

ISOVITEXIN O-GLUCOSIDES FROM *AQUILEGIA VULGARIS* L.

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Abstract: 4'-O- β -glucopyranoside of isovitexin from the leaves with stems and 4'-O- δ -glucopyranoside of 2''-O- β -glucopyranosylisovitexin from the flowers of *Aquilegia vulgaris* L. were isolated. The structures of the compounds were determined by chemical and spectroscopic methods.

Keywords: *Aquilegia vulgaris* L.; Ranunculaceae; 4'-O-glucosyl-6-C-glucosylapigenin; 4'-2''-di-O-glucosyl-6-C-glucosylapigenin, isolation, identification.

Aquilegia vulgaris L. has been used in folk medicine against liver disorders in the therapy of jaundice especially. An ethanolic extract from the leaves with stems of columbine possesses of hepatoprotective activity (1). This extract, contains flavonoids (a principal flavonoid component – isocytiside in concentration 3,0%) and other phenolic compounds. Test on *A. vulgaris* L., that were carried out in the Department of Pharmacognosy, resulted in identification of flavonoid C- and O-glycosides. During these works, it occurred that the leaves and stems of columbine contain different flavonoids than flowers. The main compound of flavonoids in leaves and stems was apigenin 4'-methyl ether 6-C-glucoside (isocytiside), its 7-O-glucoside and X''-O-glucoside as well as isoorientin, orientin, apigenin 7-O-rutinoside, apigenin 7-O-glucoside and apigenin. The flowers contain mainly: kaempferol 3,7-O-diglucoside, kaempferol 3,7,4'-O-triglucoside, and kaempferol 4',7-O-diglucoside, kaempferol 3-O-glucoside, kaempferol 7-O-glucoside also apigenin 6-C-glucoside (isovitexin) (2, 3, 4). The aim of the present study was the isolation and identification of next two C-, O-glucosylflavones from *A. vulgaris* L..

EXPERIMENTAL

Plant material, extraction and isolation

Plant material was described in previous works (1997, 1999). The dried plant material (100 g of leaves with stems and 50 g of flowers) were extracted with hot MeOH. The extracts were concentrated with hot water and filtered. The clear brown filtrate was successively extracted with CHCl₃, EtOAc and EtOAc-MeOH (9:1). The water layer was subjected to CC polyamide (Woelm)

with H₂O and H₂O-MeOH gradient. Fractions were further separated by preparative paper chromatography on Whatman No. 3 (HOAc-H₂O 15:85). Final purification of compounds was executed on a Sephadex LH-20 (Pharmacia) column in MeOH as solvent. 70 mg of amorphous pale yellow powder **X** from the leaves and stems and 43 mg of amorphous yellow powder **VI** from the flowers of columbine were obtained.

Identification

NMR spectra were recorded on Varian – 300 spectrometer operating at 300 and 75 MHz for proton and carbon, respectively. TMS as int. standard, chemical shifts (ppm) and *J* (Hz).

PC was carried out on Whatman No. 1 paper using solvent systems: (a) HOAc – H₂O (15:85); (b) EtOAc – HCOOH – H₂O (10:2:3 upper layer). Chromatograms were observed in UV light ($\lambda=366$ nm) before and after spraying with 1% AlCl₃ in MeOH, 0.5% β -aminoethyl ester of diphenylboric acid (Naturstoffreagenz A) in MeOH. PPC was performed on Whatman No. 3 paper using (a) as a solvent system. TLC cellulose in (c) *n*-PrOH – EtOAc – H₂O (7:2:1), aniline phthalate for sugar analysis.

Hydrolysis procedure: 1 mg of compounds **VI** and **X** were heated with 1M HCl at 100°C for 4 h. The hydrolysate were extracted with EtOAc. The water phase was controlled for sugars (TLC, c) and organic phase analyzed for flavonoids (PC a, c; PPC a). Enzymatic hydrolysis: 1 mg of **VI** and **X** were incubated at 40° with β -glucosidase (Koch-Light) for 24 h.

4'-O- β -glucopyranosyl-2''-O- β -glucopyranosylisovitexin **VI**.

PC R_f=0.72 (a); 0.19 (b)

UV $_{\lambda_{max}}$ nm: MeOH 273, 322; +NaOAc 282, 340;

+NaOAc/H₃BO₃ 278, 320; +NaOMe 273, 322 (with decrease in intensity); +AlCl₃ 281, 360, 380; +AlCl₃/HCl 281, 330, 380.

¹H NMR (DMSO - d₆) δ: 8.06 (*d*, *J*=9.0 Hz, H-2', 6'); 7.20 (*d*, *J*=9.2 Hz, H-3', 5'); 6.90 (*s*, H-8); 6.56 (*s*, H-3); 5.03 (*d*, *J*=6.81 Hz, H-1'''); 4.60 (*d*, *J*=8.7 Hz, H-1''); 4.34 (*d*, *J*=7.0 Hz, H-1'''''); 3.82-3.18 (*m*, sugar protons).

¹³C NMR (DMSO - d₆) δ: 181.9 (C-4); 163.3 (C-2); 162.9 (C-7); 160.7 (C-4'); 160.2 (C-5); 156.1 (C-9); 128.2 (C-2', 6'); 123.7 (C-1'); 116.5 (C-3', 5'); 108.8 (C-6); 104.2 (C-3); 104.0 (C-10); 94.8 (C-8); 6 - C - glc: 70.9 (C-1''); 82.7 (C-2''); 76.4 (C-3''); 69.7 (C-4''); 81.7 (C-5''); 60.9 (C-6''); 4' - O - glc: 99.7 (C-1'''); 73.1 (C-2'''); 76.8 (C-3'''); 69.0 (C-4'''); 77.0 (C-5'''); 61.2 (C-6'''); 2'' - O - glc: 97.4 (C-1'''''); 74.2 (C-2'''''); 75.7 (C-3'''''); 68.7 (C-4'''''); 76.8 (C-5'''''); 62.0 (C-6''''');

4'-O-β-glucopyranosylisovitexin **X**.

PC R_f=0.70 (a); 0.46 (b)

UV_{λmax} nm: MeOH 272, 338; +NaOAc 283, 371; +NaOAc/H₃BO₃ 279, 342; +NaOMe 285, 380 (with decrease in intensity); +AlCl₃ 287, 348, 385; +AlCl₃/HCl 287, 345, 385

¹H NMR (DMSO - d₆) δ: 8.06 (*d*, *J*=9.0 Hz, H-2', 6'); 7.20 (*d*, *J*=8.9 Hz, H-3', 5'); 6.89 (*s*, H-8); 6.57 (*s*, H-3); 5.03 (*d*, *J*=6.9 Hz, H-1'''); 4.59 (*d*, *J*=9.89, H-1''); 3.80-3.17 (*m*, sugar protons).

¹³C NMR (DMSO - d₆) δ: 181.9 (C-4); 163.3 (C-2); 162.8 (C-7); 160.5 (C-4'); 160.1 (C-5); 156.1 (C-9); 128.0 (C-2', 6'); 123.7 (C-1'); 116.5 (C-3', 5'); 108.8 (C-6); 103.6 (C-3); 103.4 (C-10); 93.6 (C-8); 6 - C - glc: 72.9 (C-1''); 70.5 (C-2''); 78.8 (C-3''); 70.0 (C-4''); 81.4 (C-5''); 61.5 (C-6''); 4' - O - glc: 99.7 (C-1'''); 73.0 (C-2'''); 77.0 (C-3'''); 69.5 (C-4'''); 77.0 (C-5'''); 60.5 (C-6''').

RESULTS AND DISCUSSION

Methanol extracts were received from the leaves with stems and flowers of *A. vulgaris* L. which were then vaporized, precipitated using hot water and filtered. The filtrate was then successively extracted with CHCl₃, EtOAc and EtOAc-MeOH (9:1). The EtOAc and EtOAc-MeOH extracts contained flavonoids, which the course of separation and identification was described earlier (2, 3, 4). Chromatographic separation of the aqueous fraction resulted in obtaining two C-, O-glycosylflavones: **VI** from flowers and **X** from leaves with stems.

Compounds of **VII** and **X** were characterized by brown fluorescence in UV light (λ=366 nm)

which did not alter under NH₃ vapor, what indicated that C-5 contained free group of OH, and in C-4' substituted in both compounds (5).

Analysis of UV spectrum of methanol solutions of **VI** and **X** compounds and diagnostic shifts confirmed free groups OH in C-5, substituted in C-4' and indicated free group OH in C-7 position and lack of ortho-dihydroxyl group in ring B (5).

Acid hydrolysis **VI** and **X** yielded glucose (co-TLC, system a) and a mixture of two isomeric C-glycosylflavones: **A** and **B** which were Wessely Moser rearrangement products; (PC analysis, system a: R_f=0.48 **A**; 0.27 **B** and system b: R_f=0.62 **A**; 0.44 **B**). The compounds **A** and **B** were separated by PC on Whatman No. 3 in system a. The compounds were eluted with MeOH, subjected to co-chromatography (R_f values were in agreement with isovitexin and vitexin) and UV analysis. Bathochromic shift of **A** and **B**, compounds after addition of NaOMe between 55-65 nm together with the increase of intensity, indicated that in C-4' there is also OH group, liberated during hydrolysis. During the above analysis it was discovered, that **VI** and **X** compounds are substituted by glucose in the position C-4' which is connected with O-glycosidic linkage. Both compounds hydrolysed under the influence of β-glucosidase what indicated β-configuration of O-glucosides linkages.

More information was found following analysis of ¹H and ¹³C NMR spectrum. In the ¹H NMR spectrum of **VI** and **X** the doublets were observed at δ=8.06 ppm with coupling constants *J*=9.0 Hz and the doublets at δ=7.20 ppm (*J*=8.9-9.2 Hz), corresponded to the H-2', 6' protons and the H-3', 5' protons, respectively. Signals at δ 6.90 ppm (**VI**) and δ 6.89 (**X**) corresponded to the protons H-8, a singlets at δ 6.56 (**VI**) and δ 6.57 (**X**) corresponded to the protons H-3. Lack of signals for H-6 in the spectrum of both compounds indicated substitution in this position. In the range of δ=4.34-5.03 ppm three signals for **VI** and two signals for **X** were attributed to the anomeric protons of sugars. The coupling constants of *J*=6.81-9.89 Hz indicated the β-configuration of the glycosidic linkages.

The position of attachment of sugars was deduced from the ¹³C NMR spectral data.

C-6 glucosylation in **VI** and **X** resulted from downfield shift of C-6 signals do δ=108.8 ppm (Δδ=10 ppm); this signal in unsubstituted apigenin appears at 98.8 ppm (6, 7).

A downfield shift of C-1' signal (δ=123.7 ppm) and an upfield shift of the C-4' signal (δ=160.7 ppm in **VI** and 160.5 ppm in **X**) in

comparison with isovitexin (C-1', $\delta=121.04$; C-4', $\delta=160.94$) showed that the two glucose moieties were linked to C-4' by the *O*-glycosidic linkage in both compounds (6, 7). The C-2'' signal of 6-C-glucose in ^{13}C NMR spectrum VI was shifted downfield by 12.3 ppm in comparison with isovitexin and appeared at $\delta=82.7$ ppm, while the adjacent anomeric carbon underwent an upfield shift of 2.0 ppm at $\delta=70.9$ ppm therefore, the linkage of the third glucose molecule in VI proved to be at the C-2''. Hence, the structure of VI, obtained from the flowers was determined to be 4'-*O*- β -glucopyranosyl-2''-*O*-glucopyranosylisovitexin and compounds X separated from leaves and stems proved to be 4'-*O*- β -glucopyranosylisovitexin.

The compounds VI and X do not occur often in the plant kingdom. Isovitexin 4'-*O*-glucoside was separated from few plants genus *Gentiana* (sp. Gentianaceae) (8), from genus *Briza* (Graminae) (9), *Cerastium arvense* (Caryophyllaceae) (10); however 4'-*O*-glucoside of -2''-*O*-glucosylisovitexin for the first time was separated from *Gentiana asclepiadea* (11).

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Received: 26.02.2001