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Phenolic compounds from *Usnea baileyi* (Stirt.) Zahlbr growing in Lam Dong province

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Abstract:

This study entails a continuation of the phytochemical study regarding the lichen *Usnea baileyi* collected in Lam Dong province. Eight compounds, 8'-*O*-methylprotocetraric acid (1), protocetraric acid (2), virensic acid (3), subvirensic acid (4), barbatic acid (5), diffractaic acid (6), 4-*O*-demethylbabartic acid (7), and atranorin (8), were isolated using various chromatographic methods. Their chemical structures were elucidated through spectroscopic analysis as well as through a comparison of their data with that in the literature.

Keywords: **depside, depsidone, lichen, phenolic compound, *Usnea baileyi.***

Classification number: **2.2**

Introduction

The genus *Usnea* encompasses over 350 species across the world [1]. They produce diverse lichen metabolies which are endowed with various bioactivities. The fruticose lichen *Usnea baileyi* has proliferated in Lam Dong province, Vietnam. Our previous study concerning this lichen precipitated the isolation of several depsidones from the ethyl acetate [2]. The present research reports the isolation and structure elucidation of eight phenolic compounds (**1-8**) from the remaining fractions of the ethyl acetate and dichloromethane extracts (Fig. 1).

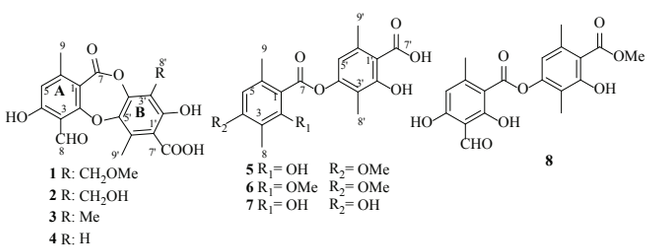

Fig. 1. Chemical structures of 8'-O-methylprotocetraric acid (1), protocetraric acid (2), virensic acid (3), subvirensic acid (4), babartic acid (5), diffactaic acid (6), 4-O-demethylbabartic acid (7), and atranorin (8).

Materials and methods

General experimental procedures

The NMR spectra were measured on Bruker Advance (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers. Proton chemical shifts were referenced to the solvent residual signal of CD_3SOCD_3 at δ_H 2.50 and of $CDCl_3$ at δ_H 7.26. The ^{13}C NMR spectra were referenced to the central peak of CD_3SOCD_3 at δ_C 39.52 and of $CDCl_3$ at δ_C 77.16. The HR-ESI-MS were recorded on a HR-ESI-MS Bruker micrOTOF Q-II. All NMR and HR-ESI-MS spectra were recorded in the Chemistry Department, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. Thin layer chromatography (TLC) was conducted

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on precoated silica gel 60 F_{254} or silica gel 60 RP-18 $F_{254}S$ (Merck Millipore, Billerica, Massachusetts, USA), and spots were visualised as a result of spraying with 10% H_2SO_4 solution followed by heating.

Plant material

Thalli of lichen *U. baileyi* were collected from the bark of trees at Tam Bo mountain, Di Linh district, Lam Dong province, Vietnam in May 2015. The scientific name of this lichen was authenticated by Ms. Natwida Dangphui and Assistant Professor Dr. Ek Sangvichien of Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

Extraction and isolation

The air-dried lichen powder (800.0 g) was macerated with acetone (3x10 l) at room temperature. The filtered solution was then evaporated to dryness to yield 80.0 g of crude acetone extract. This extract was washed three times by acetone to obtain a precipitate P (23.8 g). The remainder of the solution was further concentrated to afford the crude acetone extract (56.2 g).

The precipitate P (23.8 g) was subjected to silica gel CC and eluted with a solvent system of CH_2Cl_2: MeOH: AcOH (9.0: 0.2: 0.06) to afford three fractions, P1 (10.7 g), P2 (7.2 g), and P3 (5.8 g). Fraction P3 (5.8 g) was fractioned by CC and eluted with CH_2Cl_2: MeOH: AcOH (9.5: 0.5: 0.07) to afford P3.1 (1.8 g) and P3.2 (3.9 g). Purification of P3.1 (1.8 g) by CC led to the isolation of compounds **1** (4.6 mg), **2** (8.0 mg), and **3** (6.5 mg).

The crude acetone extract (56.2 g) was applied to silica gel quick column and eluted with CH_2Cl_2, EtOAc, acetone and MeOH to obtain four extracts, DC (31.2 g), EA (9.6 g), Ac (6.5 g), and Me (4.6 g), respectively. The EA extract was washed by acetone (3x100 ml) to obtain the precipitate EA-P (1.0 g) and a filtrated solution. The solution was then evaporated to dryness to induce fraction EA-L (7.8 g). The solvent system of CH_2Cl_2: MeOH: AcOH (9.0:0.2:0.06) was then applied for the entire purification process of fraction EA-L. Three fractions EA-L1-3 were obtained by subjecting fraction EA-L to column chromatography. Purifying the fraction EA-L2 (1.2 g) by CC resulted in two compounds, namely **5** (14.1 mg) and **6** (18.4 mg). The extract DC was fractionated by CC and eluted with a gradient of *n*-hexane: EtOAc (8:2-0:10) to obtain four fractions DC1-4, respectively. Applying CC on fraction DC1 (7.8 g) with the mobile phase of *n*-hexane: EtOAc: AcOH (9.0:1.0:0.1) produced five fractions, DC1.1-5. Compound **8** (6.2 mg) and **4** (15.3 mg) were isolated from the purification of DC1.2 (0.6 g), while **7** (5.2 mg) was obtained from the purification of DC1.4.2 using silica gel column chromatography with the same solvent system of *n*-hexane: EtOAc: AcOH (7.5:2.5:0.06).

- 8′-*O*-methylprotocetraric acid (1). White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 1;

- Protocetraric acid (2). White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 1;

- Virensic acid (3). White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 1;

- Subvirensic acid (4). White amorphous powder; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 1;

- Babartic acid (5). White colorless needle; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 2;

- Diffractaic acid (**6**). White colorless needle; the 1H and 13C NMR (DMSO-d_6) spectroscopic data, see Table 2;

- 4-*O*-demethylbabartic acid (7). White colorless needle; the ^{1}H and ^{13}C NMR (DMSO-d_6) spectroscopic data, see Table 2;

- Atranorin (**8**). White colorless needle; the ^{1}H and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 2.

Results and discussion

Compound **1** was obtained as a white amorphous powder. The ^{1}H NMR and HSQC spectra of **1** demonstrated the presence of one formyl (δ_H 10.55, 1H, s), one aromatic proton (δ_H 6.78, 1H, s), one oxymethylene group (δ_H 4.43, 2H, s), one methoxy group (δ_H 3.19, 3H, s), and two methyl groups (δ_H 2.45, 3H, s and 2.34, 3H, s). The ^{13}C NMR spectrum in accordance with HSQC spectrum confirmed the presence of 19 carbons comprising one aldehyde carbon (δ_C 191.8), two carboxyl carbons (δ_C 170.4 and 161.3), 12 aromatic carbons (δ_C 164.4, 163.8, 158.2, 151.8, 145.1, 141.2, 131.2, 116.9, 115.5, 115.1, 112.3, and 111.8), one oxygenated methylene carbon (δ_C 62.4), one methoxy group (δ_C 57.3), and two methyls (δ_C 21.3 and 14.4). HMBC cross peaks of both H-5 (δ_H 6.78) and 3-CHO (δ_H 10.55) to C-3 (δ_C 112.3), H-5 to C-9 (δ_C 21.3) and H_3-9 (δ_H 2.45) to C-1 (δ_C 111.8), C-5 (δ 116.9) and C-6 (δ 151.8) defined the connectivity through C-3–C-4–C-5–C-6–C-1 in the A-ring (see Fig. 2). In addition, the cross peaks of H_3-9′ (δ_H 2.34) to C-1′ (δ_C 115.5), C-5′ (δ_C 141.2), and C-6′ (δ_C 131.2) confirmed its position in the B-ring. The ^{1}H NMR chemical shift of H_2-8′ along with the HMBC cross peaks of H_2-8′ to C-2′ (δ_C 158.2), C-3′ (δ_C 115.1), and C-4′ (δ_C 145.1) determined the linkage of this group at C-3. The comparison of NMR data of **1** and those of 8′-*O*-methylprotocetraric acid [3] indicated that they were identical; therefore, **1** was elucidated as 8′-*O*-methylprotocetraric acid.

Table 1. ^{1}H and ^{13}C NMR of 1-4[a].

Position	1		2		3		4	
	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C
1		111.8		111.9		111.9		111.8
2		164.4		164.8		164.0		164.1
3		112.3		112.7		112.3		112.3
4		163.8		163.9		163.8		163.8
5	6.78 s	116.9	6.82 s	115.6	6.83 s	115.1	6.83 s	117.0
6		151.8		152.2		152.1		152.0
7		161.3		161.5		161.3		161.5
8	10.54 s	191.8	10.58 s	191.9	10.59 s	191.7	10.57 s	191.7
9	2.45 s	21.3	2.42 s	21.5	2.43 s	21.4	2.42 s	21.4
1′		111.5		111.9		111.9		111.8
2′		158.2		155.9		155.1		156.2
3′		115.1		117.5		115.7	6.67 s	105.8
4′		145.1		144.5		144.7		144.5
5′		141.2		140.7		141.8		141.0
6′		131.2		127.5		127.6		128.8
7′		170.4		170.3		170.8		168.2
8′	4.43 s	62.4	4.64 s	52.9	2.14 s	9.3		
9′	2.34 s	14.4	2.41 s	14.4	2.41 s	14.3	2.27 s	14.0
OCH_3	3.19	57.3						

[a]: these were recorded in DMSO-d_6.

Compound **2** was obtained as a white amorphous powder. Both its ^{1}H and ^{13}C NMR spectroscopic data were similar to those of **1**; the only difference was the absence of the methoxy moiety (δ_H 3.19 and δ_C 57.3, 8′-OMe in **1**), which demonstrated the replacement of 8′-OH for 8′-OMe in the B-ring of **2**. The comparison of NMR data of **2** with those of protocetraric acid [3] illustrated that they were identical; therefore, **2** was elucidated as protocetraric acid.

Compound **3** was isolated as a white amorphous powder. Examination of the ^{1}H NMR and ^{13}C NMR spectra of **3** revealed signal patterns resembling those of **2**, with the exception of the replacement of the methyl group (δ_H 2.14 and δ_C 9.3, 8′-Me) rather than the oxygenated methylene moiety (δ_H 4.64 and δ_C 52.9, 8′-CH_2OH) in the B-ring. The comparison of NMR data of **3** with those of virensic acid [3] demonstrated that they were identical; accordingly, **3** was elucidated as virensic acid.

Compound **4** was yielded as a white amorphous powder. The 1D NMR data of **4** were reminiscent of that of **3** (Tables 1 and 2); the primary difference was the presence of H-3 (δ_H 6.83, 1H, s) in lieu of the methyl 8′-Me (δ_H 2.05, 3H, s and δ_C 9.3, 8′-Me). The NMR data of **4** were identical to that of subvirensic acid [4]. Combined, the chemical structure of **4** was elucidated as subvirensic acid.

Compound **5** was isolated as a white amorphous powder. The ^{1}H NMR and HSQC spectra of **5** demonstrated the presence of one hydroxy proton (δ_H 10.74, 1H, s), two aromatic protons (δ_H 6.68, 1H, s and 6.60, 1H, s), one methoxy group (δ_H 3.86, 3H, s), and four methyl groups (δ_H 2.57, 2.48, 2.00, 1.99, 3H for each, s). The ^{13}C NMR spectrum combined with HSQC spectrum revealed the presence of 19 carbons comprising two carbonxyl carbons (δ_C 173.1 and 168.6), 12 aromatic carbons (δ_C 161.3, 161.1, 159.5, 151.8, 139.0, 139.0, 115.9, 115.7, 111.4, 110.0, 107.0, and 106.3.8), one methoxy group (δ_C 55.7), and four methyls (δ_C 23.0, 22.7, 9.04, and 7.99). HMBC cross peaks of both H-5 (δ_H 6.60) and H_3-OMe (δ_H 3.86) to C-4 (δ_C 161.3) and both H-5 and H_3-9 (δ_H 2.57) to C-1 (δ_C 107.0), C-5 (δ_C 106.3), and C-6 (δ_C 139.0) defined the positions of these groups (Fig. 2). Moreover, the HMBC cross peaks of 2-OH (δ_H 10.74) to C-1, both 2-OH and H_3-8 (δ_H 2.00) to C-2 (δ_C 159.5) and C-3 (δ_C 110.0) totally defined the connectivity through C-1–C-2–C-3–C-4–C-5–C-6 in the A-ring. Furthermore, the HMBC correlations of both H-5′ (δ_H 6.68) and H_3-9′ (δ_H 2.48) to C-1′ (δ_C 111.4), C-5′ (δ_C 115.9), and C-6′ (δ_C 139.0) and both H-5′ and H_3-8′ (δ_H 1.99) to C-3′ (δ_C 115.7) and C-4′ (δ_C 151.8) defined the system through C-3′–C-4′–C-5′–C-6′–C-1′ in the B-ring. The ^{13}C NMR chemical shift of C-7 (δ_C 168.6) and C-4′ characterised for the ester linkage between C-7 and C-4′ of a depside scaffold. The comparison of NMR data of **5** with those of barbatic acid [5] indicated that they were identical; **5** was elucidated as barbatic acid.

Compound **6** was obtained as colorless needle. The ^{1}H and ^{13}C NMR spectrum of **6** were highly similar to those of **5**, with the exception of the absence of 2-OH (δ_H10.74, 1H, s), replaced by the methoxy group (δ_H 3.68 and δ_C 61.8) in **6**. The NMR data of **6** resembled that of diffractaic acid [6]. Therefore, **6** was elucidated as diffractaic acid.

Compound **7** was obtained as colorless needle. The ^{1}H and ^{13}C NMR spectrum of **7** were identical to that of **5**; the sole difference was the absence of the 4-OMe group (δ_H 3.86, 3H, s) rather than one hydroxy group at (δ_H 11.13, 1H, s) in **7**. The NMR data of **7** closely resembled that of 4-*O*-demethylbabartic acid [7]. Consequently, **7** was elucidated as 4-*O*-demethylbabartic acid.

Table 2. ^{1}H and ^{13}C NMR of 5-8.

Position	5[a]		6[a]		7[a]		8[b]	
	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C	δ_H, J(Hz)	δ_C
1		107.0		119.3		108.6		108.7
2		159.5		156.4		160.7		169.2
3		110.0		116.4		110.9		110.4
4		161.3		161.3		161.9		167.7
5	6.68 s	106.3	6.45 s	108.5	6.63, s	110.9	6.51, s	116.2
6		139.0		134.8		139.0		152.2
7		168.6		165.5		169.2		169.8
8	2.00 s	8.0	1.90 s	8.7	1.94, s	8.0	10.36, s	194.0
9	2.57 s	22.7	2.23 s	19.5	2.44, s	22.7	2.69, s	25.7
2-OMe			3.68 s	61.8				
4-OMe	3.86 s	55.7	3.60 s	55.8				
2-OH	10.74 s				11.13, s		12.54, s	
4-OH							12.50, s	
1′		111.4		111.5		111.6		103.0
2′		161.1		159.5		161.3		163.0
3′		115.7		116.0		115.7		116.9
4′		151.8		152.2		151.7		152.6
5′	6.60 s	115.9	6.62 s	115.7	6.36, s	115.9	6.40, s	113.0
6′		139.0		139.0		139.0		140.0
7′		173.1		173.1		173.1		172.3
8′	1.99 s	9.0	1.98 s	8.9	1.94 s	9.1	2.10 s	9.5
9′	2.48 s	23.0	2.34 s	22.8	2.44 s	23.5	2.54 s	24.1
2′-OH					10.33 s		11.94 s	
COOMe							3.97 s	52.3

[a]: these were recorded in DMSO-d_6.
[b]: these were recorded in $CDCl_3$.

Compound **8** was obtained as colorless needle. The ^{1}H and ^{13}C NMR spectra of **8** closely resembled those of **7,** with two differences. Firstly, the 3-Me group (δ_H 1.94, 3H, s, H_3-8) in **7** was replaced by the formyl proton (δ_H 10.36, 1H, s). Secondly, the presence of one additional methoxy group at δ_H 3.98 and δ_C 52.4 suggested the methyl ester at C-7′. The NMR data of **7** closely resembled that of atranorin [8]. Therefore, **8** was confirmed as atranorin.

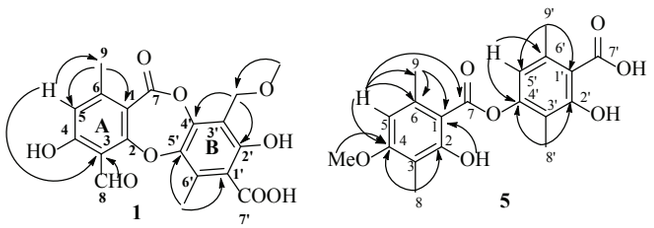

Fig. 2. Key HMBC correlations of 1 and 5.

8′-*O*-methylprotocetraric acid (**1**), virensic acid (**3**), barbatic acid (**5**), diffractaic acid (**6**), and 4-*O*-demethylbabartic acid (**7**) were discovered for the first time from *Usnea baileyi.* It should be noted that this is the first time that subvirensic acid (**4**) was isolated from the genus *Usnea* [9].

Conclusions

From *Usnea baileyi* collected in Lam Dong province, eight phenolic compounds were isolated and elucidated, including 8′-*O*-methylprotocetraric acid (**1**), protocetraric acid (**2**), virensic acid (**3**), subvirensic acid (**4**), barbatic acid (**5**), diffractaic acid (**6**), 4-*O*-demethylbabartic acid (**7**), and atranorin (**8**).

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The authors declare that there is no conflict of interest regarding the publication of this article.

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