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

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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.[1] Previous phytochemical studies of *P. emblica* resulted in the isolation of tannins, flavonoids, and acids.[1-4] 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 ($[M+Na]^+$, calcd. 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 ^{1}H- and ^{13}C-NMR data of **1** revealed 32 carbon signals due to 17 quaternary carbons, 12 methines and 3 methylenes. Among them, the ^{13}C-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),[5,6] and had the same β configuration glycoside, 2 gallates, a cyclohexane, and a furan ring, which were confirmed by the ^{1}H–^{1}H 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 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, 19, 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,[6,7] 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

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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 R_f 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.

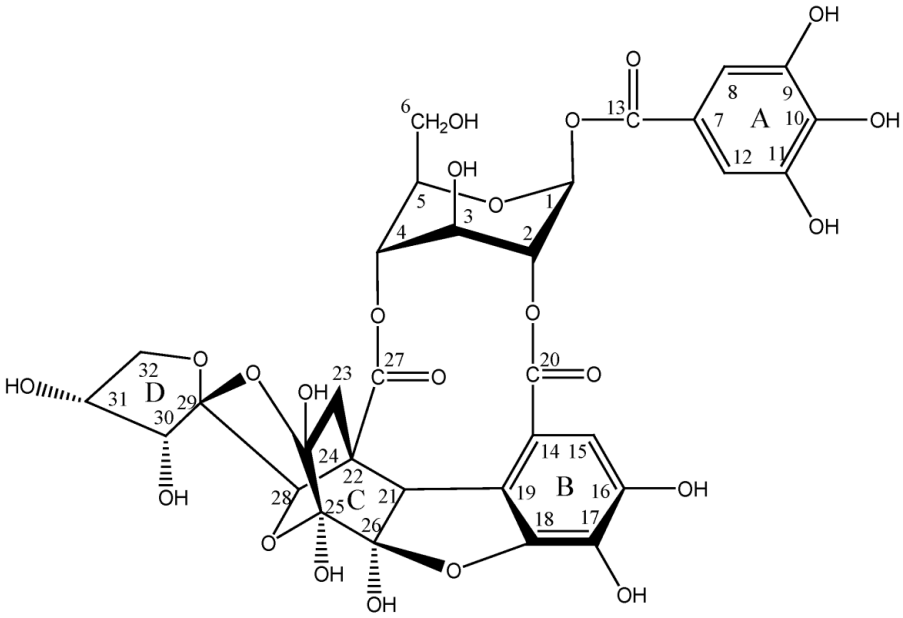

Fig. 1. Structure of compound **1**.

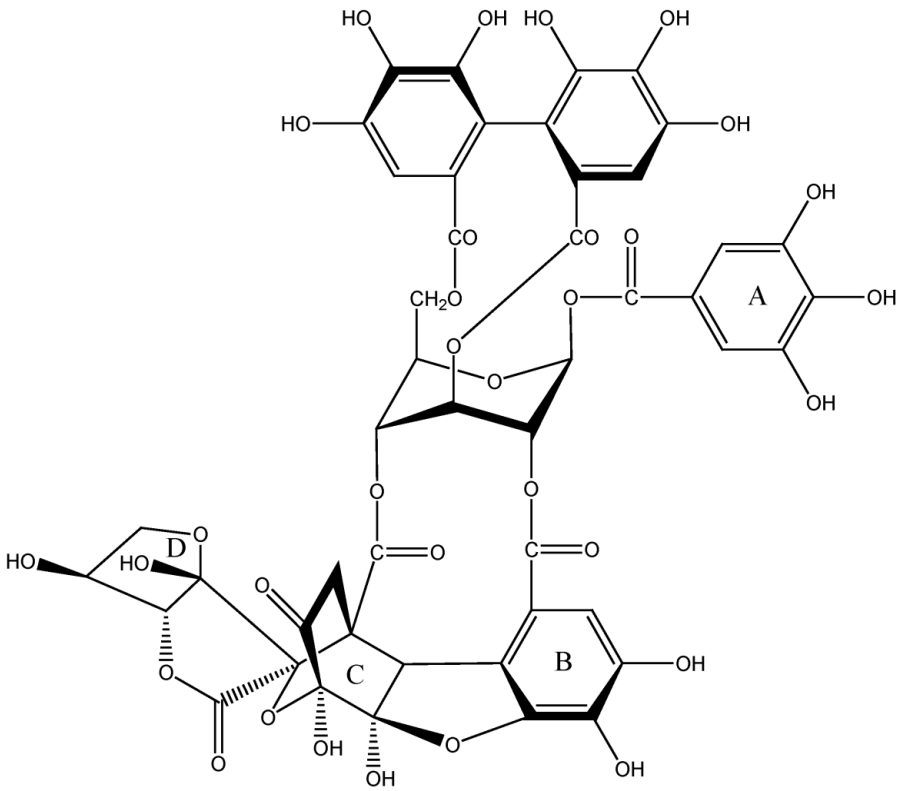

Fig. 2. Structure of Elaeocarpusin.

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 LCQ^{DECA} spectrometers. Column chromatography was carried out on silica gel (Marine Chemical Factory, Qingdao,

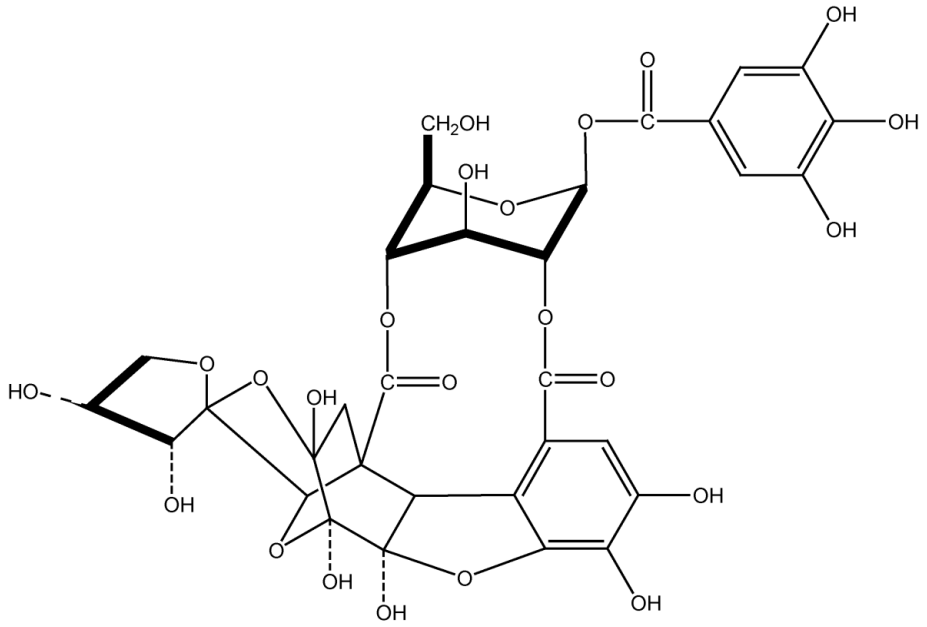

Fig. 3. The key ^{1}H-^{1}H COSY correlations of compound **1**.

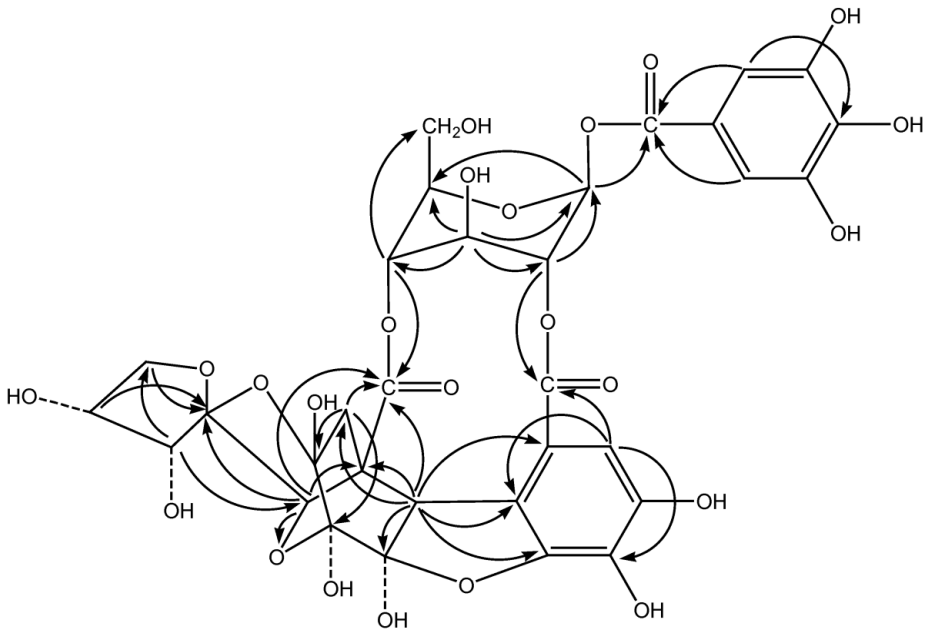

Fig. 4. The key HMBC correlations of compound **1**.

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 (2 kg, 200-300 mesh) eluted with petroleum ether-acetone eluents (50:1→1:1). The Fr.4 (1.8 g) was subjected to column chromatography over silica gel, eluted with petroleum ether-acetone (50:1→1:1) gradient system to afford **5** (110 mg), **6** (55 mg), and **7** (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 **8** (100 mg).

The n-BuOH extract was evaporated under reduced pressure to yield 100 g residue. This residue was chromatographed over macropore adsorptive resin eluted with 30% ethanol to afford Fr.6. Fr.6 (2 g) was subjected to column chromatography over silica gel with chloroform-methanol (15:1→1:1) gradient system to give **9** (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, $[\alpha]_D^{20}$ -54° (*c* 0.12, MeOH); HR-ESI-MS *m/z*: 805.1059 $[M+Na]^+$ (calcd. for $C_{32}H_{30}NaO_{23}$, 805.1070); ESI-MS: *m/z* 805 $[M+Na]^+$; IR (KBr) ν_{max} 3357, 1710 and 1614 cm^{-1}. ^{1}H-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), 4.54-4.56 (1H, m, H-3), 4.33 (1H, t, *J* = 6.5 Hz, H-5), 4.20 (1H, dd, *J* = 10, 5.6 Hz, H_a-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, H_2-6), 3.92 (1H, dd, *J* = 10, 3 Hz, H_b-32), 2.68, 1.57 (each 1H, d, *J* = 14 Hz, H_2-6). ^{13}C-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).

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