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SHORT COMMUNICATION

A new depsidone of *Lobaria pulmonaria* with acetylcholinesterase inhibition activity

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Abstract
The phytochemical investigation conducted on a foliose lichen, *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae), led to the isolation of a new depsidone in the form of its diacetate derivative which showed a moderate acetylcholinesterase inhibition activity (1 µg) *in vitro*. This is the first record of identified depsidone structure in searching for these inhibitors.
Keywords: Lichen, secondary metabolite, biological activity

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Introduction

Most of the secondary metabolites which are so characteristic of lichens are derived from acetyl-polymalonyl pathway. Indeed, the large majority of depsides, depsidones, dibenzofurans, usnic acids and depsones are peculiar to lichens[1].

Depsidones have an ether linkage in addition to the ester linkage of the depsides, resulting in a rigid polycyclic system. Therefore, they are based on an 11*H*-dibenzo[*b,e*][1,4]dioxepin-11-one ring system[2].

As part of our continuing search on acetylcholinesterase (AChE) inhibitors[3], a phytochemical investigation was conducted on a foliose lichen, *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae). The chemical nature of *L. pulmonaria* has been studied by various authors including C. P. Culberson who, when reviewing *Lobaria* species, referred to it as having an interesting distribution of varieties and chemical types containing different combinations of β-orcinol depsidones[4,5].

Materials and methods

Biological material

The lichen *L. pulmonaria* (L.) Hoffm. (Lobariaceae) 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).

General experimental procedures

^{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 was carried out on precoated silica gel 60 F254 (0.25 mm, Merck, Darmstadt, Germany). LRMS and HRMS were recorded on a JEOL JMS D-300 and an AEI MS-50, respectively.

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 (1:1) extract (5.81 g) was dissolved in H_2O (50 mL) and partitioned

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sequentially with $CHCl_3$ (3 × 50 mL) and *n*-BuOH (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 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 the system of petroleum ether–diethyl ether (gradient, starting from petroleum–ether/diethyl ether 1:1) to yield a mixture of acetylated depsidones (24 mg), which was further separated with thin-layer chromatography using $CHCl_3$ to give a new depsidone in the form of its diacetate derivative (3 mg; 0.0043% of dry weight).

A new depsidone (Figure 1): white, amorphous powder; IR (ATR) ν_{max} 1781, 1743, 1702, 1606 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.41 (1H, s, H-9), 7.35 (1H, s, H-6′), 6.79 (1H, s, H-5), 4.00 (3H, s, $0CH_3$-4), 2.58 (3H, s, H-8), 2.43 (3H, s, $0COCH_3$-1′), 2.30 (3H, s, H-8′), 2.23 (3H, s, $0COCH_3$-2′); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 186.4 (CHO, C-9), 168.9 (OCO, C-2′), 168.4 (OCO, C-1′), 164.6 (C, C-4), 163.9 (COO, C-7), 160.2 (C, C-2), 151.7 (C, C-6), 148.9 (C, C-2′), 144.7 (C, C-1′), 142.7 (C, C-4′), 134.1 (C, C-5′), 90.8 (C, C-6′), 116.2 (C, C-3′), 114.8 (C, C-1), 114.4 (CH, C-3), 112.2 (CH, C-5), 56.7 (C, $0CH_3$-4), 22.4 (CH_3, C-8), 20.7 ($OCOCH_3$, C-2′), 20.4 ($OCOCH_3$, C-2′), 10.3 (CH_3, C-8′); ESIMS m/z 416 $[M + H]^+$ (calcd for $C_{21}H_{19}O_9$ 415,1029).

Biological assay

Acetylcolinesterase inhibition was performed dissolving the sample in MeOH at a concentration of 1 mg/mL. 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), 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[6]. It is a simple and rapid bioautographic enzyme assay. The test relies on the cleavage by AChE 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 AChE (1000 U in 150 mL of Tris-hydrochloric acid buffer pH 7.8) was obtained, which was stabilized 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. Acetylcolinesterase inhibition activity was detected by a white spot on a purple background after 1–2 min.

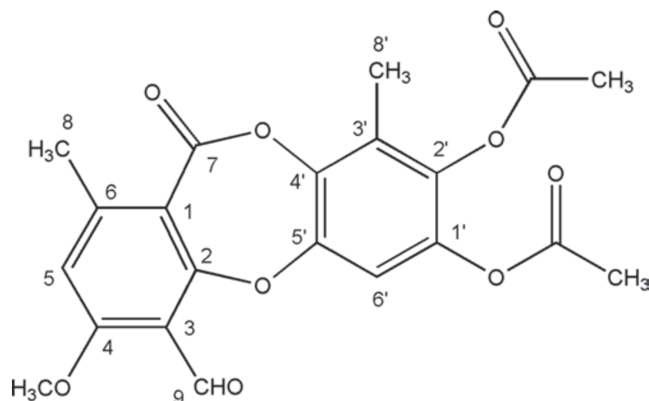

Figure 1. A new depsidone in the form of its diacetate derivative.

Results and discussion

High-resolution mass spectrometry established the molecular formula of a new depsidone as $C_{21}H_{18}O_9$. Its structure followed from 1-D and 2-D NMR spectra. The ^{1}H-NMR spectrum showed five three-proton singlets between δ 2.0 and 4.0, two one-proton singlets between δ 6.7 and 7.4, and an aldehyde proton at δ 10.41; two C-methyl resonances (δ 2.23 and 2.43) were specific signals of the identified depsidone molecule. The ^{13}C-NMR and DEPT spectra exhibited 21 carbon signals. A combination of 2-D NMR experiments (COSY, NOESY, HSQC and HMBC) allowed us to assign all signals in the ^{1}H- and ^{13}C- NMR spectra.

The AChE inhibition test[6,7] showed a moderate activity (1 µg) for a new depsidone in the form of its diacetate derivative. In comparison, the alkaloid galantamine[8] inhibited the enzyme at 0.01 µg, while the same mixture of depsidones in its acetylated and methylated forms were also active, 0.5 and 2 µg, respectively[9,10]. On the other hand, the mixture in its natural nonmodified form as well as isolated stictic acid (common depsidone for *Lobaria* sp.) did not show any activity in the AChE test on TLC plate.

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Declaration of interest

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