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Study on chemical constituents of the lichen *Parmotrema sancti-angelii* (Lynge) Hale. (Parmeliaceae)

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Abstract

Lichens are fungal and algal/cyanobacterial symbioses resulting in the production of specific metabolites. *Parmotrema sancti-angelii* (Lynge) Hale is a lichen which has not been chemically and biologically studied well. From the lichen collected in Vietnam, colour reactions for identification of lichen substances (+K red, +P yellow, -C, +KC red) suggested the presence of quinones, depsides and xanthones containing two free hydroxyl groups in meta-position, depsides and depsidones containing an aldehyde group. Chemical constituent study led to the isolation of three compounds, including methyl β-orcinolcarboxylate (**1**), salazinic acid (**2)** and atranorin (**3**). Their structures were confirmed unambiguously by X-ray diffraction, spectroscopic data and compared with those in references. This is the first report of salazinic acid distribution in such lichen.

Keywords. Parmeliaceae, *Parmotrema sancti-angelii*, X-ray, NMR, salazinic acid.

1. INTRODUCTION

Lichens, a symbiotic relationship between fungi and photosynthetic algae (and/or cyanobacteria). The symbiosis leads to the production of typical secondary metabolites. Most of them are polyphenolic compounds with depside and depsidone structures [1]. Lichen substances have many biological activities, including antibiotic, antibacterial, antiviral, anti-inflammatory, analgesic, antipyretic, anti-proliferative and other activities [2]. *Parmotrema* is a large genus in the Parmeliaceae with approximately 350 species of foliose lichens and a high level of diversity in the tropical areas of the world. There are few reports focusing on biological activity and chemical composition of *Parmotrema sancti-angelii* (Lynge) Hale. Recently, atranorin, lecanoric acid, α-collactolic acid, some monocyclic aromatic compounds and bicyclo compounds were reported in such lichen [3]. Three phenolic compounds atranorin, lecanoric acid and α-collactolic acid demonstrated moderate to strong bactericidal activity [4].

The aim of this paper is general identification of lichen substances by spot tests on upper cortex with useful lichen reagents (K, C, KC, P). From lichen *Parmotrema sancti-angelii*, collected in Lam Dong province, Vietnam, three compounds methyl β-orcinolcarboxylate (**1**), salazinic acid (**2)** and atranorin (**3**) were isolated by rapid and efficient purification. Although (**2**) has already been reported for some species of this genus, it is the first report for *P. sancti-angelii*. Their chemical structures were elucidated by X-rays, NMR spectroscopic data analysis and comparison with those reported in the literature.

2. EXPERIMENTAL

2.1. Lichen material

Parmotrema sancti-angelii (Lynge) Hale was collected in Lam Dong province, Vietnam on November 2011. The scientific name was identified by Prof. Joël Boustie, Faculty of Pharmacy, University of Rennes 1, France. A voucher specimen (No Par-0913) was deposited in the herbarium of the Department of Chemistry, Can Tho University of Medicine and Pharmacy, Can Tho City, Vietnam.

2.2. General experimental procedures

The NMR experiments were performed on a Bruker DMX 300 spectrometer. HRMS-ESI were carried out on a MICROMASS ZabspecTOF spectrometer for electrospray ionization. The crystal data was collected on a Enraf-Nonius FR590-kappa diffractometer with a CCD area detector and graphite monochromated MoKα radiation. The structure was solved using direct methods, refined with the Shelx software package and expanded using Fourier techniques. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in structure factor calculations from their location in difference maps. C-bound H atoms were treated as riding in geometrically idealized positions, with Uiso (H) = kUeq (C), where k = 1.5 for the methyl groups, which were allowed to rotate around their C—C bond, and 1.2 for all other C bound H atoms. Computing Softwares for: Data Collection, Cell Refinement and Data Reduction: COLLECT/HKL2000. Structure solution: SHELX-S97. Structure Refinement: SHELXL2012; CRYSTALBUILDER. Molecular Graphics: ORTEP-III; MERCURY.

Spot tests were carried out with reagents K (10 % KOH), C (30 % potassium hypochlorite) and K followed by C (KC), P (5% *p*-phenylenediamine in ethanol).

Column chromatography was performed on normal phase silica gel (40-63 μm, Keselgel 60, Merck 7667). TLC was performed on Kieselgel 60F254 plates (Merck) and spots were visualized under UV light or sprayed with vanillin (0.5 g vanillin in 80 mL sulfuric acid and 20 mL ethanol), then heated. All solvents used were purchased from Chemsol, purity ≥ 99.0 %.

2.3. Extraction and isolation

The dried, crushed lichen material (60 g) was extracted with acetone at 60°C and then concentrated under reduced pressure. While the acetone extract was evaporated, the precipitate occurred and was filtered off (810 mg). The precipitate after recrystallized was subjected to a silica gel column and eluted with petroleum ether:ethyl acetate (95:5) to yield atranorin (**3**, 4.8 mg), methyl β-orcinolcarboxylate (**1**, 6.9 mg) and salazinic acid (**2**, 420 mg).

Compound **1.** White prisms (acetone); 1D- and 2D-NMR spectral data: see table 1.

Compound **2.** Colorless needles (acetone); HRMS-ESI: *m/z* = 389.0522 $[M+H]^+$ (calcd. for $C_{18}H_{13}O_{10}$ 389.0508); 1D- and 2D-NMR spectral data: see table 2.

Compound **3.** Colorless needles; HRMS-ESI: *m/z* 397.0899 $[M+Na]^+$ (calcd. for $C_{19}H_{18}O_8$).

3. RESULTS AND DISCUSSION

Figure 1: The results spot tests on the thallus *P. sancti-angelii*

Spot tests on upper cortex showed a deep red with K, yellow with P, no reaction to C (figure 1) but the thallus turned red when K and C were applied. The results suggested the presence of quinones, depsides and xanthones containing two free hydroxyl groups in *meta*-position, depsides and depsidones containing an aldehyde group in the thallus [1]. By a rapid step analysis, colour reactions gave useful hints for the presence of certain functional groups of a lichen substance and also for classification of lichens.

Compound **1** was a monocyclic aromatic compound. The ^{1}H-NMR spectrum displayed signals of two methyl groups at δ_H 2.10 ppm (3H, *s*) and 2.46 ppm (3H, *s*), one methoxy group at δ_H 3.92 ppm (3H, *s*), one aromatic methine proton at δ_H 6.21 ppm (1H, *s*), two phenolic –O**H** at δ_H 5.09 ppm (1H, *s*) and 12.05 ppm (1H, *s*). The ^{13}C-NMR spectrum showed one carbonyl ester group (δ_C 172.7), one methoxy group (δ_C 52.0), two methyl groups (δ_C 7.8 and 24.2), six aromatic methine carbons (δ_C 105.4, 108.6, 110.7, 140.3, 158.1 and 163.3). The HMQC and HMBC correlations (table 1) as well as comparison with the reported data [1] confirmed the structure of **1** as methyl β-orcinolcarboxylate.

Table 1: The NMR data of **1** (300 MHz, $CDCl_3$)

Position	δ_H	δ_C	HMBC (H→ C)
1		105.4	
2		158.1	
3		108.6	
4		163.3	
5	6.21 (1H, *s*)	110.7	1, 3, 9
6		140.3	
7		172.7	
8	2.10 (3H, *s*)	7.8	2, 3, 4
9	2.46 (3H, *s*)	24.2	1, 5, 6
OCH_3	3.92 (3H, *s*)	52.0	7
OH-2	12.05 (1H, *s*)		1, 2, 3
OH-4	5.09 (1H, *s*)		3, 4

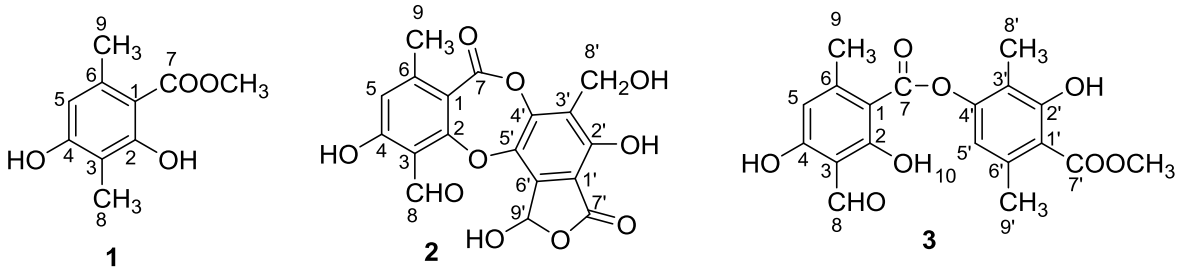

Figure 2: Structures of compounds **1**, **2**, and **3** isolated from *P. sancti-angelii*

Compound **2**, obtained as colorless needles, had the molecular formula $C_{18}H_{12}O_{10}$ as determined by HRMS-ESI. The ^{1}H-NMR spectrum in DMSO-d_6 showed signals of two phenolic –O**H** at δ_H 8.29 ppm (1H, *brs*) and 12.06 ppm (1H, *s*), one aldehydic proton at δ_H 10.46 ppm (1H, *s*), one aromatic methine proton at δ_H 6.88 ppm (1H, *s*), one methine proton at δ_H 6.79 ppm (1H, *brs*), one benzyloxy proton at δ_H 4.64 ppm (2H, *s*) and one aromatic methyl at δ_H 2.45 ppm (3H, *s*). The ^{13}C-NMR spectrum of **2** showed 18 signals closely related to salazinic acid. The HSQC and HMBC correlations (table 2) as well as comparison with the reported data [1] confirmed the structure of **2** as salazinic acid. Such compound has previously been described in some others species of genera *Parmotrema*, *Ramalina siliquosa*, *Parmelia reticulata* [5-7]. Here, compound **2** was isolated for the first time in *P. sancti-angelii*.

The structure of **3** was determined by X-ray diffraction as atranorin (figure 3). Molecular formula: $C_{19}H_{18}O_8$. Chemical formula weight: 374.33. Symmetry cell setting: Monoclinic Symmetry space group name H-M P 21/n. Symmetry space group name Hall -P 2yn. Cell length a (Å) 10.929(3). Cell length b (Å) 10.976 (3). Cell length c (Å) 14.843 (3). Cell angle alpha 90. Cell angle beta 109.745(12). Cell angle gamma 90. Cell volume (Å^3) 1675.7(7) [8-10].

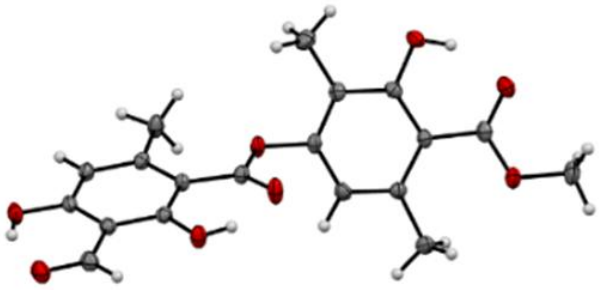

Figure 3: Structure of **3** by X-ray diffraction

4. CONCLUSION

From the lichen *Parmotrema sancti-angelii* (Lynge) Hale collected in Vietnam, spot tests on upper cortex suggested the presence of quinones, depsides and xanthones containing two free hydroxyl groups in *meta*-position, depsides and depsidones containing an aldehyde group in the thallus. Three compounds were isolated and

determined structures. To the best of our knowledge, this is the first report on the occurrence of salazinic acid in this species. Further studies on its chemical constituents are in progress.

Table 2: The NMR data of **2** (300 MHz, DMSO-d_6)

Position	δ_H	δ_C	HMBC (H→ C)
1		111.9	
2		165.9	
3		110.7	
4		164.0	
5	6.88 (1H, *s*)	117.4	1, 3, 9
6		152.8	
7		160.3	
8	10.48 (1H, *s*)	192.7	4
9	2.45 (3H, *s*)	21.4	1, 5, 6
1'		109.6	
2'		152.2	
3'		123.5	
4'		148.2	
5'		137.3	
6'		138.1	
7'		163.5	
8'	4.64 (2H, *s*)	52.6	2', 3', 4'
9'	6.79 (1H, *brs*)	94.8	
OH-2'	12.06 (1H, *s*)		
OH-4	8.29 (1H, *brs*)		

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