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Ellagi- and gallotannins from *Punica granatum* heartwood

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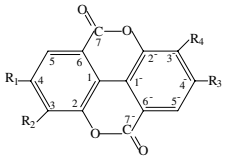
From the heartwood of *Punica granatum* L., the new 3′-*O*-methyl-3,4-methylenedioxyellagic acid, as well as eight known ellagitannins and gallotannins have been isolated and characterized. The structures were established by chromatography, chemical degradation and UV spectroscopy and confirmed by MS and NMR spectroscopy.

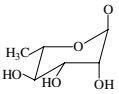
1. Introduction

Punica granatum L. (Punicaceae) a small tree native to the Mediterranean region, known in Egypt as "Romman" is used in traditional medicine [1, 2]. Previous phytochemical investigations were focused on the leaves [3, 4] and bark [5–7] of this species to identify ellagitannins, gallotannins and flavone glycosides.

The present work describes the isolation and identification of the new ellagic acid derivative **1** and ellagic acid 4-*O*-α-L-rhamnopyranoside (**2**) which was isolated the first time from this species and the second time in nature [8] in addition to 6-*O*-galloyl-(α/β)-*O*-glucopyranose (**3**), 6-*O*-galloyl-2,3-(S)-hexahydroxydiphenoyl-(α/β)-D-glucopyranose (**4**), corilagin (**5**), 3,3′-di-*O*-methylellagic acid (**6**), ellagic acid (**7**), gallic acid (**8**) and methyl gallate (**9**).



1: R_1 —— R_2 = OCH_2O, R_3 = OH, R_4 = OCH_3



2: R_1 = , $R_2 = R_3 = R_4$ = OH

2. Investigations, results and discussion

The dry powder of *Punica granatum* heartwood was extracted with aqueous ethanol (80%) under reflux. The extract obtained was concentrated in vacuo and examined by 2D-PC, whereby a mixture of ellagitannins and gallotannins was detected due to positive results with spray reagents specific for ellagi- and gallotannins [9]. The concentrated extract was applied to polyamide CC using water/ethanol mixtures with decreasing polarity which afforded fractions containing ellagitannins and gallotannins. Further fractionation was achieved by means of consecutive microcrystalline cellulose and Sephadex LH-20 columns. Elution with aqueous ethanol and/or n-butanol saturated with water afforded the known tannins **2**–**9** along with the new ellagic acid derivative **1**. Their structures were elucidated by established chromatographic and spectroscopic methods especially ESI-MS and NMR techniques.

Compound **1** was isolated as a white amorphous powder which showed a single spot on PC exhibiting buff fluorescence under short UV light and characteristic UV spectral data of a methoxy ellagic acid derivative (UV λ_{max} (MeOH): 247, 270 sh, 353 sh, 368) [10]. The molecular formula of **1** was determined by the presence of a molecular ion peak at m/z 227 $(M-H)^-$ in negative ESI.

The ^{1}H NMR spectrum of **1** showed the presence of two aromatic protons at δ 7.50 ppm (s; H-5) and δ 7.51 (s; H-5′) assigned to the ellagic acid moiety and singlets at δ 6.37 ppm integrated to two protons assigned to methylenedioxy group and at δ 4.04 ppm to one methoxy group. The assignments of the recorded proton resonances were confirmed by COSY-NMR measurement.

In ^{13}C NMR, compound **1** displayed 16 distinct carbon signals but some of these signals were separated by only a few tenths of a ppm. Five quaternary carbons appear in the region of 110–116 ppm (110.94, 111.06, 112.03, 112.57 and 115.96) to assign unequivocally the carbon signals. Two carbonyl carbons at δ 158.21 and 157.57 due to α, β-unsaturated lactones, two protonated aromatic carbons at δ 112.03 and 103.83, one methylene carbon at δ 104.28 and methoxyl carbon at δ 60.94 were revealed.

Finally the structure was confirmed by its HMBC correlation, the patterns of H–C correlations were H-5 (δ 7.50) to C-1, C-3, C-4, C-6 and C-7; H-5′ (δ 7.51) to C-1′, C-3′, C-4′, C-6′ and C-7′; $-CH_2-$ (δ 6.37) to C-3′ and C-4′ and CH_3-O- (δ 4.08) to C-3. Thus **1** was unambiguously identified as 3′-*O*-methyl-3,4-methylenedioxyellagic acid.

Compound **2** was isolated as yellowish white amorphous powder, in PC giving mauve fluorescence under short UV light changing to lemon yellow with ammonia and characteristic UV spectral data of an ellagic acid derivative (λ_{max} (MeOH): 255, 350 sh, 362) [11, 12]. Acid hydrolysis of **2** resulted in ellagic acid and L-rhamnose (CoP). Accordingly, the structure was expected to be an ellagic acid *O*-rhamnoside. Confirmation of the structure was achieved by ^{1}H NMR, whereby the spectrum showed in the aromatic region two singlets at δ pp 7.7 and 7.45, each integrated to one proton and assigned to H-5 and H-5′ respectively. The downfield shift of H-5 resonance revealed that the rhamnoside moiety was located at C-4, the presence of an *O*-α-L-rhamnosyl moiety was deduced from three resonances of H-1″, H-2″ and CH_3-6'' as δ 5.45 (1 H, d, J = 1.7 Hz), 4.0 (1 H, dd, J = 3.3, 1.7 Hz) and 1.12 (3 H, d, J = 5.9 Hz) respectively. Thus **2** was unambiguously identified as ellagic acid 4-*O*-α-L-rhamnopyranoside.

3. Experimental

3.1. Equipment

UV-analyses were run on a Shimazu UV-240 spectrometer and 4 ml quartz cells (1 cm optical pathway). Analytically pure MeOH was used. ESI-MS spectrometry was measured on a double focusing sector field Finnigan MAT95 mass spectrometer Bremen, Germany). NMR (^{1}H and ^{13}C) analyses were measured on JEOL EX-270 MHz and Varian Mercury 300 MHz

spectrometers relative to TMS. PC was carried out on Whatman No.1 sheets using solvent systems A (6% AcOH) and B (n-BuOH−AcOH−H_2O 4 : 1 : 5, top layer).

3.2. Plant material

Punica granatum heartwood was collected from a mature tree, growing in Giza, Egypt in June 1995, and identified by Prof. Dr. L. Boulos, National Research Center, Cairo, Egypt.

3.3. Extraction and isolation

The dry powder of *P. granatum* heartwood (2 kg) was defatted with $CHCl_3$, and extracted with EtOH (80%) which yielded a dry extract (110 g) on removal of the solvent. This extract was chromatographed on a polyamide 6S column (Riedel-De Haen AG, Seelze, Hannover, Germany), being eluted with H_2O followed by EtOH mixtures of decreasing polarity. The obtained major tannin fractions were further fractionated using successive microcrystalline cellulose and Sephadex LH-20 columns to give pure **1** (25 mg) and **2** (8 mg).

3.4. 3′-O-Methyl-3,4-di-O-methylenedioxyellagic acid (**1**)

Rf-values: 0.12 and 0.83 in solvent A and B, respectively. UV λ_{max} (MeOH) : 247, 270 sh, 353 sh, 386. ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 7.50 (1 H, s, H-5), 7.51 (1 H, s, H-5′), 6.37 (2 H, s, $-CH_2-$), 4.04 (3 H, s, $-OCH_3-$). ^{13}C NMR: δ ppm 158.21 (C-7), 157.57 (C-7′), 152.7 (C-4′), 150.02 (C-4), 141.50 (C-3′), 140.19 (C-2′), 138.27 (C-3), 130.99 (C-2), 115.96 (C-1), 112.57 (C-1′), 112.03 (C-5′), 111.06 (C-6′), 110.94 (C-6), 104.28 ($-CH_2-$), 103.83 (C-5), 60.94 (CH_3-O-).

3.5. Ellagic acid 4-O-α-L-rhamnopyranoside (**2**)

Rf-values: 0.21 and 0.42 in solvent A and B, respectively. UV λ_{max} (MeOH): 255, 350 sh, 362. ^{1}H NMR (270 MHz, DMSO-d_6): δ ppm 7.7 (1 H, s, H-5); 7.45 (1 H, s, H-5′); 5.45 (1 H, d, J = 1.7 Hz, H-1″); 4.0 (1 H, dd, J = 3.3 & 1.7 Hz, H-2″); 1.12 (3 H, d, J = 5.9 Hz, CH_3–6″); 3.0–3.8 (remaining sugar protons). Acid hydrolysis of 2: A solution of 4 mg in 10 ml 2 N HCl (MeOH−H_2O, 1 : 1) was refluxed at 100 °C for 2 h. After cooling, the reaction mixture was extracted with EtOAc. The aqueous hydrolysate was neutralized, concentrated and then examined by CoPC to prove the presence of ellagic acid and rhamnoside.

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