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A new depsidone from the lichen *Usnea ceratina* Arch

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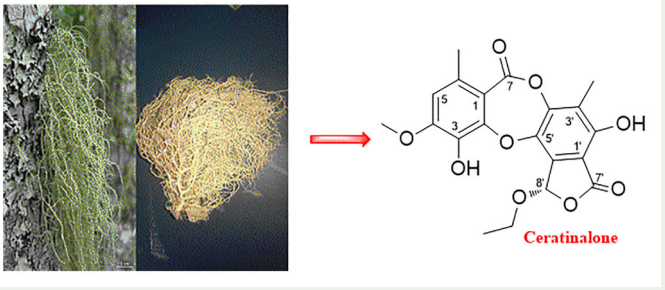
ABSTRACT

Chemical investigation of the lichen *Usnea ceratina* Arch led to the isolation of five depsidones, including one new compound ceratinalone **(1)** along with four known compounds bailesidone **(2),** stictic acid **(3)**, 8′-*O*-methylstictic acid **(4)** and 8′-*O*-ethylstictic acid **(5)**. The structures were determined by analysis of their MS and NMR data as well as by comparison with literature values. Compounds **1** and **4** were evaluated the cytotoxic activity against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer), HepG2 (liver hepatocellular carcinoma), and MCF-7 (human breast cancer) cell lines, showing the moderate activity.



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1. Introduction

Lichens are symbiotic associations comprised of a fungus and a photobiont partner (green alga and/or cyanobacteria). This unique organism produces numerous metabolites endowed with various biological activities (Müller 2001; Boustie and Grube 2005). Depsidone, an unique scaffold, showed diverse biological activities such as capacity of blocking UV rays (Huneck and Yoshimura 1996; Russo et al. 2008), antioxidant (Chomcheon et al. 2009; Ismed et al. 2017), antifungal (Millot et al. 2017), inhibition

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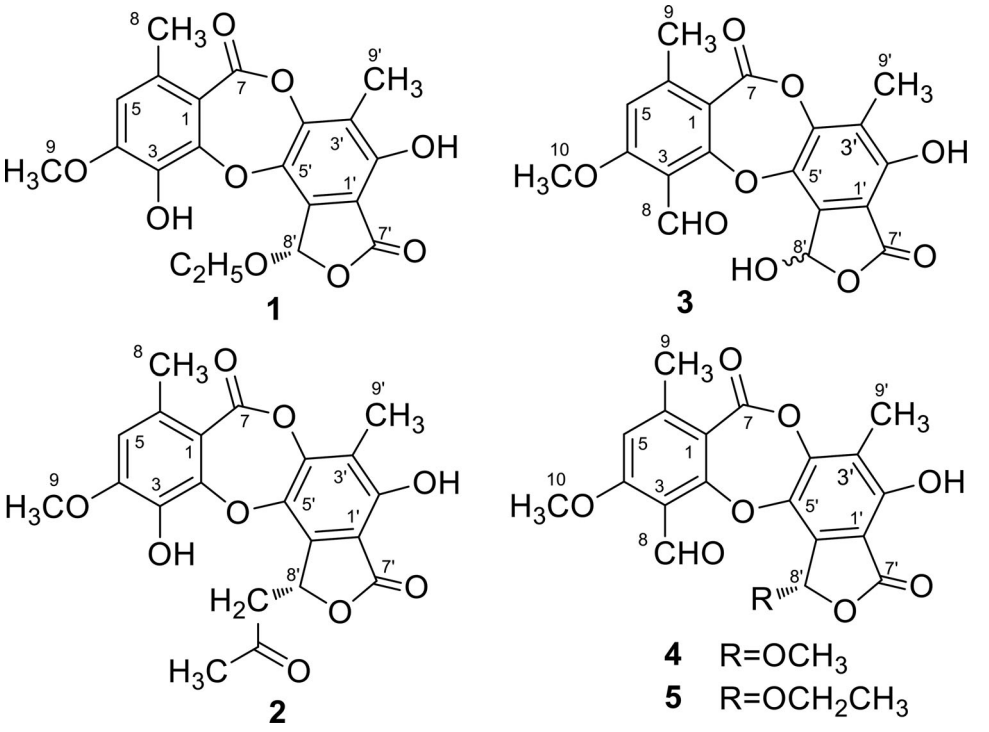

Figure 1. Structures of isolated compounds 1–5.

against a number of malignant cancer cells, antiviral, antimicrobial, as well as enzyme inhibition (Nakazawa et al. 1962; Huneck 1999; Boustie and Grube 2005; Pittayakhajonwut et al. 2006; Micheletti et al. 2009; Honda et al. 2010; Boustie et al. 2011; Nishanth et al. 2015; Rankivic and Kosanic 2015). Our recent phytochemical investigations revealed the wealth of the chemical diversity sheltered by Vietnamese lichen species, that sometimes comprised unprecedented scaffolds (Duong et al. 2015; 2018; 2018; 2019; Sichaem et al. 2019; Duong et al. 2020; Phan et al. 2020). Some Vietnamese lichens belonging to the genus *Usnea* have been investigated, i.e., *Usnea aciculifera* and *U. baileyi* (Tuong et al. 2014; Nguyen et al. 2018). Chemical data of the native lichen *Usnea ceratina* growing in Vietnam are scarce. In this study, we reported the isolation and structural elucidation of five depsidones including one new depsidone and four known compounds from the lichen *Usnea ceratina* Arch. The known compounds were bailesidone (**2**) (Nguyen et al. 2018), stictic acid (**3**) (Lohézic-Le Dévéhat et al. 2007), 8′-*O*-methylstictic acid (**4**) (Kathirgamanathar et al. 2005), and 8′-*O*-ethylstictic acid (**5**) (Ingolfsdottir et al. 1986) (Figure 1). The cytotoxic activity against HeLa, NCI-H460, HepG2 and MCF-7 cancer cell lines of compounds **1** and **4** was evaluated.

2. Results and discussion

Compound **1** was obtained as white amorphous powder. Its molecular formula was determined to be $C_{20}H_{18}O_9$ from its HRESIMS ion at *m/z* 425.0827 $[M+Na]^+$

(calcd for $C_{20}H_{18}O_9Na$, 425.0849). The ^1H NMR spectrum displayed signals for one aromatic proton (δ_H 6.87, s), one acetal proton (δ_H 6.72, s), a methoxy group (δ_H 3.92, s), an ethoxy group (δ_H 4.00, 2H, q, $J = 7.0$ Hz and 1.32, 3H, t, $J = 7.0$ Hz), and two methyl groups at δ_H 2.23 and 2.39 (3H each, *s*). The ^{13}C NMR and HSQC spectra in accordance with MS data exhibited 20 carbon signals including two carboxyl carbons (δ_C 161.9 and 168.9), one acetal carbon (δ_C 102.5), one methoxy group (δ_C 56.7), one ethoxy group (δ_C 67.0 and 15.3), two methyl groups (δ_C 9.2 and 20.3), and 12 sp^2 carbons in the range 108–153 ppm.

HMBC cross-peaks of proton H-5 (δ_H 6.87) with carbons C-1 (δ_C 114.8), C-3 (δ_C 136.1), and C-4 (δ_C 152.9), of the methyl protons at δ_H 2.39 (H_3-8) with carbon signals at δ_C 114.8 (C-1), 112.3 (C-5), and 134.0 (C-6) defined the connectivity through C-1-C-6 and further validated the A-ring of β-orcinol depsidone scaffold (Duong et al. 2015; 2020). This was also strengthened by the NOESY correlations of H-5 with both 4-OCH_3 and H_3-8. In the B-ring, HMBC correlations from H-8′ to carbon signals at δ_C 108.9 (C-1′), 140.0 (C-6′), 168.9 (C-7′), and 67.0 (C-10′) indicated the presence of a γ-lactone moiety in **1** (Figures S2–S4), further backed up by NOESY correlation between H-8′ and H-10′. Detailed comparison of NMR data of **1** with those of menegazziaic acid (Kathirgamanathar et al. 2005) showed the high similarities except for the replacement of an ethoxy group in **1** for the methoxy group. β-Orcinol depsidones bearing the ethoxy-subtituted lactone moiety was found in lichens, i.e., 8′-*O*-ethylstictic acid (Ingolfsdottir et al. 1986). Altogether, compound **1** was elucidated as shown in Figure 1.

Depsidones containing a B-ring fused with a γ-butyrolactone moiety had the absolute configuration assigned as *S*-configuration using the electronic circular dichroism (ECD) spectrum (Nguyen et al. 2017; 2018). Negative Cottons effects (CE) of compounds **1–2** and **4–5** were highly similar, indicating their same absolute configuration. These CEs were reminiscent to those of lobarientalone A (Nguyen et al. 2017) or bailesidone (Nguyen et al. 2018), indicating the (8′*S*) configuration of **1**. In contrast, no CEs were found for compound **3**, indicating its racemic status. The slight difference of CEs of **2** with those of **1**, **4**, and **5** might come from the different substituents at C-8′.

The ethyl ether groups at C-8′ of **1** and **5** are not common in nature. The etherification would appear when using ethanol during the extraction or isolation. However, in our scenario, ethanol has not been used during the isolation, proposing that **1** and **5** were not artifactual compounds. In order to confirm that the new compound **1** occurred naturally in the original lichen, TLC experiments using standard solvent systems were conducted to define the presence of compound **1** in the acetone extract (Figures S9–S10). In the acetone extract TLC profiles (see Figures S9–S10), the isolated compounds having the ethyl ether group, **1** and **5** were clearly evident, indicating their natural occurrence.

The isolated compounds **1** and **4** were evaluated for the cytotoxic activity against MCF-7, HeLa, HepG2, and NCI-H460 by using the sulfohodamine B colorimetric assay with camptothecin as the positive control (Skehan et al. 1990). Both compounds exhibited moderate activity against four cell lines. Compound **4**

revealed stronger activity against the HeLa cell with the IC_{50} value of 15.61 ± 0.27 μg/mL (Table S3).

3. Experimental

3.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) using residual solvent signals as internal references: DMSO-d_6 at δ_H 2.50, δ_C 39.51, chloroform-*d* at δ_H 7.26 and δ_C 77.16, and acetone-d_6 at δ_H 2.05, δ_C 29.84 and 206.26. The HR–ESI–MS was recorded on a HR–ESI–MS MicrOTOF–Q mass spectrometer on a LC-Agilent 1100 LC-MSD Trap spectrometer. Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F254 or silica gel 60 RP–18 F254S (Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

3.2. Plant material

The thalli of the lichen *Usnea ceratina* Arch were separated from the bark of various trees at 109 m altitude at Paksong town (15°11′00″ N, 106°14′00″ E), Paksong district, Champasack province, Laos in April 2015. The scientific name of the plant was authenticated by Dr. Harrie J. M. Sipman, Freie Universitaet, Berlin, Germany. A voucher specimen (US–B030) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University – Ho Chi Minh City, Vietnam.

3.3. Extraction and isolation

The fresh lichen thalli (1.60 kg) were cleaned under running tap water and air-dried. The ground powder (1.15 kg) was macerated with acetone at room temperature. After filtration, the solvent was evaporated under reduced pressure to dryness to afford the crude acetone residue (163.0 g). This crude extract was subjected to silica gel solid phase extraction and eluted consecutively with chloroform, ethyl acetate, acetone and methanol to provide chloroform (C, 60.0 g), ethyl acetate (EA, 2.0 g), acetone (Ace, 55.0 g) and methanol (M, 3.5 g) fractions, respectively.

Fraction C (60.0 g) was applied to silica gel column chromatography (CC) and eluted with the solvent systems of *n*-hexane–chloroform (stepwise, 9:1 to 0:10) to give 5 sub-fractions, coded C1 (13.58 g), C2 (8.53 g), C3 (6.59 g), C4 (5.74 g), and C5 (5.05 g), then eluted with chloroform–methanol (stepwise, 9:1 to 0:10) to give 4 sub-fractions, namely C6 (4.83 g), C7 (3.82 g), C8 (1.98 g), and C9 (1.04 g). The sub-fraction C5 (5.05 g) was rechromatographed by silica gel CC, eluted with *n*-hexane–chloroform (8:2, v/v) to give two compounds, **3** (5.0 mg) and **4** (6.0 mg). The sub-fraction C6 (4.83 g) was applied to silica gel CC, eluted with *n*-hexane–chloroform (7:3) to afford **5** (6.5 mg) and **1** (6.0 mg). The same manner was applied to the sub-fraction C7 (3.82 g), eluted with *n*-hexane–chloroform (5:5), to afford **2** (6.0 mg).

3.3.1. Ceratinalone (1)

White amorphous powder. HR-ESI-MS *m/z* 425.0827 $[M + Na]^+$ (calcd for $C_{20}H_{18}O_9Na$, 425.0849). ^1H NMR (500 MHz, Acetone-d_6) δ_H 8.12 (1H, *brs*, 3-O**H**), 6.87 (1H, *s*, H-5), 6.72 (1H, *s*, H-8′), 4.00 (2H, *q*, $J = 7.0$ Hz, H-10′), 3.92 (3H, *s*, 4-OC**H$_3$**), 2.39 (3H, *s*, H-9), 2.23 (3H, *s*, H-9′) and 1.32 (3H, *t*, $J = 7.0$ Hz, H-11′). ^{13}C NMR (125 MHz, Acetone-d_6) δ_C 168.9 (C-7′), 162.0 (C-7), 153.0 (C-2, C-4), 152.6 (C-2′), 150.6 (C-4′), 136.2 (C-6′), 136.0 (C-3), 134.1 (C-6), 133.8 (C-5′), 121.3 (C-3′), 114.8 (C-1), 112.4 (C-5), 108.9 (C-1′), 102.6 (C-8′), 67.1 (C-10′), 56.8 (4-O**C**H$_3$), 20.4 (C-8), 15.4 (C-11′) and 9.3 (C-9′).

In order to check that the new metabolites **1** and **5** were not artifacts of the isolation procedure, the extraction process using acetone was conducted again on a new sample of lichen. A new lichen sample (100 mg) was cut into small pieces and extracted with acetone (5 mL) at ambient temperature for 24 h. Each filtered solution was blown by a fan at room temperature to afford a concentrated solution (0.5 mL). The acetone extract was spotted on an analytical TLC plate and run against acetone solutions of the two compounds **1** and **5** using standard solvent systems (Duong et al. 2017): A (toluene: dioxane:AcOH, 180: 45: 5, v/v/v) and C (toluene: AcOH, 170: 30, v/v). Spots were visualized by UV prior to spraying the plate with vanillin/5% aqueous H_2SO_4 solution followed by heating.

3.5. Biological assay

Compounds **1** and **4** were subjected to a cytotoxic evaluation against HeLa (human epithelial carcinoma), MCF–7 (human breast cancer), HepG2 (liver hepatocellular carcinoma) and NCI-H460 (human lung cancer) cell lines. The tested samples were performed at a concentration of 100 μg/mL using sulforhodamine B (SRB) assay with camptothecin as the positive control. The details were similar to those presented in our previous paper (Duong et al. 2015).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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