A New Ellagitannin from the Fruit of *Phyllanthus emblica* L.

Chun-Bin Yang†, Fan Zhang‡, Mei-Cai Deng†, Guang-Yun He†, Jian-Min Yue‡ and Run-Hua Lu†,*

†Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, P. R. China
‡Chinese Medicine College, Xinjiang Medical University, Urumqi 830054, P. R. China

A new ellagitannin along with eight known compounds has been isolated from the ethanol extract of the fruit of *Phyllanthus emblica* L. The chemical structure of the new compound was established as Phyllanthunin (1) by HR-ESI-MS, 1D and 2D NMR spectroscopic analysis.

**Keywords:** *Phyllanthus emblica*; Ellagitannin; Phyllanthunin.

**INTRODUCTION**

The fruit of *Phyllanthus emblica* L., is of great importance in Asiatic medicine, not only as an antiscorbutic, but in the treatment of diverse ailments such as jaundice, dyspepsia, and cough. Previous phytochemical studies of *P. emblica* resulted in the isolation of tannins, flavonoids, and acids. During our systematic search for pharmacologically and structurally interesting substances from traditional Chinese medicine, a new ellagitannin, Phyllanthunin (1, Fig. 1), together with eight known compounds were isolated from the ethanol extract of the fruit. The known compounds were stearic acid (2), β-sitosterol (3), daucosterol (4), ethyl gallate (5), lauric acid (6), cinnamic acid (7), ellagic acid (8), and gallic acid (9). This paper describes their isolation and structure elucidation.

**RESULTS AND DISCUSSION**

Compound 1 was obtained as white powder. The molecular formula, C_{32}H_{30}O_{23}, was deduced from HR-ESI-MS protonated molecular ion at m/z 805.1059 ([M+Na]^+) calculated 805.1070. Broad IR absorption bands at 3357, 1710 and 1614 cm\(^{-1}\) indicated the presence of hydroxyl group, ester group, and aromatic ring, respectively. The ^1H and ^13C-NMR data of 1 revealed 32 carbon signals due to 17 quaternary carbons, 12 methines and 3 methylenes. Among them, the ^13C-NMR spectra exhibited typical signals rising from 3 ester groups (δ_C 170.4, 165.5, and 165.1), 2 aromatic rings (δ_C 145.2 (s), 144.9 (s), 143.9 (s), 137.7 (s), 119.1 (s), 118.6 (s), 113.8 (d), 110.5 (s), and 109.1 (d)), and a sugar unit [δ_C 92.1 (d)]. Two aromatic proton signals at δ_H 7.32 (s, 1H) and δ_H 7.14 (s, 2H) indicated that an aromatic ring was symmetrical.

All these data suggested that 1 was an ellagitannin possessing the skeleton of Elaeocarpusin (Fig. 2), and had the same β configuration glycoside, 2 gallates, a cyclohexane, and a furan ring, which were confirmed by the ^1H–^1H COSY spectrum (Fig. 3). In the HMBC experiment (Fig. 4), correlations between H-1 (δ_H 6.36) and H-2 (δ_H 5.31) with δ_C 165.1 and δ_C 165.5 confirmed the presence of 2 gallates attaching to C-1 and C-2, respectively. One ester group was assigned to C-4 according to the HMBC correlations between H-4 (δ_H 4.86) and δ_C 165.1 and δ_C 165.5 confirmed the presence of 2 gallates attaching to C-1 and C-2, respectively. One ester group was assigned to C-4 according to the HMBC correlations between H-4 (δ_H 4.86) and δ_C 170.4. The locations of the cyclohexane between ester group and ring B were established by the HMBC correlations between H-21 (δ_H 4.83) with C-14, 18, 22, 23, 26, and 27 (δ_C 118.6, 143.9, 110.5, 52.6, 31.4, 97.9, and 170.4). Furthermore, the correlations in HMBC between H-28 (δ_H 4.98) with C-22, 23, 25, 27, 29, 30 (δ_C 52.6, 31.4, 98.0, 170.4, 108.8, and 76.5), and correlation between H-30 (δ_H 4.07) with C-28 (δ_C 76.2) revealed the furan ring attaching to C-28. Comparison of the NMR data of 1 with those of Elaeocarpusin showed that 30-OH and 31-OH had α configuration. According to reports, an ether ring could be established between 29-OH and ring C. From eighteen degrees of unsaturation, an ether ring must be between C-24 and C-29 according to space

This work was supported by the “Western Light” Joint Research Program of the Chinese Academy of Sciences

* Corresponding author. Tel: +86-28-85245800; E-mail: lurh@cib.ac.cn
configuration. Finally, the structure of 1 was finally elucidated unambiguously by HMBC experiment (Fig. 4).

Known compounds were identified by comparison of their spectroscopic data with literature values or comparison of the mixed melting point and Rf value with authentic samples as follows: stearic acid (2), β-sitosterol (3),7 daucosterol (4),8 ethyl gallate (5),9 lauric acid (6), cinnamic acid (7), ellagic acid (8), and gallic acid (9),4,9 respectively.
Among them, 2, 5, 8 and 9 were the main constituents and 2, 4, 5 and 6 are reported for the first time from this plant.

**EXPERIMENTAL SECTION**

**General Experimental Procedures**

Melting points were determined using an XRC-1 melting point apparatus. Optical rotation was measured on a Perkin-Elmer 341 polarimeter at 589 nm. IR spectrum was measured on a Perkin-Elmer FT-IR spectrometer. NMR spectra were obtained on a Bruker Avance 600 spectrometer using TMS as an internal standard. HR-ESI-MS was acquired on Bruker BioTOF Q and ESI-MS on Finnigan LCQDECA spectrometers. Column chromatography was carried out on silica gel (Marine Chemical Factory, Qingdao,
China) and Sephadex LH-20 (Pharmacia).

**Plant Material**

The fruits of *P. emblica* (CDLR 040416) were purchased from Hehuachi Chinese traditional medicine Market (Chengdu, P. R. China).

**Extraction and Isolation**

Dried fruits of *P. emblica* (3 kg) were cut into small pieces and extracted (× 3) with ethanol (95%) at room temperature to afford a dark-brown residue (370 g) upon removal of the solvent under reduced pressure. The ethanol extract was suspended in water and partitioned successively with petroleum ether, ethyl acetate, and n-BuOH (2.0 L × 3 each).

The petroleum ether fraction was chromatographed over silica gel (200-300 mesh, 320 g) column with eluents of increasing polarity [petroleum ether/acetone (50:1→1:1)] to afford Fr.1-Fr.3 according to TLC analysis. Compounds 2 (400 mg), 3 (100 mg), and 4 (80 mg) were obtained from Fr. 1 (0.6 g), Fr. 2 (0.2 g), and Fr. 3 (0.1 g) by recrystallization from acetone, methanol, and methanol, respectively.

The ethyl acetate extract (10 g) was divided into 2 fractions (Fr.4-Fr.5) by chromatograph on silica gel column with petroleum ether/acetone (50:1→1:1) (55 mg), and 6 (100 mg). The remaining part of Fr.5 (2.5 g) was subjected to column chromatography over silica gel eluted with chloroform-methanol (30:1→1:1) gradient system to afford 5 (110 mg), 6 (55 mg), and 7 (100 mg). The n-BuOH extract (115 mg) and 1 (50 mg); the last compound was further purified by Sephadex LH-20 eluted with MeOH.

**Phyllanthunin (1)**

White powder (MeOH), mp 107-109 °C, [α]D20 = -54° (c 0.12, MeOH); HR-ESI-MS m/z: 805.1059 [M+Na]⁺ (calcd. for C32H30NaO23, 805.1070); ESI-MS: m/z 805 [M+Na]⁺; IR (KBr) νmax 3357, 1710 and 1614 cm⁻¹. ¹H-NMR (ppm, MeOD), δ 7.32 (1H, s, H-15), 7.14 (2H, s), 6.36 (1H, d, J = 3.5 Hz, H-1), 5.31 (1H, t, J = 1.7 Hz, H-2), 4.98 (1H, s, H-28), 4.86 (1H, d, J = 3.5 Hz, H-4), 4.83 (1H, s, H-21), 4.54-4.46 (1H, m, H-3), 4.33 (1H, t, J = 6.5 Hz, H-5), 4.20 (1H, dd, J = 10, 5.6 Hz, H₃-32), 4.18 (1H, m, H-31), 4.07 (1H, s, H-30), 4.03, 3.96 (each 1H, dd, J = 11, 6.5 Hz, H₂-6), 3.92 (1H, dd, J = 10, 3 Hz, H₄-32), 2.68, 1.57 (each 1H, d, J = 14 Hz, H₂-6). ¹³C-NMR (ppm, MeOD), δ 170.4 (C-27, s), 165.5 (C-20, s), 165.1 (C-13, s), 145.2 (C-9, 11, s), 144.9 (C-16, s), 143.9 (C-18, 3), 139.1 (C-10, s), 137.7 (C-17, s), 119.1 (C-7, s), 118.6 (C-14, s), 113.8 (C-15, d), 110.5 (C-19, s), 109.1 (C-8, 12, d), 108.8 (C-29, s), 98.0 (C-25, s), 97.9 (C-26, s), 97.5 (C-24, s), 92.1 (C-1, d), 80.8 (C-31, d), 78.1 (C-5, d), 76.5 (C-30, d), 76.2 (C-28, d), 74.2 (C-32, t), 73.2 (C-2, d), 70.9 (C-4, d), 62.1 (C-3, d), 62.1 (C-6, t), 52.6 (C-22, s), 50.9 (C-21, d), 31.4 (C-23, t).

**ACKNOWLEDGMENTS**

Funding from the “Western Light” Joint Research Program of the Chinese Academy of Sciences is gratefully acknowledged.

Received February 16, 2007.

**REFERENCES**