SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:2 (2014) 199-202

records of natural products

Cholinesterase Inhibiting Activity and A New Piperidine Alkaloid from *Lobelia laxiflora* L. Roots (Campanulaceae)

Enas H. Abdel Rahman and Azza R. Abdel Monem*

Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.

(Receive December 27, 2012; Revised June 4, 2013; Accepted, January 10, 2014)

Abstract: The total alkaloidal fraction of *Lobelia laxiflora* L. roots was tested for cholinesterase inhibiting activity using spectrophotometric method. The IC_{50} value of the alkaloidal fraction recorded was close to that of eserine (286.3 µg/mL and 270 µg/mL, respectively). This biologically active alkaloidal fraction was subjected to a phytochemical study to isolate and identify its major constituents. Two piperidine alkaloids, N-methyl-2(2'-methoxybutyl),6(2"-hydroxybutyl)-Δ^3-piperidine (**1**) and N-methyl-2(2'-hydroxybutyl),6(2"-hydroxybutyl)-Δ^3-piperidine (**2**), were isolated. The structures of the two compounds were established based on their spectral data, including MS, ^{1}H- and ^{13}C-NMR, COSY, HMQC and HMBC spectral experiments. Compound (**1**) is a new natural compound while compound (**2**) was previously isolated from the aerial parts of the same plant.

Keywords: *Lobelia laxiflora*; piperidine alkaloids; cholinesterase inhibiting activity.

1. Plant Source

The whole plant of *Lobelia laxiflora* L. was collected from a nursery at Saft El-laban, Giza, Egypt, in April 2011 and was kindly identified by Dr. Mohamed el Gebaly, National Research Institute, Doki, Giza, Egypt and Madam Treze Labib, head of taxonomist, El-Orman Garden, Giza, Egypt. A voucher specimen was kept in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt (012.10.32).

2. Previous Studies

Neurological disorders like Alzheimer's or Parkinson's diseases represent a public health problem. Thus, the discovery of effective agents for the treatment of these diseases is one of the major challenges in herbal medicine [1,2]. Campanulaceae is a family of about 70 genera and 2000 species, most are tropical or subtropical herbs, a few trees or shrubs. Genus *Lobelia* (200-300 species), belonging to subfamily Lobelioideae which has sometimes considered as a separate family (Lobeliaceae) [3], has a long history of therapeutic usage ranging from respiratory stimulant and

* Corresponding author: E-Mail: azzaramy@yahoo.com

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/19/2014 EISSN:1307-6167

emetic to tobacco smoking cessation [4,5]. This genus characterized by the presence of piperidine alkaloid, which constitute a large class of compounds having various biological activities [6]. Lobeline appears to be the most biologically active alkaloid of *Lobelia* plants.

The most promising bioactivity of lobeline concerns its use in the treatment of CNS diseases [5]. A previous study showed that lobeline improves memory in rodents, probably due to its involvement in cholinergic mechanisms of neurotransmission [7], which claimed to be the most common cause of Alzheimer's disease [8]. *Lobelia laxiflora* L. is a perennial blooming bush, 1 m in height, spread in Central America. A previous study on the aerial parts of this plant resulted in isolation of three new piperidine alkaloids. The isolated alkaloids showed anti-inflammatory activity [9]. This work was carried out on the alkaloidal fraction of *L. laxiflora* L. roots to evaluate its possible cholinesterase inhibiting activity and to isolate and identify the biologically active alkaloids.

3. Present Study

Acetylcholinesterase (Electric-eel EC 3.1.1.7), acetylthiocholine iodide and 5,5´-dithiobis[2-nitrobenzoic-acid] (DTNB) were purchased from Sigma (St. Louis, MO, USA). Buffers and other chemicals were of analytical grade. Acetylcholinesterase inhibiting activity was measured according to a slightly modified spectrophotometric method [10]. Acetylthiocholine iodide was used as substrate, while DTNB was used for the measurement of cholinesterase activity. 100 mM sodium phosphate buffer (pH 8.0, 140 µL), DTNB (10 µL), test solution (20 µL) and acetylcholinesterase (20 µL) were mixed and incubated for 15 minutes (25º C). The reaction was then initiated by the addition of acetylthiocholine iodide (10 µL). The hydrolysis of acetylthiocholine iodide was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide at a wavelength of 412 nm (15 min). Sample was dissolved in DMSO. All the reactions were performed in triplicate in 96-well micro title plates and monitored in a *SpectraMax 340* (Molecular Devices, USA) spectrometer. The concentrations of the sample that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC_{50}) were determined by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values. The IC_{50} values were then calculated using a software program (GraphPad Prism, version 5.01, Inc., 2007, San Diego California USA).

The alkaloidal fraction exhibited a potent cholinesterase inhibiting activity, and its recorded IC_{50} value was found to be equal to 286.3 µg/mL comparing to 270 µg/mL for eserine as a reference standard.

Dried powdered roots of *Lobelia laxiflora* L. (160 g) were extracted with a mixture of EtOH (95%)-H_2O (9:1, v/v) till exhaustion. The combined extracts were evaporated under reduced pressure ($\leq 60^oC$) to give 2 g of brown residue. The residue was acidified with 3% HCl (50 mL) and left overnight at room temperature then filtered. The acidic solution was alkalinized with 25% NH_4OH then extracted with $CHCl_3$ (4 x 50 mL). The $CHCl_3$ extract was evaporated under reduced pressure to obtain the alkaloidal fraction (350 mg). This fraction (250 mg) was chromatographed on successive silica gel columns using gradient elution with $CHCl_3$:MeOH mixtures to obtain compounds **1** (32 mg) and **2** (15 mg).

Precoated silica gel plates 60 F 254 (E-Merck) were used for TLC using S_1 [$CHCl_3$:MeOH:NH_4OH (9:1:2 v/v/drops)] as solvent system. The chromatograms were visualized by spraying with Dragendorff's spray reagent.

Compound 1: Brown resinous substances; R_f = 0.5 (TLC, S_1); MS *m/z*: 255 $[M]^+$; ^{1}H-NMR ($CDCl_3$, δ ppm): 0.86 (3H, t, *J*=7.5 Hz, H-4"), 0.92 (3H, t, *J*=7.5 Hz, H-4'), 1.21 (2H, m, 3' a and 3" a), 1.45 (4H, m, H-3' b, 3" b, 1' a and 1" a,), 1.80 (2H, m, H-1' b and 1" b), 2.08 (2H, m, H-5), 2.62 (3H, s, N-C$\underline{H_3}$), 2.83 (3H, s, OC$\underline{H_3}$), 3.62 (1H, m, H-6), 3.76 (1H, m, H-2"), 3.96 (1H, m, 2'), 4.05 (1H, m, H-2), 5.37 (1H, dd, *J*=1.9, 10 Hz, H-3), 5.77 (1H, m, H-4). ^{13}C-NMR ($CDCl_3$, δ ppm): 9.59 (C-4"), 10.2 (C-4'), 26.43 (N-CH_3), 26.64 (C-5), 30.58 (C-3"), 30.88 (C-3'), 38.23 (C-1"), 38.90 (C-1'), 41.86 (OCH_3), 62.48 (C-6), 69.86 (C-2), 70.23 (C-2'), 71.36 (C-2"), 124.28 (C-3), 125.66 (C-4).

Compound 2: Brown resinous substances; R_f = 0.45 (TLC, S_1); MS *m/z*: 241 $[M]^+$; ^{1}H-NMR ($CDCl_3$, δ ppm): 0.88 (3H, t, *J*=7.5 Hz, H-4"), 0.90 (3H, t, *J*=7.5 Hz, H-4'), 1.18 (2H, m, 3' a, H-3" a), 1.42 (4H, m, H-3' b, 3" b, 1' a and 1" a), 1.71 (2H, m, H-1' b and 1" b),), 2.04 (2H, m, H-5), 2.33 (3H, s, N-CH_3), 3.65 (1H, m, H-6), 3.71 (2H, m, H-2' and H-2"), 4.17 (1H, m, H-2), 5.39 (1H, dd, *J*=1.9, 10 Hz, H-3), 5.75 (1H, m, H-4). ^{13}C-NMR ($CDCl_3$): 9.67 (C-4"), 9.71 (C-4'), 23.68 (N-CH_3), 25.79 (C-5), 30.72 (C-3"), 30.91 (C-3'), 37.95 (C-1"), 38.66 (C-1'), 60.19 (C-6), 68.08 (C-2), 71.14 (C-2"), 72.31 (C-2'), 125.55 (C-4), 126.19 (C-3).

Spectral analysis was carried out in Institute of Organic Chemistry, University of Gottingen, Germany. Mass spectrum was operated on LTQ-Orbitrap Velos spectrometer. NMR analysis was performed using ^{1}H-NMR (300 MHz), ^{13}C-NMR (75 MHz): Bruker Avance spectrophotometer.

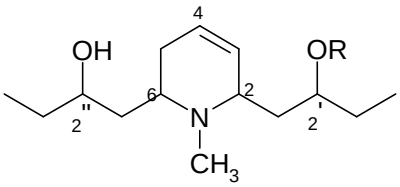
Mass spectrum of compound **1** showed peaks at *m/z* 255, corresponding to molecular formula $C_{15}H_{29}NO_2$ and two fragments at 182 $[M\text{-}C_4H_9O]^+$ and 168 $[M\text{-}C_5H_{11}O]^+$ obtained after cleavage of one of the two side chains. A fragment at *m/z* 96 is typical for N-methylated-Δ^3-piperidine. NMR spectrum showed signals at δ_H 3.96 and 3.76 each (1H, m) and at δ_C 71.36 and 70.23 indicate the presence of two oxygenated tertiary carbons. Singlet at δ_H 2.83 (3H) assigned for a methyl of a methoxy group. According to HSQC this methoxy group was located on a carbon at δ_C 41.86. Signals at δ_C 69.86 and 62.48 corresponding to two carbons neighbored to N atom (C-2 and C-6). Their corresponding protons were displayed at δ_H 4.17 and 3.65, respectively. Singlet at δ_H 2.62 (3H) corresponding to N-C$\underline{H}_3$ which located on carbon at δ_C 26.43 according to HSQC. Δ^3 is represented by signals of two olefinic protons at δ_H 5.77 and 5.37 and their respective carbons at δ_C 124.28 and 125.66. By comparing the spectral data of compound **1** with the published data of similar compounds [9,11,12], it was identified as N-methyl-2(2'-methoxybutyl), 6(2"-hydroxybutyl)-Δ^3-piperidine, which is a new natural compound. The identification was confirmed by 2D-NMR spectral analysis including HSQC, HMBC and COSY.

Compound **2** showed $[M]^+$ at *m/z* 241 corresponding to molecular formula $C_{14}H_{27}NO_2$ and is typical to $[M]^+$ of compound **1** after removal of CH_3 of the methoxy group. NMR spectral data of compound **2** showed the absence of the signals at δ_H 2.83 (OC$\underline{H}_3$) and δ_C 41.86 (O$\underline{C}H_3$). Thus this compound was identified as N-methyl-2(2'-hydroxybutyl), 6(2"-hydroxybutyl)- Δ^3-piperidine. This compound was previously isolated from the aerial parts of the same plant [9].

The alkaloidal fraction of *Lobelia laxiflora* L. roots showed potent cholinesterase inhibiting activity comparing to eserine. Previous studies recorded similar activity for lobeline [7]. Thus, this observed bioactivity could be attributed to the piperidine alkaloid content of the root, which was isolated during this course of study. This result suggests the use of *Lobelia laxiflora* L. as a herbal remedy for treatment of neurological disorders like Alzheimer's disease. Further pharmacological and toxicological studies are recommended to establish this finding.

Acknowledgment

The authors are sincerely gratitude to Dr. Mohamed Nabil, Institute of Pharmaceutical Biology, Technical University of Braunschweing, for performing the spectral analysis. Also, we thank Dr. Mariane George Tadrros, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University, for performing cholinesterase inhibiting activity.



Compound	1	2
R	CH_3	H

Figure 1. Structures of Compounds **1** and **2**

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] M. W. Holladay, M. J. Dart and J. K. Lynch (1997). Neuronal nicotinic acetylcholine receptors as targets for drug discovery, *J. Med. Chem.* **4**, 4169-4194.

[2] G. K. Lloyd and M. Williams (2000). Neuronal nicotinic acetylcholine receptors as novel drug targets, *J. Pharm. Exp. Ther.* **292**, 461-467.

[3] W.C. Evans (2002). *Trease and Evans Pharmacognosy*. W. B. Saunders, 15 Ed., Edinburgh, London, New York, Philadelphia, St Louis, Sydney, Toronto, p 35.

[4] A. R. Buchhalter, R. V. Fant and J. E. Henningfield (2008). Novel pharmacological approaches for treating tobacco dependence and withdrawal current status, *Drugs.* **68:8**, 1067-1088.

[5] X. F. Francois and L. Jacques (2004). History, chemistry and biology of alkaloids from *Lobelia inflata*, *Tetrahedron.* **60**, 10127-10153.

[6] A. D. Elbein and R. J. Molyneux (1987). *In alkaloids: Chemical and Biological Perspectives.* S.W. Pelleetier, Ed., Wiley, New York, Vol. **5**, p 1-54.

[7] M. W. Decker, M. J. Majchrzak and D. J. Anderson (1992). Effect of nicotine on spatial memory deficits in rats with septal lesions, *Brain Res.* **572**, 281-285.

[8] P. A. Newhouse , A. Potter, M. Kelton and J. Corwin (2001). Nicotinic treatment of Alzheimer's disease, *Biol. Psychiatry.* **49**, 268-278.

[9] S. Philipov, R. Istatkova, N. Ivanovska, P. Denkova, K. Tosheva, H. Navas and J. Villegas (1998). Phytochemical study and anti-inflammatory properties of *Lobelia laxiflora* L, *Z. Naturforsch.* **53c**, 311-317.

[10] G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol.* **7**, 88-95.

[11] H. C. Krebs and H. Ramiarantsoa (1998). Piperidine alkaloids and other constituents of *Dialypetalum floribundum*, *Phytochemistry.* **48:5**, 911-913.

[12] V. U. Ahmad, A. Kamal and M. S. Ali (2002). Aspertins A-D: Further Piperidine Alkaloids from *Andrachne aspera* (Euphorbiaceae), *Turk J Chem.* **26**, 245-250.