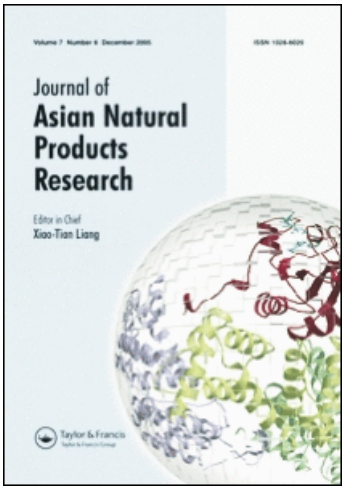

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 11 August 2010

To cite this Article Zhao, Ying-Hong , Deng, Tie-Zhong , Chen, Yu , Liu, Xiang-Ming and Yang, Guang-Zhong(2010) 'Two new triterpenoids from *Lycopodium obscurum* L.', Journal of Asian Natural Products Research, 12: 8, 666 — 671
To link to this Article: DOI: 10.1080/10286020.2010.493881
URL: http://dx.doi.org/10.1080/10286020.2010.493881

Journal of Asian Natural Products Research
Vol. 12, No. 8, August 2010, 666–671

ORIGINAL ARTICLE

Two new triterpenoids from *Lycopodium obscurum* L.

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(*Received 9 February 2010; final version received 12 May 2010*)

Two new onoceranoid triterpenoids, (3α,8β,14α,21β)-26,27-dinoronocerane-3,8,14,21-tetrol (**1**) and 26-nor-8β-hydroxy-α-onocerin (**2**), were isolated from *Lycopodium obscurum* L. Their structures were elucidated on the basis of spectroscopic analyses.

Keywords: *Lycopodium*; *Lycopodium obscurum* L.; onoceranoid triterpenoid; Lycopodiaceae

1. Introduction

Serratane-type triterpenoids represent a large family of plant constituents obtained from the club moss belonging to Lycopodiaceae. These structurally diverse pentacyclic triterpenes possess unusual skeletons with a seven-membered C-ring, and a C=C double bond between C (14) and C (15), and O-functionalities at C (3) and C (21) [1]. Many *Lycopodium* plants contain α-onocerin, and it was deduced that serratane-type triterpenoids came from single protonation of α-onocerin [2]. Serratane-type triterpenoids exhibit well-documented pharmacological properties, such as cancer chemopreventive activity [3] and inhibitory effects against *Candida albicans* secreted aspartic proteases [4]. *Lycopodium obscurum* L. has been used in China as a traditional folk medicine for the treatment of contusion, dysmenorrhea, quadriplegia, arthritic pain [5], and has been reported to be a source of serratenes [6,7]. During our search for biologically active secondary metabolites, we investigated the chemical constituents of *L. obscurum* L. Here, we describe the isolation and structural elucidation of two new naturally occurring triterpenoids (Figure 1).

2. Results and discussion

Compound **1** was isolated as a white amorphous powder. The molecular formula of **1** was deduced as $C_{28}H_{50}O_4$, which was confirmed by HR-EI-MS at *m/z* 432.3591 corresponding to a fragmental ion $[M - H_2O]^+$. The ^{1}H NMR spectrum of **1** displayed the presence of six tertiary methyls [δ_H 0.93 (s), 1.27 (s), 1.40 (s)], two coinciding equatorial oxygenated methines [δ_H 3.68 (2H, br s, H-3, 21)], and two coinciding equatorial oxygenated methines [δ_H 4.36 (2H, br s, H-8, 14)]. The ^{13}C NMR and DEPT spectra of **1** displayed 14 carbon signals, including two oxygenated methines (δ_C 75.3, 66.5), three methyls, five methylenes, two methines, as well as

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.493881
http://www.informaworld.com

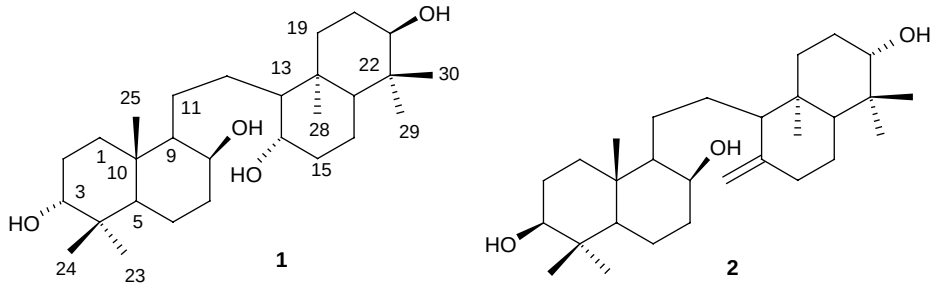

Figure 1. Structures of compounds **1** and **2**.

two quaternary carbons, suggesting the presence of a symmetric structure within this molecule. A comparison of ^{13}C NMR spectral data of **1** with those of the known compound (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol, isolated from *Lycopodium japonicum* [8], revealed similar carbon signals, except for the chemical shifts of C(1) or (19), C(3) or (21), and C(5) or C(17). This suggested that the plane structure of **1** should be the same as that of (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol, except for the orientation of C(3) or (21)-OH. A comparison of ^{13}C NMR spectral data of **1** with those of (3β,8β,14α,21α)-26,27-dinoronocerane-3,8,14,21-tetrol indicated that C (3) or C (21) containing the axial hydroxyl group in **1** was more shielded (δ_C 75.3) than the latter containing the equatorial one (δ_C 78.4). The chemical shifts of C (1) or C (19) and C (5) or C (17) were also shielded by about 3.5 and 7.6 ppm, respectively, in **1** due to the γ-gauche interaction [9]. The assumption was further supported by the NOE correlations between Me (24) or Me (29) (δ_H 0.93 (s)) and H-3 or H-21 (δ_H 3.68 (br s)), and the small coupling constant between H-2 and H-3 (Figure 2). From the above data, the structure of **1** was established as (3α,8β,14α,21β)-26,27-dinoronocerane-3,8,14,21-tetrol.

Compound **2**, which was obtained as a white amorphous powder, exhibited an $[M + Na]^+$ ion peak at *m/z* 469.3671 in the HR-FAB-MS, corresponding to the molecular formula $C_{29}H_{50}O_3$. Its ^{1}H NMR spectrum showed six tertiary methyls [δ_H 0.69 (3H, s), 0.75 (3H, s), 0.77 (3H, s), 0.91 (3H, s), 0.96 (6H, s)], three oxygenated methines [δ_H 3.13 (1H, dd, J = 11.4, 4.2 Hz), 3.18 (1H, dd, J = 10.2, 6.0 Hz), 3.92 (1H, br s)], and one exomethylene [δ_H 4.83 (1H, s), 4.64 (1H, s)]. The ^{13}C NMR and DEPT spectra of **2** displayed 29 carbon signals, including an exomethylene (δ_C 149.3, 107.8), three oxygenated methines (δ_C 79.7, 79.5, 67.9), six methyls, 10 methylenes, four methines, as well as four quaternary carbons. All the above data suggested that **2** should be tetracyclic triterpenoids having OH and six

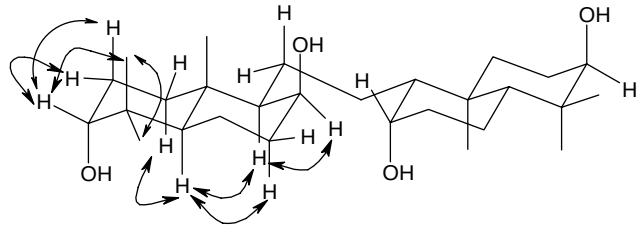

Figure 2. Key ROESY correlations of compound **1**.

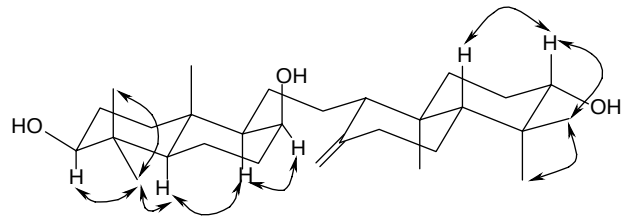

Figure 3. Key ROESY correlations of compound **2**.

tertiary Me groups, such as α-onocerin [10]. On comparison of the ^{13}C NMR spectral data of **2** with those of 26-nor-8-oxo-α-onocerin [7], it was found that except for the presence of one more oxygenated methine (δ_C 67.9) instead of the carbonyl group (δ_C 211.7) in **2**, the ^{13}C NMR spectral data of **2** were nearly superimposed on those of 26-nor-8-oxo-α-onocerin. Thus, the carbonyl group in 26-nor-8-oxo-α-onocerin was replaced by the hydroxyl group in **2**. Three hydroxyl groups of **2** were located at C (3), C (8), and C (21), which were further supported by the HMBC correlations between H-3/C-2 (δ_C 28.6), C-4 (δ_C 40.0), and C-24 (δ_C 16.6); H-8/C-7 (δ_C 36.8) and C-9 (δ_C 56.2); and H-21/C-20 (δ_C 30.8), C-22 (δ_C 40.2), C-29 (δ_C 16.3), and C-30 (δ_C 28.9). The relative configuration of **2** was derived from the ROESY spectrum. The ROESY correlations of H-3/H_3-23 and H-8/H-9 (Figure 3) suggested that 3-OH and 8-OH were β-oriented. The 21-OH was deduced to be in the α-position, which was based on the ROESY correlations of H-21/H_3-30. Thus, the structure of compound **2** was established as 26-nor-8β-hydroxy-α-onocerin.

Compounds **1** and **2** were evaluated for their *in vitro* cytotoxic activities against KB (human oral carcinoma) cancer cell lines using the MTT assay method. Compound **2** exhibited weak cytotoxic activity with an IC_{50} value of 32.5 μM using fluorouracil as a positive control (IC_{50} = 15.9 μM). Compound **1** was inactive against tested cell lines.

3. Experimental

3.1 General experimental procedures

IR was carried out on a Nicolet NEXUS-6700 instrument. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. NMR spectra were run on Bruker AM-400 and 600 spectrometers with TMS as an internal standard. EI-MS and HR-EI-MS were measured with a Finnigan MAT 95 instrument. FAB-MS and HRFABMS were carried out on a VG Autospec-3000 spectrometer. Thin-layer chromatography was performed on silica gel 60 GF_{254}, and column chromatography (CC) was carried out using silica gel (200–300 mesh) from Qingdao Haiyang Chemical Group Co., Qingdao, China and C_{18} reverse-phase silica gel from YMC Co. Ltd Kyoto, Japan.

3.2 Plant material

The whole plant of *L. obscurum* L. was collected from Jianshi County, Hubei Province, China, and identified by Prof. Dingrong Wan, College of Pharmacy, South Central University for Nationalities.

3.3 Extraction and isolation

The air-dried whole plant of *L. obscurum* L. (12.3 kg) was powdered and then extracted with MeOH (25 liters) three times at room temperature. The MeOH extract (1.65 kg) was suspended in 3% tartaric acid/H_2O (pH = 3) and then partitioned with EtOAc. The EtOAc extract (890 g) was suspended in 90% H_2O/MeOH and then successively parti-

tioned with petroleum ether (PE), EtOAc, and *n*-BuOH. The EtOAc extract (324 g) was subjected to CC (silica gel, PE:acetone 9:1, 8:2, 7:3, 1:1, 3:7, 0:1, v/v) to give nine fractions (Fr.1–Fr.9). Fr.5 (10.9 g) was subjected to CC (silica gel, $CHCl_3$/acetone 1:0→1:1) to give five sub-fractions (Fr.5.1–Fr.5.5). Fr.5.4 (5.5 g) was subjected to CC (ODS, H_2O/MeOH 9:1→1:9) to give compound **1** (20 mg). Fr.5.3 (2.2 g) was subjected to CC (silica gel, cyclohexane/EtOAc 9:1 → 1:1) to give four subfractions (Fr.5.3.1–Fr.5.3.4). Fr.5.3.2 (350 mg) was subjected to CC (ODS, H_2O/MeOH 7:3 → 3:7) to give compound **2** (40 mg).

*3.3.1 (3α,8β,14α,21β)-26,27-Dinoronocerane-3,8,14,21-tetrol (**1**)*

A white amorphous powder; $[\alpha]_D = +24.5$ ($c = 0.200$, MeOH); ^{1}H NMR and ^{13}C NMR spectral data see Table 1; EI-MS *m/z* (rel. int.): 432 ($[M]^+ - H_2O$, 2), 414 ($[M]^+ - 2H_2O$, 29), 399 (34), 193 (46), 175 (100), 161 (32), 147 (27), 135 (44),

Table 1. ^{1}H NMR, ^{13}C NMR, and HMBC spectral data of compound **1** (in C_5D_5N, δ in ppm).

No.	^{1}H NMR	^{13}C NMR	HMBC
1	1.65 (1H, m) 1.89 (1H, m)	32.8	C (2), C (10)
2	1.81 (1H, m) 2.07 (1H, m)	26.1	C (4), C (10)
3	3.68 (1H, br s)	75.3	C (1), C (5), C (24)
4	–	38.2	
5	1.85 (1H, m)	49.4	C (10), C (23), C (25)
6	1.52 (1H, m) 1.96 (1H, m)	17.4	
7	1.69 (1H, m) 2.22 (1H, m)	36.6	
8	4.36 (1H, br s)	66.5	C (10)
9	1.32 (1H, m)	54.6	C (10), C (11)
10	–	38.1	
11	1.53 (1H, m) 2.11 (1H, m)	22.9	
12	1.53 (1H, m) 2.11 (1H, m)	22.9	
13	1.32 (1H, m)	54.6	
14	4.36 (1H, br s)	66.5	
15	1.69 (1H, m) 2.22 (1H, m)	36.6	
16	1.52 (1H, m) 1.96 (1H, m)	17.4	
17	1.85 (1H, m)	49.4	
18	–	38.1	
19	1.65 (1H, m) 1.89 (1H, m)	32.8	
20	1.81 (1H, m) 2.07 (1H, m)	26.1	
21	3.68 (1H, br s)	75.3	
22	–	38.2	
23	1.27 (3H, s)	29.2	C (3), C (4), C (5), C (24)
24	0.93 (3H, s)	22.7	C (3), C (4), C (5), C (23)
25	1.40 (3H, s)	16.6	C (1), C (5), C (9), C (10)
28	1.40 (3H, s)	16.6	
29	0.93 (3H, s)	22.7	
30	1.27 (3H, s)	29.2	

Table 2. ^{1}H NMR, ^{13}C NMR, and HMBC spectral data of compound **2** (in CD_3OD, δ in ppm).

No.	^{1}H NMR	^{13}C NMR	HMBC
1	0.82 (1H, m)	38.5	
	1.78 (1H, m)		
2	1.48 (2H, m)	28.6	
3	3.13 (1H, dd, $J = 11.4$, 4.2 Hz)	79.5	C (2), C (4), C (24)
4	–	40.0	
5	0.81 (1H, m)	56.1	C (4), C (6), C (10), C (24)
6	1.45 (1H, m)	18.0	
	1.75 (1H, m)		
7	1.41 (1H, m)	36.8	
	1.94 (1H, m)		
8	3.92 (1H, br s)	67.9	C (7), C (9)
9	0.86 (1H, m)	56.2	C (8), C (10), C (11)
10	–	40.3	
11	1.28 (1H, m)	23.1	
	1.53 (1H, m)		
12	1.16 (1H, m)	24.2	
	1.65 (1H, m)		
13	1.55 (1H, m)	57.9	C (12), C (14), C (18)
14	–	149.3	
15	1.97 (1H, m)	39.0	C (14)
	2.38 (1H, d, $J = 13.2$ Hz)		
16	1.30 (1H, m)	25.2	
	1.68 (1H, m)		
17	1.13 (1H, m)	56.6	C (16), C (18), C (22), C (30)
18	–	39.5	
19	1.58 (2H, m)	38.6	
20	1.34 (2H, m)	30.8	
21	3.18 (1H, dd, $J = 10.2$, 6.0 Hz)	79.7	C (20), C (22), C (29), C (30)
22	–	40.2	
23	0.96 (3H, s)	28.9	C (3), C (4), C (24)
24	0.91 (3H, s)	16.6	C (3), C (4), C (23)
25	0.75 (3H, s)	16.2	C (1), C (5), C (10)
27	4.64 (1H, s)	107.8	C (13), C (14), C (15)
	4.83 (1H, s)		
28	0.69 (3H, s)	15.2	C (17), C (18)
29	0.77 (3H, s)	16.3	C (21), C (22), C (30)
30	0.96 (3H, s)	28.9	C (22), C (29)

121 (36), 107 (29), 95 (38), 81 (32), 69 (30), 55 (21); HR-EI-MS *m/z*: 432.3591 ($[M]^+ - H_2O$) (calcd for $C_{28}H_{48}O_3$, 432.3604), 414.3515 ($[M]^+ - 2H_2O$) (calcd for $C_{28}H_{46}O_2$, 414.3498).

*3.3.2 26-Nor-8β-hydroxy-α-onocerin (**2**)*

A white amorphous powder; $[\alpha]_D = +30.4$ ($c = 0.395$, MeOH); IR (KBr) ν_{max} (cm^{-1}): 3383, 2933, 2851, 1643, 1465, 1391, 1089, 1030, 929, 894. ^{1}H NMR and ^{13}C NMR spectral data see Table 2; FAB-MS: *m/z* 469 $[M + Na]^+$; HR-FAB-MS: *m/z* 469.3671 $[M + Na]^+$ (calcd for $C_{29}H_{50}O_3Na$, 469.3657).

Acknowledgement

This work was financially supported by the Academic Team of South Central University for Nationalities: Ethno-Medicine Research and Development (XTZ09010).

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