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SESQUITERPENOIDS FROM *HOMALOMENA PIERREANA* ENGL.

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ABSTRACT

Chemical investigation of *Homalomena pierreana* Engl. resulted in the isolation of six known sesquiterpenoids, namely 1*β*,4*β*,6*α*-trihydroxyeudesmane (**1**), 1*β*,4*β*,7*α*-trihydroxyeudesmane (**2**), oplodiol (**3**), bullatantriol (**4**), homalomenol A (**5**), and oplopanone (**6**). Their structures were elucidated by 1D and 2D NMR spectral interpretation.

Keywords: Homalomena pierreana, Araceae, sesquiterpenoid, bullatantriol, 1*β*,4*β*,6*α*-trihydroxyeudesmane, 1*β*,4*β*,7*α*-trihydroxyeudesmane, oplopanone, oplodiol, homalomenol A.

1. INTRODUCTION

Homalomena is a genus of flowering plants of the family Araceae. It is estimated to be composed of approximately 80 to 150 species. *Homalomena* species are primarily found in southern Asia and the southwestern Pacific. In Vietnam, *Homalomena* plants were used to treat rheumatism, joint pain, twitching, numbness. Currently, *Homalomena* species are used as drugs to treat rheumatic diseases, reduce pain, and stimulate digestion. *Homalomena pierreana* Engl. (Araceae) is distributed in some districts of Quang Ngai and Quang Nam provinces such as Tra My, Tra Nam, Phuoc Son, Phuoc Thanh, Phuoc Hiep... This plant is classified to other species by: Leaf blade rounded or truncated at base (*H. pierreana* Engl.); versus leaf blade sagittate at base of *H. occulta* (Lour.) Schott., *H. aromatica* (Sprengel) Schott., *H. gigantea* Engl. & K. Krause, and *H. tonkinensis* Engl.). During our survey at Tarin dam (Thuong Nhat commune), Mo water fall (Huong Loc commune), Nam Dong district for collecting the *Homalomena* samples within the frame of Vietnam-USA protocol project, we found and collected the species *H. pierreana* that was previously described and recorded in Vietnam Red Book 2007, Part II:

plant [1]. In this paper, we report the isolation and structure elucidation of six sesquiterpenoids from the methanol extract of the *Homalomena pierreana* rhizomes.

2. EXPERIMENTAL

2.1. General methods

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker AM500 (Billerica, MA, USA) FT-NMR spectrometer. TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany), YMC RP-18 (30–50 μm, Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan), and Diaion HP-20 (Sigma-Aldrich, USA) resins. TLC used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F_{254S} plates (1.15685.0001, Merck), and compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3–5 min.

2.2. Plant materials

Samples of *H. pierreana* were collected at Bach Ma National Park, Thua Thien - Hue, Vietnam, in May 2013, and identified by Prof. Ninh Khac Ban. The voucher specimen was deposited at the Department of Biological Resources, Institute of Marine Biochemistry, VAST, Vietnam.

2.3. Extraction and isolation

Dried powder of *H. pierreana* rhizomes (1.5 kg) was extracted three times with MeOH under ultrasonic conditions. The obtained solutions were filtered, combined, and concentrated under reduced pressure to yield a methanol extract (200 g). This residue was suspended in water (4.0 L) and partitioned in turn with *n*-hexane, ethyl acetate (3 × 4.0 L) to obtain *n*-hexane extract (65.0g), EtOAc extract (40.0g), and water layer. The EtOAc extract was next subjected to YMC CC and eluted with MeOH/H_2O 1:2 to give 5 fractions (E1−E5). The E1 fraction (650 mg) was first subjected to silica gel CC eluting with *n*-hexane:acetone 2.5:1, and further separated by YMC RP-18 CC with MeOH:H_2O 1:2 to afford compound **2** (22 mg). The E3 fraction (830 mg) was subjected to silica gel CC (*n*-hexane:acetone 2:1) to afford compound **4** (13 mg) and the HAE3.1 subfraction was further separated by silica gel CC (*n*-hexane:acetone 2:1) to afford compound **1** (15 mg). The E4 fraction (2.5 g) was subjected to YMC RP-18 CC to give HAE4.1 and HAE4.2 fractions. The HAE4.1 fraction was then subjected to Sephadex LH-20 CC eluted with MeOH:H_2O 1:1 to afford compound **6** (10 mg). The HAE4.2 fraction was further purified by silica gel CC (dichloromethane:ethyl acetate 5:1) to afford compounds **3** (12 mg) and **5** (10 mg).

*1β,4β,6α-trihydroxy-eudesmane (**1**):* White crystal, mp. 186 - 188°, $[\alpha]_D$ +42° (MeOH, *c* 0.25). ^{1}H NMR (500MHz, CD_3OD): δ (ppm) 3.16 (1H, dd, *J* = 4.0, 11.5 Hz, H-1), 1.52 (1H, m, H_a-2), 1.94 (1H, dd, *J* = 3.0, 11.5 Hz, H_b-2), 1.56 (1H, dd, *J* = 12.5, 4.0 Hz, H_a-3), 1.65 (1H, dt, *J* = 12.5, 2.5 Hz, H_b-3), 1.04 (1H, d, *J* = 10.0 Hz, H-5), 3.87 (1H, t, *J* = 10.0 Hz, H-6), 1.31 (1H, m, H-7), 1.31 (1H, m, H_a-8), 1.49 (1H, m, H_b-8), 1.01 (1H, m, H_a-9), 1.88 (1H, dt, *J* = 13.0, 3.0 Hz, H_b-9), 2.33 (1H, td, *J* = 7.0, 2.0 Hz, H-11), 0.95 (3H, d, *J* = 7.0 Hz, H-12), 0.89 (3H, d, *J* = 7.0 Hz, H-13), 0.99 (3H, s, H-14), 1.43 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

*1β,4β,7α-trihydroxy-eudesmane (**2**):* White crystal, mp. 135 - 141°, $[\alpha]_D$ −7.2° (MeOH, *c* 0.25). ^{1}H NMR (500MHz, CD_3OD): δ (ppm) 3.24 (1H, dd, *J* = 4.0, 12.0 Hz, H-1), 1.54 (1H, H_a-2), 1.94 (1H, m, H_b-2), 1.54 (1H, H_a-3), 1.72 (1H, m, H_b-3), 1.56 (1H, H_a-6), 1.66 (1H, d, *J* = 10.0 Hz, H_b-6), 1.62 (1H, d, *J* = 7.0 Hz, H-11), 0.98 (3H, d, *J* = 7.0 Hz, H-12), 0.98 (3H, d, *J* = 7.0 Hz, H-13), 0.99 (3H, s, H-14), 1.31 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

*Oplodiol (**3**):* White crystal, mp. 106-107°, $[\alpha]_D$ −52° ($CHCl_3$, *c* 0.25). ^{1}H NMR (500MHz, CD_3OD): δ (ppm) 3.24 (1H, dd, *J* = 4.0, 12.0 Hz, H-1), 1.26 (1H, dd, *J* = 5.0, 12.0 Hz, H-5), 5.33 (1H, m, H-7), 1.80 (1H, dd, *J* = 17.0 Hz, H_a-9), 2.08 (1H, dd, *J* = 6.0, 17.0 Hz, H_b-9), 2.21 (1H, t, *J* = 6.5 Hz, H-11), 1.05 (3H, d, *J* = 7.5 Hz, H-12), 1.05 (3H, d, *J* = 7.5 Hz, H-13), 0.96 (3H, s, H-14), 1.16 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

*Bullatantriol (**4**):* White crystals, mp. 174 - 175°, $[\alpha]_D$ +82.8° (MeOH, *c* 0.25). ^{1}H NMR (500MHz, CD_3OD): δ (ppm) 3.31 (1H, d, *J* = 4.0 Hz, H-1), 1.57 (1H, m, H_a-2), 1.87 (1H, dq, H_b-2), 1.48 (1H, dd, *J* = 5.0, 14.0 Hz, H_a-3), 1.64 (1H, m, H_b-3), 0.94 (1H, d, *J* = 10.0 Hz, H-5), 2.27 (1H, qd, Hz, H-6), 1.36 (1H, dd, *J* = 10.0, 14.0 Hz, H_a-7), 2.11 (1H, d, *J* = 14.0 Hz, H_b-7), 1.38 (1H, m, H_a-8), 2.10 (1H, H_b-8), 1.59 (1H, H_a-9), 1.19 (1H, t, *J* = 7.0 Hz, H_b-9), 1.24 (3H, s, H-12), 1.25 (3H, s, H-13), 1.03 (3H, s, H-14), 1.29 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

*Homalomenol A (**5**):* Oil, $[\alpha]_D$ + 33.2° ($CHCl_3$, *c* 0.25). ^{1}H NMR (500 MHz, CD_3OD): δ (ppm) 3.34 (1H, d, *J* = 5.0 Hz, H-1), 1.55 (1H, m, H_a-2), 1.88 (1H, m, H_b-2), 1.44 (1H, td, *J* = 14.0, 5.0 Hz, H_a-3), 1.65 (1H, m, H_b-3), 1.02 (1H, d, *J* = 11.5 Hz, H-5), 3.05 (1H, m, H-6), 5.12 (1H, dt, *J* = 10.0, 1.0 Hz, H-7), 1.20 (1H, m, H_a-8), 2.08 (1H, m, H_b-8), 1.69 (3H, s, H-12), 1.67 (3H, s, H-13), 1.05 (3H, s, H-14), 1.11 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

*Oplopanone (**6**):* White crystal, mp. 96 - 97°, $[\alpha]_D$ −16° ($CHCl_3$, *c* 0.25). ^{1}H NMR (500MHz, CD_3OD): δ (ppm) 1.42 (1H, H_a-2), 1.77 (1H, m, H_b-2), 2.74 (1H, m, H-6), 1.54 (1H, H_a-8), 2.03 (1H, m, H_b-8), 0.70 (3H, d, *J* = 7.0 Hz, H-12), 0.93 (3H, d, *J* = 7.0 Hz, H-13), 1.17 (3H, s, H-14), 2.21 (3H, s, H-15). ^{13}C NMR (125MHz, CD_3OD) see Table 1.

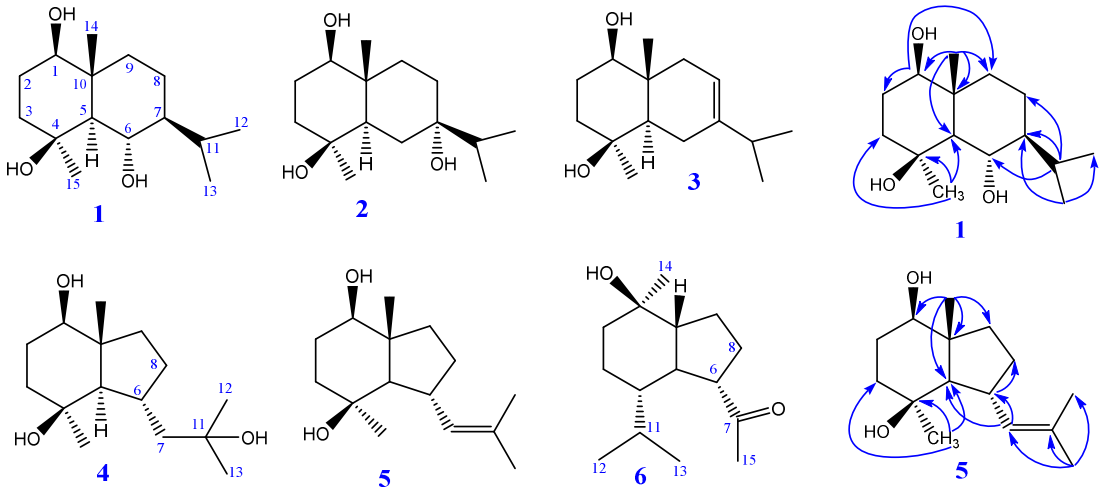

Figure 1. The structures of compounds **1**–**6** and key HMBC correlations of compounds **1** and **5.**

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white crystals. The peaks at δ_H 0.99 (3H, s) and 1.43 (3H, s) in the ^{1}H NMR spectrum indicated the presence of two tertiary methyl groups. The proton signals at δ_H 0.95 (3H, d, *J* = 7.0 Hz) and 0.89 (3H, d, *J* = 7.0 Hz) indicated the presence of an isopropyl group. Two oxymethine protons at δ_H 3.16 (1H, dd, *J* = 4.0, 11.5 Hz) and 3.87 (1H, d, *J* = 10.0 Hz) were correlated to the carbon resonances at δ_C 80.07 (C-1) and 69.99 (C-6), respectively, in the HSQC spectra. The ^{13}C NMR spectrum revealed 15 signals including four methyl groups, two oximethine carbons at δ_C 80.07 (C-1), 69.99 (C-6), and one oxygenated quaternary carbon at δ_C 72.75 (C-4). The HMBC spectrum indicated some main cross-peaks of proton H-1 (δ_H 3.16) with C-2/C-9/C-10, H-11 (δ_H 2.33) with C-6/C-7/C-8/C-12/C-13, H-14 (δ_H 0.99) with C-1/C-5/C-9/C-10, and H-15 (δ_H 1.43) with C-3/C-4/C-5. The equatorial position of the hydroxyl group at C-1 was supported by the coupling constants of H-1 (*J* = 4.0 and 11.5 Hz) in the ^{1}H NMR spectrum [2]. Another coupling constant (*J* = 10.0 Hz) that was attributed to H-6 implicated a trans-diaxial spin-spin relation between H-6 and H-7 protons. Assuming a chair–chair conformation as frequently found for eudesmanolides with ab-oriented axial angular methyl group, and considering the equatorial arrangement of the three hydroxyl groups at C-1, C-4, and C-6 [3]. Based on above spectroscopic analysis, compound **1** was designed to be 1*β*,4*β*,6*α*-trihydroxy-eudesmane [4].

Table 1. ^{13}C NMR spectral data of compounds **1**–**6** and reported compounds.

C	$^a\delta_C$	**1**	$^b\delta_C$	**2**	$^c\delta_C$	**3**	$^d\delta_C$	**4**	$^e\delta_C$	**5**	$^f\delta_C$	**6**
1	80.1	80.07	80.5	80.53	80.0	80.80	80.7	80.70	80.0	80.88	73.2	73.46
2	27.6	27.64	40.1	27.67	40.7	27.59	28.6	28.63	36.7	28.69	41.9	42.82
3	42.4	42.39	40.6	40.64	39.7	40.38	42.0	41.97	40.7	41.66	22.9	23.90
4	72.8	72.75	72.1	72.09	71.0	71.46	72.4	72.45	71.8	72.25	49.4	50.79
5	57.8	57.79	46.1	46.10	46.5	47.92	60.3	60.27	59.1	60.70	46.7	47.99
6	70.0	69.99	29.3	30.07	26.9	24.15	33.0	33.01	35.0	36.10	55.7	56.89
7	53.0	53.01	74.8	74.83	142.1	143.32	52.1	52.14	132.2	134.29	211.6	214.70
8	19.4	19.43	30.1	29.35	116.2	117.25	33.4	33.43	29.6	30.81	28.7	29.77
9	39.2	39.18	27.7	35.77	23.2	42.01	40.2	40.19	28.0	40.14	25.2	26.50
10	42.1	42.06	35.8	40.06	37.9	38.95	48.1	48.10	47.2	48.48	57.0	57.98
11	27.0	26.99	40.5	40.50	35.1	36.35	72.6	72.62	128.7	129.06	29.4	30.70
12	21.7	21.69	17.5	17.51	21.3	21.68	30.0	29.97	18.1	18.24	15.5	16.00
13	16.1	16.07	17.4	17.38	21.9	22.25	30.3	30.27	25.8	25.98	21.8	22.27
14	13.9	13.87	12.2	12.18	11.8	12.16	15.0	15.01	14.2	14.84	20.1	19.96
15	34.5	34.47	29.9	29.87	29.9	29.89	32.0	31.98	30.7	30.85	29.4	29.66

$^a\delta_C$ of 1*β*,4*β*,6*α*-trihydroxy-eudesmane [4], $^b\delta_C$ of 1*β*,4*β*,7*α*-trihydroxy-eudesmane [2], $^c\delta_C$ of oplodiol [6], $^d\delta_C$ of bullatantriol [5], $^e\delta_C$ of homalomenol A [2], $^f\delta_C$ of oplopanone [6].

Compounds **2**, **3** were also obtained as white crystals. Their ^{13}C NMR data suggested that these compounds have the same carbon skeleton as compound **1**. However, the ^{13}C NMR spectrum of **2** indicated the presence of one oxymethine carbon at δ_C 80.53 (C-1), and two oxygenated quaternary carbons at δ_C 72.09 (C-4) and 74.83 (C-7) instead of two oximethine carbons and one oxygenated quaternary carbon as in compound **1**. Thus, compound **2** was

identified as 1β,4β,7α-trihydroxy-eudesmane which was isolated from *Homalomena aromatica* [2]. Compound **3** has been determined as oplodiol on the basis of NMR spectral data and direct comparison with the reference compound isolated from *Pulicaria paludosa* [7]. On the basis of 2D NMR data, some chemical shifts reported for **2** and **3** must be revised as shown in table 1.

Compound **4** was obtained as a white crystal. The ^{1}H and ^{13}C NMR features were typical for a sesquiterpene. The ^{1}H NMR spectrum indicated the presence of four tertiary methyl groups [δ_H 1.03 (H-14), 1.24 (H-12), 1.25 (H-13), and 1.29 (H-15), each 3H, s], the methine proton attached to oxygen-bearing carbon displayed a doublet peak at δ_H 3.31 (1H, d, J = 4.0 Hz). The ^{13}C NMR spectrum revealed 15 signals including four methyl groups, one oxymethine carbon at δ_C 80.07 (C-1) and two oxygenated quaternary carbons at δ_C 72.45 (C-4) and 72.68 (C-11). Characteristic ^{13}C NMR signals at δ_C 80.07 (CH), 72.45 (C), 72.68 (C), 48.10 (C), and 15.01 (CH_3) (Table 1) suggested a eudesmane skeleton with one secondary and two tertiary hydroxyl groups. The NMR data showed general features similar to those of bullatantriol, an oppositane-type sesquiterpenoid previously isolated from *Chimonathus praecox* and *Homalomena aromatica* [2, 5]. Thus, compound **4** was identified as bullatantriol [5].

The other compounds were determined as homalomenol A (**5**) and oplopanone (**6**) by direct comparison of their spectroscopic data (1D, 2D NMR) with the literature values [2, 6]. In conclusion, six known sesquiterpenoids were isolated and structurally elucidated from *Homalomena pierreana*. This is the first report of these compounds from this species.

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TÓM TẮT

CÁC HỢP CHẤT SESQUITECPENOID PHÂN LẬP ĐƯỢC TỪ CÂY *HOMALOMENA PIERREANA* ENGL.

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Bằng các phương pháp sắc ký kết hợp, sáu hợp chất sesquitecpenoid, 1*β*,4*β*,6*α*-trihydroxyeudesmane (**1**), 1*β*,4*β*,7*α*-trihydroxyeudesmane (**2**), oplodiol (**3**), bullatantriol (**4**), homalomenol A (**5**) và oplopanone (**6**), được phân lập từ cặn chiết metanol của cây *Homalomena pierreana*. Cấu trúc hóa học của các hợp chất **1**–**6** được xác định bằng các phương pháp phổ như phổ cộng hưởng từ hạt nhân (NMR) một chiều và hai chiều, kết hợp so sánh với các số liệu đã được công bố. Đây là lần đầu tiên các hợp chất này được phân lập từ loài *H. pierreana*.

Từ khóa: Homalomena pierreana, sesquiterpenoid, bullatantriol, 1*β*,4*β*,6*α*-trihydroxyeudesmane, 1*β*,4*β*,7*α*-trihydroxyeudesmane, oplopanone, oplodiol, homalomenol A.