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Research Article

Praesalide E, a new phenolic compound from the lichen *Parmotrema praesorediosum* (nyl.) Hale

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ABSTRACT

Introduction: *Parmotrema praesorediosum* (Nyl.) Hale is widely distributed in the south of Vietnam. To contribute to finding new compositions as well as increasing the phytochemical knowledge of *Parmotrema* species from which we could further study to find some new biologically potential compounds, we have done systematic research on *Parmotrema praesorediosum* growing in Vietnam, and we have been reported twelve novel phenolic compounds. This paper reported the isolation and structural determination of two compounds as the result of the continuous study on the chloroform extract of *Parmotrema praesorediosum*. **Method:** The crude extract was obtained by the maceration method of dried power of *Parmotrema praesorediosum* lichen in methanol. This extract was then separated by the solid-phase extraction and eluted with petroleum ether, chloroform, ethyl acetate, acetone, and methanol in turn to give the corresponding extracts. Two compounds were isolated from chloroform extract by silica gel column chromatography. Their chemical structures were determined by the NMR and HR-ESI-MS data analysis. Moreover, the chemical structure of **1** was also deduced from its methylated product. The cytotoxicity of compound **2** against HeLa, NCI-H460 and, MCF-7 cell lines was done using the Sulforhodamine B method. **Results:** Two isolated compounds, consisting of praesalide E **(1)** and usenamin A (**2**), and one methylated product from **1** were identified. Compound **2** showed no toxicity against all three tested cell lines at the concentration of 100 μg/mL. **Conclusion:** To the best of our knowledge, **1** was a new compound, and compound **2** was known to present in *Parmotrema* genus for the first time.

Key words: *Parmotrema praesorediosum*, lichen, cytotoxicity, praesalide.

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INTRODUCTION

The studies of drugs originated from natural products or based on natural product models have been studied a lot because of their high effectiveness and few side effects. Lichens, symbiotic associations between fungi and algae, contain unique substances such as depsides, depsidones, diphenyl ethers… Therefore, lichens are attractive for pharmacological and chemical studies[1–3], whereas lichens habited in Vietnam, a tropical region, have not much been chemically studied. To contribute to finding new compositions as well as increasing the phytochemical knowledge of *Parmotrema* spieces, we have done systematic research on lichen substances from the Vietnamese flora and reported twelve novel phenolic compounds, including eight diphenyl ethers, three phthalide derivatives, and one monoaromatic compound[4–8] from *Parmotrema praesorediosum* (Nyl.) Hale, which is widely distributed in the south of Vietnam. Herein, we continuously report the isolation and structural elucidation of a new phenolic praesalide E **(1)** and usenamin A (**2**) and a cytotoxic ability against HeLa, NCI-H460, and MCF-7 cell lines of compound **2** as well. Their chemical structures (Figure 1) were unambiguously determined by analyzing 1D and 2D NMR and high-resolution ESI mass spectroscopic data and comparing their NMR data with the ones published in the literature.

MATERIALS AND METHODS

General experimental procedures

Column chromatography was performed on silica gel (Merck) (230-400 Mesh). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel GF_{254} (Merck), visualized by vanillin, followed by heating. NMR spectra were acquired on Bruker 500 Avance III at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. The HR-ESI-MS spectra were recorded on a Bruker microOTOF Q-II in the Center Analysis Laboratory of the University of Science, Vietnam National University (VNU)– Ho Chi Minh City. The optical rotations were measured on Krüss (German) digital polarimeter. All solvents were used for extraction and purification from ChemSol manufacturer and redistillation.

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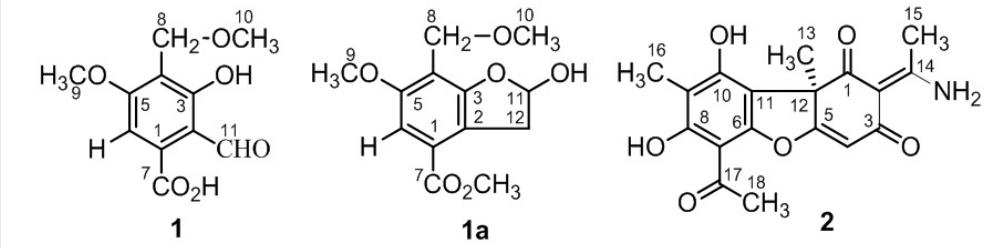


Figure 1: The chemical structures of isolated and methylated compounds **1**, **1a**, and **2.**

Plant material

The lichen *Parmotrema praesorediosum* (Nyl.) Hale was collected at Nam Cat Tien National Forest Reserve and Intermediate Zones, Nam Cat Tien Village, Tan Phu District, Dong Nai Province, Vietnam. The scientific name of the lichen was authenticated by Dr. Vo Thi Phi Giao, Faculty of Biology, University of Science, VNU HCM. A voucher specimen (No US–B020) was deposited in the Herbarium of the Department of Organic Chemistry, Faculty of Chemistry, University of Science, VNU HCM.

Extraction and isolation

5.0 Kg of the fresh lichen thalli were cleaned under running tap water and air-dried. The ground powder (3.0 kg) was exhaustedly extracted with methanol by the method of maceration. The filtrated solution was evaporated at reduced pressure. While the methanolic solution was evaporated, a precipitate appeared and was filtered off, and then the solution was continued to evaporate to dryness. The resulting was the precipitate (9.0 g) and the crude methanolic residue (450.0 g). The methanolic residue (450.0 g) was subjected to silica gel solid-phase extraction and eluted consecutively with petroleum ether, chloroform, ethyl acetate, acetone, and methanol in turn at room temperature to afford petroleum ether extract (E1 25.0 g), petroleum ether extract (E2 15.0 g), chloroform extract (C, 105.0 g), ethyl acetate extract (EA, 50.0 g), acetone extract (AC, 45.0 g) and methanol extract (M, 37.0 g).

The chloroform extract was subjected to silica gel column chromatography, eluted with the solvent system of petroleum ether – ethyl acetate (0-100% of ethyl acetate) to obtain twenty-three fractions from C1 to C23. Fraction C16 (4.2 g) was rechromatographed, eluted with petroleum ether-chloroform (5:5) to give the compound **2** (15.0 mg). Fraction C20 (23.9 g) was repeatedly subjected to silica gel column chromatography, eluted with *n*-hexane–diethyl ether (2:8) and chloroform-methanol (98:2) to afford compound **1** (15.7 mg).

Methylation of 1

TMS-CH_2N_2 in *n*-hexane were added to a solution of **1** (12.0 mg) in Et_2O (1 mL) and MeOH (0.5 mL). The mixture was stirred at room temperature for 1 hour and 15 mins. After termination by diluted acetic acid in MeOH, the reaction mixture was concentrated *in vacuo*, and the residue was purified by preparative TLC (*n*-hexane-Et_2O, 2:8) to yield compound **1a** (4.1 mg).

Cytotoxicity inhibitory activities

The testing of cytotoxic activities against the MCF-7 (breast cancer cell line), HeLa (cervical cancer cell line), and NCI-H460 (lung cancer cell line) was done using the Sulforhodamine B method (SBR assay), described by Skehan with camptothecin as a positive control[9]. This experiment was described in more detail by Nguyen *et al.*[10] and was done at the Department of Molecular Biology, Faculty of Biology, University of Science, VNU HCM.

RESULTS

From the chloroform extract of the lichen *P. praesorediosum* at Nam Cat Tien National Forest Reserve and Intermediate Zones, Dong Nai Province, two compounds, **1** (15.7 mg) and **2** (15.0 mg), were isolated. 4.1 mg of **1a** was synthesized and purified from 12.0 mg of **1**. Their physical properties and spectroscopic data were performed as following and in **Table 1**.

Praesalide E (**1**): Yellow solid. HR-ESI-MS: *m/z* 241.0704 $[M+H]^+$ and *m/z* 263.0523 $[M+Na]^+$. ^1H and ^{13}C-NMR (DMSO-d_6) data: see **Table 1**. Selected HMBC correlations: see **Figure 2**.

Compound 1a: Yellow solid. $[\alpha]_D^{21}$ +6.6 (*c* 0.85, $CHCl_3$). IR(KBr) ν_{max} cm^{-1}: 3477, 1725, 1626, 1462, 1238. HR-ESI-MS: *m/z* 269.1022 $[M+H]^+$ and *m/z*

291.0840 $[M+Na]^+$. ^1H and ^{13}C-NMR ($CDCl_3$) data: see **Table 1**. Selected COSY, HMBC and NOESY correlations: see **Figure 2**.

(+)-(12*R*)-Usenamin A (**2**): Yellow solid, $[\alpha]_D^{23}$+852 (*c* 0.001, MeOH); HR-ESI-MS: *m/z* 366.0938 $[M+Na]^+$. The ^1H-NMR ($CDCl_3$): δ_H 13.35 (1H, s, 8-OH), 11.76 (1H, s, 10-OH), 5.82 (1H, s, H-4), 2.68 (3H, s, H-18), 2.62 (3H, s, H-15), 2.09 (3H, s, H-16), 1.70 (3H, s, H-13). ^{13}C-NMR ($CDCl_3$): δ_C 199.0 (C-1), 102.4 (C-2), 190.8 (C-3), 102.7 (C-4), 174.9 (C-5), 155.9 (C-6), 101.6 (C-7), 163.7 (C-8), 108.4 (C-9), 158.3 (C-10), 105.0 (C-11), 57.4 (C-12), 31.9 (C-13), 175.5 (C-14), 26.6 (C-15), 7.6 (C-16), 200.8 (C-17), 31.4 (C-18). Selected HMBC correlations: see **Figure 2**.

Compound **2** was tested the cytotoxic activity against three cell lines HeLa (human epithelial carcinoma), NCI-H460 (human lung cancer), and MCF-7 (human breast cancer) at the concentration of 100 μg/mL. The percentages of inhibition of cell growth (I %) against HeLa, NCI-H460, and MCF-7 were 18.9 $\pm$ 3.4, 6.0 $\pm$ 0.1, and 3.7 $\pm$ 1.2, respectively, which showed very low cytotoxicity against all tested cell lines at the concentration of 100 μg/mL.

DISCUSSION

Compound **1** was isolated as a yellow solid. Its molecular formula was determined as $C_{11}H_{12}O_6$ based on the protonated ion peak at *m/z* 241.0704 $[M+H]^+$, (Calcd. for $C_{11}H_{12}O_6$+H, 241.0712) and the *sodiated* adduct at *m/z* 263.0523 $[M+Na]^+$, (calcd. for $C_{11}H_{12}O_6$+Na, 263.0531). The ^1H-NMR spectrum of **1** showed one chelated hydroxyl proton (1H, δ_H 12.47, *s*), one aldehydic proton (1H, δ_H 10.42, *s*), only one aromatic methine proton (1H, δ_H 6.86, s) of a pentasubstituted benzene ring. At a higher magnetic field, the presence of methylene protons at δ_H 4.40 (2H, s, H-8) showing HMBC cross-peaks to methoxy carbon at δ_C 57.3 (C-10) constructed the -CH_2-O-CH_3 group in its chemical structure. The rest methoxy proton at δ_H 3.87 (3H, s, H-9) and the proton H-8 showed HMBC correlations to the same oxygenated aromatic carbon at δ_C 162.9 (C-5), which suggested two adjacent positions on benzene ring of these two groups. From the molecular formula of $C_{11}H_{12}O_6$, its degree of unsaturation were calculated to be 6, including 4 of the benzene ring, 1 of aldehyde group, and the rest of a carboxyl carbon which was determined via the observation of a signal at δ_C 168.4 (C-7) on the ^{13}C-NMR spectrum. All above analyses, as well as 12 hydrogen atoms in its molecular, inferred that **1** possessed one benzene ring with five substitutions including -OH, -CHO, -OCH_3, -CH_2OCH_3, -COOH. However, the ^{13}C-NMR spectrum of **1** displayed only six carbon signals, and the HMBC spectrum did not show enough correlation to determine its chemical structure unambiguously. Therefore, it could be deduced via its methylated product.

Methylation of **1** by diazomethane afforded **1a** to be as a yellow solid. The HR-ESI-MS spectrum of **1a** displayed the pseudo molecular ion peak at *m/z* 269.1022 $[M+H]^+$, (Calcd. for $C_{13}H_{16}O_6$+H, 269.1025) and *m/z* 291.0841 $[M+Na]^+$, (calcd. for $C_{13}H_{16}O_6$+Na, 291.0845), which approved its molecular formula of $C_{13}H_{16}O_6$. The combination of 1D and 2D-NMR data analysis showed that **1a** possessed similar structure to **1**, including a pentasubstituted benzene ring [5 quaternary aromatic carbons (δ_C 158.8, 158.5, 126.5, 120.4, 113.1) and one aromatic methine (δ_H 7.09, s; δ_C 104.1)], a methoxy group [C-9 (δ_H 3.87, s; δ_C 56.2)], a methoxymethylene group [C-8 (δ_H 4.52, d, J = 10.5 Hz, 4.56, d, J = 10.5 Hz; δ_C 63.1), C-10 (δ_H 3.37, s; δ_C 58.2)], a carboxyl group [C-7 (δ_C 166.6)]. The positions of the carboxyl, methoxymethylene, and methoxy groups were determined at C-1, C-4, C-5 of benzene ring, respectively, via HMBC correlations as shown in **Figure 2**. The differences between **1** and **1a** were the absence of the chelated hydroxyl proton and the aldehydic proton in **1a**. Meanwhile, it displayed one more methoxy group [C-13 (δ_H 3.90, s; δ_C 52.1)], one more methylene group [C-12 (δ_H 3.37, dd, J = 18.0, 2.5 Hz and 3.57, dd, J = 18.0, 6.5 Hz; δ_C 38.9)] and one more hemiacetalic group [C-11 (δ_H 6.15, dd, J = 6.5, 2.5 Hz; δ_C 101.8). The methoxy group attached to carboxyl carbon (C-7) was confirmed by HMBC correlation of this methoxy proton and C-7. The COSY cross-peak between the acetalic proton and the methylene protons as well as HMBC correlations of these protons to the same aromatic carbons at δ_C 120.4 (C-2) and 158.5 (C-3) suggested the appearance of a benzofuran skeleton. The NOESY interaction from the acetalic proton to the methoxy protons of the methoxymethylene group suggested the positions of the acetal carbon and the methylene carbon in the benzofuran moiety (**Figure 2**). Complete analysis of the 2D-NMR data for **1a** resulted in its formulation, as shown in **Figure 1**.

From the chemical structure of **1a**, the one of **1** was deduced via a proposed retrosynthesis as presented in Scheme 1. The conversion of the carboxylic acid group (C-7) in **1** to a methyl ester in **1a** by diazomethane is a simple reaction. The formation of the furan ring could be explained as follows: firstly, diazomethane reacted with the aldehyde group of **1** to

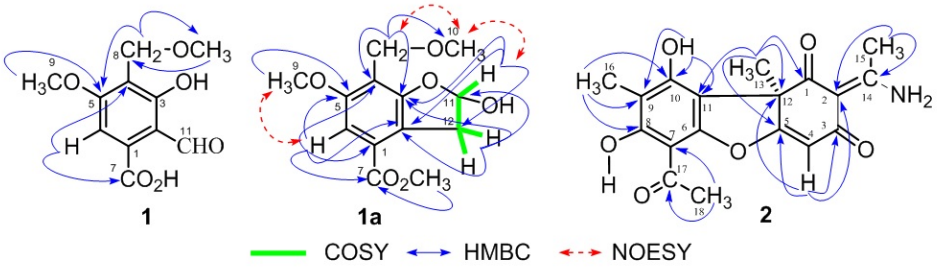


Figure 2: Selected HMBC and NOESY correlations of **1**, **1a**, and **2**

form a homologated aldehyde in a modification of the Buchner–Curtius–Schlotterbeck reaction[11]. The next reaction has been extended to form a hemiacetal by a nucleophilic addition between the pair of free electrons on the phenolic hydroxyl group and the aldehyde group. Furthermore, re-analyses of HMBC spectrum for **1** revealed that H-6 (δ_H 6.86) correlated with a carbon signal at δ_C 113.3 (C-4) and the methoxy group H_3-9 (δ_H 3.87) as well as the methylene protons H_2-8 (δ_H 4.40) correlated with an oxygenated aromatic carbon signal at δ_C 162.9 (C-5), confirming the presence of aromatic carbons C-4 and C-5 (**Figure 2**). These results suggested the chemical structure of **1** as 2-formyl-3-hydroxy-5-methoxy-4-methoxymethylbenzoic acid, namely praesalide E.

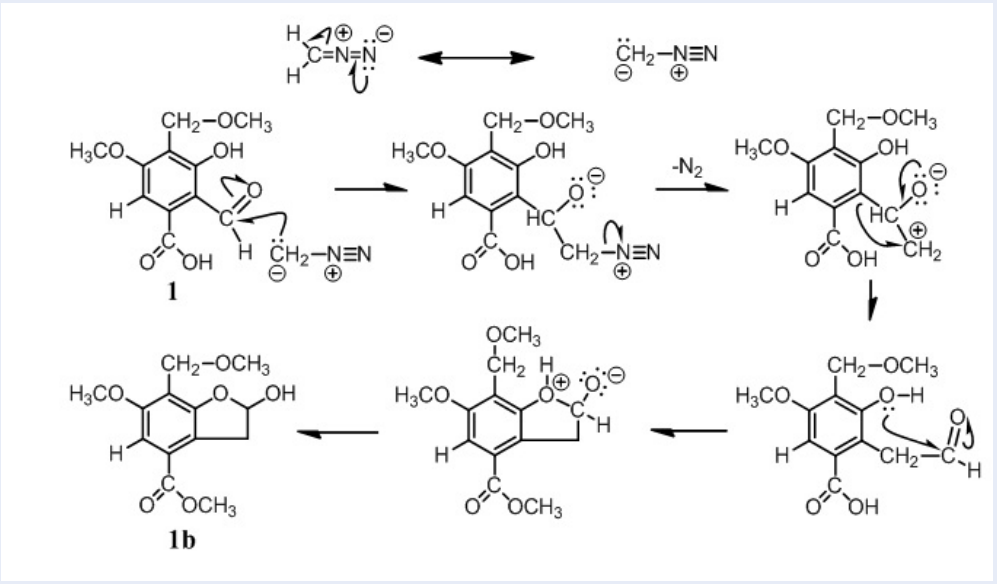
Compound **2** was obtained as a yellow solid. Its molecular formula was determined as $C_{18}H_{17}O_6N$ through its pseudo molecular ion peak at *m/z* 366.0938 $[M+Na]^+$ (calcd. 366.0954 for $C_{18}H_{17}O_6N$+Na) in the HR-ESI–MS spectrum. The ^{1}H-NMR spectrum exhibited signals of two chelated hydroxyl groups at δ_H 11.76 (1H, *s*, 10-O**H**), and 13.35 (1H, *s*, 8-O**H**); four methyl groups at δ_H 1.70 (3H, *s*, H-13), 2.09 (3H, *s*, H-16), 2.62 (3H, *s*, H-15), and 2.68 (3H, *s*, H-18); and an aromatic proton at δ_H 5.82 (1H, *s*, H-4). These protons enabled the identification of **2** possessing the 9b*H*-dibenzofurandione moiety. The ^{13}C-NMR spectrum showed 18 carbons including three keto carbonyl carbons at δ_C 199.0 (C-1), 190.8 (C-3), and 200.8 (C-17); nine olefinic carbons at δ_C 101.6 (C-7), 102.6 (C-4), 102.4 (C-2), 105.0 (C-11), 108.4 (C-9), 158.3 (C-10), 155.9 (C-6), 163.7 (C-8), and 174.9 (C-5) among them five carbons were oxygenated; one saturated quaternary carbon at δ_C 57.4 (C-12); signals of four methyl groups at δ_C 7.6 (C-16), 26.6 (C-15), 31.4 (C-18), and 31.9 (C-13). The rest carbon signal appearing at δ_C 175.5 (C-14) was assigned for an enamine group which was demonstrated by the presence of a nitrogen atom in the molecule and the HSQC and HMBC data analysis. Some selected HMBC correlations of **2** were presented in Figure 2, which approved its plana chemical structure. The absolute configuration of the unique chiral center C-12 was suggested to be *R* due to the biosynthetic aspect that **2** and two similar compounds, usnic acid and isousnic acid possessing 12-*R* configuration[12], were isolated from the same material and were all dextrorotary. Additionally, the comparison of these spectroscopic data of **2** with those of (+)-(*12R*)-usenamin A in the literature[13] showed good compatibility.

CONCLUSION

From the continuous phytochemical investigation of the chloroform extract of the lichen *Parmotrema praesorediosum*, two phenolic compounds were isolated, consisting of praesalide E (**1**) and usenamin A (**2**). Their chemical structures were established primarily by NMR, MS spectroscopic data, optical rotations analysis as well as deduced from the methylated product. Compound **1** was a new compound, while compound **2** was isolated from *Parmotrema* genus for the first time. Compound **2** had no toxicity against all three tested cell lines, HeLa, NCI-H460, and MCF-7, at the concentration of 100 μg/mL.

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
COSY: Correlation spectroscopy
HSQC: Heteronuclear single quantum coherence
HMBC: Heteronuclear multiple bond correlation
NOESY: Nuclear Overhauser Effect Spectroscopy
s: singlet
brs: broad singlet
d: doublet
dd: doublet of doublets



Scheme 1: Mechanism for the methylation of **1**

Table 1: NMR data of the compounds 1 and 1a

No.	1*			1a#		
	δ_H	J (Hz)	δ_C	δ_H	J (Hz)	δ_C
1						126.5
2						120.4
3						158.5
4			113.3			113.1
5			162.9			158.7
6	6.86			7.09		104.1
7			168.4			166.6
8	4.40		61.1	4.56	d (10.5)	63.1
				4.52	d (10.5)	
9	3.87		56.0	3.87		56.2
10	3.21		57.3	3.37		58.2
11	10.42	brs		6.15	dd (6.5, 2.5)	101.8
12				3.38	dd (18.0, 2.5)	38.9
				3.57	dd (18.0, 6.5)	
13				3.90		52.1
3-OH	12.47					

Note: *: dimethylsulfoxide-d_6 #: chloroform-*d*

COMPETING INTEREST

The authors declare no competing financial interest.

AUTHORS' CONTRIBUTION

Huynh B.L.C has contributed to conducting experiments, acquisition of data, and interpretation of data. Nguyen T. A. T., Vo T. N., Pham N. K. T. interpreted NMR and MS data as well as searched the bibliography. Nguyen T. H. T gave final approval of the manuscript to be submitted.

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