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Study on the chemical constituents of *Dacrydium elatum* and their cytotoxic activity

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Abstract: From an ethyl acetate extract of twigs and barks of *Dacrydium elatum* a new diterpenoid named dacrydianone (**1**), together with lambertic acid (**2**), three ecdysteroids: 20-hydroxyecdysone (**3**), ajugasterone C (**4**), ponasterone A (**5**), and daucosterol (**6**), has been isolated. Their structures were elucidated by an extensive analysis of the UV/Vis, FT-IR, MS and NMR spectra as well as comparison with those in published literature. The EtOAc extract and the isolated compounds **1–5** were evaluated for their cytotoxicity on four cancer cell lines: breast cancer (MCF-7), lung cancer (Lu-1), liver cancer (HepG2) and human oral cancer (KB). The EtOAc extract and compound **2** showed a medium activity on four tested cancer cell lines, while compounds **3** and **5** exhibited weak activity on these cell lines. Compounds **1** and **4** did not show activity on the tested cell lines.

Keywords: cytotoxicity; dacrydianone; *Dacrydium elatum*; diterpenoids; ecdysteroids.

1 Introduction

Dacrydium elatum (Roxb.) Wall. ex Hook. (syn. *D. pierrei* Hick) [Podocarpaceae] is a tree up to 30 m in height, about 80 cm in diameter and widely distributed in Vietnam between the altitude of 900 and 2500 m [1]. This plant is a threatened, endemic species of Vietnam. It is also found in the Southeast Asian countries and China [2, 3]. Its valuable wood is used in house construction, handicraft and essential oil production. In Vietnamese folk medicine, this plant is used for the treatment of dysentery, stomach pain and rheumatism [3, 4]. However, there were no reports on chemical constituents and biological activities of *D. elatum* in the literature until now. Here we report the isolation, structural elucidation and cytotoxicities of the extract and of the isolated constituents from this plant collected in the highland Lam Dong province, Vietnam. A new compound, named dacrydianone **1**, together with five known compounds: lambertic acid (**2**), 20-hydroxyecdysone (**3**), ajugasterone C (**4**), ponasterone A (**5**) and daucosterol (**6**), has been isolated from the twigs and barks of *D. elatum*. The cytotoxic activity of the EtOAc extract and that of five isolated compounds on the cancer cell lines: breast cancer (MCF-7), lung cancer (Lu-1), liver cancer (HepG2) and human oral cancer (KB), have been evaluated.

2 Results and discussion

Compound **1** ($[\alpha]_D^{25} = +225.3$; $c = 0.1$, MeOH) was obtained as a white powder. Its negative electrospray ionization-mass spectrometry (ESI-MS) spectrum contained a pseudo molecular ion peak at $m/z = 313$ $[M-H]^-$, and its positive high-resolution (HR)-ESI-MS spectrum showed a peak at $m/z = 315.1966$ $[M+H]^+$ (calc. 315.1954 for $C_{20}H_{27}O_3$). This suggested a molecular formula of $C_{20}H_{26}O_3$ for compound **1** with eight double-bond equivalents. The FT-IR spectrum of **1** indicated the presence of a hydroxyl and a conjugated carbonyl group by the absorptions at $\tilde{\nu} = 3375$ ($-OH$) and 1652 ($-C=O$) cm^{-1} . The UV/Vis spectrum showed the absorptions at $\lambda_{max} = 274$ nm ($\log \epsilon = 3.844$) and 314 ($\log$

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Table 1: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) data for dacrydianone (**1**) in CDCl_3 .

Position	1		
	δ_{C}	δ_{H} (J in Hz)	HMBC
1	33.43	1.73–1.81 (m) 2.40–2.44 (m)	2, 3, 5, 20
2	17.49	1.73–1.81 (m) 1.91–2.00 (m)	1, 10
3	37.71	1.91–2.00 (m) 1.56–1.68 (m)	1, 2
4	36.07		
5	143.06		
6	143.86		
7	180.17		
8	125.86		
9	147.86		
10	40.63		
11	123.64	7.34 (s)	8, 10, 13
12	141.83		
13	151.73		
14	110.83	7.47 (s)	7, 9, 12, 13
15	27.84	3.33 (sept, 7.0)	11, 12, 13, 16, 17
16	22.28	1.27 (d, 7.0)	15
17	22.53	1.29 (d, 7.0)	15
18	27.50	1.44 (s)	3, 4, 5
19	28.05	1.44 (s)	3, 4, 5
20	34.88	1.52 (s)	1, 5, 9, 10
OH-6		7.03 (s)	5, 6, 7
OH-13		5.43 (brs)	

$\varepsilon=3.8467$), suggesting for a conjugated aromatic system in its molecule. Its NMR spectrum showed the presence of an isopropyl group with the signals at $\delta_{\text{H}}=1.27$ (3H, d, $J=7.0$ Hz), 1.29 (3H, d, $J=7.0$ Hz) and 3.33 ppm (1H, sept, $J=7.0$ Hz), together with two methyl singlets at $\delta_{\text{H}}=1.44$ (6H, $2 \times \text{CH}_3$, s) and 1.52 (3H, s), as well as two aromatic

proton singlets at $\delta_{\text{H}}=7.34$ (1H, s) and 7.47 ppm (1H, s). The ^{13}C NMR spectrum of compound **1** indicated the signals of 20 carbons, including 5 methyl, 3 methylene, 3 methine and 9 tertiary carbons. The detailed assignments of all protons and carbon atoms of **1** were carried out by an extensive analysis of its NMR spectra [^1H , ^{13}C , heteronuclear single-quantum coherence, heteronuclear multiple-bond correlation (HMBC) and nuclear Overhauser effect spectroscopy (NOESY)] shown in Table 1. The positions of protons H-11 and H-14 were confirmed by the correlations between H-11 ($\delta_{\text{H}}=7.34$ ppm) and C-8 ($\delta_{\text{C}}=125.86$ ppm), C-10 ($\delta_{\text{C}}=40.63$ ppm), C-13 ($\delta_{\text{C}}=151.73$ ppm); and between H-14 ($\delta_{\text{H}}=7.47$ ppm) and C-7 ($\delta_{\text{C}}=180.17$ ppm), C-9 ($\delta_{\text{C}}=147.86$ ppm), C-12 ($\delta_{\text{C}}=141.83$ ppm), C-13 ($\delta_{\text{C}}=151.73$ ppm) in the HMBC spectrum. Besides, the HMBC correlations of H-1 β ($\delta_{\text{H}}=2.40\text{--}2.44$ ppm) with C-2 ($\delta_{\text{C}}=17.49$ ppm), C-3 ($\delta_{\text{C}}=37.71$ ppm), C-5 ($\delta_{\text{C}}=143.06$ ppm), C-20 ($\delta_{\text{C}}=34.88$ ppm) and H-20 ($\delta_{\text{H}}=1.52$ ppm) with C-1 ($\delta_{\text{C}}=33.43$ ppm), C-5 ($\delta_{\text{C}}=143.06$ ppm), C-9 ($\delta_{\text{C}}=147.86$ ppm), C-10 ($\delta_{\text{C}}=40.63$ ppm) were also observed. In the HMBC spectrum, the hydroxyl proton 6-OH ($\delta_{\text{H}}=7.03$ ppm) showed correlation with C-6 ($\delta_{\text{C}}=143.86$ ppm), C-5 ($\delta_{\text{C}}=143.06$ ppm) and C-7 ($\delta_{\text{C}}=180.17$ ppm), suggesting the location of a hydroxyl group at C-6 (Fig. 1). These data supported a partial structure of Δ 5,6-hydroxy, 7-keto in ring B of compound **1**. The positions of the isopropyl group at C-12 and the hydroxyl group at C-13 were confirmed by the correlation of H-15 ($\delta_{\text{H}}=3.33$ ppm) with C-11 ($\delta_{\text{C}}=123.64$ ppm), C-12 ($\delta_{\text{C}}=141.83$ ppm), C-13 ($\delta_{\text{C}}=151.73$ ppm), C-16 ($\delta_{\text{C}}=22.28$ ppm) and C-17 ($\delta_{\text{C}}=22.53$ ppm) in the HMBC spectrum. In agreement with this proposed structure, the NOESY spectrum of **1** showed important correlations of H-15 ($\delta_{\text{H}}=3.33$ ppm) with H-11 ($\delta_{\text{H}}=7.34$ ppm), H-16 ($\delta_{\text{H}}=1.27$ ppm) and H-17 ($\delta_{\text{H}}=1.29$ ppm). Furthermore, the

Fig. 1: Selected NOESY (left) and HMBC correlations (right) of dacrydianone (**1**).

Fig. 2: Chemical structure of dacrydianone (1).

NOE were also observed between H-1 β (δ_H =2.40–2.44 ppm) and H-2 β (δ_H =1.91–2.00 ppm), H-11 (δ_H =7.34 ppm), H-20 (δ_H =1.52 ppm) as well as between H-3 β (δ_H =1.56–1.68 ppm) and H-2 β (δ_H =1.91–2.00 ppm), H-18, H-19 (overlapping at δ_H =1.44 ppm) and H-11 (δ_H =7.34 ppm) to H-15 (δ_H =3.33 ppm) and H-20 (δ_H =1.52 ppm) (Fig. 1). All the NMR spectroscopic data of **1** (1D, 2D) presented differences to those data reported for similar compounds montbretol [5] and salvinolone [6], where the two aromatic protons are located at positions 11, 14 and 6, 14, respectively. The axial position of the methyl group at C-10 of compound **1** is based on the biogenetic pathway for the terpenoids. In conclusion, the structure of the new compound **1**, as given in Fig. 2, is a rearranged arbutane diterpenoid and named dacrydianone.

The other five known compounds were identified as lambertic acid (**2**) [7], three ecdysteroids: 20-hydroxy ecdysone (**3**) [8, 9], ajugasteron C (**4**) [10], ponasteron A (**5**) [8, 9], and daucosterol (**6**) [11]. The structures of them were determined by comparison of their FT-IR, MS, NMR (1D, 2D) spectra with those reported in the literature (Fig. 3). All these compounds were isolated for the first time from *D. elatum*.

The EtOAc extract and compounds **1**–**5** were tested for their cytotoxicity on four human cancer cell lines: KB (human oral cancer), MCF-7 (breast cancer), LU-1 (lung cancer) and HepG2 (liver cancer) according to the methods in [12]. Ellipticine and 10% aqueous dimethyl sulfoxide (DMSO) were used as positive and negative control, respectively. The EtOAc extract and compound **2** exhibited a moderate activity, while compounds **3** and **5** showed weak cytotoxic activity. The other compounds did not show activity in this test (Table 2).

3 Experimental section

3.1 General experimental procedures

The optical rotation $[\alpha]_D^{25}$ was measured with an IRP 8000 from Kruss Optronic GmbH (Germany). IR spectra (KBr)

Fig. 3: The isolated compounds (1–6) from *D. elatum*.

Table 2: Cytotoxic activity of compounds 1–5.

Compounds	IC ₅₀ (μg mL ⁻¹)			
	MCF7	Lu-1	HepG2	KB
1	>100	>100	>100	>100
2	58.45 ± 5.46	52.78 ± 6.24	70.86 ± 6.96	51.36 ± 3.08
3	99.13 ± 7.95	94.37 ± 4.00	95.93 ± 3.22	93.13 ± 6.24
4	>100	>100	>100	>100
5	68.34 ± 8.15	97.17 ± 7.26	62.87 ± 5.43	86.13 ± 5.32
EtOAc extract	25.21 ± 1.32	29.27 ± 2.66	41.21 ± 4.68	25.16 ± 2.13
Ellipticine	0.44 ± 0.06	0.43 ± 0.01	0.46 ± 0.02	0.41 ± 0.05

were recorded on an IMPACT 410 Nicolet (Germany), and UV/Vis spectra on an UV-2450 (Shimadzu, Kyoto, Japan). NMR spectra: Bruker Avance 500 spectrometer (Karlsruhe, Germany). ¹H: 500 MHz, ¹³C: 125 MHz; TMS was used as an internal standard. ESI-MS: LC-MSD-Trap-SL Varian spectrometer (USA); HR-ESI-MS: Thermo Scientific LTQ Orbitrap XL (USA). Silica gel (230–400 mesh; Merck, Darmstadt, Germany), Sephadex LH-20 gel (Sigma Aldrich, St. Louis, MO, USA) and RP-18 resins (11.7 nm; YMC Co., Ltd., Kyoto, Japan) were used for column chromatography (CC). Pre-coated silica gel 60 F₂₅₄ plates (0.25 mm, Merck) and RP-18 F_{254s} plates (0.25 mm; Merck) were used for thin layer chromatography (TLC). TLC spots were visualized under UV light by spraying with 5% H₂SO₄ in alcohol followed by heating.

3.2 Plant material

Dacrydium elatum was collected in Lam Dong province, Vietnam, in August 2012 and identified by Dr. Nguyen Tien Hiep, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. A voucher specimen (No. CPC 4708) was deposited at the Institute of Chemistry, VAST.

3.3 Extraction and isolation

The dried and powdered twigs and barks of *D. elatum* (2.2 kg) were extracted with *n*-hexane, ethyl acetate and methanol at room temperature, successively. The solvents were removed under reduced pressure to give the corresponding extracts of 2.6, 13.0 and 120 g. The ethyl acetate extract (13.0 g) was subjected to silica gel CC (*n*-hexane–EtOAc 90:10 → 0:100) to obtain 11 fractions (F.1–F.11). Fraction F.4 (1.52 g) was further separated by silica gel

CC (*n*-hexane–EtOAc 90:10 → 80:20) to obtain 10 fractions (F.4.1–F.4.10). Fraction F.4.2 (167 mg) was separated on a silica gel column (*n*-hexane–EtOAc 98:2 → 90:10) to obtain compound **1** (6 mg). Fraction F.4.9 was subjected to a silica gel CC (*n*-hexane–EtOAc 90:10 → 80:20) to afford compound **2** (15 mg). Fraction F.5 (270 mg) was purified on a Sephadex LH-20 column (MeOH 100%) to give compound **6** (12 mg). Fraction F. 6 (710 mg) was chromatographed on Sephadex LH-20 (MeOH 100%) and then on silica gel (EtOAc–MeOH 98:2) to afford compound **5** (11 mg). Fraction F.9 (830 mg) was repeatedly chromatographed on Sephadex LH-20 (MeOH 100%) to obtain compound **3** (14 mg). Fraction F.10 (770 mg) was chromatographed on Sephadex LH-20 (MeOH 100%) and then on silica gel (CH₂Cl₂–MeOH 95:5 → 90:10) to give compound **4** (16 mg).

Dacrydianone (**1**): white amorphous solid. *R*_f = 0.5 (*n*-hexane–EtOAc 9:1). $-\left[\alpha\right]_D^{25} = +225.3$ (*c* = 0.1, MeOH). –IR (KBr): $\tilde{\nu}$ = 3375 (–OH), 3060 (aromatic), 2923 (–CH₂, CH₃), 1652 (conjugated C=O), 1556 (C=C). –UV/Vis (MeOH): $\lambda_{\max}$ (log ϵ) = 314 nm (3.85), 274 nm (3.84). –MS ((–)-ESI): *m/z* = 313 [M–H][–]. –HRMS ((+)-ESI): *m/z* = 315.1966 [M+H]⁺ (calcd. 315.1954 for C₂₀H₂₇O₃). ¹H and ¹³C NMR data (see Table 1).

3.4 Cytotoxic assay

The cytotoxicity evaluation of compounds **1–5** was carried out on the cancer cell lines HepG2, MCF-7, KB, LU using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) method [12]. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and cultured at a density of 6 × 10⁴ cells per mL per well in a 96-well microtiter plate. Five different concentrations (128.0, 32.0, 8.0, 2.0, 0.5 μg mL⁻¹) of each tested compound dissolved in DMSO were added to each well. Each

concentration was tested in triplicate. After incubation at 37°C in 5% CO₂ for 48 h, MTT (0.2 mg mL⁻¹) was added to each well and incubated for another 4 h. The liquid in the wells was removed, and DMSO (200 µL) was added to each well. Ellipticine was used as positive control. The absorbance was recorded on a microplate reader at a wavelength of 570 nm (Genious Tecan, Grödig, Austria). Cell viability was measured and IC₅₀ values were calculated (TABLE CURVE 2Dv4, USA).

4 Supplementary information

The HR-ESI-MS, ESI-MS, 1D (¹H, ¹³C, DEPT) and 2D (HSQC, HMBC, NOESY) spectra of compound **1** are given as Supplementary information available online (<https://doi.org/10.1515/znb-2018-0214>).

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References

- [1] P. A. Zuidema, M. Vlam, P. D. Chien, *Trees* **2011**, 25, 29.
- [2] P. H. Ho, *Vietnam Flora*, Vol. I, The Youth Publication, Ho Chi Minh City, Vietnam, **2000**, p. 227.
- [3] V. V. Chi, *Dictionary of Vietnamese Medicinal Plants*, Ho Chi Minh City, Vietnam, **1999**, p. 562.
- [4] P. Venkatalakshmi, V. Vadivel, P. Brindha, *Int. J. Res. Ayurveda Pharm.* **2016**, 7, 130.
- [5] A. Ulubelen, G. Topcu, *J. Nat. Prod.* **1992**, 55, 441.
- [6] L. Z. Lin, G. Blasko, G. A. Cordel, *Phytochemistry* **1989**, 28, 177.
- [7] J. D. P. Campello, S. F. Fonseca, C. J. Chang, E. Wenkert, *Phytochemistry* **1975**, 14, 243.
- [8] K. Vokac, M. Budesnksky, J. Harmatha, J. Kohoutova, *Phytochemistry* **1998**, 49, 2109.
- [9] A. Suksamrarn, A. Jankam, B. Tarnchompoo, S. Putchakarn, *J. Nat. Prod.* **2002**, 65, 1194.
- [10] A. Guerriero, F. Pietra, *Comp. Biochem. Physiol.* **1985**, 80B, 277.
- [11] S. Mouffok, H. Haba, C. Lavaud, C. Long, M. Benkhaled, *Rec. Nat. Prod.* **2012**, 6, 292.
- [12] T. Mosmann, *J. Immunol. Methods* **1983**, 65, 55.

Supplementary Material: The online version of this article offers supplementary material (<https://doi.org/10.1515/znb-2018-0214>).