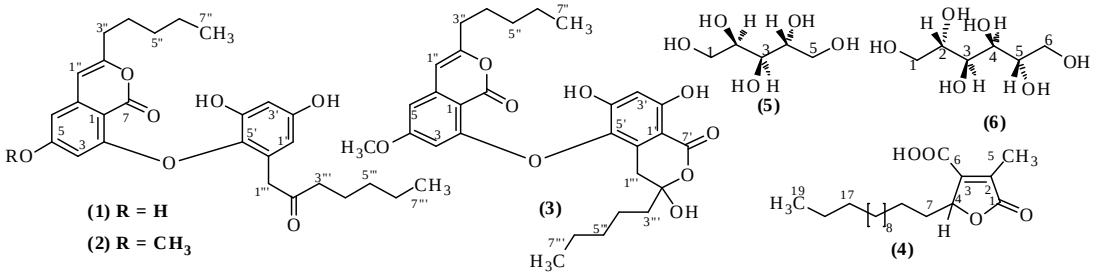
COLLATOLIC ACID DERIVATIVES FROM LICHEN *PARMOTREMA PLANATILOBATUM (*HALE*)* HALE (PARMELIACEAE)

DUONG THUC HUY*, TRAN THI THANH THUY**

ABSTRACT

*Continuation of the phytochemical analysis of the lichen Parmotrema planatilobatum (Hale) Hale resulted in the isolation of six compounds. On the basis of spectroscopic evidences (NMR, MS), the structures were established as 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-hydroxy-3-pentyl-1H-isochromen-1-one **(1)**, 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-methoxy-3-pentyl-1H-isochromen-1-one **(2)**, β-collatolic acid **(3),** lichesterinic acid **(4)**, D-arabinitol **(5)**, D-mannitol **(6)**. This is the first time these compounds are reported in Parmotrema planatilobatum (Hale) Hale.*

Keywords: Parmeliaceae, Parmotrema planatilobatum, diphenyl ethers, γ-lactone aliphatic acid, alditols



TÓM TẮT

Một số dẫn xuất của collatolic acid từ loài Parmotrema planatilobatum (Hale) Hale (Parmeliaceae)

*Những nghiên cứu tiếp theo về thành phần hóa học của loài địa y Parmotrema planatilobatum (Hale) Hale đã cho kết quả cô lập được sáu hợp chất. Cấu trúc của các hợp chất được xác định bằng các phương pháp phổ nghiệm (NMR, MS) như sau: 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-hydroxy-3-pentyl-1H-isochromen-1-one **(1)**, 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-methoxy-3-pentyl-1H-isochromen-1-one **(2)**, β-collatolic acid **(3),** lichesterinic acid **(4)**, D-arabinitol **(5)**, D-mannitol **(6)**. Đây là lần đầu tiên các hợp chất này được tìm thấy trong loài địa y Parmotrema planatilobatum (Hale) Hale.*

Từ khóa: Parmeliaceae, Parmotrema planatilobatum, diphenyl ethers, γ-lactone aliphatic acid, alditols.

* MSc, PhD. Student, University of Education, Ho Chi Minh City
** BSc, University of Education, Ho Chi Minh City

1. Introduction

Lichen metabolites are some of the potential natural product sources that exhibit manifold bioactivities.[10] The biological activities of lichen substances have been reviewed extensively.[2,8]

Previous studies on the chemical constituents of the lichen *Parmotrema planatilobatum* (Hale) Hale have resulted in the isolation of some aromatic compounds.[3,4] This paper describes the isolation and structural elucidation of six compounds: 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-hydroxy-3-pentyl-1*H*-isochromen-1-one (**1**), 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-methoxy-3-pentyl-1*H*-isochromen-1-one (**2**), *β*-collatolic acid (**3**), lichesterinic acid **(4)**, D-arabinitol **(5)**, and D-mannitol **(6)**.

Figure 1. *Parmotrema planatilobatum* (Hale) Hale

2. Experimental

General experimental procedures

The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). HR-MS were recorded on a Bruker micrOTOF Q-II. All instruments are available in the Central Laboratory for Analysis, University of Science, Vietnam National University - Ho Chi Minh City.

Plant material

Parmotrema planatilobatum (Hale) Hale was collected on the bark of *Pinus dalatensis* at Lam Dong province, Vietnam. The scientific name was identified by MSc. Vo Thi Phi Giao, Faculty of Biology, University of Science, VNU-HCMc. A voucher specimen (No US-B023) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, Vietnam National University – HCM City.

Extraction and isolation

The clean, air-dried and ground material (350 g) was extracted by methanol at room temperature and the filtrated solution was concentrated under the reduced pressure. While the methanolic solution was evaporated, a precipitate (**P**, 2.0 g) occurred and was filtered off. The rest solution was evaporated to obtain crude methanol extract (63 g). This crude extract was applied to silica gel solid phase extraction, consecutively eluted with four solvent systems of petroleum ether (60–90 ^{o}C) 100%, petroleum ether–ethyl acetate (20–50%), petroleum ether–ethyl acetate (60–100%) and methanol to afford corresponding extracts: extract **E** (1.5 g), extract **EA1** (22.1 g), extract **EA2** (12.1 g) and extract **M** (10.2 g). The extracted solutions were evaporated until dryness in a rotary evaporator at approx. 40 °C.

Extract **EA1** (22.1 g) was applied to silica gel column chromatography, eluting with hexane–ethyl acetate–acetone (9:1:0.5) to give five fractions, **EA1.1** (7.9 g), **EA1.2** (2.7 g), **EA1.3** (1.4 g), **EA1.4** (2.1 g), and **EA1.5** (1.5 g). Fraction **EA1.1** (7.9 g) was rechromatographed, eluting with chloroform– methanol (9:1) to give two subfractions, **EA1.1.1 (**3.5 g**)** and **EA1.1.2 (**2.0 g**)**. Fraction **EA1.1.1** was applied to the reversed phase C_{18} column chromatography to afford four compounds, **1** (7.9 mg), **2** (5.8 mg), **3** (14.7 mg), and **4** (5.9 mg). Fraction **EA1.5** (1.5 g) was applied to column chromatography, eluting with chloroform: methanol (C: M, 9:1) to give two fractions, **EA1.4.1 (**495.7 mg**)** and **EA1.4.2 (**197.8 mg**)**. Fraction **EA1.4.1** was purified to afford two compounds, **5** (295.7 mg) and **6** (795.6 mg).

- **8-[2,4-Dihydroxy-6-(2-oxoheptyl)phenoxy]-6-hydroxy-3-pentyl-1*H*-isochromen-1-one (1):** Colorless needles (acetone). HR-ESI-MS, negative mode: $[M-H]^-$ *m/z* 481.2222 [calcd for ($C_{28}H_{34}O_7$ – H), 481.2226]. The ^{1}H and ^{13}C-NMR data (acetone-d_6): see Table 1. HMBC correlations: see Figure 2. These spectroscopic data were suitable with those reported in the literatures.[9]

- **8-[2,4-Dihydroxy-6-(2-oxoheptyl)phenoxy]-6-methoxy-3-pentyl-1*H*-isochromen-1-one (2**): White amorphous powder. HR-ESI-MS, positive mode: $[M+Na]^+$ *m/z* 491.2041 [calcd for ($C_{27}H_{32}O_7$ +Na), 491.2046]. The ^{1}H and ^{13}C-NMR data (acetone-d_6): see Table 1. HMBC correlations: see Figure 2. These spectroscopic data were suitable with with those reported in the literatures.[5]

- **β-Collactolic acid (3)**: Colorless crystals (acetone). HR-ESI-MS, positive mode: $[M+Na]^+$ *m/z* 549.2080 [calcd for ($C_{29}H_{34}O_9$+Na) 549.2100]. The ^{1}H and ^{13}C-NMR data (acetone-d_6): see Table 1. These spectroscopic data were suitable with those reported in the literatures.[7]

- **Lichesterinic acid (4)**: White amorphous powder. ^{1}H-NMR data (acetone-d_6): 5.13 (1H, *m*, H-4), 2.12 (3H, *d*, J=2.0 Hz, H-5), 1.60 (1H, *m*, H-7a), 1.38 (1H, *m*, H-7b), 1.28-1.38 (20H, *m*, H-8 to H-18), 0.87 (3H, *t*, J=7.0 Hz, H-5). ^{13}C-NMR data (Acetone-d_6): 172.7 (C-1), 163.5 (C-6), 149.3 (C-3), 137.0 (C-2), 82.2 (C-4), 33.5 (C-7), 32.3 (C-17), 29.0-30.0 (C-9 to C-16), 25.1 (C-8), 23.3 (C-18), 14.6 (C-19), 10.3 (C-5). These spectroscopic data were suitable with those reported in the literature.[8]

- **D-Arabinitol (5):** White amorphous powder. The ^{1}H and ^{13}C-NMR data (CD_3OD): see Table 2. These spectroscopic data were suitable with with those reported in the literature.[1]

- **D-Mannitol (6):** White amorphous powder. The ^{1}H and ^{13}C-NMR data (CD_3OD): see Table 2. These spectroscopic data were suitable with those reported in the literature.[1]

3. Results and discussions

Compound **(1)** was isolated as colorless crystals. Its molecular formula was determined to be $C_{28}H_{34}O_7$ by HR-ESI-MS (negative mode) quasi-molecular ion peak at *m/z* 481.2222 $[M-H]^-$ showing 8 degrees of unsaturation. The ^1H NMR spectrum (Table 1) displayed resonances of five aromatic protons including two pairs of doublets [δ 6.60 (1H, *d*, J=2.5 Hz, H-5), 6.11 (1H, *d*, J=2.5 Hz, H-3)]; [6.46 (1H, *d*, J=2.5 Hz), 6.31 (1H, *d*, J= 3.0 Hz)] and a singlet proton at δ 6.36 (1H, *s*). These findings indicated that **(1)** possessed two aromatic rings. In addition, the ^1H NMR spectrum also exhibited one methoxy group [δ 3.78 (3H, *s*)], one downfield methylene group [δ 3.54 (2H, *s*)] and two C_5-side chains [2.49 (2H, *t*, J=7.5 Hz), 1.69 (2H, *m*), 1.38 (2H, *m*), 1.37(2H, m), 0.90 (3H, *t*, J=7.5 Hz); 2.36 (2H, *t*, J=7.5 Hz), 1.37 (2H, *m*), 1.17 (2H, *m*), 1.08 (2H, *m*), 0.80 (3H, *t*, J=7.5 Hz)]. The ^{13}C NMR spectrum in accordance with HSQC spectrum revealed the presence of 28 carbons: one ketone group at δ 207.3 (>C=O), one carboxyl group at δ 162.6 (-COO), five oxygenated aromatic carbons (δ 166.2, 159.3, 156.4, 151.2, 133.9), three substituted carbons of which one was attached to the carbonyl group (δ 143.1, 130.8, 103.7), five methine carbons (δ 110.2, 104.0, 103.6, 102.3, 101.6), one methoxy group (δ 56.1) and two C_5-side chains with ten high field signals.

In the A-ring, the HMBC spectrum showed correlations of the proton at δ 6.36 (H-1") with the carbons at δ 159.8 (C-2"), 143.1 (C-6), 103.7 (C-1), 102.3 (C-5), and the methylene group (δ 33.8, C-3") of the C_5-side chain. Moreover, the protons H_2-3" correlated with the carbons C-1", C-2", C-4", and C-5". It indicated that the A-ring possessed an isocoumarin skeleton. Additionally, two aromatic protons (δ 6.60 & 6.11) and the methoxy protons correlated with the same carbon at δ 166.2, suggesting the methoxy group was at C-4.

In the B-ring, the methylene group H_2-1"' (δ_H 3.54, δ_C 44.5) correlated with the ketone group (δ 207.3), indicating that the B-ring possessed the 2-oxoheptyl chain on the basis of HMBC correlations. In addition, the HMBC correlations of H-3' to C-2', C-4', and C-5' and of H-1' to C-2', C-3', C-5', and C-6' confirmed the structure of the B-ring. According to HMBC correlations and comparison to those reported in the literature,[9] **(1)** was elucidated to be 8-[2,4-dihydroxy-6-(2-oxoheptyl)phenoxy]-6-methoxy-3-pentyl-1*H*-isochromen-1-one.

Compound **(2)** was isolated as colorless crystals. The molecular formula of **(2)** as $C_{27}H_{32}O_7$ determined by HR-ESI-MS quasi-molecular ion peak at *m/z* 491.2041 showed that **(2)** possessed one less methylene unit than **(1)**. The ^1H and ^{13}C NMR spectra (Table 1) of **(2)** were similar to those of **(1)** except of the disappearance of a methoxy group. This indicated the presence of a hydroxyl group instead of the methoxy group at C-4 in **(2)**. It was supported by the HSQC and HMBC correlations (Figure 2). Therefore, **(2)** was determined to be 8-(2,4-dihydroxy-6-(2-oxoheptyl)phenoxy)-6-hydroxy-3-pentyl-1*H*-isochromen-1-one.[5]

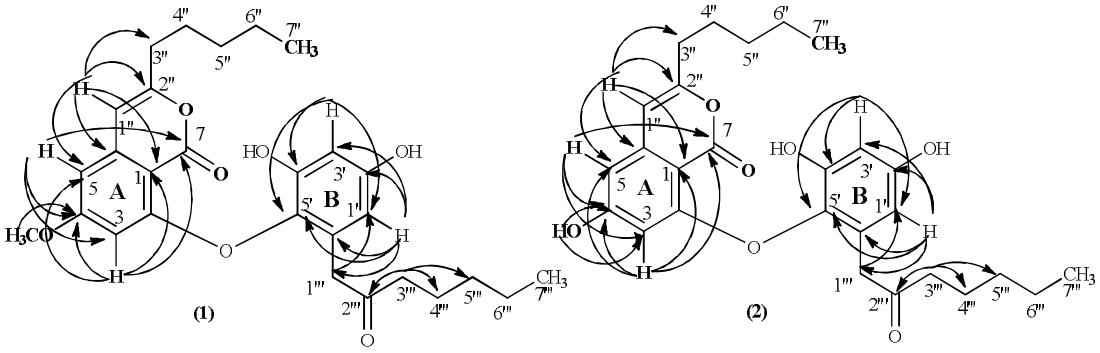


Figure 2. *Chemical structure and selected HMBC correlation of (1) and (2)*

Table 1. *NMR spectral data of (1), (2), and (3)*

Positions	(1)		(2)		(3)	
	δ_H, *J (Hz)*	δ_C	**δ_H**, *J (Hz)*	δ_C	**δ_H**, *J (Hz)*	δ_C
1		103.7		103.9		103.8
2		159.3		159.4		162.0
3	6.11 (*d*, 2.5)	101.6	6.07 (*d*, 2.5)	102.1	6.26 (*brs*)	101.4
4		166.2		164.6		166.4
5	6.60 (*d*, 2.5)	102.3	6.44 (*d*, 2.5)	102.7	6.67 (*s*)	103.0
6		143.1		143.0		143.3
7		162.6		163.0		159.5
1’	6.31 (*d*, 3.0)	110.2	6.32 (*d*, 3.0)	110.0		101.4
2’		156.5		156.3		160.0
3’	6.46 (*d*, 2.5)	104.0	6.46 (*d*, 3.0)	104.8	6.48 (*s*)	103.2
4’		151.2		150.4		157.9
5’		133.9		133.8		133.2
6’		130.8		130.8		nd
7’						166.4
1’’	6.36 (*s*)	103.6	6.27 (*s*)	103.4	6.40 (*s*)	103.8

2''		159.8		159.5		160.2
3''	2.49 (*t*, 7.5)	33.8	2.47 (*t*, 7.5)	33.8	2.52 (*t*, 7.5)	33.8
4''	1.69 (*m*)	27.3	1.68 (*m*)	27.3	1.72 (*m*)	27.3
5''	1.38 (*m*)	31.9	1.37 (*m*)	32.0	1,40 (*m*)	31.9
6''	1.37 (*m*)	24.0	1.38 (*m*)	23.1	1,38 (*m*)	23.9
7''	0.90 (*t*, 7.5)	14.2[a]	0.90 (*t*, 7.5)	14.2	0.91 (*t*, 7.5)	14.4[b]
1'''	3.54 (*s*)	44.5	3.53 (*s*)	44.3	3.0 (*m*)	32.4
2'''		207.3		207.3		103.3
3'''	2.36 (*t*, 7.5)	42.6	2.37 (*t*, 7.5)	42.6	1.90 (brs)	41.0
4'''	1.37 (*m*)	23.0	1.38 (*m*)	23.0	1.38 (*brs*)	23.0[c]
5'''	1.08 (*m*)	32.0	1.10 (*m*)	31.9	1.28 (*brs*)	32.5
6'''	1.17 (*m*)	23.0	1.18 (*m*)	23.0	1.28 (*brs*)	23.1[c]
7'''	0.80 (*t*, 7.5)	14.0[a]	0.81 (*t*, 7.5)	14.2	0.85 (*t*, 7.5)	14.2[b]
4-OH			9.29 (*s*)			
4-OCH_3	3.78 (*s*)	56.1			3.80 (*s*)	56.3
2'-OH			8.44 (*s*)		11.35 (*s*)	
4'-OH					9.84 (*s*)	
2'''-OH						

NMR spectra were recorded in acetone-d_6; [a, b, c]: interchangeable assignments; nd: not determined

Table 2. *NMR spectral data of (5) and (6)*

N	(5) (CD_3OD)		(6) (CD_3OD)	
	δ_H, *J* (Hz)	**δ_C**	**δ_H, *J* (Hz)**	**δ_C**
1	3.82 (*dd*, 11.0, 6.0) 3.64 (*dd*, 11.0, 6.0)	64.9	3.81 (*dd*, 11.5, 3.5) 3.63 (*dd*, 11.5, 6.0)	64.2
2	3.90 (*td*, 6.5, 1.5)	73.1	3.69 (*ddd*, 8.0, 6.0, 3.5)	73.1
3	3.55 (*dd*, 8.0, 1.5)	72.5	3.77 (*d*, 8.0)	71.5
4	3.71 (*ddd, 8.0, 6.0, 3.5*)	72.0	3.77 (*d*, 8.0)	71.5
5	3.64 (*dd*, 11.5, 6.0) 3.64 (*dd*, 11.5, 3.5)	65.0	3.69 (*ddd*, 8.0, 6.0, 3.5)	73.1
6			3.81 (*dd*, 11.5, 3.5) 3.63 (*dd*, 11.5, 6.0)	

4. Conclusion

From the lichen *Parmotrema planatilobatum* collected from Lam Dong province, Vietnam, six compounds were successfully isolated and their chemical structures were elucidated. This is the first time these compounds are known in *Parmotrema planatilobatum*. Further studies on this lichen are in progress.

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