New Phenolic Constituents from the Fruit Juice of Phyllanthus emblica

Ying-Jun ZHANG, Takashi TANAKA, Chong-Ren YANG, and Isao KOUNO

Faculty of Pharmaceutical Sciences, Nagasaki University; Bunkyo Machi 1–14, Nagasaki 852–8521, Japan and Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China.

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Six new phenolic constituents, \(2\)-malic acid 2-\(O\)- (1), mucic acid 2-\(O\)- (5), mucic acid 1,4-lactone 2-\(O\)- (6), 5-\(O\)- (8), 3-\(O\)- (10), and 3,5-di-\(O\)- (11) gallates, were isolated from the fruit juice of Phyllanthus emblica together with their methyl esters (2—4, 7, 9), and their structures were determined by spectral and chemical methods. Compounds 5, 6, and 8, the major phenolic constituents of the juice, were present as an equilibrium mixture in aqueous solution.

Key words Phyllanthus emblica; Euphorbiaceae; organic acid gallate; mucic acid

Phyllanthus emblica L. (Euphorbiaceae) is a shrub or tree growing in subtropical and tropical areas of the People’s Republic of China, India, Indonesia, and the Malay Peninsula. The whole plant, especially the fruit has been used as an anti-inflammatory and antipyretic drug in many local traditional medicines: Chinese herbal medicine, Tibetan medicine, and Ayurvedic medicine. Several reports about this plant have shown that the fruits are rich in vitamin C, mucic acid, and tannins. In our previous work, a novel highly oxygenated norbisabolane together with its methyl ester and generated norbisabolane were isolated from the roots of the same plant along with 15 tannins and related compounds. As part of our continuing studies on the plant, we report herein the isolation and structural elucidation of new phenolic constituents, \(2\)-malic acid 2-\(O\)- (1), mucic acid 2-\(O\)- (5), mucic acid 1,4-lactone 2-\(O\)- (6), 5-\(O\)- (8), 3-\(O\)- (10), 3,5-di-\(O\)- (11) gallates, and their methyl esters (2—4, 7, 9) from the fruit juice of P. emblica.

Results and Discussion

A 60% aqueous acetone extract of the powdered form of the fruit juice of P. emblica was subjected to MCI gel CHP 20P column chromatography to afford five fractions. Fraction I obtained by elution with \(H_2O\) was further chromatographed successively over Sephadex LH-20, MCI gel CHP20P, Toyopearl HW-40F, and Cosmosil 75C18 OPN to afford compounds 1—11 and three known compounds. By comparison of the physical and spectral data with those of authentic samples, the known compounds were identified as gallic acid, chebulic acid, and 1-O-galloyl-\(\beta\)-D-glucose, respectively.

Compound 1 was obtained as a white amorphous powder and showed a dark blue coloration with ferric chloride reagent. Its molecular formula was assigned as \(C_{11}H_{10}O_9\) on the basis of the \(^{13}\text{C}\)-NMR spectral data, the negative-ion FAB-MS \([m/z\ 285, (M–H)^–]\), and elemental analysis. The \(^{13}\text{C}\)-NMR spectrum of 1 showed signals due to two carboxyl carbons at \(\delta 170.9\) and 170.6, an oxygen bearing methine at \(\delta 69.4\) and a methylene at \(\delta 36.5\), along with a set of carbons arising from a galloyl group. Hydrolysis of the galloyl ester with tannase yielded gallic acid and \(2\)-malic acid (10), 3-\(O\)- (10), 3,5-di-\(O\)- (11) gallates, and their methyl esters (2—4, 7, 9) from the fruit juice of P. emblica.

\begin{center}
\includegraphics[width=0.5\textwidth]{chart1.png}
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Chart 1

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respectively. In addition, correlations of a doublet methine proton at δ 5.55 (H-2) with the C-1 and the carboxyl carbon (δ 166.7) of galloyl group indicated that 2 was a 2-O-gallate of 2,3,4,5-tetrahydroxyladipic acid (mucic acid) dimethyl ester. The dimethyl ester was obtained by hydrolysis of 2 with tannase and identified as mucic acid dimethyl ester by direct comparison with an authentic sample. Accordingly, the structure of 2 was established as mucic acid dimethyl ester 2-O-gallate.

Compounds 3 and 4 showed the same (M+H)+ ion peak at m/z 377 in the positive-ion FAB-MS, which was 14 mass units less than that of 2 and suggested the lack of a methoxyl group. The 1H- and 13C-NMR spectral data of 3 and 4 closely resembled those of 2, except for the absence of one of the two methoxyl signals. The presence of a mucic acid core in their molecules was confirmed by tannase hydrolysis followed by methylation affording mucic acid dimethyl ester. In the HMBC spectra of 3 and 4, H-2 (δ 5.58 for 3, δ 5.55 for 4) was correlated with C-1 (δ 170.6 for 3, δ 170.2 for 4) and the carboxyl carbon of galloyl group (δ 166.2 for 3, δ 166.3 for 4). However, the methoxyl proton (δ 3.71) of 3 was correlated with the C-6 (δ 174.6), while the methoxyl proton (δ 3.73) of 4 was coupled with the C-1 (δ 170.2). This observation indicated that these two compounds were positional isomers differing in the location of their methoxyl group. Hence, the structures of 3 and 4 were established as mucic acid 6-methyl ester 2-O-gallate (3) and mucic acid 1-methyl ester 2-O-gallate (4), respectively.

The molecular formula of compound 5 was assigned as C17H12O5 based on the 13C-NMR spectral data, the negative-ion FAB-MS [m/z 361, (M-H-)], and elemental analysis. Chemical shifts and coupling patterns of the 1H-NMR spectrum of 5 as well as its 13C-NMR spectral data were very similar to those of 2-4, except for the absence of methyl signals, indicating that this compound is the free acid form of 2-4. Thus, the structure of 5 was determined as mucic acid 2-O-galate.

Compound 6 was obtained as a white amorphous powder. Its 1H-, 13C-NMR, and HSQC spectra revealed the presence of two carboxyl groups (δ 171.0, 173.0), four oxygen-bearing methines (δc 82.0, 75.5, 72.4, 68.2, corresponding to δH 4.76 (dd, J = 1.8, 8.4 Hz), 5.97 (d, J = 8.7 Hz), 4.87 (dd, J = 8.7, 8.4 Hz), 4.49 (d, J = 1.8 Hz), respectively), and a galloyl group [δH 7.18 (2H, s)]. The composition of 6 was similar to that of 5; however, their chemical shifts and coupling constants were significantly different. The core alcohol of 6 was shown to be mucic acid because it became an equilibrium mixture with 5 in aqueous solution and even in the NMR sample tube (acetone-d6+D2O). The positive-ion FAB-MS of 6 showed the (M+H)+ ion peak at m/z 345, which was 18 mass units less than that of 5, indicating that 6 is a lactone form of 5. Among the above-mentioned four methine protons, two doublet signals at δ 5.97 and 4.49 were assignable to H-2 or H-5 of the mucic acid core. In the HMBC spectrum, one of the doublet signals at δ 5.97 was correlated with two carboxyl signals at δ 171.0 and 166.5, the latter being assignable to the carboxyl carbon of the galloyl group, indicating that the galloyl group was located at this position. Furthermore, the chemical shift of the other doublet signal indicated that this position was not acylated. Since the lactone formation usually occurs at γ (1,4-lactone) or δ (1,5-lactone) position, this observation suggested that the mucic acid moiety in 6 existed as a 1,4-lactone form. Although locations of the two esters could not be determined by the HMBC experiment, this was achieved by comparison of the 1H-NMR spectrum with that of d- saccharic acid 1,4-lactone (12), which is a C-4 epimer of mucic acid with the same relative configurations at C-2 and C-3 positions. The H-2 signal of 12 appeared as a doublet signal with large coupling constant (J5,6 = 8.7 Hz, J5,3 = 2.8 Hz), and the J value coincided with that observed for the methine signal at δ 5.97 of 6 where the galloyl group was located. This indicated that the galloyl group of 6 was attached to the hydroxyl group adjacent to the lactone carbonyl carbon. Based on the above evidence, the structure of 6 was concluded to be mucic acid 1,4-lactone 2-O-gallate.

The 1H-NMR spectrum of compound 7 was almost superimposable on that of 6, except for the appearance of an additional methyl signal at δ 3.81(s). The positive-ion FAB-MS of 7 showed the (M+H)+ ion peak at m/z 359, which was 14 mass units more than that of 6, indicating the occurrence of a methyl group in 7. Accordingly, 7 was determined as mucic acid 1,4-lactone 6-methyl ester 2-O-gallate.

Compound 8 was obtained as a white amorphous powder. Its molecular composition C13H12O7 was identical to that of 6. Coupling patterns of four methine signals observed in the 1H-NMR spectrum were similar to those of 6; however, the H-5 of 8 resonated at lower field [δ 5.57 (d, J = 2.1 Hz)] compared with that of 6 [δ 4.49 (d, J = 1.8 Hz)], and instead, the H-2 was shifted to higher field [δ 4.71 (d, J = 8.7 Hz)] for 8, δ 5.97 (d, J = 8.7 Hz) for 6]. This observation clearly indicated that the galloyl group was attached to C-5 in 8, which was also supported by the lower field shift of C-5 (δ 74.4) and the upper field shift of C-2 (δ 74.0) compared to those of 6 [δ 68.2 (C-5), δ 75.5 (C-2)]. According to the above evidence, the structure of 8 was established as mucic acid 1,4-lactone 5-O-gallate.

Compound 9 exhibited the (M+H)+ ion peak at m/z 359, and 1H-NMR spectrum was very similar to that of 8 except for occurrence of an additional methyl signal at δ 3.80, suggesting that 9 was the methyl ester of 8. Therefore, 9 was determined to be mucic acid 1,4-lactone 6-methyl ester 5-O-gallate.

Compound 10 was found to be an isomer of 6 and 8 by analysis of the 13C-NMR spectral data and the result of positive-ion FAB-MS [m/z 343, (M-H-)]. The 1H-NMR spectrum showed a large downfield shift of H-3 [δ 5.73 (dd, J = 7.2, 6.6 Hz)] instead of H-2 of 6, indicating that the galloyl group was attached to the C-3 position in 10. Thus, the structure of 10 was established as mucic acid 1,4-lactone 3-O-gallate.

Compound 11 was obtained as a white amorphous powder, and its 1H- and 13C-NMR spectra revealed the presence of two galloyl groups (δ 7.23, 7.17), and a mucic acid core. This was supported by the positive-ion FAB-MS showing the (M-H-1) peak at m/z 495. In the 1H-NMR spectrum, the signals due to H-3 and H-5 were appeared at the lower field [δ 5.62 (dd, J = 7.5, 6.9 Hz) and δ 5.65 (d, J = 1.8 Hz), respectively], while H-2 and H-4 signals remained at the upper field [δ 5.10 (d, J = 7.5 Hz), δ 5.14 (dd, J = 1.8, 6.9 Hz)]. Hence, the structure of 11 was determined to be mucic acid 1,4-lactone 3,5-di-O-gallate.
These mucic acid gallates were unstable in the aqueous solution and became an equilibrium mixture. Actually, treatment of 2 with H2O-MeOH (4:1) at room temperature for 36 h afforded compounds 2–9, and 5 also gave a mixture of compounds 2–9 under similar conditions, confirming the equilibrium between these compounds (Fig. 1). Taking the equilibrium into account, the fact that these gallates are optically active implied the occurrence of an enantiospecific glycosylation at C-2 position of the optically inactive mucic acid in P. emblica. Interestingly, the mucic acid core of 8 corresponds to the enantiomer of 6.

Most of the methyl esters were probably artifacts generated during the separation; however, direct analysis of the 60% aq. acetone extract of the juice by TLC and HPLC indicated the occurrence of small amounts of the methyl esters.

Because of the equilibrium, the purification of the mucic acid gallates was extremely difficult, and the absolute configuration has not been determined. Although isolation yields were not high, HPLC analysis showed that compounds 5, 6, and 8 were the major phenolic constituents of the juice together with 1-O-galloyl-β-d-glucose, and these galloyl esters may play an important role as antioxidants in the juice together with vitamin C.

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. 1H- and 13C-NMR spectra were recorded with Varian Unity plus 500 and 400 MHz, respectively. Coupling constants were expressed in Hz, and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. MS were recorded on a JEOL JMS-DX303 spectrometer. Column chromatographies were performed with MCI-gel CHP 20P (75–150 μm, Mitsubishi Chemical Co.), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co., Ltd.), Toyopearl HW-40F (37–70 μm, Tosoh Co.), Cosmosil 75C2-OPN (Nacalai Tesque, Inc.), and Chromatorex ODS column (150 mm, Tosoh Co.). Tautomeric states of the esters were determined by 1H- and 13C-NMR spectra recorded with Varian Unity plus 500 MHz.

Hydrolysis of 4 with Tannase

A solution of 4 (25 mg) in water (1 ml) was incubated with tannase (2 mg) at room temperature overnight. The reaction mixture was subjected to Chromatorex ODS column chromatography with H2O-MeOH (1:0—0:1 and then 50% acetone) to give mucic acid dimethyl ester (3.2 mg). Off-white amorphous powder, [α]D 25 +51.0° (c = 0.41, MeOH). 1H-NMR (aceton- d6, 500 MHz) δ: 3.75 (1H, s, OCH3), 3.79 (3H, s, OCH3), 4.10 (1H, dd, J = 2.0, 10.0 Hz, H-3), 4.62 (1H, dd, J = 1.5, 15.0 Hz, H-5), 5.55 (1H, d, J = 2.0 Hz, H-2), 7.24 (2H, s, galloyl H-2, 6). 13C-NMR (aceton-d6, 125 MHz) δ: 52.5 (O-CH3), 52.7 (O-CH3), 70.6 (C-3), 71.0 (C-5), 71.9 (C-1), 73.2 (C-4), 110.0 (C-2, C-6), 120.2 (C-1), 135.2 (C-4), 157.7 (C-3′, 5′), 166.7 (C-7′), 170.6 (C-1′), 174.8 (C-6′). FAB-MS m/z: 391 (M+H)+. Anal. Calcd for C19H22O10: C, 60.11; H, 5.68. Found: C, 60.0; H, 5.56.

Hydrolysis of 5 with Tannase

A solution of 5 (25 mg) in water (1 ml) was incubated with tannase (2 mg) at room temperature overnight. The reaction mixture was subjected to Chromatorex ODS column chromatography with H2O-MeOH (1:0—0:1 and then 50% acetone) to give gallic acid (3 mg) and 1-malic acid (8 mg). White amorphous powder, [α]D 25 +34.0° (c = 0.2, MeOH). 1H-NMR (aceton-d6, 500 MHz) δ: 3.75 (1H, s, OCH3), 3.79 (3H, s, OCH3), 4.10 (1H, dd, J = 2.0, 10.0 Hz, H-3), 4.62 (1H, dd, J = 1.5, 15.0 Hz, H-5), 5.55 (1H, d, J = 2.0 Hz, H-2), 7.24 (2H, s, galloyl H-2, 6). 13C-NMR (aceton-d6, 125 MHz) δ: 52.5 (O-CH3), 52.7 (O-CH3), 70.6 (C-3), 71.0 (C-5), 71.9 (C-1), 73.2 (C-4), 110.0 (C-2, C-6), 120.2 (C-1), 135.2 (C-4), 157.7 (C-3′, 5′), 166.7 (C-7′), 170.6 (C-1′), 174.8 (C-6′). FAB-MS m/z: 391 (M+H)+. Anal. Calcd for C19H22O10: C, 60.11; H, 5.68. Found: C, 60.0; H, 5.56.
Mucic Acid 1,4-Lactone 3,5-Di-O-gallate (11) White amorphous powder, \([\delta]_{D}^{22} 96.5^\circ (c=0.20, 
MeOH); \) 1H-NMR (acetone-\(d_{6}, 300 \text{ MHz}\)) \(\delta\): 5.10 (1H, \(\text{d}, J=7.5 \text{ Hz}, 
H-2), 5.14 (1H, \text{dd}, \(J=1.8, 6.9 \text{ Hz}, 
H-4), 5.62 (1H, \text{dd}, \(J=7.5, 6.9 \text{ Hz}, 
H-3), 5.65 (1H, \text{d}, \(J=1.8 \text{ Hz}, 
H-5), 7.17, 7.23 (\text{each } 2H, 
galloyl H-2'), 2', 6', 6'). 13C-NMR (acetone-\(d_{6}, 75 \text{ MHz}\)) \(\delta\): 71.1 (C-5), 72.4 (C-2), 76.3 (C-3), 79.1 (C-4), 110.1 (galloyl C-2', 2', 6', 6'), 120.2, 119.8 (C-1', 1'), 138.3 (C-4', 4'), 146.0 (C-3', 3', 5', 5'), 166.6, 165.6 (C-7', 7'). Analyzed for C\(_{22}H_{18}O_{12}\): C, 43.47; H, 3.91. Found: C, 43.42; H, 4.22.

Equilibrium of Compounds 2—9 A solution of 2 (150 mg) in H\(_2\)O/MeOH (4:1, 10 ml) was left at room temperature for 36 h. Repeated chromatography of the mixture over Sephadex LH-20 and Cosmosil 75C\(_{8}\) OPN column afforded compounds 3 (20 mg), 4 (17 mg), 5 (30 mg), 6 (10 mg), 7 (3 mg), 8 (30 mg), 9 (7 mg), and a recovery of 2 (14 mg). These compounds were identified by comparison of the \(1^H\), \(13C\)-NMR spectral data and the \([\delta]_{D}\) values with those of the authentic samples. A similar experiment was also carried out for compound 5: a solution of 5 (1 mg) in H\(_2\)O/MeOH (4:1, 10 ml) was left at room temperature for 48 h and it became a mixture of compounds 2—9, which were identified directly by TLC comparison with the authentic samples (benzene–ethyl formate–formic acid, 1:7:1).

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References