Flavonol Glycosides and Iridoids from *Asperula lilaciflora*

by **Hasan Kırmızıbekmez***[a]), **Hilal Bardakcı**[a]), **Milena Masullo**[b]), **Özge Kamburoğlu**[c]), **Gonca Eryılmaz**[c]), **Galip Akaydın**[d]), **Erdem Yeşilada**[a]), and **Sonia Piacente**[b])

[a]) Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, TR-34755, Kayışdağı, İstanbul (phone: +90-216-5780000-3050; fax: +90-216-5780068; e-mail: hasankbekmez@yahoo.com)

[b]) Salerno University, Department of Pharmaceutical and Biomedical Sciences, Via Ponte Don Melillo, IT-84084 Fisciano, Salerno

[c]) Yeditepe University, Faculty of Pharmacy, TR-34755, Kayışdağı, İstanbul

[d]) Hacettepe University, Department of Biology Education, TR-06800, Beytepe, Ankara

A new flavonol glycoside, quercetin 3-*O*-[6'''-*O*-3,5-dihydroxycinnamoyl-*β*-glucopyranosyl-(1→2)]-*β*-galactopyranoside (named lilacifloroside; **1**) and a new iridoid **2** (named asperulogenin), were isolated from the aerial parts of *Asperula lilaciflora* in addition to eight known secondary metabolites, *i.e.*, quercetin, kaempferol, quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-*β*-galactopyranoside, quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-arabinopyranoside, asperuloside, deacetylasperulosidic acid, asperulosidic acid methyl ester, and chlorogenic acid. The structures were elucidated on the basis of extensive 1D- and 2D-NMR experiments as well as MS data. Compound **1** contains the rare 3,5-dihydroxycinnamoyl moiety in its structure. This work constitutes the first phytochemical study of the title plant.

Introduction. – *Asperula* L., with a total of 183 species, is one of the most important genera in the family Rubiaceae [1]. There are *ca.* 40 *Asperula* species growing wild in Turkey; 19 are endemic [2]. Some members of *Asperula* are utilized for enhancing the volume of the urine, for the treatment of constipation, as well as tonic in Anatolian folk medicine [3]. Previous phytochemical studies on *Asperula* revealed that the genus contains flavonoids [4], iridoids [4][5], and anthraquinones [6] as the major secondary metabolites. In this study, we have examined the constituents of *A. lilaciflora*, an endemic species to Turkey. Several chromatographic studies of the MeOH extract of the aerial parts of *A. lilaciflora* led to the isolation of a new flavonol glycoside, named lilacifloroside (=quercetin 3-*O*-[6'''-*O*-3,5-dihydroxycinnamoyl-*β*-glucopyranosyl-(1→2)]-*β*-galactopyranoside; **1**) as well as a new iridoid **2** (named asperulogenin), along with eight known secondary metabolites.

Results and Discussion. – The H_2O-soluble portion of the crude MeOH extract prepared from the aerial parts of *Asperula lilaciflora* was subjected to a series of column-chromatographic separations (polyamide, SiO_2, C_{18}-MPLC, and *Sephadex LH-20*) to obtain two new secondary metabolites, **1** and **2**, along with eight known compounds (*Fig. 1*).

The known compounds were identified as quercetin, kaempferol, quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-arabinopyranoside [7], quercetin 3-*O*-*β*-glucopyranosyl-(1→2)-*β*-galactopyranoside [8], asperuloside [9], deacetylasperulosidic acid [10], asper-

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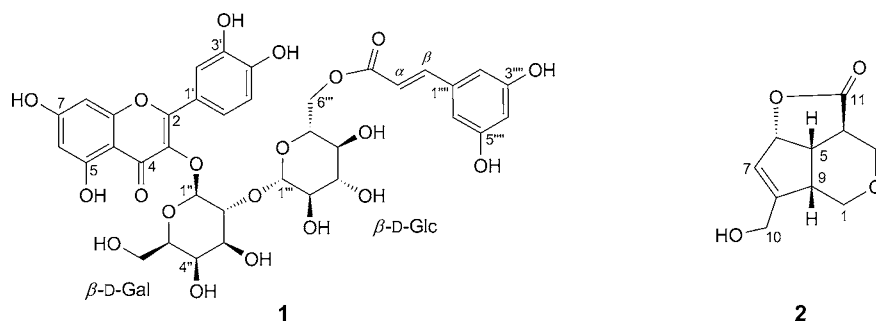

Fig. 1. *Compounds* **1** *and* **2** *isolated from* Asperula lilaciflora

ulosidic acid methyl ester [11], and chlorogenic acid [12] by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as yellow amorphous powder. The molecular formula $C_{36}H_{36}O_{20}$ was deduced from ESI-MS (*m/z* 811 ($[M+Na]^+$)) and NMR data. The ^{1}H-NMR spectrum of **1** (*Table 1*) displayed the signals at δ(H) 7.71 (*d*, $J = 1.8$), 7.69 (*dd*, $J = 1.8, 8.2$) and 6.89 (*d*, $J = 8.2$) as *ABX* system. These signals taken together with the two '*meta*-coupled' signals at δ(H) 6.26 and 6.17 (*d*, $J = 1.7$, each) were indicative of a quercetin unit [13]. Moreover, AB_2-system signals at δ(H) 6.83 (br. *s*, 1 H), 6.67 (*t*, $J = 2.3$, 2 H), as well as *AX*-type '*trans*-coupled' resonances at δ(H) 7.35 and 6.03 (*d*, $J = 15.7$, each) were observed in the aromatic region of the ^{1}H-NMR spectrum. These signals, along with the corresponding C-atom resonances (δ(C) 168.8, 149.3 (2 C), 146.6, 127.2, 122.4 (2 C), 114.6, 114.1) were consistent with a 3,5-dihydroxycinnamoyl moiety [14]. The two anomeric H- and C-atom resonances (δ(H) 5.17 (*d*, $J = 7.7$); δ(C) 101.0; and δ(H) 4.78 (*d*, $J = 7.2$); δ(C) 105.5) indicated a diglycosidic structure. The complete assignments of all H- and C-atom resonances of the disaccharide unit, based on extensive 2D-NMR experiments (COSY, HSQC, and HMBC (*Table 1* and *Fig. 2*)) revealed the presence of β-galactopyranosyl and β-glucopyranosyl units. The presence of a β-glucopyranosyl-(1 → 2)-β-galactopyranosyl disaccharide moiety was deduced

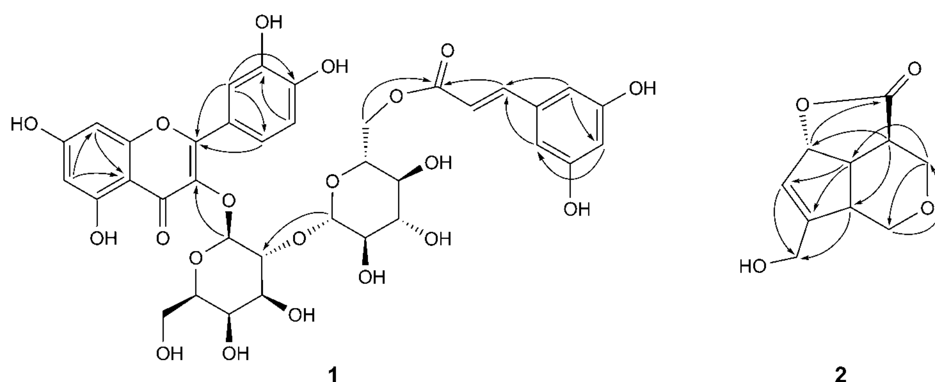

Fig. 2. *Key HMBCs* (H → C) *of compounds* **1** *and* **2**

Table 1. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp.; in CD_3OD)*, and HMBCs of* **1**[a])

Position	δ(H)[a])	δ(C)[a])	HMBC (H → C)
Aglycone			
2	–	157.8	
3	–	135.1	
4	–	179.0	
5	–	162.8	
6	6.17 (*d*, *J* = 1.7)	99.5	C(8), C(10)
7	–	165.7	
8	6.26 (*d*, *J* = 1.7)	94.3	C(6), C(9), C(10)
9	–	158.1	
10	–	105.1	
1′	–	123.6	
2′	7.71 (*d*, *J* = 1.8)	116.9	C(2), C(4′), C(6′)
3′	–	145.6	
4′	–	149.5	
5′	6.89 (*d*, *J* = 8.2)	115.8	C(3′)
6′	7.69 (*dd*, *J* = 1.8, 8.2)	123.2	C(2′)
Gal			
1″	5.17 (*d*, *J* = 7.7)	101.0	C(3)
2″	4.05 (*dd*, *J* = 7.7, 9.4)	81.9	C(1‴)
3″	3.71 (*dd*, *J* = 2.6, 9.4)	74.9	
4″	3.84 (*d*, *J* = 2.6)	69.7	
5″	3.40 – 3.44 (*m*)	76.6	
6″	3.61 (*dd*, *J* = 11.5, 5.9), 3.52 (*dd*, *J* = 3.5, 11.5)	61.5	
Glc			
1‴	4.78 (*d*, *J* = 7.2)	105.5	C(2″)
2‴	3.47 (*dd*, *J* = 7.2, 7.9)	75.7	
3‴	3.47 – 3.50[b])	77.4	
4‴	3.42[b])	71.5	
5‴	3.68 – 3.72 (*m*)	74.9	
6‴	4.46 (*dd*, *J* = 1.9, 11.7), 4.41 (*dd*, *J* = 6.1, 11.7)	64.4	C=O
Acyl			
1⁗	–	127.2	
2⁗/6⁗	6.67 (*t*, *J* = 2.3)	122.4	C(4⁗), C(β)
3⁗/5⁗	–	149.3	
4⁗	6.83 (br. *s*)	114.6	C(2⁗), C(6⁗)
H–C(α)	6.03 (*d*, *J* = 15.7)	114.1	C(β)
H–C(β)	7.35 (*d*, *J* = 15.7)	146.6	C(α), C(2⁗), C(6⁗)
C=O	–	168.8	

[a]) Assignments are based on COSY, HSQC, and HMBC experiments. [b]) Overlapped signals.

from the downfield shift of C(2″) (δ(C) 81.9) of galactose, which was further confirmed by the long-range correlation H–C(2″) (δ(H) 4.05)/C(1‴) (δ(C) 105.5) and *vice versa* in the HMBC spectrum. The glycosidation site of the disaccharide moiety to the aglycone was found to be C(3)–OH due to the cross-peak observed between the

H–C(1″) (δ(H) 5.17) of inner sugar (galactose) and the C(3) (δ(C) 135.1) of the flavonol moiety in the HMBC spectrum. Besides these findings, the H- and the C-atom signals of the CH_2OH group of the glucose were shifted downfield, indicating that the acylation site was C(6‴). This was also confirmed by the strong HMBC between the CH_2(6‴) (δ(H) 4.46 and 4.41) of glucose and C=O (δ(C) 168.8) of the 3,5-dihydroxycinnamoyl moiety. Accordingly, compound **1** was identified as quercetin 3-*O*-[6‴-*O*-3,5-(dihydroxycinnamoyl)-β-glucopyranosyl-(1 → 2)]-β-galactopyranoside. To the best of knowledge, **1** is being reported for the first time and named lilacifloroside.

Compound **2** was obtained as amorphous colorless powder. The molecular formula was determined as $C_{10}H_{12}O_4$, with five degrees of unsaturation, by positive-ion mode ESI mass spectrometry (*m/z* 197.0 ($[M+H]^+$)) and NMR data.

The ^{1}H-NMR spectrum (*Table 2*) of **2** displayed signals of one olefinic H-atom (δ(H) 5.83 (br. *s*)), three CH_2–O group (δ(H) 4.26 (*d*, *J* = 15.0), 4.17 (*d*, *J* = 15.0), 3.94 (*dd*, *J* = 10.8, 4.5), 3.85 (*dd*, *J* = 10.8, 3.6), 3.83 (*dd*, *J* = 11.3, 4.1), and 3.72 (*dd*, *J* = 11.3, 6.9)), one CH–O group (δ(H) 5.43 (br. *d*, *J* = 7.1), as well as three CH groups (δ(H) 3.35 (*dd*, *J* = 14.2, 6.4), 3.12 (*dd*, *J* = 11.0, 6.9), and 2.94 – 2.99 (*m*)) H-atom signals. Its ^{13}C-NMR spectrum displayed ten resonances including those of a C=O group (δ(C) 180.1) and two olefinic C-atoms (δ(C) 153.3 and 124.6). Detailed analysis of 1D- and 2D-NMR spectra (COSY, HSQC, and HMBC (*Table 2* and *Fig. 2*)) suggested that **2** had a C_{10} iridoid skeleton composed of cyclopentapyran ring system. The H- and C-atom signals attributed to 3 and 4 were compatible with the iridoids lacking of a C(3)=C(4) bond between these C-atoms as in the case of macedonine [15]. The location of the C=O group was unambiguously assigned to be C(4) by the strong HMBCs CH_2(3)/C(11) and H–C(5)/C(11). The deshielding of H–C(6) (δ(H) 5.43), and the long-range correlation between H–C(6) and C(11), along with an additional degree of unsaturation revealed the presence of a lactone ring between the C(11)OOH and OH group at C(6) as in the case of asperuloside [9]. The relative configuration of **2** was elucidated by ROESY spectrum in which significant ROEs H_β–C(5)/H_β–C(9), H_β–C(7)/H_β–C(6), H_β–C(3)/H_β–C(5), and H_β–C(5)/H_β–C(1) indicated that these H-

Table 2. *^{1}H- and ^{13}C-NMR Data* (600 and 150, resp.; in CD_3OD), *and HMBCs of* **2**[a]). Atom numbering as indicated in *Fig. 1.*

Position	δ(H)[a]	δ(C)[a])	HMBC (H → C)
1	3.83 (*dd*, *J* = 11.3, 4.1, H_α), 3.72 (*dd*, *J* = 11.3, 6.9, H_β)	60.7	C(3), C(9)
3	3.94 (*dd*, *J* = 10.8, 4.5, H_α), 3.85 (*dd*, *J* = 10.8, 3.6, H_β)	62.7	C(1), C(4), C(5)
4	2.94 – 2.99 (*m*)	45.7	C(3), C(5), C(6), C(9)
5	3.35 (*dd*, *J* = 14.2, 6.4)	43.6	C(3), C(7), C(8)
6	5.43 (br. *d*, *J* = 7.1)	87.8	C(4), C(11)
7	5.83 (br. *s*)	124.6	C(5), C(10)
8	–	153.3	
9	3.12 (*dd*, *J* = 11.0, 6.9)	49.7	C(1), C(4), C(10)
10	4.26 (*d*, *J* = 15.0), 4.17 (*d*, *J* = 15.0)	60.4	C(8), C(7)
11	–	180.1	

[a]) Assignments are based on COSY, HSQC, HMBC, and ROESY experiments.

atoms were positioned on the same side. On the other hand, H_{α}–C(3) correlated with H_{α}–C(4). Moreover, the absence of ROE correlation between H–C(4) and H–C(6) supported the proposed relative configuration. Consequently, compound **2** was elucidated as 2a,4a,5,7,7a,7b-hexahydro-4-(hydroxymethyl)-2,6-dioxacyclopenta[*cd*]-inden-1-one and given the trivial name asperulogenin.

Iridoids are useful taxonomic markers particularly in dicotyledon families. Although a few iridoid glycosides have been reported from the genus *Asperula*, no non-glycosidic iridoids or dihydroiridoids (lacking C(3)=C(4) bond) have been isolated from this genus so far. Compound **2** is also an unusual iridoid lacking a hemiacetal group at C(1). There exist a few compounds with quite similar structural feature like macedonine [15] and 1-dehydroxy-3,4-dihydroaucubigenin [16]. Concerning the isolated flavonol glycosides, compound **1** can be regarded as a unique flavonoid due to the presence of 3,5-dihydroxycinnamoyl moiety in its structure. This acyl unit has only been encountered in the structures of anthocyanins [14] and triterpenes [17] up to now. Furthermore, an arabinose-bearing diglycosidic flavonoid is being reported for the first time from *Asperula*. A similar quercetin derivative containing the same diglycoside moiety, acylated by ferulic acid, was reported from *Carrichtera annua* (Brassicaceae) [18]. The phylogenetic works on the Rubiaceae revealed that the genus *Asperula* is not monophyletic [19]. Thus, the compounds obtained in this study such as lilacifloroside (**1**), asperulogenin (**2**), and quercetin 3-*O*-β-glucopyranosyl-(1 $\rightarrow$ 2)-arabinopyranoside might be utilized to support the phylogenetic studies and may contribute to the chemotaxonomy of the genus *Asperula*, particularly to its *Cynanchicae* section in which *A. lilaciflora* is placed.

Experimental Part

General. TLC: SiO_2-Coated (silica gel *60 F_{254}*; *Merck*) aluminum plates; eluents, $CHCl_3$/MeOH/H_2O 80 : 20 : 2, 70 : 30 : 3, and 61 : 32 : 7; and AcOEt/MeOH/H_2O 100 : 10 : 5; visualization by spraying with 1% vanillin/H_2SO_4 soln., followed by heating at 105° for 2 – 3 min. Column chromatography (CC): silica gel 60 (SiO_2, 0.063 – 0.200 mm; *Merck*, DE-Darmstadt), *Polyamide* (*Fluka*) and *Sephadex LH-20* gel (*Fluka*). Medium-pressure liquid chromatography (MPLC): *Combi Flash Companion* (*Isco*), *Redi step* columns (*LiChroprep C_{18}*; 130 and 43 g; *Teledyne Isco*). Optical rotations: *JASCO DIP 1000* polarimeter. UV Spectra: *HP Agilent 8453* spectrophotometer; λ_{max} in nm. IR Spectra (KBr): *Perkin-Elmer 2000* FT-IR spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AMX-600* instruments (600 (^{1}H) and 150 MHz (^{13}C)) with XWIN NMR 3.6 software package; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Finnigan* TSQ 7000 in MeOH; positive ion mode; in *m*/*z*.

Plant Material. Asperula lilaciflora BOISS. (Rubiaceae) was collected from Ermenek, Karaman, Turkey, in July 2010. The plant material was identified by one of us (*G. A.*). A voucher specimen (Akaydın 13361) was deposited with the Herbarium of Education, Hacettepe University, Ankara, Turkey.

Extraction and Isolation. The air-dried and powdered aerial parts of *A. lilaciflora* (315 g) were first left to maceration in MeOH (2 × 2.2 l; 4 d each) and then extracted at 45° for 4 h. The pooled MeOH extracts were evaporated to dryness (34 g, 10.8%), and the residue was suspended in H_2O (75 ml) and partitioned with $CHCl_3$ (3 × 75 ml). The H_2O fraction (20 g) was subjected to CC (*Polyamide*; MeOH/H_2O 0 – 100%): *Frs. A – H*. *Fr. B* (10.7 g) was dissolved in H_2O (15 ml) and partitioned with BuOH (4 × 15 ml). The BuOH fraction (1.05 g) was separated by MPLC (*LiChroprep C_{18}*, MeOH/H_2O 0 $\rightarrow$ 70%): *Frs. B_1 – B_{11}*. *Fr. B_2* (51 mg) was further purified by CC (SiO_2; $CHCl_3$/MeOH/H_2O 90 : 10 : 0 $\rightarrow$ 61 : 32 : 7): **2** (2 mg) and deacetylasperulosidic acid (4 mg). *Fr. B_6* (134 mg) was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 95 : 5 : 0 $\rightarrow$ 80 : 20 : 1): asperuloside (50 mg). Similarly, *Fr. B_9* (54.5 mg) was purified by using

the same technique: asperulosidic acid methyl ester (3 mg). *Fr. E* (290 mg) was subjected to MPLC (*LiChroprep C_{18}*; MeOH/H_2O 0 → 90): quercetin 3-*O*-*β*-glucopyranosyl-(1 → 2)-*β*-galactopyranoside (20 mg). *Fr. G* (430 mg) was separated by CC (SiO_2, CH_2Cl_2/MeOH/H_2O 95 : 5 : 0 → 50 : 40 : 10): kaempferol (1 mg), quercetin (2 mg) and *Fr. G_7*. *Fr. G_7* (90 mg) was further purified by CC (*Sephadex LH-20*; MeOH): quercetin 3-*O*-*β*-glucopyranosyl-(1 → 2)-arabinopyranoside (3 mg). Finally, *Fr. H* (433 mg) was subjected to MPLC (*LiChroprep C_{18}*; MeOH/H_2O 15 → 100): chlorogenic acid (50 mg) and *Fr. H_4*. Compound **1** (5 mg) was obtained from *Fr. H_4* (94 mg) by CC (SiO_2; CH_2Cl_2/MeOH/H_2O 90 : 10 : 1 → 80 : 20 : 2).

Lilacifloroside (= *Quercetin 3*-O-*[6‴*-O-*3,5-Dihydroxycinnamoyl-β-glucopyranosyl-(1 → 2)]-β-galactopyranoside* = *2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4*H-*chromen-3-yl 2*-O-*{6*-O-*[(2*E*)-3-(3,5-Dihydroxyphenyl)prop-2-enoyl]-β-*D-*glucopyranosyl}-β-*D-*galactopyranoside*; **1**): Yellowish amorphous powder. $[\alpha]_D^{24} = +11.3$ ($c = 0.1$, MeOH). UV (MeOH): 222 (4.38), 254 (4.29), 337 (4.29). IR (KBr): 3412, 1710, 1654, 1606, 1498, 1448, 1360, 1269, 1197, 1168, 1076. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS (pos.): 811.3 ($[M + Na]^+$, $C_{36}H_{36}NaO_{20}^+$).

Asperulogenin (= *(2a*R*,4a*S*,7a*R*,7b*S*)-2a,4a,5,7,7a,7b-Hexahydro-4-(hydroxymethyl)-1*H-*2,6-dioxacyclopenta[*cd*]inden-1-one*; **2**): Amorphous powder. $[\alpha]_D^{24} = +10.4$ ($c = 0.1$, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS (pos.): 197.0 ($[M + H]^+$, $C_{10}H_{13}O_4^+$).

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