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SHORT COMMUNICATION

Acetylcholinesterase inhibition activity of acetylated depsidones from *Lobaria pulmonaria*

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As part of our ongoing project of new acetylcholinesterase inhibitors from lower marine and terrestrial species, a phytochemical investigation was conducted on a foliose lichen, *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae), from Bosnia and Herzegovina. The study led to the isolation of a mixture of acetylated depsidones which showed a moderate activity (0.5 μg) in the acetylcholinesterase inhibition test on Thin-layer chromatography plate. Our results indicate for the first time the significance of depsidones, highly specific metabolites from lichen species, in searching for these inhibitors which still represent the best drugs currently available for the management of Alzheimer's disease.

Keywords: lichen; *Lobaria pulmonaria*; depsidones; acetylcholinesterase inhibition activity; Alzheimer's disease

1. Introduction

Lobaria pulmonaria (L.) Hoffm. (Ascomycotina, Peltigerales, Lobariaceae) is a foliose lichen with broad lobes and a greenish, reticulate upper surface with deep hollows (González, Barrera, Pérez, & Padrón, 1994). The chemical composition of *L. pulmonaria* has been studied by various authors including Culberson (1969) who, when reviewing *Lobaria* species, referred to it as having an interesting distribution of varieties and chemical types containing different combinations of ß-orcinol depsidones.

The acetylcholinesterase enzyme (AChE) is involved in the metabolic hydrolysis of acetylcholine at cholinergic synapses in the central and peripheral nervous system. The abnormal activity of this enzyme is one factor responsible for Alzheimer's disease, the most common cause of senile dementia in later life. Although Alzheimer's disease is primarily a disease of the elderly, it can strike victims as young as 30 years. The disease destroys neurons in the brain (including cholinergic receptors) and is associated with the appearance of plaques and tangles of

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nerve fibres. AChE inhibitors are still the best drugs currently available for the management of this disease (Graham, 2005).

Our continuing search on AChE inhibitors (Pejin, Iodice, Tommonaro, & De Rosa, 2008) led to the isolation of a mixture of depsidones from the lichen *L. pulmonaria*. Here, we report the AChE inhibition of the acetylated derivatives of these secondary metabolites.

2. Results and discussion

The AChE inhibition test (Marston, Kissling, & Hostettmann, 2002; Rhee, van de Meent, Ingkaninaa, & Verpoorte, 2001) showed a moderate activity (0.5 μg) for the mixture. In comparison, the alkaloid galantamine used clinically for the treatment of Alzheimer's disease (Scott & Goa, 2000) inhibited the enzyme at 0.01 μg. Because most inhibitors of AChE are alkaloids that often possess several side effects (Graham, 2005) it is important to search for new AChE inhibitors not belonging to this structural class.

3. Experimental

3.1. *General*

^{1}H- and ^{13}C-NMR spectra were recorded at the NMR Service of the Institute for Biomolecular Chemistry of National Council Research of Italy (CNR-ICB) on a Bruker Avance-400 spectrometer operating 400 and 100 MHz, respectively, using an inverse probe fitted with a gradient along the Z-axis, in $CDCl_3$, using the solvent signal as an internal standard. Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany). Acetylcholinesterase, 1-naphthyl acetate and the rest of the reagents used in the biological tests were obtained from Sigma Chemicals (St. Louis, MO). Fast Blue B salt was from Fluka (Milano, Italy).

3.2. *Plant material*

The lichen *Lobaria pulmonaria* (L.) Hoffm. was collected from *Fagus sylvatica* on the mountain Zelengora (Bosnia and Herzegovina) in July 2009. Voucher specimen has been deposited in the Herbarium of the Institute of Botany, University of Belgrade, Serbia (BEOU 5997).

3.3. *Extraction and isolation*

Before extraction the lichen was carefully inspected for contaminants. Air-dried parts of *L. pulmonaria* (70 g) were ground and extracted three times with $CHCl_3$, $CHCl_3$-MeOH 1 : 1, MeOH, and MeOH-H_2O 1 : 1, respectively, (500 mL each) at room temperature, for up to 1 day each, with the extractives pooled and then evaporated *in vacuo*. The dried $CHCl_3$-MeOH extract 1 : 1 (5.81 g) was dissolved in H_2O (50 mL) and partitioned sequentially with $CHCl_3$ (3 × 50 mL) and MeOH (3 × 50 mL). The crude insoluble coloured residue (0.46 g), obtained after the partition, was classified

as fraction rich in epsilons, by means of its spectroscopic data and typical chromatographic profile. In order to further characterise the residue, it was dissolved in 5 mL of pyridine and 1 mL of acetic anhydride (77 mg) and was refluxed for 2 h. After evaporating to dryness, the residue was chromatographed on a silica gel column and eluted with gradient of diethyl-ether (starting from petroleum-ether/diethyl-ether 1 : 1) to yield a mixture of acetylated depsidones (24 mg), which was hard to separate with traditional chromatographic methods. The mixture needs to be characterised further and an adequate procedure is expected to remove all impurities which could have relevant effects on AChE, and the possibility of whether or not some agent other than depsidones could be contributing to the AChE inhibition in the residue.

3.4. *Biological assay*

Acethylcolinesterase inhibition was performed dissolving the sample (a mixture of acetylated depsidones) in MeOH at a concentration of 1 mg mL^{-1}. From this main solution was performed a serial dilution in order to obtain lower concentration of the sample (0.1; 0.01; 0.001 mg mL^{-1}), and 10 μL of each solution was applied to TLC plates to test 10, 1, 0.1 and 0.01 μg of the sample to detect the minimum concentration that inhibited AChE. Galanthamine was used as positive control. The assay was carried out as described by Marston et al. (2002). It is a simple and rapid bioautographic enzyme assay. The test relies on the cleavage by acetylcholinesterase of 1-naphthyl acetate to form 1-naphthol, which in turn reacts with Fast Blue B salt to give a purple-coloured diazonium dye. Briefly, a stock solution of acetylcholinesterase (1000 U in 150 mL of Tris-hydrochloric acid buffer pH 7.8) was obtained, which was stabilised adding bovine serum albumin (150 mg). A 10 μL aliquot of each solution of the sample was applied to the TLC plates, dried to remove the solvent and then sprayed with enzyme stock solution. For incubation of the enzyme, the plate was kept at 37°C for 20 min in a humid atmosphere. For the detection of the enzyme, solutions of 1-naphthyl acetate (250 mg in 100 mL of EtOH) and of Fast Blue B salt (400 mg in 160 mL of distilled H_2O) were mixed and sprayed onto the plate. Acethylcolinesterase inhibition activity was detected by a white spot on a purple background after 1–2 min.

4. Conclusion

Our results indicate for the first time the significance of depsidones in searching for AChE inhibitors which could be lead structures for new drugs for Alzheimer's disease.

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