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Acetylcholinesterase Inhibitive Activity-Guided Isolation of Two New Alkaloids from Seeds of *Peganum Nigellastrum* Bunge by an *In Vitro* TLC- Bioautographic Assay

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Acetylcholinesterase inhibitors (AChEIs) currently form the basis of the newest drugs available for the treatment of Alzheimer's disease. For the aim of screening effective AChEIs, the methanol extracts of the seeds of genus *Peganum* were found to show significant inhibitory activity of acetylcholinesterase enzyme (AChE) using an *in vitro* TLC-bioautographic assay. In further studies to seed of *P. nigellastrum* Bunge, activity-guided fractionation led to the isolation of two new alkaloids nigellastrine I (9) and nigellastrine II (10), and along with eight known alkaloids, vasicinone (1), vasicine (2), harmine (3), deoxyvasicinone (4), deoxyvasicine (5), harmaline (6), harmol (7), harman (8), in which harmol and harman were first isolated from species *P. nigellastrum* Bunge. As active constituents, all compounds showed good inhibitory activities against AChE. The results of *in vitro* semi-quality TLC-bioautographic assay showed that harmine, harmaline and harmol displayed a similar AChE inhibitive activities comparing to galanthamine. These results indicated that these alkaloids in *P. nigellastrum* Bunge could be a potent class of AChEIs.

Key words: *Peganum nigellastrum* Bunge, Acetylcholinesterase, TLC-bioautographic assay, Alzheimer's disease, Alkaloid, Nigellastrine I, Nigellastrine II

INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, which primarily affects the elderly population, and is estimated to account for 50-60 percent of dementia cases in those over 65 years of age (Francis et al., 1999). AD is a highly prevalent neurodegenerative disease, characterized initially by selective loss of cholinergic neurons in the basal forebrain (Whitehouse et al., 1982), followed by cognitive and behavioral impairments that progressively disrupt activities of daily living, leading to institutionalization and eventually death (Blennow et al., 2006). Cholinergic neurotransmission dysfunction in the brain contributes to the salient cognitive decline in AD. The loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter, acetylcholine. Acetylcholinesterase inhibitors (AChEIs) currently form the basis of the newest drugs available for the management of this disease. AChEIs are general chemical classes, such as physostigmine, galanthamine, tacrine and heptylphysostigmine, and have been tested for the symptomatic treatment of AD (Becker et al., 1988; Fulton and Benfield, 1996). However, the nonselectivity of these drugs, and their limited efficacy, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are some of the severe limitations to their therapeutic success (Bores et al., 1996; Forette et al., 1999). It is necessary for other studies on the AChEIs

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derived from medicinal plants or from designing and development of synthesis.

Genus *Peganum* (*Zygophy1laceae*), including six species and one variety, is perennial plant native of the central Asia region but now grows wild and discontinuously in Africa, the middle east, India, south America, Mexico, and southern USA. It was reported that three species, *P. harmala* Linn, *P. multisectum* (Maxim) Bobr, and *P. nigellastrum* Bge, grow wild in northwest China (Ma and Wang, 1998; Ma and Li, 1996; Wang et al., 2002). In China, the entire plant and seeds of *P. harmala* Linn, listed in the Uygur Drug Standard of Ministry of Public Health (Pharmacopoeia Commission, 1998), have been used as traditional folk medicinal substances in the Xinjiang Uygur autonomous region and in the Mongolian autonomous region. It has been used to treat diseases such as cough, asthma, rheumatoid arthritis and swelling pain, etc. Previous studies on the plants from the genus *Peganum* have reported the isolation of various alkaloids and its biological properties such as psychopharmacological and behavioral effects in the brain (Airaksinen and Kari, 1981; Pfau and Skog, 2004), cytotoxicity and antitumour activities (Lamchouri et al., 2000; Lamchouri et al., 1999; Sobhani et al., 2002;), affinity to benzodiazepine receptors (Glennon et al., 2000; Husbands et al., 2001), tremoregenesis (Lutes et al., 1988), cardiovascular actions (Aarons et al., 1977), antimicrobial activity (Ahmad et al., 1992), anti-parasitic activity (Di Giorgio et al., 2004), and strong reversible inhibition of monoamine oxidase (MAO) (Kim et al., 1997; Schwarz et al., 2003).

Despite a number of studies on genus *Peganum*, few have regarded the active principals and AChE inhibitory activity. In continuation of our on-going study to search the bioactive constituents of seeds of genus *Peganum* by a rapid bioautographic assay on TLC plates developed for the screening of AChE inhibition by plant extracts (Marston et al., 2002), it was found that the alkaloids fraction showed potential inhibitory effects on the AChE activity. Herein, the isolation and structure elucidation of two new alkaloids (**9**, **10**) and 8 known alkaloids vasicinone (**1**), vasicine (**2**), harmine (**3**), deoxyvasicinone (**4**), deoxyvasicine (**5**), harmaline (**6**), harmol (**7**), harman (**8**) from seeds of *P. nigellastrum* Bunge, as well as the AChE inhibitory of the isolated compounds are reported.

MATERIALS AND METHODS

Materials

The seeds of genus *Peganum, P. harmala* Linn, *P. multisectum* (Maxim) Bobr, and *P. nigellastrum* Bunge, and a probable *P.* variety were collected in wild in Xinjiang, Ningxi, Gansu, inner Mongolia and Shaanxi provinces, China, in september 2006. All of these plant materials were authenticated by professor Chang-hong Wang and the voucher specimens were deposited at the herbarium of the Shanghai R&D center for standardization of traditional chinese medicine, Shanghai, China.

Acetylcholinesterase from electric eel (EC3.1.1.7) was purchased from Sigma (St. Louis, MO; product no. C3389). Bovine serum albumin, fast blue B salt, Tris, and 1-naphthyl acetate were obtained from Sigma. Galanthamine was obtained from Shanghai R&D centre for standardization of Chinese medicines. All other chemical solvents used for isolation were of analytical grade. Column chromatography (CC) and preparative-TLC were carried out using precoated silica gel 60 F_{254} (0.5 mm, obtained from Qingdao Ocean Chemical Co.). C_{18}-ODS (Merck) and Sephadex LH-20 (Amersham Biosciences, GE Health Care) were used. NMR Spectra: at 500 MHz for ^{1}H and at 125 MHz for ^{13}C on a Bruker AV-500 spectrometer. ESI-MS and HR-ESI-MS: LCQ Deca XP^{plus} (Thermo Finnigan) and Finigan MAT95 spectrometers, respectively.

Samples test and fraction screening on AChE inhibitory activity

Samples preparation and TLC analysis: In each case, 0.5 g seeds of *P. harmala* Linn, *P. multisectum* (Maxim) Bobr, and *P. nigellastrum* Bunge, and a probable *P.* variety were extracted overnight with 90% methanol (15 mL) before ultrasound extraction 20 min, and filtration, respectively.

In order to establish active fraction by the TLC bioautographic assay, 10 and 1 μL of the four stock solutions in methanol were applied onto the TLC plate A and plate B respectively, and migrated with ethyl acetate-methanol-ammonia (10:1.5:0.5) in duplicates. The developed plate A was firstly inspected under ultraviolet light (366 nm) and then was colorated by spraying Dragendorff's reagent (potassium heptaiodobismuthate solution) and plate B was introduced a bioautographic assay (Marston et al., 2002), respectively.

TLC bioautographic assay: The TLC bioautographic assay was carried out as described previously (Marston et al., 2002) by some modification. AChE (1000 U) was dissolved in 150 mL of 0.05 M Tris-hydrochloric acid buffer at pH 7.8; bovine serum albumin (150 mg) was added to the solution in order to stabilize the enzyme during the bioassay. The stock solution was kept at 4°C. TLC plates were eluted with methanol in order to wash them, and were thoroughly