THE DEPSIDONES FROM USNEA ACICULIFERA

MỘT SỐ DEPSIDONE TỪ LOÀI ĐỊA Y USNEA ACICULIFERA

Vo Thi Nga[1], Tuong Lam Truong[2], Nguyen Kim Phi Phung[2]
[1] Ho Chi Minh City Univerisity of Technology and Education
[2] University of Science, National University – Ho Chi Minh City

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ABSTRACT

The lichen is an association between a fungus and a phytosynthetic symbiont, generally green algae or cyanobacteria. Lichens have a number of practical applications, especially as sources of medicinal substances. Usnea aciculifera Vain, a lichen growing widely and abundantly on the bark of Pinus dalatensis at Lam Dong Province, Vietnam, has not yet been chemically and pharmaceutically studied in Vietnam. Our chemical study on Usnea aciculifera lead to the isolation of three depsidones, including norstictic acid (1), stictic acid (2) and 8-O-methylstictic acid (3). Their chemical structures were elucidated by 1D and 2D NMR. Lichen substances such as depsides or depsidones are of interest as natural antibiotics. The presence of norstictic acid (1) and stictic acid (2) in high yield of around 0.13% and 0.15%, respectively; and possessing the ability in inhibiting cell growth of human colon adenocarcinoma HT-29 cells showed that this species seems to become a potential medicinal resource.

Keywords: *lichen; Usnea aciculifera; depsidone; stictic acid; norstictic acid.*

TÓM TẮT

Địa y là một dạng kết hợp giữa nấm và một loại sinh vật có thể quang hợp, như tảo xanh. Địa y có rất nhiều ứng dụng, đặc biệt trong y dược. Usnea aciculifera Vain, là một loài địa y rất phổ biến, sống bám trên thân cây thông Pinus dalatensis ở tỉnh Lâm Đồng, Việt Nam. Hiện nay vẫn ít nhóm quan tâm nghiên cứu trên loài địa y này. Từ nghiên cứu trên loài địa y Usnea aciculifera chúng tôi đã cô lập được ba depsidone, gồm norstictic acid (1), stictic acid (2) và 8-O-methylstictic acid (3). Cấu trúc hóa học của chúng được biện giải bằng phổ cộng hưởng từ hạt nhân một chiều và hai chiều. Các hợp chất địa y như depside hoặc depsidone từng được biết đến như những chất kháng sinh tự nhiên. Sự hiện diện của norstictic acid và stictic acid với hàm lượng khá cao, lần lượt là 0.13% và 0.15%; và khả năng ức chế tế bào ung thư tuyến ruột kết ở người HT-29 của stictic acid [6] có thể mở ra hướng ứng dụng loài địa y này như một nguồn dược liệu tiềm năng.

Từ khóa: *lichen; Usnea aciculifera; depsidone; stictic acid; norstictic acid.*

1. INTRODUCTION

A lichen is an association between a fungus and a phytosynthetic symbiont, generally green algae or cyanobacteria. [1] Lichens have a number of practical applications, especially as sources of medicinal substances. Lichen metabolites are some of the potential natural product resources that exhibit manifold bioactivities including antibiotic, antiviral, analgesic, antipyretic, antiproliferative and cytotoxic

effects. Lichen substances such as depsides or depsidones are of interest as natural antibiotics. [2] Although over 1,000 lichen metabolites with various biological activities have been reported, *Usnea aciculifera* has not yet been chemically and biologically studied in Vietnam. In our previous work on *Usnea aciculifera*, some depsides icluding barbatinic acid, diffractaic acid and aciculiferin A, were found with a strong cytotoxic activity against three cancer cell lines such as MCF-7, NCI-H460 and HeLa cell lines.[3]

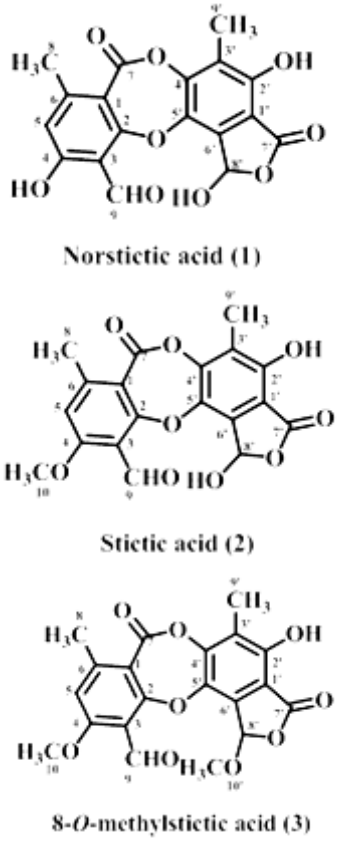

***Figure 1**. Chemical structures of the isolated depsidones.*

In this research, we will discuss the isolation and elucidation of three depsidones from *Usnea aciculifera* (Figure 1).

2. MATERIALS AND METHODS

2.1 Materials

Usnea aciculifera was collected on the bark of the *Pinus dalatensis* at Lam Dong Province, Vietnam in May 2011. The material was identified by Dr. HarrieSipman, Freie University of *Berlin*, Germany. A voucher specimen (No US–B029) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University–Ho Chi Minh City.

2.2 Apparatus

NMR spectra were measured on a Bruker Avance III spectrometer, at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C-NMR, using residual solvent signal as internal reference: DMSO-d_6 δ_H 2.50, δ_C 39.5.

2.3 Methods

The clean, air-dried and ground material (1.5 kg) was macerated by methanol at room temperature and concentrated under reduced pressure to afford the crude methanolic extract (400 g). This extract was subjected to silica gel column chromatography using gradient elution of n-hexane–ethyl acetate then ethyl acetate–methanol (99:1–1:1) to give seven fractions from A1 to A7. Fraction A2 (45.3 g) was applied to column chromatography and eluted in stepwise with solvent systems of n-hexane – ethyl acetate (85:15 to 60:40) to give 5 fractions (A2.1–A2.5).

Fraction A2.3 (3.5 g) was applied to column chromatography and eluted in stepwise with solvent systems of n-hexane – ethyl acetate (70:30 to 20:80) to give three compounds including **1** (2,000.0 mg), **2** (2,200.0 mg) and **3** (193.0 mg).

Norstictic acid (**1**): White powder, mp. 286 – 287 ^{o}C. ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6) data as seen in Table 1.

Stictic acid (**2**): White powder, mp. 270 – 272 ^{o}C. ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6) data as seen in Table 1.

8-*O*-methylstictic acid (**3**): White powder, mp. 248 – 249 ^{o}C. ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6) data as seen in Table 1.

3. RESULTS AND DISCUSSIONS

3.1 Structural elucidation of compound 1

The ^{1}H NMR spectrum of **1** exhibited eight proton signals including two methyl groups attached to aromatic rings at δ 2.20 (H-9') and 2.44 (H-8), one hemiacetal proton at δ 6.78 (H-8'), one aromatic proton at δ 6.84 (H-5), two hydroxyl groups at 10.16 (4-OH) and 8.26 (8'-OH), one formyl group at δ 10.45 (C-9) and one chelated hydroxyl group to a neighboring carboxyl group at δ 12.04 (2'-OH). The presence of only one aromatic proton at δ 6.84 (H-5) was supportive of the ether bridge between C-2 and C-5' leading to a depsidone skeleton.[4]

The ^{13}C-NMR spectrum of compound **1** showed 18 carbons including 12 aromatic carbons, two carboxyl group signals at δ 163.6, 160.3 and one hemiacetal carbon at δ 95.0. It showed two methyl signals at δ 9.6 (3'-CH_3), 21.4 (6-CH_3) and one formyl group at δ 192.8 (C-9). The positions of these groups were confirmed by HMBC correlations (Figure 2).

In the HMBC experiment, the aromatic proton at δ 6.84 (H-5) showed HMBC correlations with C-1 (δ 111.8), C-4 (δ 164.0), 6-CH_3 (δ 21.4) and C-9 (δ 192.8). The proton of formyl group at δ 10.45 showed HMBC correlations with C-3 (δ 110.6), C-4 (δ 164.0) and C-5 (δ 117.4) established the position of the CHO group at C-3. The two methyl groups could be linked at C-6 and C-3' by correlations from H-8 to C-1, C-5, C-6 and H-9' to C-2', C-3'and C-4'

The spectral data were suitable to the published ones [5] therefore, the chemical structure of compound **1** was ditermined as norstictic acid.

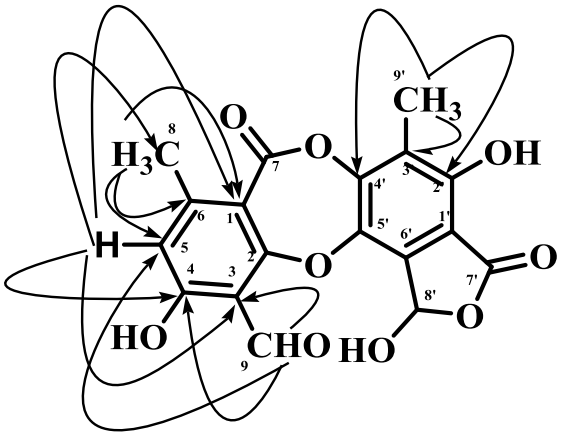

Figure 2. *Key HMBC correlations in norstictic acid (**1**).*

3.2 Structural elucidation of compound 2

Compound **2** was obtained as white powder. The ^{1}H NMR spectrum of **2** was similar to the one of compound **1**, except the presence of a singlet signal at δ 3.94 (3H, s, 4-OCH_3) for an additional methoxy group. This was also supported by ^{13}C-NMR according to the presence of methoxy group at δ 56.7 ppm. The position of this methoxy was assigned at C-4 due to the HMBC correlation between this proton and C-4 (δ 163.0 ppm).

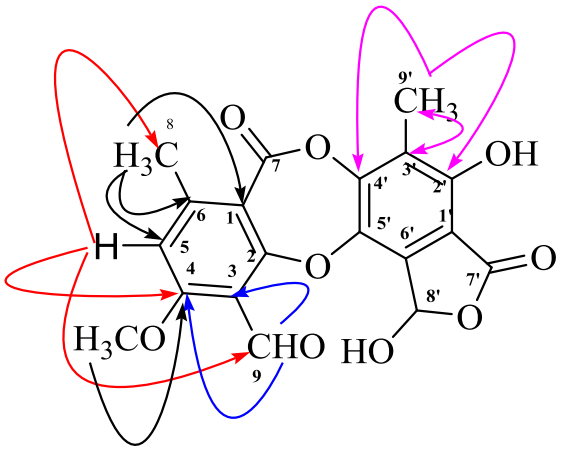

Figure 3. *Key HMBC correlations in stictic acid (**2**)*

The structure of compound **2** was demonstrated by HMBC correlation (Figure

3). The above NMR data analysis and comparison with those in literature [6] suggested that compound **2** was static acid.

3.3 Structural elucidation of compound 3

Compound **3** was obtained as white powder. The ^{1}H-NMR in DMSO-*d6* of **3** gave signals for two methyl groups (δ 2.19, 2.50), one each for methoxy (δ 3.92) and aldehyde (δ 10.40) attached to aromatic rings at almost the same chemical shifts given by **2**, whereas an additional methoxy signal at δ 3.44 was given by **3** in place of a signal for a lactol at δ 8.19 observed in **2**. The ^{13}C-NMR also showed the presence of an additional methoxy group at δ 56.8 ppm. The position of this methoxy was assigned at C-8' due to the HMBC correlation between this proton and C-8' (δ 100.0 ppm).

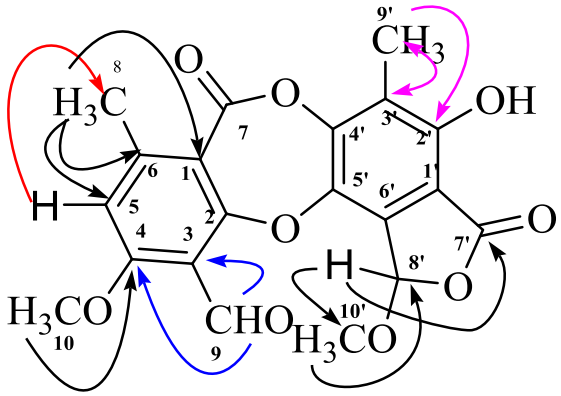

Figure 4. *Key HMBC correlations in 8-O-methylstictic acid (**3**)*

The structure of compound **3** was confirmed by HMBC correlations (Figure 4). The above NMR data analysis and comparison with those in literature [7] suggested that compound **3** was 8-*O*-methylstictic acid.

4. CONCLUSSION

Usnea aciculifera Vain, lichen growing widely and abundantly on the bark of *Pinus dalatensis* at Lam Dong Province, Viet Nam, has not yet been chemically and pharmaceutically studied. Our chemical study on *Usnea aciculifera* lead to the isolation and the structure elucidation of three depsidones, including norstictic acid (**1**), stictic acid (**2**) and 8-*O*-methylstictic acid (**3**) by 1D and 2D NMR analysis. Although norstictic acid has been isolated from *Usnea densirostra* and *Usnea angulata*, it was isolated for the first time in the species *Usnea aciculifera*. Stictic acid has been demonstrated the ability in inhibiting cell growth of human colon adenocarcinoma HT-29 cells. There was a remarkable result that norstictic acid (**1**) and stictic acid (**2**) presented in high yield of around 0.13% and 0.15%, respectively, comparing to the dried material of *Usnea aciculifera*. This species of lichen seems to become a potential medicinal resource.

ACKNOWLEDGMENTS

Thanks go to Ho Chi Minh City University of Technology and Education for its support in this research work.

Table 1: *NMR spectroscopic data of compounds **1**, **2** and **3***

Position	Compound **1** (DMSO-d_6)		Compound **2** (DMSO-d_6)		Compound **3** (DMSO-d_6)	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		111.8		113.0		113.3
2		166.2		162.3		162.5
3		110.6		114.3		114.4
4		164.0		163.0		162.9
5	6.84 (1H, *s*)	117.4	7.11 (1H, *s*)	112.7	7.10 (1H, *s*)	113.0
6		152.3		150.8		152.4
7		160.3		160.6		160.7
8	2.44 (3H, *s*)	21.4	2.52 (3H, *s*)	21.4	2.50 (3H, *s*)	21.6
9	10.45 (1H, *s*)	192.8	10.49 (1H, *s*)	186.6	10.40 (1H, *s*)	186.8
10	-	-	3.94 (3H, *s*)	56.7	3.92 (3H, *s*)	56.8
1’		109.2		109.1		109.0
2’		152.1		151.8		151.1
3’		120.9		120.7		121.8
4’		147.9		147.9		148.4
5’		137.4		135.8		133.2
6’		135.8		137.4		137.7
7’		163.6		166.3		166.0
8’	6.78 (1H, *brs)*	95.0	6.63 (1H, *s*)	95.0	6.47 (1H, *s*)	100.0
9’	2.20 (3H, *s*)	9.6	2.22 (3H, *s*)	9.5	2.19 (3H, *s*)	9.8
10’	-	-	-	-	3.44 (3H, *s*)	57.0
2’-OH	12.04 (1H, *brs*)		10.14 (1H, *s*)		-	-
4-OH	10.16 (1H, *brs*)		-		-	-
8’-OH	8.26 (1H, *s*)		8.19 (1H, *s*)		-	-

REFERENCES

[1] E. J. W. Barrington, Arthur J. Willis, *The biology of lichens: Contemporary biology*, 2nd edition, Edward Arnold, *London*, 1974.

[2] Podterob AP, *Chemical composition of lichens and their medical applications*, Pharmaceutical Chemistry Journal, 42**,** pp. 582–588, 2008.

[3] Tuong Lam Truong, Vo Thi Nga, Huynh Thi Minh Suong, Nguyen Kim Phi Phung, *The bioactive depsides from Usnea aciculifera*, Vietnam Journal of Chemistry, 53(2e1), pp. 94-98, 2015.

[4] P. Papadopoulou, O. Tzakou, C. Vagias, P. Kefalas, and V. Roussis, *β-Orcinol Metabolites from the Lichen Hypotrachyna revoluta*, Molecules, 12, pp. 997-1005, 2007.

[5] M. E. Mendiondo, J. D. Coussio, Usnic, *norstictic and salazinic acids from Usnea densirostra and Usnea angulata*, Phytochemistry, 11, pp. 424, 1972.

[6] Boris Pejin, Camine Iodice, Gordana Bogdanovic, Vesna Kojic, Vele Tesevic, *Stictic acid inhibits cell growth of human colon adenocarcinoma HT-29 cells*, Arabian Journal of Chemistry, pp. 1-3, 2013.

[7] Siegfried Huneck, Raffaele Tabacchi, *Ψ-esters of depsidones with a lactole ring*, Phytochemistry, 26 (4), pp. 1131-1138, 1987.

Corresponding author:
PhD. Vo Thi Nga
Faculty of Chemical and Food Technology, HCMC University of Technology and Education
E-mail: ngavt@hcmute.edu.vn