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# Phenolic Compounds from *Scutellaria pontica*

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From the aerial parts of *Scutellaria pontica*, a phenolic glucoside, 3,5-dihydroxyphenyl β-D-glucopyranoside (**1**); as well as a *C*-glucosyl flavon, isovitexin (**2**); flavones and flavone glycosides, 5-hydroxy-7,3′,4′-trimethoxyflavone (**3**); apigenin (**4**); apigenin-7-*O*-β-D-glucopyranoside (**5**); and apigenin-7-*O*-β-D-glucopyranoside-4′-*O*-methylether (**6**) were isolated in addition to two phenylethanoid glycosides, martynoside (**7**) and verbascoside (= acteoside) (**8**). The structures of the isolated compounds were established on the basis of spectroscopic evidence.

**Key Words:** *Scutellaria pontica*, Lamiaceae, 3,5-dihydroxyphenyl β-D-glucopyranoside, isovitexin, 5-hydroxy-7,3′,4′-trimethoxyflavone, apigenin, apigenin-7-*O*-β-D-glucopyranoside, apigenin-7-*O*-β-D-glucopyranoside-4′-*O*-methylether, martynoside, verbascoside (= acteoside).

## Introduction

*Scutellaria pontica*, C. Koch. (Sect. Salviifoliae) (Lamiaceae) is a mat-forming perennial herb, which is widely distributed in the mountains of north-eastern Anatolia, growing on grassy slopes and banks, and rocky outcrops at elevations of 1800-2700 m[1]. In a previous report, the isolation of neoclerodane diterpenoids was described from the title plant[2]. The current study describes the isolation and structure elucidation of the phenolic glucoside, 3,5-dihydroxyphenyl β-D-glucopyranoside (**1**); together with a *C*-glucosyl flavone, isovitexin (**2**); two flavones, 5-hydroxy-7,3′,4′-trimethoxyflavone (**3**); apigenin (**4**); two flavone glucosides, apigenin-7-*O*-β-D-glucopyranoside (**5**); and apigenin-7-*O*-β-D-glucopyranoside-4′-*O*-methylether (**6**); as well as two phenylethanoid glycosides, martynoside (**7**) and verbascoside (= acteoside) (**8**); from the aerial parts of *S. pontica*.

## Experimental

**General Experimental Procedures:** UV ($\lambda_{max}$, nm) spectra were obtained with a Hitachi HP-8452 A spectrophotometer. FAB-MS was recorded in an NBA matrix, in the positive ion mode, on a JEOL JMS-DX300 mass spectrometer. $^{1}$H (500 MHz) and $^{13}$C NMR (125 MHz) spectra were recorded on a JEOL

JNM-A500 FT-NMR spectrometer, in DMSO-$d_6$ or $CD_3OD$ with tetramethylsilane (TMS) as an internal standard. Chemical shifts were given in ppm and coupling constants ($J$) in Hz. Complete proton and carbon assignments were based on 1D ($^1$H, $^{13}$C and DEPT) and 2D ($^1$H-$^1$H COSY, $^1$H-$^{13}$C HMQC and HMBC) NMR experiments. Reversed-phase liquid chromatography (RP-VLC) was performed on a cartridge, packed with LiChroprep RP-18 (20 g). For open column chromatography (CC), Polyamide (Macherey Nagel MN SC-6), Kieselgel 60 (0.063-0.200 mm, Merck) and Sephadex LH-20 (Fluka) were used. Medium-pressure liquid chromatography (MPLC) was performed on a Labomatic glass column (26x460 mm, i.d.), packed with LiChroprep RP-18, using a Lewa M5 peristaltic pump. 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°C for 1-2 min.

**Plant Material:** *Scutellaria pontica* C. Koch. (Lamiaceae) was collected from the vicinity of Çağırankaya, İkizdere, Rize, Turkey, in July 1998. Voucher specimens have been deposited at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 98-085).

**Extraction and Prepurification:** The air-dried and finely powdered aerial parts of *S. pontica* (500 g) were extracted with $Me_2CO$ (3x2000 ml) at 40°C. After filtration of the $Me_2CO$ extract, the residual plant material was dried at room temperature and extracted with MeOH (3x1000 ml) at 40°C. The combined methanolic extract was evaporated under reduced pressure and 44 g of MeOH extract was obtained. The crude methanolic extract was then dissolved in $H_2O$ (250 ml) and the water-insoluble material was removed by filtration. The filtrate was then extracted with *n*-BuOH (4x150 ml) to give the *n*-BuOH extract (14 g). The *n*-BuOH extract was fractionated over a polyamide column, eluting with $H_2O$ and gradient MeOH-$H_2O$ mixtures (25% to 100%, each 500 ml) to afford 12 main fractions (Frs. A-L).

**Isolation of the Compounds:** An aliquot of fr. A (750 mg) was fractionated over RP-VLC, using MeOH-$H_2O$ (40% and 50% MeOH, each 50 ml) as eluent to give eight fractions (Frs. $A_1$-$A_8$). Fr. $A_8$ (319 mg) was applied to MPLC. Elution with MeOH-$H_2O$ mixtures (10%, 20%, 25% and 30%, each 100 ml) yielded **1** (24.7 mg). Fr. E (1.2 g) was further separated by silica gel (120 g) CC with $CH_2Cl_2$-MeOH-$H_2O$ mixtures (80:20:1, 80:20:2 and 70:30:3, each 500 ml) to afford six fractions (Frs. $E_1$-$E_6$). Fr. $E_2$ (240.5 mg) was rechromatographed on a silica gel (25 g) column eluting with $CH_2Cl_2$-MeOH mixture (90:10, 700 ml) to yield three fractions (Frs. $E_{2a}$-$E_{2c}$). Fr. $E_{2c}$ (130.5 mg) was applied to MPLC and elution with MeOH-$H_2O$ gradients (15% to 60%) gave **7** (41.82 mg). Fr. $E_5$ (112.3 mg) was further chromatographed over silica gel (15 g) with $CH_2Cl_2$-MeOH-$H_2O$ mixture (80:20:1, 100 ml) as eluent to yield fractions $E_{5a}$-$E_{5c}$. Fr. $E_{5b}$(46.9 mg) was purified over Sephadex with MeOH to afford **8** (16.1 mg). An aliquot of fr. G (1.0 g) was rechromatographed on a silica gel column (100 g). Elution with $CH_2Cl_2$-MeOH-$H_2O$ mixtures (80:20:1, 80:20:2, 70:30:3 and 60:40:4, each 400 ml) yielded six fractions (Frs. $G_1$-$G_6$). Fr. $G_4$ (14.17 mg) was applied to Sephadex CC with MeOH to give **2** (5 mg). Fr. H (738 mg) was fractionated on RP-VLC with MeOH-$H_2O$ mixtures (40% and 50%, each 50 ml) to yield five fractions (Frs. $H_1$-$H_5$). Fr. $H_3$ (203.5 mg) was subjected to silica gel CC (20 g) using $CH_2Cl_2$-MeOH-$H_2O$ mixtures (85:15:1 and 80:20:1, each 300 ml) as eluent to give a mixture of **4**, **5** and **6** (25.4 mg). This mixture was not separated by CC or TLC with any solvent system. Fr. L (102 mg) was fractionated over Sephadex with MeOH to afford **3** (4.8 mg).

**3,5-dihydroxyphenyl $\beta$-D-glucopyranoside (1)**: $C_{12}H_{16}O_8$ (mol.wt. 288); FAB-MS: $m/z$ 289

$[M+H]^+$; UV $\lambda_{max}$ (MeOH) nm: 228; $^1$H NMR (DMSO-d$_6$, 500 MHz): aglycon moiety: $\delta$ 6.06 (2H, *d*, *J* = 2.1 Hz, H-2 and H-6), 5.99 (1H, *t*, *J* = 2.1 Hz, H-4); glucose moiety 4.89 (1H, *d*, *J* = 7.6 Hz, H-1′), 3.50-3.25 (4H, *m*, H-2′,3′,4′,5′), 3.77 (1H, *dd*, *J* = 12.5 and 2.1 Hz, H-$6'_b$), 3.59 (1H, *dd*, *J* = 12.5 and 5.0 Hz, H-$6'_a$);$^{13}$C NMR (DMSO-d$_6$, 125 MHz,): aglycon moiety: $\delta$ 159.41 (*s*, C-1), 158.47 (*s*, C-3 and C-5), 98.37 (*d*, C-4), 97.01 (*d*, C-2 and C-6); glucose moiety 100.69 (*d*, C-1′), 76.89 (*d*, C-5′), 76.30 (*d*, C-3′), 73.61 (*d*, C-2′), 70.20 (*d*, C-4′), 61.36 (*t*, C-6′).

**Isovitexin (2)**: $C_{21}H_{20}O_{10}$ (mol.wt. 432); FAB-MS: $m/z$ 433 $[M+H]^+$; UV $\lambda_{max}$ (MeOH) nm: 270, 334; (NaOMe) nm: 275, 328, 397; ($AlCl_3$) nm: 260sh, 278, 305, 351, 382; ($AlCl_3$+HCl) nm: 260sh, 278, 301, 344, 381; (NaOAc) nm: 279, 302, 385; (NaOAc/$H_3BO_3$) nm: 273, 345; $^1$H NMR ($CD_3OD$, 500 MHz): aglycon moiety: $\delta$ 7.84 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.92 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.60 (1H, *s*, H-3), 6.50 (1H, *s*, H-8); glucose moiety $\delta$ 4.89 (1H, *d*, *J* = 10.1 Hz, H-1″), 3.50-3.25 (4H, *m*, H-2″,3″,4″,5″), 3.87 (1H, *dd*, *J* = 11.9 and 2.2 Hz, H-$6''_b$), 3.73 (1H, *dd*, *J* = 11.9 and 5.4 Hz, H-$6''_a$); $^{13}$C NMR ($CD_3OD$, 125 MHz): aglycon moiety: $\delta$ 184.04 (*s*, C-4), 166.21 (*s*, C-2), 165.33 (*s*, C-7), 162.86 (*s*, C-4′), 162.04 (*s*, C-5), 158.79 (*s*, C-9), 129.45 (*d*, C-2′ and C-6′), 123.14 (*s*, C-1′), 117.08 (*d*, C-3′ and C-5′), 109.29 (*s*, C-6), 104.98 (*s*, C-10), 103.87 (*d*, C-3), 95.34 (*d*, C-8); glucose moiety: $\delta$ 82.62 (*d*, C-5″), 80.16 (*d*, C-3″), 75.31 (*d*, C-1″), 72.59 (*d*, C-2″), 71.79 (*d*, C-4″), 62.86 (*t*, C-6″).

**5-hydroxy-7,3′,4′-trimethoxyflavone (3)**: $C_{18}H_{16}O_6$(mol. wt. 328); FAB-MS: $m/z$ 329 $[M+H]^+$; UV $\lambda_{max}$ (MeOH) nm: 242, 250sh, 270, 290sh, 341; (NaOMe) nm: 277, 310, 370; ($AlCl_3$) nm: 260, 275, 297, 358, 385; ($AlCl_3$+HCl) nm: 260, 276, 295sh, 345, 381sh; (NaOAc) nm: 276, 315, 358; (NaOAc/$H_3BO_3$) nm: 271, 340; $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ 7.84 (1H, *dd*, *J* = 8.8 and 4.0 Hz, H-6′), 6.93 (1H, *dd*, *J* = 8.8 and 2.4 Hz, H-5′), 6.62 (1H, *s*, H-3), 6.58 (1H, *dd*, *J* = 4.0/2.4 Hz, H-2′), 6.45 (1H, *d*, *J* = 2.1 Hz, H-8), 6.21 (1H, *d*, *J* = 2.1 Hz, H-6), 3.93 (3H, *s*, 4′-OMe), 3.88 (3H, *s*, 7-OMe), 3.87 (3H, *s*, 3′-OMe); $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ 184.25 (*s*, C-4), 166.18 (*s*, C-2), 164.30 (*s*, C-3′), 164.29 (*s*, C-7), 163.83 (*s*, C-5), 162.67 (*s*, C-4′), 152.08 (*s*, C-9), 129.50 (*d*, C-6′), 123.34 (*s*, C-1′), 117.08 (*d*, C-5′), 105.20 (*s*, C-10), 103.89 (*s*, C-3), 103.82 (*d*, C-2′), 100.16 (*d*, C-6), 95.78 (*d*, C-8), 62.00 (*q*, 4′-OMe), 61.00 (*q*, 3′-OMe), 56.13 (*q*, 7-OMe).

**Apigenin (4)**: $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ 7.83 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.92 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.83 (1H, *d*, *J* = 2.1 Hz, H-6), 6.71 (1H, *d*, *J* = 2.1 Hz, H-8), 6.58 (1H, *s*, H-3); $^{13}$C NMR ($CD_3OD$, 125 MHz): $\delta$ 180.45 (*s*, C-4), 164.94 (*s*, C-5), 164.41 (*s*, C-2), 162.60 (*s*, C-4′), 160.72 (*s*, C-9), 160.16 (*s*, C-7), 129.30 (*d*, C-2′ and C-6′), 123.14 (*s*, C-1′), 117.06 (*d*, C-3′ and C-5′), 109.39 (*s*, C-10), 106.57 (*d*, C-3), 104.83 (*d*, C-6), 99.34 (*d*, C-8).

**Apigenin-7-*O*-$\beta$-D-glucopyranoside (5)**: $^1$H NMR ($CD_3OD$, 500 MHz): aglycon moiety: $\delta$ 7.83 (2H, *d*, *J* = 8.8 Hz, H-2′ and H-6′), 6.92 (2H, *d*, *J* = 8.8 Hz, H-3′ and H-5′), 6.83 (1H, *d*, *J* = 2.1 Hz, H-6), 6.71 (1H, *d*, *J* = 2.1 Hz, H-8), 6.58 (1H, *s*, H-3), glucose moiety: $\delta$ 4.90 (1H, *d*, *J* = 7.6 Hz, H-1″), 3.50-3.25 (4H, *m*, H-2″,3″,4″,5″), 3.87 (1H, *dd*, *J* = 11.9 and 2.2 Hz, H-$6''_b$), 3.73 (1H, *dd*, *J* = 11.9 and 5.4 Hz, H-$6''_a$); $^{13}$C NMR ($CD_3OD$, 125 MHz): aglycon moiety: $\delta$ 180.45 (*s*, C-4), 166.25 (*s*, C-5), 164.41 (*s*, C-2), 162.60 (*s*, C-4′), 160.72 (*s*, C-9), 160.16 (*s*, C-7), 129.51 (*d*, C-2′ and C-6′), 123.32 (*s*, C-1′), 117.10 (*d*, C-3′ and C-5′), 109.39 (*s*, C-10), 106.57 (*s*, C-3), 104.83 (*d*, C-6), 99.34 (*d*, C-8); glucose moiety: $\delta$ 105.11 (*d*, C-1″), 78.66 (*d*, C-5″), 77.58 (*d*, C-3″), 74.83 (*d*, C-2″), 71.80 (*d*, C-4″), 62.61 (*t*, C-6″).

**Apigenin-7-*O*-$\beta$-D-glucopyranoside-4′-*O*-methylether (6)**: $^{1}$H NMR ($CD_3OD$, 500 MHz): aglycon moiety: $\delta$ 7.83 (2H, *d*, $J$ = 8.8 Hz, H-2′ and H-6′), 6.92 (2H, *d*, $J$ = 8.8 Hz, H-3′ and H-5′), 6.83 (1H, *d*, $J$ = 2.1 Hz, H-6), 6.71 (1H, *d*, $J$ = 2.1 Hz, H-8), 6.58 (1H, *s*, H-3); glucose moiety: $\delta$ 4.99 (1H, *d*, $J$ = 7.6 Hz, H-1″), 3.50-3.25 (4H, *m*, H-2″,3″,4″,5″), 3.85 (1H, *dd*, $J$ = 12.2 and 2.0 Hz, H-6$''_b$), 3.88 (3H, *s*, OMe), 3.73 (1H, *dd*, $J$ = 12.2 and 6.4 Hz, H-6$''_a$); $^{13}$C NMR ($CD_3OD$, 125 MHz): aglycon moiety: $\delta$ 180.45 (*s*, C-4), 166.25 (*s*, C-5), 164.41 (*s*, C-2), 162.60 (*s*, C-4′), 160.72 (*s*, C-9), 160.16 (*s*, C-7), 129.51 (*d*, C-2′ and C-6′), 123.32 (*s*, C-1′), 117.10 (*d*, C-3′ and C-5′), 109.39 (*s*, C-10), 106.57 (*s*, C-3), 104.83 (*d*, C-6), 99.34 (*d*, C-8), 56.54 (*q*, OMe); glucose moiety: $\delta$ 105.11 (*d*, C-1″), 78.49 (*d*, C-5″), 77.35 (*d*, C-3″), 74.78 (*d*, C-2″), 71.29 (*d*, C-4″), 62.32 (*t*, C-6″).

**Martynoside (7)**: $^{1}$H NMR ($CD_3OD$, 500 MHz) data superimposable with those reported in the literature[3].

**Verbascoside (= acteoside) (8)**: $^{1}$H NMR ($CD_3OD$, 500 MHz) data superimposable with those reported in the literature[4].

## Results and Discussion

A serial chromatographic separations of the water soluble part of the methanolic extract obtained from the over-ground parts of *S. pontica*, on polyamide followed by RP-VLC, MPLC, silica gel and Sephadex CC resulted in the isolation of compounds **1-8** (Fig. 1).

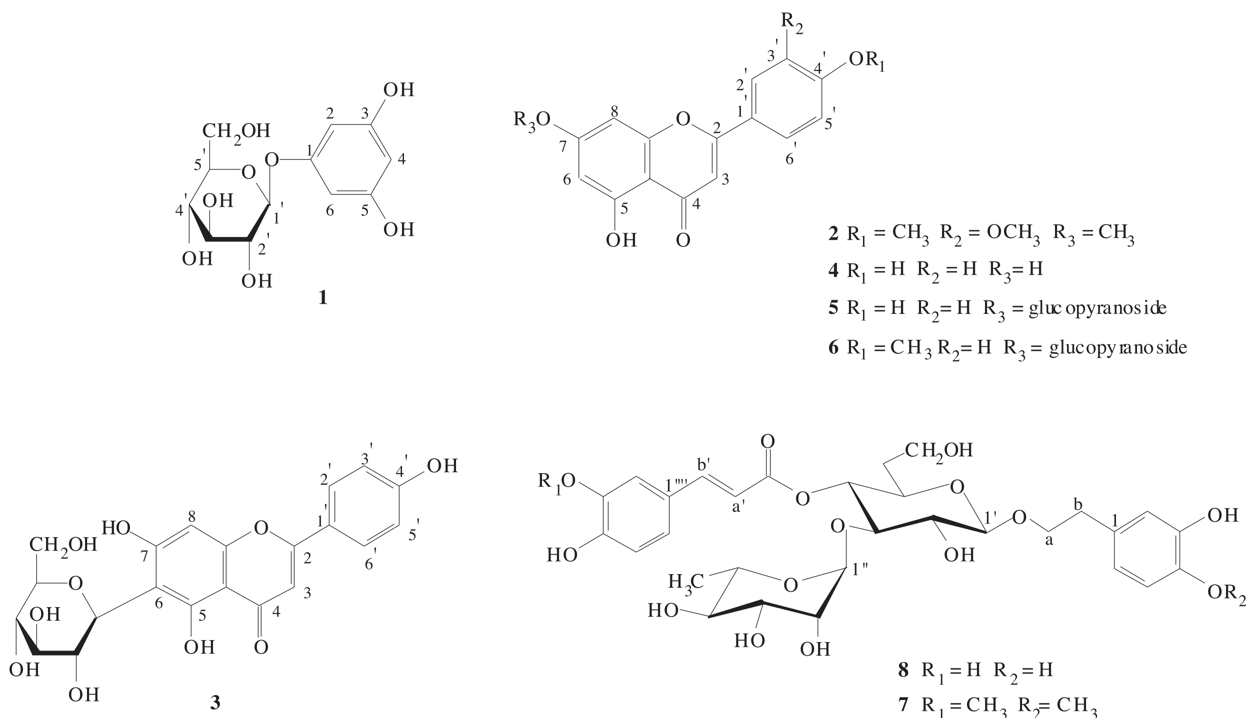


**Figure 1.** Phenolic compounds isolated from *S. pontica*

Compound **1** was isolated as an amorphous powder. The molecular formula of **1** was determined to be $C_{12}H_{16}O_8$ on the basis of the FAB-MS ($m/z$ 289 $[M+H]^+$) and $^{13}$C NMR data. The $^{1}$H NMR spectrum of **1** displayed the signals arising from aromatic ($\delta_H$ 6.06, 2H, *d*, $J$ = 2.1 Hz; 5.99, 1H, *t*, $J$= 2.1 Hz) and

sugar moieties. The anomeric proton signal at $\delta_H$ 4.89 (1H, *d*, $J = 7.9$ Hz) was consistent with the presence of a $\beta$-D-glucopyranose unit in **1**. In the $^{13}C$ NMR spectrum of compound **1**, ten carbon resonances, two of which were in double integrities and six of which could be assigned to a $\beta$-D-glucopyranosyl moiety, were observed. The complete analysis of the NMR data was based on the results of $^{1}H$-$^{1}H$ COSY, $^{1}H$-$^{13}C$ HMQC and $^{1}H$-$^{13}C$ HMBC (Fig. 2) experiments. In the $^{1}H$ NMR spectrum of **1**, the 2H doublet resonance at $\delta_H$ 6.06 ($J = 2.1$ Hz), which correlated with the carbon resonance at $\delta_C$ 97.01 (*d*) in the HMQC spectrum, was attributed to H-2 and H-6 of the phenyl moiety. Likewise, the triplet resonance at $\delta_H$ 5.99 ($J = 2.1$ Hz) showing an HMQC correlation to the carbon signal at $\delta_C$ 98.37 (*d*), was assigned to H-4 of the phenyl moiety. $^{1}H$-$^{13}C$ HMBC cross-peaks observed between H-6/C-2, H-6/C-4, H-2/C-4, H-2/C-6, H-4/C-2 and H-4/C-6 confirmed the proposed structure. The $^{13}C$ NMR resonance at $\delta_C$ 159.41 (*s*) was assigned to the C-1 atom of the aglycon on the basis of the $^{1}H$-$^{13}C$ long-range correlations observed from H-2 and H-6 to C-1. The carbon resonance at $\delta_C$ 158.47 (*s*) was shown in double integrity and an HMBC cross-peak observed from H-4 to this carbon resonance indicated that this carbon resonance was assignable to the C-3 and C-5 atoms of the aromatic moiety. Furthermore, the chemical shift values of C-3 and C-5 required the tertiary hydroxyl functions to be present at these positions. The site of glucosidation was established to be the C-1 position of the aglycon due to the hetero-nuclear long-range coupling between the anomeric proton ($\delta_H$ 4.89) of the glucose unit and the C-1 ($\delta_C$ 159.41) atom of the aglycon. Based on the above-mentioned NMR data, the structure of **1** was established as 3,5-dihydroxyphenyl $\beta$-D-glucopyranoside.

Compound **2** was isolated as a yellow amorphous powder. The FAB mass spectrum of **2** in positive ion mode gave a quasi-molecular ion peak $[M+H]^+$ at $m/z$ 433, compatible with the molecular formula $C_{21}H_{20}O_{10}$. Its UV absorptions in MeOH and the shifts observed after the addition of shift reagents were consistent with the presence of a 5,7,4′-trihydroxyflavone structure in **2**[5,6]. The $^{1}H$ and $^{13}C$ NMR spectra of compound **2** exhibited resonances due to aromatic systems and a $\beta$-D-glucopyranose moiety. The $^{13}C$ NMR signals of **2** were assigned with the help of an HMQC experiment. The connectivities of the molecular fragments were established by a hetero-nuclear multiple-bond correlation experiment (HMBC) (Fig. 2). The singlet $^{1}H$ NMR resonance at $\delta_H$ 6.50 was assigned to the H-8 proton of the A-ring, due to the long-range correlations observed from H-8 to C-10 ($\delta_C$ 104.98), C-7 ($\delta_C$ 165.33) and C-9 ($\delta_C$ 158.79). Two doublets at $\delta_H$ 7.84 and 6.92 (each 2H, $J = 8.8$ Hz) were characteristic of the H-2′/6′ and H-3′/5′ protons, respectively, of the *para*-substituted B-ring. A singlet proton resonance at $\delta_H$ 6.60, which correlated to the carbon resonance at $\delta_C$ 103.87 (*d*) in the HMQC spectrum, was assigned to H-3 of the aglycon. HMBC correlations observed from H-3 to C-2 ($\delta_C$ 166.21), C-10 ($\delta_C$ 104.98) and C-1′ ($\delta_C$ 123.14) supported this assumption. On the other hand, both the chemical shift value of the anomeric carbon atom ($\delta_C$ 75.31) and the coupling constant value of H-1′ ($J = 10.1$ Hz) indicated that the linkage of the glucose was through a *C*-bond[5,7]. The information concerning the linkage of the sugar moiety was obtained from the HMBC spectrum. A prominent long-range correlation between the anomeric proton ($\delta_H$ 4.89) of the glucose unit and C-6 ($\delta_C$109.29) of the aglycon showed the attachment of the sugar moiety at the C-6 position. Further proof for this assignment came from the HMBC cross-peaks observed from H-1′ to C-5 ($\delta_C$ 162.04) and C-7 ($\delta_C$ 165.33). Based on the NMR data and comparison of the data given in the literature, the structure of compound **2** was identified as isovitexin[5−8].

Compound **3** was isolated as a yellowish amorphous powder. The FAB-MS ($m/z$ 329 $[M+H]^+$) and $^{13}C$ NMR data indicated the molecular formula of **3** to be $C_{18}H_{16}O_6$. The UV spectrum in MeOH and shifts

observed with the diagnostic reagents[5,6] showed the presence of a 5,7,3′,4′-tetrasubstituted flavone structure. The complete assignment of the $^{1}$H and $^{13}$C NMR data was based on the 2D NMR ($^{1}$H-$^{1}$H-COSY, $^{1}$H-$^{13}$C-HMQC and HMBC) experiments. In the $^{1}$H NMR spectrum, the doublet signals at $\delta_H$ 6.21 ($J$ = 2.1 Hz) and 6.45 ($J$ = 2.1 Hz), which correlated to the carbons at $\delta_C$ 100.16 (*d*) and 95.78 (*d*), respectively, in the HMQC spectrum, were characteristic of the two *meta*-related 6- and 8- protons with a 5,7-disubstituted A-ring of a flavonoid. The double-doublets at $\delta_H$ 7.84 ($J$ = 8.8 and 4.0 Hz) and 6.93 ($J$ = 8.8 and 2.4 Hz), which were coupled in the $^{1}$H-$^{1}$H COSY spectrum, were readily attributed to H-6′ and H-5′, respectively, of the B-ring. Therefore, the $^{1}$H NMR signal at $\delta_H$ 6.58 (*dd*, $J$ = 4.0 and 2.4 Hz) was assigned to H-2′. The singlet at $\delta_H$ 6.62 was attributed to H-3 on the basis of the HMBC correlations from H-3 to C-2 ($\delta_C$ 166.18) and C-10 ($\delta_C$ 105.20) (Fig. 2). Additionally, the $^{1}$H NMR spectrum of compound **3** exhibited three singlets at $\delta_H$ 3.93 (3H), 3.88 (3H) and 3.87 (3H), showing the existance of three methoxyl groups. However, in the $^{13}$C NMR spectrum, the signals of two methoxyl groups appeared at downfield ($\delta_C$ 62.00 and 61.00), indicating that these methoxyl groups were di-*ortho* substituted[7,9]. The position of the methoxyl groups were determined by the $^{1}$H-$^{13}$C HMBC experiment as follows. The signal at $\delta_H$ 3.88 correlated with the carbon signal at $\delta_C$ 164.29 (C-7), whereas the proton resonances at $\delta_H$ 3.87 and 3.93 showed hetero-nuclear long-range correlations with the carbon signals at $\delta_C$ 164.30 (C-3′) and 162.67 (C-4′), respectively. The structure of **3** was, therefore, determined to be 5-hydroxy-7,3′,4′-trimethoxyflavone.

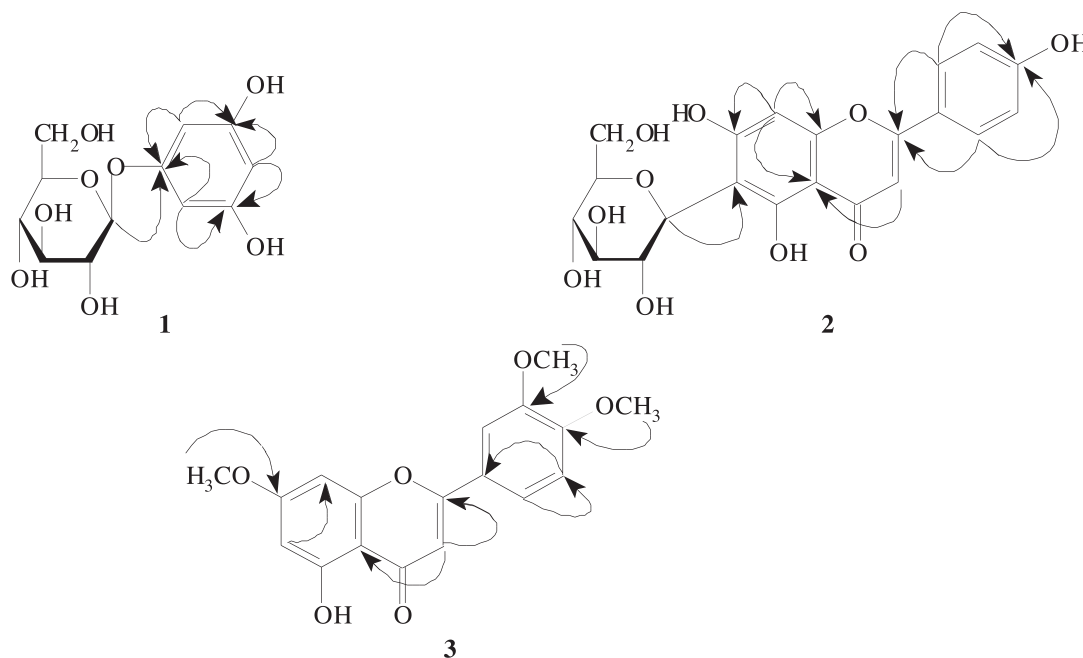


**Figure 2.** Selected HMBC correlations for **1**, **2** and **3**

Compounds **4-6** were obtained as a mixture. Although most NMR resonances were in double or triple integrities. the chemical shifts in the $^{1}$H and $^{13}$C NMR spectra of **4-6** could be assigned from 2D ($^{1}$H-$^{1}$H COSY, $^{1}$H-$^{13}$C HMQC and HMBC) experiments. The NMR data revealed that **4-6** were all flavonoid compounds.

In the $^{1}$H NMR spectrum of **4**, the aromatic proton signals of two *m*-coupled doublets at $\delta_H$ 6.83 and 6.71 (each,$J$ = 2.1 Hz) showing HMQC correlations to the carbon resonances at $\delta_C$ 104.83 (*d*) and 99.34 (*d*),

respectively, were attributed to the H-6 and H-8 of the A-ring. Two vicinally coupled doublets at $\delta_H$ 7.83 and 6.92 (each, 2H, $J$ = 8.8 Hz) showed long-range couplings with the $^{13}$C NMR signal at $\delta_C$ 162.60 (C-4′) and, therefore, were assigned to H-2′/6′ and H-3′/5′, respectively, of the B-ring. Additionally, a singlet at $\delta_H$ 6.58 was ascribed to H-3. The assignment of H-3 was confirmed by its hetero-nuclear long-range correlations to C-2 ($\delta_C$ 164.41) and C-1′ ($\delta_C$ 123.14). The $^{13}$C NMR resonance at $\delta_C$ 160.16, which showed HMBC correlations with H-6 and H-8, was attributed to C-7. Based on the above-mentioned data and comparison of $^1$H and $^{13}$C NMR data with those given in the literature, the structure of compound **4** was identified as apigenin[5−7].

The $^1$H and $^{13}$C NMR spectra of compound **5** were almost identical to those of **4**. However, additional resonances arising from a $\beta$-D-glucopyranose unit ($\delta_H$ 4.90, 1H, *d*, $J$ = 7.6 Hz, H-1″; $\delta_C$ 105.11, *s*, C-1″) were observed in the NMR data of **5**. An HMBC correlation observed from H-1″ to C-7 ($\delta_C$ 160.16) of the A-ring showed the attachment of the glucose unit at C-7. Since the chemical shift value of C-1″ and the coupling constant of H-1″ were suggestive of a *O*-glucosidation[5−7], the structure of **5** was established as apigenin-7-*O*-$\beta$-D-glucopyranoside.

Likewise, the proton and carbon chemical shifts due to the aglycon and sugar moieties of **6** were in good agreement with those of **5**, indicating a similar substructure and glucosidation pattern. In addition, the $^1$H NMR spectrum of **6** displayed a methoxyl singlet at $\delta_H$ 3.88, which correlated to the quaternary carbon resonance at $\delta_C$ 162.60 (C-4′) in the HMBC spectrum. Based on this evidence, the methoxyl group was assumed to be located at C-4′ of the B-ring. Thus, the structure of **6** was identified as apigenin-7-*O*-$\beta$-D-glucopyranoside-4′-*O*-methylether[5−7].

The structures of the phenylethanoid glycosides were identified as martynoside[3] (**7**) and verbascoside (= acteoside)[4] (**8**) on the basis of comparison of their $^1$H NMR data with those given in the literature.

## Conclusion

In this study, eight phenolic compounds (**1**-**8**) were isolated and identified from the over-ground parts of *S. pontica*. To the best of our knowledge, this is the first report on the isolation of 3,5-dihydroxyphenyl $\beta$-D-glucopyranoside (**1**) from the genus *Scutellaria*. To date, the isolation of *C*-glucosylflavones from *Scutellaria* species are limited in number and only two di-*C*-glycosylflavones, chrysin-6-*C*-$\beta$-D-glucoside-8-*C*-$\alpha$-L-arabinoside and chrysin-6-*C*-$\alpha$-L-arabinoside-8-*C*-$\beta$-D-glucoside, were reported from *S. baicalensis*[10]. Therefore, the isolation of isovitexin (**2**) from *S. pontica* is the second report of the isolation of a *C*-glucosylflavone from *Scutellaria* species. In addition, this is the first case of the isolation of 5-hydroxy-7,3′,4′-trimethoxyflavone (**3**), apigenin (**4**), apigenin-7-*O*-$\beta$-D-glucopyranoside (**5**) and apigenin-7-*O*-$\beta$-D-glucopyranoside-4′-*O*-methylether (**6**) from *S. pontica*.

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