VIETNAM JOURNAL OF CHEMISTRY VOL. 53(2e) 103-106 APRIL 2015
DOI: 10.15625/0866-7144.2015-2e-024

TRITERPENES FROM THE LEAVES OF *Glochidion obliquum*

Vu Kim Thu[1], Le Thi Kieu Anh[2], Dang Ngoc Quang[2], Nguyen Van Thang[3], Hoang Le Tuan Anh[3], Nguyen Xuan Nhiem[3], Dan Thi Thuy Hang[3], Chau Van Minh[3], Phan Van Kiem[3,*]

[1]*Faculty of Basic Science, Hanoi University of Mining and Geology*

[2]*Faculty of Chemistry, Hanoi National University of Education*

[3]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST)*

Received 23 January 2015; Accepted for Publication 15 March 2015

Abstract

Using combined chromatographic methods, six tritrerpenes, 5β,6β-epoxytaraxeran-3β-ol (**1**), 3β-hydroxyglutin-5-ene (**2**), friedelan-3-one (**3**), lupeol (**4**), glochidonol (**5**), and glochidone (**6**) were isolated from the methanol extract of the leaves of *Glochidion obliquum* Decne. Their structures were elucidated by 1D- and 2D-NMR spectra and in comparison with those reported in the literature. Of which, compound **1** was reported from nature for the first time.

Keywords. *Glochidion obliquum*, triterpene, 5β,6β-epoxytaraxeran-3β-ol, 3β-hydroxyglutin-5-ene, friedelan-3-one.

1. INTRODUCTION

Glochidion is a large genus of the Euphorbiaceae family, comprising more than 250 species in the world. *Glochidion obliquum* Decne is a shrub or small tree distributed throughout India, Malaysia, Indonesia, and Cambodia. In Vietnam, this plant was distributed in Lang Son, Phu Tho, Vinh Phuc, Tay Ninh, Dong Nai, and Kien Giang. The leaves of *G. obliquum* have been used in the folk medicine to treat sarcoptic acariasis [1]. A number of phytochemical studies of genus *Glochidion* has reported the isolation of oleanane saponins [2], lupanes and flavonoids [3]. In addition, oleanane saponins showed cytotoxic activities [4]. Previously, chemical investigation of *G. obliquum* confirmed the presence of flavonoids and triterpenes [5]. As a part of our phytochemical studies of *G. obliquum*, we report herein the isolation, structural elucidation of six triterpenes from this plant.

2. MATERIAL AND METHODS

2.1. Plant material

The leaves of *Glochidion obliquum* Decne were collected in Phucyen, Vinhphuc, Vietnam in December, 2012 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (GO1212) was deposited at the Herbarium of the Institute of Marine Biochemistry.

2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR), and chemical shifts (δ) are reported in ppm using TMS as an internal standard. Column chromatography (CC) was performed on silica gel 230÷400 mesh (0.040÷0.063 mm, Merck) or YMC RP-18 resins (30÷50 μm, Fujisilisa Chemical Ltd.). Thin layer chromatography was performed on DC Alufolien Kieselgel 60 F254 (Merck) or RP-18 F_{254s} (Merck) plates. Compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 5 minutes.

2.3. Extraction and isolation

The dried leaves of *G. obliquum* (4.2 kg) were powdered and extracted three times with hot methanol (50 °C) to give the methanol extract (210.3 g), which was then suspended in water and extracted in turn with *n*-hexane, dichloromethane, and ethyl acetate, giving corresponding extracts: *n*-hexane (87.2 g, GOH), dichloromethane (55.0 g, GOD), and ethyl acetate (12.0 g, GOE), and water layers (35.5 g, GOW).

The dichloromethane layer (GOD 55.0 g) was chromatographed on a silica gel column using stepwise gradient elution of *n*-hexane - ethyl acetate (20:1, 10:1, 5:1, 2.5:1, v/v) to yield four sub-fractions, GOD1—GOD4. The GOD1 fraction was chromatographed on a silica gel column eluting with *n*-hexane - acetone (2:1, v/v) to give two smaller fractions, GOD1A and GOD1B. The GOD1A fraction was chromatographed on a RP-18 column eluting with acetone - water (3.5:1, v/v) to obtain compounds **1** (14 mg) and **2** (21 mg). The GOD1B fraction was further chromatographed on a silica gel column eluting with dichloromethane - ethyl acetate (10:1, v/v) to give **3** (10 mg). The GOD3 sub-fraction was chromatographed on a silica gel column using n-hexane - acetone (4:1, v/v) to give two smaller fractions, GOD3A and GOD3B. The GOD3A sub-fraction was further separated on a RP-18 column eluting with methanol - water (6:1, v/v) to obtain compound **6** (7.5 mg). Compound **4** was yielded from GOD3B fraction through chromatography on a RP-18 column eluting with methanol - water (5:1, v/v).

The ethyl acetate layer GOE (12.0 g) was directly separated on a silica gel column with *n*-hexane - acetone (2:1, v/v) to obtain two fractions, GOE1 and GOE2. The GOE1 fraction was further chromatographed on a silica gel column eluting with chloroform - acetone (3:1, v/v) to give two smaller fractions, GOE1A and GOE1B. Compound **5** (8 mg) was yielded from GOE1A fraction by chromatography on a silica gel column eluting with *n*-hexane-ethyl acetate (1:1, v/v).

5*β*,6*β*-Epoxytaraxeran-3*β*-ol (**1**): White amorphous powder; $[\alpha]_D^{25}$: +40.5 (*c*=0.1, $CHCl_3$); mp: 205-206°C; $C_{30}H_{50}O_2$; MW: 442.4; ^{1}H- and ^{13}C-NMR data, see table 1.

3*β*-Hydroxyglutin-5-en (**2**): White amorphous powder; $[\alpha]_D^{25}$: +10.3 (*c*=0.1, $CHCl_3$); mp: 210-213°C; $C_{30}H_{50}O$; MW: 426.4; ^{1}H-NMR (500 MHz, $CDCl_3$) typical signals at δ_H: 0.85 (s, H-25), 0.95 (s, H-29), 0.99 (s, H-30), 1.00 (s, H-27), 1.04 (s, H-23), 1.09 (s, H-26), 1.14 (s, H-24), 1.16 (s, H-28), 3.47 (br d, *J* = 12.0 Hz, H-3), and 5.63 (d, *J* = 6.0 Hz, H-6); ^{13}C-NMR data, see table 1.

Friedelan-3-one (**3**): White amorphous powder; $[\alpha]_D^{25}$: −27.8 (*c*=0.1, $CHCl_3$) mp 262-264°C; $C_{30}H_{50}O$; MW: 426.4; ^{1}H-NMR (500 MHz, in $CDCl_3$) typical signals at δ_H 0.72 (3H, s, H-24), 0.87 (3H, s, H-25), 0.95 (3H, s, H-30), 1.00 (6H, s, H-26, H-27), 1.05 (3H, s, H-29), 1.18 (3H, s, H-28), and 0.88 (3H, d, *J* = 6.5 Hz, H-23); ^{13}C-NMR data: see Table 1.

Compounds **4**, **5**, and **6,** see Ref. [3].

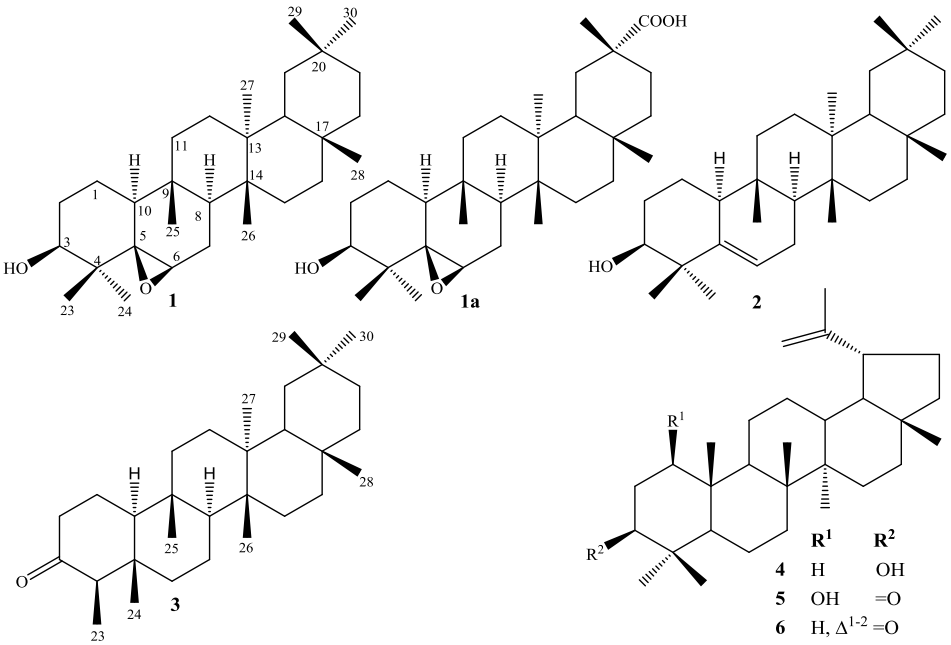

Figure 1: Chemical structures of **1**−**6** and **1a**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined as $C_{30}H_{50}O_2$ by the ESI-MS at *m/z* 443 $[M + H]^+$ and ^{13}C-NMR. The ^{1}H-NMR spectrum of **1** showed the following signals: eight tertiary methyl groups at δ_H 0.86, 0.87, 0.94, 0.98 × 2, 1.01, 1.12, and 1.15 (each 3H, s); two oxymethine protons at δ_H 3.49 (br d, *J* = 10.0 Hz) and 3.12 (d, *J* = 5.5 Hz). The

Table 1: The NMR data for compounds **1-3** and reference compounds

C	1			2		3	
	δ_C [#]	δ_C [a,b]	δ_H [a,c] (mult., *J* = Hz)	δ_C [*]	δ_C [a,b]	δ_C [$]	δ_C [a,b]
1	18.3	17.73	1.51 (m)/1.80 (m)	18.50	18.23	22.28	22.29
2	30.3	29.97	1.29 (m)	27.48	27.85	41.53	41.53
3	76.0	77.41	3.49 (br d, 10.0)	76.57	76.36	213.17	213.24
4	39.1	39.49	-	41.06	40.84	58.24	58.24
5	64.9	65.18	-	141.88	141.66	42.14	42.16
6	52.2	53.28	3.12 (d, 5.5)	122.30	122.07	41.30	41.31
7	21.9	21.20	1.81 (m)	23.86	23.67	18.24	18.25
8	45.1	47.45	1.32 (m)	47.69	47.48	53.12	53.12
9	35.1	34.78	-	35.01	34.88	37.45	37.46
10	49.3	48.03	1.72 (dd, 3.5, 12.0)	49.95	49.74	59.50	59.50
11	35.3	32.99	1.26 (m)/1.45 (m)	33.37	33.15	35.64	35.64
12	29.4	29.39	1.86 (m)	30.60	30.38	30.50	30.51
13	39.6	37.90	-	38.09	37.87	39.71	39.71
14	39.3	38.72	-	39.56	39.34	38.31	38.31
15	29.3	35.17	1.24 (m)/1.37 (m)	34.85	34.64	32.78	32.79
16	36.8	35.92	1.36 (m)/1.54 (m)	35.34	35.12	36.01	36.02
17	30.6	30.07	-	30.34	30.12	29.99	30.01
18	45.0	43.05	1.55 (m)	43.33	43.11	42.81	42.81
19	30.7	35.34	1.29 (m)/1.46 (m)	35.54	36.05	35.35	35.36
20	40.7	28.25	-	28.48	28.26	28.18	28.18
21	30.5	32.36	1.19 (m)/1.31 (m)	32.34	32.12	32.44	32.43
22	36.9	39.06	0.92 (m)/1.51 (m)	39.20	38.98	39.24	39.26
23	25.4	25.19	1.12 (s)	29.18	28.97	6.81	6.82
24	20.9	20.41	0.86 (s)	25.68	25.45	14.65	14.66
25	18.3	17.08	0.87 (s)	16.43	16.21	17.94	17.95
26	15.9	19.54	1.01 (s)	18.64	18.42	20.25	20.26
27	17.8	18.49	0.98 (s)	19.85	19.63	18.65	18.66
28	31.9	32.12	0.98 (s)	32.57	32.40	32.09	32.10
29	32.8	34.64	0.94 (s)	34.76	34.54	35.01	35.02
30	182.7	31.99	1.15 (s)	32.27	32.05	31.77	31.79

[a]Recorded in $CDCl_3$, [b]125 MHz, [c]500 MHz; [#]δ_C of triptocallic acid [6]. [*]δ_C of 3β-hydroxyglutin-5-ene [7], [$]$\delta_C$ of friedelan-3-one [8].

^{1}H- and ^{13}C-NMR data of **1** were similar to those of triptocallic acid C except for the disappearence of carboxylic group at C-20 [6]. The HMBC correlations between H-23 (δ_H 1.12)/H-24 (δ_H 0.86) and C-3 (δ_C 77.41)/C-4 (δ_H 39.49)/C-5 (δ_C 65.18); H-6 (δ_H 3.12) and C-4 (δ_C 39.49)/C-5 (δ_C 65.18), confirmed the presence of the hydroxyl and epoxy groups at C-3 and C-5/C-6. The confirguration of hydroxyl group at C-3 was proved by the large coupling constant of H-2 and H-3, *J* = 10.0 Hz. Moreover, the NOESY observations between H-6 (δ_H 3.12) and H-24 (δ_H 0.86) indicated that the configuration of epoxy group at C-3 was β. From the above evidence, the structure of **1** was elucidated to be 5β,6β-epoxitaraxeran-3β-ol. Compound **1** was synthesized from alnus-5-ene-3β-ol [9]. This is the first report from nature.

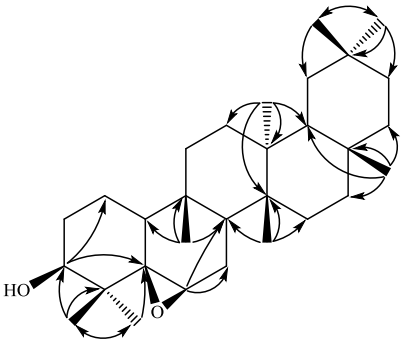

Figure 2: The key HMBC correlations of **1**

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was determined as

$C_{30}H_{50}O$ by the ESI-MS *m/z* at 427 $[M + H]^+$ and ^{13}C-NMR spectrum. The ^1H-NMR spectrum of compound

2 showed the signals: eight methyl groups at δ_H 0.85, 0.95, 0.99, 1.00, 1.04, 1.09, 1.14, and 1.16 (each 3H, s); one oxymethine proton at δ_H 3.47 (br d, J = 12.0 Hz); one olefinic proton at δ_H 5.63 (d, J = 6.0 Hz). The ^{13}C-NMR and DEPT spectra of **2** exhibited the signals of 30 carbons, including seven quaternary at δ_C 28.48, 30.34, 34.88, 37.87, 39.34, 40.84, and 141.66); five methine at δ_C 43.33, 47.48, 49.74, 76.36, and 122.07; ten methylene at δ_C 18.23, 23.67, 27.85, 30.38, 32.34, 33.15, 34.64, 35.34, 35.54, and 39.20; and eight methyl carbons at δ_C 16.43, 18.64, 19.85, 25.68, 29.18, 32.27, 32.57, and 34.76. The ^1H- and ^{13}C-NMR data of **2** were similar to those of **1** except for an additional double bond at C-5/C-6. The ^{13}C chemical shifts of the double bond at δ_C 122.07 and 141.66, suggested the position of the double bond at C-5/C-6. Moreover, the NMR spectroscopic data of compound **2** were similar to those of 3*β*-hydroxyglutin-5-ene [7]. Thus, compound **2** was elucidated to be 3*β*-hydroxyglutin-5-ene. This compound was isolated from genus *Glochidion* for the first time.

The ^1H-NMR sectrum of compound **3** showed the signals for seven tertiary methyl groups at δ_H 0.72, 0.87, 0.95, 1.00×2, 1.05, 1.18 (each 3H, s), and one secondary methyl group at δ_H 0.88 (d, J = 6.5 Hz), suggesting the presence of friedelane skeleton. The ^{13}C-NMR and DEPT spectra of **3** showed the signals of 30 carbons, including seven quaternary at δ_C 28.18, 30.01, 37.46, 38.31, 39.71, 42.16, and 213.24; four methine at δ_C 42.81, 53.12, 58.24, and 59.50; eleven methylene at δ_C 18.25, 22.29, 30.51, 32.43, 32.79, 35.36, 35.64, 36.02, 39.26, 41.31, and 41.53; and eight methyl carbons at δ_C 6.82, 14.66, 17.95, 18.66, 20.26, 31.79, 32.10, and 35.02. The analytical NMR data of **3** confirmed that compound **3** was friedelan-3-one [8], a compound isolated from *Glochidion* genus for the first time.

By similar way, compounds **4**-**6** were identified as lupeol, glochidonol, and glochidone, respectively [10, 11]. These compounds were also reported in our previous chemial investigations of *Glochidion eriocarpum* [3].

Acknowledgement. *This research was supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2012.24.*

REFERENCES

1. V. V. Chi. *The Dictionary of Medicinal Plants in Vietnam.* Hanoi: Medical Publishing House, 2012.
2. P. V. Kiem, V. K. Thu, P. H. Yen, N. X. Nhiem, N. H. Tung, N. X. Cuong, C. V. Minh, H. T. Huong, J. H. Hyun, H. K. Kang, Y. H. Kim. *New triterpenoid saponins from Glochidion eriocarpum and their cytotoxic activity*, Chemical and Pharmaceutical Bulletin, **57**, 102-105 (2009).
3. V. K. Thư, P. V. Kiệm, P. H. Yến, H. T. Hương, H. T. M. Nhung, N. T. Quỳ, N. X. Cường, N. H. Nam, C. V. Minh. *Các hợp chất lupan phân lập từ cây bòn bọt (Glochidion eriocarpum Champ.)*, Tạp chí Dược học, **50**, 39-43
4. N. X. Nhiem, V. K. Thu, P. V. Kiem, C. V. Minh, B. H. Tai, T. H. Quang, N. X. Cuong, P. H. Yen, H.-J. Boo, H. K. Kang, Y. H. Kim. *Cytotoxic oleane-type triterpene saponins from Glochidion eriocarpum.* Archives of Pharmacal Research, **35**, 19-26 (2012).
5. T. D. Thang, P. C. Kuo, C. S. Yu, Y. C. Shen, T. M. Hoa le, T. Van Thanh, Y. H. Kuo, M. L. Yang, T. S. Wu. *Chemical constituents of the leaves of Glochidion obliquum and their bioactivity,* Archives of Pharmacal Research, **34**, 383-389 (2011).
6. K. Nakano, Y. Oose, Y. Takaishi. *A novel epoxy-triterpene and nortriterpene from callus cultures of Tripterygium wilfordii*, Phytochemistry, **46**, 1179-1182 (1997).
7. K. H. Lee, S. U. Choi, K. R. Lee. *Sesquiterpenes from Syneilesis palmata and their cytotoxicity against human cancer cell lines in vitro*, Archives of Pharmacal Research, **28**, 280-284 (2005).
8. A. Hisham, G. J. Kumar, Y. Fujimoto, N. Hara. *Salacianone and salacianol, two triterpenes from Salacia beddomei*, Phytochemistry, **40**, 1227-1231 (1995).
9. M. Tori, M. Takai, Y. Matsumoto, Y. Moriyama, T. Tsuyuki, T. Takahashi, A. Itai, Y. Iitaka. *Epoxidation of alnus-5-en-3β-yl acetate and conversion of dendropanoxide into daturadiol.* Chemistry Letters, **12**, 527-530 (1983).
10. H. Fuchino, T. Satoh, N. Tanaka. *Chemical evaluation of Betula species in Japan. I. Constituents of Betula ermanii*, Chemical and Pharmaceutical Bulletin, **43**, 1937-1942 (1995).
11. P. Puapairoj, W. Naengchomnong, A. Kijjoa, M. M. Pinto, M. Pedro, M. S. Nascimento, A. M. Silva, W. Herz. *Cytotoxic activity of lupane-type triterpenes from Glochidion sphaerogynum and Glochidion eriocarpum two of which induce apoptosis*, Planta Medica, **71**, 208-213 (2005).

Corresponding author: **Phan Van Kiem**
Institute of Marine Biochemistry, Vietnam Academy of Science and Technology
18, Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

E-mail: phankiem@yahoo.com.