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A new phenolic compound from the lichen *Parmotrema praesorediosum* (Nyl.) Hale

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Abstract

Chemical investigation of the lichen *Parmotrema praesorediosum* (Nyl.) Hale led to isolate six phenolic compounds including praesorediosic (**1**), orcinol (**2**), orselinic acid (**3**), lecanorin (**4**), isolecanoric acid (**5**) and virensic acid (**6**). Among them, compound **1** appeared to be found for the first time in the nature. The structure of these compounds was elucidated by spectroscopic analyses of HRESIMS and NMR as well as the comparison of their NMR data with those in the literature. These compounds were evaluated for their cytotoxicity using sulforhodamine-B assay against HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer), HepG2 (liver hepatocellular carcinoma) and MCF-7 (human breast cancer) cell lines.

Keywords. Lichen, *Parmotrema praesorediosum*, phenolic, praesorediosic, cytotoxic activity.

1. INTRODUCTION

Parmotrema praesorediosum (Nyl.) Hale (Parmeliaceae) was belonged to lichens. Many compounds isolated from lichens showed diverse biological activities such as antibacterial, antifungal, antibiotic, anticancer and so on.[1]

However, there has not much studied on chemical constituent and bioactivities on this lichen yet. Our previous studies on chemical constituents from this lichen species displayed many compounds classified as γ–butyrolactone, diphenyl ether, ester of haematommic acid and usnic acid derivatives.[2,3]

This study reported the continuous isolation of one new compound and five known ones as well as their cytotoxicity against four cancer cell lines (HeLa, NCI-H460, HepG2 and MCF-7).

2. MATERIALS AND METHODS

2.1. General

NMR spectra were measured on a Bruker Avance 500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR), with chemical shift data reported in ppm relative to the used solvent. The HR-ESI-MS spectra were measured on a Bruker microOTOF Q-II equipment at the Central Analytical Laboratory of the University of Science, Vietnam National University - Ho Chi Minh City (US-VNU HCMC).

2.2. Plant material

The lichen *Parmotrema praesorediosum* was collected at Nam Cat Tien village, Tan Phu district, Dong Nai province, Vietnam. The lichen's scientific name was authenticated by MSc. Vo Thi Phi Giao, Faculty of Biology, University of Science, US-VNU HCMC. A voucher specimen (No US–B020) was deposited in the Herbarium of Department of Organic Chemistry, Faculty of Chemistry, US-VNU HCMC.

2.3. Extraction and isolation

Lichen thallis was washed, dried and ground into powder (3.0 kg). The crude extract was prepared by maceration method in methanol at room temperature and was then evaporated the filtrated methanolic solution at the reduced pressure. During the

evaporation process to prepare the crude extract, a precipitate was appeared and was filtered out. These resulted in 9.0 g of the precipitate and 450.0 g of methanolic crude residue. This was then separated into six fractions by subjected to silica gel solid phase extraction and eluted with petroleum ether, chloroform, ethyl acetate, acetone and methanol in turn. Six extracts were obtained including petroleum ether E1 (25.0 g), petroleum ether E2 (15.0 g), chloroform (105.0 g), ethyl acetate (50.0 g), acetone (45.0 g) and methanol (37.0 g).

The acetate extract (50.0 g) was applied to silica gel column chromatography, eluted with chloroform–methanol (9:1-5:5) to give 7 fractions (from EA.1 to EA.7). Subfraction EA.2 (5.7 g) was silica gel rechromatographed and eluted with chloroform–methanol (9:1) to give **2** (5.0 mg). Subfraction EA.5 (2.9 g) was silica gel rechromatographed and eluted with chloroform-methanol (9:1) to give compound **3** (18.1 mg), **4** (7.3 mg) and **5** (10.3 mg).

The acetone extract (45.0 g) was applied to silica gel column chromatography and eluted with ethyl acetate-methanol (9:1-5:5) to give 6 fractions (from AC.1 to AC.6). Subfraction AC2 (0.4 g) was applied to column chromatography, eluting with chloroform-acetone-acetic acid (95:5:3 drops) to yield **6** (5.2 mg). Subfraction AC.4 (8.74 g) was silica gel rechromatographed and eluted with chloroform-acetone-acetic acid (8:2:3 drops) to give compound **1** (5.5 mg).

Praesorediosic (1): White powders. HR-ESI-MS (positive mode) *m/z* 211.0559 $[M+H]^+$ (calcd. for $C_{10}H_{10}O_5$+H, 211.0607). The ^{1}H, ^{13}C-NMR (DMSO), see table 1.

Orcinol (2): Colorless needles, mp. 107 ^{o}C. HR-ESI-MS (positive mode) *m/z* 125.0601 $[M+H]^+$ (calcd. for $C_7H_8O_2$+H, 125.0603). The ^{1}H-NMR (Acetone-d_6): δ 8.06 (2H, *s*), 6.16 (3H, *s*), 2.15 (3H, *s*). ^{13}C-NMR (Acetone-d_6): δ 108.2 (C-1), 159.2 (C-2), 100.5 (C-3), 159.2 (C-4), 108.2 (C-5), 140.4 (C-6), 21.4 (C-7).

Orselinic acid (3): Colorless needles, mp. 184 ^{o}C. HR-ESI-MS (negative mode) *m/z* 167.0346 $[M-H]^-$ (calcd. for $C_8H_8O_4$-H, 167.0345). The ^{1}H, ^{13}C-NMR (Acetone-d_6), see table 1.

Lecanorin (4): Colorless needle, mp. 196 ^{o}C. HR-ESI-MS (positive mode) *m/z* 297.0715 $[M+Na]^+$ (calcd. for $C_{15}H_{14}O_5$+Na, 297.0739). The ^{1}H, ^{13}C-NMR (Acetone-d_6), see table 1.

Isolecanoric acid (5): Colorless needles, mp. 184 ^{o}C. HR-ESI-MS (positive mode) *m/z* 341.0633 $[M+Na]^+$ (calcd. for $C_{16}H_{14}O_7$+Na, 341.0638). The ^{1}H-NMR (Acetone-d_6): δ 6.53 (1H, *d*, *J* = 2.5), 6.49 (1H, *d*, *J* = 2.5), 6.29 (1H, *d*, *J* = 2.5), 6.22 (1H, *d*, *J* = 2.5), 2.60 (3H, *s*), 2.54 (3H, *s*). ^{13}C-NMR (Acetone-d_6): δ 108.5 (C-1), 164.3 (C-2), 101.9 (C-3), 164.5 (C-4), 112.9 (C-5), 144.6 (C-6), 171.0 (C-7), 23.5 (C-8), 116.4 (C-1'), 153.5 (C-2'), 106.5 (C-3'), 166.2 (C-4'), 116.2 (C-5'), 144.8 (C-6'), 175.3 (C-7'), 24.2 (C-8').

Virensic acid (6): Colorless crystal, mp. 246 ^{o}C. HR-ESI-MS (negative mode) *m/z* 357.0657 $[M-H]^-$ (calcd. for $C_{18}H_{14}O_8$-H 357.0611). The ^{1}H, ^{13}C-NMR (Acetone-d_6), see table 1.

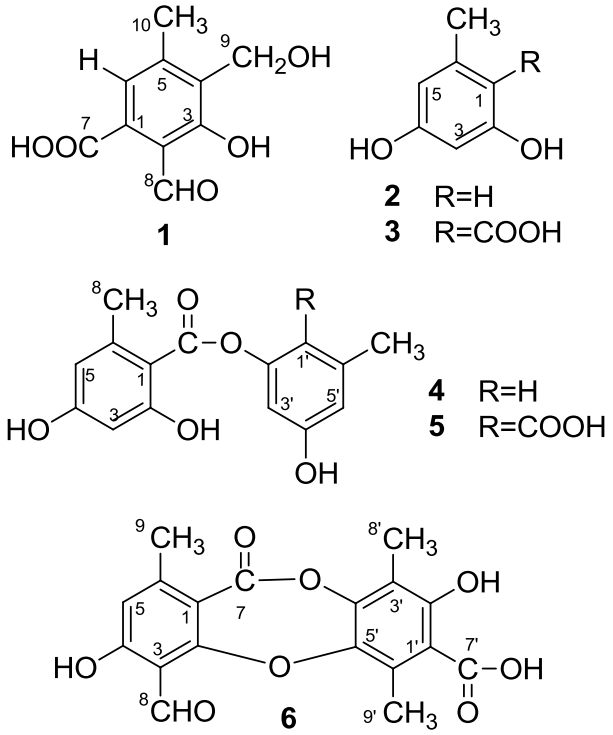

Figure 1: Chemical structure of isolated compounds

2.4. Cytotoxicity inhibitory activities

The cytotoxic activities against the MCF-7, HeLa, HepG2 and NCI-H460 human cell lines of six compounds were evaluated using the Sulforhodamine B method (SBR assay), described by Skehan with the presence of a positive control, camptothecin.[4]

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. Its molecular formula was determined as $C_{10}H_{10}O_5$ through its pseudomolecular ion peak at *m/z* 211.0559 $[M+H]^+$ in the HR-ESI-MS spectrum. The ^{1}H-NMR spectrum data of compound **1** showed signals of two hydroxyl protons at δ_H 12.09 (1H, *s*), and 8.27 (1H, *s*), a formyl group at δ_H 10.44 (1H, *s*), an aromatic proton at δ_H 6.87 (1H, *s*), a methylene

group at δ_H 4.62 (2H, *s*) and a methyl group at δ_H 2.45 (3H, s). The ^{13}C-NMR spectra displayed 10 carbons, including a formyl group (δ_C 192.8), a carbon carboxyl (δ_C 164.0), a methylene carbon (δ_C 52.6), a methyl carbon (δ_C 21.4) and six aromatic carbons in the zone of 110-161 ppm (table 1).

The HMBC spectrum observed cross peak from the methyl protons at δ_H 2.45 (H-10) to carbon signals C-4 (δ_C 112.0), C-5 (δ_C 152.3), and C-6 (δ_C 117.3), and from the methylene protons at δ_H 4.62 (H-9) to carbon signals C-3 (δ_C 160.3), and C-5 (δ_C 152.3), suggesting attachment of the hydroxymethyl group to the benzene ring at C-4. Moreover, the HMBC correlations from aromatic proton at δ_H 6.87 (H-6) to aromatic carbon C-1 (δ_C 110.6), methyl carbon C-10 (δ_C 21.4) and carboxyl carbon C-7 (δ_C 164.0), as well as the formyl proton at δ_H 10.44 (H-8) to aromatic carbons at C-1 (δ_C 110.6) and C-3 (δ_C 160.3), indicated that the carboxyl group was joined to C-1 (figure 2). Consequently, the structure of **1** was proposed to be 2-formyl-3-hydroxy-4-(hydroxymethyl)-5-methylbenzoic acid. Compound **1** was a new compound isolated from natural lichen and was named praesorediosic.

Compound **3** was obtained as colorless needles. The similar NMR data of **3** with those of **1** suggested that they had the same basic framework with the exception of the presence of an aromatic proton instead of a formyl group and a hydroxymethyl group in the molecule. The position of the carboxyl group was established through HMBC correlations from the methyl protons at δ_H 2.51 (H-8) to carbon signals C-1 (δ_C 105.1), C-5 (δ_C 112.3), and C-6 (δ_C 145.1), from the chelated hydroxyl proton at δ_H 12.14 to carbon signals C-1 (δ_C 105.1), C-2 (δ_C 163.6), C-3 (δ_C 101.7) and from the aromatic proton at δ_H 6.29 (H-5) to carbon signals C-1 (δ_C 105.1), C-3 (δ_C 101.7) and C-4 (δ_C 167.4) (figure 2). The comparison of NMR data of **3** with those of orselinic acid in the literature[4,5] showed good compatibility. Therefore, the structure of compound **3** was suggested as orselinic acid.

Compound **4** was a depside. Its molecular formula was determined as $C_{15}H_{14}O_5$ through its pseudomolecular ion peak at *m/z* 297.0715 $[M+Na]^+$ in the HR-ESI–MS spectrum. The ^{1}H-NMR spectrum data of compound **4** showed signals of three hydroxyl protons at δ_H 11.31 (1H, *s*), 9.33 (1H, *s*), 8.60 (1H, *s*), five aromatic protons at δ_H 6.29 (1H, *d*, *J* = 2.5), 6.38 (1H, *d*, *J* = 2.5), 6.58 (2H, *s*), and 6.63 (1H, *s*), and two methoxyl groups at δ_H 2.29 (3H, *s*), and 2.59 (3H, *s*). The ^{13}C-NMR spectrum showed 15 carbon signals, consisting of two methyl carbon signals (δ_C 21.4 and 24.4), twelve aromatic carbons (δ_C 101-167 ppm), and one carboxyl carbon signal (δ_C 171.1) (table 1).

The above NMR and HR-ESI-MS analysis as well as 2D NMR data of **4** showed that it could be a depside which was combined by **2** and **3** through an ester bridge (figure 2). Thus the structure of **4** was assigned as lecanorin.[6]

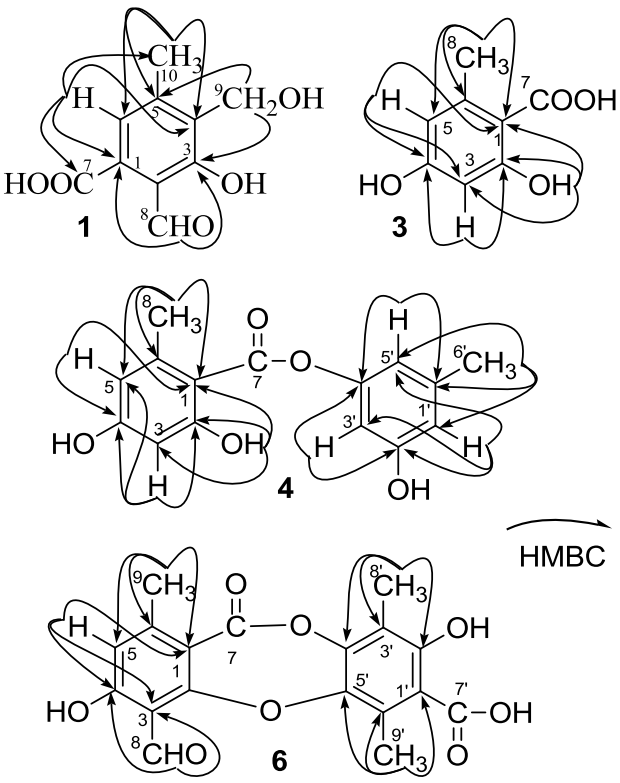

Figure 2: Key HMBC correlations of **1**, **3**, **4** and **6**

Compound **6** was a depsidone. The molecular formula of **6** was established as $C_{18}H_{14}O_8$ by HR-ESI-MS spectrum (*m/z* 357.0657 $[M-H]^-$). The ^{1}H-NMR spectrum showed a chelated hydroxyl proton, a formyl group, an aromatic proton at δ_H 6.81 (1H, *s*), and three methyl groups at δ_H 2.20, 2.51 and 2.70 (3H each, *s*).

The ^{13}C-NMR spectrum of **6** revealed 18 carbon signals, including three methyl carbon signals (δ_C 9.3, 15.6 and 22.1), twelve aromatic carbons (δ_C 111-166 ppm), one carboxyl carbon signal (δ_C 173.4) and a formyl group (δ_C 194.4) (table 1).

The positions of the methyl groups were determined via HMBC correlations of the methyl protons at δ_H 2.70 (H-9) with carbons C-1 (δ_C 113.8), C-5 (δ_C 118.1), and C-6 (δ_C 154.6), from the methyl protons at δ_H 2.51 (H-9') to carbons C-1' (δ_C 115.4), C-5' (δ_C 143.3), and C-6' (δ_C 131.2) and from methyl protons at δ_H 2.20 (H-8') to carbons C-2' (δ_C 160.1), C-3' (δ_C 117.1) and C-4' (δ_C 147.8) (figure 2).

Table 1: NMR data of compounds **1**, **3**, **4** and **6**

No.	Compound **1**[(a)]		Compound **3**[(b)]		Compound **4**[(b)]		Compound **6**[(b)]	
	δ_H (ppm) *J* (Hz)	δ_C (δ ppm)	δ_H (ppm) *J* (Hz)	δ_C (δ ppm)	δ_H (ppm) *J* (Hz)	δ_C (δ ppm)	δ_H (ppm) *J* (Hz)	δ_C (δ ppm)
1		110.6		105.1		105.1		113.8
2		132.8		163.6		166.8		161.7
3		160.3	6.22 (*d*, 2.5)	101.7	6.29 (*d*, 2.5)	101.8		111.9
4		112.0		167.4		164.0		165.8
5		152.3	6.29 (*d*, 2.5)	112.3	6.38 (*d*, 2.5)	112.8	6.81 (*s*)	118.1
6	6.87 (*s*)	117.3		145.1		144.7		154.6
7		164.0		174.5		171.1		166.0
8	10.44 (*s*)	192.8	2.51 (*s*)	24.4	2.59 (*s*)	24.4	10.78 (*s*)	194.4
9	4.62 (*s*)	52.6					2.70 (*s*)	22.1
10	2.45 (*s*)	21.4						
2-OH			12.14 (*s*)		11.31 (*s*)			
3-OH	12.09 (*s*)							
4-OH			9.17 (*s*)		9.33 (*s*)		12.25 (*s*)	
9-OH	8.27 (*s*)							
1′					6.63 (*s*)	114.7		115.4
2′						159.1		160.1
3′					6.58 (*s*)	107.4		117.1
4′						151.9		147.8
5′					6.58 (*s*)	114.4		143.3
6′						141.1		131.2
7′					2.29 (*s*)	21.4		173.4
8′							2.20 (*s*)	9.3
9′							2.51 (*s*)	15.6
2′-OH					8.60 (*s*)			

[(a)]DMSO; [(b)]Acetone-d_6.

Table 2: Inhibition (%) of cytotoxic activities against four cancer cell lines of isolated compounds

Compound	Inhibition of Cell Growth (I %)			
	MCF-7	HeLa	NCI-H460	HepG2
1	**58.44±2.59**	27.88±7.98	8.53±1.11	-2.85±10.45
2	25.35±2.90	1.47±5.20	10.36±3.73	-19.28±9.13
3	20.89±6.91	-3.43±4.24	2.01±3.02	-18.13±1.27
4	29.04±4.55	33.86±2.45	9.24±0.67	-35.93±7.50
5	49.21±2.73	15.18±2.56	19.70±3.56	-14.34±6.91
6	36.76±3.36	**53.44±3.54**	21.24±2.67	8.80±5.84
Camptothecin	58.2±3.30	77.6±0.60	41.2±2.40	52.27±0.58

Based on the above evidences and the comparision between NMR data of **6** and those reported in the literature,[5] the chemical structure of **6** was elucidated as virensic acid.

All six isolated compounds were tested the cytotoxic activities against four cell lines HeLa, NCI-H460, HepG2 and MCF-7 at the concentration of 100 μg/mL. Camptothecin was tested at the concentration of 0.01 μg/mL for MCF-7 and NCI-H 460, 0.07 μg/mL for HepG2, and of 1 μg/mL for HeLa.

The results of this essay were, expressed as a percentage of cell growth inhibition (I%), presented in table 2. The compound **1** showed potential inhibitive activity against MCF-7 cell line with %I about 56-61 %, while **6** showed potential inhibitive activity against HeLa cell line with %I about 50-57 %.

4. CONCLUSION

From the lichen *Parmotrema praesorediosum* (Nyl.) Hale, collected at Nam Cat Tien village, Tan Phu district, Dong Nai province, Vietnam, six organic compounds had been isolated and elucidated. Although **2-6** had been known in other species but this is the first time they are reported in this lichen. Compound **1** was a new one in the nature. These compounds were also first-time evaluated for cytotoxic activity against HeLa, NCI-H460, HepG2 and MCF-7 cell lines.

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