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Polyphenols of Egyptian Rosaceae plants – two new flavonoid glycosides from *Sanguisorba minor* Scop.

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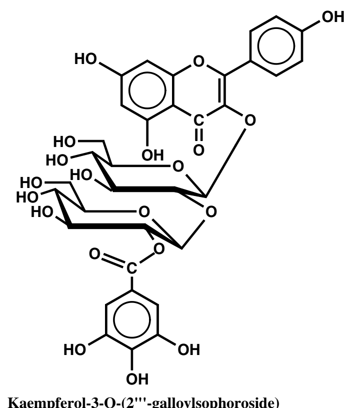
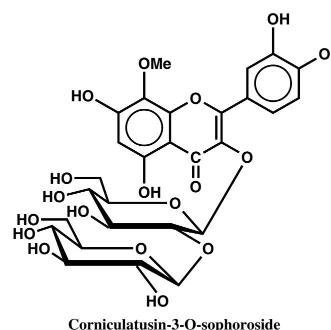
Two new flavonol glycosides, 8-methoxyquercetin-3-*O*-β-glucosyl-(1‴–2″)-*O*-β-glucoside and kaempferol-3-*O*-[2‴-galloyl-*O*-β-glucosyl-(1‴–2″)-*O*-β-glucoside)], together with five known quercetin and kaempferol 3-*O*-mono-glycosides, were isolated and identified from the aerial parts of *Sanguisorba minor* Scop. (Rosaceae). All structures were determined by routine methods of analysis and confirmed mostly by negative ESI-MS, ^{1}H- and ^{13}C NMR.

1. Introduction

Many members of Rosaceae predominantly produce a variety of polyphenolic conjugates which includes beside, ellagitannins [1, 2] several flavonoid glycosides. These flavonoids mainly are apigenin-*C*-glycosyls [3, 4], quercetin and kaempherol 3-*O*-glycosides and 3-*O*-galloylated glycosides [5]. They also, include 8-methoxykaempferol-3-*O*-neohesperidoside, 3-*O*-glucoside [6], 3-*O*-sophoroside [7]. Rosaceae is represented in Egypt by seven species, belonging to six different genera [8]. They provide extracts which are used in traditional medicine for treating cardiac insuffeciency corresponding to stages I & II, viral infection diseases, diarrhea and enuresis [9]. In previous studies of the phenolic conjugates of Egyptian Rosaceae medicinal plants, the isolation and characterization of a di-*C*-β-cellobiosylapigenin, together with 13 other phenolics, from the whole plant of *Cotoneaster orbicularis* Schltdl. [3], and of quercetin-3-*O*-galloylgalactoside, among 10 additional phenolics from the whole *Rosa arabica* plant [5], as well as the isolation and characterization of a diacetyl-2″-*O*-α-rhamnosylvitexin in addition to 5 flavonoid conjugates from the leaves of *Crataegus sinaica* Boiss. [4], have been reported. In continuation of these studies isolated and identified, along with the known glycosides, kaempherol-3-*O*-β-glucuronide (**3**), 3-*O*-β-galactide (**4**), quercetin-3-*O*-β-glucuronide (**5**), -3-*O*-β-galactoside (**6**) and -3-*O*-β-arabinoside (**7**) the new conjugates, 8-methoxyquercetin-3-*O*-β-glucopyranosyl-(1‴–2″)-β-glucopyranoside, or corniculatusin-3-O-β-sophoroside (**1**) and kaempferol-3-*O*-[2‴-galloyl-β-glucosyl-(1‴–2″)-β-glucoside], or kaempferol-3-*O*-(2‴-galloyl-β-sophoroside) (**2**), from the aqueous ethanolic whole plant extract of *Sanguisorba minor* Scop.



Kaempferol-3-O-(2'''-galloylsophoroside)



Corniculatusin-3-O-sophoroside

2. Investigations, results and discussion

Compounds **1–7** were isolated from an aqueous ethanolic (3 : 1) extract of the ground aerial parts of *S. minor* by applying repeated Sephadex LH-20 column and preparative paper chromatography (PPC). The known compounds **3–7** showed chromatographic, UV absorption, hydrolytic, negative ESI-MS, ^{1}H- and ^{13}C NMR results identical with those of kaempferol-3-*O*-β-galactopyranoside [10], kaempferol-3-*O*-β-glucupyranoronide [11], quercetin-3-*O*-β-galactopyranoside [12], quercetin-3-*O*-β-glucupyranoronide [11] and quercetin-3-*O*-β-arabinopyranoside [13], respectively.

Compound **1** was obtained as a brownish yellow amorphous powder. The molecular weight (M_r) was determined as 656 (negative ESI-MS, $[M-1]^-$ ion at *m/z* 655). Direct measurements of the ESI-MS at collision induced dissociation voltage (CID) [14] led to formation of fragment ions at *m/z* 640, 493, 475, 331.07, 315,07, 303.07 and 166, a pattern of fragmentation which when incorporated with the above given analytical data would be best interpreted in terms of a 3,8-di-*O*-substited gossypetin structure in which the substituent at position 8 is a methyl group and that at position 3 is a di-*O*-hexoside. This view was supported by acid hydrolysis of **1**, (aqueous 2 N HCl, 100 °C, 2 h) to yield 8-methoxyquercetin, corniculatusin [15] ($R_f s$, UV spectral, -ve ESI-MS, ^{1}H and ^{13}C NMR data) and glucose (Co-PC). Partial β-glucosidase hydrolysis of **1** (0.5 ml of the enzyme in 0.05 M acetate buffer, pH 5.1, 37 °C) gave an intermediate which was isolated from an ethyl acetate extract of the hydrolysate by PPC and was then identified as corniculatusin-3-*O*-β-glucoside [16]. Partial acid hydrolysis of **1** (0.1 N aqueous HCl, 100 °C, 5 min) gave the same intermediate, corniculatusin 3-*O*-glucoside (Co-PC). Cosequently, compound **1** must be corniculatusin 3-*O*-glucosylglucoside. ^{1}H NMR of **1** confirmed this conclusion and showed, in the recorded

spectrum (DMSO-d_6), two anomeric sugar proton resonances at δ 5.68 (*d*, $J = 8$ Hz) assignable to the 1″-β-anomeric glucosyl proton, and at δ 4.61 (*d*, $J = 8$ Hz) attributable to the 1‴-β-anomeric glucoside proton. It also showed a singlet at δ 3.81, assignable to a methoxyl protons and in the aromatic region, a singlet at δ 6.22, a doublet (J = 7.5 Hz) at 6.90, a doublet (J = 2.5 Hz) at 7.60 and a double doublet (*J* = 7.5 and 2.5 Hz) at 7.64, assignable to the protons of the 8-OMe, 6-H, 5′-H, 2′-H and 6′-H, respectively in the corniculatusin moiety of **1**. Further confirmation of the achieved structure of **1** was received through ^{13}C NMR analysis. The spectrum exhibited in the aromatic region a pattern of carbon resonances consistent with 3-*O*-substituted corniculatusin (see Experimental). It also showed a group of 12 carbon resonances in the sugar region, thus indicatig a di-*O*-glycosylation. The anomeric sugar carbon resonances were recognized at δ 98.6 (C-1″), and at 103.9 (C-1‴). The carbon resonance localized in this spectrum, at δ 83.2 was assigned to the glucosylated C-2″ glucoside carbon, being shifted downfield due to glucosylation of its geminal hydroxyl group (α effect), (in comparison with the corresponding resonances in the spectra of flavonol-3-*O*-β-glucoside [17]). That *O*-glycosylation in **1** is present at C-3 of the flavonol moiety which was confirmed by the upfield shift of this carbon resonance and the accompanying large downfield shift of the C-2 resonance of the same moiety [all in comparison with the corresponding resonances in the spectrum of the aglycone corniculatusin (see Experimental)]. Other glucosyl and glucoside carbon resonances, in the received spectrum possessed chemical shift values which were in close agreement with structure of **1** as corniculatosin-3-*O*-β-glucopyranosyl-(1‴2″)-β- glucopyranoside, or corniculatusin-3-*O*-β-sophoroside.

Compound **2** was isolated as an amorphous light yellow powder, which appeared to be a kaempferol derivative with a substituted 3-hydroxyl group as could be concluded from its chromatographic behavior and from its UV spectra in methanol and with diagnostic reagents [14, 15]. Acid hydrolysis of **2** gave kaempferol (Co-PC), gallic acid (isolated by PPC from an ethyl acetate extract of the hydrolysate, Co-PC, UV spectra, ^{1}H and ^{13}C NMR) and glucose (Co-PC). The compound was recovered unchanged on enzymic hydrolysis with β-glucosidase and exhibited on negative ESI-MS analysis a molecular ion peak at $[M-H]^-$ *m/z* 761 corresponding to a molecular weight (M_r) of 762. These data suggested a kaempferol-3-monogalloylated-diglucoside in which the galloyl moiety is esterifying one of the hydroxyl groups of the terminal glucose moiety. The disaccharide was then obtained from **2**, by alkaline hydrogen peroxide oxidation and was identified to be sophoroside (Co-PC) [7]. Structure elucidation of **2** was achieved by ^{1}H and ^{13}C NMR spectroscopic analysis (DMSO-d_6). The received spectra unambiguously identified the compound as kaempferol derivative bearing a 3-*O*-substituent. The number and characteristic chemical shifts of the ^{13}C glycosidic signals indicated the presence of two glucopyranose moieties in which the hydroxyl groups of the C-6 and C-4 carbons were unsubstituted [17]. The relatively downfield resonance at δ ppm 83.16 is unequivocally attributable to the glucosylated C-2″, while that at 74.8 is assignable to the galloylated C-2‴ in the terminal glucose moiety. Both moieties had β-glycosidic linkages. This followed from the magnitude of the vicinal proton couplings (J = 8 Hz) of the anomeric ptotons in the ^{1}H spectrum and from the chemical shift values of the resonances of the anomeric carbons in the ^{13}C spectrum [δ ppm 101.94 (C-1‴), 98.75 (C-1″)]. The recognizable upfield shift (Δδ = 3.38 ppm) of the resonanace of C-1‴, in comparison with the corresponding resonance in the spectrum of kaempferol 3-*O*-β-sophoroside [17], is obiviously due to the galloylation sited at its vicinal carbon C-2‴. Galloylation at that position also resulted in a downfield shift of the resonances, in the ^{1}H spectrum, of the H-2‴ to δ ppm 5.12 (*t*, *J* = 8 Hz) The symetrical galloyl moiety itself revealed its presence in the ^{13}C NMR spectrum by its characteristic battern of resonances (see Experimental). That the sophorose moiety is linked to the kaempferol hydroxyl group number 3 followed from the lowfield location (δ ppm 5.62) of the anomeric glucoside proton resonance and unambiguously confirmed by the upfield of the aglycone C-3 resonance and the accompanying large downfield shift of the resonance of the *ortho*-carbon C-2 (all in comparison with the corresponding chemical shifts in the spectrum of kaempferol itself) [11]. Hence, The structure of **2** was determined as kaempferol-3-*O*-[2‴-galloyl-β-glucopyranosyl-(1‴ 2″)-*O*-β-glucopyranoside].

3. Experimental

3.1. Instruments and materials

NMR: Jeol YH 300 spectrometer, 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR), respectively. ^{1}H resonances were measured relative to TMS and ^{13}C NMR resonances to DMSO-d_6 and converted to TMS scale by adding 39.5. ESI-MS: Micromass Quattro-LC triple quadrupole mass spectrometer equipped with a "Z-Spray" electrospray ion source. UV: UV/V Shimazu spectrometer. PC (descending): Whatman no. 1 paper, using solvent systems: (1) H_2O; (2) 15% HOAc; (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 and solvent 3 for preparative paper chromatographic (PPC) isolation, on Whatman no. 3MM paper. Fresh herbs of *Sanguisorba minor* growing wild near El-Ariesh, north of Sinai proper, Egypt were collected in November, 2000 and authenticated by Dr. M. El Gibali, National Research Centre (NRC), Dokki, Cairo, Egypt. A voucher specimen has been deposited at the herbarium of the NRC.

3.2. Extraction, isolation and purification

The collected plant specimen was exhaustively extracted with EtOH−H_2O (3 : 1). The concentrated extract was separately applied to polyamide (6S Riedel De Haen AG-Seelze-Hannover) columns and eluted with H_2O, followed by H_2O−MeOH mixtures of decreasing polarities, to yield eleven fractions (I–XI). Compound **1** was isolated from fraction IV (eluted by 30% MeOH) by PPC, using BAW, Compound **2**, **3** and **5** from fraction V (eluted by 40% MeOH) by repeated Sephadex LH-20 column fractionation using *n*-BuOH saturated with H_2O for elution. Compounds (**4** & **6**) from fraction VI (eluted by 50% MeOH) by Sepadex LH-20 (Pharmacia) column using and H_2O−MeOH mixture (7 : 3) as an eluent, (**7**) from VI (eluted by 50%) by sephadex LH-20, using H_2O−MeOH mixture (1 : 1) for elution.

3.3. New natural products

3.3.1. Corniculatusin-3-O-β-glucopyranosyl-(1‴–2″)-β-glucopyranoside (1)

M_r 656, -ve ESI-MS [M-H]$^-$: 655; $R_f$values: 0.51 (H_2O), 0.75 (HOAc), 0.50 (BAW). UV (λ_{max} nm): MeOH 257, 272, 328, 358 (inflection); NaOAc 279, 320, 382; NaOAc + H_3BO_3 265, 300 (inflection), 374; $AlCl_3$ 277, 305 (inflection), 360, 438; NaOMe 277, 330, 415. Complete acid hydrolysis of **1** gave glucose (Co-PC), and corniculatusin [$R_f s$: 02 (H_2O), 04(HOAc), 48 (BAW); UV (λ_{max} nm): MeOH; M_r 332, -ve ESI-MS, [M-H]$^-$: 331; ^{1}H NMR: δ ppm 3.83 (*s*, 8-OMe), 6.24 (*s*, H-6), 6.89 (*d*, J = 8 Hz, H-5′), 7.52 (*d,d,* J = 8 and 2.5 Hz, H-6′), 7.64 (*d*, J = 2.5 Hz, H-2′); ^{13}C NMR: δ ppm 60.49 (OMe-8), 146.66 (C-2), 135.77 (C-3), 176.9 (C-4), 156.1 (C-5), 98.7 (C-6), 156.22 (C-7), 127.38 (C-8), 148.55 (C-9), 103.76 (C-10), 124.35 (C-1′), 115.48 (C-2′), 145.07 (C-3′), 147.38 (C-4′), 115.5 119.92 (C-6′)]. Partial acid or β-glucosidase hydrolysis of **1** gave corniculatusin-3-*O*-β-glucopyranoside [$R_f s$: 0.32 (H_2O), 0.45 (HOAc), 0.52 (BAW); UV (λ_{max} nm): MeOH 258, 269, 357; M_r 494, -ve ESI-MS, [M-1]$^-$ 493; Normal acid hydrolysis yielded glucose and corniculatusin]; ^{1}H NMR of (**1**): β-glucosyl moiety: δ ppm 4.61 (*d*, *J* = 8 Hz, H-1‴), 3.0–3.80 (*m*, other glucosyl protons), β-glucoside moiety: 5.68 (*d*, J = 8 Hz, H-1″), 3.0–3.80 (*m*, other glucoside protons), corniculatusin

moiety: δ ppm 3.81 (OMe-8), 6.22 (*s*, H-6), 7.90 (*d,* J = 7.5 Hz, H-5), 7.6 (*d*, *J* = 2.5 Hz, H-2′), 7.64 (*d,d,* J = 7.5 and 2.5 Hz, H-6′). ^{13}C NMR of **1**: corniculatusin moiety: δ ppm 156.5 (C-2), 133.7 (C-3), 177.9 (C-4), 155.7 (C-5), 98.6 (C-6), 157.2 (C-7), 128.3 (C-8), 148.9 (C-9), 103.8 (C-10), 122.4 (C-1′), 115.9 (C-2′), 145.7 (C-3′), 148.9 (C-4′), 116.4 (C-5′), 121.7 (C-6′); β-glucosyl moiety: δ ppm 103.9 (C-1‴), 74.9 (C-2‴), 77.0 (C-3‴), 70.0 (C-4‴), 77.3 (C-5‴), 61.4 (C-6‴); β-glucoside moiety: δ ppm 98.6 (C-1″), 83.2 (C-2″), 78.0 (C-3″), 70.0 (C-4″), 77.4 (C-5″), 61.38 (C-6″).

3.3.2. Kaempferol-3-O-[2″-galloyl-β-glucopyranosyl-(1‴−2″)-O-β-glucopyranoside] (**2**)

M_r 762, -ve ESI-MS [M-H]$^-$: 761; $R_f$values: 0.57 (H_2O), 0.75 (HOAc), 0.59 (BAW). UV (λ_{max} nm): MeOH 267, 295 (inflection), 354; NaOAc 270, 302 (inflection); NaOAc + H_3BO_3 266, 297, 352; $AlCl_3$ 268, 303 (inflection), 355; NaOMe 274, 325m 403. Normal acid hydrolysis of **2** gave glucose (Co-PC), gallic acid [$R_f s$: 0.53 (H_2O), 0.59 (HOAc), 0.77 (BAW); UV (λ_{max} nm): MeOH 272; ^{1}H NMR: δ ppm 7.0 (*s*, H-3‴ and H-5‴); ^{13}C NMR: 120.6 (C-1‴), 108.8 (C-2‴ and C-6‴), 145.5 (C-3‴ and C-5‴), 138.1 (C-4‴)] and kaempferol [$R_f s$: 0.02 (H_2O), 0.04 (HOAc), 0.88 (BAW). Hydrogen peroxide oxidation: The released sugar obtained by H_2O_2 treatment, for 12 h at room temprature, of an alkaline solution of **2** was co-chromatographed against sophorose [R_f: 0.57(BAW)]. ^{1}H NMR of **2**: kaempferol moiety: δ ppm 6.22 (*d*, J = 2.5 Hz, H-6), 6.44 (*d, J* = 2.5 Hz, H-8), 6.98 (*d,* J = 7.5 Hz, H-3 & H-5), 8.05 (*d,* J = 7.5, H-2 & H-6; β-glucosyl moiety: δ ppm 4.80 (*d*, J = 8 Hz, H-1‴), 5.12 (*t*, J = 8 Hz, H-2‴), 3.2–4.0 (*m,* H-3‴, H-4‴, H-5‴ and 2 H-6‴); β-glucoside moiety: δ ppm 5.62 *d*, *J* = 8 Hz, H-1″), 3.2–4 (*m,* remaining glucoside protons); galloyl moiety: δ ppm 6.92 (*s*, H-2‴ and H-6‴). ^{13}C NMR of **7**: Kaempferol moiety: δ ppm 156.5 (C-2), 132.4 (C-3), 176.1 (C-4), 156.9 (C-5), 98.3 (C-6), 160.8 (C-7), 129.1 (C-8), 147.9 (C-9), 103.0 (C-10), 122.3 (C-1′), 130.5 (C-2′ & C-6′), 115.8 (C-3′ & C-5′), 160.1 (C-4′), 60.3 (OMe-8); β-glucosyl moiety: δ ppm 101.9 (C-1‴), 74.8 (C-2‴), 74.6 (C-3‴), 69.9 (C-4‴), 78.0 (C-5‴), 61.2 (C-6‴); β-glucoside moiety: δ ppm 98.7 (C-1″), 83.16 (C-2″), 76.6 (C-3″), 69.7 (c-4″), 76.6 (C-5″), 61.3 (C-6″); galloyl moiety: δ ppm 119.4 (C-1⁗), 10807 (C-2⁗ and C-6⁗), 145.48 (C-3⁗ and C-5⁗), 138.6 (C-4⁗), 165.8 (C-7⁗).

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