Science & Technology Development Journal, 2022; 25(2):2373-2380

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Natural Science / Research article

Chemical constituents of the lichen *Usnea lapponica* Vain., Parmeliaceae

Nguyen Huu Tri, Nguyen Thi My Dung*

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ABSTRACT

Introduction The lichen *Usnea lapponica* Vain. belonging to the *Usnea* genus (family Parmeliaceae) grow hanging from tree branches in the damp forest at Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province. In addition, this lichen has not yet been chemically and biologically studied. The primary goal of the present work was to study the chemical constituents of the lichen *Usnea lapponica* Vain. **Methods**: A dried powder of thalli *Usnea lapponica* Vain. was extracted by maceration with MeOH at ambient temperature to prepare the crude MeOH extract. This crude extract was subjected to silica gel column chromatography with gradient polar solvent including *n*-hexane, $CHCl_3$, EtOAc, and MeOH to separate into different polar fractions. The chemical structures of the isolated compounds were elucidated through the interpretation of their 1D and 2D NMR and HRESIMS data**. Results**: In this paper, we reported the isolation of six known compounds, including two depsides lecanorin **(1)** and isolecanoric acid **(2)**, two depsidones norstictic acid **(3)** and methylstictic acid **(4)**, an ergosterol, 22*E*,24*R*-5α,6α-epoxyergosta-8,22-diene-3β,7α-diol **(5)**, and lupeol **(6)**. **Conclusion**: This is the first time that these compounds have been reported from *Usnea lapponica* Vain.

Key words: Usnea lapponica, depside, depsidone, ergosterol, lupeol

INTRODUCTION

Lichens are complex symbiotic associations between fungi and algae that are important constituents of many ecosystems. The production of various unique extracellular secondary metabolites known as lichen substances is the result of this symbiosis. These compounds possess a wide range of biological activities, including antimicrobial, antifungal, antiviral, and anticancer activities.[1] Historically, some lichen species have been used for the food, dye, cosmetics, and folk medicine industries. Among them, the genus *Usnea* (Parmeliaceae; lichenized Ascomycetes) is the largest genus of fruticose lichen, with more than 360 species, and is widely distributed from polar zones to tropical areas in the world.[2] The lichen *Usnea lapponica* Vain. belonging to the *Usnea* genus grow hanging from tree branches in the damp forest at Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province. According to Philippe Clerc[3], this lichen was suggested to synonymy with *Usnea perplexans* Stirt. However, Kristiina Mark[4] and his partner proposed synonymization of *Usnea subtiliserilis* and under *Usnea lapponica*. In addition, this lichen has not yet been chemically and biologically studied. The primary goal of the present work was to study the chemical constituents of the lichen *Usnea lapponica* Vain.

MATERIALS AND METHODS

General Experimental Procedures.

The solvents utilized, including *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methanol (MeOH) (purity $\geq$ 99.0%), were purchased from Chemsol company (Vietnam).

NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) and Bruker 400 Avance spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). $CDCl_3$ and DMSO-d_6 were used both as a solvent and as an internal reference at δ_H 7.26 and 2.50 and δ_C 77.2 and 39.5, respectively. The HRESIMS data were obtained using a Bruker microOTOF Q-II. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 or silica gel 60 RP–18 F254S (Merck Millipore, Billerica, Massachusetts, USA), visualized by vanillin–H_2SO_4 solution and heating. Gravity column chromatography separations were performed with silica gel 60 (0.040–0.063 mm) (HiMedia, Mumbai, India) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

Lichen Material

Thalli of the studied lichen (Figure 1) were separated from tree branches and bark of various old trees in

Sai Gon University, HCMC.

Correspondence

Nguyen Thi My Dung, Sai Gon University, HCMC.

Email: nguyenthimydung@sgu.edu.vn

History

- Received: 2022-02-10
- Accepted: 2022-04-04
- Published: 2022-5-11

DOI : 10.32508/stdj.v25i2.3896

Check for updates

Cite this article : Tri N H, Dung N T M. **Chemical constituents of the lichen *Usnea lapponica* Vain., Parmeliaceae**. *Sci. Tech. Dev. J., 2022;* 25(2):2373-2380.

a damp forest at Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province, Vietnam, in August 2018 and authenticated by Dr Vo Thi Phi Giao, Faculty of Biology, University of Science, Vietnam National University - Ho Chi Minh City. A voucher specimen, coded US-B040-DUNG, was deposited at the laboratory of the Faculty of Environmental Science, Saigon University.

Figure 1: Lichen *Usnea lapponica* Vain.

Extraction and isolation

The cleaned, air-dried, and ground material (1.9 kg) was extracted by maceration with MeOH at ambient temperature, and the filtrated solution was evaporated under reduced pressure to afford the crude MeOH extract (510 g).

This crude extract was separated by flash column chromatography (CC), first eluted with *n*-hexane to afford the *n*-hexane extract (58.9 g), then with a gradient of EtOAc and MeOH (stepwise, 10:0, 9:1, 8:2, and 5:5) to afford four fractions, EA1 (110.4 g), EA2 (98.2 g), EA3 (65.3 g), and EA4 (89 g), and finally with MeOH to afford the MeOH residue (65.2 g).

Fraction EA1 was subjected to silica gel CC and eluted with *n*-hexane-$CHCl_3$ (stepwise, 9:1, 8:2, 7:3, and 5:5) to give 4 subfractions from EA1-1 to EA1-4. Subfraction EA1-1 was subjected to silica gel CC, eluted with *n*-hexane-$CHCl_3$ (stepwise, 9:1, 8:2, 7:3, 5:5, and 0:10) and further rechromatographed twice with the same procedure to give six compounds, including **(1)** (32 mg), **(2)** (44 mg), **(3)** (10 mg), **(4)** (26 mg), **(5)** (29 mg), and **(6)** (7 mg).

RESULTS

The chemical investigation on the thalli of lichen *Usnea lapponica* Vain. collected in Lam Dong Province obtained six compounds using efficient separation techniques. These compounds were isolated from the EA1 fraction of EtOAc extract, and their structures are shown in Figure 2. The spectral properties of these compounds, including ^1H and ^{13}C NMR data, were identified and compared to those previously described in the literature.

Lecanorin (1): white amorphous powder. HRESIMS (positive mode) *m/z* 297.0736 $[M+Na]^+$ (calcd. for $C_{15}H_{14}O_5Na$, 297.0739). Rf: 0.4 (silica gel plate; $CHCl_3$-MeOH, 95:5). The ^1H and ^{13}C NMR (recorded at 500 MHz for ^1H and 125 MHz for ^{13}C in DMSO– d_6), see Table 1.

Isolecanoric acid (2): white amorphous powder. HRESIMS (positive mode) *m/z* 341.0605 $[M+Na]^+$ (calcd. for $C_{16}H_{14}O_7Na$, 341.0637). Rf: 0.5 (silica gel plate; $CHCl_3$-MeOH, 90:10). The ^1H and ^{13}C NMR (recorded at 500 MHz for ^1H and 125 MHz for ^{13}C in DMSO– d_6), see Table 1.

Norstictic acid (3): white amorphous powder. HRESIMS (positive mode) *m/z* 395.0348 $[M+Na]^+$ (calcd. for $C_{18}H_{12}O_9Na$, 395.0379). Rf: 0.5 (silica gel plate; $CHCl_3$-MeOH, 95:5). The ^1H and ^{13}C NMR (recorded at 400 MHz for ^1H and 100 MHz for ^{13}C in DMSO– d_6), see Table 2.

Methylstictic acid (4): white amorphous powder. HRESIMS (positive mode) *m/z* 423.0699 $[M+Na]^+$ (calcd. for $C_{20}H_{16}O_9Na$, 423.0692). Rf: 0.5 (silica gel plate; $CHCl_3$, 100%). The ^1H and ^{13}C NMR (recorded at 500 MHz for ^1H and 125 MHz for ^{13}C in $CDCl_3$), see Table 2.

22*E*,24*R*-5α,6α-epoxyergosta-8,22-diene-3β,7α-diol (5): white amorphous powder. HRESIMS (positive mode) *m/z* 451.3169 $[M+Na]^+$ (calcd. for $C_{28}H_{44}O_3Na$, 451.3188). Rf: 0.4 (silica gel plate; $CHCl_3$-MeOH, 95:5). The ^1H and ^{13}C NMR (recorded at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$), see Table 3.

Lupeol (6): white amorphous powder. HRESIMS (positive mode) *m/z* 449.3772 $[M+Na]^+$ (calcd. for $C_{30}H_{50}ONa$, 449.3759). Rf: 0.4 (silica gel plate; $CHCl_3$, 100%). The ^1H and ^{13}C NMR (recorded at

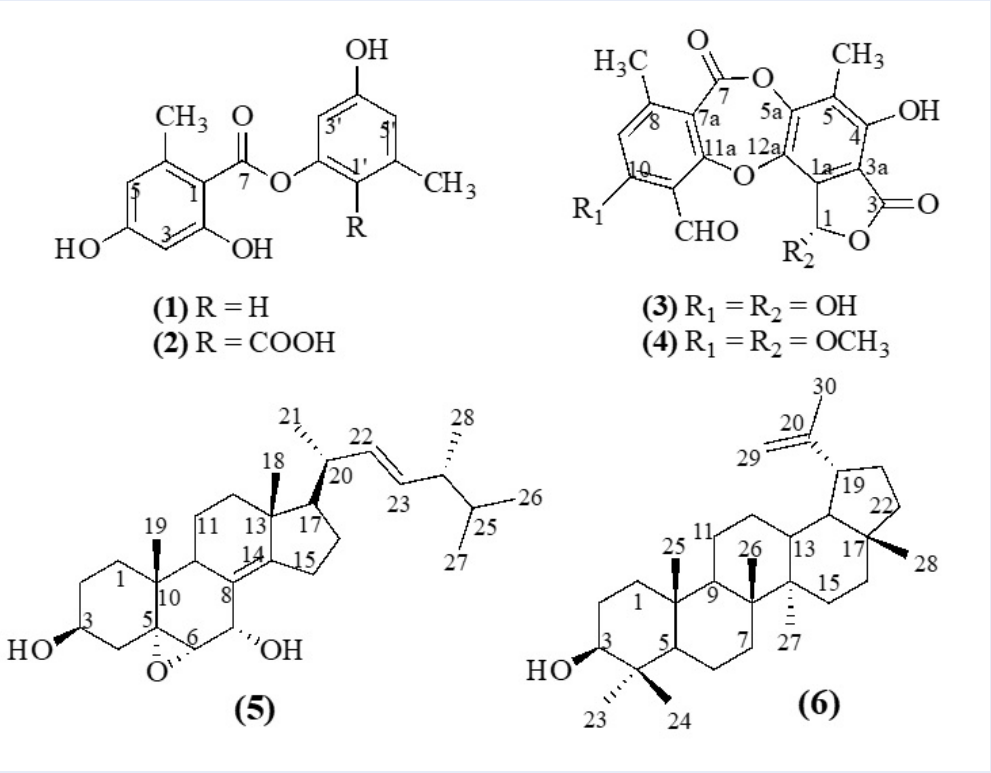

Figure 2: The chemical structures of isolated compounds from *Usnea lapponica* Vain.

500 MHz for ^{1}H and 125 MHz for ^{13}C in DMSO– d_6), see Table 3.

DISCUSSION

Compound **(1)** was obtained as a white amorphous powder. Its molecular formula, $C_{15}H_{14}O_5$, was determined from its HRESIMS at *m/z* 297.0736 $[M+Na]^+$ (calcd. for $C_{15}H_{14}O_5Na$, 297.0739). The ^{1}H NMR data showed the presence of two singlets at δ_H 2.35 (3H) and 2.24 (3H) for two methyl groups, four singlets of five aromatic protons at δ_H 6.22 for protons H-3 and H-5 and at δ_H 6.51, 6.46, and 6.41 for protons H-1', H-5' and H-3', respectively. The ^{13}C NMR spectrum showed signals of 15 carbons corresponding to two methyl groups at δ_C 21.0 and 21.5 (6'-CH_3 and 6-CH_3), five aromatic methines at δ_C 100.5; 106.2; 109.9; 112.9, and 113.5 (C-3; C-3'; C-5; C-1', and C-5', respectively), seven quaternary carbons including four oxygenated carbons at δ_C 108.3; 139.7; 140.3; 151.1; 158.0; 160.3, and 161.1 (C-1; C-6'; C-6; C-2'; C-4'; C-2, and C-4, respectively), and one carboxyl carbon at δ_C 167.7 (C-7). The key HMBC correlations from 6-CH_3 to C-1; C-5, and C-6 together with from 6'-CH_3 to C-1'; C-5', and C-6' disclosed the position of these methyl groups at C-6 and C-6'. The exact location of the aromatic protons was also established based on 2D NMR. These spectroscopic data, along with its HRESIMS data at *m/z* 297.0736 $[M + Na]^{+,}$ were consistent with the published data.[5] Consequently, the structure of **(1)** was established as lecanorin (Figure 2).

Compound **(2)**, giving the molecular formula $C_{16}H_{14}O_7$ by HRESIMS at *m/z* 341.0605 $[M+Na]^+$ (calcd. for $C_{16}H_{14}O_7Na$, 341.0637), a white amorphous powder was obtained. Its ^{1}H NMR spectrum showed two singlets at δ_H 2.36 (3H) and 2.54 (3H) for two methyl groups, a pair of doublets for two *m*-substituted aromatic protons at δ_H 6.32 (1H, *d*, J = 2.0 Hz, H-5′) and 6.24 (1H, *d*, J = 2.0 Hz, H-3′) and a singlet at δ_H 6.22 (2H, H-3 and H5). The ^{13}C NMR spectrum was similar to that of **(1)** except for the presence of one more carboxyl carbon at δ_C 171.8 (C-7'). The position of the aromatic protons and the substituted functional groups were established based on HMBC correlations. These spectroscopic data were compatible with those published in the literature.[6] Therefore, the structure of **(2)** was suggested to be isolecanoric acid. (Figure 2).

Compound 3 was isolated as a white amorphous powder and identified as norstictic acid, as shown in

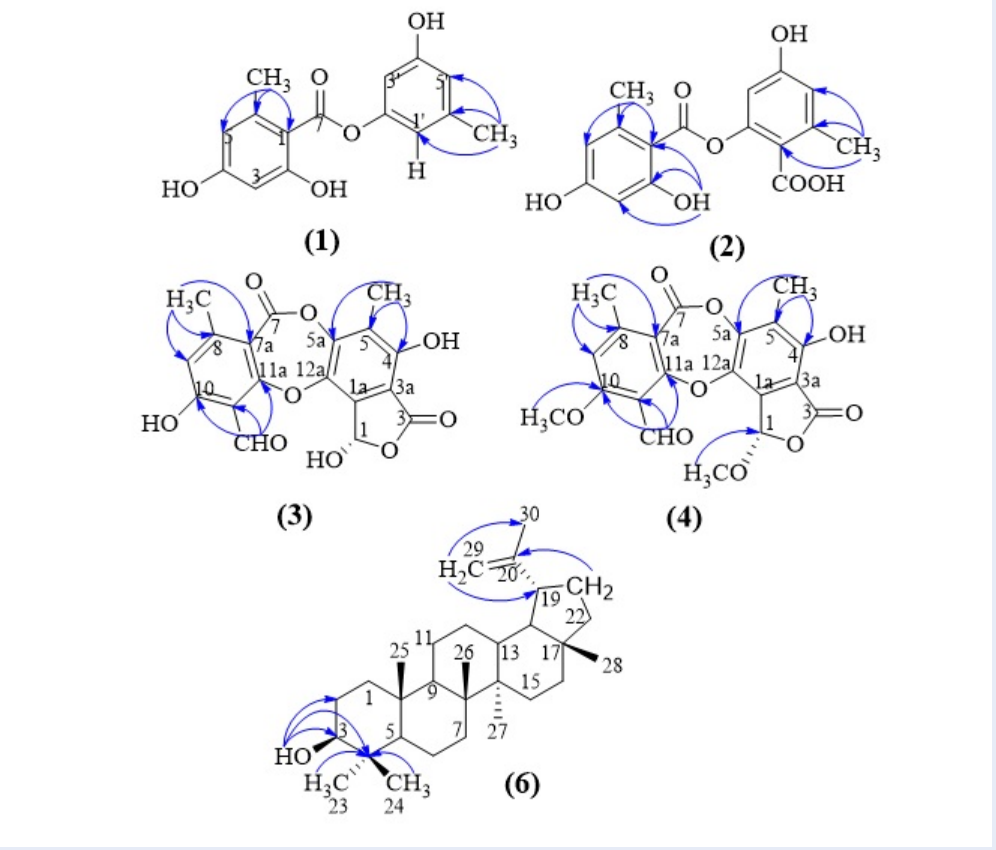

Figure 3: Key HMBC correlations observed for **(1)** – **(4)** and **(6)**.

Figure 2, by comparison of its physical and spectral data with the literature[7]. The HRESIMS showed an $[M+Na]^+$ peak at m/z 395.0348, which revealed the molecular formula of $C_{18}H_{12}O_9$. The ^1H and ^{13}C NMR spectra of **(3)** (Table 2) showed signals of two methyl groups [δ_H 2.21 (3H, *s*); 2.45 (3H, *s*); δ_C 9.6; 21.4, respectively], an aldehyde group [δ_H 10.46 (1H, *s*); δ_C 192.8], and a lactone group (δ_C 163.6). Additionally, the presence of two methine groups [δ_H 6.79 (1H, *s*); δ_C 95.0, and δ_H 6.85 (1H, *s*); δ_C 117.4] and an ester group supported the ether bridge between C-11a and C-12a, leading to a depsidone skeleton. The HMBC correlations (Figure 3) between these protons and carbons confirmed the structure of **(3)**.

Compound 4 was isolated as a white amorphous powder. The ^1H and ^{13}C NMR spectra of **(4)** (Table 2) showed signals assignable to two methoxy groups [δ_H 3.97 (3H, *s*); 3.70 (3H, *s*); δ_C 58.0; 56.8, respectively], a lactone group (δ_C 169.7), two methyl groups [δ_H 2.30 (3H, *s*); 2.56 (3H, *s*); δ_C 9.3; 22.4, respectively], an aldehyde group [δ_H 10.50 (1H, *s*); δ_C 187.0], an aromatic proton [δ_H 6.74 (1H, *s*); δ_C 112.1], and a methine proton [δ_H 6.40 (1H, *s*); δ_C 103.1]. Furthermore, the HRESIMS of **(4)** showed a pseudomolecular ion peak at m/z 423.0699 $[M+Na]^+$, which revealed the molecular formula of $C_{20}H_{16}O_9$. Its ^1H and ^{13}C NMR resonances were similar to those of **(3)** except for two additional methoxy groups at C-1 [δ_C 3.70 (3H, *s*); δ_C 56.8] and C-10 [δ_H 3.97 (3H, *s*); δ_C 58.0] in **(4)**. The HMBC correlations from 1-OCH_3 (δ_H 3.70) to C-1 and from H-10 (δ_H 3.97) to C-10 confirmed the location of two additional methoxy groups. By comparing these data with those in the literature,[8] **(4)** it was suggested to be methylstictic acid.

Compound **(5)** was isolated as a white amorphous powder. The ^1H NMR spectrum contained four doublet signals for secondary methyl groups at δ_H 1.01 (3H, *d*, J = 6.8 Hz, H-21), 0.90 (3H, *d*, J = 6.8 Hz, H-28), 0.82 (3H, *d*, J = 6.4 Hz, H-26), 0.81 (3H, *d*, J = 6.4 Hz, H-27) and two singlets of two tertiary methyls at δ_H 1.24 (3H, *s*) and 0.57 (3H, *s*). The signal at δ_H 3.93 (1H, *m*) was assigned to the proton on carbon sp^3 bearing a hydroxyl group. Additionally, the signal of

Table 1: ^{1}H and ^{13}C NMR data of (1) and (2) [(a)]

Pos.	(1)[(b)]		(2)[(b)]	
	δ_H, (J)	δ_C	δ_H, (J)	δ_C
1	-	108.3	-	108.1
2	-	160.3	-	160.6
3	6.22 s	100.5	6.22 s	100.5
4	-	161.1	-	161.2
5	6.22 s	109.9	6.22 s	110.0
6	-	140.3	-	140.4
7	-	167.7	-	167.6
1'	6.51 s	112.9	-	116.3
2'	-	151.1	-	151.3
3'	6.41 s	106.2	6.24 d (2.0)	107.2
4'	-	158.0	-	165.7
5'	6.46 s	113.5	6.32 d (2.0)	112.5
6'	-	139.7	-	142.2
7'	-	-	-	171.8
6-CH_3	2.35 s	21.5	2.36 s	21.6
6'-CH_3	2.24 s	21.0	2.54 s	23.0
2-OH	10.38 s	-	10.46 s	-
4-OH	10.00 s	-	9.59 s	-
4'-OH	10.01 s	-	-	-

[(a)] *Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.* [(b)] *Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C in DMSO–d_6.*

two olefinic protons at δ_H 5.18 (2H, *dd*, *J* = 15.6; 7.6 Hz, H-22 and H-23) of a cholestane skeleton were also observed. The ^{13}C NMR spectrum showed the presence of 28 carbon signals, including four olefinic carbon signals at δ_C 135.7 (C-22), 134.6 (C-9), 132.2 (C-23), and 127.1 (C-8) and four oxygenated carbons at δ_C 68.7 (C-3), 67.2 (C-7), 65.8 (C-5), and 62.7 (C-6). Furthermore, the molecular formula of **(5)** was determined to be $C_{28}H_{44}O_3$ through the sodium adduct ion at *m/z* 451.3169 $[M+Na]^+$ in the HRESIMS spectrum. On the basis of the above results, compound **(5)** was determined to be 22*E*,24*R*-5α,6α-epoxyergosta-8,22-diene-3β,7α-diol.[9]

Compound **(6)** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed the presence of singlet signals for seven tertiary methyl groups at δ_H 0.65, 0.76, 0.77, 0.87, 0.91, 0.99, and 1.64. A doublet of triplets for one proton at δ_H 2.37 assigned to H-19β is characteristic of lupeol. The H-3 proton displayed a multiplet at δ_H 2.97, while a pair of doublet signals of two protons at δ_H 4.68 and 4.54 were assigned to olefinic protons at H-29. The structural assignment of **(6)** lupeol was further substantiated by the characteristic signals of lupeol at δ_C 76.8 (C-3), 109.6 (C-29) and 150.2 (C-20) in its ^{13}C NMR experiments. The molecular formula of **(6)** was determined to be $C_{30}H_{50}O$ through the sodium adduct ion at *m/z* 449.3772 $[M+Na]^+$ in the HRESIMS spectrum. The structure of **(6)** was confirmed through HSQC and HMBC experiments (Figure 3). On the basis of the above results, compound **(6)** was identified as lupeol.[10]

CONCLUSION

In the investigation of the chemical constituents of lichen *Usnea lapponica* Vain. collected in Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province, six known compounds were isolated, including lecanorin **(1)**, isolecanoric acid **(2)**, norstictic acid **(3)**, methylstictic acid **(4)**, 22*E*,24*R*-5α,6α-epoxyergosta-8,22-diene-3β,7α-diol **(5)** and lupeol

Table 2: ^{1}H and ^{13}C NMR data of (3) and (4) [(a)]

Position	(3)[(b)]		(4)[(c)]	
	δ_H, (J)	δ_C	δ_H, (J)	δ_C
1	6.79, s	95.0	6.40, s	103.1
1a		135.8		138.8
3		163.6		169.7
3a		109.2		107.9
4		152.1		152.6
5		120.9		121.3
5a		147.9		149.6
7		160.3		160.9
7a		111.8		114.5
8		152.3		151.5
9	6.85, s	117.4	6.74, s	112.1
10		164.0		163.7
11		110.6		115.1
11a		166.2		163.0
12a		137.4		132.2
11-CHO	10.46, s	192.8	10.50, s	187.0
1-OCH_3		-	3.70, s	56.8
1-OH	8.26, s	-	-	-
10-OCH_3	-	-	3.97, s	58.0
10-OH	10.17, s	-	-	-
5-CH_3	2.21, s	9.6	2.30, s	9.3
8-CH_3	2.45, s	21.4	2.56, s	22.4
4-OH	12.04, s	-	7.90, s	-

[(a)] Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.[(b)] Recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C in DMSO–d_6.[(c)] Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.

(6). This is the first time these compounds have been reported in the lichen *Usnea lapponica* Vain.

ABBREVIATIONS

^{1} H- NMR: Proton nuclear magnetic resonance

^{13} C NMR: Carbon-13 nuclear magnetic resonance

$CDCl_3$: Deuterochloroform

CC: Column chromatography

d: doublet

dd: doublet of double

ddd: doublet of double of double

m: multiplet

DMSO: dimethyl sulfoxide (CD_3SOCD_3)

EtOH: Ethanol (C_2H_5OH)

EtOAc: Ethyl acetate ($CH_3COOCH_2CH_3$)

HMBC: Heteronuclear multiple bond correlation

HRESIMS: High-resolution electrospray ionization mass spectrometry

MeOH: Methanol (CH_3OH)

brs: Broad singlet

s: singlet

TLC: Thin layer chromatography

COMPETING INTERESTS

The authors declare no competing financial interest.

Table 3: ^{1}H and ^{13}C NMR data of (5) and (6) [a]

Pos.	(5)[b]		(6)[c]	
	δ_H	δ_C	δ_H	δ_C
1		31.0		38.3
2		29.8		27.1
3	3.93 m	68.7	2.97 m	76.8
4		39.3		38.5
5		65.8		54.9
6	3.30 d (2.4)	62.7		17.8
7	4.21 brs	67.2		33.8
8		127.1		40.4
9		134.6		49.8
10		38.1		36.7
11		23.5		20.4
12		35.8		24.7
13		42.3		37.6
14		49.7		42.4
15		24.0		27.0
16		29.1		35.1
17		53.8		42.5
18	0.57 s	11.4		47.8
19	1.24 s	22.9	2.37 ddd (17.0; 11.0; 6.0)	47.4
20		40.5		150.2
21	1.01 d (6.8)	21.1	1.87 m; 1.24 m	29.2
22	5.18 dd (15.6; 7.6)	135.7		39.6
23	5.18 dd (15.6; 7.6)	132.2	0.87 s	28.1
24		43.0	0.65 s	15.7
25		33.2	0.76 s	15.9
26	0.82 d (6.4)	19.8	0.99 s	15.8
27	0.81 d (6.4)	20.1	0.91 s	14.3
28	0.90 d (6.8)	17.8	0.77 s	17.9
29	-	-	4.68 d (2.5) 4.54 d (2.5)	109.6
30	-	-	1.64 s	19.0
3-OH	-	-	4.25 d (5.5)	-

[a] *Chemical shifts (δ) are expressed in ppm, and J values are presented in Hz.* [b] *Recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$.* [c] *Recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C in DMSO–d_6.*

AUTHORS' CONTRIBUTIONS

Nguyen Huu Tri and Nguyen Thi My Dung contributed to conducting the experiments, obtaining the data and writing the manuscript. Nguyen Thi My Dung (corresponding author) has contributed a significant explanation of the data and revised the manuscript.

ACKNOWLEDGMENTS

Sai Gon University funded this research under grant number CS2020-53.

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