CHEMICAL CONSTITUENTS OF THE LICHEN *PARMOTREMA TSAVOENSE* (KROG & SWINSCOW) KROG & SWINSCOW (PARMELIACEAE)

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ABSTRACT

*Two major depsidones protocetraric acid **(1)**, 8'-O-methylprotocetraric acid **(2)**, virensic acid (**3**), two aliphatic acids (+)-prasorediosic acid **(4)**, (+)-vinaprasorediosic acid A **(5)**, and along with common lichen metabolites atranorin **(6)**, methyl haematommate **(7)**, methyl β-orsellinate **(8)**, methyl orsellinate **(9)**, zeorin **(10)** were isolated from the lichen Parmotrema tsavoense (Krog & Swinscow) Krog & Swinscow. Their chemical structures were established by 1D NMR, 2D NMR, high resolution ESI–MS spectroscopic analysis and comparison with those reported in the literatures. The lichen Parmotrema tsavoense has not been studied on phytochemistry.*

Keywords: Parmotrema tsavoense, depsidone, aliphatic acid, monocyclic compound.

TÓM TẮT

Thành phần hóa học của loài địa y Parmotrema tsavoense (Krog & Swinscow) Krog & Swinscow (Parmeliaceae)

*Hai hợp chất depsidone chính protocetraric acid **(1)**, 8'-O-methylprotocetraric acid **(2)**, virensic acid **(3)**, hai acid béo (+)-prasorediosic acid **(4)**, (+)-vinaprasorediosic acid A **(5)**, và cùng với các hợp chất địa y phổ biến khác atranorin **(6)**, methyl haematommate **(7)**, methyl β-orsellinate **(8)**, methyl orsellinate **(9)**, zeorin **(10)** đã được cô lập từ loài địa y Parmotrema tsavoense (Krog & Swinscow) Krog & Swincow. Cấu trúc hoá học của chúng được xác định bằng các phương pháp phổ nghiệm cũng như so sánh với các tài liệu tham khảo. Loài địa y Parmotrema tsavoense chưa được nghiên cứu về hóa thực vật.*

Từ khóa: Parmotrema tsavoense, depsidone, aliphatic acid, monocyclic compound.

1. Introduction

Depsidones and γ-lactone aliphatic acids are bioactive lichen metabolites (Huneck S. 1997).[6] They possess the antivirus and enzyme inhibitory activities according to Boustie & Grube (2007),[1] Boustie *et al.* (2010),[2] Muller (2001).[14] The previous phytochemical studies of the lichens *Parmotrema* genera growing in Vietnam indicate that they contain various depsidones and γ-lactone aliphatic acids (Huynh B. L. C., 2014, 2016).[8,9] *Parmotrema tsavoense* (Krog & Swinscow) Krog & Swinscow has not been studied on phytochemisty.

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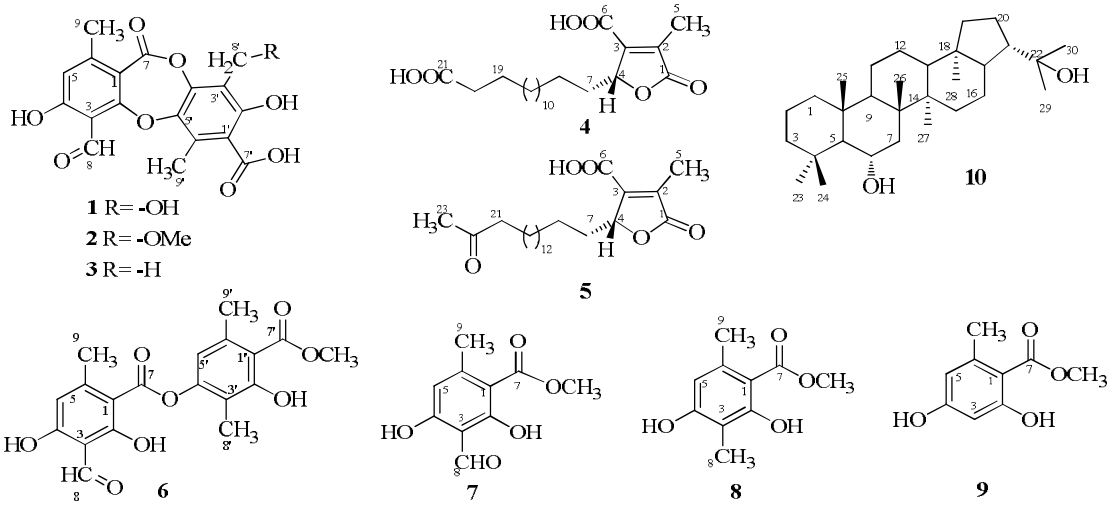

Figure 1. *Chemical structures of* ***1-10***

In this paper, we reported the isolation of ten known compounds protocetraric acid **(1)**, 8'-*O*-methylprotocetraric acid **(2)**, virensic acid **(3)**, (+)-prasorediosic acid **(4)**, (+)-vinaprasorediosic acid A **(5)**, atranorin **(6)**, methyl haematommate **(7)**, methyl *β*-orsellinate **(8)**, methyl orsellinate **(9)**, zeorin **(10)** from the lichen *Parmotrema tsavoense*. Their chemical structures were elucidated by spectroscopic data analysis and comparison with those reported in the literature.

2. Experimental

General experimental procedures

Figure 2. *Parmotrema tsavoense on rock*

The NMR spectra were measured on a Bruker Avance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) and Varian Mercury-400 Plus NMR (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers with TMS as internal standard. Proton chemical shifts were referenced to the solvent residual signal of $CDCl_3$ at δ_H 7.26, of CD_3COCD_3 at δ_H 2.05, of DMSO-d_6 at δ_H 2.50. The ^{13}C–NMR spectra were referenced to the central peak of $CDCl_3$ at δ_C 77.1, of CD_3COCD_3 at δ_C 29.4, of DMSO-d_6 at δ_C 39.5. The HR–ESI–MS were recorded on a Bruker micrOTOF Q-II. TLC was carried out on precoated silica gel 60 F_{254} or silica gel 60 RP–18 F_{254}S (Merck) and spots were visualized by spraying with 30% H_2SO_4 solution followed by heating. Gravity column chromatography was performed with Silica gel 60 (0.040–0.063 mm, Himedia).

Plant material

Parmotrema tsavoense (Krog & Swinscow) Krog & Swinscow was collected on the surface of rocks on Ta Cu mountain, Binh Thuan province (August-September 2012). Its scientific name was determined by Dr. Wetchasart Polyiam, Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand. A voucher specimen (No US-B027) was deposited in the herbarium of the Department of Organic Chemistry, University of Science.

Extraction and isolation

The clean, air-dried and ground material (1350 g) was extracted by methanol at ambient temperature, and the filtrated solution was concentrated under reduced pressure. While the methanolic solution was being evaporated, a precipitate (79.7 g) occurred and was filtered off. The rest of the solution was evaporated to dryness to obtain a crude methanol extract (249.8 g). This crude extract was applied to normal phase silica gel column chromatography, eluted with the solvent system of hexane–ethyl acetate (9:1) to afford fraction **P1** (9.9 g). Consecutive elution of the column with the same solvent system but increasing polarity (8:2, 7:3, 6:4, 5:5, 4:6) yielded five fractions, **P2** (2.8 g), **P3** (3.3 g), **P4** (3.1 g), **P5** (16.1 g), and **P6** (9.9 g), respectively. Finally, the remaining residue was eluted with ethyl acetate–methanol in the ratios (9:1) and (0:10), respectively, to afford two fractions, **P7** (5.1 g) and **M** (80.1 g). The precipitate was washed many times with acetone to afford compound **1** (19.8 g) and a washed solution (48.1 g). A part of the washed solution (1.1 g) was evaporated to dryness and applied to column chromatography to afford compound **2** (29.9 mg). A part of the extract **P1** (1.0 g) was applied to silica gel column chromatography, eluted with hexane–ethyl acetate–acetic acid (9:1:0.02) to give four compounds, **6** (295.7 mg), **7** (98.9 mg), **8** (199.1 mg), and **9** (99.8 mg). Fraction **P3** (3.3 g) was rechromatographed, eluted with hexane–ethyl acetate–acetic acid (9:1:0.02) to give three fractions, **P3.1** (0.1 g), **P3.2** (0.8 g), and **P3.3** (1.1 g). Fraction **P3.1** was rechromatographed, eluted with hexane–ethyl acetate–acetic acid (9:1:0.02) to afford two compounds **3** (3.7 mg) and **10** (19.7 mg). Fraction **P3.3** was purified by column chromatography, eluted with hexane–chloroform–ethyl acetate (1:1:1) to afford compound **4** (10.9 mg). Fraction **P5** (16.1 g) was subjected to silica gel column chromatography with hexane–ethyl acetate–acetic acid (9:1:0.02) as eluent to obtain four fractions, **P5.1** (1.1 g) and **P5.2–5.4** (10.7 g). Applying fraction **P5.1** to silica gel column chromatography yielded two fractions, **P5.1.1** (0.2 g) and **P5.1.2** (0.2 g). Fraction **P5.1.2** was further chromatographed to afford compound **5** (4.8 mg).

- **Protocetraric acid (1):** White amorphous powder. The ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data (DMSO-d_6): see Table 1. HMBC correlations: see Figure 3. These spectroscopic data were suitable with those reported in the literature.[3]

- **8'-*O*-Methylprotocetraric acid (2)**: White amorphous powder. The ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data (DMSO-d_6): see Table 1. HMBC correlations: see

Figure 3. These spectroscopic data were suitable with with those reported in the literatures.[6]

- **Virensic acid (3):** White amorphous powder. The ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) data (DMSO-d_6): see Table 1.. These spectroscopic data were suitable with those reported in the literature.[6]

- (+)-**Prasorediosic acid (4):** White amorphous powder. $[\alpha]_D^{23}$ + 439 (*c* 0.13, ethanol). HR-ESI-MS, negative mode: *m/z* 381.2265 $[M-H]^-$ (calcd. for $C_{21}H_{34}O_6$–H, 381.2277). The ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) data (Acetone-d_6): see Table 2. These spectroscopic data were suitable with with those reported in the literature.[9]

- (+)-**Vinaprasorediosic acid A (5):** White amorphous powder. + 59 (*c* 0.28, ethanol). HR-ESI-MS, positive mode: *m/z* 417.2610 $[M+Na]^+$ (calcd. for $C_{23}H_{38}O_5$–H, 417.2617). The ^{1}H- (500 MHz) and ^{13}C-NMR data (125 MHz) (DMSO-d_6): see Table 2. These spectroscopic data were suitable with with those reported in the literature.[9]

- Atranorin (**6**): Colorless needles (acetone). The ^{1}H-NMR (500 MHz)data ($CDCl_3$) were suitable with with those reported in the literature.[4,13] Methyl haematommate (**7**): Colorless needles (chloroform). The ^{1}H-NMR (500 MHz) data ($CDCl_3$) were suitable with with those reported in the literature.[4,12] Methyl *β*-orsellinate (**8**): Pale-green crystals (chloroform). The ^{1}H-NMR (500 MHz) data (Acetone-d_6) were suitable with with those reported in the literature.[4,7] Methyl orsellinate (**9**): White needles (chloroform). The ^{1}H-NMR (500 MHz) data (Acetone-d_6) were suitable with with those reported in the literature.[4,11] Zeorin (**10**): White amorphous powder. The ^{1}H-NMR (400 MHz) data ($CDCl_3$) were suitable with with those reported in the literature.[5,10] See Supporting information.

3. Results and discussion

Compound **1** was isolated as an amorphous powder. Its ^{1}H and ^{13}C spectra revealed the presence of one aromatic methine (δ_H 6.83, δ_C 117.0), one aldehyde group (δ_H 10.59, δ_C 191.7), two methyls (δ_H 2.43, δ_C 21.3, C-9; δ_H 2.40, δ_C 14.3, C-9'), one hydroxymethylene group (δ_H 4.60, δ_C 52.9), two carboxyl groups (δ_C 170.1, 163.9), and eleven aromatic quaternary carbons. From these data, **1** was presumed to be a depsidone. In the A-ring, the HMBC spectrum showed cross peaks of protons H-5 and 6-CH_3 to carbons C-6 and C-1. Moreover, protons H-5 and H-8 also correlated to C-3 and C-4. Thus, the structure of the A-ring was determined. In the B-ring, the hydroxymethylene showed the ^{3}J correlation to carbon C-2' and the second methyl group also showed the ^{4}J correlation to this carbon in the HMBC spectrum. Accordingly, the B-ring was elucidated (Fig. 3). Comparison of NMR data of **1** with the ones in the literature (Brandao L. F. G. et al., 2013)[3] confirmed that **1** was protocetraric acid.

Compound **2** was isolated as a white amorphous powder. Comparison of the 1D- and 2D-NMR data of **2** and **1 (**as shown in Table 1 and Figure 3) indicated that they

were analogous, except for the presence of an additional methoxy group at δ_H 3.22. Besides, this methoxy group showed a cross peak to C-8' on the basis of HMBC correlations. This indicated that the hydroxymethylene group was methyl etherificated. Comparison of the NMR data of **2** with those in the literature (Huneck S., 1997)[6] indicated that **2** was 8'-*O*-methylprotocetraric acid**.**

Table 1. *NMR data (DMSO-d_6) of* ***1-3***

	1[a]		**2**[a]		**3**[b]	
N	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		112.4		112.5		112.3
2		161.2		161.5		161.3
3		111.8		112.5		111.8
4		163.8		164.4		163.8
5	6.83 (*s*)	117.0	6.84 (*s*)	117.6	6.83 (*s*)	117.0
6		152.0		152.2		152.0
7		163.9		164.6		164.0
8	10.59 (*s*)	191.7	10.58 (*s*)	192.0	10.59 (*s*)	191.7
9	2.43 (*s*)	21.3	2.42 (*s*)	21.8	2.43 (*s*)	21.4
1'		116.6		116.5		115.7
2'		155.0		155.9		155.3
3'		118.6		116.3		115.1
4'		144.5		146.0		144.7
5'		141.7		142.6		141.8
6'		129.4		131.4		127.6
7'		170.1		170.8		170.8
8'	4.60 (*s*)	52.9	4.50 (*s*)	62.8	2.14 (*s*)	9.3
9'	2.40 (*s*)	14.3	2.40 (*s*)	15.0	2.40 (*s*)	14.3
8'-OCH_3		115.6	3.22 (*s*)	57.9		

[a] recorded in 500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR
[b] recorded in 400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR

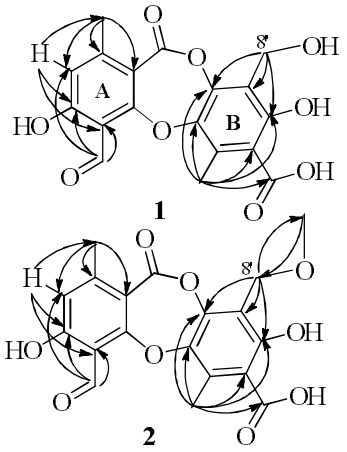

Figure 3. *HMBC correlations of* ***1*** *and* ***2***

Compound **3** was isolated as a white amorphous solid. The NMR spectral data of **3** contained all signals as found in **1**, except for the replacement of the hydroxymethylene group H_2-8′ (δ_H 4.60, δ_C 52.9) as in **1** by the methyl group H_3-8′ (δ_H 2.14, δ_C 9.3). This resulted in the slightly differences of ^{13}C chemical shift values in the B-ring. Comparison of NMR data of **3** with the ones in the literature (Huneck S., 1996)[6] confirmed that **3** was virensic acid.

Compound **4** was isolated as a white amorphous powder. The ^{1}H NMR spectrum revealed one *multiplet* oxymethine (δ_H 5.15), one *doublet* methyl at δ_H 2.03. Moreover, the ^{1}H NMR spectrum exhibited one broad *singlet* signal at δ_H 1.28 with high intensity together with one *triplet* methylene at δ_H 2.18 characteristic of the γ-lactone skeleton with a long chain alkyl group.[6,9] The chemical shift of the *triplet* methylene at δ_H 2.18 in accordance with the presence of the ^{13}C carboxyl signal at δ_C 174.3 indicated the presence of the fragment -CH_2–**CH_2**–COOH. These findings were consistent with the molecular formula, $C_{21}H_{34}O_6$. Accordingly, **4** was elucidated to be prasorediosic acid (Huynh B.L.C, 2016, Huneck S., 1997).[6,9] Moreover, the specific rotation of **4** is dextrorotary, similarly to that of (+)-prasorediosic acid (Huynh B.L.C, 2016),

indicating the (4*R*) configuration of **4**. Consequently, **4** was determined to be (+)-prasorediosic acid.

Compound **5** was isolated as a white amorphous powder. Comparison of the NMR data between **5** and **4** indicated that they were very similar, except for the presence of the acetyl group at (δ_H 2.04, δ_C 28.9, 208.3, H_3C-C=O) and the absence of the *triplet* highfield methyl of **5** at δ_H 0.87. This difference was supported by the molecular formula of **5**, $C_{23}H_{38}O_5$. Moreover, the NMR data as well as the specific rotation of **5** were very similar to (+)-vinaprasorediosic acid A (Huynh B.L.C, 2016),[9] indicating that the absolute configuration of C-4 was (*R*). Accordingly, **5** was elucidated as (+)-vinaprasorediosic acid A.

Table 2. *NMR data of* ***4*** *and* ***5***

N	**4**[a]		N	**5**[b]	
	δ_H, J (Hz)	**δ_C**		**δ_H, J (Hz)**	**δ_C**
1		172.4	1		172.7
2		134.2	2		nd
3		149.8	3		nd
4	5.15 (*m*)	80.9	4	5.13 (*m*)	80.3
5	2.03 (*d*, 2.0)	10.3	5	2.02 (*d*, 2.0)	10.2
6		163.5	6		163.3
7	1.55 (*m*), 1.20 (*m*)	31.6	7	1.42 (*m*)	31.3
8	1.21 (*m*)	24.5	8	1.21 (*m*)	24.8
9-17	1.21 (*m*)	28.0–29.0	9-19	1.21 (*m*)	28.0–-29.0
18	1.48 (*m*)	23.1	20	1.52 (*m*)	23.1
19	1.99 (*m*)	24.1	21	2.37 (*t*, 7.0)	42.6
20	2.18 (*t*, 7.0)	33.4	22		208.3
21		174.3	23	2.04 (*s*)	28.9

[a] recorded in acetone-d_6 , [b] recorded in DMSO-d_6, nd: not determined

4. Conclusion

Ten known compounds were isolated from the lichen *Parmotrema tsavoense* collected in Binh Thuan province. Two depsidones protocetraric acid **(1)**, 8'-*O*-methylprotocetraric acid **(2)** were isolated as major components of the lichen. This is the first time these ten compounds are reported in *Parmotrema tsavoense.* Further studies on this lichen are in progress.

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