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Flavonol Glycosides from *Asperula arvensis* L.

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From the aerial parts of *Asperula arvensis* L. 9 known flavonol glycosides, namely quercetin (**1**), isoquercitrin [= quercetin 3-O-β-glucopyranoside] (**2**), hyperin [= quercetin 3-O-β-galactopyranoside] (**3**), quercetin 7-O-β-galactopyranoside (**4**), quercetin 4′-O-β-galactopyranoside (**5**), isorhamnetin 3-O-β-galactopyranoside (**6**), isorhamnetin 5-O-β-galactopyranoside (**7**), dihydrokaempferol 7-4′-dimethylether 3-O-β-glucopyranoside (**8**) and isorhamnetin 3-O-α-rhamnopyranosyl (1‴ → 6″)-β-glucopyranosid (**9**), were isolated. The structures of the compounds were elucidated by high field 1D and 2D NMR and ESI-MS spectroscopies.

Key Words: *Asperula arvensis*, Rubiaceae, flavonol glycosides, quercetin, isoquercitrin, hyperin, quercetin 7-O-β-galactopyranoside, quercetin 4′-O-β-galactopyranoside, isorhamnetin 3-O-β-galactopyranoside, isorhamnetin 5-O-β-galactopyranoside, dihydrokaempferol 7-4′-dimethylether 3-O-β-glucopyranoside, isorhamnetin 3-O-α-rhamnopyranosyl (1‴ → 6″)-β-glucopyranosid.

Introduction

There are 41 *Asperula* species (Rubiaceae) in the flora of Turkey[1]. Flowering shoots of *Asperula odorata* L. are used in folk medicine as a diuretic and tonic and against diarrhea[2]. Iridoid glycosides[3−4], cardenolides[5], flavonoids[6−7] and anthraquinone glycosides[8−10] have been reported from several *Asperula* species. However, no work has been reported on the chemical constituents of *Asperula arvensis.* The present study describes the isolation and structure elucidation of 9 flavonol glycosides (1-9) from the aerial parts of *Asperula arvensis* L.

Experimental

General Experimental Procedures: The UV (MeOH) spectra were recorded on a Varian Cary 3E. spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX 300 operating at 300 MHz

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and 500 MHz for proton and 75.5 for carbon using TMS as internal standard. The solvents used were methanol and DMSO-d_6. ESI-MS was performed on a Finnigan MAT 95 spectrometer. Silica gel 60 (0.063-0.200 mm, Merck) and Sephadex LH-20 (Fluka) were used for open column chromatographic separations. TLC was carried out on pre-coated Kieselgel 60 F_{254} aluminum sheets (Merck) and compounds were detected under UV (254 nm) fluorescence and spraying with 1% vanillin-H_2SO_4 reagent, followed by heating at 105 ^oC for 1-2 min.

Plant Material: *Asperula arvensis* L. (Rubiaceae) was collected from Erzurum, between the towns of Ilıca and İspir (1900 m), in July 2002. A voucher specimen has been deposited in the Herbarium of the Biology Department, Atatürk University, Erzurum, Turkey (ATA.HERB. 9741).

Extraction and Prepurification: Air-dried aerial parts of the plant (600 g) were extracted 3 times with MeOH at 40 ^oC (3 x 2 L). After filtration, the combined extracts were evaporated under vacuum to dryness (90 g). The residue was suspended in H_2O (200 mL) and partitioned with $CHCl_3$ (4 x 200 ml). The aqueous layer (50 g) was subjected to a column of Sephadex LH 20 eluting with MeOH to yield 6 main fractions: Frs. A-F. (Fr. A: 6.5 g, Fr. B: 3.2 g, Fr. C: 2.7 g, Fr. D: 1.8 g, Fr. E: 1.1 g, Fr. F: 1.4 g).

Isolation of the Compounds: Fr. C was eluted with MeOH from the Sephadex LH 20 column and was separated by preparative TLC using $CHCl_3$-MeOH-H_2O (61:32:7) mixtures as developing solvent to yield 7 main fractions: Frs. C_1-C_7. C_3 was purified by preparative TLC using EtOAc-HCOOH-AcOH-H_2O (100:11:11:27) solvent to give compound **9** (7 mg). C_4 was purified by preparative TLC using EtOAc-HCOOH-AcOH-H_2O (100:11:11:27) solvent to give compounds **2** (5.5 mg) and **3** (10 mg). C_5 was subjected to a column of Sephadex LH 20 eluting with MeOH to give compound **5** (6 mg). C_6 was subjected to a column of Sephadex LH 20 eluting with MeOH to give compound **6** (7.4 mg). C_7 was subjected to a column of Sephadex LH 20 eluting with MeOH to give compound **8** (3.2 mg). Fraction F was eluted with MeOH from the Sephadex LH 20 column and was separated by preparative TLC using $CHCl_3$-MeOH-H_2O (61:32:7) mixtures as developing solvent to yield 6 fractions: Frs. F_1-F_6. They were applied to repeated silica-gel ($CHCl_3$-MeOH-H_2O, 80:20:2) and Sephadex LH 20 (MeOH) column chromatography to give compounds **1** (5.9 mg), **4** (4 mg), and **7** (10 mg).

Acid Hydrolysis of 3 and 7: Compound **3** in a mixture of 8% HCl (2 mL) and MeOH (20 mL) was refluxed at 100 °C for 2 h. The reaction mixture was evaporated in vacuo to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized product was subjected to TLC analyses on silica gel with EtOAc-MeOH-H_2O-HOAc (57:13:13:17). The chromatogram was sprayed with a Thymol-EtOH-conc.H_2SO_4(0.5 g:95 mL:5 mL) reagent and heated at 110 °C. The same procedure was used for compound **7**. The sugars were identified as galactose after comparison with authentic samples for compounds **3** and **7**.

Results

Quercetin (1): $C_{15}H_{10}O_7$ (mol.wt. 302.3); negative ion ESI-MS m/z 301 $[M-H]^-$; ^{1}H NMR (DMSO, 300 MHz): δ 6.17 (1H, *d*, J = 2.0 Hz, H-6), 6.37 (1H, *d*, J = 2.0 Hz, H-8), 6.87 (1H, *d*, J = 8.0 Hz, H-5′), 7.62 (1H, *dd*, J = 2.0, 7.5 Hz, H-6′), 7.73 (1H, *d*, J = 2.0 Hz, H-2′); ^{13}C NMR (MeOH, 75 MHz): Table.

Quercetin 3-O-β-glucopyranoside (isoquercitrin) (2): $C_{21}H_{20}O_{12}$ (mol.wt. 464); positive ion ESI-MS m/z 487 $[M+Na]^+$; negative ion ESI-MS m/z 463 $[M-H]^-$;^{1}H NMR (MeOH, 300 MHz): δ 6.10 (1H, *d*, J = 2.0 Hz, H-6), 6.26 (1H, *d*, J = 2.0 Hz, H-8), 6.85 (1H, *d*, J = 8.0 Hz, H-5′), 7.57 (1H, *dd*, J = 2.0,

7.5 Hz, H-6′), 7.70 (1H, *d*, J = 2.0 Hz, H-2′), 5.10 (1H, *d*, J = 7.7 Hz, H-1″), 3.30-3.80 (6H, *m*, H-2″, H-3″, H-4″, H-5″, H-6″); ^{13}C NMR (MeOH, 75 MHz): Table.

Quercetin 3-O-β-galactopyranoside (hyperin) (3): $C_{21}H_{20}O_{12}$ (mol.wt. 464); positive ion ESI-MS m/z 487 $[M+Na]^+$; negative ion ESI-MS m/z 463 $[M-H]^-$; ^{1}H NMR (MeOH, 300 MHz): δ 6.12 (1H, *d*, J = 1.9 Hz, H-6), 6.30 (1H, *d*, J = 1.9 Hz, H-8), 6.85 (1H, *d*, J= 8.0 Hz, H-5′), 7.57 (1H, *dd*, J = 2.0, 7.5 Hz, H-6′), 7.82 (1H, *d*, J = 2.0 Hz, H-2′), 5.04 (1H, *d*, J = 7.6 Hz, H-1″), 3.82 (1H, *m*, H-2″), 3.54 (1H, *m*, H-3″), 3.85 (1H, *d*, J = 2.0 Hz, H-4″), 3.45 (1H, *m*, H-5″), 3.58 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 3.65 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

Quercetin 7-O-β-galactopyranoside (4): $C_{21}H_{20}O_{12}$ (mol.wt. 464); positive ion ESI-MS m/z 487 $[M+Na]^+$, 951 $[2M+Na]^+$, negative ion ESI-MS m/z 463 $[M-H]^-$, 927 $[2M-H]^-$; ^{1}H NMR (MeOH, 300 MHz): δ 6.19 (1H, *d*, J = 2.0 Hz, H-6), 6.38 (1H, *d*, J = 2.0 Hz, H-8), 6.86 (1H, *d*, J= 8.0 Hz, H-5′), 7.58 (1H, *dd*, J = 2.0 and 7.5 Hz, H-6′), 7.82 (1H, *d*, J = 2.0 Hz, H-2′), 5.14 (1H, *d*, J = 7.6 Hz, H-1″), 3.45-3.75 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.84 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 4.20 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

Quercetin 4′-O-β-galactopyranoside (5): $C_{21}H_{20}O_{12}$ (mol.wt. 464); positive ion ESI-MS m/z 487 $[M+Na]^+$, 951 $[2M+Na]^+$, negative ion ESI-MS m/z 463 $[M-H]^-$, 927 $[2M-H]^-$; ^{1}H NMR (MeOH, 300 MHz): δ 6.12 (1H, *d*, J = 2.0 Hz, H-6), 6.29 (1H, *d*, J = 2.0 Hz, H-8), 6.85 (1H, *d*, J = 8.0 Hz, H-5′), 7.57 (1H, *dd*, J = 2.0 and 7.5 Hz, H-6′), 7.82 (1H, *d*, J = 2.0 Hz, H-2′), 5.04 (1H, *d*, J = 7.6 Hz, H-1″), 3.35-3.75 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.83 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 4.21 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

Isorhamnetin 3-O-β-galactopyranoside (6): $C_{22}H_{22}O_{12}$ (mol.wt. 478); positive ion ESI-MS m/z 501 $[M+Na]^+$, negative ion ESI-MS m/z 477 $[M-H]^-$, 955 $[2M-H]^-$; ^{1}H NMR (MeOH, 300 MHz): δ 6.19 (1H, *d*, J = 2.0 Hz, H-6), 6.39 (1H, *d*, J = 2.0 Hz, H-8), 6.89 (1H, *d*, J = 8.0 Hz, H-5′), 7.57 (1H, *dd*, J = 2.0 and 7.5 Hz, H-6′), 8.02 (1H, *d*, J = 2.0 Hz, H-2′), 3.95 (3H, *s*, OMe), 5.32 (1H, *d*, J = 7.6 Hz, H-1″), 3.40-3.70 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.82 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 4.21 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

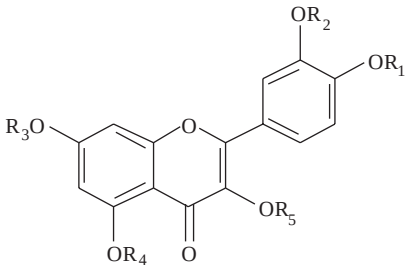
Isorhamnetin 5-O-β-galactopyranoside (7): $C_{22}H_{22}O_{12}$ (mol.wt. 478); positive ion ESI-MS m/z 501 $[M+Na]^+$; negative ion ESI-MS m/z 477 $[M-H]^-$; ^{1}H NMR (MeOH, 500 MHz): δ 6.13 (1H, *d*, J = 2.0 Hz, H-6), 6.30 (1H, *d*, J = 2.0 Hz, H-8), 6.88 (1H, *d*, J = 8.0 Hz, H-5′), 7.57 (1H, *dd*, J= 2.0 and 7.5 Hz, H-6′), 7.90 (1H, *d*, J = 2.0 Hz, H-2′), 3.94 (3H, *s*, OMe), 5.22 (1H, *d*, J = 7.6 Hz, H-1″), 3.35-3.75 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.83 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 4.21 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

Dihydrokaempferol 7,4′-dimethyl ether 3-O-β-glucopyranoside (8): $C_{23}H_{26}O_{11}$ (mol.wt. 478); positive ion ESI-MS m/z 501 $[M+Na]^+$, 979 $[2M+Na]^+$, negative ion ESI-MS m/z 477 $[M-H]^-$, 955 $[2M-H]^-$; ^{1}H NMR (MeOH, 500 MHz): δ 5.34 (1H, *d*, J = 11.0 Hz, H-2), 4.21 (1H, *d*, J = 11.0 Hz, H-3), 6.16 (1H, *d*, J = 2.0 Hz, H-6), 6.30 (1H, *d*, J = 2.0 Hz, H-8), 6.88 (1H, *d*, J = 7.5 Hz, H-5′), 6.90 (1H, *d*, J = 7.5 Hz, H-3′), 7.60 (1H, *dd*, H-6′), 7.60 (1H, *m*, H-2′), 3.94 (3H, *s*, 4′-OCH_3), 3.96 (3H, *s*, 7-OCH_3), 5.27 (1H, *d*, J = 7.7 Hz, H-1″), 3.40-3.65 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.73 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6′$_a$), 3.83 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6′$_b$); ^{13}C NMR (MeOH, 75 MHz): Table.

Isorhamnetin 3-O-α-rhamnopyranosyl (1‴ → 6″)-β-glucopyranoside (isorhamnetin 3-O-rutinoside) (9): $C_{28}H_{32}O_{16}$ (mol.wt. 624); negative ion ESI-MS m/z 623 $[M-H]^-$; ^{1}H NMR (MeOH, 300 MHz): δ 6.15 (1H, *d*, J = 2.0 Hz, H-6), 6.30 (1H, *bs*, H-8), 6.90 (1H, *d*, J = 7.5 Hz, H-5′), 7.20 (1H, *dd*, J = 2.0, 7.5 Hz, H-6′), 7.75 (1H, *d*, J = 2.0 Hz, H-2′), 3.94 (3H, *s*, 3′-OCH_3), 5.15 (1H, *d*, J = 7.7 Hz, H-1″), 4.50 (1H, *d*, J = 1.8 Hz, H-1‴), 3.20-3.70 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.20-3.70 (4H, *m*, H-2‴, H-3‴, H-4‴, H-5‴), 3.72 (1H, *dd*, J = 11.0 and 7.0 Hz, H-6″$_a$), 3.80 (1H, *dd*, J = 11.0 and 4.0 Hz, H-6″$_b$), 1.09 (3H, *d*, J = 6.3 Hz, CH_3-6‴); ^{13}C NMR (MeOH, 75 MHz): Table.

Table. ^{13}C NMR (MeOH, 75 MHz) data of compounds **1-9.**

Position	1	2	3	4	5	6	7	8	9
Aglycone									
2	147.9	158.0	158.7	149.5	150.0	156.2	143.0	83.0	156.5
3	137.2	135.1	135.2	135.7	135.6	133.9	140.1	71.4	133.8
4	177.3	178.9	178.9	177.4	178.8	176.5	170.3	197.8	170.4
5	162.5	163.2	163.0	162.4	162.7	161.3	159.5	163.4	159.5
6	99.3	101.4	101.3	100.1	101.2	100.0	101.4	96.1	102.5
7	165.7	167.3	167.2	165.2	165.2	164.2	163.6	167.0	163.8
8	94.4	95.4	95.7	94.8	95.6	94.7	95.6	94.0	95.8
9	158.2	158.6	158.5	158.1	158.7	156.5	158.3	162.6	158.3
10	104.4	105.2	105.0	105.0	104.8	105.2	104.8	103.1	104.8
1′	124.1	123.0	122.2	126.2	129.8	123.6	123.6	129.7	128.6
2′	116.0	116.2	116.0	116.0	116.0	114.5	114.5	129.2	114.5
3′	146.2	145.9	146.2	145.5	146.5	148.4	148.5	116.2	148.5
4′	148.7	149.5	148.5	148.2	146.0	146.2	146.6	159.6	146.7
5′	116.2	117.4	117.6	117.7	117.6	115.9	115.9	113.6	116.2
6′	121.6	122.7	122.9	122.9	122.8	123.6	123.1	129.3	124.0
7-OCH_3								55.9	
3′-OCH_3						56.9	56.9		56.7
4′-OCH_3								56.2	
Glc 1″		101.4						104.0	102.5
2″		74.3						76.0	75.9
3″		76.8						78.9	77.4
4″		70.3						71.4	71.6
5″		77.5						78.5	78.2
6″		61.3						62.5	69.8
Gal 1″			105.8	105.4	105.8	104.4	104.8		
2″			73.2	73.2	73.2	73.2	73.2		
3″			75.2	75.1	75.2	75.1	75.1		
4″			70.0	70.0	70.0	70.0	70.0		
5″			77.1	77.2	77.2	77.3	77.2		
6″			61.9	62.0	61.9	62.2	62.1		
Rha1‴									102.0
2‴									72.1
3‴									72.3
4‴									73.9
5‴									71.6
6‴									18.1



	R_1	R_2	R_3	R_4	R_5
1	H	H	H	H	H
2	H	H	H	H	glc
3	H	H	H	H	gal
4	H	H	gal	H	H
5	gal	H	H	H	H
6	H	CH_3	H	H	gal
7	H	CH_3	H	gal	H
9	H	CH_3	H	glc and rha	H

Figure 1. Structure of compounds 1-7 and 9.

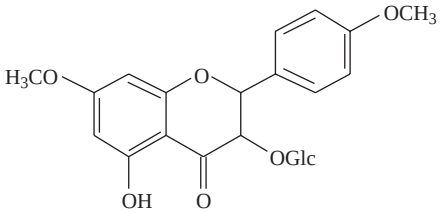


Figure 2. Structure of compound 8.

Discussion

Compound **1** was isolated as a yellow amorphous powder. The negative ESI-MS of **1** gave a quasi-molecular ion peak $[M\text{-}H]^-$ at m/z 301, compatible with the molecular formula $C_{15}H_{10}O_7$. Its UV absorptions in MeOH were consistent with the presence of a 3, 5, 7, 3′, 4′- pentahydroxyflavone structure[11]. The ^{1}H- and ^{13}C-NMR spectra of compound **1** exhibited resonances due to aromatic systems. The ^{13}C–NMR signals of **1** were assigned with the help of an HMQC experiment. In the ^{1}H-NMR spectrum of **1**, the aromatic region exhibited an ABX system at δ 7.73 (1H, *d*, J = 2.0 Hz, H-2′), 7.62 (1H, *dd*, J = 2.0 and 7.5 Hz, H-6′), and 6.87 (1H, *d*, J = 8.0 Hz, H-5′) due to a 3′, 4′ disubstitution of ring B and a typical *meta*-coupled pattern for H-6 and H-8 protons (δ 6.17 and 6.37, *d*, J = 2.0 Hz). The ^{13}C–NMR spectrum of **1** showed the presence of 15 aromatic carbon signals. Based on the NMR data and comparison of the data given in the literature, the structure of compound **1** was identified as quercetin[12−13].

Compounds **2**, **3**, **4**, and **5** were isolated as yellow amorphous powders. The negative ESI-MS of these gave a quasi-molecular ion peak $[M\text{-}H]^-$ at m/z 463, compatible with the molecular formula $C_{21}H_{20}O_{12}$. In the UV spectral analyses these compounds gave a typical MeOH spectrum of quercetin derivatives[11]. The ^{1}H- and ^{13}C–NMR spectra showed the presence of a quercetin moiety and sugar residue whose aglycone parts were the same as those of compound **1**. However, other spectroscopic evidence indicated that compound **2** contained glucose, while compounds **3**, **4** and **5** contained galactose as sugar parts. An anomeric proton signal of compound **2** appeared at δ_H 5.10 (*d*, J = 7.7 Hz, H-1″) and the resonances in the region of δ_H 3.30-3.80 (6H, *m*, H-2″, H-3″, H-4″, H-5″,H-6″) together with the corresponding carbon resonances inferred from the HSQC spectrum suggested the presence of β-glucopyranose units. In the HMBC spectrum, a crosspeak between C-3 and H-1″ established the linkage point quercetin and sugar moieties. The structure of compound **2** was identified as quercetin 3-O-β-glucopyranoside [12−13]. The anomeric proton resonances of compounds **3**, **4** and **5** were observed at δ_H 5.04 (*d*, J= 7.6 Hz, H-1″, δ_C 105.8), 5.14 (1H, *d*, J = 7.6 Hz, H-1″, δ_C 105.4) and 5.04 (1H, *d*, J = 7.6 Hz, H-1″, δ_C 105.8). By a comparison of the ^{13}C-NMR

data of the sugar moiety in **3**, **4** and **5** with that of galactose, it was determined to be galactose. In the HMBC spectra of **3**, **4** and **5**, crosspeaks between C-3 and H-1″, C-7 and H-1″, C-4′ and H-1″ established the linkage point quercetin and sugar moieties. Therefore, compounds **3**, **4** and **5** were characterized as quercetin 3-O-β-galactopyranoside[14], quercetin 7-O-β-galactopyranoside[15] and quercetin 4′-O-β-galactopyranoside[16], respectively.

Compounds **6-7** were isolated as yellow amorphous powders. They exhibited UV absorptions confirming their phenolic nature. On UV spectral analyses, these compounds gave typical MeOH spectra of quercetin derivatives[11]. In the ^{1}H-NMR spectrum of each compound, the aromatic region exhibited an ABX system due to a 3′, 4′ disubstitution of ring B and a typical *meta*-coupled pattern for H-6 and H-8 protons. The presence of the methoxy groups of **6** and **7** were supported by δ_H 3.95 and δ_H 3.94, δ_c 56.97 and δ_c 56.92 signals, respectively. Anomeric proton signals of both compounds were observed at δ 5.32 (*d*, $J = 7.6$ Hz) and 5.22 (*d*, $J = 7.6$ Hz). The ^{13}C-NMR signals of **6** and **7** were assigned with the help of an HMQC experiment. The positions of a methoxy group and 2 glycosidic residues were deduced from cross peaks between C-3′/OMe3′, C-3/H-1″ and C-5/H-1″ in the HMBC spectra. Therefore, compound **6** was characterized as isorhamnetin 3-O-β-galactopyranoside[17] while compound **7** was characterized as isorhamnetin 5-O-β-galactopyranoside[18] .

Compound **8** was isolated as a yellow amorphous powder. Its negative ESI-MS gave a quasi-molecular ion peak [M-H]$^-$ at m/z 477, compatible with the molecular formula C_{23} $H_{26}O_{11}$. On UV spectral analysis, this compound gave a typical MeOH spectrum of a dihydrokaempferol derivative[11]. The ^{1}H- and ^{13}C–NMR spectra of **8** showed the presence of 2 aromatic systems linked by –CH-CH-CO- chain. The aromatic protons were recorded at δ 6.16 and 6.30 as *meta*-related doublets ($J = 2.0$ Hz) for H-6 and H-8, and at δ 7.60 (*m*), δ 6.90 (*d*, $J = 7.5$ Hz), δ 6.88 (*d*, $J = 7.5$ Hz) and δ 7.60 (*dd*) for H-2′, H-3′, H-5′ and H-6′ respectively. The ^{1}H–NMR spectrum showed 2 doublets at δ 4.21 and 5.34 ($J = 11.0$ Hz) characteristic of trans H-2/H-3 protons in a dihydroflavonol. The presence of 2 methoxy groups was supported by δ_H 3.94 and δ_H 3.96, δ_c 56.20 and δ_c 55.90 signals. An anomeric proton signal of **8** was observed at δ 5.27 (*d*, $J = 7.7$ Hz). By a comparison of the ^{13}C-NMR data of the sugar moiety in **8** with that of galactose, the sugar moiety was determined to be galactose. The connectivities of the molecular fragments were established by a hetero-nuclear multiple-bond correlation experiment (HMBC). Compound **8** was characterized as dihydrokaempferol 7,4′-dimethyl ether 3-O-β-glucopyranoside.

Compound **9** was isolated as a yellow amorphous powder. Its negative ESI-MS gave a quasi-molecular ion peak [M-H]$^-$ at m/z 623, compatible with the molecular formula $C_{28}H_{32}O_{16}$. On UV spectral analysis, this compound gave a typical MeOH spectrum of a quercetin derivative[11]. The ^{1}H- and ^{13}C-NMR spectra of compound **9** showed the expected signals in the aromatic region for the isorhamnetin aglycone. The ^{1}H-NMR of **9** showed 2 doublets at δ_H 5.15 (1H, $J = 7.7$ Hz, H-1″) and δ_H 4.50 (1H, $J = 1.8$ Hz, H-1‴), suggesting 2 anomeric protons of a sugar moiety. The anomeric proton signals were consistent with the β configuration of a glucose, and α configuration of a rhamnose. The presence of the methoxy group was supported by δ_H 3.94 (3H, s) and δ_C 56.75 signals. The positions of a methoxy group and 2 glycosidic residues were deduced from cross peaks between C-3′/OMe3′, C-3/H-1″ and C-6″/H-1‴ in the HMBC spectra. Compound **9** was characterized as isorhamnetin 3-O-α-rhamnopyranosyl (1‴ $\rightarrow$ 6″)-β-glucopyranoside[19].

Conclusion

In this study, 9 known flavonoids (**1-9**) were isolated and identified from the aerial parts of *Asperula arvensis.*

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