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Research Article

Phenolic compounds from the lichen *Parmotrema cristiferum* (Taylor) Hale

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ABSTRACT

Introduction: Lichens are fungal and algal or cyanobacterial symbioses resulting in the production of specific metabolites with a great variety of effects such as antimycobacterial, antiviral, anti-inflammatory, antipyretic and antiproliferative. Parmotrema cristiferum (Taylor) Hale, a species of foliose lichen, was collected at Champasack Province, Laos in April 2015. This paper describes the isolation and structure elucidation of nine compounds isolated from this lichen. **Methods:** Phytochemical investigations of the chloroform extract of the lichen P. cristiferum led to the isolation of nine pure compounds. Their chemical structures were elucidated by extensive HR-ESI-MS and 1D and 2D-NMR spectroscopic analysis, and comparison with previously published data. **Results:** From the studied lichen, nine compounds, orcinol (**1**), orsellinic acid (**2**), atranol (**3**), methyl β-orsellinate (**4**), atranorin (**5**), diffractaic acid (**6**), lecanoric acid (**7**), bailesidone (**8**), and tinctorinone (**9**) were isolated, structurally elucidated and evaluated for their α-glucosidase inhibitory activity. **Conclusions:** Among isolated compounds, except **2**, **4,** and **7,** the remaining six compounds were known for the first time from the lichen *P. cristiferum*. All these compounds were inactive in the α-glucosidase enzyme inhibitory assay.

Key words: Parmotrema cristiferum (Taylor) Hale, depside, depsidone, phenolic, α-glucosidase

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia. There are many and diverse therapeutic strategies in the management of type 2 diabetes. Predominantly herbal drugs have been widely used globally for diabetic treatment over thousands of years due to their traditional acceptability and lesser side effects. Therefore, screening of α-amylase and α-glucosidase inhibitors in medicinal plants has received much attention.[1]

Parmotrema cristiferum (Taylor) Hale is a foliose lichen; belonging to the genus *Parmotrema,* one of the largest genera of the Parmeliaceae family.[2] Previous studies of this lichen reported that its methanolic extract had various pharmacological activities such as antimicrobial, antioxidant, and insecticidal activities.[3] However, metabolites possessing antidiabetic activity unexplored well as no chemical data of this lichen are reported. Therefore, to search for new classes of α-glucosidase inhibitors, we nine compounds' isolation and structural elucidation from the chloroform extract of the lichen *Parmotrema cristiferum* (Taylor) Hale and the α-glucosidase inhibitory activity of these compounds.

MATERIALS AND METHODS

General experimental procedures

The HR–ESI–MS was recorded on an HR–ESI–MS MicrOTOF–Q mass spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker Avance 500 spectrometer. Chemical shifts are expressed in ppm using a residual solvent signal as internal reference ($CDCl_3$ δ_H 7.26, δ_C 77.2). α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (750 UN) and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acarbose and dimethyl sulfoxide were obtained from Merck. Other chemicals were of the highest grade available.

Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F254 or silica gel 60 RP–18 F254S (Merck) and the isolated compounds were visualized by spraying with 5% vanillin solution followed by heating. Gravity column chromatography was performed on silica gel 60 (0.040–0.063 mm, Himedia).

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Plant material

The lichen thalli of *Parmotrema cristiferum* (Taylor) Hale was collected at 109 m altitude at Paksong town (15°11¢00¢¢N, 106°14¢00¢¢E), Paksong district, Champasack province, Laos in April 2015. The botanical species of *Parmotrema cristiferum* (Taylor) Hale was authenticated by Dr. Vo Thi Phi Giao, Faculty of Biology, University of Science, National University — Ho Chi Minh City.

Extraction and isolation

The thallus material (1.2 kg) was washed underflow of tap water and then was air-dried at ambient temp to obviate thermally induced decomposition before being ground into a fine powder. The ground powder sample (0.8 kg) was macerated by acetone at room temperature to afford a crude acetone extract (79.0 g). This crude was subjected to silica gel solid-phase extraction and eluted consecutively with *n*-hexane, chloroform, ethyl acetate, acetone, and methanol at room temperature and then each type of eluants was evaporated at reduced pressure to afford *n*-hexane (6.39 g), chloroform (30.48 g), ethyl acetate (32.57 g), acetone (7.82 g), and methanol (1.64 g) extracts, respectively.The chloroform extract (30.48 g) was applied to silica gel column chromatography and eluted with solvent systems of *n*-hexane:chloroform (stepwise, 9:1 to 0:10) to give five fractions, coded **C1** (1.18 g), **C2** (11.59 g), **C3** (15.49 g), **C4** (1.10 g), **C5** (1.12 g). Fraction **C1** (1.18 g) was repeatedly chromatographed followed by preparative TLC, eluted with *n*-hexane:chloroform (5:5 to 0:10) to afford four compounds coded **1** (18.0 mg), **2** (7.2 mg), **3** (12.3 mg), and **4** (64.0 mg). Fraction **C2** (11.59 g) was silica gel rechromatographed and eluted with chloroform:ethyl acetate (6:4 to 0:10) to give three compounds: **5** (95.3 mg), **6** (13.8 mg), and **7** (7.3 mg). Fraction **C3** (15.49 g) was selected for further fractionation by silica gel CC using an isocratic mobile phase consisting of chloroform:methanol (9:1 to 5:5) to obtain six subfractions (**C3.1** - **C3.6**). Further fractionation of **C3.2** (586.0 mg) was applied to a silica gel CC using chloroform:methanol (98:2) to afford **8** (10.0 mg), and **9** (5.3 mg).

α -Glucosidase inhibition assay

The inhibitory activity of α-glucosidase was determined as our previous assay.[4] Reaction mixture consisting of 60 mL of phosphate-buffered solution (100 mM, pH 6.8), 20 mL of test samples at different concentrations, and 20 mL of *a*-glucosidase (0.3 IU.mL^{-1}) was injected into a 96 well plate and incubated at 37 °C for 10 minutes. Then, 100 mL of *p*-nitrophenyl-α-D-glucopyranoside (200 mM) solution was added to this mixture in a phosphate buffer. Next, incubate the reaction mixture at 37 °C for 30 minutes. Finally, add 50 mL of NaOH solution (50 mM) to this mixture. The absorbance of *p*-nitrophenol (*p*NP) was measured at 405 nm on the ELX800 (BIOTEX). Acarbose was used as the positive control.

RESULTS

By using efficient separation techniques, the chemical investigation on the chloroform extract of the lichen P. cristiferum led to the isolation of nine compounds. Their chemical structures were elucidated by 1D and 2D NMR as well as the HR-ESI-MS. They were four phenolic compounds, orcinol (**1**), orsellinic acid (**2**), atranol (**3**), methyl β-orsellinate (**4**), three depsides, atranorin (**5**), diffractaic acid (**6**), lecanoric acid (**7**), and two depsidones, bailesidone (**8**), and tinctorinone (**9**). Their ^{1}H and ^{13}C-NMR data of compounds 1-4 were presented in Tables 1 and 3; of compounds 5-9 were presented in Tables 2 and 3; and the following characteristics.

- Orcinol (**1**): Colorless needles, mp. 106-108 ^{o}C. HR-ESI-MS (positive mode) *m/z* 125.0601 $[M+H]^+$ (calcd. for $C_7H_8O_2$ 125.0602).
- Orsellinic acid (**2**): Colorless needles, mp. 184-185 ^{o}C. HR-ESI-MS (positive mode) *m/z* 169.0491 $[M+H]^+$ (calcd. for $C_8H_8O_4$+H, 169.0501).
- Atranol (**3**): Faint yellow needles, mp. 124 ^{o}C. HR-ESI-MS (positive mode) *m/z* 153.0536 $[M+H]^+$ (calcd. for $C_8H_8O_3$+H, 153.0551).
- Methyl β-orsellinate (**4**): Colorless needles, mp. 143-144 ^{o}C. HR-ESI-MS (positive mode) *m/z* 197.0814 $[M+H]^+$ (calcd. for $C_{10}H_{12}O_4$+H, 197.0811).
- Atranorin (**5**): Prisms, mp. 196 ^{o}C. HR-ESI-MS (negative mode) *m/z* 373.0881 $[M-H]^-$ (calcd. for $C_{19}H_{18}O_8$-H, 373.0923).
- Diffractaic acid (**6**): White amorphous powder, mp. 189-190 ^{o}C. HR-ESI-MS (positive mode) *m/z* 375.1437 $[M+H]^+$ (calcd. for $C_{20}H_{22}O_7$+H, 375.1443).
- Lecanoric acid (**7**): Colorless needles, mp. 184 ^{o}C. HR-ESI-MS (negative mode) *m/z* 317.0672 $[M-H]^-$ (calcd. for $C_{16}H_{14}O_7$-H, 317.0661).
- Bailesidone (**8**): White amorphous powder, $[\alpha]_D^{29}$ = 0 {$[\alpha]_D^{25}$ 47.4 (*c* 0.7, $CHCl_3$)}.[5] HR-ESI-MS (positive mode) *m/z* 415.1025 $[M+H]^+$ (calcd. for $C_{21}H_{18}O_9$+H, 415.1029).

- Tinctorinone (**9**): White amorphous powder. $[\alpha]_D^{29}$ = 0. HR-ESI-MS (positive mode) *m/z* 415.1025 $[M+H]^+$ (calcd. for $C_{21}H_{18}O_9$+H, 415.1029).

DISCUSSION

The structures of nine isolated compounds were elucidated based on modern physicochemical methods such as HR-MS and 1D and 2D NMR compared their data with those in the literature. The compounds possessing a similar framework were structurally discussed.

Compound **1** was isolated as colorless needles. Its molecular formula was determined as $C_7H_8O_2$ through its molecular ion peak at *m/z* 125.0601 $[M+H]^+$ in the HR-ESI-MS spectrum. The ^{1}H-NMR data of **1** gave one methyl group, three aromatic methine protons, and two hydroxyl protons (Table 1). The ^{13}C-NMR spectrum showed five signals, including one methyl group, three aromatic methines, and three quaternary aromatic carbons (Table 3). The good compatibility of its NMR and MS data with those of orcinol in the literature[6,7] proposed that compound **1** was orcinol.

Compound **2** was obtained as colorless needles. The similarity in the NMR data (Tables 1 and 3) just with the replacement of one aromatic proton in the **1** by a carboxyl group in **2** suggested that it was orselinic acid.[6,7] This was supported by the pseudomolecular ion peak at *m/z* 169.0491 $[M+H]^+$ in the HR-ESI-MS spectrum which fitted well with the molecular formula of $C_8H_8O_4$. Therefore, **2** was orselinic acid.

Compound **3** was quickly identified as atranol because it possessed similar NMR data (Tables 1 and 3) with those in the literature.[6,8] The position of the aldehyde group was clarified by HMBC cross-peaks of its proton (δ_H 10.23) to C-3 (δ_C 109.3) and C-2, 4 (δ_C 163.2) (Figure 2). Its molecular formula $C_8H_8O_3$ was determined through the pseudomolecular ion peak at *m/z* 153.0536 $[M+H]^+$.

The comparison NMR data of **1** and **4** showed that the latter possessed one more methoxycarbonyl (δ_H 3.71, δ_C 173.5, 52.1, COO-CH_3) and one methyl group (δ_H 1.82, δ_C 8.1, C-9). The positions of these two groups were evidenced by HMBC cross-peaks of the methoxy protons at δ_H 3.71 (7-OCH_3) with the carboxyl carbon at δ_C 173.5 (C-7), and of the methyl protons (δ_H 1.82) with adjacent aromatic carbons at δ_C 164.1 (C-2), 109.5 (C-3) and 161.0 (C-4). Based on the compatibility of the NMR and HR-ESI-MS data of **4** with the literature,[9] **4** was methyl β-orsellinate.

Compound **5** was preliminarily proposed as a depside via the comparison of its NMR data (Tables 2 and 3) with corresponding depsides in the literature. A close search showed that **5** was composed of the combination of **3** and **4** moieties. This observation was well supported by its HMBC pattern (Figure 1) and HR-ESI-MS spectrum (negative mode) with a pseudomolecular ion peak at *m/z* 373.0881 $[M-H]^-$ (calcd. for $C_{19}H_{18}O_8$-H, 373.0923). Thus, the structure of **5** was assigned as atranorin.[8]

Compound **6** was obtained as a white amorphous powder. A careful search of the NMR data (Tables 2 and 3) showed that **6** was also a depside and composed of two moieties of β-orsellinic acid (the carboxylic acid of **4**). This was proved by the HR-ESI-MS spectrum (positive mode) with a pseudomolecular ion peak at *m/z* 375.1437 $[M+H]^+$ (calcd. for $C_{20}H_{22}O_7$+H, 375.1443), by HMBC experiment (Figure 1), and by the similar NMR data with those of diffractaic acid.[10,11] Finally, the chemical structure of **6** was determined to be diffractaic acid.

Compound **7** was isolated as colorless needles. In the same above argument, the similarity in the spectral data (Tables 2 and 3) suggested that **7** was composed of two moieties of orsellinic acid (**2**). The HR-MS spectrum (*m/z* 317.0672 $[M-H]^-$, calcd. for $C_{16}H_{14}O_7$-H, 317.0661) and good accordance in the NMR data[12] (Tables 1 and 2) proposed **7** as lecanoric acid.

Compound **9** was isolated as a white amorphous powder. The comparison NMR data of **9** and **8** (Tables 2 and 3) showed that they were depsidones with a lactone ring, with some differences. HMBC experiment of **9** confirmed that its lactone moiety possessed one acetal carbon (δ_H 7.10, δ_C 102.4) and one ethoxy side-chain [δ_H 3.97, δ_C 66.2 (O-**CH**$_2$-CH_3), and δ_H 1.34 (t, 7.0 Hz), δ_C 15.6 (O-CH_2-**CH**$_3$]. In addition, its A ring contained a further hydroxymethylene [δ_H 4.96 (*dd*, 11.5, 8.5 Hz), 4.79 (*dd*, 11.5, 8.5 Hz), δ_C 53.0, C-9]. The comparison of these NMR data with those of tinctorinone, a depsidone in the lichen *Parmotrema tinctorum*[13] showed the compatibility. The specific optical rotation data of **9** was zero, thus it was a racemic mixture of tinctorinone.

The inhibitory assay against α-glucosidase for isolated compounds was evaluated. The results (Table 4) indicated that these compounds (1-9) were inactive.

CONCLUSION

From the chloroform extract of the lichen *Parmotrema cristiferum,* collected at Paksong district,

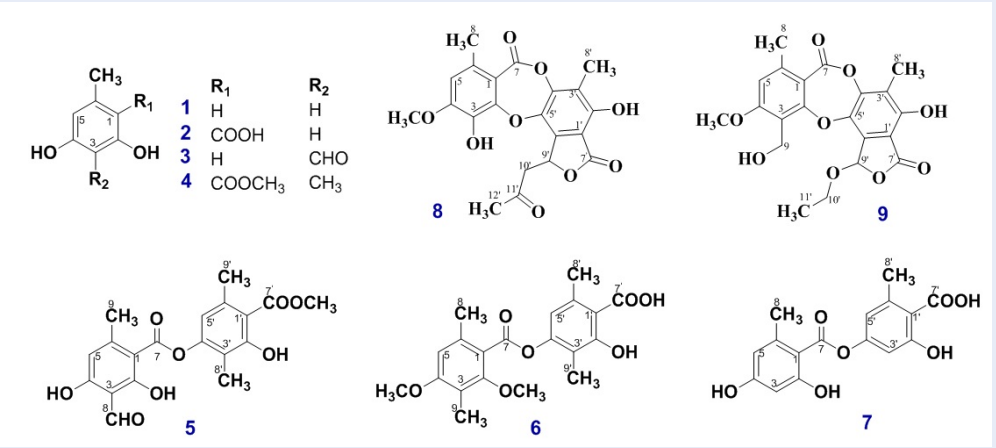

Figure 1: Structures of all isolated compounds

Table 1: ^{1}H-NMR (Acetone-d_6) data of compound 1 -4

No.	1	2	3	4
1	6.15 s		6.23 s	
3	6.15 s	6.29 (d, 2.0)		
5	6.15 s	6.22 (d, 2.5)	6.23 s	6.14 s
7	2.15 s		2.20 s	
8		2.51 s	10.23 s	2.21 s
9				1.82 s
$COOCH_3$				3.71 s
2-OH	8.02 s		10.85 s	11.73 s
4-OH	8.02 s		10.85 s	9.02 s

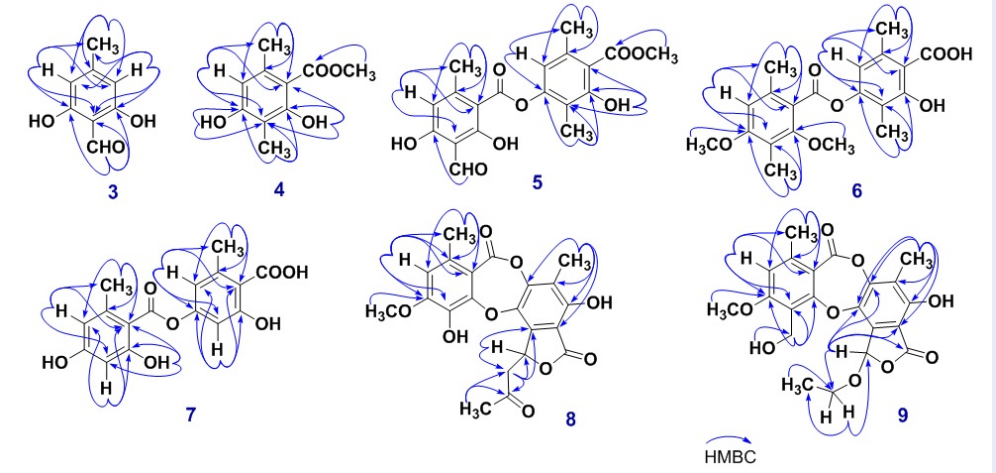

Figure 2: Keys HMBC of isolated compounds

Table 2: ^{1}**H-NMR data of compound 5 -9**

No.	$5^{(b)}$	$6^{(a)}$	$7^{(a)}$	$8^{(c)}$	$9^{(a)}$
3			6.14 (d, 2.5)		
5	6.42 s	6.76 s	6.23 (d, 1.5)	6.60 s	6.95 s
8	10.21 s	2.45 s	2.44 s	2.44 s	2.49 s
9a	2.39 s	2.14 s			4.96 (dd, 11.5, 8.5)
9b					4.79 (dd, 11.5, 4.5)
2-OCH_3		3.85 s			
4-OCH_3		3.91 s		3.92 s	3.94 s
2-OH			11.10 s		
9-OH					4.16 (dd, 8.5, 4.5)
3’			6.44 (d, 2.5)		
5’	6.65 s	6.57 s	6.36 (d, 2.5)		
8’	2.04 s	2.13 s	2.49 s	2.26 s	2.25 s
9’	2.34 s	2.64 s		6.15 (dd, 8.5, 3.0)	7.10 s
10’a				3.05 (dd, 17.5, 8.5)	4.04 m
10’b				3.61 (dd, 17.5, 3.0)	3.97 m
11’					1.34 (t, 7.0)
12’				2.29 s	
7’-OCH3	3.88 s				
2’-OH	10.51 s				

(a) Acetone-d_6 ; (b) DMSO-d_6 ; (c) $CDCl_3$

Champasack province, Laos, using various chromatographic methods, nine phenolic compounds were isolated. They were orcinol (**1**), orsellinic acid (**2**), atranol (**3**),methyl β-orsellinate (**4**), atranorin (**5**), diffractaic acid (**6**), lecanoric acid (**7**), a racemic mixture of bailesidone (**8**), and a racemic mixture of tinctorinone (**9**). Except for compounds **2**, **4**, and **7**,[14] the remaining six compounds were reported for the first time in this species. All nine compounds were evaluated against α-glucosidase enzyme but none of them were active. Further research on the remaining extracts is continued.

ABBREVIATIONS

HR-ESI-MS: High resolution- Electrospray ionization-Mass spectrometry

^{1} **H-NMR:** Proton Nuclear Magnetic Resonance

^{13} **C-NMR:** Carbon-13 Nuclear Magnetic Resonance

HMBC: Heteronuclear Multiple Bond Correlation

s: singlet

d: doublet

dd: doublet of doublets

t: triplet

m: multiplet

COMPETING INTEREST

The authors declare no competing financial interest.

AUTHORS’ CONTRIBUTION

Huynh B.L.C, Ngo T.T.D contributed in conducting experiments, acquisition of data, interpretation of data. Nguyen T.N.V, Nguyen M.A.N, Tran T.B interpreted NMR and MS data as well as searched the bibliography. Nguyen T.L.T, Bui T. T did the biological assay. Nguyen K.P.P. gave the final correction for the manuscript

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Table 3: ^{13}**C-NMR data of compound 1 -9**

No.	$1^{(a)}$	$2^{(a)}$	$3^{(a)}$	$4^{(a)}$	$5^{(b)}$	$6^{(a)}$	$7^{(a)}$	$8^{(c)}$	$9^{(a)}$
1	108.4	104.8	108.4	104.9	110.7	121.3	104.9	114.3	114.2
2	159.4	163.4	163.2	164.1	163.5	157.8	166.6	147.6	160.1
3	100.7	101.5	109.3	109.5	107.9	117.8	101.8	134.4	119.5
4	159.4	167.1	163.2	161.0	161.6	160.8	159.1	150.9	162.9
5	108.4	112.1	108.4	111.5	109.0	109.1	112.8	110.9	112.3
6	140.6	144.9	151.6	140.5	149.0	135.9	144.6	134.4	145.9
7	21.5	174.2	22.4	173.5	164.6	166.6	167.9	161.5	162.0
8		24.2	194.3	24.2	193.9	20.0	24.3	20.7	21.4
9				8.1	21.2	9.0			53.0
2-OCH_3						62.3			
4-OCH_3						56.2		56.6	56.7
$COOCH_3$				52.1					
1’					115.3	113.1	116.2	107.2	109.1
2’					157.4	163.9	165.3	152.5	152.8
3’					116.3	116.8	108.4	119.1	121.3
4’					151.4	153.3	153.0	149.9	150.4
5’					115.7	116.1	115.5	137.9	139.7
6’					136.6	141.5	144.6	136.2	133.9
7’					169.7	175.4	170.6	170.9	169.1
8’					9.3	9.4	23.9	9.1	9.3
9’					21.1	23.9		76.9	102.4
10’								46.7	66.2
11’								204.3	15.6
12’								30.7	
7’-OCH_3					52.3				

(a) Acetone-d_6 ; (b) DMSO-d_6 ; (c) $CDCl_3$

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Table 4: α-Glucosidase inhibitory activity of isolated compounds

No.	Compound and coded number	IC50 (μM) $\pm$ SD
1	Orcinol (1)	1523.5 $\pm$ 4.0
2	Orselinic acid (2)	441.0 $\pm$ 3.0
3	Atranol (3)	599.2 $\pm$ 2.6
4	Methyl β-orselinate (4)	360.0 $\pm$ 1.5
5	Atranorin (5)	262.8 $\pm$ 1.0
6	Diffractaic acid (6)	419.6 $\pm$ 0.5
7	Lecanoric acid (7)	579.7 $\pm$ 1.6
8	Bailesidone (8)	234.5 $\pm$ 0.7
9	Tinctorinone (9)	304.2 $\pm$ 0.7
10	Acarbose (Positive control)	209.7 $\pm$ 0.3

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