SOME TRITERPENOIDS AND STEROIDS FROM THE LICHEN LOBARIA ORIENTALIS, LOBARIACEAE.

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ABSTRACT

Chemical investigation of the ethyl acetate extract of the lichen *Lobaria orientalis*, Lobariaceae, collected in Vietnam, afforded six known compounds, including four triterpenoids, zeorin (1), 16*β*-acetoxyhopane-6*α*,22-diol (2), retigeric acid A (3), retigeric acid B (4), together with two steroids, ergosterol-5α,8α-peroxide (5) and cerevisterol (6). Their chemical structures were elucidated by spectroscopic data analysis and comparison with those reported in the literature. The cytotoxic activities of some compounds against liver hepatocellular carcinoma (HepG2), human lung cancer (NCI-H460), human epithelial carcinoma (HeLa) and human breast cancer (MCF-7) cell lines were evaluated. Compound 2 exhibited good cytotoxicity against MCF-7 cancer cell line with IC_{50} value of 21.10 µg/mL. This is the first time compounds (1), (2), (6) are reported in the *Lobaria* genus.

Keywords: Lobaria orientalis, lichen, triterpenoid, steroid, HepG2, NCI-H460, HeLa, MCF-7.

1. INTRODUCTION

Lichens, symbiotic products of mycobiont (fungal partner) and photobiont (algal partner), are known to produce a various range of secondary metabolites which possessed a wide range of biological activities including antibiotic, antifungal, antiviral, antitumor, anticancer, etc.[1 - 4] The *Lobaria* genus was known to contain compounds such as depsidones (stictic and norstictic acids), didepsides (tenuiorin), tridepside (gyrophoric acid), and triterpenes such as retigeric acids A and B.[2, 6, 8] *Lobaria orientalis* has not yet been chemically studied. In this paper, we reported the isolation and structural elucidation of four known triterpenoids, zeorin **(1)**, 16*β*-acetoxyhopane-6*α*,22-diol **(2)**, retigeric acid A **(3)**, retigeric acid B **(4)**, together with two known steroids, ergosterol-5α,8α-peroxide (**5**), cerevisterol (**6**) which were isolated from the lichen

Lobaria orientalis. The cytotoxic activities against liver hepatocellular carcinoma (HepG2), human lung cancer (NCI-H460), human epithelial carcinoma (HeLa) and human breast cancer (MCF-7) cell lines were evaluated on five compounds **1**–**3** and **5**–**6**.

2. MATERIALS AND METHODS

2.1. General experimental procedures

The NMR spectra were measured on a Bruker Avance III spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C), 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, 2.50 and δ_C 77.2, 39.5. The HRESIMS were obtained using a Bruker microOTOF Q-II. Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm, Silicycle).

2.2. Lichen material

Thalli of the studied lichen were separated from bark of various old trees in Bidoup Nui Ba National Park, Dam Rong district, Lam Dong province, Vietnam in July – August 2012. The species was determined as *Lobaria orientalis* (Asahina) Yoshim by Dr. Robert Lücking (Department of Botany. The Field Museum. Illinois USA). A voucher specimen (No US–B034) was deposited at the Herbarium of the Department of Organic Chemistry, University of Science, National University – Ho Chi Minh City – Vietnam.

2.3. Extraction and purification of compounds

Before extraction, the lichen was carefully inspected for contaminants. Air-dried parts of *L. orientalis* (1,400.0 g) were ground and extracted with MeOH (4 x 10 L) by the maceration method at ambient temperature, and the filtrated solution was concentrated under reduced pressure to give MeOH residue (170.0 g). This crude extract was separated by Quick Column Chromatography (QCC), firstly eluted with *n*-hexane to afford the *n*-hexane extract (15.5 g), secondly with a gradient of EtOAc and MeOH (stepwise,10:0, 9:1, 8:2 and 5:5) to afford four EtOAc fractions, EA1 (13.5 g), EA2 (8.3 g), EA3 (7.7 g) and EA4 (86.5 g) and finally, with MeOH to afford the MeOH residue M (24.5 g). Fraction EA1 was applied to silica gel column chromatography, eluted with *n*-hexane–$CHCl_3$ (3:7) and then with $CHCl_3$ to give **(1)** (15 mg), **(2)** (5 mg) and (**5**) (17 mg). Fraction EA3 was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH (stepwise, 98:2, 95:5 and 90:10) to give three compounds **(3)** (30 mg), **(4)** (100 mg), and **(6) (**11 mg).

3. RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous powder. The ^{1}H NMR spectrum (Table 1) revealed signals of eight methyl singlets at δ_H 0.70 (3H, s, H-28), 0.81 (3H, s, H-25), 0.91 (3H, s, H-27), 0.94 (3H, s, H-24), 0.97 (3H, s, H-26), 1.03 (3H, s, H-29), 1.07 (3H, s, H-30), and 1.11(3H, s, H-23), one methine proton indicative of a secondary alcoholic function at δ_H 3.74 (1H, *ddd, J* = 7.0, 13.0, 19.0 Hz, H-6), one methine proton at δ_H 2.10 (1H, *dd* , *J*= 9.6, 19.2 Hz, H-21). The comparison of these spectroscopic data of **1** with those of zeorin in the literature [7]. showed good compatibility. Therefore, **1** was hopan-6α,22-diol or zeorin.

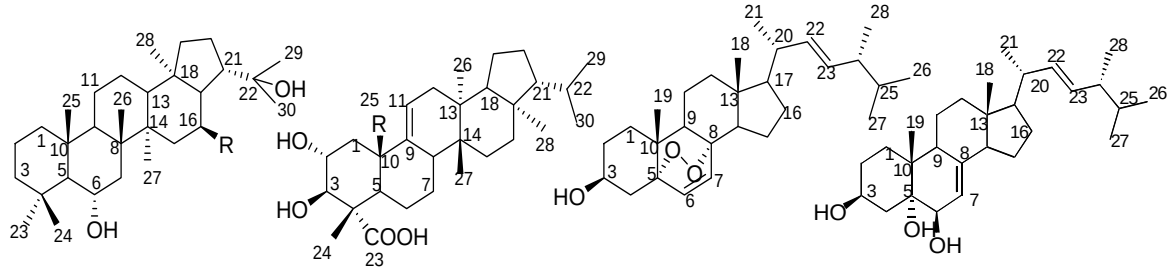
Compound **2** was isolated as a white amorphous powder. The NMR spectral data of compounds **2** and **1** were similar, except for the presence of one additional acetoxy group [(δ_H 5.21, 1H, *dt, J* = 4.5, 9.5 Hz, H-16 and δ_H 2.07, 3H, *s*, 16–$OCOCH_3$) and (δ_C 21.7 and 170.0)]. The coupling constants of H-16 values were *J*ae= 4.5 Hz and *J*aa = 9.5 Hz, indicating the *β*-orientation of the 16-$OCOCH_3$ group. The comparison of spectral data of **2** with those reported the literature [7] suggested that it was 16*β*-acetoxyhopan-6*α*,22-diol.

Compound **3** was isolated as a white amorphous powder. The ^{1}H NMR spectrum of **3** showed seven signals of methyl group including five singlets at δ_H 0.69, 0.73, 0.80, 1.03 and 1.07, two doublets at δ_H 0.81 (3H, *d*, *J* = 7.0 Hz), and 0.87 (3H, *d*, *J* = 6.5 Hz) and one methine proton at δ_H 5.35 (1H, *dd*, *J* = 2.5, 7.0 Hz, H-11). The ^{13}C NMR spectrum of **3** showed 30 signals including two sp^2 carbons at δ_C 116.0 and 150.4, one carboxyl carbon at δ_C 177.7. These spectroscopic data were compatible with the published ones, [5] therefore, **3** was retigeric acid A.

Compound **4** was isolated as a white amorphous powder. The spectral data of **3** and **4** were similar, except that a methyl group in **3** was replaced by a carboxylic group in **4**. A comparison of the ^{13}C NMR data (Table 1) of **4** with those in the literature [5] showed good compatibility, therefore, **4** was retigeric acid B.

Compound **5** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed four doublets of secondary methyls at δ_H 1.00 (3H, *d*, J = 7.0 Hz, H-21), 0.84 (3H, *d*, J = 8.0 Hz, H-26), 0.82 (3H, *d*, J = 7.5 Hz, H-27), 0.91 (3H, *d*, J = 7.0 Hz) and two singlets of two tertiary methyls at δ_H 0.82 and 0.89. The signal at δ_H 3.97 (1H, *m*) was assigned to the proton on carbon bearing a hydroxyl group. Moreover, signals of two olefinic proton at δ_H 5.15 (1H, *dd*, *J* = 8.0, 15.0 Hz, H-22), 5.23 (1H, *dd*, *J* = 7.5, 15.5 Hz, H-23) and of methine protons at δ_H 6.23 (1H, *d*, *J* = 8.5 Hz, H-6), 6.50 (1H, *d*, *J* = 8.5 Hz, H-7) of a cholestane skeleton were also observed. The ^{13}C NMR (Table 2) spectrum showed the presence of 28 carbon signals including four olefinic carbon signals at δ_C 135.6 (C-6), 135.4 (C-22), 132.5 (C-23) and 130.9 (C-7), and three oxygenated carbons at δ_C 82.3 (C-5), 79.6 (C-8) and 66.6 (C-3). Furthermore, the molecular formula of **5** was determined as $C_{28}H_{44}O_3$ through the sodium adduct ion at *m/z* 451.3183 $[M + Na]^+$ in the HRESIMS spectrum. On the basis of above results, **5** was 5*α*,8*α*-epidioxy-24-norcholesta-6,22-dien-3*β*-ol. [8].

Compound **6** was isolated as a white amorphous powder. The spectral NMR data of **6** and **5** were somewhat similar (Table 2). Analysis of the spectral data of **6** and the comparison with the ones in the literature [8] suggested that **6** was cerevisterol.



(1) R = H **(3)** R = CH_3 **(5)** **(6)**

(2) R = $OCOCH_3$ **(4)** R = COOH

Table 1. ^{1}H NMR Spectroscopic Data for **1**–**4**.

Position	**1**[a]		**2**[b]		**3**[c]		**4**[c]	
	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1		40.0	-	40.5	2.11 *m*	47.9	1.00 *m*	46.1
2		18.1	-	18.6	3.43 *s*	67.3	4.50 *m*	67.9
3		43.6	-	43.9	3.43 *s*	79.0	3.46 *m*	79.0
4		33.4		33.8		51.9		51.9
5	0.72 *d* (16.0)	60.0	0.81 *d* (10.5)	61.3	1.90 *m*	40.8	2.05 *dd* (12.5, 8.0)	41.5
6	3.74 *ddd* (7.0, 13.0, 19.0)	66.6	3.94 *ddd* (4.5, 10.5, 15.0)	69.2	-	19.4	-	20.1
7	-	44.7	-	45.6	-	17.2	1.83 *m*	17.7
8	-	42.2	-	43.2	2.02 *m*	38.5	-	38.7
9		49.4		49.4		150.4		142.7
10		38.6		39.4		39.9		47.7
11	-	21.3	-	21.0	5.35 *dd* (2.5, 7.0)	116.0	5.50 *d* (5.5)	120.3
12		23.7		23.6		36.3		36.4
13		49.0		48.9		36.2		36.1
14		41.5		44.2		37.2		37.7
15		34.0		41.3		28.7		28.9
16	-	20.6	5.21*dt* (4.5, 9.5)	73.4	-	42.4	-	35.6
17		53.9		57.1		35.6		42.5
18		43.6		46.9		51.4		51.4
19		41.0		41.7		19.7		19.7
20		26.1		28.1		27.7		27.8
21	2.10 *dd* (9.6, 19.2)	50.4	2.51 *dd* (9.5, 19.5)	52.0	0.97 *m*	59.0	-	59.0
22		71.6		73.3		30.2		30.3
23	1.11 *s*	36.7	1.11 *s*	36.8	-	177.7	12.13 *s*	176.9
24	0.94 *s*	22.0	1.12 *s*	22.2	1.03 *s*	11.9	-	10.6
25	0.81 *s*	16.8	0.87 *s*	17.3	1.07 *s*	26.1	12.13 *s*	175.9
26	0.97 *s*	18.1	1.01 *s*	18.4	0.69 *s*	13.7	0.80 *s*	13.8
27	0.91 *s*	17.0	1.05 *s*	17.1	0.80 *s*	15.2	0.66 *s*	15.4
28	0.70 *s*	15.9	0.85 *s*	18.3	0.73 *s*	15.6	0.73 *s*	15.5
29	1.03 *s*	29.0	1.15 *s*	27.5	0.81 *d* (7.0)	21.9	0.81 *d* (5.0)	22.9
30	1.07 *s*	30.8	1.15 *s*	30.5	0.87 *d* (6.5)	22.8	0.86 *d* (4.5)	22.0
2-OH	-		-		4.40 *s*		4.65 *s*	
3-OH	-		-		-		2.60 *dd* (3.0, 4.0)	
6-OH	3.91 *d* (8.0)		-		-		-	
22-OH	3.85 *s*		-		-		-	
23-OH	-		-		-		12.12 *s*	
25-OH	-		-		-		12.12 *s*	
16-CO-				170.0				
16-OCOCH$_3$	-		2.07 *s*	21.7	-		-	

[a]Recorded at 400 MHz (^{1}H-NMR) and 100 MHz (^{13}C NMR) in DMSO-d_6.

[b]Recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$.

[c]Recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C NMR) in DMSO-d_6Chemical shifts (δ) are expressed in ppm.

Five isolated compounds (**1-2**, **4-6)** were *in vitro* evaluated for their cytotoxic potential against HeLa, HepG2, NCI-H460 and MCF–7 cancer cell lines using the Sulforhodamine B method with camptothecin as the positive control. Among them, **2** exhibited good and **4, 6** showed moderate cytotoxicities against MCF–7 cancer cell line with IC_{50} values of 21.10, 85.45 and 94.57 μg/mL, respectively (Tables 3 and 4).

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data for **5–6**.

Position	**5**[b]		**6**[c]	
	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1	-	34.9	-	32.4
2	-	30.3	-	31.2
3	3.97 *m*	66.6	3.76 *m*	65.9
4	-	37.1	-	40.2
5	-	82.3	-	74.4
6	6.23 *d* (8.5)	135.6	3.37 *s*	72.1
7	6.50 *d* (8.5)	130.9	5.08 *dd* (3.0, 5.5)	119.4
8	-	79.6	-	139.6
9	-	51.3	-	42.2
10	-	37.1	-	36.6
11	-	23.6	-	21.3
12	-	39.5	-	38.9
13	-	44.7	-	43.0
14	-	51.9	-	54.1
15	-	20.8	-	22.6
16	-	28.8	-	27.7
17	-	56.4	-	55.3
18	0.82 *s*	18.3	0.54 *s*	12.0
19	0.89 *s*	13.0	0.91 *s*	17.7
20	-	39.8	-	39.9
21	1.00 *d* (7.0)	23.6	0.99 *d* (6.5)	20.9
22	5.15 *dd* (8.0, 15.0)	135.4	5.17 *dd* (8.0, 15.0)	135.4
23	5.23 *dd* (7.5, 15.5)	132.5	5.24 *dd* (7.0, 15.0)	131.4
24	-	42.9	-	42.0
25	-	33.2	-	32.4
26	0.84 *d* (8.0)	19.8	0.81 *d* (6.5)	19.4
27	0.82 *d* (7.5)	20.0	0.80 *d* (6.5)	19.7
28	0.91 *d* (7.0)	17.7	0.89 *d* (7.0)	17.2
3-OH	-	-	4.22 *d* (5.5)	-
5-OH	-	-	3.58 *s*	-
6-OH	-	-	4.49 *d* (5.5)	-

[b]Recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$..
[c]Recorded at 500 MHz (^{1}H-NMR) and 125 MHz (^{13}C NMR) in DMSO-d_6
Chemical shifts (δ) are expressed in ppm

Table 3. Cell growth inhibitory effects of some isolated compounds.

Compound[a)]	Inhibition of cell growth (%)			
	MCF-7	HeLa	NCI-H460	HepG2
1	31.26 ± 3.05	9.36 ± 6.45	22.15 ± 2.10	-3.40 ± 4.92
2	93.76 ± 1.56	92.96 ± 1.86	96.02 ± 0.82	97.34 ± 2.15
4	60.54 ± 2.73	69.05 ± 2.52	49.97 ± 2.62	37.32 ± 1.06
5	51.67 ± 4.52	16.32 ± 7.35	31.2 ± 0.97	1.20 ± 1.86
6	59.43 ± 3.30	53.67 ± 3.31	29.51 ± 5.87	9.12 ± 4.09
Camptothecin	45.37 ± 2,78	48.14 ± 0,67	80.75 ± 1,97	56.96 ± 1,61

[a)] The compounds were tested at the concentration of 100 μg/mL.
[b)] The presented data are means of three experiments ± SD.
[c)] Camptothecin was tested at the concentration of 0.01 μg/mL for NCI-H460, 0.05 μg/mL for MCF-7, 0.07 μg/mL for HepG2, and of 1 μg/mL for HeLa.

Table 4. The IC_{50} values against MCF-7 cancer cell line of compounds **2**, **4** and **6**.

Compound	**2**	**4**	**6**
IC_{50} (μg/mL)	21.10 ± 0.38	85.45 ± 0.99	94.57 ± 4.91

4. CONCLUSIONS

Six known compounds zeorin **(1)**, 16*β*-acetoxyhopane-6*α*,22-diol **(2)**, retigeric acid A **(3)**, retigeric acid B **(4)**, ergosterol-5α,8α-peroxide (**5**), cerevisterol (**6**) were isolated for the first time from the lichen *Lobaria orientalis*. The cytotoxic activities of five compounds **1**–**3** and **5**–**6** against some cancer cell lines were evaluated and among them, **2** showed good cytotoxicity against MCF-7 cancer cell lines with IC_{50} value of 21.10 μg/mL.

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TÓM TẮT

CÁC HỢP CHẤT TRITERPENOID VÀ STEROID
TỪ ĐỊA Y *LOBARIA ORIENTALIS*, LOBARIACEAE

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Từ cao ethyl acetate của loài địa y *Lobaria orientalis* đã cô lập được sáu hợp chất gồm bốn hợp chất thuộc nhóm triterpenoid là zeorin **(1)**, 16*β*-acetoxyhopane-6*α*,22-diol **(2)**, retigeric acid A **(3)** và retigeric acid B **(4)** và hai hợp chất thuộc nhóm sterol là ergosterol-5α,8α-peroxide (**5**) và cerevisterol (**6**). Khảo sát thử nghiệm hoạt tính gây độc tế bào trên bốn dòng tế bào ung thư là HeLa, HepG2, NCI-H460 and MCF–7 của năm hợp chất **1-2** và **4-6** cho thấy hợp chất **2** ức chế mạnh đối với tế bào ung thư vú MCF-7 với giá trị IC_{50} là 21,10 µg/mL.

Từ khóa: Lobaria orientalis, lichen, triterpenoid, steroid, HepG2, NCI-H460, HeLa, MCF-7.