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Research Article

CHEMICAL CONSTITUENTS OF *n*-HEXANE EXTRACT OF THE VIETNAMESE LICHEN *PARMOTREMA CRISTIFERUM*

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ABSTRACT

Chemical data of the lichen Parmotrema cristiferum are scarce. Three compounds albifolione (1), zeorin (2), and atranorin (3) were isolated from the n-hexane extract of the lichen Parmotrema cristiferum collected in Lam Dong Province. Multiple chromatographic methods were applied. Their chemical structures were elucidated by comparing their spectroscopic data with reported data in the literature. Three compounds were known in the first time from the Parmotrema cristiferum .

***Keywords*:** atranorin*;* diterpenoid; *Parmotrema cristiferum;* zeorin

1. Introduction

Vietnamese lichens have possessed the wealth of the chemical diversity (Duong et al. 2015, Duong et al. 2017, Duong et al. 2018a, Duong et al. 2018b). The genus *Parmotrema* growing in Vietnam have produced over 30 novel and bioactive compounds (Duong et al. 2021, Devi et al. 2020). The lichen *Parmotrema cristiferum* (Taylor) Hale occurred popularly in Lam Dong province, Vietnam. Previous biological studies of this lichen reported that its methanolic extract had various pharmacological activities such as antifungal (Rajan and Vinayaka, 2016), antimicrobial, antioxidant and insecticidal (Kekuda et al. 2015) activities. Only one chemical study was reported, providing eight compounds: cristiferides A-B, 2,4-dihydroxyphthalide, lecanoric acid, orsellinic acid, 5-chloroorsellinic acid, methyl haematommate, and methyl β-orsellinate (Pham et al. 2022). Among them, cristiferide B and methyl haematommate revealed potent inhibition toward alpha-glucosidase with IC_{50} values of 72.66 μM and 48.73 μM, respectively. As part of a continuing investigation of Vietnamese lichens, the non-polar fraction of lichen *P. cristiferum* was investigated. In this paper, the isolation and structural elucidation of three compounds: albifolione (**1**), zeorin (**2**), and atranorin (**3)** from the lichen *P. cristiferum* are reported. Their structures were elucidated from spectroscopic data and comparison with literature data.

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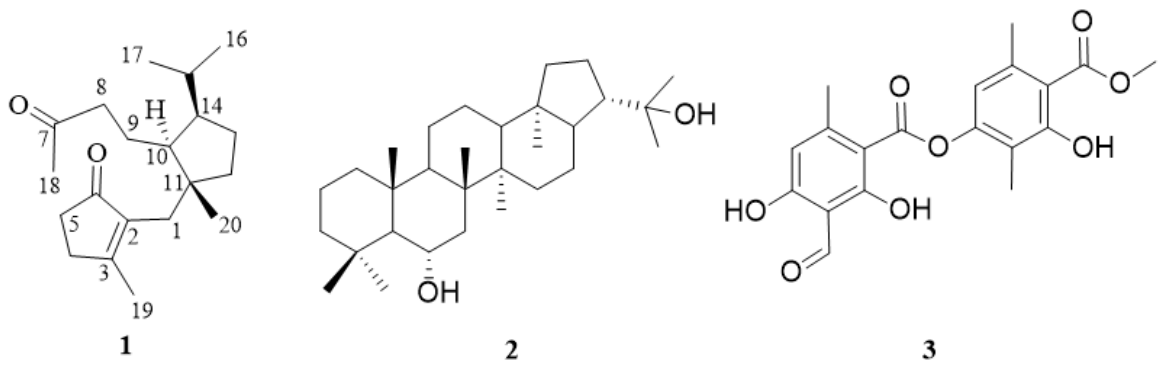

Figure 1. *Chemical structures of isolated compounds* ***1-3***

2. Experiment

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance spectrometer (500 MHz or 400 MHz for ^{1}H–NMR and 125 MHz or 100 MHz for ^{13}C–NMR) in acetone-d_6, chloroform-d, dimethylsulfoxide-d_6, or methanol-d_4. Thin-layer chromatography was carried out on silica gel 60 (Merck, 40-63 μm), and spots were visualized by spraying with 10% H_2SO_4 solution, followed by heating.

2.2. Plant material

The thallus of lichen *P. cristiferum* was collected in Duc Trong district, Lam Dong province, Vietnam in March 2020. The scientific name of the lichen was determined by Dr. Thi-Phi-Giao Vo, Faculty of Biology, Ho Chi Minh University of Science, National University–Ho Chi Minh City. A voucher specimen (UE-L006) was deposited in the herbarium of the Department of Organic Chemistry, Ho Chi Minh University of Education

2.3. Extraction and isolation

The clean, air-dried, and ground material (1.1 kg) was macerated in EtOAc at room temperature (10 L x 5 times, every 12 hours) and the filtrated solution was concentrated under reduced pressure to afford the crude EtOAc extract (330 g). The crude extract was suspended in water and successively liquid-liquid partitioned into *n*-hexane (H, 29.2 g), n-hexane: EtOAc (5:5) (HEA, 48.7 g), and EtOAc (EA, 157.5 g) to afford corresponding extracts. The H extract (29.2 g) was applied to silica gel column chromatography (CC) using a mobile phase of *n*-hexane-EtOAc (stepwise, 20:1, 15:1, 10:1, 1:1, 0:1 v/v) to give fractions H1- H10. Washing fraction H1 (1.1 g) by methanol provided compound **2** (89 mg) Fraction H3 (2.9 g) was applied to Sephadex LH-20 gel (CC), eluted with MeOH to obtain fractions H3.1-H3.5. Fraction H3.5 (689 mg) was washed with acetone (5 x 100 mL) to leave the solid (compound **3**, 141 mg). Fraction **H4** (1.6 g) was applied to silica gel CC, eluted with *n*-hexane-$CHCl_3$-EtOAc (10:10:1, v/v/v) to afford five fractions **H4.1-5**. Fraction **H4.3** (121 mg) was applied to C18-reverse-phase silica gel CC, eluted with MeOH-H_2O (12:1, v/v) to afford compound **1** (3.5 mg).

- **Albifolione (1)**. Colorless oil. ^{1}H-NMR (500 MHz, chloroform-*d*, δ ppm, *J* in Hertz): 2.51-2.56 (2H, *m*, H-4), 2.49 (1H, *m*, H-8b), 2.37-2.42 (2H, *m*, H-5), 2.36 (1H, *m*, H-8a), 2.24 (1H, *d*, 13.0, H-1a), 2.20 (1H, *d*, 13.0, H-1b), 2.12 (3H, *s*, H-18), 2.06 (3H, *s*, H-19), 2.04 (1H, *m*, H-14), 1.60 (1H, *m*, H-9b), 1.58 (1H, *m*, H-15), 1.45 (1H, *m*, H-12a), 1.45 (1H, *m*, H-10), 1.36 (1H, *m*, H-12b), 1.31 (1H, *m*, H-9a), 1.24 (1H, *m*, H-13), 0.91 (3H, *d*, 6.5, H-16), 0.89 (3H, *d*, 7.0, H-17), 0.87 (3H, *s*, H-20). ^{13}C-NMR (125 MHz, chloroform-*d*, δ ppm): 35.4 (C-1), 139.7 (C-2), 172.4 (C-3), 32.1 (C-4), 34.5 (C-5), 210.6 (C-6), 209.5 (C-7), 45.1 (C-8), 19.5 (C-9), 48.4 (C-10), 48.4 (C-11), 37.3 (C-12), 27.7 (C-13), 50.6 (C-14), 29.9 (C-15), 22.3 (C-16), 22.8 (C-17), 30.3 (C-18), 19.0 (C-19), 23.9 (C-20).
- **Zeorin** (**2**). While amorphous powder. ^{1}H-NMR (500 MHz, chloroform-*d*, δ ppm, *J* in Hertz): 3.96 (1H, *td*, 11.0, 4.0 , H-6), 2.24 (1H, *m*, H-21), 1.18 (3H, *s*, H-23), 1.18 (3H, *s*, H-30), 1.14 (3H, *s*, H-29), 1.03 (3H, *s*, H-26), 1.01 (3H, *s*, H-24), 0.97 (3H, *s*, H-27), 0.87 (3H, *s*, H-25), 0.82 (1H, *d*, 11.5, H-5), 0.76 (3H, *s*, H-28). ^{13}C-NMR (125 MHz, chloroform-*d*, δ ppm): 40.5 (C-1), 18.6 (C-2), 43.9 (C-3), 33.7 (C-4), 61.2 (C-5), 69.5 (C-6), 45.5 (C-7), 43.0 (C-8), 49.9 (C-9), 39.5 (C-10), 20.8 (C-11), 24.1 (C-12), 49.6 (C-13), 42.0 (C-14), 34.5 (C-15), 21.2 (C-16), 54.1 (C-17), 44.2 (C-18), 41.4 (C-19), 26.7 (C-20), 51.2 (C-21), 74.4 (C-22), 36.9 (C-23), 22.2 (C-24), 17.2 (C-25), 18.4 (C-26), 17.2 (C-27), 16.2 (C-28), 28.8 (C-29), 30.9 (C-30).
- **Atranorin** (**3**). While amorphous powder. ^{1}H-NMR (500 MHz, chloroform-*d*) and ^{13}C-NMR (125 MHz, chloroform-*d*) data were identical with those reported in the literature.

3. Results and discussion

Compounds **1** was isolated as a colorless oil. ^{1}H-NMR spectrum in a combination of HSQC spectrum indicated the presence of two doublet proton of a methylene group at δ_H 2.19 (*d*, J = 13.0 Hz) and 2.24 (*d*, J = 13.0 Hz), two other methylenes in the range of δ_H 2.37-2.56, three methines at δ_H 1.44, 1.58, and 2.04, five methyls at δ_H 0.87 (s), 0.89 (*d*, J = 7.0 Hz), 0.91 (*d*, J = 6.5 Hz), 2.06 (*s*), and 2.12 (*s*). JMOD and HSQC spectra revealed two carbonyl carbons at δ_C 209.7 (C-7) and 210.4 (C-6), two substituted olefinic carbons at δ_C 139.8 (C-2) and 172.3 (C-3) and a quaternary carbon at δ_C 48.4 (C-11). JMOD spectrum also showed seven methylenes at δ_C 19.5 (C-9), 27.6 (C-13), 32.1 (C-4), 34.5 (C-5), 35.4 (C-1), 37.2 (C-12), and 45.3 (C-8), five methyls at δ_C 19.0 (C-19), 22.3 (C-16), 22.8 (C-17), 23.9 (C-20) and 30.3 (C-18), three methines at δ_C 29.9 (C-15), 48.3 (C-10) and 50.6 (C-14). HMBC spectrum of **1** showed the correlations of the methylene group at δ_H 2.51-2.56 (H_2-4) to carbons at δ_C 210.6 (C-6), 139.7 (C-2), 172.4 (C-3), 34.5 (C-5) and 19.0 (C-19); of the second methylene group at δ_H 2.37-2.42 (H_2-5) to carbons C-3, C-4, and C-6; and of the methyl at δ_H 2.06 (H_3-19) to carbons C-2, C-3, and C-4, indicating the presence of A-ring (Figure 2). The methylene group at δ_H 2.20 và 2.24 (H_2-1) gave HMBC correlations with three carbons C-2, C-3, C-6 of the A-ring and four carbons at δ_C 48.4 (C-10), 48.4 (C-11), 37.3 (C-12), 23.9 (C-20) of the B-ring, indicating the connection between A-ring and B-ring

at C-1. HMBC correlations of the methyl at δ_H 0.87 (H-20) with carbons C-1, C-10, C-11, C-12 indicated its position in the B-ring. The isopropyl group was determined at C-14 due to HMBC correlations of the methyls at δ_H 0.91 (H_3-16) and δ_H 0.89 (H-17) to carbons at δ_C 29.9 (C-15) and 50.6 (C-14). The 3-oxobut-1-yl moiety was defined by HMBC correlations of the methyl at δ_H 2.12 (H_3-18) to carbons at δ_C 209.5 (C-7) and 45.1 (C-8); of the methylene H_2-8 at δ_H 2.36 and 2.49 to carbons at δ_C 209.5 (C-7) and 19.5 (C-9). This group was defined to locate at C-10 based on HMBC correlations of H_2-8 to C-10. NOESY correlations of H-10 (δ_H 1.44) with H-14 (δ_H 2.04) and of H-10 (δ_H 1.44) with H_2-1 (δ_H 2.20, 2.24) indicated their same orientation (Figure 2). This indicated that H_3-20 (δ_H 0.87) and the isopropyl group were syn-facial. NMR data of **1** with those of albifolione (Liu et al. 2012) showed a similarity (Table 1), indicating that **1** was albifolione (Figure 1). This compound was found for the first time in the genus *Parmotrema*.

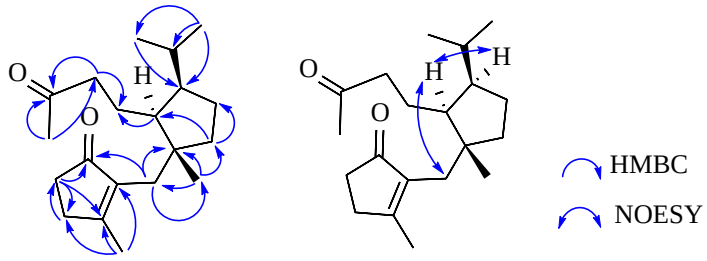

Figure 2. Selected HMBC and NOESY correlations of **1**

Table 1. *Comparison of NMR data of* ***1*** *and albifolione*

No	**1**		**Albifolione (Liu et al. 2012)**	
	δ_H[a] ppm, *J* (Hz)	δ_C[a] ppm	δ_H[b] ppm, *J* (Hz)	δ_C[b] ppm
1	2.24 (1H, *d*, *J* = 13.0 Hz) 2.20 (1H, *d*, *J* = 13.0 Hz)	35.4	2.26 (1H, *d*, *J* = 13.2 Hz) 2.22 (1H, *d*, *J* = 13.2 Hz)	34.9
2	-	139.7	-	139.3
3	-	172.4	-	172.0
4	2.51-2.56 (2H, *m*)	32.1	2.52-2.55 (2H, *m*)	31.8
5	2.37-2.42 (2H, *m*)	34.5	2.37-2.41 (2H, *m*)	34.2
6	-	210.6	-	210.1
7	-	209.5	-	209.4
8	2.36 (*m*) 2.49 (*m*)	45.1	2.35-2.38 (*m*) 2.51-2.53 (*m*)	44.9
9	1.31 (*m*) 1.60 (*m*)	19.5	1.56-1.60 (*m*) 1.34-1.39 (*m*)	19.0
10	1.44 (*m*)	48.4	1.47-1.50 (*m*)	47.9
11	-	48.4	-	47.9
12	1.45 (*m*) 1.36 (*m*)	37.3	1.45-1.47 (*m*) 1.35-1.39 (*m*)	36.7

13	1.24 (2H, *m*)	27.7	1.25-1.30 (1H, *m*)	27.2
14	2.04 (*m*)	50.6	2.03-2.06 (*m*)	50.1
15	1.58 (*m*)	29.9	1.60-1.63 (*m*)	29.6
16	0.91 (3H, *d*, *J* = 6.5 Hz)	22.3	0.93 (3H, *d*, *J* = 6.5 Hz)	22.0
17	0.89 (3H, *d*, *J* = 7.0 Hz)	22.8	0.90 (3H, *d*, *J* = 6.5 Hz)	22.4
18	2.12 (3H, *s*)	30.3	2.14 (3H, *s*)	30.0
19	2.06 (3H, *s*)	19.0	2.09 (3H, *s*)	18.7
20	0.87 (3H, *s*)	23.9	0.89 (3H, *s*)	23.5

a: ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHZ) was measured in $CDCl_3$

b: ^{1}H NMR (400 MHz), 13C NMR (100 MHZ) was measured in $CDCl_3$

4. Conclusions

From the *n*-hexane extract of the lichen *Parmotrema cristiferum*, three compounds albifolione (**1**), zeorin (**2**), and atranorin (**3**) were isolated Their chemical structures were elucidated by comparing their spectroscopic data with reported data in the literature. Three compounds were known in the first time from the *Parmotrema cristiferum*. Further studies on this species are in the progress.

❖ ***Conflict of Interest:*** *Author have no conflict of interest to declare.*

REFERENCES

Bodo, B., & Molho, D. (1980). Structure of the isomuronic and neuropogolic acids, new aliphatic acids, from the lichen Neuropogon trachycarpus. *Phytochemistry*, *19*(6), 1117-1120.

Devi, A. P., Duong, T. H., Ferron, S., Beniddir, M. A., Dinh, M. H., ... Pogam, P. L. (2020). Salazinic acid-derived depsidones and diphenylethers with α-glucosidase inhibitory activity from the lichen *Parmotrema dilatatum*. *Plan Med.*, *86*(16), 1216-1224.

Duong, T. H., Chavasiri, W., Boustie, J., & Nguyen, K. P. P. (2015). New meta-depsidones and diphenyl ethers from the lichen *Parmotrema tsavoense* (Krog & Swinscow) Krog & Swinscow, Parmeliaceae. *Tetrahedron*, *71*(52), 9684-9691.

Duong, T. H., Huynh, B. L. C., Chavasiri, W., Chollet-Krugler, M., Nguyen, V. K., ... Nguyen, K. P. P. (2017). New erythritol derivatives from the fertile form of *Roccella montagnei*. *Phytochemistry*, *137*, 156-164.

Duong, T. H., Ha, X. P., Chavasiri, W., Beniddir, M. A., Genta-Jouve, G., ... Le Pogam, P. (2018). Sanctis A–C: three racemic procyanidin analogues from the lichen *Parmotrema sancti-angelii*. *Eur. J. Org. Chem.*, *19*, 2247-2253.

Duong, T. H., Beniddir, M. A., Genta-Jouve, G., Aree, T., Chollet-Krugler, M., ... Le Pogam, P. (2018). Tsavoenones A–C: unprecedented polyketides with a 1, 7-dioxadispiro [4.0. 4.4] tetradecane core from the lichen *Parmotrema tsavoense*. *Org. Biomol. Chem.*, *16*, 5913-5919.

Duong, T. H., Nguyen, V. K., Sichaem, J., Tran, T. N., Do, T. H., ... Huynh, B. L. C. (2021). Reticulatin, a novel C 43 -spiroterpenoid from the lichen *Parmotrema reticulatum* growing in Vietnam. *Natural Product Research*, 1-8.

Kekuda, T., Dhanya, R., Dhatri, R., Sunita, C., Onkarappa, R., & Vinayaka, K. (2015). Radical scavenging, antimicrobial and insecticidal efficacy of *Parmotrema cristiferum* and *Dirinaria applanata*. *Sci Technol Arts Res J, 4*(1), 95-102.

Liu, N., Guo, D. X., Wang, S. Q., Wang, Y. Y., Zhang, L.,... Lou, H. X. (2012). Bioactive sesquiterpenoids and diterpenoids from the liverwort *Bazzania albifolia*. *Chemistry & biodiversity*, *9*(10), 2254-2261.

Pham, N. K. T., Nguyen, H. T., Dao, T. B., Vu-Huynh, K. L., Nguyen, T. Q., ... Duong, T. H. (2022). Two new phenolic compounds from the lichen *Parmotrema cristiferum* growing in Vietnam. *Nat Prod Res, 36*, 3865-3871.

Rajan, V. P., Gunasekaran, S., Ramanathan, S., Murugaiyah, V., Samsudin, M. W., & Din, L. B. (2016). Biological activities of four *Parmotrema* species of Malaysian origin and their chemical constituents. *J Appl Pharm Sci., 6*(8), 36-43.

THÀNH PHẦN HÓA HỌC CỦA CAO *n*-HEXANE CỦA LOÀI ĐỊA Y *PARMOTREMA CRISTIFERUM* SINH TRƯỞNG Ở VIỆT NAM

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TÓM TẮT

*Có rất ít nghiên cứu hóa học trên loài địa y Parmotrema cristiferum. Ba hợp chất albifolione (**1**), zeorin (**2**), và atranorin (**3**) được cô lập từ cao n-hexane của loài địa y Parmotrema cristiferum sinh trưởng ở tỉnh Lâm Đồng. Các phương pháp sắc kí khác nhau đã được sử dụng. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm cũng như so sánh với dữ liệu trong các tài liệu tham khảo. Cả ba hợp chất lần đầu tiên được tìm thấy trong loài địa y Parmotrema cristiferum.*

***Từ khóa*:** atranorin*;* diterpenoid; *Parmotrema cristiferum;* zeorin