 300-mg level. These phenanthrenes showed characteristic UV absorption maxima at 263.0, 279.6, 304.5, and 318.4 nm; ^{1}H-NMR signals for H-9 and H-10 as an AB system at δ ca. 7.45 and 7.56 (J_{AB} = 9.4 Hz);[10,11] and EIMS spectra displaying the base peak (fragment ion **A**) at *m*/*z* 58 + 14 *n* (*n* = 0 from **3a**; *n* = 1 from **4a**;

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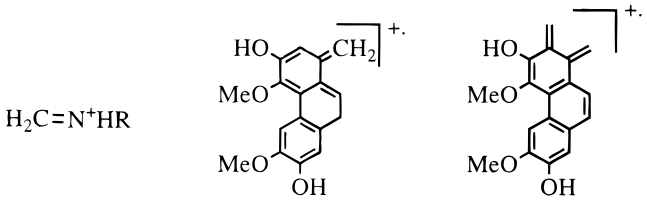


A **B** *m/z* 284 **C** *m/z* 296

Figure 1. Most significant fragment ions in the mass spectra of **1a**−**10a** (ions **A** and **B**) and **11**−**19** (ion **C**).

$n = 2$ from **5a** and **6a**), 70 (from **7a**), and 120 (from **8a**), from a β-cleavage fragmentation.[10]

Mannich reaction of these secondary bases (**3a**−**8a**) with HCHO under acetate buffer conditions (pH 5.76)[4] gave the target products **13**−**18** in near quantitative yield. The ^{1}H-NMR spectra of these products showed the two-proton singlet of H-2a at δ ca. 3.54, and the EIMS spectra showed a common fragment ion (**C**) at *m/z* 296 (Figure 1) from a *retro* Diels−Alder fragmentation process, thus confirming the structures of these products.

N-Isopropyllaurolitsine (**9**) could not be obtained by reacting laurolitsine with isopropyl bromide, probably due to the steric effect of the isopropyl group to C-7 of the aporphine moiety. To overcome this steric problem, the key intermediate for the Mannich reaction, *N*-isopropylsecolaurolitsine (**9a**), $[M]^+$ at *m/z* 355, *N*-^{i}Pr at δ 1.13 (6H, d, $J = 6.3$ Hz) and 2.93 (1 H, septet, $J = 6.3$ Hz), was prepared by reductive *N*-alkylation of secolaurolitsine (**2a**) (Me_2CO−$NaBH_4$) (89.8% yield). Compound **2a**, $[M]^+$ at *m/z* 313, was prepared from *N*-acetylaurolitsine (**10**), which was obtained from *N*-acetylation of **2** with Ac_2O, by acid solvolysis (concd HCl−MeOH = 3:4, reflux, 1 d, 87.0% yield).[12] During the preparation of **2a**, *N*-acetylsecolaurolitsine (**10a**), ^{1}H NMR δ 1.92 (s, 3H, NH*Ac*), was also isolated from a reaction with shorter reaction time (1 h). This suggests that **10a** may be the intermediate of **2a**, and the mechanism of the acid solvolysis could be drawn as shown in Figure 2, suggesting the Hofmann-like ring opening, facilitated by the aid of an acyl group.

Under experimental conditions similar to those used for the preparation of **13**−**18**, **9a** gave **19**. Along with the characteristic ^{1}H-NMR signals of litebamine-like compounds, the EIMS spectrum of **19** showed the same major fragment ion (**C**) at *m/z* 296 as the other litebamine *N*-homologues (Figure 1), and ion **A** at *m/z* 72 confirming the assigned structure.

This practical method not only gives a high yield of *N*-alkylnorlitebamines (>80% in the last two steps) but also simplifies the workup procedures inasmuch as a sole product was obtained in each step. In addition, the purification of the last two-step products, by recrystallization or washing, was very facile.

The anti-AChE activity of these compounds was evaluated by the colorimetric method.[13] The results indicated that the litebamine *N*-homologues (**13**−**19**) were more active than their respective secoaporphine intermediates (**3a**−**6a**, and **9a**) (Table 1). Particularly, those having a bulky group at the nitrogen showed large differences in their inhibitory activity, such as 6.80 μM (**19**) vs 72.02 μM (**9a**). Molecular modeling studies revealed that the distance between N and O^3 (ca. 4.288 Å) in the litebamine *N*-homologues is close to that of the corresponding atoms in acetylcholine of the extended conformation (ca. 3.815 Å).[14] This might explain the higher anti-AChE activity of these compounds. The repulsion between the *N*-substituent and the aromatic nucleus in secoaporphines **3a**−**6a** and **9a**, resulting in an N and O^3 distance unsuitable for binding to the active site of AChE, might account for their low anti-AChE activity. This study also indicated that the C_3 or C_4 *N*-substitution in the *N*-alkylated norlitebamines possessed the optimal anti-AChE activity; however, a planar C_3 *N*-substitution (i.e., allyl group) gave a lower such activity (15.80 μM for **17** vs 7.21 μM for **14**). The quaternary compound **20**, obtained from a treatment of **14** with methyl iodide, was about two and half times more potent than **14**, indicating the increased affinity of the quaternary ammonium to the binding site of AChE. This study discloses a part of the structure−activity relationship of litebamine derivatives toward AChE and will be of value for the development of anti-AChE agents of isoquinoline-type skeleton.

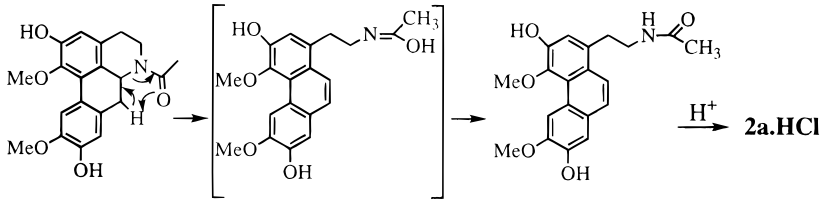
Experimental Section

General Experimental Procedures. The physical data of the prepared compounds were obtained from the following instruments: Fisher-Johns melting point apparatus (uncorrected); Perkin−Elmer 1760-X IR FT spectrometer; Hitachi 150−20 UV; JEOL JMX-HX110 (HREIMS) and Finnigan TSQ-700 (EIMS) mass spectrometers; Bruker AC-80 and AMX-400 spectrometers using solvent peak as reference standard.

Preparation of *N*-Alkyllaurolitsines (3−6) and *N*-Benzyllaurolitsine (8). The preparation of *N*-ethyllaurolitsine (**3**) is given as an example. The mixture of crude laurolitsine (**2**) (6.0 g, ca. 25% purity), DMF (60 mL), K_2CO_3 (2.82 g), and EtI (2.30 mL, 22.4 mmol) was stirred at 50−55 °C for 6 h under nitrogen. The resulting suspension, after removal of the organic solvent under reduced pressure, was diluted with H_2O (300 mL) and adjusted to pH 8.0 with 25% ammonia water. The mixture was partitioned against $CHCl_3$ (200 mL × 3). The combined $CHCl_3$ layers were dried (anhydrous Na_2SO_4) and evaporated. The residue was purified by Si gel chromatography (1−3% MeOH in $CHCl_3$) to give pure **3** (1.21 g): mp 175−177 °C; ^{1}H-NMR ($CDCl_3$) δ 7.86 (1H, s, H-11), 6.81 (1H, s, H-8), 6.61 (1H, s, H-3), 3.89 (3H, s, 10-OMe), 3.56 (3H, s, 1-OMe), 3.06 (2H, m, *N*-C*H*$_2$CH$_3$), 1.11 (3H, t, $J = 7.2$ Hz, *N*-CH$_2$C*H*$_3$); EIMS (70 eV) *m/z* 341 $[M]^+$ (100), 326 (66), 284 (26).

Under the similar experimental conditions, crude **2** (6.20 g) with 1-bromopropane (2.62 mL, 30.5 mmol) yielded **4** (1.45 g), crude **2** (5.10 g) with butyl bromide (2.70 mL, 25.9 mmol) yielded **5** (1.23 g), crude **2** (5.30 g) with isobutyl bromide (2.70 mL, 28.5 mmol) yielded **6** (1.25 g), and crude **2** (2.00 g) with benzyl chloride (0.4 mL, 1.54 mmol) yielded **8** (580 mg).

***N*-Propyllaurolitsine (4):** mp 162−166 °C; ^{1}H-NMR ($CDCl_3$) δ 7.86 (1H, s, H-11), 6.82 (1H, s, H-8), 6.61 (1H, s, H-3), 3.89 (3H, s, 10-OMe), 3.56 (3H, s, 1-OMe), 3.20 (1H, dd, $J = 13.6$, 3.9 Hz, H-7α), 3.12 (1H, ddd, $J = 11.4$, 5.9, 1.5 Hz, H-5β), 3.01 (1H, ddd, $J = 17.0$, 11.4, 5.9 Hz, H-4β), 2.94 (1H, dd, $J = 13.6$, 3.9 Hz, H-6a), 2.87 (1H, ddd, $J = 13.0$, 9.8, 6.4 Hz) and 2.40 (1H, m) (*N*-C*H*$_2$C$_2$H$_5$), 2.62 (1H, br dd, $J = 17.0$, 3.8 Hz, H-4α),



10 **10a**

Figure 2. Proposed mechanism of acid solvolysis of *N*-acylaporphine **10**.

Table 1. Inhibitory Effect of Secolaurolitsines (**3a**−**6a** and **9a**), Litebamine *N*-Homologues (**11**, **13**−**20**) on Electric Eel AChE

secoaporphine intermediates	IC_{50} (μM)[a]	litebamine *N*-homologues	IC_{50} (μM)[a]
		11	22.00
3a	27.22	**13**	14.45
4a	107.34	**14**	7.21
5a	110.67	**15**	7.79
6a	61.99	**16**	6.51
		17	15.80
		18	10.67
9a	72.02	**19**	6.80
		20	2.79

[a] The IC_{50} values were calculated from the dose−response curve of at least four concentrations of each tested compound that gave 10−90% inhibition of the AChE activity.

2.52 (1H, dd, J = 13.6, 13.6 Hz, H-7β), 2.43 (1H, ddd, J = 11.4, 11.4, 3.8 Hz, H-5α), 1.57 (2H, m, *N*-CH_2CH_2-CH_3), 0.94 (3H, t, J = 7.4 Hz, *N*-$C_2H_4CH_3$); COSY-45 data, δ 3.20 to δ 2.94 and 2.52; δ 3.12 to δ 3.01, 2.62, and 2.43; δ 3.01 to δ 3.12, 2.62, and 2.43; δ 2.62 to δ 3.12, 3.01, and 2.43; δ 2.94 to δ 3.20 and 2.52; δ 2.87 to δ 2.40 and 1.57; δ 2.40, to δ 2.87 and 1.57, δ 1.57 to δ 2.87, 2.40 and 0.94; EIMS (70 eV) *m*/*z* 355 $[M]^+$ (100), 340 (51), 326 (46), 284 (21), 163 (21), 72 (13).

***N*-Butyllaurolitsine (5):** mp 98−100 °C; ^{1}H-NMR δ 7.86 (1H, s, H-11), 6.82 (1H, s, H-8), 6.62 (1H, s, H-3), 3.91 (3H, s, 10-OMe), 3.56 (3H, s, 1-OMe), 2.88 (1H, m) and 2.43 (1H, m) (*N*-$CH_2C_3H_7$), 1.53 (2H, m, *N*-$CH_2CH_2C_2H_5$), 1.38 (2H, m, *N*-$C_2H_4CH_2CH_3$), 0.94 (3H, t, J = 7.2 Hz, *N*-$C_3H_6CH_3$); EIMS (70 eV) *m*/*z* 369 $[M]^+$ (100), 354 (51), 338 (19), 326 (60), 297 (14), 284 (36).

***N*-Isobutyllaurolitsine (6):** mp 84−86 °C; ^{1}H-NMR ($CDCl_3$) δ 7.86 (1H, s, H-11), 6.82 (1H, s, H-8), 6.62 (1H, s, H-3), 3.90 (3H, s, 10-OMe), 3.55 (3H, s, 1-OMe), 2.88 (1H, m) and 2.48 (1H, m) (*N*-$CH_2iC_3H_7$), 1.85 [1H, m, *N*-$CH_2CH(CH_3)_2$], 0.93 [6H, d, J = 7.2 Hz, *N*-$CH_2CH(CH_3)_2$]; EIMS (70 eV) *m*/*z* 369 $[M]^+$ (56), 326 (100), 297 (19), 284 (6), 277 (28), 263 (27), 163 (12).

***N*-Benzyllaurolitsine (8):** mp 96−98 °C; ^{1}H-NMR ($CDCl_3$) δ 7.88 (1H, s, H-11), 7.33 (5H, m, *N*-$CH_2C_6H_5$), 6.83 (1H, s, H-8), 6.62 (1H, s, H-3), 4.30 (1H, d) and 3.32 (1H, d) (J_{AX} = 13.7 Hz, *N*-$CH_2C_6H_5$), 3.91 (3H, s, 10-OMe), 3.57 (3H, s, 1-OMe); EIMS (70 eV) *m*/*z* 403 $[M]^+$ (100), 388 (46), 372 (16), 324 (28), 284 (39), 269 (16), 120 (16), 91 (70).

Preparation of *N*-Allyllaurolitsine (7). The mixture of crude laurolitsine (**2**) (10.0 g, ca. 25% purity, ca. 8 mmol), DMF (40 mL), $KHCO_3$ (2.82 g), and allyl bromide (0.5 mL, 5.78 mmol) was stirred at 50−55 °C for 6 h under nitrogen. The resulting suspension, after removal of organic solvent under reduced pressure, was diluted with H_2O (300 mL) and adjusted to pH 8.0 with ammonia water. The mixture was partitioned against $CHCl_3$ (200 mL × 3). The combined $CHCl_3$ layers were dried (Na_2SO_4), and after removal of the solvent, the extract was purified by Si gel chromatography (1% MeOH in $CHCl_3$) to give pure **7** (825 mg, 2.34 mmol): mp 175−180 °C; ^{1}H-NMR ($CDCl_3$) δ 7.86 (1H, s, H-11), 6.81 (1H, s, H-8), 6.62 (1H, s, H-3), 5.96 (1H, ddt, J = 17.2, 10.1, 6.6 Hz, *N*-CH_2CH=CH_2), 5.26 (1H, br d, J = 17.2 Hz, *N*-CH_2CH=CH_EH_Z), 5.19 (1H, br d, J = 10.1 Hz, *N*-CH_2CH=CH_EH_Z), 3.90 (3H, s, 10-OMe), 3.56 (3H, s, 1-OMe), 3.05 (2H, br d, J = 6.6 Hz, *N*-CH_2CH=CH_2); EIMS (70 eV) *m*/*z* 353 $[M]^+$ (100), 338 (72), 326 (46), 284 (21), 163 (21), 72 (13).

Preparation of *N*-Ethyl- (3a), *N*-Propyl- (4a), *N*-Butyl- (5a), *N*-Isobutyl- (6a), *N*-Allyl- (7a), and *N*-Benzylsecolaurolitsines (8a). The preparation of *N*-benzylsecolaurolitsine (**8a**) is given as an example. The mixture of **8** (350 mg, 0.86 mmol), EtOH (10 mL), and 1 M $NH_4OAc_{(aq)}$ (10 mL) was heated under reflux overnight and cooled to room temperature. The pure crystalline product **8a** (265 mg, 75.7% yield) was collected by suction. **8a**: mp 88−92 °C; ^{1}H-NMR ($CDCl_3$) δ 9.12 (1H, s, H-5), 7.70 (1H, d, J = 9.1 Hz, H-10), 7.39 (1H, d, J = 9.1 Hz, H-9), 7.26 (1H, s, H-8), 7.12 (1H, s, H-2), 4.04 (3H, s, 6-OMe), 3.84 (3H, s, 4-OMe), 3.83 (2H, s, *N*-$CH_2C_6H_5$), 3.24 (2H, m, H-11), 3.03 (2H, m, H-12); EIMS (20 eV) *m*/*z* 403 $[M]^+$ (12), 284 (100), 269 (9), 120 (54), 91 (14).

Under a similar experimental condition, **3** (500 mg, 1.47 mmol) yielded **3a** (450 mg, 90.0% yield), **4** (500 mg, 1.41 mmol) yielded **4a** (484 mg, 96.8% yield), **5** (500 mg, 1.36 mmol) yielded **5a** (483 mg, 96.6% yield), **6** (500 mg, 1.36 mmol) yielded **6a** (475 mg, 95.0% yield), and **7** (500 mg, 1.42 mmol) yielded **7a** (490 mg, 98.0% yield). Melting points of **3a**−**7a** are as follows: 154−156 °C (**3a**), 164−168 °C (**4a**), 148−150 °C (**5a**), 106−110 °C (**6a**), and 165−170 °C (**7a**). For the other physical and spectral data (^{1}H-NMR and MS), see Lee et al.[5]

Preparation of Secolaurolitsine (2a). The mixture of crude **2** (10.0 g), DMF (60 mL), and Ac_2O (2 mL) was stirred at room-temperature overnight in a sealed tube, and the solvent was evaporated under reduced pressure to give an amorphous residue that was recrystallized from MeOH (50 mL) to give pure *N*-acetyllaurolitsine (**10**) (3.60 g): mp 272−274 °C; ^{1}H-NMR (MeOH-d_4) δ 8.06 (1H, s, H-11), 6.77 (s) and 6.72 (s) (1H, H-8), 6.61 (1H, s, H-3), 3.89 (3H, s, 10-OMe), 3.58 (3H, s, 1-OMe), 2.20 (s) and 2.19 (s) (3H, *N*Ac); EIMS (20 eV) *m*/*z* 355 $[M]^+$ (88), 296 (74), 283 (100), 269 (42), 240 (18).

The solution of **10** (500 mg, 1.41 mmol), MeOH (8 mL), and 37% HCl (6 mL) was stirred under reflux for 24 h in a sealed tube. The cooled solution, after removal of organic solvent under reduced pressure, yielded a brownish solid residue that was washed with H_2O to

give pure secolaurolitsine hydrochloride (**2a.HCl**) (430 mg, 87.3% yield): mp 236−238 °C; ^{1}H-NMR (MeOH-d_4) δ 9.12 (1H, s, H-5), 7.76 (1H, d, J = 9.1 Hz, H-10), 7.53 (1H, d, J = 9.1 Hz, H-9), 7.20 (1H, s, H-8), 7.13 (1H, s, H-2), 4.05 (3H, s, 6-OMe), 3.84 (3H, s, 4-OMe), 3.37 (2H, m, H-11), 3.24 (2H, m, H-12); mp of **2a** 166−170 °C; EIMS of **2a** (20 eV) m/z 313 $[M]^+$ (95), 284 (100), 269 (44); HREIMS m/z 313.1324 (calcd for $C_{18}H_{19}NO_4$, 313.1314).

An experiment with a shorter reaction time (1 h) than that of **10** gave an additional product, *N*-acetylsecolaurolitsine (**10a**): R_f 0.34 (10% MeOH−$CHCl_3$); mp 219−221 °C; ^{1}H-NMR (MeOH-d_4) δ 9.11 (1H, s, H-5), 7.79 (1H, d, J = 9.1 Hz, H-10), 7.43 (1H, d, J = 9.1 Hz, H-9), 7.18 (1H, s, H-8), 7.05 (1H, s, H-2), 4.06 (3H, s, 6-OMe), 3.84 (3H, s, 4-OMe), 3.45 (2H, m, H-11), 3.18 (2H, m, H-12), 1.92 (3H, s, *N*Ac); EIMS (20 eV) m/z 355 $[M]^+$ (100), 296 (78), 283 (35), 263 (12); HREIMS m/z 355.1437 (calcd for $C_{20}H_{21}NO_5$, 355.1420). A treatment of **10** (100 mg, 0.28 mmol) with 50% H_2SO_4 (5 mL) in a sealed tube at 80 °C for 3.5 h also yielded **10a** (50 mg, 50%) after neutralization of the reaction mixture, which was purified through an Amberlite XAD-2 column, washed with H_2O, and eluted with MeOH.

Preparation of *N*-Isopropylsecolaurolitsine (9a). To a solution of **2a.HCl** (200 mg, 0.57 mmol), MeOH (5 mL) and acetone (0.5 mL) was added $NaBH_4$ (200 mg) portionwise and the resulting suspension was stirred for 2 h at room temperature. The reaction mixture, after the removal of organic solvents, was diluted with water (30 mL) and was adjusted to pH 8.0 with 25% ammonia water. The mixture was passed over an Amberlite XAD-2 column (40 g) washed with distilled water (300 mL) to remove the inorganic salt, then was eluted with MeOH (250 mL). Evaporation of the solvent afforded pure **9a** (182 mg, 89.8% yield): mp 135−140 °C; ^{1}H-NMR (MeOH-d_4) δ 9.12 (1H, s, H-5), 7.73 (1H, d, J = 9.1 Hz, H-10), 7.44 (1H, d, J = 9.1 Hz, H-9), 7.18 (1H, s, H-8), 7.08 (1H, s, H-2), 4.04 (3H, s, 6-OMe), 3.84 (3H, s, 4-OMe), 3.21 (2H, m, H-11), 2.95 (2H, m, H-12), 2.93 [1H, septet, J = 6.3 Hz, *N*-C*H*$(CH_3)_2$], 1.13 [6H, d, J = 6.3 Hz, *N*-CH$(CH_3)_2$]; EIMS (20 eV) m/z 355 $[M]^+$ (18), 284 (100), 269 (8), 72 (44); HREIMS m/z 355.1749 (calcd for $C_{21}H_{23}NO_4$, 355.1783).

Preparation of *N*-Ethyl- (13), *N*-Propyl- (14), *N*-Butyl- (15), *N*-Isobutyl- (16), *N*-Allyl- (17), *N*-Benzyl- (18), and *N*-Isopropylnorlitebamines (19). The preparation of **13** is given as an example. The mixture of *N*-ethylsecolaurolitsine (**3a**) (300.0 mg, 0.88 mmol), MeOH (20 mL), 0.1 M AcOH (8 mL), 1 M NaOAc (8 mL), and 37% HCHO (1 mL) in a 100-mL round-bottom flask was stirred at room temperature for 5 h. After removal of MeOH, the aqueous residue was diluted with distilled H_2O (20 mL) and partitioned against $CHCl_3$ (20 mL × 3). The combined $CHCl_3$ layers were dried (Na_2SO_4) and evaporated to give crude **13**. The residue was washed with $CHCl_3$ and MeOH to give pure **13** (289.0 mg, 93.0% yield): mp 154−156 °C; ^{1}H-NMR (DMSO-d_6) δ 8.90 (1H, s, H-5), 7.61 (1H, d, J = 9.1 Hz, H-10), 7.43 (1H, d, J = 9.1 Hz, H-9), 7.18 (1H, s, H-8), 3.92 (3H, s, 6-OMe), 3.72 (3H, s, 4-OMe), 3.51 (2H, s, H-2a), 3.06 (2H, ψt, J = 5.8 Hz, H-11), 2.72 (2H, ψt, J = 5.8 Hz, H-12), 2.55 (2H, q, J = 7.1 Hz, *N*-CH_2-CH_3), 1.30 (3H, t, J = 7.1 Hz, *N*-CH_2CH_3); EIMS (20 eV) m/z 354 (22), 353 $[M]^+$ (100), 338 (22), 296 (68), 281 (16), 140 (15); HREIMS m/z 353.1617 (calcd for $C_{21}H_{23}NO_4$, 353.1627).

Under a similar experimental condition, **4a** (300 mg, 0.85 mmol) yielded **14** (284 mg, 91.6% yield), **5a** (300 mg, 0.81 mmol) yielded **15** (282.4 mg, 91.0% yield), **6a** (300 mg, 0.81 mmol) yielded **16** (280 mg, 90.4% yield), **7a** (400 mg, 1.13 mmol) yielded **17** (385 mg, 93.1% yield), **8a** (51.6 mg, 128 μmol) yielded **18** (50.5 mg, 95.0% yield), and **9a** (51.6 mg, 145 μmol) yielded **19** (44.1 mg, 82.9% yield).

***N*-Propylnorlitebamine (14):** mp 175−177 °C; ^{1}H-NMR (MeOH-d_4) δ 9.12 (1H, s, H-5), 7.59 (1H, d, J = 9.1 Hz, H-10), 7.56 (1H, d, J = 9.1 Hz, H-9), 7.17 (1H, s, H-8), 4.02 (3H, s, 6-OMe), 3.76 (3H, s, 4-OMe), 3.78 (2H, s, H-2a), 3.15 (2H, m, H-11), 2.96 (2H, m, H-12), 2.59 (2H, t, J = 7.4 Hz, *N*-CH_2C$_2$H$_5$), 1.53 (2H, m, *N*-CH_2CH_2CH_3), 0.94 (3H, t, J = 7.4 Hz, *N*-CH_2-CH_2CH_3); EIMS (20 eV) m/z 368 (23), 367 $[M]^+$ (97), 338 (100), 296 (58), 281 (16); HREIMS m/z 367.1779 (calcd for $C_{22}H_{25}NO_4$, 367.1783).

***N*-Butylnorlitebamine (15):** mp 115−118 °C; ^{1}H-NMR (DMSO-d_6) δ 8.91 (1H, s, H-5), 7.60 (1H, d, J = 9.1 Hz, H-10), 7.43 (1H, d, J = 9.1 Hz, H-9), 7.19 (1H, s, H-8), 3.93 (3H, s, 6-OMe), 3.70 (3H, s, 4-OMe), 3.47 (2H, s, H-2a), 3.04 (2H, m, H-11), 2.72 (2H, m, H-12), 2.50 (2H, m, *N*-CH_2C$_3$H$_7$), 1.52 and 1.33 (2H each, m, *N*-CH_2CH_2CH_2CH_3), 0.89 (3H, t, J = 7.3 Hz, *N*-C$_3$H$_7$CH_3); EIMS (20 eV) m/z 382 (24), 381 $[M]^+$ (100), 366 (10), 355 (10), 339 (40), 338 (92), 296 (55), 281 (8); HREIMS m/z 381.1946 (calcd for $C_{23}H_{27}NO_4$, 381.1940).

***N*-Isobutylnorlitebamine (16):** mp 120−124 °C; ^{1}H-NMR (MeOH-d_4) δ 8.92 (1H, s, H-5), 7.61 (1H, d, J = 9.0 Hz, H-10), 7.44 (1H, d, J = 9.0 Hz, H-9), 7.19 (1H, s, H-8), 3.93 (3H, s, 6-OMe), 3.71 (3H, s, 4-OMe), 3.54 (2H, s, H-2a), 3.04 (2H, m, H-11), 2.70 (2H, m, H-12), 2.27 (2H, d, J = 7.2 Hz, *N*-CH_2*i*C$_3$H$_7$), 1.90 [1H, m, *N*-CH_2C*H*$(CH_3)_2$], 0.88 [6H, d, J = 6.5 Hz, *N*-CH_2CH-$(CH_3)_2$]; EIMS (20 eV) m/z 382 (8), 381 $[M]^+$ (33), 339 (22), 338 (100), 296 (4); HREIMS m/z 381.1939 (calcd for $C_{23}H_{27}NO_4$, 381.1940).

***N*-Allylnorlitebamine (17):** mp 151−154 °C; ^{1}H-NMR (MeOH-d_4) δ 9.12 (1H, s, H-5), 7.67 (1H, d, J = 9.0 Hz, H-10), 7.45 (1H, d, J = 9.0 Hz, H-9), 7.19 (1H, s, H-8), 4.04 (3H, s, 6-OMe), 3.78 (3H, s, 4-OMe), 3.77 (2H, s, H-2a), 3.22 (2H, ψt, J = 5.8 Hz, H-11), 2.90 (2H, ψt, J = 5.8 Hz, H-12), 3.29 (2H, br d, J = 6.6 Hz, *N*-CH_2-CH=CH_2), 6.02 (1H, ddt, J = 17.2, 10.2, 6.6 Hz, *N*-CH_2C*H*=CH_2), 5.37 (1H, br d, J = 17.2 Hz, *N*-CH_2-CH=CH_EH$_Z$), 5.30 (1H, br d, J = 10.2 Hz, *N*-CH_2-CH=CH_EH$_Z$); EIMS (20 eV) m/z 366 (22), 365 $[M]^+$ (100), 350 (16), 296 (58), 281 (14); HREIMS m/z 365.1650 (calcd for $C_{22}H_{23}NO_4$, 365.1627).

***N*-Benzylnorlitebamine (18):** mp 130−132 °C; ^{1}H-NMR (DMSO-d_6) δ 8.92 (1H, s, H-5), 7.63 (1H, d, J = 9.1 Hz, H-10), 7.46 (1H, d, J = 9.1 Hz, H-9), 7.39 (1H, s, H-8), 7.39 (5H, m, *N*-CH_2C$_6$H$_5$), 3.94 (3H, s, 6-OMe), 3.70 (3H, s, 4-OMe), 3.73 (2H, s, *N*-CH_2C$_6$H$_5$), 3.58 (2H, s, H-2a), 3.10 (2H, m, H-11), 2.80 (2H, m, H-12); EIMS (20 eV) m/z 416 (14), 415 $[M]^+$ (58), 400 (22), 296 (78), 281 (48), 210 (10), 165 (18), 91 (100); HREIMS m/z 415.1774 (calcd for $C_{26}H_{25}NO_4$, 415.1784).

***N*-Isopropylnorlitebamine (19):** mp 225−227 °C; ^{1}H-NMR (DMSO-d_6) δ 9.03 (1H, s, H-5), 7.68 (1H, d, J

= 9.0 Hz, H-10), 7.45 (1H, d, J = 9.0 Hz, H-9), 7.19 (1H, s, H-8), 4.04 (3H, s, 6-OMe), 3.79 (3H, s, 4-OMe), 3.87 (2H, s, H-2a), 3.23 (2H, m, H-11), 2.97 (2H, m, H-12), 2.98 [1H, m, *N*-C*H*(CH_3)$_2$], 1.25 [1H, d, J = 6.5 Hz, *N*-CH(C*H*$_3$)$_2$]; EIMS (20 eV) *m/z* 368 (13), 367 $[M]^+$ (87), 352 (100), 310 (25), 296 (37), 281 (22), 165 (23); HREIMS *m/z* 367.1777 (calcd for $C_{22}H_{25}NO_4$, 367.1784).

Preparation of *N*-Propylnorlitebamine *N*-Methoiodide (20). The mixture of *N*-propylnorlitebamine (**14**) (50 mg, 136 μmol), CH_3CN (4.0 mL), and MeI (4.0 mL) was stirred at 40 °C for 6 h. After evaporation of the solvents, the precipitate was washed with MeOH (5 mL) to give a pure product (**20**) (61.2 mg, 92.5%): ^{1}H-NMR (DMSO-d_6) δ 8.94 (1H, s, H-5), 7.68 (1H, d, J = 9.1 Hz, H-10), 7.59 (1H, d, J = 9.1 Hz, H-9), 7.26 (1H, s, H-8), 4.69 (1H, d) and 4.63 (1H, d) (J_{AB} = 16.2 Hz) (H-2a), 3.96 (3H, s, 6-OMe), 3.80 (2H, m, H-12), 3.75 (3H, s, 4-OMe), 3.45 (4H, m, H-11 and *N*-C*H*$_2$C$_2$H$_5$), 3.16 (3H, s, *N*-Me), 1.91 (2H, m, *N*-CH$_2$C*H*$_2$CH$_3$), 1.03 (3H, t, J = 7.4 Hz, *N*-C$_2$H$_4$C*H*$_3$); EIMS (70 eV) *m/z* 381 $[M - HI]^+$ (8), 367 $[M - MeI]^+$ (12), 338 $[M - C_3H_7I]^+$ (18), 324 (24), 296 (30), 281 (32), 142 $[MeI]^+$ (100), 127 (58).

Assay of antiAChE Activity. The enzymatic activity of AChE (EC 3.1.1.7) purified from electric eel (type V−S, Sigma Co.) was determined by the colorimetric method[13] using acetylcholine iodide as substrate and 5,5′-dithio-(bis-2-nitrobenzoic acid) (DTNB) as a coupler. Briefly, AChE (0.125 U) was incubated in phosphate buffer (0.1 M, 1 mL, pH 8.0) containing DTNB (0.3 mM) and test samples for 2 min at 37 °C. The reaction was initiated by adding acetylcholine (0.5 mM), and the activity was measured spectrophotometrically at 412 nm as a function of time using a Beckman (Fullerton, CA) DU-650 spectrophotometer. All operations were performed in the dark due to the photosensitivity of the AChE.

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