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Chemical Investigation of the Constitutive Phenolics of *Rosa arabica*; the Structure of a New Dimeric Phenolic Glycoside

Ahmed M. A. Souleman* and Amani M. D. El-Mousallamy#

National Research Centre, Dokki, Cairo, Egypt,
#Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, Egypt

Abstract – The aqueous ethanolic whole plant extract of *Rosa arabica* was found to contain the new natural dimeric phenolic compound, ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnopyranoside, **9**, along with ten known phenolic metabolites (**1-8**, **10** and **11**). Structures of all compounds (**1-11**) were established by routine methods of analysis and confirmed by FAB-MS, ^{1}H and ^{13}C NMR spectral analysis.

Key words – *Rosa arabica*, Rosaceae, dimeric phenolic glycoside, ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnopyranoside, FAB-MS, NMR.

Introduction

Rosa arabica Crep. is a thorny glabbrous shrub which grows wild in the southern part of Sinai proper, Egypt (Täckholm, 1974). It belongs to the genus Rosa which possesses potential medicinal properties (Bisset, ed., 1994) and is known to synthesise and accumulate a wide variety of flavonoids, including the unique 2-phenoxychromones (Hashidoko *et al.*, 1991), in addition to gallo- and ellagi-tannins (Hashidoko, 1995). The plant known in Egypt as "Ward barri", is used as a fragrant and antidiarrhoeal agent (Boulos, 1983) but it has not been investigated, previously for its constitutive phenolics. In the present communication we report on the isolation and characterisation from an aqueous alcoholic *R. arabica* whole plant extract of a new dimeric phenolic glycoside, ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnopyranoside, together with ten known phenolics, gallic acid (**1**), quercetin 3-*O*-rutinoside (**2**), 3-*O*-β-galactopyranoside (**3**), 3-*O*-β-glucopyranoside (**4**), 3-*O*-α-Rhamnopyranoside (**5**), 3-*O*-β-(6"-galloyl)-galactopyranoside (**6**), kaempferol 3-*O*-β- glucopyranoside (**7**), kaempferol 3-*O*-β-galactopyranoside (**8**), quercetin (**10**) and kaempferol (**11**).

Results and Discussion

The meal of the dried whole plant material of *Rosa arabica* was exhaustively extracted with aqueous ethanol (3:1). Compounds **1-11** were individually isolated and purified from the received extract through polyamide column fractionation, using H_2O-EtOH solvent systems of decreasing polarities as eluents, followed by Sephadex LH-20 column chromastography and H_2O or *n*-BuOH saturated with H_2O for elution. The known compounds **1-8**, **10** and **11** gave chromatographic, UV spectral properties (Table 1), FAB-MS, ^{1}H and ^{13}C NMR data identical with those reported for gallic acid **1** (Nawwar *et al.*, 1982); quercetin 3-*O*-rutinoside **2**, quercetin 3-*O*-β-glucopyranoside **4**; quercetin 3-*O*-α-rhamnopyranoside **5** (Nawwar *et al.*, 1984); quercetin 3-*O*-β-galactopyranoside **3**; kaempferol 3-*O*-β-glucopyranoside **7**; kaempferol 3-*O*-β-galactopyranoside **8**; quercetin **10** and kaempferol **11** (Barakat *et al.*, 1997); quercetin 3-*O*-β-(6"-galloyl)-galactopyranoside **6** (Barakat, 1985).

The new compound **9** was isolated as a white amorphous powder of molecular weight = 448 amu as shown by negative FAB-MS ([M-H]$^-$ at *m/z* = 447). The chromatographic and UV spectral analysis of **9** (Table 1) suggested a conjugate of ellagic acid dimethyl ether (Nawwar *et al.*, 1982 and Souleman *et al.*, 1998). This view was supported by normal aqueous acid hydrolysis of the compound (2N HCl, 3 hours, 100°) to yield ellagic acid 3,3'-dimethyl ether and rhamnose (CoPC). The former was extracted from the aqueous hydrolysate by ethyl acetate, dried *in vacuo* and its identity was further

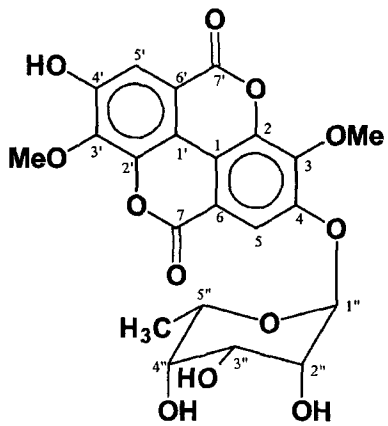
*Author for correspondence.

Table 1. Chromatographic and UV data of compounds **1-11**

Compd No.	Chromatographic properties R_fs(x100)			UV Spectral data λ_{max} (nm)				
	H_2O	HOAc	BAW	MeOH	NaOAc	NaOAc-H_3BO_3	$AlCl_3$	NaOMe
1	53	59	78	272				
2	65	65	55	255, 267* 360	255*, 270 278	261, 380	266, 300* 364*, 420	270, 330 403
3	11	44	58	258, 355	273, 358	272, 380	262, 405	265, 410
4	08	41	60	258, 356	274, 362	272, 380 420*	268, 430	275, 474
5	20	52	72	259*, 297* 348	276, 372	272, 382	268, 352 408	270, 355 402
6	08	45	56	260, 295* 356	272, 295* 360	265, 300 375	275, 300* 425	275, 330 410
7	20	44	68	267, 353	273, 355	271, 355	272, 408	275, 310 402
8	23	46	65	267, 351	274, 355	272, 355	272, 405	275, 312 405
9	29	48	57	244, 252* 345, 380				
10	00	07	72	255, 268* 370	254*, 276 375	272, 388	270, 360* 440	252*, 320 -dec
11	00	09	85	268, 369	270, 310 375	270, 320 370	270, 305* 360, 430	278, 316 416-dec.

*: inflection
dec.: decomposition

proved by UV spectral, EI-MS, ^1H and ^{13}C NMR analysis (see Experimental). Consequently, **9** must be ellagic acid 3,3'-dimethyl ether mono-rhamnoside. The final structure of the compound was then achieved through NMR spectral analysis. The ^1H NMR spectrum (DMSO-d_6, room temp.) exhibited



Compound 9: Ellagic acid 3,3'-dimethylether 4-*O*-α-rhamnopyranoside.

the characteristic proton resonance pattern of ellagic acid 3,3'-dimethyl ether bearing an *O*-substituent at its position number 4 [δ 7.82 (s, H-5), 7.55 (s, H-5'), 4.2 (s, OMe-3) and 4.16 (s, OMe-3')]. The additional proton resonances which appeared in the aliphatic region of this spectrum could be identified as an α-rhamnopyranoside substituent. This followed from the doublet resonance at δ 5.2 (1H, d, J=2 Hz), assignable to an anomeric rhamnose proton as well as from the intense doublet at δ 0.92 (3H, d, J=6 Hz), atributable to the methyl rhmnoside protons. Other sugar proton resonances appeared as a broad multiblet (δ 3.2-4.1) overlapped with hydroxyl and H_2O proton resonances, thus further confirming the structure of compound **9** as ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnoside. In the ^{13}C NMR spectrum, the presence of the 4-*O*-substituted ellagic acid 3,3'-dimethyl ether moiety was evidenced by the distinct 14 carbon resonances, in the aromatic region of the spectrum, between δ 111.2 and 159.1. The chemical shift values of these signals were closely similar to those reported for the carbon resonances of the same moiety in ellagic acid 3,3'-dimethyl ether 4-*O*-β-glu-

copyranoside, characterised before from the roots of *Tamarix nilotica* (Nawwar *et al.*, 1982). The presence of an α-rhamnoside moiety followed from the resonance in the methyl region at δ 17.9 and from that at δ 100.5, assignable to the α-anomeric rhamnose carbon. The remaining four resonances, in the sugar region (see Experimental) possessed chemical shift values which agree well with those reported for the rhamnoside carbons C-2 upto C-5 in *O*-α-rhamnoside derivatives, e.g. flavone rhamnosides (Harborne, 1994). The weight of the above given evidences finally confirmed the identity of compound **9** to be ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnopyranoside, which represents, to the best of our knowledge a new natural product.

Experimental

General – For NMR analysis, a Jeol EX-270 NMR spectrometer, 270 MHz for ^{1}H NMR and 67.5 MHz for ^{13}C NMR, was used with superconducting magnet from Oxford and 5 mm Dual probehead for ^{1}H- and ^{13}C-NMR analysis. Typical conditions: spectral width = 4000 Hz for ^{1}H and 15000 Hz for ^{13}C, 32 K data points and a flip angle of 45°. The UV spectra were taken in MeOH and with shift reagents diagnostic for flavonoids (Harborne *et al.*, 1975 and Mabry *et al.*, 1969) using Shimadzu UV-240 spectrometer. FAB-MS (negative mode) were measured using MM 7070 spectrometer (VG analytical). PC was carried out on Whatman No. 1 paper using solvent systems: [1] H_2O; [2] HOAc-H_2O (15:85); [3] BAW (*n*-BuOH-HOAc-H_2O) 4:1:5, upper layer); [4] C_6H_6-*n*-BuOH-H_2O-pyridine (1:5:3:3, upper layer). Solvents 3 and 4 were used for sugar analysis.

Plant material – A fresh shrub sample of *Rosa arabica* growing wild in Saint Cathrine, south of Sinai proper, Egypt was collected in November, 1997 and authenticated by Dr. I. El-Garf, Department of Botany, Faculty of Science, Cairo University. A voucher specimen is deposited in the Herbarium of the NRC, Cairo.

Extraction, isolation and identification – An aqueous EtOH extract (3:1) of the fresh shrub collected sample (3 kg), concentrated *in vacuo*, was applied to a polyamide 6S CC (Riedel-De Haen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O-EtOH mixts. Of decreasing polarities to yield eleven fractions (I-XI) which were individually subjected to 2D-PC. Compound **1** (179 mg) was isolated pure from the H_2O-EtOH (90:10) fraction by repeated crystallisation (x3) from H_2O, while compound **2** (134 mg) was separated pure from the (70:30) fraction after Sephadex LH-20 column chromatography, using H_2O as an eluent. Compounds **3** (152 mg) and **4** (89 mg) were individually isolated pure from the (40:60) fraction by applying repeated Sephadex LH-20 column fractionation, using *n*-BuOH saturated with H_2O for elution. Individual pure samples of **5** (102 mg) and **6** (98 mg) were obtained from the (30:70) fraction also by repeated fractionation over Sephadex LH-20 column, using *n*-BuOH saturated with H_2O. Compounds **7** (64 mg) and **8** (43 mg) each was separated pure from the (20:80) fraction by refractionation over polyamide column, using solvent system, toluene-MeOH-H_2O (60:38:2) for elution. Compound **9** (94 mg) was separated pure from the (10:90) fraction by repeated crystallisation (x3) from H_2O-EtOH (70%). Compounds **10** (66 mg) and **11** (71 mg) each was obtained pure by polyamide column fractionation of the last EtOH fraction, using ethyl acetate saturated with H_2O as an eluent.

Ellagic acid 3,3'-dimethyl ether 4-*O*-α-rhamnopyranoside (9) – R_fs: Table 1. UV (MeOH) λ_{max}: Table 1. Normal aqu. acid hydrolysis of **9**, (37 mg refluxed with 10 ml 2 N HCl at 100° 3 hours) yielded ellagic acid 3,3;-dimethyl ether **9'**. **9'**: R_fs: Table 1. UV (MeOH) λ_{max}: Table 1. EI-MS: *m/z*: 330 (100) $[M]^+$, 315 (38), 286 (10), 259 (3), 231 (3.5), 203 (5), 103 (6). ^{1}H-NMR: δ 10.52 (broad s, OH-4 & OH-4'), 7.5 (2H, s, H-5 & H-5'), 4.02 (s, OMe-3 & OMe-3'). ^{13}C-NMR: δ 111.7 (C-1 & C-1')), 141.3 (C-2 & C-2'), 140.3 (C-3 & C-3'), 152.9 (C-4 & C-4'), 111.5 (C-5 & C-5'), 112.1 (C-6 & C-6'), 158.4 (C-7 & C-7'), 60.9 (OMe-3 & OMe-3'). ^{1}H-NMR of the parent compound **9**: δ 7.82 (1H, s, H-5), 7.57 (1H, s, H-5'), 5.25 (1H, d, J=2 Hz, H-1"), 4.14 (3H, s, OMe-3), 4.06 (3H, s, OMe-3'), 3.34-4.04 (m, rhamnoside proton signals overlapped with hydroxyl and water proton resonances). ^{13}C-NMR of **9**: δ 114.9 (C-1), 141.6 (C-2), 142.1 (C-3), 151.4 (C-4), 112.8 (C-5), 113.3 (C-6), 159.2 (C-7), 111.5 (C-1'), 141.5 (C-2'), 140.7 (C-3'), 152.1 (C-4'), 112.5 (C-5'.), 112.3 (C-6'), 159.2 (C-7'), 100.5 (C-1"), 70.2 (C-2"), 70.4 (C-3"), 71.8 (C-4"), 69.9 (C-5"), 17.9 (C-Me).

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