SOME HOPANES AND ESGOSTANES FROM LICHEN *PARMOTREMA SANCTI-ANGELII* (LYNGE) HALE (PARMELIACEAE)

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ABSTRACT

*Five known hopane-type triterpenes, zeorin **(1)**, 6α-acetoxyhopane-22-ol **(2)**, leucotylin **(3)**, 16β-acetoxyhopane-6α,22-diol **(4)**, 6α-acetoxyhopane-16β,22-diol **(5)**, along with two esgostane-type sterols, 5α,8α-esgosterol peroxide (**6**), brassicasterol (**7**) were isolated from the lichen Parmotrema sancti-angelii (Lynge) Hale. Their chemical structures were elucidated by spectroscopic data analysis and comparison with those reported in the literature. This is the first time these compounds are reported in Parmotrema sancti-angelii (Lynge) Hale.*

Keywords: *Parmotrema sancti-angelii*, hopane-type triterpenes, esgostane-type sterols

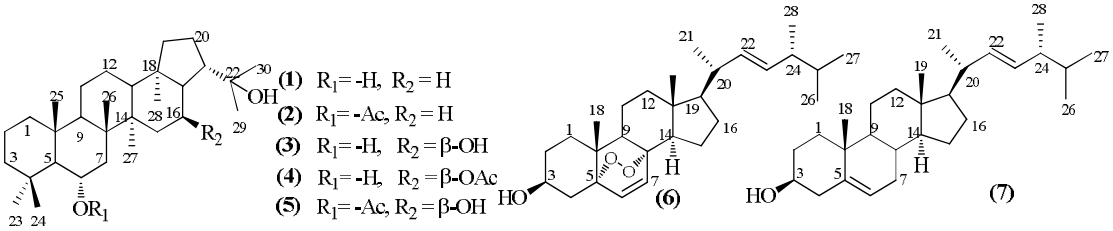

Figure 1. *Chemical structures of 1-7*

TÓM TẮT

Một số hopane và esgostane cô lập từ loài địa y Parmotrema sancti-angelii (Lynge) Hale (Parmeliaceae)

*Năm hợp chất triterpene khung hopane zeorin **(1)**, 6α-acetoxyhopane-22-ol **(2)**, leucotylin **(3)**, 16β-acetoxyhopane-6α,22-diol **(4)**, 6α-acetoxyhopane-16β,22-diol **(5)** và hai hợp chất sterol khung esgostane được cô lập từ loài địa y Parmotrema sancti-angelii (Lynge) Hale. Cấu trúc hóa học của chúng được xác định bằng các phương pháp phổ nghiệm cũng như so sánh với các tài liệu tham khảo. Đây là lần đầu tiên các hợp chất này được tìm thấy trong loài địa y Parmotrema sancti-angelii (Lynge) Hale.*

Từ khóa: *Parmotrema sancti-angelii*, hopane-type triterpenes, esgostane-type sterols.

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1. Introduction

Lichens produce large concentrations of bioactive compounds (Huneck 2001).[5] The bioactivities and pharmaceutical potential of lichen metabolites have been reviewed extensively according to Boustie & Grube (2007),[2] Boustie *et al.* (2010),[3] Muller (2001).[8] Previous studies on the chemical constituents of *Parmotrema sancti-angelii* reported the presence of some compounds demonstrating bactericidal activity (Verma 2011),[10] but triterpenes have not been reported yet.

Figure 2. *Parmotrema sancti-angelii (Lynge) Hale*

In this paper, the crude acetone extract of the lichen *Parmotrema sancti-angelii* was subjected to silica gel column chromatography to afford seven compounds, zeorin (**1**),[7] 6α-acetoxyhopane-22-ol (**2**),[4] leucotylin (**3**),[1] 16β-acetoxyhopane-6α,22-diol (**4**),[4] 6α-acetoxyhopane-16β,22-diol (**5**),[4] along with 5α,8α-esgosterol peroxide (**6**),[6] brassicasterol (**7**)[9] (Figure 1). Their chemical structures were elucidated by spectroscopic data analysis and comparison with those reported in the literature.

2. Experimental

General experimental procedures

The NMR spectra were measured on a Bruker Avance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) and Varian Mercury-400 Plus NMR (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers with TMS as internal standard. Proton chemical shifts were referenced to the solvent residual signal of $CDCl_3$ at δ_H 7.26, of CD_3COCD_3 at δ_H 2.05, of CD_3OD at δ_H 3.31. The ^{13}C–NMR spectra were referenced to the central peak of $CDCl_3$ at δ_C 77.1, of CD_3COCD_3 at δ_C 29.4, of CD_3OD at δ_C 49.0. The HR–ESI–MS were recorded on aBruker micrOTOF Q-II. TLC was carried out on precoated silica gel 60 F_{254} or silica gel 60 RP–18 $F_{254}S$ (Merck) and spots were visualized by spraying with 30% H_2SO_4 solution followed by heating. Gravity column chromatography was performed with Silica gel 60 (0.040–0.063 mm, Himedia).

Plant material

Parmotrema sancti-angelii (Lynge) Hale was collected on the bark of tea trees *Camellia sinensis* at Bao Loc city, Lam Dong province, Vietnam (07/2013–09/2013) and the scientific name was identified by Dr. Harrie J. M. Sipman, Botanic Garden and Botany Museum Berlin-Dahlem, Freie University, Berlin, Germany. A voucher specimen (No US-B021) was deposited in the herbarium of the Department of Organic

Chemistry, Univeristy of Science, Vietnam National University - Ho Chi Minh City, Vietnam.

Extraction and isolation

The clean, air-dried and ground material (950 g) was extracted by maceration with acetone at ambient temperature, and the filtrated solution was evaporated under reduced pressure to afford the crude acetone extract (145.1 g). The crude acetone extract (145.1 g) was dissolved in hot acetone (45 ^{o}C) to obtain two parts, the solution and the insoluble powder (**P**, 30.0 g). The solution was evaporated to afford the acetone extract (110.4 g). This one was applied on normal phase silica gel column chromatography, eluted with the solvent system of hexane–ethyl acetate (9:1) to afford **H0** extract (6.1 g). Continuous elution of the column with the same solvent systems but increasing polarity (8:2), (7:3), (6:4), (4:6), and (3:7) yielded five fractions, **H1** (2.1 g), **C** (15.4 g), **EA1** (4.5 g), **EA2** (5.1 g), and **EA3** (9.8 g), respectively.

Fraction **H0** (6.1 g) was applied to silica gel column chromatography, eluted with hexane–ethyl acetate (9: 1) to give four fractions, **H0.1** (3.3 g) and **H0.2**–**H0.4** (1.9 g). Fraction **H0.1** (3.3 g) was separated into four fractions, **H0.1.1**–**H01.3** (1.5 g) and **H0.1.4 (**1.0 g**)** by silica gel column chromatography with chloroform 100% as the eluent. Fraction **H0.1.4** was fractionated by column chromatography to provide two fractions **H0.1.4.1** (695.8 mg) and **H0.1.4.2** (119.5 mg). Fraction **H0.1.4.1** was chromatographed to give **2** (6.8 mg) and **7** (3.9 mg). Fraction **H0.1.4.2** was applied to preparative TLC with the solvent system of chloroform– methanol (100:4) to afford **4** (10.1 mg) and **5** (5.2 mg).

Fraction **H1** (2.1 g) was applied to silica gel column chromatography, eluting with hexane–ethyl acetate–acetic acid (9:1:0.5) to give five fractions, **H1.1** (1.1 g), **H1.2**–**H1.4** (0.5 g) and **H1.5** (197.3 mg). Fraction **H1.1** (1.1 g) was further chromatographed, eluting with hexane–ethyl acetate–acetone (9:1:0.5) to give two fractions, **H1.1.1** (200.2 mg) and **H1.1.2** (498.4 mg). Fraction **H1.1.1** was purified to afford **6** (19.8 mg). Washing fraction **H1.1.2** three times by acetone yielded **1** (99.2 mg). Fraction **H1.5** (197.3 mg) was applied to preparative TLC with the solvent system of hexane–ethyl acetate–methanol (7:3:0.4) to yield **3** (4.9 mg).

- **Zeorin (1):** White amorphous powder. The ^{1}H- (400 MHz) NMR data ($CDCl_3$): 0.82 (1H, d, J=11.0 Hz, H-5), 3.96 (1H, td, J=10.8, 4.0 Hz, H-6), 2.17 (1H, m, H-21), 1.19 (3H, s, H-23), 1.00 (3H, s, H-24), 0.85 (3H, s, H-25), 1.03 (3H, s, H-26), 0.96 (3H, s, H-27), 0.75 (3H, s, H-28), 1.14 (3H, s, H-29), 1.18 (3H, s, H-30). The ^{13}C-NMR (100 MHz) data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with with those reported in the literature.[7]

- **6*α*-Acetoxyhopane-22-ol (2):** White amorphous powder. The ^{1}H- (500 MHz) NMR data ($CDCl_3$): 1.13 (1H, d, J=11.5 Hz, H-5), 5.23 (1H, dt, J=11.0, 7.5 Hz, H-6), 2.21 (1H, m, H-21), 1.04 (3H, s, H-23), 0.86 (3H, s, H-24), 0.93 (3H, s, H-25), 1.10

(3H, s, H-26), 0.98 (3H, s, H-27), 0.77 (3H, s, H-28), 1.18 (3H, s, H-29), 1.21 (3H, s, H-30), 2.04 (AcO-). The ^{13}C- (125 MHz) NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with with those reported in the literature.[4]

- **Leucotylin (3):** White amorphous powder. The ^{1}H- (500 MHz) NMR data (Acetone-d_6): 0.87 (1H, d, J=10.5 Hz, H-5), 3.93 (1H, m, H-6), 4.06 (1H, ddd, J=11.5, 9.5, 7.5 Hz, H-16), 1.48 (1H, m, H-17), 2.50 (1H, m, H-21), 1.19 (3H, s, H-23), 1.02 (3H, s, H-24), 0.91 (3H, s, H-25), 1.09 (3H, s, H-26), 1.07 (3H, s, H-27), 0.81 (3H, s, H-28), 1.26 (3H, s, H-29), 1.13 (3H, s, H-30). The ^{13}C- (125 MHz) NMR data (Acetone-d_6): see Table 1. These spectroscopic data were suitable with with those reported in the literatures.[1]

- **16*β*-Acetoxyhopane-6*α*,22-diol (4):** White amorphous powder. The ^{1}H- (500 MHz) NMR data (CD_3OD): 0.86 (1H, d, J=11.5 Hz, H-5), 3.93 (1H, td, J=11.0, 4.0, H-6), 5.29 (1H, ddd, J=12.5, 9.5, 4.5 Hz, H-16), 1.74 (1H, m, H-17), 2.44 (1H, m, H-21), 1.14 (3H, s, H-23), 1.00 (3H, s, H-24), 0.91 (3H, s, H-25), 1.08 (3H, s, H-26), 1.12 (3H, s, H-27), 0.88 (3H, s, H-28), 1.12 (3H, s, H-29), 1.14 (3H, s, H-30). The ^{13}C- (125 MHz) NMR data (CD_3OD): see Table 1. These spectroscopic data were suitable with with those reported in the literature.[4]

- **6*α*-Acetoxyhopane-16*β*,22-diol (5):** White amorphous powder. The ^{1}H- (500 MHz) NMR data (CD_3OD): 1.18 (1H, d, J=11.5 Hz, H-5), 5.26 (1H, td, J=11.0, 4.0 Hz, H-6), 4.07 (1H, td, J=11.0, 4.0, H-16), 1.51 (1H, m, H-17), 2.50 (1H, m, H-21), 1.15 (3H, s, H-23), 0.87 (3H, s, H-24), 0.97 (3H, s, H-25), 1.08 (3H, s, H-26), 1.06 (3H, s, H-27), 0.82 (3H, s, H-28), 1.26 (3H, s, H-29), 1.13 (3H, s, H-30). The ^{13}C- (125 MHz) NMR data (CD_3OD): see Table 1. These spectroscopic data were suitable with with those reported in the literature.[4]

- **5*α*,8*α*-Esgosterol peroxide (6):** White amorphous powder. The ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data ($CDCl_3$): see Table 2. These spectroscopic data were suitable with with those reported in the literature.[6]

- **Brassicasterol (7):** White amorphous powder. The ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR data ($CDCl_3$): see Table 2. These spectroscopic data were suitable with with those reported in the literature.[9]

3. Results and discussion

Compound **3** was isolated as a white amorphous powder. The ^{1}H NMR spectrum showed eight methyl singlets at δ 1.19 (3H, s, H-23), 1.02 (3H, s, H-24), 0.91 (3H, s, H-25), 1.09 (3H, s, H-26), 1.07 (3H, s, H-27), 0.81 (3H, s, H-28), 1.26 (3H, s, H-29), 1.13 (3H, s, H-30), two methine protons indicative of secondary alcoholic functions at δ 3.93 (1H, m, H-6) and 4.06 (1H, ddd, J=11.5, 9.5, 7.5 Hz, H-16). Interpretation of the ^{13}C NMR and HSQC data of **(1)** revealed the presence of 30 carbon signals, including two oxygenated methines at δ_C 65.8 (C-16) and 67.1 (C-6), five quaternary carbons, five methines, nine methylenes, and eight methyl groups (Table 1). The missing carbon

signal C-22 was determined according to HMBC correlations of H-21, H-29, and H-30 to C-22. The ^{1}H and ^{13}C NMR chemical shifts in the ring AB region showed close similarity to those of zeorin.[7] Detailed spectroscopic comparison between **3** and **1** deduced that they were very similar, except for the presence of one additional oxymethine group (δ_H 4.06, ddd, J=11.5, 9.5, 7.5 Hz) instead of the methylene group at C-16 in **1**. The upfield methine H-5 (δ_H 0.87, d, J = 10.5 Hz) exhibited 1,2-diaxial ^{1}H-^{1}H coupling to a secondary alcohol methine (δ_H 3.93, m, H-6). That meant the hydroxyl group at C-6 was at α-orientation. The location of the second oxymethine H-16 was achieved by the analysis of HMBC data. Key HMBC correlations of H-15 to C-16 and C-17; of H-17 to C-16, C-18, C-21, and C-22; and of H-21 to C-17, C-20, C-22 and C-30 indicated the hydroxyl group at C-16 (**Fig. 2**). In addition, the coupling constants of H-16 exhibited two 1,2-diaxial coupling values J_{aa}= 12.5 Hz and 9.5 Hz, indicating the β-orientation of 16-OH group. Consequently, **3** was elucidated to be hopan-6α,16β,22-triol or leucotylin.[1]

Compound **4** was isolated as a white amorphous powder. Detailed spectroscopic comparison between **4** and **3** deduced that they were very similar, except for the presence of one additional acetylated methine group [δ_H 5.29, ddd, J=12.0, 9.5, 4.5 (-CH-O); δ_H 2.00 (CH_3-C=O)] instead of the oxymethine at C-16 in **3**. This acetylated methine showed diaxial coupling to H-15β and H-17 with the coupling constants J_{aa} 12.0 and 9.5 Hz suggesting the β-orientation of acetyl group. HMBC correlations of H-15 to C-16 and C-17, H-16 to the carboxyl group, of H-17 to C-15, C-16, C-18, C-21, C-22, and C-28, and of H-21 to C-17, C-18, C-20, and C-22 also confirmed the position of acetyl group at C-16. Furthermore, four spin systems drawn with bold bonds along with H-15, H-16, H-17, and H-21 were established on the basis of ^{1}H-^{1}H COSY spectrum, also confirming the position of 16-OAc (**Fig. 2**). The NMR spectroscopic data of **4** was similar to those of 16β-acetoxyhopan-6α,22-diol.[4] Accordingly, **4** was unambiguously elucidated to be 16β-acetoxyhopan-6α,22-diol.

Compound **5** was isolated as a white amorphous powder. Detailed spectroscopic comparison between **5** and **3** indicated that they were very assembly, except for the replacement of one oxymethine at δ_H 3.93 in **3** by one acetylated methine group 5.26 (1H, td, J=11.0, 4.0 Hz, H-6) in **5**. Detailed analysis of the coupling constants of this acetylated methine group indicated that it coupled with two adjacent axial protons (J_{aa} =11.0 Hz and 11.0 Hz), suggesting the β-orientation of the hydroxyl group. HMBC correlation analysis also supported the positions of the hydroxyl group at C-16 and the acetyl group at C-6. In particular, HMBC correlations of H-17 to C-16, C-18, C-21, and C-28 and of H-21 to C-17, C-18, C-20 and C-22 confirmed the hydroxyl group at position C-16 (**Fig. 2**). Moreover, the HMBC correlations between H-6 (δ_H 5.26) to C-5, C-10, and the carboxyl carbon and between H-7 to C-5 and C-6 indicated the position of the acetyl group. The NMR spectroscopic data of **5** was similar to those of 6α-acetoxyhopan-16β,22-diol.[7] Thus, **5** was elucidated to be 6α-acetoxyhopan-16β,22-diol.[4]

Table 1. *^{13}C-NMR spectral data of* ***1-5***

N	(1)[a]	(2)[a]	(3)[b]	(4)[c]	(5)[c]
	δ_C	δ_C	δ_C	δ_C	δ_C
1	40.5	40.4	41.1	41.6	41.3
2	18.6	18.5	18.3	19.6	19.4
3	43.9	43.7	43.7	44.9	44.6
4	33.7	33.3	33.4	34.6	34.3
5	61.2	58.6	60.0	61.7	59.7
6	69.5	72.2	67.1	69.3	73.1
7	45.5	41.2	41.1	45.8	44.6
8	43.0	42.9	43.5	44.0	43.9
9	49.9	49.5	49.1	50.6	50.2
10	39.5	39.7	40.0	40.4	40.7
11	20.8	21.2	20.9	22.1	22.1
12	24.1	24.1	23.3	24.7	24.6
13	49.6	50.0	48.6	49.8	50.8
14	42.0	42.2	42.5	45.1	45.2
15	34.5	34.5	43.9	42.2	42.1
16	21.2	22.2	65.8	72.9	68.0
17	54.1	54.1	60.9	58.9	61.9
18	44.2	44.1	45.4	47.2	46.7
19	41.4	41.4	40.0	42.8	42.7
20	26.7	26.7	26.3	28.2	28.6
21	51.2	51.2	50.9	51.8	52.3
22	74.4	74.0	74.0	74.0	74.7
23	36.9	36.5	36.5	37.1	37.0
24	22.2	22.3	21.6	22.5	22.5
25	17.2	17.2	16.5	17.7	17.7
26	18.4	18.2	17.6	18.6	18.8
27	17.2	17.2	16.5	17.6	17.6
28	16.2	16.2	17.6	18.4	18.6
29	28.8	28.9	27.0	27.3	27.3
30	30.9	31.1	30.3	31.5	30.8
AcO-		22.0		21.8	22.0
		170.5		172.6	172.3

NMR spectra were recorded in $CDCl_3$,[a] acetone-d_6,[b] CD_3OD.[c]

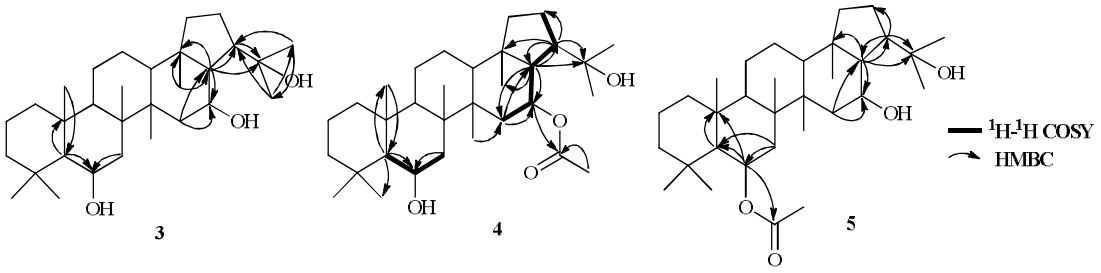

Figure 3. *Key COSY and HMBC correlations of **3**, **4**, and **5***

Table 2. *NMR spectral data of **6** and **7***

N	**6 ($CDCl_3$)**		**7 ($CDCl_3$)**	
	δ_H, J (Hz)	δ_C	δ_H, J (Hz)	δ_C
1	1.97 (*m*, H-1eq) 1.70 (*dt*, 13.5, 3.5, H-1ax)	34.9		37.3
2	1.56 (*m*) 1.84 (*m*)	30.2		29.9
3	3.97 (*m*, H-3ax)	66.5	3.53 (*m*, H-3ax)	72.0
4	2.10 (*ddd*, 13.5, 5.0, 2.0, H-4eq) 1.95 (*m*, H-4ax)	37.1		39.9
5		82.3		140.8
6	6.24 (*d*, 8.5)	135.6	5.35 (*brd*, 5.0)	121.8
7	6.50 (*d*, 8.5)	130.9		32.1
8		79.6		31.9
9	1.50 (*m*)	51.3		50.3
10		37.1		37.3
11	1.52 (*m*) 1.23 (*m*)	23.6		23.2
12	1.97 (*m*) 1.25 (*m*)	39.3		40.3
13		44.6		42.5
14	1.58 (*m*)	51.7		56.3
15	1.67 (*m*) 1.39 (*m*)	20.6		24.3
16	1.85 (*m*) 1.37 (*m*)	28.9		28.6
17	1.23 (*m*)	56.2		57.0
18	0.88 (*s*)	18.3	0.66 (s)	19.5
19	0.81 (*s*)	13.0	1.01 (s)	12.2
20	2.02 (*m*)	39.9		39.9
21	1.00 (*d*, 6.5)	21.0	1.01 (*d*, 6.5)	20.1
22	5.14 (*dd*, 15.5, 7.7)	135.4	5.14(*m*)	136.0
23	5.22 (*dd*, 15.5, 7.7)	132.5	5.19 (*m*)	132.0
24	1.85 (*m*)	42.9		43.0

25	1.49 (*m*)	33.2		33.1
26	0.82 (*d*, 7.0)	19.8	0.81 (d, 7.0)	21.2
27	0.83 (*d*, 7.0)	20.1	0.83 (d, 7.5)	21.1
28	0.91 (*d*, 6.5)	17.7	0.91 (d, 7.0)	19.7

4. Conclusion

From the lichen *Parmotrema sancti-angelii* collected from Lam Dong province, Vietnam, five triterpenes and two sterols were successfully isolated and their chemical structures were elucidated. This is the first time these compounds are known in *Parmotrema sancti-angelii*. Further studies on this lichen are in progress.

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