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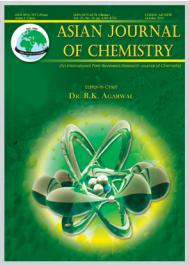

NOTE

Acetylcholinesterase Inhibition Activity of a Mixture of Methylated Depsidones from the Lichen *Lobaria pulmonaria*

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A phytochemical investigation conducted on a folise lichen, *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae), from Bosnia and Herzegovina led to a mixture of methylated depsidones which showed acetylcholinesterase inhibition activity (2 μg) in the acetylcholinesterase inhibition test on thin-layer chromatography plate. Present results indicate for the first time the potential of methylated depsidones in searching for these inhibitors, which still represent the best drugs currently available for the management of Alzheimer's disease.

Key Words: Lobariaceae, Phytochemistry, Depsidones, Alzheimer's disease.

Lobaria pulmonaria (L.) Hoffm. (Ascomycotina, Peltigerales, Lobariaceae) is a folise lichen with broad lobes and a greenish, reticulate upper surface with deep hollows[1]. The chemical composition of *L. pulmonaria* has been studied by various authors including Culberson who, when reviewing Lobaria species, referred to it as having an interesting distribution of varieties and chemical types containing different concentrations of β-orcinol depsidones[2].

Today a wide range of secondary metabolites from lichen species are recognized as having medicinal value[3]. Our continuing search on acetylcholinesterase (AChE) inhibitors[4,5] led to a mixture of acetylated depsidones from the lichen *L. pulmonaria*. Here, we report the AChE inhibition of the same mixture of depsidones in methylated form. The lichen *L. pulmonaria* 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).

^{1}H and ^{13}C NMR spectra were recorded at the NMR Service of the Institute of 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 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 obtained from Fluka (Milan, Italy).

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 partioned sequentially with $CHCl_3$ (3 × 50 mL) and MeOH (3 × 50 mL). The crude insoluble colored 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 characterize the residue, it was methylated with diazomethane (35 mg). After evaporation 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 methylated depsidones (21 mg), which was hard to separate with traditional chromatographic methods. The AChE inhibition test was carried out as described[6,7]. The assay showed weaker acetylcholinesterase inhibition activity (2 μg) for the methylated mixture than its acetylated form (0.5 μg)[5]. In comparison, the alkaloid galantamine used clinically for the treatment of Alzheimer's disease inhibited the enzyme at 0.01 μg[8].

To our best of knowledge, these results indicate for the first time the AChE inhibition activity of methylated depsidones as a mixture highlighting its potential in searching for new AChE inhibitors. Because most of these inhibitors are alkaloids that often possess several side effects[9], it is important to search for new AChE inhibitors not belonging to this structural class. However, the mixture needs to be characterized 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.

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