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A NEW LICHEN DEPSIDONE FROM *L OBARIA PULMONARIA*

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The continuation of our phytochemical survey of *Lobaria pulmonaria* (L.) Hoffm. (Lobariaceae), has led to the isolation and identification of a new lichen depsidone (**1**) characterised by the normal analytical and spectroscopic techniques. Unlike previously isolated depsidone (**2**) from the same species, the compound **1** has not showed activity in the acetylcholinesterase inhibition test on Thin-layer chromatography plate.

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1. Introduction

Lichens are by definition symbiotic organisms, usually composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont, which is most often either a green alga or cyanobacterium. The majority of organic compounds found in lichens are secondary metabolites of the fungal component, which are deposited on the surface of the hyphae rather than within the cells. These products are usually insoluble in water and can be only extracted with organic solvents. Of the acetyl-polymalonyl derived compounds, aromatic products are especially well represented, the most characteristic being depsides, depsidones, dibenzofurans, usnic acids and depsones. Other aromatic compounds of acetate-polymalonate origin, such as the chromanes, xanthones, and antraquinones are often identical or analogous to products of non-lichen forming fungi or higher plants [1].

Lobaria pulmonaria (L.) Hoffm. (Ascomycotina, Peltigerales, Lobariaceae) is a foliose lichen with broad lobes and a greenish, reticulate upper surface with deep hollows [2]. The genus *Lobaria* is represented by a variety species, in which *L. pulmonaria* has been extensively studied and ß-Orcinol depsidones were found to be main constituents [3].

As part of our ongoing project on natural products of lower marine and terrestrial organisms [4,5], a phytochemical investigation has been continued on the lichen *L. pulmonaria*.

2. Experimental

2.1. 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-

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400 spectrometer operating 400 and 100 MHz, respectively, using an inverse probe fitted with a gradient along the Z-axis, in CDCl3, using the solvent signal as an internal standard. Thin-layer chromatography was carried out on pre-coated 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.

2.2. Plant material

The lichen *Lobaria 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).

2.3. 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 (1:1) extract (5.81 g) was dissolved in H_2O (50 mL) and partioned 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 characterise the residue, it was chromatographed on Sephadex LH-20 column (20 mg) and eluted with the system of methylene chloride/methanol 1:1 to yield the fraction with a new depsidone (5 mg, 0.0071% of dry weight).

New depsidone **1** (Figure 1): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.44 (1H, s, H-9), 10.24 (1H, s, H-8'), 10.24 (1H, s, H-7'), 7.10 (1H, s, H-5), 3.92 (3H, s, 0CH_3-4), 2.50 (3H, s, H-8), 2.19 (3H, s, H-9'); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 186.7 (CHO, C-9), 170.6 (COOH, C-8'), 170.6 (COOH, C-7'), 163.2 (C, C-4), 163.2 (COO, C-7), 162.4 (C, C-2), 160.9 (C, C-2'), 147.9 (C, C-4'), 144.8 (C, C-6), 137.4 (C, C-6'), 136.2 (C, C-5'), 120.5 (C, C-3'), 114.4 (C, C-3), 114.4 (C, C-1'), 113.1 (C, C-1), 112.8 (CH, C-5), 56.8 (C, 0CH_3-4), 21.6 (CH_3, C-8), 9.6 (CH_3, C-9'). ESIMS m/z 403 $[M+H]^+$ (calcd for $C_{19}H_{15}O_{10}$ 403.3096).

2.4. Biological Assay

Acetylcholinesterase inhibition test 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. Galantamine was used as positive control. The assay was carried out as described Marston, Kissling, & Hostettmann [6]. 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. Acetylcholinesterase inhibition activity was detected by a white spot on a purple background after 1-2 min.

3. Results and discussion

High-resolution mass spectrometry established the molecular formula of a new lichen depsidone **1** ($C_{19}H_{14}O_{10}$, Figure 1). The structure followed from 1-D and 2-D NMR spectra. The ^{1}H-NMR spectrum showed three three-proton singlets between δ 2.0 and 4.0, one-proton singlet between δ 7.0 and 10.0, carboxylic protons at δ 10.24 and an aldehyde proton at δ 10.44; C-carboxylic resonances were specific signals of the identified depsidone molecule. The ^{13}C-NMR and DEPT spectra exhibited 19 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.

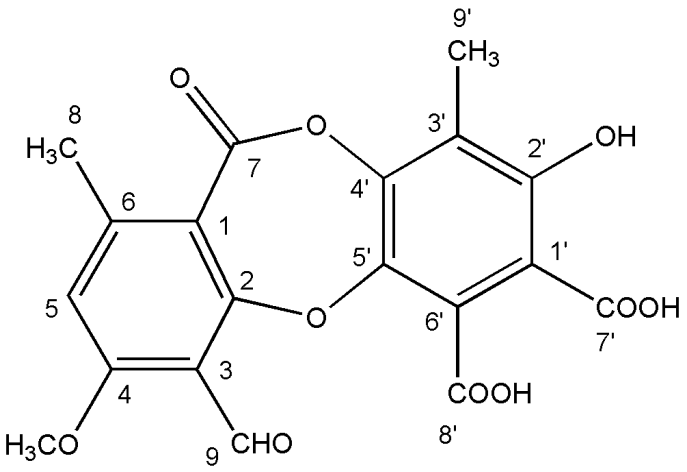

Fig. 1. A new lichen depsidone

The AChE inhibition test [6,7] showed no activity for the compound **1**. In comparison, the alkaloid galantamine used clinically for the treatment of Alzheimer's disease [8] inhibited the enzyme at 0.01 μg, while a new depsidone **2** isolated from the same lichen in the form of its diacetate derivative ($C_{21}H_{18}O_{9}$, Figure 2) was active at 1 μg [9]. According to our best knowledge, it is the only identified depsidone with acetylcholinesterase inhibitory activity till date. On the other hand, AChE inhibitors are still considered the best drugs currently available for the management of Alzheimer's disease [10]. Because most inhibitors of AChE are alkaloids that often possess several side effects, it is important to search for new AChE inhibitors not belonging to this structural class.

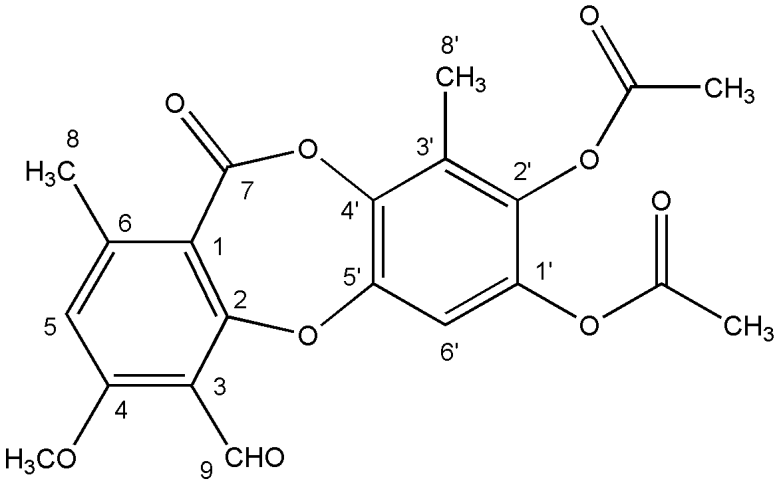

Fig. 2. Previously isolated depsidone in the form of its diacetate derivative

4. Conclusion

Our results indicate the significance of covalent modification (acetylation) of depsidone structure in the AChE inhibition.

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