

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

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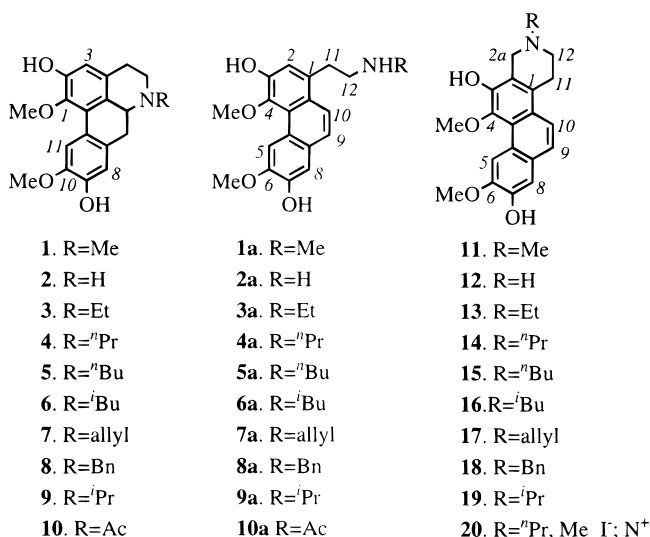
Litebamine *N*-homologues were easily prepared from lauroiltsine, generally via three reaction steps (*N*-alkylation, solvolysis with 1 M NH<sub>4</sub>OAc under reflux, and the Mannich reaction) in more than 80% overall yield. Among the prepared compounds, *N*-propyl-, *N*-isobutyl-, and *N*-isopropylnorlitebamines exhibited moderate antiacetylcholinesterase activity (IC<sub>50</sub> ca. 7.0 μM), while the corresponding *N*-metho salt of *N*-propylnorlitebamine showed potent activity (IC<sub>50</sub> 2.70 μM).

Litebamine (**11**) is a novel phenanthrene alkaloid isolated from the wood of *Litsea cubeba*.<sup>1</sup> It possesses antiplatelet aggregation activity through the inhibition of thromboxane B<sub>2</sub> formation induced by arachidonic acid in washed rabbit platelets (15 μM, 77% inhibition).<sup>2</sup> Recently, it was demonstrated to possess activity against acetylcholinesterase (AChE), with an IC<sub>50</sub> value of 22.0 μM.<sup>3</sup> 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.

### Results and Discussion

A three-step preparation of litebamine (**11**) from boldine (**1**) via a biogenetic approach has been developed in our laboratory.<sup>4</sup> 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<sub>4</sub>OAc in EtOH–H<sub>2</sub>O under reflux, which afforded **1a** in high yield (>90%).<sup>5</sup> 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.<sup>6</sup> Based on these surveys, we used the solvolysis method to prepare the key intermediates, the *N*-alkylsecolaurolitsines (**3a–10a**). These alkylsecoaporphines were prepared from lauroiltsine (**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.<sup>7</sup>

*N*-Alkylsecolaurolitsines (**3–6**) and *N*-benzylsecolaurolitsine (**8**) were prepared from crude **2** via direct reaction with the corresponding halide under reflux in alkaline conditions.<sup>8</sup> *N*-Allylsecolaurolitsine (**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



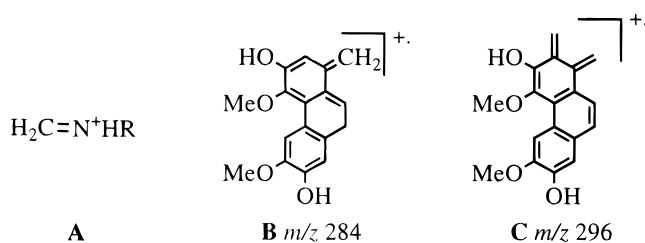
*N*-allylation and subsequent Hoffman-like degradation reaction can occur if more reagent is added. These two successive reactions gave *N,N*-diallylsecoboldine, which is almost TLC-inseparable from the desired product **7**. The diallylsecoboldine displays a [M]<sup>+</sup> at *m/z* 393 and a base peak at *m/z* 110, corresponding to fragment ion **A** (Figure 1), with R = allyl and H replaced by another allyl group. The <sup>1</sup>H-NMR spectra of **3–8** showed *N*-alkyl, *N*-allyl, or *N*-benzyl signals in addition to that of lauroiltsine, 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,<sup>9</sup> supporting their structures. Solvolysis of these 2-hydroxyaporphines with 1 M NH<sub>4</sub>OAc in EtOH–H<sub>2</sub>O (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; <sup>1</sup>H-NMR signals for H-9 and H-10 as an AB system at δ ca. 7.45 and 7.56 (*J*<sub>AB</sub> = 9.4 Hz);<sup>10,11</sup> 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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**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  $\beta$ -cleavage fragmentation.<sup>10</sup>

Mannich reaction of these secondary bases (**3a–8a**) with HCHO under acetate buffer conditions (pH 5.76)<sup>4</sup> gave the target products **13–18** in near quantitative yield. The <sup>1</sup>H-NMR spectra of these products showed the two-proton singlet of H-2a at  $\delta$  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*-Isopropyllauroilsine (**9**) could not be obtained by reacting lauroilsine 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]<sup>+</sup> at *m/z* 355, *N*-<sup>*i*</sup>Pr at  $\delta$  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<sub>2</sub>CO–NaBH<sub>4</sub>) (89.8% yield). Compound **2a**, [M]<sup>+</sup> at *m/z* 313, was prepared from *N*-acetyllauroilsine (**10**), which was obtained from *N*-acetylation of **2** with Ac<sub>2</sub>O, by acid solvolysis (concd HCl–MeOH = 3:4, reflux, 1 d, 87.0% yield).<sup>12</sup> During the preparation of **2a**, *N*-acetylsecolaurolitsine (**10a**), <sup>1</sup>H NMR  $\delta$  1.92 (s, 3H, NHAc), 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 <sup>1</sup>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.<sup>13</sup> 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  $\mu$ M (**19**) vs 72.02  $\mu$ M (**9a**). Molecular modeling studies revealed that the distance between N and O<sup>3</sup> (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).<sup>14</sup> 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<sup>3</sup> 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<sub>3</sub> or C<sub>4</sub> *N*-substitution in the *N*-alkylated norlitebamines possessed the optimal anti-AChE activity; however, a planar C<sub>3</sub> *N*-substitution (i.e., allyl group) gave a lower such activity (15.80  $\mu$ M for **17** vs 7.21  $\mu$ M 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.

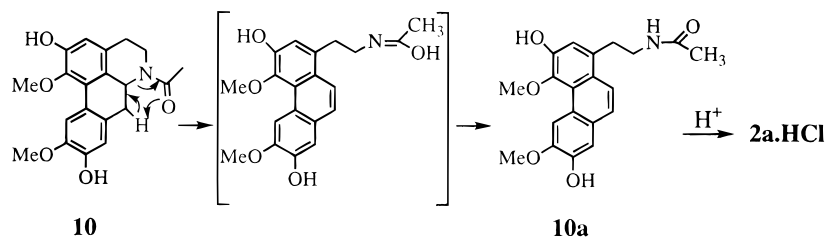
## Experimental Section

**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*-Alkyllauroilsines (3–6) and *N*-Benzyllauroilsine (8).** The preparation of *N*-ethylauroilsine (**3**) is given as an example. The mixture of crude lauroilsine (**2**) (6.0 g, ca. 25% purity), DMF (60 mL), K<sub>2</sub>CO<sub>3</sub> (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<sub>2</sub>O (300 mL) and adjusted to pH 8.0 with 25% ammonia water. The mixture was partitioned against CHCl<sub>3</sub> (200 mL  $\times$  3). The combined CHCl<sub>3</sub> layers were dried (anhydrous Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residue was purified by Si gel chromatography (1–3% MeOH in CHCl<sub>3</sub>) to give pure **3** (1.21 g): mp 175–177 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  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*-CH<sub>2</sub>CH<sub>3</sub>), 1.11 (3H, t,  $J = 7.2$  Hz, *N*-CH<sub>2</sub>CH<sub>3</sub>); EIMS (70 eV) *m/z* 341 [M]<sup>+</sup> (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*-Propyllauroilsine (4):** mp 162–166 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  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-7 $\alpha$ ), 3.12 (1H, ddd,  $J = 11.4, 5.9, 1.5$  Hz, H-5 $\beta$ ), 3.01 (1H, ddd,  $J = 17.0, 11.4, 5.9$  Hz, H-4 $\beta$ ), 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*-CH<sub>2</sub>C<sub>2</sub>H<sub>5</sub>), 2.62 (1H, br dd,  $J = 17.0, 3.8$  Hz, H-4 $\alpha$ ),



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

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

secoaporphine intermediates	IC <sub>50</sub> (μM) <sup>a</sup>	litebamine <i>N</i> -homologues	IC <sub>50</sub> (μM) <sup>a</sup>
		<b>11</b>	22.00
<b>3a</b>	27.22	<b>13</b>	14.45
<b>4a</b>	107.34	<b>14</b>	7.21
<b>5a</b>	110.67	<b>15</b>	7.79
<b>6a</b>	61.99	<b>16</b>	6.51
		<b>17</b>	15.80
		<b>18</b>	10.67
<b>9a</b>	72.02	<b>19</b>	6.80
		<b>20</b>	2.79

<sup>a</sup> The IC<sub>50</sub> 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,  $N\text{-CH}_2\text{CH}_2\text{-CH}_3$ ), 0.94 (3H, t,  $J = 7.4$  Hz,  $N\text{-C}_2\text{H}_4\text{CH}_3$ ); 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]<sup>+</sup> (100), 340 (51), 326 (46), 284 (21), 163 (21), 72 (13).

***N*-Butyllauroilitsine (5):** mp 98–100 °C; <sup>1</sup>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) ( $N\text{-CH}_2\text{C}_3\text{H}_7$ ), 1.53 (2H, m,  $N\text{-CH}_2\text{-CH}_2\text{C}_2\text{H}_5$ ), 1.38 (2H, m,  $N\text{-C}_2\text{H}_4\text{CH}_2\text{CH}_3$ ), 0.94 (3H, t,  $J = 7.2$  Hz,  $N\text{-C}_3\text{H}_6\text{CH}_3$ ); EIMS (70 eV)  $m/z$  369 [M]<sup>+</sup> (100), 354 (51), 338 (19), 326 (60), 297 (14), 284 (36).

***N*-Isobutyllauroilitsine (6):** mp 84–86 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) 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) ( $N\text{-CH}_2\text{iC}_3\text{H}_7$ ), 1.85 (1H, m,  $N\text{-CH}_2\text{CH}(\text{CH}_3)_2$ ), 0.93 [6H, d,  $J = 7.2$  Hz,  $N\text{-CH}_2\text{CH}(\text{CH}_3)_2$ ]; EIMS (70 eV)  $m/z$  369 [M]<sup>+</sup> (56), 326 (100), 297 (19), 284 (6), 277 (28), 263 (27), 163 (12).

***N*-Benzyllauroilitsine (8):** mp 96–98 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ 7.88 (1H, s, H-11), 7.33 (5H, m,  $N\text{-CH}_2\text{C}_6\text{H}_5$ ), 6.83 (1H, s, H-8), 6.62 (1H, s, H-3), 4.30 (1H, d) and 3.32 (1H, d) ( $J_{\text{AX}} = 13.7$  Hz,  $N\text{-CH}_2\text{C}_6\text{H}_5$ ), 3.91 (3H, s, 10-OMe), 3.57 (3H, s, 1-OMe); EIMS (70 eV)  $m/z$  403 [M]<sup>+</sup> (100), 388 (46), 372 (16), 324 (28), 284 (39), 269 (16), 120 (16), 91 (70).

**Preparation of *N*-Allyllauroilitsine (7).** The mixture of crude lauroilitsine (**2**) (10.0 g, ca. 25% purity, ca. 8 mmol), DMF (40 mL), KHCO<sub>3</sub> (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<sub>2</sub>O (300 mL) and adjusted to pH 8.0 with

ammonia water. The mixture was partitioned against CHCl<sub>3</sub> (200 mL × 3). The combined CHCl<sub>3</sub> layers were dried (Na<sub>2</sub>SO<sub>4</sub>), and after removal of the solvent, the extract was purified by Si gel chromatography (1% MeOH in CHCl<sub>3</sub>) to give pure **7** (825 mg, 2.34 mmol): mp 175–180 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) 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,  $N\text{-CH}_2\text{CH}=\text{CH}_2$ ), 5.26 (1H, br d,  $J = 17.2$  Hz,  $N\text{-CH}_2\text{CH}=\text{CH}_E\text{H}_Z$ ), 5.19 (1H, br d,  $J = 10.1$  Hz,  $N\text{-CH}_2\text{CH}=\text{CH}_E\text{H}_Z$ ), 3.90 (3H, s, 10-OMe), 3.56 (3H, s, 1-OMe), 3.05 (2H, br d,  $J = 6.6$  Hz,  $N\text{-CH}_2\text{CH}=\text{CH}_2$ ); EIMS (70 eV)  $m/z$  353 [M]<sup>+</sup> (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*-Benzylsecolauloritsines (8a).** The preparation of *N*-benzylsecolauloritsine (**8a**) is given as an example. The mixture of **8** (350 mg, 0.86 mmol), EtOH (10 mL), and 1 M NH<sub>4</sub>OAc(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; <sup>1</sup>H-NMR (CDCl<sub>3</sub>) 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,  $N\text{-CH}_2\text{C}_6\text{H}_5$ ), 3.24 (2H, m, H-11), 3.03 (2H, m, H-12); EIMS (20 eV)  $m/z$  403 [M]<sup>+</sup> (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 (<sup>1</sup>H-NMR and MS), see Lee et al.<sup>5</sup>

**Preparation of Secolauroilitsine (2a).** The mixture of crude **2** (10.0 g), DMF (60 mL), and Ac<sub>2</sub>O (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*-acetylsecolauloritsine (**10**) (3.60 g): mp 272–274 °C; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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, NAc); EIMS (20 eV)  $m/z$  355 [M]<sup>+</sup> (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<sub>2</sub>O to

give pure secolaurolitsine hydrochloride (**2a.HCl**) (430 mg, 87.3% yield): mp 236–238 °C; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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 (20 eV) *m/z* 313 [M]<sup>+</sup> (95), 284 (100), 269 (44); HREIMS *m/z* 313.1324 (calcd for C<sub>18</sub>H<sub>19</sub>NO<sub>4</sub>, 313.1314).

An experiment with a shorter reaction time (1 h) than that of **10** gave an additional product, *N*-acetylsecolaurolitsine (**10a**): *R*<sub>f</sub> 0.34 (10% MeOH–CHCl<sub>3</sub>); mp 219–221 °C; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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]<sup>+</sup> (100), 296 (78), 283 (35), 263 (12); HREIMS *m/z* 355.1437 (calcd for C<sub>20</sub>H<sub>21</sub>NO<sub>5</sub>, 355.1420). A treatment of **10** (100 mg, 0.28 mmol) with 50% H<sub>2</sub>SO<sub>4</sub> (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<sub>2</sub>O, 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<sub>4</sub> (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; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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*-CH(CH<sub>3</sub>)<sub>2</sub>], 1.13 [6H, d, *J* = 6.3 Hz, *N*-CH(CH<sub>3</sub>)<sub>2</sub>]; EIMS (20 eV) *m/z* 355 [M]<sup>+</sup> (18), 284 (100), 269 (8), 72 (44); HREIMS *m/z* 355.1749 (calcd for C<sub>21</sub>H<sub>23</sub>NO<sub>4</sub>, 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<sub>2</sub>O (20 mL) and partitioned against CHCl<sub>3</sub> (20 mL × 3). The combined CHCl<sub>3</sub> layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to give crude **13**. The residue was washed with CHCl<sub>3</sub> and MeOH to give pure **13** (289.0 mg, 93.0% yield): mp 154–156 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) 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, *ψ*t, *J* = 5.8 Hz, H-11), 2.72 (2H, *ψ*t, *J* = 5.8 Hz, H-12), 2.55 (2H, q, *J* = 7.1 Hz, *N*-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 (3H, t, *J* = 7.1 Hz, *N*-CH<sub>2</sub>-CH<sub>3</sub>); EIMS (20

eV) *m/z* 354 (22), 353 [M]<sup>+</sup> (100), 338 (22), 296 (68), 281 (16), 140 (15); HREIMS *m/z* 353.1617 (calcd for C<sub>21</sub>H<sub>23</sub>NO<sub>4</sub>, 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; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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<sub>2</sub>C<sub>2</sub>H<sub>5</sub>), 1.53 (2H, m, *N*-CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.94 (3H, t, *J* = 7.4 Hz, *N*-CH<sub>2</sub>-CH<sub>2</sub>CH<sub>3</sub>); EIMS (20 eV) *m/z* 368 (23), 367 [M]<sup>+</sup> (97), 338 (100), 296 (58), 281 (16); HREIMS *m/z* 367.1779 (calcd for C<sub>22</sub>H<sub>25</sub>NO<sub>4</sub>, 367.1783).

***N*-Butylnorlitebamine (**15**):** mp 115–118 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) 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<sub>2</sub>C<sub>3</sub>H<sub>7</sub>), 1.52 and 1.33 (2H each, m, *N*-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 0.89 (3H, t, *J* = 7.3 Hz, *N*-C<sub>3</sub>H<sub>7</sub>CH<sub>3</sub>); EIMS (20 eV) *m/z* 382 (24), 381 [M]<sup>+</sup> (100), 366 (10), 355 (10), 339 (40), 338 (92), 296 (55), 281 (8); HREIMS *m/z* 381.1946 (calcd for C<sub>23</sub>H<sub>27</sub>NO<sub>4</sub>, 381.1940).

***N*-Isobutylnorlitebamine (**16**):** mp 120–124 °C; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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<sub>2</sub>iC<sub>3</sub>H<sub>7</sub>), 1.90 [1H, m, *N*-CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>], 0.88 [6H, d, *J* = 6.5 Hz, *N*-CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>]; EIMS (20 eV) *m/z* 382 (8), 381 [M]<sup>+</sup> (33), 339 (22), 338 (100), 296 (4); HREIMS *m/z* 381.1939 (calcd for C<sub>23</sub>H<sub>27</sub>NO<sub>4</sub>, 381.1940).

***N*-Allylnorlitebamine (**17**):** mp 151–154 °C; <sup>1</sup>H-NMR (MeOH-*d*<sub>4</sub>) 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, *ψ*t, *J* = 5.8 Hz, H-11), 2.90 (2H, *ψ*t, *J* = 5.8 Hz, H-12), 3.29 (2H, br d, *J* = 6.6 Hz, *N*-CH<sub>2</sub>-CH=CH<sub>2</sub>), 6.02 (1H, ddt, *J* = 17.2, 10.2, 6.6 Hz, *N*-CH<sub>2</sub>CH=CH<sub>2</sub>), 5.37 (1H, br d, *J* = 17.2 Hz, *N*-CH<sub>2</sub>-CH=CH<sub>E</sub>H<sub>Z</sub>), 5.30 (1H, br d, *J* = 10.2 Hz, *N*-CH<sub>2</sub>-CH=CH<sub>E</sub>H<sub>Z</sub>); EIMS (20 eV) *m/z* 366 (22), 365 [M]<sup>+</sup> (100), 350 (16), 296 (58), 281 (14); HREIMS *m/z* 365.1650 (calcd for C<sub>22</sub>H<sub>23</sub>NO<sub>4</sub>, 365.1627).

***N*-Benzylnorlitebamine (**18**):** mp 130–132 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) 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<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 3.94 (3H, s, 6-OMe), 3.70 (3H, s, 4-OMe), 3.73 (2H, s, *N*-CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 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]<sup>+</sup> (58), 400 (22), 296 (78), 281 (48), 210 (10), 165 (18), 91 (100); HREIMS *m/z* 415.1774 (calcd for C<sub>26</sub>H<sub>25</sub>NO<sub>4</sub>, 415.1784).

***N*-Isopropylnorlitebamine (**19**):** mp 225–227 °C; <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) 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,  $N\text{-CH}(\text{CH}_3)_2$ ], 1.25 [1H, d,  $J = 6.5$  Hz,  $N\text{-CH}(\text{CH}_3)_2$ ]; EIMS (20 eV)  $m/z$  368 (13), 367 [M]<sup>+</sup> (87), 352 (100), 310 (25), 296 (37), 281 (22), 165 (23); HREIMS  $m/z$  367.1777 (calcd for  $\text{C}_{22}\text{H}_{25}\text{NO}_4$ , 367.1784).

**Preparation of *N*-Propylnorlitebamine *N*-Methiodide (20).** The mixture of *N*-propylnorlitebamine (14) (50 mg, 136  $\mu\text{mol}$ ),  $\text{CH}_3\text{CN}$  (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%): <sup>1</sup>H-NMR ( $\text{DMSO-}d_6$ )  $\delta$  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) ( $J_{\text{AB}} = 16.2$  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  $N\text{-CH}_2\text{C}_2\text{H}_5$ ), 3.16 (3H, s, *N*-Me), 1.91 (2H, m,  $N\text{-CH}_2\text{CH}_2\text{CH}_3$ ), 1.03 (3H, t,  $J = 7.4$  Hz,  $N\text{-C}_2\text{H}_4\text{CH}_3$ ); EIMS (70 eV)  $m/z$  381 [M - H]<sup>+</sup> (8), 367 [M - MeI]<sup>+</sup> (12), 338 [M -  $\text{C}_3\text{H}_7\text{I}$ ]<sup>+</sup> (18), 324 (24), 296 (30), 281 (32), 142 [MeI]<sup>+</sup> (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<sup>13</sup> 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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