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Alkaloids with Acetylcholinesterase Inhibitory Activities from *Crinum latifolium* L.

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Abstract – A new crinane-type alkaloid, 6-epihydroxypowelline (**1**), together with six known alkaloids, lycorine (**2**), 2-*O*-acetyllycorine (**3**), deacetylbowdensine (**4**), 1-epideacetylbowdensine (**5**), 8-demethyl-3-oxomaritidine (**6**), and (-)-marithamine (**7**) were isolated from the whole parts of the *Crinum latifolium* L. in Vietnam. The structure identification of all compounds was determined by 1D, 2D-NMR as well as HR-ESI-MS spectroscopic techniques. The absolute configuration of these compounds was established by the ECD data. In addition, *in vitro* inhibition of acetylcholinesterase (AChE) activities was assessed for all isolated alkaloids. All alkaloids had AChE inhibitory effects, with IC_{50} values ranging from 32.65 ± 2.72 to 212.76 ± 8.30 μM and compound **3** displayed the strongest inhibition of AChE, with IC_{50} values of 32.65 ± 2.72 μM (in comparison to the reference drug, galanthamine, which had an IC_{50} of 2.40 ± 0.45 μM).
Keywords – *Crinum latifolium* L., Alkaloids, Acetylcholinesterase inhibitor, Alzheimer's disease

Introduction

The *Crinum* genus belongs to the Amaryllidaceace family, which includes approximately 100 species found primarily in tropical, subtropical, and temperate regions such as Africa, America, Australia, and southern Asia.[1,2] In traditional medicine, extracts of *Crinum* species have been used to treat a variety of diseases, including anti-asthmatics, anti-tumor, emetics, laxatives, expectorants, antipyretics, diuretics, tonics, diaphoretics, anti-malarial, anti-aging, and galactagogues.[1,2] Previous research has shown that alkaloids are important chemical constituents of the *Crinum* genus, with a diverse variety of biological activity. Lycorine and galanthamine, for example, appear to have antibacterial, antifungal, antimalarial, antioxidant, anti-diabetic, and cytotoxic properties. Galanthamine is used to treat Alzheimer's disease by inhibiting the enzyme acetylcholine esterase (AChE).

Crinum latifolium L., also known as "Trinh nu hoang cung" in Vietnam, is a herbaceous perennial blooming plant that grows in warm, tropical climates, primarily in the Americas, Australia, and Southern Asia. This herb is mostly used by Asian ethnomedics to cure ovarian and prostate malignancies, earaches, tubercles, whiteheads, rheumatism, and hydrocele. Phytochemical and biological investigations of *C. latifolium* indicate the existence of alkaloids, flavones, chalcones, coumarins, terpenes, and other minor components that revealed anti-inflammatory, anti-AChE, anti-malarial, and cytotoxic activities.[3]

Alzheimer's disease (AD) is a neurological condition that is the leading cause of human dementia.[4] Cholinergic neurotransmission is the primary technique to treating reduced cognitive function in Alzheimer's and dementia patients. AChE is one of the enzymes involved in neurotransmitter imbalance and contributes to ACh hydrolysis in the cerebral cortex and hippocampus. As a result, inhibiting AChE and BuChE is regarded a promising technique for treating Alzheimer's disease.[4]

As a part of our continued study for new AChE inhibitors

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from Vietnamese medicinal plants, we herein report the isolation and structure elucidation of crinane-(**1**, **4**–**7**) and lycorine-type alkaloids (**2**, **3**). Furthermore, the *in vitro* AChE inhibitory activities of all isolated compounds have also been undertaken.

Experimental

General experimental procedures – The NMR spectra (^{1}H, ^{13}C, and 2D NMR) were recorded using a Brucker Avance III^{TM} 600 MHz spectrometer with tetramethylsilane (TMS) serving as the internal standard. HR-ESI-MS spectra were measured by using a MicrOTOF-Q III mass spectrometer (Brucker). Column chromatography (CC) was conducted using a silica gel (Kieselgel 60, 63-200 μm, Merck), and RP-18 resins (30-50 μm, Fuji Silysia Chemical Ltd.). The process of thin-layer chromatography (TLC) was carried out using pre-coated plates with a thickness of 0.20 mm. The plates used were made of silica gel 60 F254 (Merck) and RP-18 F254S (Merck).

Plant identification – The whole plants of *C. latifolium* L. were collected in Tuyen Quang province in January 2022 and taxonomically identified by Dr. Le Tuan Anh (Mientrung Institute for Scientific Research, Vietnam National Museum of Nature). The voucher specimen (CL-200422) was deposited at the herbarium of the Center for High Technology Research and Development, VAST, Hanoi, Vietnam.

Extraction and isolation – The dried whole of *C. latifolium* (5.0 kg) was extracted with methanol three times at 50ºC (20 L × 3 h, each time) under multifunctional ultrasonic. The extracts were combined, filtered, and concentrated under reduced pressure to yield a crude methanol extract (520 g). Then, the methanol extract was further dissolved in MeOH/H_2O (2:1, v/v, 2L), adjusted to pH 2, and successively partitioned with EtOAc. Next, the water-soluble part was adjusted to pH 12–13 with NaOH, followed by the liquid-liquid extraction with CH_2Cl_2,

The alkaloidal CH_2Cl_2-soluble materials (15.1 g) were subjected to a silica gel chromatography column (CC) using a gradient solvent of CH_2Cl_2/MeOH/NH_3 (100/1/0.1 → 1/100/0.1, v/v) to yield eight fractions (3A–3D). Two sub-fractions, 7A and 7B, were obtained by subjecting fraction 3A (2.0 g) to a silica gel CC with a solvent solution of *n*-hexane/CH_2Cl_2 (5/1 → 1/1, v/v). Compound **2** (4.4 mg) was obtained by preparative thin-layer chromatography using *n*-hexane/CH_2Cl_2/NH_3 (2/1/0.1, v/v/v) to purify sub-fraction 7B (10.4 mg). Fraction 3C (501.2 mg) was further separated using a silica gel CC with a gradient solvent of CH_2Cl_2/MeOH/NH_3 (20/1/0.1 → 1/1/0.1, v/v/v) to give the two sub-fractions 9A and 9B. Compound **3** (8.0 mg) was obtained from sub-fraction 9A (80.5 mg) with elution of acetone/H_2O (2/1, v/v) on an RP-18 column. Sub-faction 9B (100.3 mg) was separated by an RP-18 column, and using an isocratic solvent system of MeOH/H_2O (1.5/1, v/v) to obtain compound **4** (10.0 mg). Three sub-fractions, 15A–15C, were obtained by placing fraction 3E (1.5 g) on a silica gel column using a solvent system of CH_2Cl_2/MeOH/NH_3 (15/1/0.1 → 1/1/0.1, v/v/v). Compound **1** (5.3 mg) was isolated from sub-factions 15B (200.5 mg) by a silica gel CC using a solvent solution of CH_2Cl_2/MeOH/NH_3 (30/1/0.1 → 5/1/0.1, v/v/v). By purifying fraction 15C (103.5 mg) on an RP-18 column and eluting with MeOH/H_2O (1.5/1, v/v), compound **6** (7.0 mg) was afforded. Two sub-fractions, 25A, and 25B, were obtained by further separating sub-fraction 3D (1.2 g) using a silica gel CC and a gradient solvent of CH_2Cl_2/MeOH/NH_3 (30/1/0.1, v/v/v). Compounds **5** (6.0 mg) and **7** (4.5 mg) were yielded from sub-fraction 25B (120.6 mg) on a reverse phase RP-18 column using MeOH/H_2O (1.5/1, v/v), and from fraction 25A (200.8 mg) on RP-18 column using a solvent system of MeOH/H_2O (2/1, v/v), respectively.

6-Epihydroxypowelline (**1**): White amorphous powder; HR-ESI-MS *m/z* 318.1348 $[M+H]^+$ (calcd. for $C_{17}H_{20}NO_5$, 318.1336); ^{1}H-NMR (CD_3OD, 600 MHz): δ 6.64 (1H, s, H-10), 6.48 (1H, d, J = 10.2 Hz, H-1), 5.88 (1H, dd, J = 10.2, 4.8 Hz, H-2), 5.84 (2H, s, -OCH_2O-), 5.34 (1H, s, H-6), 4.22 (1H, m, H-3), 3.94 (3H, s, -OCH_3), 3.91 (1H, m, H-4a), 3.36 (1H, m, H-12β), 2.91 (1H, m, H-12α), 1.95 (1H, m, H-11β), 1.88 (1H, m, H-11α), 1.88 (1H, m, H-4β), 1.78 (1H, m, H-4α); ^{13}C-NMR (CD_3OD, 150 MHz): δ 151.9 (C-9), 144.1 (C-7), 139.5 (C-10a), 136.0 (C-8), 130.4 (C-1), 129.4 (C-2), 118.2 (C-6a), 102.7 (-OCH_2O-), 98.1 (C-10), 86.1 (C-6), 63.8 (C-3), 60.2 (-OCH_3), 58.1 (C-4a), 48.4 (C-12), 45.8 (C-10b), 40.6 (C-11), 32.2 (C-4).

AChE inhibitory activity assay – AChE inhibition activities of isolated compounds were performed by the previous report.[5] The analyses were conducted using Microsoft Excel, and the data are provided as means ± SD for at least three independent experiments. The AChE inhibitory effect of each sample was expressed in terms of the IC_{50} value, as performed by using TableCurve 2Dv4 software.

Results and Discussion

Dried whole plants of *C. latifolium* L. (5.0 kg) were extracted with methanol (15 L × 3) by using a multi-functional ultrasonic extraction (at 50ºC, 3 hours each time). The extracts were combined, filtered, and concentrated at

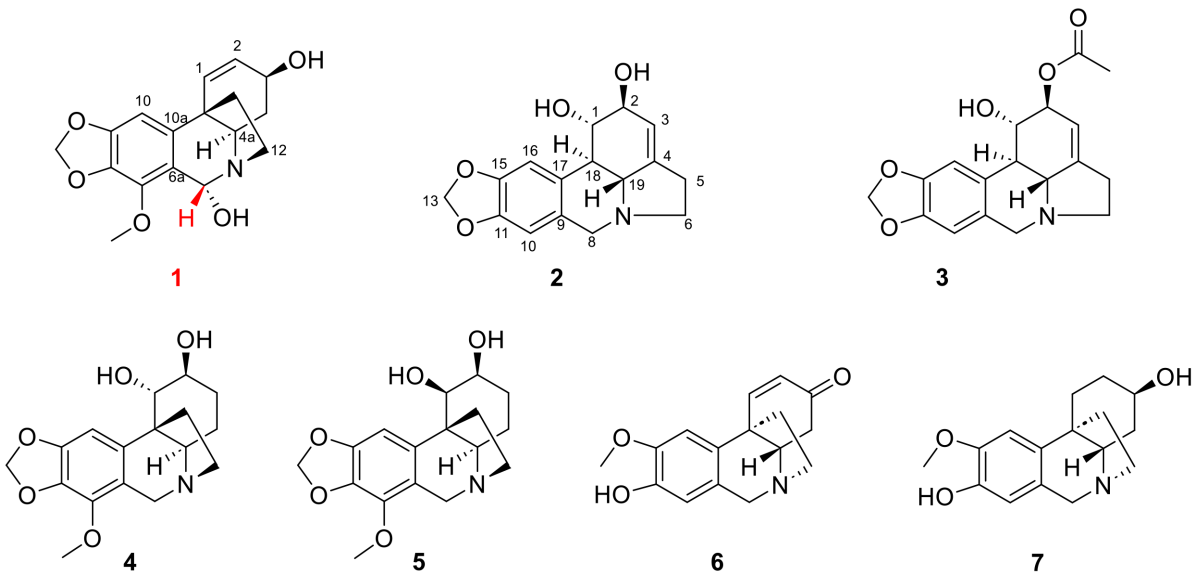

Fig. 1. Chemical structures of the isolated compounds (**1**–**7**) from the *Crinum latifolium* L.

low pressure to yield 520 g of crude methanol extract. The dichloromethane fraction of whole plants of *C. latifolium* were separated by multiple chromatography isolation techniques, resulting in one novel alkaloid (**1**) and six known compounds, lycorine (**2**),[6] 2-*O*-acetyllycorine (**3**),[7,8] deacetylbowdensine (**4**),[9] 1-epideacetylbowdensine (**5**),[10] 8-demethyl-3-oxomaritidine (**6**),[11] and (-)-marithamine (**7**)[12] identified by analyzing their NMR spectra and comparing with values in the literature (Fig. 1).

Compound **1** was obtained as a white amorphous powder; its chemical formula was determined to be $C_{17}H_{19}NO_5$ based on a pseudomolecular ion peak at *m/z* 318.1348 $[M+H]^+$ (calcd. for $C_{17}H_{19}NO_5$, 317.1263). The ^{1}H and ^{13}C-NMR data of **1** indicated that **1** was crinine-type alkaloid. The ^{1}H-NMR data indicated the presence of an aromatic proton signal [δ_H 6.64 (1H, s, H-10)]; two olefinic protons [δ_H 6.48 (1H, d, J = 10.2 Hz, H-1), and 5.88 (1H, dd, J = 10.2, 4.8 Hz, H-2)]; three methylene groups [δ_H 1.78 (1H, m, H-4α), 1.88 (1H, m, H-4β), 1.88 (1H, m, H-11α), 1.95 (1H, m, H-11β), 2.91 (1H, m, H-12α), and 3.36 (1H, m, H-12β)]; three methine protons [δ_H 5.34 (1H, s, H-6), 4.22 (1H, m, H-3), and 3.91 (1H, m, H-4a)]; a methylenedioxy group [δ_H 5.84 (1H, d, J = 1.2 Hz, -OCH_2O-), and 5.83 (1H, d, J = 1.2 Hz, -OCH_2O-)]; and three methoxy protons [δ_H 3.94 (3H, s, -OCH_3)]. The ^{13}C-NMR spectrum of **1** showed the signals of 17 carbon atoms, including of six aromatic carbons [δ_C 118.2 (C-6a), 144.1 (C-7), 136.0 (C-8), 151.9 (C-9), 98.1 (C-10), and 139.5 (C-10a)]; two olefinic carbons [δ_C 130.4 (C-1), and 129.4 (C-2)]; three methylene carbons [δ_C 32.2 (C-4), 40.6 (C-11), and 48.4 (C-12)]; three methine carbons [δ_C 86.1 (C-6), 63.4 (C-3), and 58.1 (C-4a)]; one non-protonated carbon [δ_C 45.8 (C-10b)]; one methylenedioxy carbon [δ_C 102.7 (-OCH_2O-)]; and one methoxy carbon [δ_H 3.94 (3H, s, -OCH_3)]. In addition, the key coupling constants ($J_{1,3}$ and $J_{2,3}$) and the NOESY experiment revealed that the relative configuration of **1** was the same as that of 6-hydroxypowelline (Fig. 3).[13] The ^{1}H-NMR spectrum showed the coupling constant (J = 4.8 Hz) between H-2 and H-3, and the absence of coupling between H-1 and H-3, indicate that the proton H-3 has an α-orientation.[14] Furthermore, the NOESY spectrum displayed key interactions between H-3 with H-

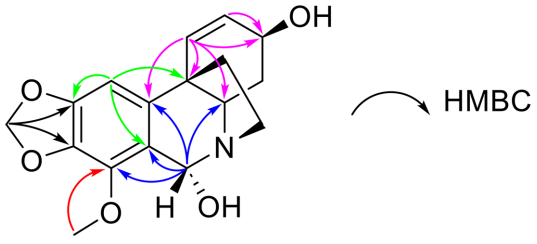

Fig. 2. Key HMBC interactions of compound **1**.

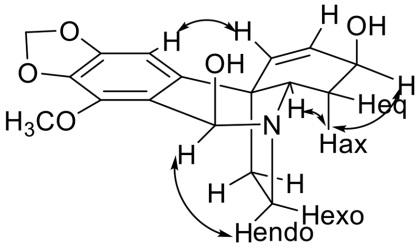

Fig. 3. Key NOESY interactions of compound **1**.

Table 1. The ^{1}H, ^{13}C-NMR data of compound **1** (CD_3OD)

No.	δ_C[a]	δ_H[b] (mult., *J* in Hz)
1	130.4	6.48 (1H, d, *J* = 10.2 Hz, H-1)
2	129.4	5.88 (1H, dd, *J* = 10.2, 4.8 Hz, H-2)
3	63.8	4.22 (1H, m, H-3)
4	32.2	1.78 (1H, m, H-4α) 1.88 (1H, m, H-4β)
4a	58.1	3.91 (1H, m, H-4a)
6	86.1	5.34 (1H, s, H-6)
6a	118.2	-
7	144.1	-
8	136.0	-
9	151.9	-
10	98.1	6.64 (1H, s, H-10)
10a	139.5	-
10b	45.8	-
11	40.6	1.88 (1H, m, H-11α) 1.95 (1H, m, H-11β)
12	48.4	2.91 (1H, m, H-12α) 3.36 (1H, m, H-12β)
-OCH_2O-	102.7	5.84 (1H, d, *J* = 1.2 Hz) 5.83 (1H, d, *J* = 1.2 Hz)
-OCH_3	60.2	3.94 (3H, s, –OCH_3)

[a]Recorded at 600 MHz, [b]Recorded at 150 MHz

4ax and between H-4ax with H-4a, suggesting the α-orientation of H-4a. β-configuration of H-6 was established by the absence of correlation between H-6 and H-4a/H*endo*. The absolute configuration of **1** was identified based on circular dichroism (CD) (Fig. S7, Supplementary material). The CD spectrum of **1** shows a negative Cotton effect signal at 294 nm ($\Delta\varepsilon$ – 0.46) and a positive signal at 245 nm ($\Delta\varepsilon$ + 0.40) (Fig. S7, Supplementary material), demonstrating the β-orientation of the 5,10b-ethanophenanthridine in the structure of compound **1**. In the NOESY spectrum of **1**, proton H-6 was α-configuration by the NOESY cross-peak from H-6 to H-12a. Therefore, compound **1** was elucidated as a new compound and named 6-epihydroxypowelline.

Acetylcholinesterase (AChE), a serine hydrolase, is required for the hydrolysis of acetylcholine (ACh), one of the most well-known neurotransmitters associated with Alzheimer's disease (AD). As a result, enzymatic suppression of AChE activity is an effective Alzheimer's disease treatment. AChE inhibitory activity was assessed for seven isolated alkaloids (**1**-**7**). The inhibitor, galanthamine, was used as a positive control. With IC_{50} values ranging from 32.65 ± 2.72 to 212.76 ± 8.30 μM, all alkaloids exhibited AChE inhibitory effects (relative to the positive control galanthamine 2.40 ± 0.45). With an IC_{50} value of 32.65 ± 2.72 μM, compound **3** exhibited the highest AChE inhibitory activity of all of them (Table 2).

Table 2. The IC_{50} values of the isolated compounds from the *Crinum latifolium* L.

Compound	IC_{50} (μM)
2	179.56 ± 2.87
3	32.65 ± 2.72
4	66.04 ± 7.05
5	87.85 ± 5.50
6	100.87 ± 18.19
7	212.76 ± 8.30
Galantamine (positive control)	2.40 ± 0.45

In summary, the alkaloid extract of the whole plants of *C. latifolium* L. led to the isolation of seven compounds, including a new alkaloid 6-epihydroxypowelline (**1**), and six known ones, including two lycorine-type alkaloids: lycorine (**2**), 2-*O*-acetyllycorine (**3**), and four crinane-type alkaloidsdeacetylbowdensine (**4**), 1-epideacetylbowdensine (**5**), 8-demethyl-3-oxomaritidine (**6**), and (-)-marithamine (**7**). All alkaloids had AChE inhibitory effects, with IC_{50} values ranging from 32.65 ± 2.72 to 212.76 ± 8.30 μM. Their effects were compared to the positive control galanthamine, which had an IC_{50} value of 2.40 ± 0.45 μM. Compound **3** had the most potent AChE inhibitory action among all tested compounds, with an IC_{50} value of 32.65 ± 2.72 μM.

Acknowledgments

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Conflicts of Interest

All of the authors declare that this research has no conflict of interest.

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