Alkaloids isolated from *Hippeastrum reticulatum* (L'Hér.) Herb. and their acetylcholinesterase inhibitory activities

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Abstract

Background: *Hippeastrum reticulatum* (L.Hér.) Herb is a species of the *Hippeastrum* Herb. genus. Screening studies have shown that this species has the ability to inhibit the enzyme acetylcholinesterase. So far, research on this species is still very limited. The purpose of this study is to provide some more informations about the chemical composition and bioactive of isolated compounds from this species. **Materials and method:** Bulbs of *Hippeastrum reticulatum* was collected in Thua Thien Hue province in May 2018. The compounds were isolated by using various chromatographic methods and their structures were identified by 1D and 2D-NMR spectroscopic methods in reference to the literature. The acetylcholinesterase inhibitory activity was determined by Ellman's microplate colorimetric method. **Results and conclusions:** Two alkaloids including N-methyltiramine (1), narciclasine-4-O-β-D-xylopyranoside (2) was isolated from bulbs of *Hippeastrum reticulatum* (L'Hér.) Herb. These compounds were isolated from *Hippeastrum* Herb. genus for the first time. Compound 2 showed moderate acetylcholinesterase inhibitory activity, with IC_{50} value of 70.06 ± 1.46 µg/mL.

Keywords*: Hippeastrum reticulatum (L'Hér.) Herb., alkaloid, N-methyltiramine, narciclasine-4-O-β-D-xylopyranoside.*

1. INTRODUCTION

Hippeastrum Herb. is a large genus of the Amaryllidaceae family of more than 90 species recorded. The species belong to this genus possess several important biological activities, such as antibacterial, antioxidant, antiviral, acetylcholinesterase inhibitors... In Vietnam, this genus has 2 species: *Hippeastrum equestre* and *Hippeastrum reticulatum*. Screening studies indicated that both species have a potent inhibitory effects of acetylcholinesterase, particularly *Hippeastrum reticulatum*. However, there are still few studies on *Hippeastrum reticulatum* species in Vietnam so far.

The aim of this study is to contribute knowledge to the chemical composition and bioactivity of isolated compounds from *Hippeastrum reticulatum*.

2. MATERIALS AND METHODS

2.1. Plant materials

The bulb of *Hippeastrum reticulatum* (L'Hér.) Herb. was picked up in May 2018 in Thua Thien Hue province, Vietnam. Its identify was confirmed by Dr. Vu Tien Chinh, Vietnam National Museum of Nature, the Vietnam Academy of Science and Technology.

2.2. Extraction and isolation

The bulb of *Hippeastrum reticulatum* (L'Hér.) Herb. was washed, dried at 50°C (12.5 kg) then powdered into powder, extracted with methanol (20 L × 3 times) by immersion at room temperature to yield extract. This extract was subjected to Diaion HP-20 column chromatography. Pass water through the column to remove water-soluble components, then elute the compounds with methanol to obtain a methanol extract (150 g).

The methanol extract was acidified with 2% HCl to pH 2 and then extracted with ethyl acetate (1 L × 3 times) to obtain ethyl acetate (EtOAc) fraction (60 g). The remaining acid solution was alkalined with NH_3 to pH 10 and then extracted with dichloromethan (CH_2Cl_2) (1L × 3 times) to obtain C fraction (30 g).

The C fraction (30g) was subjected to silica gel column chromatography, eluted with CH_2Cl_2 – methanol – H_2O (5:1:0.1, v/v/v) to obtain 5 fractions, C1-C5. Fraction C1 (4g) was subjected to reverse-phase RP-18 silica gel column chromatography eluted with aceton – H_2O (5:1, v/v) to obtain 6 fractions, C1.1-C1.6. Fraction C1.3 (600mg) was subjected to silica gel chromatography, eluted with CH_2Cl_2-methanol-NH_3 (10:1:0.1, v/v/v) to obtain 6 fractions, C1.3.1-C1.3.6. Fraction C1.3.2 (120mg) was subjected to reverse-phase RP-18 silica gel column chromatography, eluted with methanol-H_2O (3:1, v/v) to obtain compound 1 (8 mg).

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Fraction C4 (8 g) was subjected to reverse-phase RP-18 silica gel column chromatography eluted with methanol – H_2O (3:1, v/v) to obtain 5 fractions, C4.1-C4.5. Fraction C4.3 (1 g) was subjected to silica gel column chromatography, eluted with CH_2Cl_2 – methanol (10:1, v/v) to obtain 4 fractions, C4.3.1-C4.3.4. Fraction C4.3.3 (60 mg) was further purifed by Sephadex LH-20, eluted with methanol to obtain compound **2** (15 mg).

2.3. Acetylcholinesterase inhibition assay

The inhibitory activities of acetylcholinesterase (AchE) were measured using modified Ellman's method [3]. Principle of the method: ATCI (acetylthiocholine iodide) substrate is hydrolyzed by the catalysis of AChE to create thiocholine. Thiocholine reacts with DTNB solution (5,5'-dithiobis (2- nitrobenzoic acid) reagent to release yellow compound 5-thio-2-nitrobenzoic acid. Measure the absorbance of the solution formed at 405 nm to determine the AChE inhibitory activity. The positive control used is Galantamine.

The procedure of the test method (Table 1): add pH 8 buffered buffer, sample and 0.25 IU/mL AChE enzyme solution, successively, to each well of the 96-well plate. The mixture is well mixed and incubated for 15 minutes at room temperature. After that, DTNB test 2.4 mM and ATCI substrate solution 2.4 mM were added to the mixture and mixed well. Continue incubating the mixture for 15 minutes at room temperature, then the solution is measured for absorbance at 405 nm. Absorbance is measured on the ELISA Micropate Reader EMR 500 (US). Each test sample was repeated three times. The AChE inhibitory activity of the sample is calculated by the formula:

$$I\ (\%) = \frac{S}{E} \times 100$$

Where E and S were the enzymatic activities with and without the tested sample, respectively. The AChE inhibitory activity of each sample was expressed in terms of the concentration (in µg/mL) required to inhibit the hydrolysis of AchE by 50% (the IC_{50} value), which was calculated from the logarithmic dose-inhibition curve.

Table 1. The composition of the reaction evaluates the AChE inhibitory activity.

No.	Compositions	Test sample (µl)	Control sample (µl)
1	Phosphate buffer pH 8	140	140
2	Test sample	20	0
3	DMSO 10%	0	20
4	AChE 0,25 IU/ml	20	20
5	DTNB 2,4 mM	10	10
6	ATCI 2,4 mM	10	10
Total volume (µl)		200	200

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. The ^{1}H NMR spectrum of **1** in methanol-d_4 showed typical signals of four protons of para-disubstituted benzene ring at δ_H 6.79, 7.11 (each, 2H, d, J = 8.5 Hz), two methylene groups at δ_H 2.90, 3.21 (each, 2H, t, J = 7.5 Hz), and a methyl group at δ_H 2.72 (3H, s). Thus, compound **1** was determined as *N*-methyltyramine [4].

Compound **2** was obtained as a creamy needles. The ^{1}H NMR spectrum of **2** in methanol-d_4 showed typical signals of an aromatic proton at δ_H 6.79 (s), an olefinic proton at δ_H 6.22 (m), a methylenedioxy group at δ_H 6.06 (s), and an anomeric proton at δ_H 4.41 (d, J = 7.5 Hz). The signals in the range δ_H 3.2-4.4 indicated hydrogen attached to carbon containing hetero atom (O or N).

The ^{13}C NMR and HSQC spectra showed the presence of 19 carbon signals corresponding to two methylenes, ten methines and seven quaternary carbons. The presence of carbonyl carbon was clearly seen via signal at δ_C 170.5. Meanwhile, the HSQC cross-peak between two protons at δ_H 6.22 and carbon at δ_C 103.8 confirmed the appearance of methylendioxy group. In addition, the HSQC spectrum showed the protons at δ_H 6.22 (m, H-1), 4.30 (m, H-2), 4.07 (m, H-3), 4.02 (m, H-4), 4.43 (brd, J = 10.0 Hz, H-4a), 6.79 (s, H-10), 4.41 (d, J = 7.5 Hz, H-1'), 3.33 (overlapped, H-2'), 3.40 (t, J = 9.0 Hz, H-3'), 3.61 (m, H-4'), 4.05 (overlapped, H-5'a), and 3.33 (overlapped, H-5'b) correlated with carbons δ_C 123.80 (C-1), 70.3 (C-2), 72.0 (C-3), 79.6 (C-4), 52.0

(C-4a), 97.3 (C-10), 104.0 (C-1'), 74.8 (C-2'), 77.6 (C-3'), 71.0 (C-4'), 67.2 (C-5'), respectively.

In the HMBC spectrum, the correlations of H-2 to C-1/C-4/C-10b (δ_C 133.3), of H-3 to C-1/C-4a, of H-4 to C-4a, of H-4a to C-1/C-3/C-4/C-10a (δ_C 132.1)/C-10b, H-10 to C-8 (δ_C 135.9)/C-9 (δ_C 154.5)/C-6a (δ_C 107.0)/C-10a, and of methylendioxy protons to C-8/C-9 were observed (Figure 2). These evidences led to the construction of narciclasine skeleton.

The correlation of H-2' to C-3'; of H-3' to C-2'/C-4'; of H-4' to C-3'; of H-5' to C-4'/C-3' confirmed the structure of pentose moiety. The coupling constant of H-1' (J = 7.5 Hz) indicated the β-configuration of sugar unit. The series of carbon signals at δ_C 104.0, 74.8, 77.6, 71.0, 67.2 were indicative of β-D-xylopyranoside moiety [5]. The pentose sugar was further suggested to be xylopyranoside by comparison with the reported values in literature [10]. Notably, the position of D-xylopyranosyl at C-4 was confirmed by the HMBC cross-peaks H-1'/C-4, H-4/C-1'. Spectral data of compound **2** was compared with reference [2]. Based on the above evidences, compound **2** was confirmed as narciclasine-4-O-β-D-xylopyranoside.

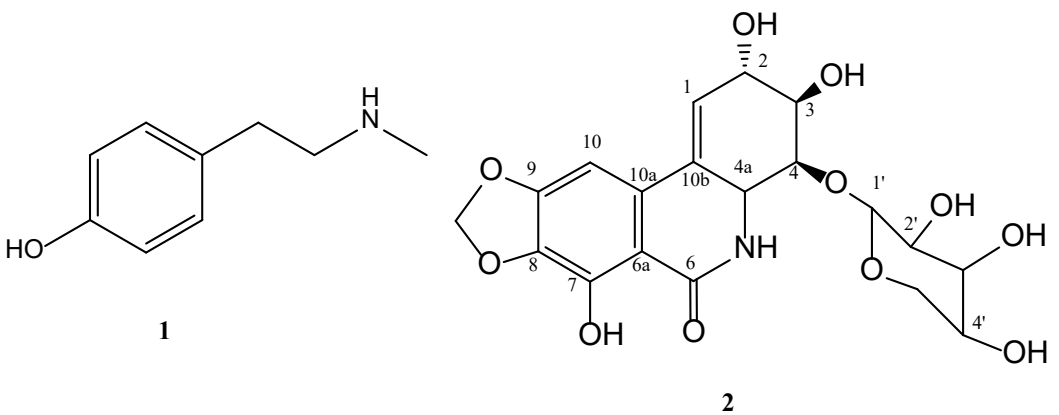

Figure 1. Structures of **1**, **2** isolated from *Hippeastrum reticulatum*.

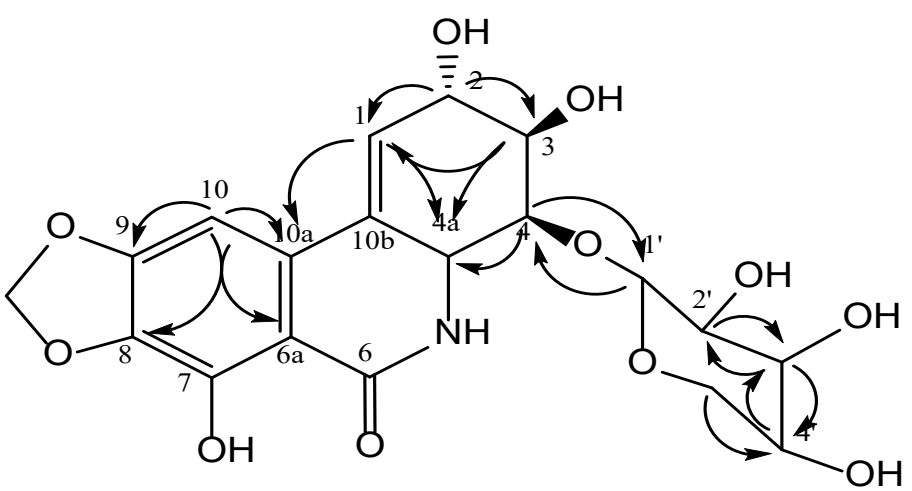

Figure 2. Key HMBC ($^1H \rightarrow ^{13}C$, arrows) correlations of **2**.

Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of **2** in methanol-d_4 [δ (ppm), *J* (Hz)]

Position	δ_C	δ_H
1	123.8	6.22 t (4.0, 6.5)
2	70.3	4.30 m
3	72.0	4.07 m
4	79.6	4.02 m
4a	52.1	4.43 m
6	170.5	-
6a	107.0	-
7	146.0	-
8	135.9	-
9	154.5	-
10a	132.1	-
10b	133.3	-
10	97.3	6.79 s
1’	104.0	4.41 (d, 7.5)
2’	74.8	3.33 m
3’	77.7	3.39 m
4’	71.1	3.61 m
5’	67.3	4.05 m
-OCH_2O-	103.8	6.06 brs

The AChE inhibitory activities of the isolated compounds were assessed at various concentrations. Galanthamine was used as a positive control. The results are summarized in Table 3. Results showed that compound **2** did not show the ability to inhibit AChE at the test concentration (500 µg / mL). Compound **1** have a moderate AChE inhibiting effect with IC_{50} values of 70,06 ± 1,46. N-Methyltyramine (1) has been isolated from a variety of plant species such as Coryphantha missouriensis (Cactaceae) [6], Pilosocereus maxonii (Rose) [7], and Acacia schweinfurthii (Leguminosae) [9]. Previous studies have shown that N-methyltyramine (1) increases blood pressure in the anaesthetized rat, relaxes guinea pig ileum and increases both the force and rate of contraction of guinea-pig right atrium by inducing the release of noradrenaline [1]. However, this is the first report of AChE inhibition activity of N-methyltyramine (1).

Table 3. *In vitro* AChE inhibitory activities of compounds **1** and **2**

Compounds	IC_{50} (µg/mL)
N-methyltyramine (**1**)	70.06 ± 1.46
Narciclasine-4-O-β-D-xylopyranoside (**2**)	> 500
Galantamine	0.33 ± 0.01

4. CONCLUSION

Two alkaloids, N-methyltiramine (**1**), narciclasine-4-O-β-D-xylopyranoside (**2**), were isolated from the bulb of *Hippeastrum reticulatum* and identified by comparison of their 1D and 2D NMR spectroscopic with those reported in the literature. These compounds were isolated from *Hippeastrum* Herb. genus for the first time. Compound **1** showed moderate inhibitory activities against AChE, with IC_{50} values of 70.06 ± 1.46 µg/mL.

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