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# Two new triterpenoids from the roots of *Phyllanthus emblica*

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ABSTRACT

Two new triterpenes, the seco-friedelane type secofriedelanophyllemblicine and the ursane-derived saponin ursophyllemblicoside were isolated from the roots of the edible fruit-producing *Phyllanthus emblica*. Their structures were unambiguously elucidated using extensive 1D and 2D NMR analyses, high resolution mass spectrometry and single-crystal X-ray crystallographic analyses along with comparison with literature data. Secofriedelanophyllemblicine represents the first 3,4-secofriedelane bearing a carboxylic acid group substituent at C-20. Ursophyllemblicoside, incorporating the rare 21α hydroxyursolic acid as a sapogenol represents the first example of saponin comprising this aglycone. Secofriedelanophyllemblicine displayed a moderate cytotoxicity against K562 and HepG2 cancer cell lines.

## 1. Introduction

The genus *Phyllanthus* is comprised in the family Phyllanthaceae although often being placed within the Euphorbiaceae [1,2]. It encompasses > 800 plant species, spanning from temperate to tropical areas, that have long been used in folk medicine for various ailments including urinary bladder disturbances, intestinal infections, diabetes, hepatitis B etc. [3]. Along with *P. reticulatus* and *P. niruri*, *P. emblica* Linn. (syn. *Emblica officinalis* Gaertn.) stands among the top three species used in traditional medicine within this privileged genus [2]. The clinical use of *P. emblica* is especially prevalent in Asia where it is regionally cultivated for its sweet and slightly astringent fruits that are known as Indian gooseberries or Amla. The fruit of *P. emblica* in particular is endowed with various pharmacological activities including antioxidant [4], vulnerary [5], anti-pyretic [6], analgesic and anti-inflammatory [7,8], chondroprotective [9], hepatoprotective [10,11], chemopreventive [12], antibacterial [13], antidiarrheic and anti-spasmodic properties [14]. The various ethnomedicinal uses and therapeutic potential of *P. emblica* were recently reviewed [15]. From a chemical viewpoint, *Phyllanthus* spp. were thoroughly investigated with > 500 specialized metabolites being reported from this genus [2], the majority of which correspond to securinine/norsecurinine type alkaloids [16], sesqui- and triterpenes, flavonoids and tannins [16]. The widespread use of *P. emblica* in folk medicine paved the way for thorough phytochemical investigations which reported the presence of norbisabolane and bisabolane sesquiterpenoid glycosides [17–19], triterpenes and sterols [20,21], some lignans [22], a variety of ellagitanins [23] and flavonoids [23,24].

The phytochemical investigation reported herein led to the isolation of two new triterpenes, namely, secofriedelanophyllemblicine (**1**) and the ursane-derived saponoside ursophyllemblicoside (**2**) along with the three formerly known ursolic acid (**3**), nobiletin (**4**) and glochidiol (**5**).

## 2. Experimental

### 2.1. General experimental procedures

NMR spectra were acquired on a Bruker Avance III spectrometer (500 MHz for $^{1}$H NMR and 125 MHz for $^{13}$C NMR). $^{1}$H and $^{13}$C chemical shifts were referenced to the solvent residual signal of $CDCl_3$ ($\delta_H$ 7.26

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and $\delta_C$ 77.1). HR-ESI-MS data were recorded on a MicroOTOF–Q mass spectrometer or an hybrid IT-TOF mass spectrometer (Shimadzu LC-MS-IT-TOF, Kyoto, Japan). Column chromatography were carried out using silica gel 40–63 μm (Merck).

### 2.2. Plant material

The roots of *Phyllanthus emblica* L. (Phyllanthaceae) were collected at Binh Thuan province, Vietnam in October 2015. The botanical sample was authenticated by Dr. Pham Van Ngot, Department of Biology, University of Pedagogy – Ho Chi Minh City, Vietnam. A voucher specimen (No US-A025) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, National University - Ho Chi Minh City, Vietnam.

### 2.3. Extraction and isolation

The dried roots were milled (9.1 kg) prior to being macerated with EtOH (3 × 10 L, each 8 h) at ambient temperature. The filtrated solution was concentrated in vacuo to afford a crude extract (259.5 g). This dry residue was subsequently reextracted using solvents of increasing polarities: *n*-hexane (**H**, 9.1 g), EtOAc (**EA**, 93.5 g) and *n*-BuOH (**B**, 50.5 g). Extract **EA** was applied to normal phase silica gel column chromatography, and eluted with a chloroform/MeOH solvent system (stepwise, 1:0 to 4:1, *v/v*) to afford five fractions: **EA1** − **EA5**. Fraction **EA2** (13.0 g) was further fractionated by column chromatography using a gradient system of *n*-hexane − EtOAc (stepwise, 4:1 to 2:3, *v/v*) to yield five subfractions (**EA2.1** − **EA2.5**). Sub-fraction **EA2.2** (1.5 g) was submitted to normal phase silica gel CC, using *n*-hexane–EtOAc (1:0 to 4:1, v/v) to afford compounds **3** (9.7 mg), **4** (5.7 mg) and **5** (12.6 mg). Likewise, fraction **EA3** (12.5 g) was fractionated using the same solvent system to isolate compounds **1** (15.8 mg) and **2** (4.5 mg).

#### 2.3.1. Secofriedelanophyllemblicine (3,4-seco-friedel-4(23)-ene-3,30-dioic acid) (1)

White amorphous solid; $[\alpha]_D^{20}$ + 350 (*c* 0.1, EtOH); UV (EtOH), $\lambda_{max}$ (log $\varepsilon$) 242 (2.28), 201 (3.52) nm; IR (KBr) $\nu_{max}$ 3399, 2917, 2849, 1502, 1487, 1383, 1236, 1122, 1045, 964, 932, 856, 831, 756, 742 $cm^{-1}$; HR-ESI-MS *m/z* 471.3472 $[M\text{-}H]^-$ (calcd. For $C_{30}H_{47}O_4$, 471.3474); $^1H$ NMR [$CD_3OD$:$CDCl_3$ (1:1)], 500 MHz, *J* in Hz) and $^{13}C$ NMR [$CD_3OD$:$CDCl_3$ (1:1)], 125 MHz) spectroscopic data, see Table 1.

#### 2.3.2. Ursophyllemblicoside (21α-hydroxy-3-O-α-L-arabinopyranosylursolic acid) (2)

White amorphous solid; $[\alpha]_D^{20}$ + 475 (*c* 0.1, EtOH); UV (EtOH), $\lambda_{max}$ (log $\varepsilon$) 220 (2.79), 202 (3.27) nm; IR (KBr) $\nu_{max}$: 3427, 2928, 1730, 1694, 1641, 1454, 1051, 871 $cm^{-1}$; HR-ESI-MS *m/z* 627.3878 $[M + Na]^+$ (calcd. For $C_{35}H_{55}O_8Na$, 627.3873). $^1H$ NMR [$CD_3OD$:$CDCl_3$ (1:1)], 500 MHz, *J* in Hz) and $^{13}C$ NMR [$CD_3OD$:$CDCl_3$ (1:1)], 125 MHz) spectroscopic data, see Table 1.

### 2.4. Single–crystal X-ray analysis of 1

White single crystals of **1** were obtained from slow evaporation of MeOH–water solution in the orthorhombic space group $P2_12_12_1$ with unit cell parameters $a$ = 13.4931(5) Å, $b$ = 13.5569(4) Å, $c$ = 14.4719(5) Å, $\alpha = \beta = \gamma = 90°$, $V$ = 2647.27(16) $Å^3$, $Z$ = 4, $D_{calc}$ = 1.186 $g/cm^3$, $MW$ = 472.68. A block single crystal of **1** with dimensions 0.37 × 0.40 × 0.48 mm was mounted on the tip of a MiTeGen microloop. X-ray diffraction experiment was performed at 100.0(2) K using a Bruker D8 QUEST CMOS detector diffractometer with MoKα radiation (λ = 0.71073 Å). A total of 29,152 reflections were collected, integrated, reduced by SAINT v8.34A, corrected for Lorentz, polarization and absorption effects, and scaled by SADABS (Bruker Software Suite, APEX2, SAINT and SADABS, 2013. Bruker AXS Inc., Madison, Wisconsin, USA) to yield 5221 independent reflections ($R_{int}$ = 0.0554). Using Olex2 [25], the structure was solved with the ShelXT structure solution program [26] using Intrinsic Phasing and refined with the ShelXL refinement package ShelXL using Least Squares minimization on $F^2$. The final $R_1(F^2)$ = 0.0374 and $wR(F^2)$ = 0.0884 for 4591 data with $F^2 > 2\sigma(F^2)$. Data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1845254). Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

**Table 1**
$^1H$ and $^{13}C$ NMR spectral data of **1** and **2** (500 and 125 MHz, in $CD_3OD$-$CDCl_3$, 1:1).

| Position | 1 | | 2 | |
|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$, *J* (Hz) | $\delta_C$ | $\delta_H$, *J* (Hz) |
| 1 | 21.0 | 1.41, *m;* 1.30, *m* | 38.4 | 1.40, *m;* 0.70, *m* |
| 2 | 36.8 | 2.17, *m* | 25.4 | 1.63, *m;* 1.42, *m* |
| 3 | 174.5 | | 89.4 | 2.93, *dd* (11.5, 4.5) |
| 4 | 150.5 | 5.52, *dd* (17.0, 10.5) | 38.6 | |
| 5 | 41.6 | | 55.3 | 0.54, *d* (11.0) |
| 6 | 41.0 | 1.31, *m;* 1.24, *m* | 17.8 | 1.28, *m;* 1.14, *m* |
| 7 | 17.4 | 1.35, *m;* 1.28, *m* | 32.9 | 1.27, *m;* 1.10, *m* |
| 8 | 52.6 | 1.22, *m* | 39.0 | |
| 9 | 39.7 | | 47.3 | 1.29, *m* |
| 10 | 57.8 | 0.79, *t* (4.0) | 36.3 | |
| 11 | 34.7 | 1.43, *m;* 1.33, *m* | 22.9 | 1.68, *m* |
| 12 | 29.1 | 1.32, *m;* 1.22, *m* | 125.2 | 5.03, *t* (3.0) |
| 13 | 37.6 | | 137.7 | |
| 14 | 38.2 | | 41.9 | |
| 15 | 32.2 | 1.39, *m* | 28.1 | 1.46, *m;* 1.14, *m* |
| 16 | 35.0 | 1.29, *m;* 1.28, *m* | 26.5 | 2.19, *m;* 1.65, *m* |
| 17 | 29.5 | | 47.6 | |
| 18 | 42.0 | 1.48, *dd* (13.0, 5.0) | 53.6 | 2.02, *d* (11.5) |
| 19 | 30.9 | 1.84, *dd* (13.0, 5.0)<br>1.20, *m* | 31.7 | 1.63, *m* |
| 20 | 39.2 | | 42.5 | 0.98, *m* |
| 21 | 27.9 | 2.35, *td* (14.5, 8.5)<br>1.25, *m* | 70.9 | 3.59, *d* (2.5) |
| 22 | 37.9 | 1.37, *m;* 1.23, *m* | 42.6 | 1.72, *m*; 1.52, *m* |
| 23 | 110.2 | 4.81, *dd* (10.5, 1.0)<br>4.79, *dd* (17.0, 1.0) | 27.4 | 0.80, *s* |
| 24 | 17.5 | 0.88, *s* | 15.8 | 0.60, *s* |
| 25 | 17.1 | 0.77, *s* | 14.9 | 0.72, *s* |
| 26 | 20.2 | 0.86, *s* | 16.5 | 0.60, *s* |
| 27 | 17.2 | 0.91, *s* | 22.4 | 0.88, *s* |
| 28 | 31.4 | 0.95, *s* | 180.3 | |
| 29 | 181.7 | | 16.5 | 0.63, *d* (6.5) |
| 30 | 31.4 | 1.15, *s* | 16.3 | 0.80, *d* (6.5) |
| 1′ | | | 105.0 | 4.11, *d* (6.0) |
| 2′ | | | 70.9 | 3.41, *dd* (8.0, 6.0) |
| 3′ | | | 72.4 | 3.36, *dd* (8.0, 3.0) |
| 4′ | | | 67.3 | 3.64, *d* (2.5) |
| 5′ | | | 64.3 | 3.66, *dd* (14.0, 4.0)<br>3.30, *dd* (14.0, 4.0)<br>3.30, *dd* (14.0, 4.0) |

### 2.5. Acid hydrolysis of 2

Acid hydrolysis was performed to obtain the sugar residue of compound **2**. A 1.3 mg aliquot of the saponin was treated with HCl 0.2 M (dioxane/$H_2O$, 1/1, v/v, 200 μL) at 95 °C. After cooling, the reaction mixture was evaporated to dryness and the residue was washed with chloroform (2 mL, 3 times) to eliminate the aglycone component. The remaining residue was dissolved in $D_2O$ for subsequent $^1H$ NMR analysis of the hydrolyzed monosaccharide.

### 2.6. Cytotoxicity assay

The cytotoxic activity of **1** was evaluated against HepG2 (liver