



A new cyclohexenone and two new phenanthrones from *Elatostema tenuicaudatum*

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A new cyclohexenone and two new phenanthrones from *Elatostema tenuicaudatum*

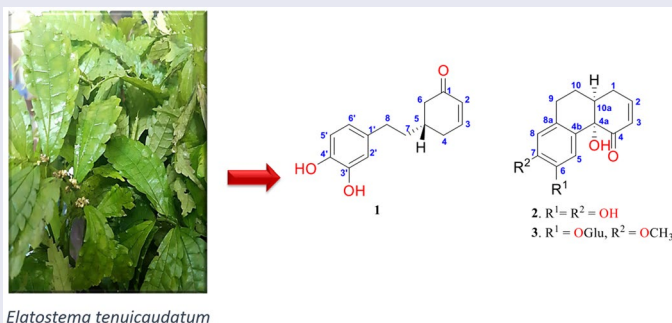
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ABSTRACT

A new cyclohexenone, 5S-(3,4-dihydroxyphenethyl)cyclohex-2-en-1-one (**1**), along with two new phenanthrones, (4aS,10aS)-1,9,10,10a-tetrahydro-4a,6,7-trihydroxy-4(4aH)-phenanthrone (**2**) and (4aS,10aS)-4a-hydroxy-7-methoxy-1,9,10,10a-tetrahydro-4(4aH)-phenanthrone 6-O- β -D-glucopyranoside (**3**) were isolated from the whole plant of *Elatostema tenuicaudatum*. The structures were determined by spectroscopic methods including 1D, 2D NMR and HR-ESI-MS. The absolute configurations of compounds **1–3** were determined by their experimental and calculated ECD spectral data. The isolates were evaluated for inhibitory activity on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophage cells, and compound **2** showed the strongest activity with an IC_{50} value of $9.5 \pm 1.2 \mu M$.

GRAPHICAL ABSTRACT



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KEYWORDS

Urticaceae; *Elatostema tenuicaudatum*; cyclohexenone; phenanthrones; NO inhibition

1. Introduction

Elatostema tenuicaudatum W.T.Wang (Urticaceae) is a small shrub, native to the southern China and the northern Vietnam. It is found in dark damp places, along streamsides, thickets, valleys and forests [1,2]. *E. tenuicaudatum* has been known as tea, vegetable and traditional medicine in Vietnam. It has been used to clear heat, detoxify, reduce swelling and treat colitis, enteritidis, diabetes and cancer. Analysis of chemical characteristics of *E. tenuicaudatum* showed the presence of hydrogenated phenanthrene glycosides [3] and cyclohexanol glucoside derivatives [4]. In our previous paper, three new dihydrophenanthrenes were isolated and identified from the whole plant of this species [5]. On continuous studies of the same extracts, three new compounds, 5S-(3,4-dihydroxyphenethyl) cyclohex-2-en-1-one (**1**), (4aS,10aS)-1,9,10,10a-tetrahydro-4a,6,7-trihydroxy-4(4aH)-phenanthrone (**2**) and (4aS,10aS)-4a-hydroxy-7-methoxy-1,9,10,10a-tetrahydro-4(4aH)-phenanthrone 6-O- β -D-glucopyranoside (**3**) (Figure 1) were isolated. Herein, the isolation, structure elucidation and NO production inhibitory activity of compounds **1**–**3** are reported.

2. Results and discussion

Compound **1** was isolated as white solid. The positive HR-ESI-MS of **1** gave quasi-molecular ion peaks at m/z 233.1169 $[M+H]^+$ and 255.0986 $[M+Na]^+$, consistent with the molecular formula of $C_{14}H_{16}O_3$. The ^1H NMR spectrum of **1** displayed an ABX system of a 1,3,4-trisubstituted benzen ring at δ_H 6.68 (1H, d, $J=7.8$ Hz, H-5'), 6.64 (1H, d, $J=1.8$ Hz, H-2'), and 6.52 (1H, dd, $J=7.8, 1.8$ Hz, H-6'); two olefinic protons at δ_H 7.12 (1H, m, H-3), 5.99 (1H, m, H-2) and nine aliphatic protons in the range δ_H 2.54–1.68. The ^{13}C NMR and HSQC spectra of **1** showed the presence of a ketone carbonyl at δ_C 202.7 (C-1), two olefinic carbons at δ_C 153.0 (C-3), 130.0 (C-2); a 1,3,4-trisubstituted benzen ring at δ_C 146.2 (C-3'), 144.3 (C-4'), 134.8 (C-1'), 120.6 (C-6'), 116.4 (C-2'), 116.3 (C-5'); four methylene carbons at δ_C 45.1 (C-6), 38.7 (C-7), 33.2 (C-4), 33.0 (C-8); and one methine carbon at δ_C 35.9 (C-5). The characteristic ^1H NMR signals at δ_H 7.12 (1H, m, H-3), 5.99 (1H, m, H-2) and ^{13}C NMR signals at δ_C 202.7 (C-1), 153.0 (C-3), 130.0 (C-2) were ascribed to an α,β -unsaturated ketone moiety. The HMBC correlations from H-3 and H-6 to C-1 and C-5 suggested the presence of the α,β -unsaturated cyclohexanone [6]. In addition, the COSY correlations between H-7 and H-8, as well as

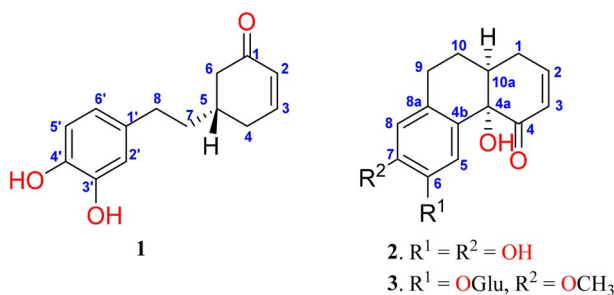


Figure 1. Chemical structures of compounds **1**–**3**.

HMBC correlations from H-7 and H-8 to C-1', from H-8 to C-2' and C-6' indicated the exist of the phenethyl moiety in **1**. The position of the phenethyl moiety at C-5 was determined by the HMBC correlations from H-7 to C-4, C-5 and C-6, from H-4 and H-6 to C-7 (Figure 2). The ECD spectrum of **1** exhibited negative Cotton effects at 312 nm ($\Delta\epsilon$: -0.13) and 234 ($\Delta\epsilon$: -1.21) which are similar with those of 5*S*-(4-methoxyphenylethyl) cyclohex-2-en-1-one [312 nm ($\Delta\epsilon$: -0.21) and 233 nm ($\Delta\epsilon$: -2.71)], indicating the same 5*S* absolute configuration [7]. Therefore, the structure of **1** was elucidated to be 5*S*-(3,4-dihydroxyphenethyl)cyclohex-2-en-1-one.

Compound **2** was isolated as brown oil and assigned the molecular formula $C_{14}H_{14}O_4$ according to the HR-ESI-MS quasi-molecular ion peak at m/z 247.0707 $[M+H]^+$ and NMR data. The dehydrated fragment $[M-H_2O+H]^+$ at m/z 229.0632 was a much more abundant ion than the corresponding protonated molecule in the mass spectrum, which indicated tertiary hydroxyl group in **2** [8]. The $^1H,^{13}C$ NMR and HSQC spectra of **2** contained signals of a tetrahydro-phenanthrone core including an α,β -unsaturated ketone moiety [δ_H 6.93 (1H, m, H-2), 6.03 (1H, m, H-3); δ_C 202.5 (C-4), 152.2 (C-2), 127.9 (C-3)], a 1,2,4,5-tetrasubstituted benzen ring [δ_H 6.60 (1H, s, H-8), 6.49 (1H, s, H-5); δ_C 147.0 (C-7), 144.7 (C-6), 129.0 (C-8a), 125.4 (C-4b), 117.0 (C-8), 115.2 (C-5)], one oxygenated quaternary carbon [δ_C 76.8 (C-4a)], three methylene groups [δ_H 2.93, 2.76 (each 1H, m, H-9), 2.48-1.74 (4H, m, H-1, H-10); δ_C 30.0 (C-1), 24.8 (C-9), 22.4 (C-10)] and one methine group [δ_H 2.49 (1H, m, H-10a), δ_C 41.5 (C-10a)]. Two hydroxyl groups were determined at C-6 and C-7 based on the HMBC correlations from H-5 and H-8 to C-6 and C-7, from H-8 to C-9 and from H-9 to C-8. The HMBC correlations from H-5, H-10a and H-3 to C-4a; from H-9 to C-10a confirmed one hydroxyl group at C-4a. The ketone group was at C-4 based on the HMBC correlations from H-2 and H-10a to C-4; and from H-10 to C-1. Connectivity of H-1/H-2, H-2/H-3, H-9/H-10 and H-1/H-10a were also elucidated by the ^1H - ^1H COSY analysis (Figure 2). Therefore, the planar structure of **2** was determined to be 1,9,10,10a-tetrahydro-4a,6,7-trihydroxy-4(4a*H*)-phenanthrone. The absolute configuration of two chiral carbons C-4a and C-10a in **2** were determined to be both *S* configurations by experimental and TD-DFT calculated ECD spectra, showing positive Cotton effects at wavelengths 200–220 nm and negative Cotton effects at wavelengths 230–250 nm (Figure 3).

Compound **3** was isolated as brown oil. Its molecular formula was deduced to be $C_{21}H_{26}O_9$ by the HR-ESI-MS quasi-molecular ion peak at m/z 445.1190 $[M+Na]^+$ and fragment ion peak at m/z 405.1278 $[M-H_2O+H]^+$. In the $^1H,^{13}C$ NMR and HSQC spectra of **3** appeared signals of a tetrahydro-phenanthrone unit similar to

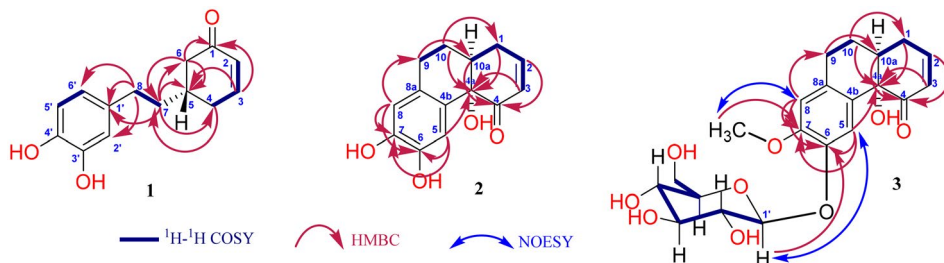


Figure 2. Selected 2D-NMR correlations for compounds **1–3**.

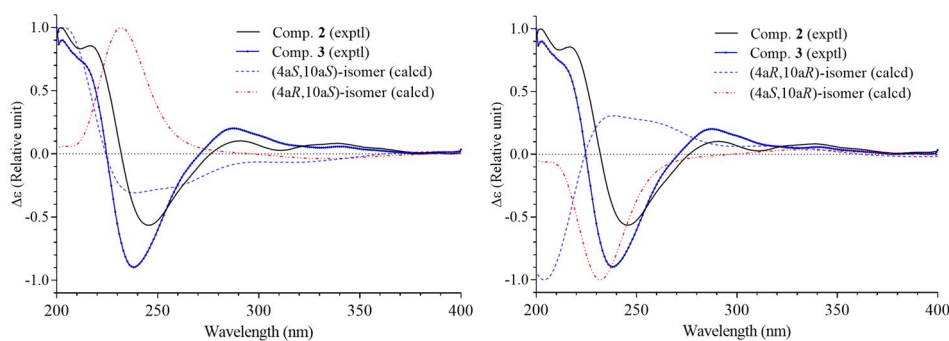


Figure 3. Experimental ECD spectra of compounds **2** and **3** in comparison with those TD-DFT calculated curves for (4aS, 10aS), (4aR, 10aS), (4aR, 10aR), (4aS, 10aR) isomers.

those of **2**, except for the presence of one methoxy group and one sugar moiety. The ^1H , ^{13}C NMR spectra of **3** contained signals of a hydrophenanthrone derivative similar to **2** including an α,β -unsaturated ketone moiety, a 1,2,4,5-tetrasubstituted benzen ring, one oxygenated quaternary carbon, three methylene groups and one methine group (Table 2). In addition, the signals of β -glucopyranosyl moiety [δ_{H} 4.72 (1H, d, $J=7.2$ Hz, H-1'); δ_{C} 103.0 (C-1'), 78.2 (C-5'), 77.8 (C-3'), 74.8 (C-2'), 71.2 (C-4'), 62.5 (C-6')] [9] and one methoxy group [δ_{H} 3.84 (3H, s), δ_{C} 56.6] were also observed. The sugar was confirmed as D-glucose by acid hydrolysis of **3**, followed by TLC comparison with authentic sample and from the positive sign of its optical rotation [10,11]. The locations of glucose moiety and methoxy group were confirmed at C-6 and C-7, respectively by the HMBC correlations from H-1' (δ_{H} 4.72) to C-6 (δ_{C} 146.1); from the methoxy protons (δ_{H} 3.84) to C-7 (δ_{C} 151.0), from H-5 (δ_{H} 6.91) and H-8 (δ_{H} 6.81) to C-6 (δ_{C} 146.1) and C-7 (δ_{C} 151.0). This inference was also verified by the NOESY correlations of H-1' (δ_{H} 4.72) and H-5 (δ_{H} 6.91); and the methoxy protons (δ_{H} 3.84) and H-8 (δ_{H} 6.81). Experimental ECD spectrum (Figure 3) of **3** is similar to those of **2**, which indicated that these compounds had the same configurations at C-4a and C-10a. Hence, the structure of **3** was elucidated to be (4aS,10aS)-4a-hydroxy-7-methoxy-1,9,10,10a-tetrahydro-4(4aH)-phenanthrone 6-*O*- β -D-glucopyranoside.

The isolates were tested for the inhibitory activity against NO production in LPS-induced RAW264.7 cells using the Griess assay with dexamethasone as positive control (IC_{50} $14.7 \pm 1.2 \mu\text{M}$). The results indicated that compounds **2** and **3** inhibited NO production with the IC_{50} values of $9.5 \pm 1.2 \mu\text{M}$ and $155.0 \pm 7.0 \mu\text{M}$, respectively, whereas compound **1** shows cytotoxic effect on RAW264.7 cells at the tested concentration.

3. Experimental

3.1. General experimental procedures

Optical rotation was measured with a Jasco P-2000 polarimeter (Jasco, Tokyo, Japan). HR-ESI-MS was acquired on a Sciex X500R QTOF mass spectrometer (Sciex, Massachusetts, USA) and Waters Xevo G3 QTOF-HRMS instrument (Waters,

Massachusetts, USA). NMR spectra were recorded on a Bruker Avance III HD 600 MHz instrument (Bruker, Rheinstetten, Germany) with tetramethylsilane as an internal standard. Electronic circular dichroism (ECD) spectrum was recorded on a Chirascan spectrometer (Applied Photophysics, England). Column chromatography was performed on silica gel 60 (40–63 μm , Merck, Darmstadt, Germany), Sephadex LH-20 (25–100 μm , Merck, Darmstadt, Germany) and RP-18 (octadecyl silica gel, 12 nm, YMC, Kyoto, Japan). Thin layer chromatography (TLC) was carried out on silica gel 60F₂₅₄ plates (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254S} plates (0.25 mm, Merck, Darmstadt, Germany). TLC spots were visualized under UV light (254 and 365 nm) and by spraying with solution of vanillin/sulfuric acid, following by heating for 3–5 min.

3.2. Plant material

The whole plant of *Elastostema tenuicaudatum* was collected in Quang Binh district, Ha Giang province, Vietnam in August 2023; and identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (EC02/2023) has been deposited at the Laboratory of Drug Research and Discovery, Institute of Chemistry, VAST, Hanoi, Vietnam.

3.3. Extraction and isolation

The dried and ground whole plant of *E. tenuicaudatum* (2.3 kg) was extracted three time with methanol-water (90:10, v/v) at room temperature. The methanol solution was concentrated under reduced pressure at 45 °C. The crude extract was suspended in H₂O and partitioned in turn between n-hexane, ethyl acetate and n-butanol, successively, to give the corresponding extracts of 28.9, 23.2 and 48.7 g, respectively.

The ethyl acetate extract (23.2 g) was chromatographed on silica gel column eluting with CH₂Cl₂–MeOH (from 95:5 to 5:1, v/v) to give seven fractions, E1–E7. Fraction E3 (738 mg) was purified on silica gel column (CH₂Cl₂–MeOH, 5:0.2, v/v), then Sephadex LH-20 column (acetone–MeOH, 4:1, v/v) to yield compound **1** (7 mg). Fraction E4 (643 mg) was purified on silica gel column (CH₂Cl₂–MeOH, 5:0.4, v/v), then on silica gel Rp-18 (MeOH–H₂O, 2:1, v/v) to afford compound **2** (4 mg).

The n-butanol extract (48.7 g) was chromatographed on a silica gel column eluted with CH₂Cl₂–MeOH–H₂O (from 5:1:0 to 2.5:1:0.1) to yield ten fractions B1–B10. Fraction B4 (836 mg) was chromatographed on silica gel column (CH₂Cl₂–MeOH–H₂O, 4:1:0.1, v/v/v), then on silica gel Rp-18 (MeOH–H₂O, 3:2, v/v) to give compound **3** (6 mg).

3.3.1. 5S-(3,4-dihydroxyphenethyl)cyclohex-2-en-1-one (**1**)

White solid; $[\alpha]_{\text{D}}^{25} +16.2$ (c 0.1, MeOH); ¹H NMR (600 MHz, CD₃OD) and ¹³C NMR (150 MHz, CD₃OD) spectral data are shown in Table 1; HR-ESI-MS: *m/z* 233.1169 [M+H]⁺ (calcd for C₁₄H₁₇O₃⁺, 233.1172), 255.0986 [M+Na]⁺ (calcd for C₁₄H₁₆NaO₃⁺, 255.0992).

Table 1. ^1H and ^{13}C NMR spectral data for compound **1**.

C	δ_{C}	δ_{H} (mult., J in Hz)	C	δ_{C}	δ_{H} (mult., J in Hz)
1	202.7		8	33.0	2.54 (2H, t, 7.8)
2	130.0	5.99 (1H, m)	1'	134.8	
3	153.0	7.12 (1H, m)	2'	116.4	6.64 (1H, d, 1.8)
4	33.2	2.51 (2H, m)	3'	146.2	
		2.15 (1H, m)			
5	35.9	2.08 (1H, m)	4'	144.3	
6	45.1	2.51 (1H, m)	5'	116.3	6.68 (1H, d, 7.8)
		2.21 (1H, dd, 16.2, 15.6)			
7	38.7	1.68 (2H, m)	6'	120.6	6.52 (1H, dd, 7.8, 1.8)

Table 2. ^1H and ^{13}C NMR spectral data for compounds **2** and **3**.

C	2		3	
	δ_{C}	δ_{H} (mult., J in Hz)	δ_{C}	δ_{H} (mult., J in Hz)
1	30.0	2.48 (1H, m)	29.9	2.48 (1H, m)
		2.34 (1H, m)		2.33 (1H, m)
2	152.2	6.93 (1H, m)	152.3	6.94 (1H, m)
3	127.9	6.03 (1H, m)	127.8	6.04 (1H, dd, 9.6, 2.4)
4	202.5		201.9	
4a	76.8		76.5	
4b	125.4		127.2	
5	115.2	6.49 (1H, s)	117.3	6.91 (1H, s)
6	144.7		146.1	
7	147.0		151.0	
8	117.0	6.60 (1H, s)	114.3	6.81 (1H, s)
8a	129.0		132.6	
9	24.8	2.93 (1H, m)	25.4	2.99 (1H, m)
		2.76 (1H, m)		2.85 (1H, m)
10	22.4	2.37 (1H, m)	22.6	2.37 (1H, m)
		1.74 (1H, m)		1.78 (1H, m)
10a	41.5	2.49 (1H, m)	41.2	2.51 (1H, m)
1'			103.0	4.72 (1H, d, 7.2)
2'			74.8	3.48 (1H, m)
3'			77.8	3.47 (1H, m)
4'			71.2	3.42 (1H, m)
5'			78.2	3.33 (1H, m)
6'			62.5	3.95 (1H, m)
				3.73 (1H, dd, 12.0, 5.4)
OCH ₃			56.6	3.84 (3H, s)

3.3.2. (4a*S*,10a*S*)- 1,9,10,10a -tetrahydro-4a,6,7-trihydroxy-4(4aH)-phenanthrone (**2**)

Brown oil; $[\alpha]_{\text{D}}^{25} +51.2$ (c 0.1, MeOH); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data are shown in Table 2; HR-ESI-MS: m/z 247.0707 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{14}\text{H}_{15}\text{O}_4^+$, 247.0965), 229.0632 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{14}\text{H}_{13}\text{O}_3^+$, 229.0859).

3.3.3. (4a*S*,10a*S*)-4a-hydroxy-7-methoxy-1,9,10,10a-tetrahydro-4(4aH)-phenanthrone 6- β -D-glucopyranoside (**3**)

Brown oil; $[\alpha]_{\text{D}}^{25} +103.8$ (c 0.1, MeOH); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) spectral data are shown in Table 2; HR-ESI-MS: m/z 445.1190 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{26}\text{NaO}_9^+$, 445.1469), 405.1278 $[\text{M} - \text{H}_2\text{O} + \text{H}]^+$ (calcd for $\text{C}_{21}\text{H}_{25}\text{O}_8^+$, 405.1544).

3.4. Acid hydrolysis of compound 3

Compound **3** (3.5 mg) was dissolved in 1 mol/L HCl–ethanol (1:1, 3 ml), and refluxed for 4 h. The acidic solution was neutralized with sodium hydroxide solution 5%, then partitioned between EtOAc and H₂O. The aqueous layer was concentrated and purified by preparative TLC silica gel 60 F₂₅₄ plates (CH₂Cl₂–MeOH–H₂O, 4:1:0.1, v:v:v) to give D-glucose ($[\alpha]_D^{25} +26.5$, c 0.06, H₂O).

3.5. Theoretical calculation of ECD spectra

Calculation was performed as previously described [12]. In brief, conformational searches and geometric equilibrium were carried out on Spartan18 program (Wavefunction Inc., Irvine, CA, USA). Two isomers (4aR,10aR) and (4aR,10aS) were submitted to conformational searches at the Molecular Mechanics MMFF. The top 20 initial stable conformers for each stereoisomer with relative energy lower than 40 kJ/mol were further optimized by DFT calculations at the B3LYP/6-31G(d,p) basic set and IEFPCM models for the solvent of methanol using Gaussian 16 program (Gaussian Inc., Wallingford, CT, USA). The optimized conformers were then subjected to TDDFT calculations at the B3LYP/6-31G(d,p) levels in the presence of methanol with IEFPCM models. The ECD spectra at 30 excited states for each conformer were collected and summed to obtain theoretical ECD spectra of calculated stereoisomers using SpecDis v1.71 software (University of Wuerzburg, Wuerzburg, Germany). The half-bands were taken at $\zeta = 0.40$ eV. The ECD spectra of corresponding enantiomers, (4aS,10aS) and (4aS,10aR), were composed by mirror image those of (4aR,10aR) and (4aR,10aS) calculated ECD curves.

3.6. Nitric oxide assay

The capability of inhibiting NO of the compounds was measured in doses of 100, 20, 4, and 0.8 µg/ml; and performed as previously described [5,13]. Dexamethasone was used as a positive control.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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