Science & Technology Development Journal, 22(1):114- 119

Original Research

Phenolic compounds from *Pamotrema dilatatum* growing in Lam Dong province

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History

- Received: 2018-11-12
- Accepted: 2019-01-04
- Published: 2019-01-26

DOI :

https://doi.org/10.32508/stdj.v22i1.1010

ABSTRACT

Introduction: Only chemical study on the lichen *Parmotrema dilatatum* was found so far in the world. The lichen Parmotrema dilatatum widely distributed in Lam Dong province, Vietnam has been studied about the isolation and elucidation of several metabolites.
Methods: Phytochemical study on the polar fractions of this lichen was carried out by using various chromatographic methods including thin-layer chromatography and normal phase silica gel chromatography.
Results: Eight phenolic compounds were isolated. The chemical structures of isolated compounds were unambiguously elucidated by NMR spectroscopy and comparison with the data in the literature. These are 8'-O-methylsalazinic acid, salazinic acid, 8'-O-methylprotocetraric acid, diffractaic acid, lecanorin, lecanoric acid, isolecanoric acid, and diorcinolic acid.
Conclusion: Among them, 8'-O-methylsalazinic acid was found as a new natural product.

Key words: depside, depsidone, diphenyl etther, Lichen, Pamotrema dilatatum, phenolic compound

INTRODUCTION

Lichen metabolites endowed with various bioactivities, especially phenolic compounds such as depsides, depsidones and diphenyl ethers[1]. Depsidones showed antiviral, antibacterial, and enzyme inhibitory activities while depsides and diphenyl ethers exhibited strong cytotoxicity toward many cancer cell lines and other potent bioactivities[2].

Vietnamese lichens have much attracted chemists to investigate new metabolites. The *Parmotrema* genera popularly growing in Vietnam, *i.e P. tsavoense*, *P. sancti-angelii*, and *P. prasorediosum* produced many new and novel compounds having interesting biological activities[3–5]. In the course of our systematic research on lichen substances from the Vietnamese lichens, we have examined *Parmotrema dilatatum*, widely distributed in the high attitude in South Vietnam. Chemical data on *Parmotrema dilatatum* are scarce with only report of isolation of two major components, salazinic acid and atranorin[6]. Herein describes the isolation of eight compounds from the polar extract of the lichen *Parmotrema dilatatum*. Their chemical structures were defined by NMR and MS spectroscopy in accordance with the data reported in the literature.

METHODS

General experimental procedures

Bruker Advance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer with TMS as internal standard recorded NMR spectra. Chemical shifts are expressed in ppm with reference of acetone-d_6 at δ_H 2.05, d$_C$ 206.26 and 29.84 and of dimethylsulfoxide-d_6 at δ_H 2.50 and δ_C 39.52. The HR–ESI–MS were recorded on a HR–ESI–MS Bruker microOTOF Q-II. TLC was carried out on precoated silica gel 60 F$_{254}$ or silica gel 60 RP–18 F$_{254}$S (Merck Millipore, Billerica, Massachusetts, USA) and spots were visualized by spraying with 10% H$_2$SO$_4$ solution followed by heating. Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm) (HiMedia, Mumbai, India).

Plant material

Lichen thalli were separated from rocks in Lam Dong province, Vietnam in August-September 2015. The scientific name was defined by Dr. Wetchasart Polyiam, Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University. A voucher specimen (No UP-002) was deposited in the herbarium of the Department of Organic Chemistry, University of Education — Ho Chi Minh City — Vietnam.

Cite this article : Huy D T. **Phenolic compounds from *Pamotrema dilatatum* growing in Lam Dong province** . *Sci. Tech. Dev. J.;* 22(1):114-119.

Extraction and isolation

The thallus material was washed under a flow of tap water, prior to being rinsed with distilled water. The lichen was air-dried at ambient temperature (35^{o}C) to avoid thermal decomposition. Clean, ground and dry material (703 g) was macerated in acetone (3x10 L) at ambient temperature, and the filtrated solution was concentrated *in vacuo* to dryness to afford a crude acetone extract (112.54 g). This crude extract was subjected to normal phase silica gel quick column chromatography, eluted consecutively with *n*-hexane, dichloromethane, ethyl acetate, acetone, and methanol to afford five extracts **PH** (3.02 g), **PC** (6.17 g), **PEA** (44.53 g), **PA** (31.71 g), and **PM** (4.91 g), respectively.

The extract **PA** was washed three times by the mixture of acetone-dichloromethane (1:1) to afford the precipitate **PAT** (22.0 g) and the solution **PAS** (9.71 g). The solution **PAS** was concentrated and chromatographed by CC, eluted with the solvent system *n*-hexane-dichloromethane-ethyl acetate-acetone-acetic acid (350:100:40:25:10) to yield six fractions, **PAS1-PAS6**. Fraction **PAS1** (1.5 g) was fractionated by CC, eluted with the same solvent system to afford three sub-fractions **PAS1.1-3.** Fraction **PAS1.1** (251 mg) was rechromatographed to afford two compounds **3** (2.3 mg) and **4** (2.3 mg). Fraction **PAS4** (3.3 g) was fractionated by CC, eluted with the solvent system *n*-hexane-dichloromethane-ethyl acetate-acetone-acetic acid (175:100:40:25:10) to afford three subfractions **PAS4.1-3**. Purifying the subfraction **PAS4.2** (700 mg) resulted in two compounds **1** (31 mg) and **2** (430 mg) **Figure 1.**

The extract **PEA** (44.53 g) was applied to CC, eluted with *n*-hexane-ethyl acetate-acetone (4:1:1) to afford ten fraction **PEA1-10**. Fraction **PEA1** (4.1 g) was fractionated by CC, eluted with the solvent system *n*-hexane-dichloromethane-ethyl acetate-acetone-acetic acid (350:100:40:25:10) to afford three fractions **PEA1.1-3**. Fraction **PEA1.3** (680 mg) was applied to preparative TLC, eluted with the previously described solvent system to afford four compounds **5** (8.7 mg), **6** (102 mg), **7** (6.3 mg), and **8** (2.3 mg) **Figure 1.**

- **8'-*O*-methylsalazinic acid (1).** White amorphous powder; HR-ESI-MS *m/z* 401.05500 [M-H]$^{-}$ (calcd for $C_{19}H_{14}O_{10}$-H, 401.05087); the ^{1}H and ^{13}C NMR (DMSO-*d6*) spectroscopic data, see **Table 1.**
- **Salazinic acid (2)**. White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-*d6*) spectroscopic data, see **Table 1.**
- **8'-*O*-methylprotocetraric acid (3)**. White amorphous powder; the ^{1}H and^{13}C NMR (DMSO-*d6*) spectroscopic data, see **Table 1.**
- **Diffractaic acid (4).** White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-*d6*) spectroscopic data, see **Table 2.**
- **Lecanorin (5)**. White amorphous powder; the ^{1}H and ^{13}C NMR (Acetone-*d6*) spectroscopic data, see **Table 2.**
- **Lecanoric acid (6).** White amorphous powder; the ^{1}H and ^{13}C NMR (Acetone-*d6*) spectroscopic data, see **Table 2**.
- **Isolecanoric acid (7).** White amorphous powder; the ^{1}H and ^{13}C NMR (Acetone-*d6*) spectroscopic data, see **Table 2.**
- **Diorcinolic acid (8).** White amorphous powder; the ^{1}H and ^{13}C NMR (Acetone-*d6*) spectroscopic data, see **Table 2.**

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder with the molecular formula was determined by HR-ESI-MS data as $C_{19}H_{14}O_{10}$. The ^{1}H NMR spectra of **1** showed the presence of one formyl (δ_H 10.60, 1H, s), one aromatic proton (δ_H 6.84, 1H, s), one methine proton (δ_H 6.62, 1H, s), one oxygenated methylene proton (δ_H 4.53, 2H, s), one methoxy group (δ_H 3.26, 3H, s), one methyl groups (δ_H 2.55, 3H, s). The ^{13}C NMR spectra showed the presence of one aldehyde carbon (δ_C 193.1), two carboxy carbon (δ_C 166.0 and 161.5), twelve aromatic carbons in the zone δ_C 100.0-166.0, one hemiacetal carbon (δ_C 95.1), two oxygenated carbons (δ_C 62.0 and 57.3), and one methyl carbon (δ_C 21.0). The HMBC correlation of the methoxy group at δ_H 3.26 (δ_C 57.3) with carbon C-8' indicated the presence of the methoxy group at this carbon. The comparison NMR spectral data of **1** have with those of 8'-*O*-methylsalazinic acid Elix *et al.* (1999) showed that they were identical. Thus, **1** was elucidated as 8'-*O*-methylsalazinic acid[7] **Figure 2.**

Compound **2** was obtained as white amorphous powder. The ^{1}H NMR and HSQC spectra of **2** showed the presence of one chelated hydroxyl group δ_H .

Compound **3** was obtained as white amorphous powder. The ^{1}H NMR and HSQC spectra of **3** showed the presence of one formyl (δ_H .

Compound **4** was isolated as obtained as colorless needle. The ^{1}H NMR spectra of **4** showed the presence of one two aromatic protons (δ_H was elucidated as diffractaic acid.

Compound **5** was obtained as white amorphous powder. The ^{1}H NMR spectra of **5** indicated the presence of one chelated hydroxy group (δ_H .

Compound **6** was obtained as white amorphous powder. NMR data of **6** was highly similar with those of

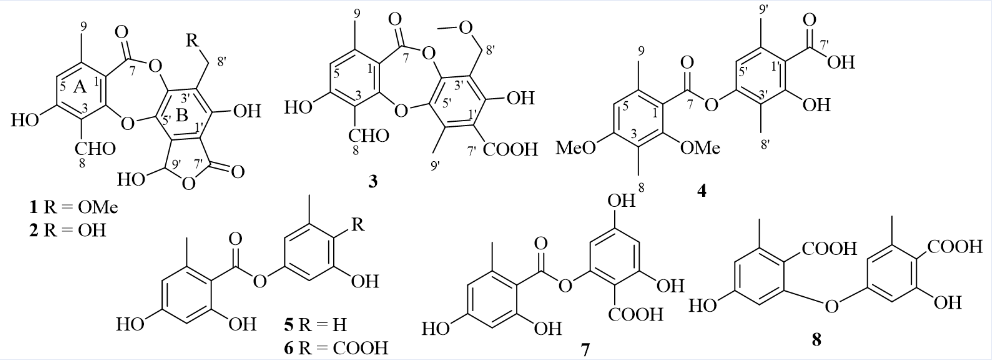

Figure 1: Chemical structures of 8'-*O*-methylsalazinic acid (**1**), salazinic acid (**2**), 8'-*O*-methylprotocetraric acid (**3**), diffactaic acid (**4**), lecanorin (**5**), lecanoric acid (**6**), isolecanoric acid (**7**), and diorcinolic acid (**8**).

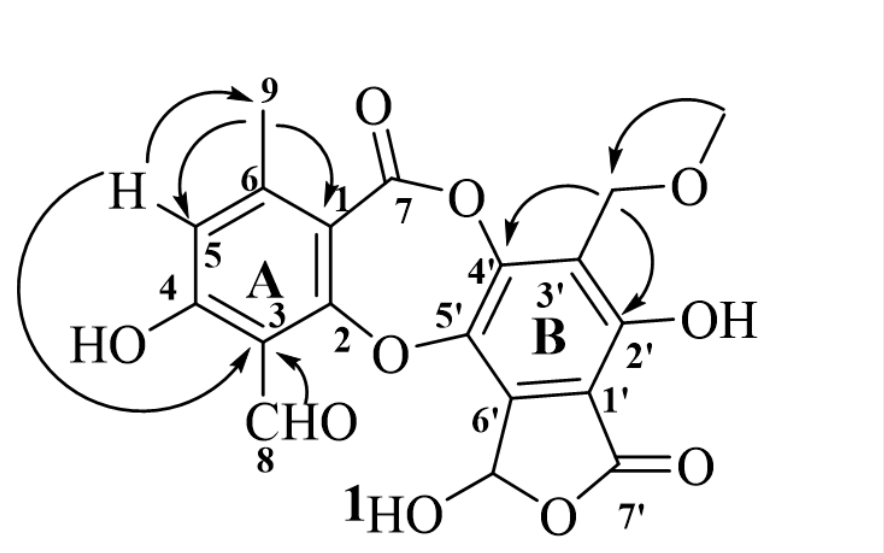

Figure 2: **Key Heteronuclear Multiple Bond Correlations of 1.**

5, except for the absence of one aromatic proton in the B-ring (δ_H was identified as lecanoric acid.

Compound **7** was obtained as white amorphous powder. The ^{1}H NMR spectra showed the presence of one chelated hydroxy group (δ_H 11.31 1H, *s*), four aromatic protons (δ_H 6.60, 1H, *s*; 6.52, 1H, *s*; 6.40, 1H, *s*; 6.32, 1H, *s*), and two methyl protons (δ_H 2.66; 3H, *s* and 2.61, 3H, *s*). NMR data of the A-ring of **7** was highly similar with those of **7**, indicating that they share the same A-ring. The differences were the signals of the B-ring, including the upfield chemical shift of C-4' (δ_C was elucidated as isolecanoric acid.

Compound **8** was obtained as white amorphous powder. The ^{1}H NMR spectra showed the presence of one chelated hydroxy group (δ_H 11.33, 1H, s), four aromatic methines (δ_H 6.53, 1H, *d*, 2.0 Hz; 6.44, 1H, *d*, 2.0 Hz; 6.38, 1H, *d*, 2.5 Hz; 6.29, 1H, *d*, 2.5 Hz), and two methyls (δ_H 2.65, 3H, s and 2.59, 3H, s). The ^{13}C NMR spectrum confirmed the presence of fifteen carbons comprising two carboxyl carbons (δ_C was elucidated as diorcinolic acid.

Salazinic acid is a representive for a rare depsidone class having a hydroxymethylene moiety in the B-ring with four compounds reported so far. Salazinic acid was isolated as a major component of the studied lichen. 8'-*O*-Methylsalazinic acid was a methylated derived from salazinic acid. Elix and coworkers (1999) converted quaesitic acid (**9**) to 8'-*O*-methylsalazinic acid (**1**) when storing quaesitic acid in methanol at ambient temperature. The transesterification occurred at C-8' of quaesitic acid led

Table 1: Nuclear magnetic resonance of compounds 1 – 3 (in DMSO-*d6*)

	1		**2**		**3**	
	δH, J(Hz)	**δC**	**δH, J(Hz)**	**δC**	**δH, J(Hz)**	**δC**
1		111.9		112.2		111.8
2		161.6		160.0		164.4
3		nd		nd		112.3
4		165.7		164.0		163.8
5	6.84, s	117.9	6.88, s	117.9	6.78, s	116.9
6		152.0		152.0		151.8
7		161.5		160.3		161.3
8	10.60, s	193.1	10.45, s	193.1	10.54, s	191.8
9	2.55, s	21.0	2.45, s	21.4	2.45, s	21.3
4- OH			12.06, s			
1’		110.8		110.6		111.5
2’		152.1		152.1		158.2
3’		125.0		123.8		115.1
4’		145.1		145.1		145.1
5’		144.7		144.7		141.2
6’		nd		137.6		131.2
7’		166.0		165.0		170.4
8’	4.53, s	62.0	4.64, s	52.8	4.43, s	62.4
9’	6.62, br	95.1	6.80, s	95.0	2.34, s	14.4
OCH_3	3.26, s	57.3			3.19, s	57.3

nd: not determined

to the formation of 8’-*O*-methylsalazinic acid (**1**) and furmaric acid (**10**) (**seeFigure 3**). In our case, the solvent methanol has not been used during experimental process thus such esterification or etherification would not happen. At this scenario, we proposed that compound **1** was isolated as a new natural product.

Honda and coworkers (1999) previously studied this lichen with the isolation and identification of two compounds atranorin and salazinic acid[6]. Thus, compounds **3**-**7** were reported in the first time from the lichen *Parmotrema dilatatum*. Orcinol-derieved diphenyl ethers which have the similar skeleton as compound **8** are quite common from the fungus source[8]. Nevertheless, diphenyl ethers seldom encountered from the *Parmotrema* genera[9]. This is the first time diphenyl ether diorcinolic acid (**8**) found in the *Parmotrema* lichens.

CONCLUSIONS

From *Pamotrema dilatatum* collected in Lam Dong province, eight phenolic compounds were isolated and elucidated, including 8’-*O*-methylsalazinic acid (**1**), salazinic acid (**2**), 8’-*O*-methylprotocetraric acid (**3**), diffactaic acid (**4**), lecanorin (**5**), lecanoric acid

Table 2: **Nuclear magnetic resonance data of compounds 4-8***

Positi	4^a		5^b		6^b		7^b		8^b	
	δH, J(Hz)	δC	δH, J(Hz)	δC	δH, J(Hz)	δC	δH, J(Hz)	δC	δH, J(Hz)	δC
1		119.3		110.6		105.5		103.9		104.1
2		156.4		164.2		162.2		163.4		165.7
3		116.4	6.28, d, 2.5	101.9	6.28, d, 2.5	100.8	6.32, brs	100.9	6.29, d, 2.5	100.8
4		161.3		159.1		162.7		165.1		163.0
5	6.45, s	108.5	6.37, d, 2.5	107.4	6.37, d, 2.5	111.8	6.40, brs	107.6	6.38, d, 2.5	111.8
6		134.8		144.7		141.6		143.8		143.8
7		165.5		174.4		167.9		165.9		169.8
8	2.23, s	19.5	2.59, s	24.4	2.61, s	22.5	2.61, s	23.7	2.59, s	23.6
9	1.90, s	8.7								
1’		111.5	6.62, t, 2.0	114.7		116.2		115.1		116.1
2’		159.5		154.5		155.9		152.2		164.4
3’		116.0	6.57, d, 2.0	114.5	6.73, d, 2.5	111.0	6.52, brs	107.6	6.53, d, 2.0	107.3
4’		152.2		152.0		151.9		163.4		151.6
5’	6.62, s	115.7	6.57, d, 2.0	112.9	6.76, d, 2.5	116.9	6.60, brs	114.3	6.44, d, 2.0	114.1
6’		139.0		141.1		137.9		143.5		143.5
7’		173.1				171.2		169.9		174.7
8’	2.34, s	22.8	2.29, s	21.4	2.65, s	21.5	2.66, s	23.0	2.65, s	22.8
9’	1.98, s	8.9								
2-OMe	3.68, s	61.8								
4-OMe	3.60, s	55.8								
2-OH			11.30, s							
2’-OH							11.31, s		11.33, s	

^{a}: *These wererecorded in DMSO-d_6*
^{b}:*These were recorded in Acetone-d_6*

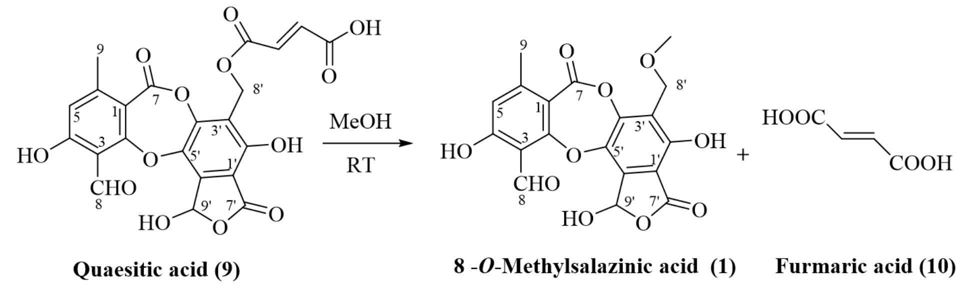

Figure 3: **The conversion of quaesitic acid 9 to 1 by Elix and coworker.**

(**6**), isolecanoric acid (**7**), and diorcinolic acid (**8**). Compound **1** was reported as a new natural product while other compounds **3**-**8** were found in the studied lichen for this first time.

ABBREVIATIONS

^{13}C NMR: Carbon-13 nuclear magnetic resonance
^{1}H NMR: Proton nuclear magnetic resonance
brs: broad singlet
CC: column chromatography
d: doublet
DMSO: Dimethyl sulfoxide (CD_3SOCD_3)
HMBC: Heteronuclear multiple bond correlation
HPLC: High-performance liquid chromatography
HSQC: Heteronuclear single quantum coherence
s: singlet
TLC: Thin layer chromatography

COMPETING INTERESTS

The authors declare no competing financial interest.

AUTHORS' CONTRIBUTIONS

Duong T. H. has contributed in conducting experiments, acquisition of data, interpretation of data, searching the bibliography and gave final approval of the manuscript to be submitted.

ACKNOWLEDGMENTS

We would like to thank Dr. Wetchasart Polyiam for lichen identification.

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