



Viet Nam National University Ho Chi Minh City, Viet Nam

Science and Technology Development Journal

ISSN: 1859-0128

Volume 21: Issue 01 June 2018

Journal homepage: <http://stdj.scienceandtechnology.com.vn>**Extraction, isolation and characterization of depsidones from *Usnea baileyi* (Stirt.) Zahlbr collected from tree barks in Tam Bo Mountain of Di Linh, Lam Dong Province, Viet Nam**¹Nguyen Van Kieu, ²Duong Thuc Huy*¹Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok, Thailand.²Department of Chemistry, Ho Chi Minh City University of Education, 280 An Duong Vuong Street, District 5, Ho Chi Minh City, Viet Nam. thuchuy84@yahoo.com**ARTICLE DETAILS****History**

Received: 05 April 2018

Accepted: 03 June 2018

Published: 20 June 2018

Keywords*Depsidones, Lichen, Menegazziaic, Stictic acid, Usnea baileyi***ABSTRACT**

Chemical data on the lichen *Usnea baileyi* are scarce. The current investigation reports the extraction, isolation and characterization of depsidones from this lichen collected in Lam Dong Province. Eight depsidones were isolated and elucidated, including methylstictic acid (**1**), stictic acid (**2**), cryptostictic acid (**3**), 8'-O-methylmenegazziaic acid (**4**), menegazziaic acid (**5**), 8'-O-methylconstictic acid (**6**), constictic acid (**7**), and hypoconstictic acid (**8**). Multi chromatographic methods comprising normal phase silica gel column chromatography and thin layer chromatography were applied to the crude acetone extract to isolate and purify compounds **1-8**. Their chemical structures were elucidated by spectroscopic analysis as well as comparing their data with ones in the literature.

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1. Introduction

Lichens are symbiotic associations comprised of a fungus and a photobiont partner (green alga and/or cyanobacteria). This unique organism produces numerous metabolites endowed with various biological activities Müller (2001); Boustie and Grube (2005). Among them, depsidone scaffold is a unique skeleton possessing many biological activities such as enzyme inhibition, cytotoxicity, and antiviral activities Müller (2001).

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Recommended citation: Kieu, Nguyen Van and Huy, Duong Thuc, (2018). **Extraction, isolation and characterization of depsidones from *Usnea baileyi* (Stirt.) Zahlbr collected from tree barks in Tam Bo Mountain of Di Linh, Lam Dong Province, Viet Nam**. *Science and Technology Development Journal*, 21(1): 24-31.

Recent phytochemical studies on Vietnamese lichen afforded novel metabolites showing good bioactivity [Duong et al. \(2017\)](#); [Nguyen et al. \(2017\)](#). The fruticose lichen *Usnea baileyi* is widely grown in high-altitude forests of Vietnam. Two previous phytochemical studies on this lichen led to the isolation of several xanthenes [Yang et al. \(1973\)](#), depsidones and depsides [Yang et al. \(1973\)](#); [Din et al. \(2010\)](#), along with usnic acid.

The current investigation reports on the extraction, isolation and characterization of depsidones from the lichen *Usnea baileyi*. Eight depsidones (**1-8**) (**Figure 1**) were isolated and structurally elucidated. Their chemical structures were elucidated by spectroscopic analysis as well as comparing their data with ones in the literature.

2. Materials — Methodology

2.1 General experimental procedures

The NMR spectra were measured on a Bruker Avance (400 MHz for ^1H 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. The ^{13}C -NMR spectra were referenced to the central peak of CD_3SOCD_3 at δ_C 39.52. Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F₂₅₄ or silica gel 60 RP-18 F₂₅₄S (Merck Millipore, Billerica, Massachusetts, USA), and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm, Merck Millipore, Billerica, Massachusetts, USA).

2.2 Plant material

Thallus of the lichen *U. baileyi* were collected from the tree barks in Tam Bo Mountain, Di Linh District, Lam Dong Province, Viet Nam in May 2015. The scientific name of this lichen was identified by Ms. Natwida Dangphui and Assistant Professor Dr. Ek Sangvichien, Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok, Thailand.

2.3 Extraction and isolation

The air-dried lichen powder (800.0 g) was macerated with acetone at room temperature (3 x 10 L), and the filtrate was evaporated until dryness to yield the crude acetone extract (80.0 g). This extract was washed three times with acetone to obtain a precipitate **P** (23.7 g). The filtrated solution was further concentrated to yield the acetone extract **Ac** (56.2 g).

The precipitate **P** (23.7 g) was subjected to silica gel column chromatography (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 **P2** was applied in the same manner as fraction **P1** to yield two fractions **P2.1** (3.2 g) and **P2.2** (3.8 g). Fraction **P2.2** was further applied on CC, eluted with *n*-hexane: CH_2Cl_2 :AcOH (9.0: 1.0: 0.1) to obtain three compounds: **1** (3.2 mg), **4** (4.2 mg) and **6** (4.0 mg).

The acetone extract **Ac** (56.2 g) was applied to fast CC, 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). Extract **EA** was washed with acetone to obtain the precipitate **EA-P** (1.0 g) and a filtrate. The solution was then evaporated to dryness to yield fraction **EA-L** (7.8 g). The precipitate **EA-P** was applied on normal phase silica gel CC, and eluted with the solvent system CH_2Cl_2 : MeOH: AcOH (9.0: 0.2: 0.06) to yield two compounds **2** (506.2 mg) and **7** (307.0 mg). Fraction **EA-L** was further separated by silica gel CC, eluted with the same solvent system to obtain three fractions **EA-L1-3**. The fraction **EA-L3** (6.5 g) was fractionated into

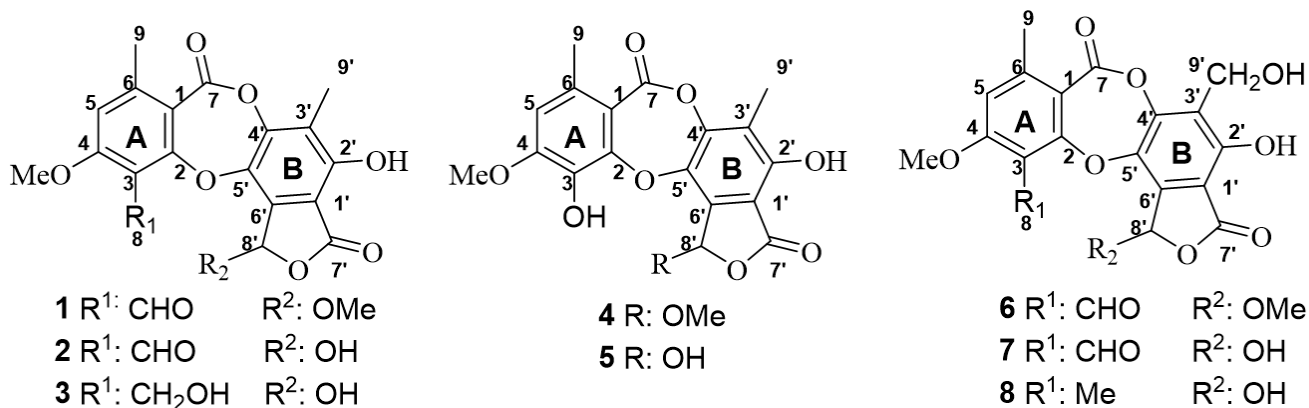


Figure 1. Chemical structures of methylstictic acid (1), stictic acid (2), cryptostictic acid (3), 8'-O-methylmenegazziaic acid (4), menegazziaic acid (5), 8'-O-methylconstictic acid (6), constictic acid (7), and hypoconstictic acid (8).

EA-L3.1-4 by CC. Purification of the fraction EA-L3.3 (1.2 g) resulted in three compounds: **3** (12.2 mg), **5** (9.5 mg), and **8** (6.8 mg).

8'-Methylstictic acid (1). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

Stictic acid (2). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

Cryptostictic acid (3). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

8'-O-Methylmenegazziaic acid (4). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

Menegazziaic acid (5). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

8'-O-methylconstictic acid (6). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

Constictic acid (7). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

Hypoconstictic acid (8). White amorphous powder; for the ¹H and ¹³C NMR (DMSO-*d*₆) spectroscopic data, see **Tables 1 and 2**.

3. Results and Discussion

Compound **1** was obtained as a white amorphous powder. The ¹H NMR and HSQC spectra of **1** showed the presence of one formyl (δ_H 10.41, 1H, s), one aromatic proton (δ_H 7.10, 1H, s), one acetal proton (δ_H 6.47, 1H, s), two methoxy groups (δ_H 3.92, 3H, s and 3.44, 3H, s), and two methyl groups (δ_H 2.49, 3H, s and 2.19, 3H, s). The ¹³C NMR spectrum combining with HSQC spectrum confirmed the presence of twenty carbons comprising one aldehyde carbon (δ_C 186.7), two carbonyl carbons (δ_C 165.9 and 160.6), twelve aromatic carbons (δ_C 162.7, 162.4, 152.4, 151.0, 148.3, 133.1, 132.6, 121.7, 114.3, 112.9, 113.2, and 108.8), one acetal carbon (δ_C 99.9), two methoxy groups (δ_C 56.8 and 56.3), and two methyls (δ_C 21.5 and 9.7). The HMBC correlations from H-5 (δ_H 7.10), 4-OCH₃ (δ_H 3.85), and 3-CHO (δ_H 10.41) to C-4 (δ_C 162.7), and from H-5 and H₃-9 (δ_H 2.49) to C-1 (δ_C 113.2, defined the connectivity through

C-3–C-4–C-5–C-6–C-1 in A-ring. Moreover, a long range HMBC correlation between H-5 and C-7 (δ_C 160.6) suggested the presence of the depsidone scaffold of **1**. The ^1H NMR chemical shift of H₃-9' along with the HMBC correlations between H₃-9' and C-2' (δ_C 151.0), C-3' (δ_C 121.7), and C-4' (δ_C 148.3) confirmed the connectivity C-2'–C-3'–C-4'. In addition, the HMBC correlations between H-9' and C-7' (δ_C 165.9) and 8'-O-Me (δ_C 56.3) led to deduction of the five-member ring lactone in the B-ring. The comparison of NMR data of **1** and those of methylstictic acid [Ramesh and Baig \(1994\)](#); [Kathirgamanathar et al. \(2005\)](#) showed that they were identical. Thus, **1** was elucidated as 8'-O-methylstictic acid.

Table 1. Proton nuclear magnetic resonance data of compounds 1 - 8*

N	1	2	3	4	5	6	7	8
	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)	$\delta\text{H, J}$ (Hz)
5	7.10 s	7.10 s	6.95 s	6.85 s	6.82 s	7.11 s	7.08 s	6.87 s
8	10.41 s	10.45 s	4.80 d, 11.2 4.62 d, 11.2		9.10 s	10.41 s	10.42 s	2.23 s
9	2.49 s	2.48 s	2.45 s	2.33 s	2.30 s	2.49 s	2.46 s	2.39 s
3-OH				9.38 s				
4-	3.92 s	3.89 s	3.87 s	3.84 s	3.81 s	3.92 s	3.88 s	3.85 s
OMe								
8'	6.47 s	6.60 br	-	6.47 s	6.70 br	6.49 s	6.62 br	6.67 br
9'	2.19 s	2.17 s	2.19 s	2.15 s	2.11 s	4.64 s	4.60 s	4.60 s
8'-		8.19 br	8.22 br		8.25 br		8.25 br	8.29 br
OH								
8'-	3.44 s			3.56 s		3.45 s		
OMe								

*These were recorded in DMSO-d₆.

Compound **2** was isolated as a white amorphous powder. The ^1H NMR and ^{13}C NMR spectrum of **2** resembled those of **1**, except for the presence of the hydroxy group (δ_H 8.19, 1H, br, 8'-OH) and the absence of the methoxy group (δ_H 3.44 and δ_C 56.3, 8'-O-Me), indicating the replacement of 8'-OH to 8'-OMe in the lactone moiety in B-ring. The comparison of NMR data of **2** with those of stictic acid [Dévéhat et al. \(2007\)](#) showed that they were identical. Thus, **2** was concluded as stictic acid.

Compound **3** was acquired as a white amorphous powder. The ^1H NMR spectrum exhibited signals for seven resonances belonging to one hydroxy signal at δ_H 8.22, two oxygenated methylene groups (δ_H 4.80, 1H, d, 11.2 and 4.62, 1H, d, 11.2), one methoxy moiety (δ_H 3.87, 3H), and two methyl groups (δ_H 2.45 and 2.19). The ^{13}C NMR spectrum displayed nineteen carbon signals, similar to those of compound **2** ([Table 2](#)), except for the appearance of the formyl group 8-CHO (δ_H 10.41, 1H, s) and the appearance of the oxygenated methylene 8-CH₂-OH (δ_C 51.3) instead of the formyl group in A-ring. The ^1H and ^{13}C NMR spectra of **3** were identical to those of cryptostictic acid [Shimada et al. \(1980\)](#). Thus, compound **3** was elucidated as cryptostictic acid.

Compound **4** was isolated as a white amorphous powder. The ^1H NMR and HSQC spectra showed the presence of one hydroxy group at δ_H 9.38 (1H, s), one aromatic proton at δ_H 6.85 (1H, s), one acetal proton at δ_H 6.47 (1H, s), two methoxy at δ_H 3.84 (3H, s) and 3.56 (3H, s), and two methyl groups at δ_H 2.33 (3H, s) and 2.15 (3H, s). In addition, the ^{13}C NMR and HSQC spectra also revealed the characteristic resonances of a depsidone skeleton including two conjugated carbonyl carbons (δ_C 166.8

and 161.5), twelve aromatic carbons, one acetal carbon (δ_C 100.8), two oxygenated carbons (δ_C 56.7 and 56.3), and two methyl groups (δ_C 19.8 and 9.5). In the HMBC spectra, correlations between H-5 (δ_H 6.85, 1H, s), 4-OMe (δ_H 3.84) and C-4 (δ_C 152.7), and of H-5, H₃-9 and C-1 (δ_C 113.3), identified the connectivity through C-1–C-6–C-5–C-4 in ring A. The absence of formyl group (3-CHO) in accordance with the ¹³C chemical shifts of C-2 (152.5), C-3 (135.0), and C-4 (152.5) defined the appearance of a hydroxyl group at C-3 with its ¹H chemical shift as δ_H 9.38, further confirmed by HMBC correlation between H-5 and C-3 (δ_C 135.0). The HMBC correlations between H₃-9' to C-2' (δ_C 152.5), C-3' (δ_C 121.2), and C-4' (δ_C 148.7), and from H-8' to C-7' (δ_C 166.8) and 8'-OMe (δ_C 56.3), indicated that the B-ring of **4** was similar with those of **1**. Detailed comparison of ¹H and ¹³C NMR data of **4** with those of 8'-O-methylmenegazziaic acid [Kathirgamanathar et al. \(2005\)](#) gave close similarity, thus identifying **4** as 8'-O-methylmenegazziaic acid.

Table 2. Carbon-13 nuclear magnetic resonance data of compounds 1 - 8*

Position	1	2	3	4	5	6	7	8
1	112.9	113.1	111.6	113.6	113.3	113.1	114.4	110.8
2	162.4	163.0	161.4	152.5	152.4	162.9	162.4	161.1
3	114.3	114.5	118.5	135.0	138.2	114.4	114.5	114.5
4	162.7	162.4	161.7	152.5	152.4	162.5	162.4	161.1
5	113.2	112.8	112.7	111.7	111.7	113.4	112.8	112.5
6	152.4	150.8	144.2	132.9	132.4	151.1	150.7	141.8
7	160.6	160.7	158.9	161.7	161.4	160.6	160.6	158.7
8	186.7	186.6	51.3			186.9	186.6	8.6
9	21.5	21.5	20.8	19.9	19.8	21.6	21.4	20.7
4-OMe	56.8	56.8	56.2	56.3	56.2	56.5	56.8	56.2
1'	108.8	109.2	109.0	109.0	109.0	109.4	113.2	109.1
2'	151.0	152.0	151.5	151.4	151.4	153.2	152.7	152.3
3'	121.7	120.8	120.3	120.4	120.4	124.0	123.2	122.1
4'	148.3	148.0	148.2	148.3	148.3	148.6	148.2	148.3
5'	132.6	137.5	137.9	132.6	132.4	135.5	138.1	137.8
6'	133.1	135.9	135.8	135.1	134.9	137.6	137.4	136.9
7'	165.9	166.3	166.5	166.8	166.5	165.6	166.2	166.1
8'	99.9	95.1	95.4	100.7	95.4	99.9	95.3	95.3
9'	9.7	9.6	9.5	9.7	9.5	53.0	52.6	52.7
8'-OMe	56.3			56.7		57.0		

*These were recorded in DMSO-d₆.

Compound **5** was isolated as a white amorphous powder. Comparison of the 1D NMR data of **5** with those of **4** (**Tables 1 and 2**) showed that they were similar, except for the absence of one methoxy group at δ_H 3.56 and δ_C 56.3 (8'-OMe), suggesting the presence of 8'-OH in the lactone moiety in B-ring instead of 8'-OMe. Comparison of NMR data of **5** with those of menegazziaic acid [Hirayama et al. \(1976\)](#) confirmed the chemical structure of **5** as menegazziaic acid.

Compound **6** was obtained as a white amorphous powder. Detailed comparison of the 1D NMR data of **6** with those of **1** showed that they were closely similar. The only difference was the replacement of an oxygenated methylene at δ_H 4.65 (δ_C 53.0) for the methyl H₃-9' (δ_H 2.19 and δ_C 9.7). The HMBC correlations between H₂-9' (4.65, 2H, s) and C-2' (δ_C 148.6), C-3' (δ_C 124.0), and C-4' (δ_C 153.2) confirmed

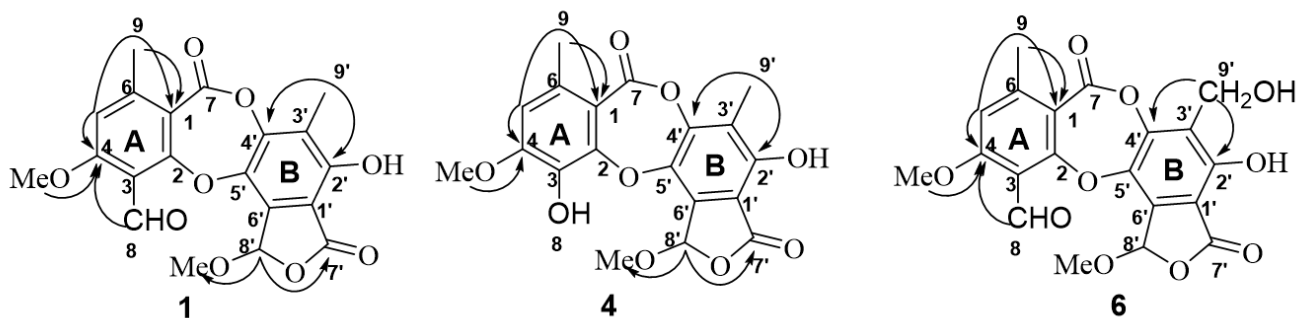


Figure 2. Key HMBC correlations of 1, 4, and 6.

its position in B-ring. The ^1H and ^{13}C NMR data of **6** were similar to those of 8'-*O*-methylconstictic acid [Papadopoulou et al. \(2007\)](#). Thus, **6** was concluded to be 8'-*O*-methylconstictic acid.

Compound **7** was isolated as a white amorphous powder. The 1D NMR data of **7** closely resembled those of **6** ([Tables 1 and 2](#)) and indicated that they have the same scaffold, except for the absence of 8'-OMe at δ_{H} 3.46 (δ_{C} 56.5). This implied that the 8'-OMe in **6** was replaced by 8'-OH in **7**. The NMR data of **7** were identical to those of constictic acid [Shimada et al. \(1980\)](#). Consequently, the chemical structure of **7** was elucidated as constictic acid.

Compound **8** was obtained as white amorphous powder. Analysis of the ^1H and ^{13}C NMR data of **8** showed similar resonances to those of **7** ([Tables 1 and 2](#)), except for the replacement of the formyl group at C-3 by the methyl group (δ_{H} 2.23, s and δ_{C} 8.6) in the A-ring. The NMR data of **8** were identical with those of hypoconstictic acid [Elix et al. \(1985\)](#). Thus, the structure of **8** was elucidated as hypoconstictic acid.

Stictic acid **2** and its derivatives **1**, **3-8** were found in many lichens belonging to *Usnea* and *Lobaria* genera [Nguyen et al. \(2017\)](#); [Dévéhat et al. \(2007\)](#). Such compounds are superoxide anion scavengers and are as potent as ascorbic acid but show no toxicity on some human cell lines [Ismed et al. \(2017\)](#). [Din et al. \(2010\)](#) reported the presence of three depsidones (salazinic acid, norstictic acid, and protocetraric acid) in *U. baileyi* by HPLC analysis without the isolation of compounds. Among the isolated compounds, **2** was reported as a major component [Din et al. \(2010\)](#) while other compounds **1**, **3-8** were found in *Usnea baileyi* for the first time.

4. Conclusion

From *Usnea baileyi* collected in the Lam Dong Province, eight compounds were isolated and elucidated, including methylstictic acid (**1**), stictic acid (**2**), cryptostictic acid (**3**), 8'-*O*-methylmenegazziaic acid (**4**), menegazziaic acid (**5**), 8'-*O*-methylconstictic acid (**6**), constictic acid (**7**), and hypoconstictic acid (**8**). Compounds **1**, **3-8** were isolated in *U. baileyi* for the first time.

5. Abbreviations

^{13}C NMR: Carbon-13 nuclear magnetic resonance; ^1H NMR: Proton nuclear magnetic resonance; CC: column chromatography ; DMSO: Dimethyl sulfoxide (CD_3SOCD_3); HMBC: Heteronuclear multiple bond correlation; HPLC: High-performance liquid chromatography; HSQC: Heteronuclear single quantum coherence ; TLC: Thin layer chromatography

6. Conflicts of interest

The authors declare no competing financial interest.

7. Author contribution

Nguyen V.K. has contributed in conducting experiments, acquisition of data and compose the manuscript. Duong T. H. made substantial contributions to interpretation of data, searching the bibliography and gave final approval of the manuscript to be submitted.

8. Acknowledgements

We are grateful to Ms. Natwida Dangphui for authentication the scientific name of the lichen.

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