

EUPOLAURIDINE ALKALOIDS OF *POLYALTHIA NEMORALIS*

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Abstract

Two eupolauridine alkaloids, eupolauridine (**1**) and 8-methoxyeupolauridine (**2**), together with a phenanthrene compound, 2,7-dihydroxy-3,6-dimethoxyphenanthrene (**3**), were isolated from the ethyl acetate extract of *Polyalthia nemoralis* barks. Their structures were elucidated on the basis of spectroscopic analysis and comparison with related known compounds. These compounds were evaluated the cytotoxicity on seven human cancer cell lines including KB, MCF7, LU-1, HepG2, LNCap, SW626 and SW480.

Keywords. *Polyalthia nemoralis*, eupolauridine alkaloid, phenanthrene.

1. INTRODUCTION

Polyalthia nemoralis scatters in the primary forests and the mountainous lands at elevations below 400 m. In Vietnam, this plant was distributed in the some northern and central provinces. The roots of this plant, called *Radix Polyalthia nemoralis*, was used in the traditional medicine for treatment of chronic gastritis and indigestion [1]. In continuation of our phytochemistry of *Polyalthia* genus, we investigated on the chemical constituent and biological activity of *Polyalthia nemoralis*. Herein, we described the isolation and structure elucidation of two alkaloids, eupolauridine (**1**), 8-methoxyeupolauridine (**2**), together with a phenanthrene compound, 2,7-dihydroxy-3,6-dimethoxyphenanthrene (**3**). These compounds were evaluated the cytotoxicity on seven human cancer cell lines including KB (human carcinoma in the mouth), MCF7 (human breast cancer), LU-1 (human lung cancer), HepG2 (human hepatoma cancer), LNCap (human prostate cancer), SW626 (human ovarian adenocarcinoma) and SW480 (human colon adenocarcinoma).

2. EXPERIMENTAL

General Experimental Procedures: Melting points were measured with a Model Thermo Scientific Mel-Tem 3.0 instrument. ESI-MS and APCI-MS were measured with a AGILENT 6120 mass spectrometer. NMR spectra were recorded by a

Bruker Avance 500 MHz instrument using TMS as internal standard.

Plant material: Barks of *Polyalthia nemoralis* were collected at Phulinh district, Hagiang province, Vietnam in August 2012 and the plant material was identified by Dr. Nguyen Quoc Binh, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology where a voucher specimen (VN-0709) was deposited.

Extraction and Isolation: Dried barks of *P. nemoralis* (1,2 kg) were ground in a hammer mill, then extracted with MeOH by percolation for 24 h at room temperature in three times. The extracts were combined and evaporated *in vacuo* and the residue was suspended in H₂O. The suspension was successively extracted with *n*-hexane and ethyl acetate to obtain *n*-hexane residue (PNH, 12.7 g) and ethyl acetate residue (PNE, 13.9 g). The PNE residue was subjected to column chromatography on silica gel using gradient elution with CH₂Cl₂-MeOH to afford 18 fractions (NE1-NE18). Fraction NE2 (0.5 g) was further separated by column chromatography on silica gel using CH₂Cl₂/MeOH (0→10 % MeOH) as eluent to yield **3** (6 mg). The fraction NE8 (0.49 g) was applied to a gel filtration column with Sephadex LH-20 using CH₂Cl₂/MeOH (1:9) as solvent, to afford **1** (32 mg). Fraction NE9 (0.2 g) was also subjected to a column chromatography on Sephadex LH-20 using CH₂Cl₂/MeOH (1:9) to afford **2** (6 mg).

Eupolauridine (1): yellow solid, mp. 125-126 °C.

^1H NMR and ^{13}C NMR data, see table 1. ESI-MS m/z : 205 $[\text{M}+\text{H}]^+$.

8-methoxyeupolauridine (2): yellow solid, mp. 137 -138 °C

^1H NMR and ^{13}C NMR data, see table 1. ESI-MS m/z : 235 $[\text{M}+\text{H}]^+$.

2,7-dihydroxy-3,6-dimethoxyphenanthrene (3): light yellow solid, mp. 140-141 °C.

^1H NMR (500 MHz, CDCl_3) δ_{H} ppm: 7.31 (2H, s, H-1 and H-8), 7.49 (2H, s, H-9 and H-10), 7.74 (2H, s, H-4 and H-5), 5.86 (2H, s, 2-OH and 7-OH), 4.11 (6H, 3-OCH₃ and 6-OCH₃).

^{13}C NMR (125 MHz, CDCl_3) δ_{C} ppm: 56.09 (3-OCH₃ and 6-OCH₃), 101.97 (C-4 and C-5), 111.56 (C-1 and C-8), 124.27 (C-4a and C-4b), 124.48 (C-9 and C-10), 126.87 (C-8a and C-10a), 144.99 (C-2 and C-7), 147.06 (C-3 and C-6).

APCI-MS m/z : 271 $[\text{M}+\text{H}]^+$.

Cytotoxicity assay

The cancer cell lines (KB, MCF7, LU-1, HepG2, LNCap, SW62 and SW480) were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/mL), streptomycin (100 $\mu\text{g}/\text{mL}$) and gentamicin (10 $\mu\text{g}/\text{mL}$). Stock solutions of compounds were prepared in DMSO/ H_2O (1/9), and cytotoxicity assays were carried out in 96-well microtiter plates against KB, MCF7, LU-1, HepG2, LNCap, SW62 and SW480 cancer cell lines (3×10^3 cells/mL) using a modification of the published method [2]. After 72 h incubation at 37 °C in air/ CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50 % of the control. Ellipticine was used as a reference compound.

3. RESULTS AND DISCUSSION

Successive chromatographies of the EtOAc crude extract of *P. nemoralis* barks on silica gel, Sephadex LH-20 yielded three compounds: the eupolauridine alkaloids **1-2** and phenanthrenoid **3**. Their structures were determined based on the NMR analysis.

Compound **1** was isolated as yellow solid, mp 125-126 °C. Its ^1H NMR signals in CDCl_3 exhibited two AB and A'B' system couplings at low field δ 7.36 (d, 1H, $J = 6.0$ Hz) and 8.64 (1H, d, $J = 6.0$

Hz), δ 7.42 (1H, d, $J = 8.0$ Hz) and 7.93 (1H, d, $J = 8.0$ Hz). The ^{13}C NMR spectrum showed resonance signals of four aromatic methine groups at δ 117.40, 122.56, 131.08 and 149.72. The signal at δ 149.72 indicated that one methine group was linked to a nitrogen atom. The remaining signals were assigned for four quaternary carbons at δ 120.72, 135.00, 139.68 and 162.66. Based on ESI-MS spectrum that exhibited a protonated molecular ion at m/z 205 $[\text{M}+\text{H}]^+$, the molecular formula was determined to be $\text{C}_{14}\text{H}_8\text{N}_2$. The molecular formula approved of the symmetry in the structure of **1**. It's mean that the signals in the ^1H NMR and ^{13}C NMR were belonged to a half of the structure. The HMBC spectrum showed the correlations between the signals at δ_{H} 8.64 (H-2) with δ_{C} 117.40 (C-3), 135.00 (C-3a) and 162.66 (C-10b), δ_{H} 7.36 (H-3) with δ_{C} 149.78 (C-2), 117.40 (C-3), 135.00 (C-3a) and 162.66 (C-10b), δ_{H} 7.93 (H-10) with δ_{C} 131.08 (C-9), 139.68 (C-10a) and 162.66 (C-10b), δ_{H} 7.42 (H-9) with δ_{C} 122.56 (C-10) and 139.68 (C-10a). Based on these correlations and literatures [3, 4], the structure of **1** was determined to be eupolauridine (Fig. 1).

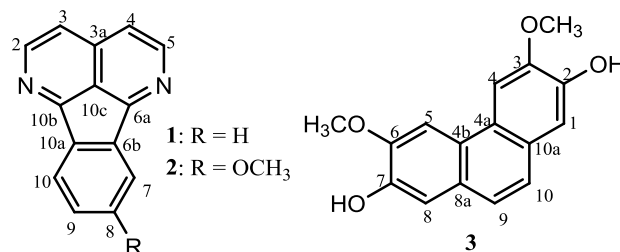


Figure 1: Structures of **1-3**

Compound **2** was obtained as yellow solid, mp 137 - 138 °C. The ^1H NMR spectrum showed the presence of an ABX coupling system of three aromatic protons at δ 6.94 (1H, dd, $J = 2.5, 8.0$ Hz), 7.57 (1H, d, $J = 2.5$ Hz) and 7.88 (1H, d, $J = 8.0$ Hz) and two other AB coupling systems at δ 8.62 (1H, d, $J = 6.0$ Hz) and 7.33 (1H, d, $J = 6.0$ Hz), δ 8.68 (1H, d, $J = 6.0$ Hz) and 7.41 (1H, d, $J = 6.0$ Hz). The remaining signal was assigned for a methoxy group at δ 3.93 (3H, s). The resonated signals in the ^{13}C NMR were concentrated in the field of aromatic carbons including seven methine groups and seven quaternary carbons. There was only one signal of methoxy group in the high field at δ 55.81. The presence of the *pseudo*-molecular ion at m/z 235 in the ESI-MS spectrum and the above spectra data confirmed the molecular formula of $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}$. The correlations between H-2 with C-3, C-3a and C-10b,

H-3 with C-2, C-4 and C-10c, H-4 with C-3, C-10c and C-5, H-7 with C-9, C-8 and C-10a, H-9 with C-10a, C-7, H-10 with C-6b, C-8 and C-10b in the HMBC spectrum approved for the eupolauridine alkaloid skeleton of **2**. The methoxy group was linked to the eupolauridine alkaloid skeleton at C-3 position. In combination of the above spectra analysis with literature [3], compound **2** was determined to be 8-methoxyeupolauridine (Fig. 1).

Compound **3** is a light yellow solid, mp 140-141 °C. The ¹H NMR showed the presence of three isolated aromatic protons at δ 7.31 (1H, s), 7.49 (1H, s) and 7.74 (1H, s), a methoxy group at δ 4.11 (3H, s). The remaining signal at δ 5.86 was assigned for an hydroxy group on the basis of correlations in the HSQC spectrum. The ¹³C NMR spectrum had the signals of three aromatic methine groups at δ 101.97, 111.56 and 124.48 and four aromatic quaternary carbons at δ 124.27, 126.87, 144.99 and 147.06. The carbon of methoxyl group was

resonated at δ 56.09. The ESI-MS exhibited a *pseudo*-molecular ion at *m/z* 269 [M-H]⁻. Based on the above spectra data the molecular formula was deduced to be C₁₆H₁₄O₄. It's mean that the structure of **3** was asymmetric and the signals in NMR spectra were only belonged to a half of the structure. The phenanthrene skeleton was determined by the correlations between H-5 with C-6, C-7, C-4b and C-8a, H-9 with C-8, C-8a and C-4b, H-8 with C-7, C-9, C-8a and C-4b in the HMBC spectrum. The methoxyl group and hydroxyl group were successively confirmed at C-6 and C-7 by the correlation between the protons of methoxyl group with C-6 and proton of hydroxyl group (δ_H 5.86) with C-7, C-6 and C-8. The structure of **3** was finally determined as 2,7-dihydroxy-3,6-dimethoxyphenanthrene (Fig. 1). There was only one report on the isolation of this compound from *Dehaasia longipedicellata* and its crystallographic structure [5].

Table 1: ¹H NMR and ¹³C NMR data of **1-2**

Position	1			2		
	δ [*] _C	δ _C	δ _H	δ _C [#]	δ _C	δ _H
2	149.8	149.78	8.64 (d, 6.0)	149.6	149.74	8.62 (d, 6.0)
3	117.3	117.40	7.36 (d, 6.0)	116.3	116.40	7.33 (d, 6.0)
3a	135.0	135.00	-	134.9	135.06	-
4	117.3	117.40	7.36 (d, 6.0)	117.8	117.86	7.41 (d, 6.0)
5	149.8	149.78	8.64 (d, 6.0)	149.7	149.84	8.68 (d, 6.0)
6a	162.7	162.66	-	162.5	162.67	-
6b	139.8	139.68	-	142.0	142.12	-
7	122.5	122.56	7.93 (d, 8.0)	109.0	109.21	7.57 (d, 2.5)
8	131.0	131.08	7.42 (d, 8.0)	162.2	162.39	-
9	131.0	131.08	7.42 (d, 8.0)	115.5	115.67	6,94 (dd, 2.5, 8.0)
10	122.5	122.56	7.93 (d, 8.0)	123.7	123.85	7.88 (d, 8.0)
10a	139.8	139.68	-	131.9	132.05	-
10b	162.7	162.66	-	162.8	162.93	-
10c	120.7	120.72	-	121.3	121.46	-
8-OCH ₃				55.7	55.81	3.93 (s)

¹H NMR was recorded in CDCl₃, 500 MHz; ¹³C NMR was recorded in CDCl₃, 125 MHz.

δ^{*}_C: ¹³C NMR data of eupolauridine in CDCl₃ at 90.56 MHz [4].

δ[#]_C: ¹³C NMR data of 8-methoxyeupolauridine in CDCl₃ at 125 MHz [3].

Compounds **1-3** were evaluated for their cytotoxicity against seven human cancer cell lines including KB, MCF7, LU-1, HepG2, LNCap, SW626 and SW480. All of three compounds exhibited cytotoxicity against tested cancer cell lines (table 2). Compound **1** was the most active with IC₅₀ value of 6.79-14.26 μg/mL.

4. CONCLUSION

Three compounds including two alkaloids, eupolauridine (**1**) and 8-methoxyeupolauridine (**2**), together with a phenanthrenoid, 2,7-dihydroxy-3,6-dimethoxyphenanthrene (**3**), were yielded from the EtOAc crude extract of *P. nemoralis* barks by

repeated chromatographies on silica gel and Sephadex LH-20. These compounds were isolated from *Polyalthia* genus for the first time. All of them

exhibited cytotoxicity against tested cancer cell lines, KB, MCF7, LU-1, HepG2, LNCap, SW626 and SW480.

Table 2: *In vitro* cytotoxic activity of 1-3

Compounds	IC ₅₀ , µg/mL						
	KB	MCF7	LU-1	HepG2	LNCap	SW626	SW480
1	45.86	35.13	32.82	37.53	37.85	39.13	33.07
2	7.34	13.82	14.26	6.79	9.77	10.76	7.25
3	18.94	17.36	18.53	14.71	19.68	21.63	14.11
Ellipticine	0.57	0.44	0.50	0.51	0.54	0.48	0.41

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