New Galloylated Flavonoid Glycosides from *Geranium stepporum* DAVIS

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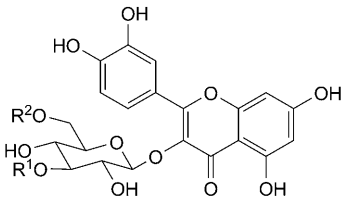
Two new galloylated flavonoid glycosides, quercetin-3-*O*-(3″-*O*-galloyl)-*β*-glucopyranoside (**1**) and quercetin-3-*O*-(3″-*O*-galloyl)-*β*-galactopyranoside (**2**) were isolated from the aerial parts of *Geranium stepporum* along with the four known flavonoids, quercetin 3-*O*-*β*-glucopyranoside (**3**), quercetin 3-*O*-*β*-galactopyranoside (**4**), quercetin 3-*O*-(6″-*O*-galloyl)-*β*-glucopyranoside (**5**) and quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside (**6**). The structure elucidation of the isolated compounds was accomplished by spectroscopic methods (UV, 1D- and 2D-NMR, and ESI-TOF-MS).

Introduction. – In the flora of Turkey, the genus *Geranium* L. (Geraniaceae) is represented by 35 species [1], some of which are traditionally used as antidiarrheal, antihemorrhoidal, antidiabetic, hemostatic, stomachic, diuretic, and for the treatment of stomach ulcers and internal bleeding [2]. The genus *Geranium* is known to contain various classes of compounds such as flavonoids [3][4], tannins [3][5] and essential oils [6]. As a part of our ongoing phytochemical studies on the polyphenolic compounds of Turkish *Geranium* species [3], we have investigated *G. stepporum* DAVIS, on which no report has been published previously. In this paper, we report the isolation and structure elucidation of the two new flavonoid glycosides quercetin-3-*O*-(3″-*O*-galloyl)-*β*-glucopyranoside (**1**) and quercetin-3-*O*-(3″-*O*-galloyl)-*β*-galactopyranoside (**2**) from the title plant, together with four known flavonoids, quercetin 3-*O*-*β*-glucopyranoside (**3**), quercetin 3-*O*-*β*-galactopyranoside (**4**), quercetin 3-*O*-(6″-*O*-galloyl)-*β*-glucopyranoside (**5**), quercetin 3-*O*-(6″-*O*-galloyl)-*β*-galactopyranoside (**6**).

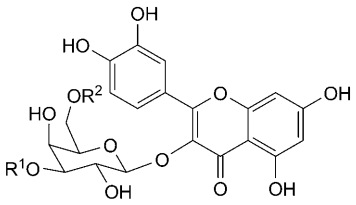
Results and Discussions. – The AcOEt soluble part of the crude MeOH extract of the aerial parts of *G. stepporum* was first fractioned by *Sephadex LH-20*, and compounds **1**–**6** were obtained by further column chromatographic (silica gel and *Sephadex LH-20*) separations.

Compound **1** was isolated as a yellow amorphous powder. The molecular formula was determined as $C_{28}H_{24}O_{16}$ by positive-ion ESI-TOF-MS ($[M+H]^+$ at 617.1133). Fragment ions were observed at *m*/*z* 303 corresponding to [aglycone + H]$^+$ and at *m*/*z* 315 corresponding to [glucose + gallic acid + H]$^+$. The UV spectrum of **1** (MeOH) exhibited maxima at 259 and 355 nm, suggesting the presence of a flavonol derivative.

The ^{1}H-NMR spectrum of **1** (*Table*) showed two *meta*-coupled signals in the aromatic region at δ(H) 6.24 ($d, J = 2.0$) and 6.44 ($d, J = 2.0$) suggesting the presence of a 5,7-dihydroxy-substituted *A* ring of the flavonoid skeleton. A *doublet* of *doublet*s at



	R^1	R^2
1	Gall	H
3	H	H
5	H	Gall



	R^1	R^2
2	Gall	H
4	H	H
6	H	Gall

Gall = galloyl

δ(H) 7.62 (*dd*, $J = 2.2, 8.0$) and two *doublet*s at δ(H) 7.75 (*d*, $J = 2.2$) and 6.92 (*d*, $J = 8.0$) which were observed as an *ABX* system revealed the 3′,4′-dihydroxy functional structure of a flavonoid *B* ring. The appearance of a sharp *singlet* H-atom signal at δ(H) 7.18 (2 H) for the magnetically equivalent H–C(2‴) and H–C(6‴) H-atoms expressed the presence of a galloyl group. The anomeric H-atom resonance at δ(H) 5.46 (*d*, $J =$ 8.0) and the signals in the region δ(H) 5.20–3.65 together with the corresponding C-resonances in the ^{13}C-NMR spectrum of **1** indicated the presence of a β-glucopyranosyl unit. The ^{13}C-NMR spectrum of **1** (*Table*) exhibited 28 signals; 15 of them were attributed to quercetin, six of them were attributed to a β-glucopyranosyl unit, while the remaining seven signals were attributed to a gallic acid moiety. In the ^{13}C-NMR spectrum of **1**, signals attributed to C(2″), C(3″), and C(4″) of the glucose residue were shifted -1.4, $+1.2$, and -1.9 ppm, respectively, comparing to those of quercetin 3-*O*-β-glucopyranoside (**3**), suggesting the galloyl moiety to be positioned at C(3″) of the glucose moiety. This assumption was supported by the HMBC correlation between H–C(3″) and C=O (galloyl). Furthermore, the HMBC correlation observed between the anomeric H-atom H–C(1″) of the glucose unit and C(3) of the quercetin moiety allowed us to assign the position of the sugar moiety at C(3). The complete assignments of **1** were achieved by 2D-NMR experiments (DQF-COSY, HMQC, and HMBC). From these data, the structure of compound **1** was established as quercetin-3-*O*-(3″-*O*-galloyl)-β-glucopyranoside.

Compound **2** was isolated as a yellow amorphous powder. ESI-TOF-MS showed the $[M+H]^+$ peak at *m*/*z* 617.1122, corresponding to the molecular formula $C_{28}H_{24}O_{16}$. The UV spectrum of **2** in MeOH showed maxima at 259 and 359 nm, suggesting it to be a flavonol derivative.

The ^{1}H- and ^{13}C-NMR spectra of compound **2** (*Table*) were similar to those of **1**, except for the signals due to the sugar moiety. Quercetin was revealed by the *meta*-coupled signals at δ(H) 6.25 and 6.44, in addition to the signals of a 1,3,4-trisubstituted aromatic ring (7.88 (*d*, $J = 2.0$), 7.64 (*dd*, $J = 2.0, 8.0$), 6.92 (*d*, $J = 8.0$)). Furthermore, the *singlet* signal at δ(H) 7.21 (2 H) for a tetrasubstituted aromatic ring was accounted for a galloyl residue. The signal of the anomeric H-atom appeared at δ(H) 5.39 (*d*, $J =$ 8.0), and the anomeric C-atom resonance was observed at δ(C) 105.3. The chemical shifts of the sugar H-atoms were assigned to H–C(1″) (δ(H) 5.39), H–C(2″) (δ(H)

Table. *^{1}H- and ^{13}C-NMR Data*[a]) *and HMBC of* **1** *and* **2**. In CD_3OD; in ppm, *J* in Hz. Asterisks (*) mark overlapping signals.

	1			**2**		
	δ(H)[b])	δ(C)[c])	HMBC (H → C)	δ(H)[b])	δ(C)[c])	HMBC (H → C)
Aglycone						
C(2)		159.1			158.9	
C(3)		135.7			135.8	
C(4)		179.6			179.6	
C(5)		163.3			163.2	
H–C(6)	6.24 (*d*, *J* = 2.0)	100.1	C(5), C(7), C(8), C(10)	6.25 (*d*, *J* = 1.8)	100.1	C(8), C(10)
C(7)		166.2			166.3	
H–C(8)	6.44 (*d*, *J* = 2.0)	94.8	C(6), C(7), C(9), C(10)	6.44 (*d*, *J* = 1.8)	94.9	C(6)
C(9)		158.6			158.6	
C(10)		105.8			105.8	
C(1′)		123.2			123.0	
H–C(2′)	7.75 (*d*, *J* = 2.2)	117.7	C(2), C(1′), C(3′), C(4′)	7.88 (*d*, *J* = 2.0)	117.9	C(2), C(1′), C(4′)
C(3′)		146.1			146.0	
C(4′)		150.0			150.2	
H–C(5′)	6.92 (*d*, *J* = 8.0)	116.2	C(3′), C(6′)	6.92 (*d*, *J* = 8.0)	116.3	C(3′), C(6′)
H–C(6′)	7.62 (*dd*, *J* = 2.2, 8.0)	123.4	C(2′), C(3′), C(4′)	7.64 (*dd*, *J* = 2.0, 8.0)	123.1	C(2′), C(4′)
Sugar						
H–C(1″)	5.46 (*d*, *J* = 8.0)	104.1	C(3)	5.39 (*d*, *J* = 8.0)	105.3	C(3)
H–C(2″)	3.77 (*dd*, *J* = 8.0, 9.2)	74.4	C(1″), C(3″)	4.21 (*dd*, *J* = 3.2, 8.0)	71.1	C(1″), C(3″)
H–C(3″)	5.20 (*t*, *J* = 9.2)	79.4	C(2″), C(4″), C=O	4.98 (*dd*, *J* = 3.2, 10)	77.7	C(2″), C=O
H–C(4″)	3.68*	69.4	C(5″), C(6″)	4.19*	67.8	C(2″)
H–C(5″)	3.40 (*ddd*, *J* = 2.0, 4.8, 8.0)	78.5		3.64*	77.2	C(6″)
CH_2(6″)	3.78*, 3.65*	62.4		3.57–3.69*	61.8	C(5″)
Galloyl						
C(1‴)		121.9			121.7	
H–C(2‴)	7.18 (*s*)	110.5	C(1‴), C(3‴), C(4‴), C=O	7.21 (*s*)	110.5	C(1‴), C(3‴), C(4‴), C=O
C(3‴)		146.6			146.6	
C(4‴)		139.9			140.1	
C(5‴)		146.6			146.6	
H–C(6‴)	7.18 (*s*)	110.5	C(1‴), C(5‴), C(4‴), C=O	7.21 (*s*)	110.5	C(1‴), C(5‴), C(4‴), C=O
C=O		168.4			168.2	

[a]) All δ(H) and δ(C) assignments are based on 2D-NMR (DQF-COSY, HMQC, HMBC). [b]) Recorded at 400 MHz. [c]) Recorded at 100 MHz.

4.21), H–C(3″) (δ(H) 4.98), H–C(4″) (δ(H) 4.19), H–C(5″) (δ(H) 3.64), CH_2(6″) (δ(H) 3.57–3.69) by DQF-COSY spectrum. All resonances of the H- and C-atoms were assigned by HMQC and HMBC experiments. The sugar was concluded to be β-galactopyranose by the ^{1}H- and ^{13}C-NMR signals and the coupling constant of H–C(1″) (J = 8.0) [7]. In the ^{13}C-NMR spectrum of **2**, signals of C(2″), C(3″), and C(4″) of the galactose residue appeared to be shifted −2.2, +2.5, and −2.4 ppm, respectively, compared to those of quercetin 3-*O*-β-galactopyranoside (**4**), suggesting that the HO–C(3″) group was acylated. HMBC between H–C(1″) and C(3) assigned the position of the β-galactopyranosyl residue at C(3) of the aglycone, while correlation between H–C(3″) and C=O (galloyl) suggested the gallic acid moiety to be attached to the β-galactopyranosyl unit at the C(3″) position. From these results, the structure of compound **2** was identified as quercetin-3-*O*-(3″-*O*-galloyl)-β-galactopyranoside.

The known flavonol glycosides, quercetin 3-*O*-β-glucopyranoside (**3**) [3][7], quercetin 3-*O*-β-galactopyranoside (**4**) [3][7], quercetin 3-*O*-(6″-*O*-galloyl)-β-glucopyranoside (**5**) [3][7], and quercetin 3-*O*-(6″-*O*-galloyl)-β-galactopyranoside (**6**) [3], were identified by comparing their 1D- and 2D-NMR spectra as well as their ESI-TOF-MS data with those published in the literature.

According to *Davis*, the *Geranium* species in Turkish flora are classified under five groups (Groups A–E) depending on their morphological characters [1a]. In our previous studies on Turkish *Geranium* species, we have isolated some galloylated (2″-*O*- and 6″-*O*-) flavonoids from *Geranium tuberosum* L. subsp. *tuberosum* (Group C), where the presence of 6″-*O*-galloylated flavonoids was reported for the first time for the genus *Geranium* [3]. *G. stepporum* is also classified in Group C [1a]. Therefore, the presence of 6″-*O*-galloylated flavonoids (**5** and **6**) in this species may have a chemotaxonomical importance for the genus *Geranium*.

Experimental Part

General. TLC: precoated *Silica gel 60* F_{254} (*Merck*) aluminum plates, elution with $CHCl_3$/MeOH/H_2O mixtures; visualization by spraying 10% H_2SO_4, followed by heating at 105° for 1–2 min. Column chromatography (CC): silica gel *60* (SiO_2; 0.063–0.200 mm; *Merck*) and *Sephadex LH-20* (*Sigma*). Optical rotations: *Rudolph Autopol-IV Automatic* polarimeter. UV Spectra: *Bio-Tek Instruments*, *M-Quant Biomolecular* spectrophotometer; λ_{max} in nm. IR Spectra: *Perkin-Elmer*, FT-IR System *Spectrum BX*, in cm^{-1}. NMR Spectra: *Bruker DRX-400* spectrometer; at 400 MHz (^{1}H) and 100 MHz (^{13}C); δ in ppm rel. to Me_4Si, J in Hz. ESI-TOF-MS: *Waters-Micromass Q-TOF Micro* instrument; in m/z.

Plant Material. *Geranium stepporum* DAVIS was collected from Kayseri, Pınarbaşı, Eğrisöğüt, in middle Anatolia, Turkey, on 6th of May 2006. A voucher specimen has been deposited with the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 06001).

Extraction and Isolation. The air-dried, powdered aerial parts of *G. stepporum* (273 g) were extracted with MeOH (4 × 2.0 l, 5 h each) at 35°, and then filtered. The combined MeOH extracts were evaporated to dryness under reduced pressure. The crude extract (43 g) was dissolved in H_2O (100 ml), and extracted with petroleum ether (40–60°; PE; 4 × 100 ml), AcOEt (6 × 100 ml), and BuOH (4 × 100 ml), successively. The AcOEt-soluble fraction (20 g) was subjected to CC (*Sephadex LH-20* H_2O/MeOH 0 → 100%) to afford 10 fractions (*Fr. A–J*) according to TLC. *Fr. E* (eluted with 30% MeOH) was subjected to CC (*Sephadex LH-20*, MeOH) to give four subfractions (*Fr.* E_{1-4}). *Fr.* E_2 was further chromatographed by CC (SiO_2, $CHCl_3$/MeOH 92:8) to yield **3** (5.0 mg) and **4** (4.0 mg). *Fr. F* (eluted with 40% MeOH) was re-chromatographed by CC (*Sephadex LH-20*, MeOH) to yield **5** (4.5 mg) and **6** (4.0 mg). *Fr. H* (eluted with 50% MeOH) was subjected to CC (*Sephadex LH-20*, MeOH), and compounds **1** (5.0 mg) and **2** (3.5 mg) were obtained.

Quercetin-3-O-*(3″*-O-*galloyl)-β-glucopyranoside* (=*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H-*chromen-3-yl 3*-O-*[(3,4,5-Trihydroxyphenyl)carbonyl]-β*-D-*glucopyranoside*; **1**). Amorphous, yellow powder. $[\alpha]_D^{20} = -15.9$ ($c = 0.1$, MeOH). UV (MeOH): 259 (4.08), 269 (4.02), 355 (3.65). IR: 3308, 1693, 1658, 1605, 1504, 1309, 1198. ^{1}H- and ^{13}C-NMR: *Table.* ESI-TOF-MS: 617.1133 ($[M+H]^+$, $C_{28}H_{25}O_{16}^+$; calc. 617.1143), 315 ([glucose + gallic acid + H]$^+$), 303 ([aglycone + H]$^+$).

Quercetin-3-O-*(3″*-O-*galloyl)-β-galactopyranoside* (=*2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H-*chromen-3-yl 3*-O-*[(3,4,5-Trihydroxyphenyl)carbonyl]-β*-D-*galactopyranoside*; **2**). Amorphous, yellow powder. $[\alpha]_D^{20} = -18.9$ ($c = 0.1$, MeOH). UV (MeOH): 259 (4.39), 272 (4.43), 359 (4.05). IR: 3306, 1702, 1660, 1604, 1500, 1307, 1191. ^{1}H- and ^{13}C-NMR: *Table.* ESI-TOF-MS: 617.1122 ($[M+H]^+$, $C_{28}H_{25}O_{16}^+$; calc. 617.1143).

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