PHENYLETHANOID AND IRIDOID GLYCOSIDES FROM *VERONICA CHAMAEDRYS L.*

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Abstract. Three phenylethanoid glycosides (1, 2, 3) and one iridoid glycoside (4) were isolated from aerial parts of *Veronica chamaedrys* L. (Scrophulariaceae) for the first time. On the basis of spectral analysis, the structures of these compounds were determined to be acteoside (3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside) (1), ehrenoside (3,4-dihydroxy-β-phenylethoxy-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside) (2), chamaedroside (3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-α-L-arabinopyranoside) (3) and aucuboside (4).

Keywords: *Veronica chamaedrys L.*, Scrophulariaceae, phenylethanoid glycoside, iridoid glycoside.

Introduction

The genus Veronica (Scrophulariaceae), which is widely distributed in Europe and Asia, especially in the Mediterranean area, is represented by 32 species in Republic of Moldova [1, 2]. Iridoid glycosides, phenylethanoid and flavonoid glycosides have been mainly reported from different Veronica species [3 – 9]. Several Veronica species are used for the treatment of cancer, influenza, hemoptysis, laryngopharyngitis, hernia, and against cough, respiratory diseases plus as an expectorant and antiscorbutic in different countries [10 – 12].

This fact and our interest in secondary metabolites of Scrophulariaceae family prompted us to perform the phytochemical investigation of the aerial parts of *Veronica chamaedrys L.* Our investigation yielded three phenylethanoid glycosides **(1, 2, 3)** and one iridoid glycoside (**4) (**fig. 1).

Results and Discussion

Compound **1** was isolated as an amorphous powder with negative optical rotation ($[\alpha]^{23}_{D}$ −81.9°, c=1.67, MeOH), whose UV spectra showed λ_{max} at 342, 321 and 304 nm indicating its polyphenolic nature. The molecular formula $C_{29}H_{36}O_{15}$ was determined by the $[M]^+$ ion peak at m/z 624 in the negative high resolution (HR) – FAB – MS.

Its structure was determined by corresponding shifts of ^{1}H and ^{13}C NMR spectral data.

The ^{13}C-NMR spectrum of **1** showed eight carbon signals of aglycone moiety, six of which were assignable to the aromatic system – benzenic ring with two hydroxy groups attached at C3 (δ 146.16 ppm) and C4 (δ 144.78 ppm). The ^{13}C-NMR spectrum of **1** showed the presence of residue of (E)-caffeic acid at δ 114.98 ppm (α'), δ 147.56 ppm (β) and carbonyl group δ 168.56 ppm. Furthermore, the caffeoyl group was positioned at C4' of the glucose on the basis of strong deshielding on the H4' proton of the inner glucose unit δ 4.92 ppm (J=9.3 Hz).

Additionally, two anomeric carbons were observed (δ C1' 103.28 and δ C1'' 103.26) indicating its biglycosidic structure containing D-glucopyranose and L-rhamnopyranose as sugar units. This biglycosidic structure was also confirmed by the ^{1}H-NMR spectrum of **1** where two signals of anomeric protons were observed at δ 4.48 (d, J=7.6 Hz) indicating β-linked D-glucopyranose and δ 5.12 (J=1.6 Hz) as α-D-rhamnopyranose.

The ^{1}H- and ^{13}C-NMR spectra of **1** observed signal of methyl group at δ 1.08 ppm and δ 18.44 ppm, respectively, belonging to ramnopyranose. Ramnopyranose residue was attached to C3' of the inner glucose on the basis of the downfield shielding of 5 ppm (δ 86.71) comparing with shift of terminal glucose. Also acyl moiety was attached to inner glucose and showed eight carbon signals, six of which revealed the presence of para-disubstituted aromatic ring, similar to that of aglycone moiety (Table 1).

From the above results, compound **1** was determined as 3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside. The structure of compound **1** was also confirmed by comparison of the data published for phenylethanoides, previously isolated from genus *Veronica L.*, and correspond to acteoside [13,14].

Compound **2** was obtained as an amorphous powder with negative optical rotation ($[\alpha]^{23}_{D}$ −58°, MeOH) and assigned the molecular formula $C_{34}H_{44}O_{19}$ by (HR) – FAB – MS (from ion peak $[M]^+$ m/z 756). The ^{1}H- and ^{13}C-NMR spectra of **2** resembled those of **1** (Table 1). The ^{13}C-NMR spectrum of **2**, however, showed additionally five signals of a characteristic terminal arabinose moiety. In the ^{1}H-NMR spectrum, the coupling constant of the anomeric proton of the arabinose moiety was J=6.7 Hz (δ 4.54, d) showing the α-configuration of this sugar. These data suggested that compound

2 had three sugar units: one β-D-glucopyranose, one α-L-rhamnopyranose and one α-L-arabinopyranose. The complete assignment of all proton and carbon resonances was based on the results of 1H-1H shift correlation spectroscopy (1H-1H COSY), 1H-13C-heteronuclear multiple quantum coherence (1H-13C-HMQC) and HMBC experiments.

From the above data, the structure of **2** was determined to be 3,4-dihydroxy-β-phenylethoxy-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside, a known compound identical to ehrenoside isolated previously from the genus *Veronica L.* [15].

Compound **3** was isolated as an amorphous powder with negative optical rotation ($[\alpha]^{22}_{D}$ −19.6°, MeOH). The molecular formula of compound **3** was determined as $C_{28}H_{34}O_{14}$ by negative high-resolution (HR) – FAB – MS. The ^{1}H- and ^{13}C-NMR spectra (Table 1) revealed the presence of aromatic system confirmed by the AB-type aromatic protons (3,4-dihydroxyphenylethyl alchohol δ 6.48, 6.63, 6.64) and two methylenes which were coupled with each other (α: δ_H 3.69, 4.05, δ_C 72.19; β: δ_H 2.79, δ_C 36.77). The signals assigned to the sugar moiety indicated the presence of an arabinose moiety. In the ^{13}C- and ^{1}H-NMR spectra, the anomeric signal was observed at δ_C 104.15 and δ_H 4.52 d (J=6.7 Hz), as an α-linked L-arabinose attached to the aglycone moiety and in C4 the residue of (E)-caffeic acid. All protons in the arabinose unit were assigned unambiguously from the correlation spectroscopy (COSY) spectrum and a heteronuclear multiple quantum coherence (HMQC) experiment correlated all proton resonances with those of the corresponding carbons in the sugar unit. The downfield signals at δ_H 4.01, belonging to H4 of arabinose suggest that the acyl moiety was attached to C4 of arabinose. The signal at δ_C 74.35, arising from C4 of the arabinose moiety (downfield shift +5ppm – glycoside effect) showed that the acyl moiety was indeed attached to C4.

Therefore, the structure of **3** was identified as 3,4-dihydroxy-β-phenylethoxy-O-[α-L-rhamnopyranosyl-(1→3)]-(4-O-caffeoyl)-α-L-arabinopyranoside, for which we proposed the name chamaedroside. Data about this compound are absent in literature.

On the basis of spectral data and phytochemical constants, compound **4** was isolated and its structure was identified as aucuboside by the comparison of its spectral data with those reported in the literature [16].

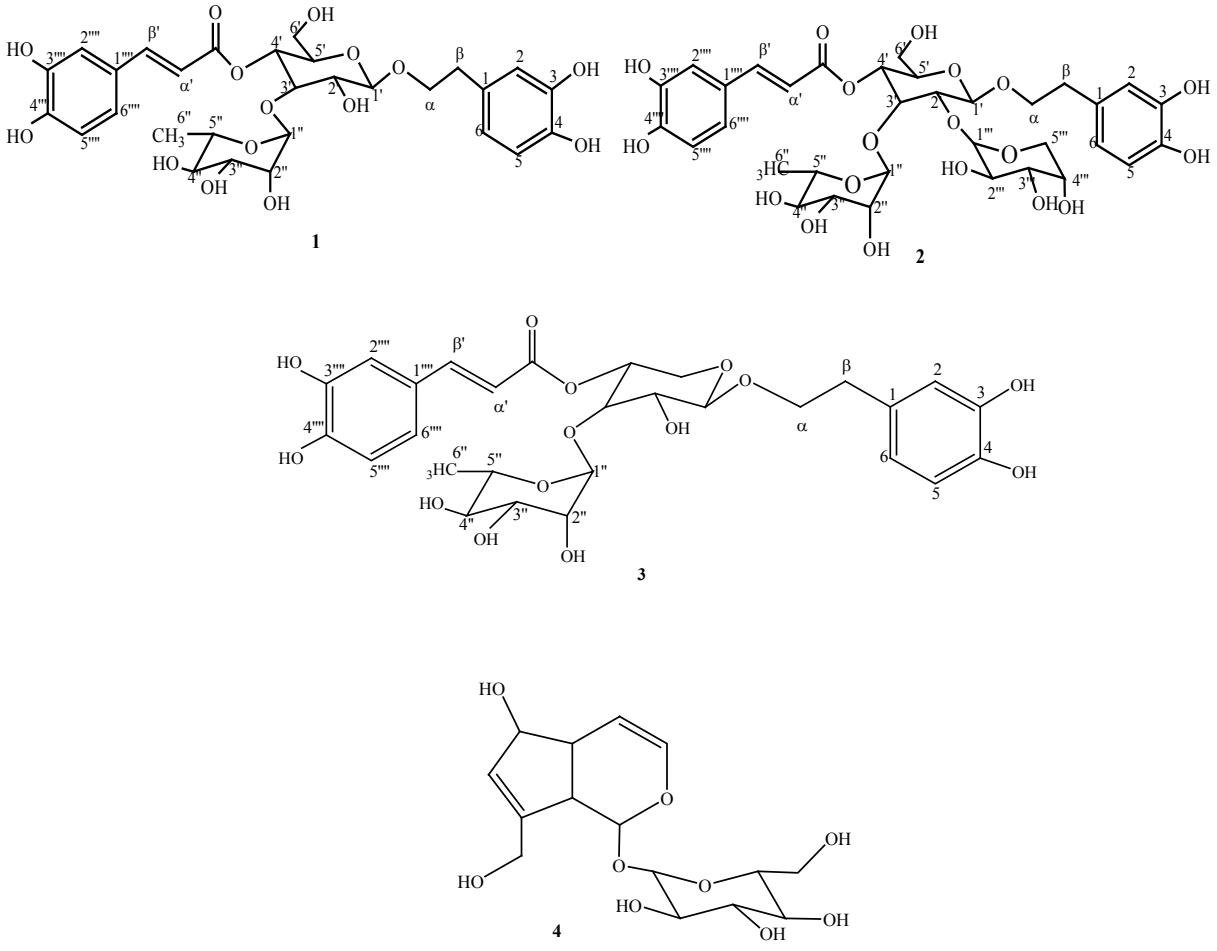

Fig. 1. Structures of isolated compounds

Table 1

^{13}C- and ^{1}H- NMR spectral data (300 MHz , CD_3OD, ppm, J in Hz) of glycosides (1-3)

	1		2		3	
Agl	C	H	C	H	C	H
1	131.71		131.92		131.87	
2	117.46	6.73 d(2.1)	117.53	6.72 d (2.1)	117.48	6.64 d (2.0)
3	146.16		146.14		146.18	
4	144.78		144.80		144.78	
5	116.37	6.67 d (7.8)	116.34	6.68 d (7.8)	116.38	6.63 d (8.0)
6	121.51	6.58 dd (8.0, 8.2)	121.53	6.59dd(7.9,2.1)	121.55	6.48 dd (2.8)
α	72.24	3.72 m	72.17	3.61 m	72.19	3.69 m
		4.07 m		4.05 m		4.05 m
β	36.71	2.79 t (7.2)	36.75	2.80 t (7.1)	36.77	2.79 m
Glc						
1’	103.28	4.48 d (7.6)	103.06	4.45 d (7.6)		
2’	73.86	3.18 t (8.9)	82.72	3.64 t (8.6)		
3’	86.71	4.11 t (9.1)	81.42	3.99 t (9.2)		
4’	70.84	4.92 t (9.3)	70.93	4.95 t (9.2)		
5’	75.79	3.56 m	75.94	3.54 t (9.3)		
6’	62.42	3.54 dd (12.0, 5.4)	62.51	3.56dd(12.1,6.0)		
		3.65 dd (12.1, 2.2)		3.61dd(12.1,2.0)		
Rha						
1’’	103.26	5.12 (1.6)	103.25	5.20 (1.5)	103.26	5.12 (1.6)
2’’	71.91	3.98 dd (3.1, 1.8)	72.16	4.01dd (3.3, 1.8)	71.91	3.98dd(3.1,1.8)
3’’	72.05	3.53 m	72.06	3.56 m	72.05	3.53 m
4’’	73.82	3.38 t (9.1)	73.86	3.31 t (9.5)	73.82	3.38 t (9.1)
5’’	70.66	3.52−3.54*	70.67	3.53−3.56*	70.66	3.52−3.54a)
6’’	18.44	1.08 d (6.4)	18.52	1.11 d (6.1)	18.44	1.08 d (6.4)
Ara						
1’’’			104.12	4.54 d (6.7)	104.15	4.52 d (6.7)
2’’’			73.03	3.61 t (9.4)	72.10	3.58 dd
3’’’			74.55	3.53−3.56*	79.68	3.96 s
4’’’			69.56	3.75 s	74.35	4.01 d
5’’’			66.86	3.24 d (12.5)	66.56	3.86dd(12.4,3.3)
				3.84dd(12.5,2.9)		3.53dd(12.4,1.9)
Acyl						
1’’’’	127.68		127.70		127.71	
2’’’’	115.26	7.08 d (1.8)	115.26	7.07 d (2.1)	115.26	7.08 d (1.8)
3’’’’	146.92		146.88		146.92	
4’’’’	149.74		149.84		149.74	
5’’’’	116.65	6.78 d (8.2)	116.85	6.78 d (8.2)	116.65	6.78 d (8.2)
6’’’’	123.16	6.98 dd (8.2, 1.6)	123.24	6.96 dd (8.2, 2.1)	123.16	6.98dd(8.2,1.6)
α’	114.98	6.31 d (15.6)	114.84	6.27 d (15.9)	114.98	6.31 d (15.6)
β’	147.56	7.56 d (16.1)	148.01	7.59 d (15.9)	147.56	7.56 d (16.1)
C=O	168.56		168.60		168.58	

*Signal patterns are unclear due to overlapping

Experimental

General experimental procedures

Melting points were measured on a Boetius table, and a specific rotation on a polarimeter on the firm Zeiss. UV spectra were recorded on UV HP 8472-A spectrometer (MeOH). NMR experiments were performed on a Bruker DRX-300 spectrometer (Bruker BioSpin GmBH, Rheinstetten, Germany) at 300 K dissolving all the samples in CD_3OD (Carlo Erba, 99.8 %). The standard pulse sequence and phase cycling were used for DQF-COSY, HSQC and HMBC spectra. The NMR data were processed using UXNMR software.

ESI-MS in the positive ion mode was performed using a Finnigan LCQ Deca ion trap instrument from Thermo Finnigan (San Jose, CA) equipped with Xcalibur software.

HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC_{18} column (300 x 7.8 mm i.d.) and a Rheodyne injector.

Column chromatography was performed over Silica gel (0.1-0.06 mm, Merck). TLC was performed on silica gel plates (Merck precoated silica gel 60 F_{254}). Solvent systems: (1) chloroform/methanol (4:1), (2) chloroform/methanol/water (65:35:3). All solvents for chromatographic separation were of analytical grade from Carlo Erba (Rodano, Italy). HPLC grade water (18 mΩ) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA).

Plant Material

The aerial parts of *Veronica chamaedrys L*. have been collected in the scientific research field of Institute of Genetics and Physiology of Plants, Academy of Sciences of Moldova in May 2006 year. The voucher specimen has been deposited by Doctor in Biology Florea V.N. in Laboratory of Natural Bioregulators under the direction of Doctor in Chemistry Kintea P. K.

Extraction and separation

Dry powdered aerial parts (600 g) were extracted three times in boiled n-buthanol saturated with water. The received n-butanol-water extract was evaporated and then purified with chloroform. After purification it was crystallised in acetone. The residue was dried in vacuum at 40°C and summary extract of glycosides has been obtained as yellow powder in 3.7 % yield. 3g of extract have been chromatographied on silica gel column (30-500mm, 60-100μm, Merck). The column was eluted with system chloroform-methanol-water (8:2:0→20:10:1) and 4ml fractions were collected. Fractions showing identical characteristics [TLC, silica gel, chloroform-methanol (4:1)] were combined. Two subfractions, (A) and (B) were obtained, which were further separated on a C_{18} column (7,8x300mm, LiChroprep RP18, 25-40μm, XTerra Waters) using a H_2O/MeOH (60-80% MeOH) isocratic. Four single compounds were isolated.

Acteoside (**1**): Amorphous powder, $[\alpha]^{23}_{D}$ −81.9° (c=1.67, MeOH). HRMS *m/z* 624.452 [calcd for $C_{29}H_{36}O_{15}$ $(M)^+$]; 488.26 $[M-136]^+$; 324.03 $[M-136-164]^+$; 179.06 $[M-136-164-145]^+$. ^{1}H NMR and ^{13}C NMR see Table 1.

Ehrenoside (**2**): Amorphous powder, $[\alpha]^{23}_{D}$ −58° (MeOH). HRMS, *m/z* 756.443 [calcd for $C_{34}H_{44}O_{19}$ $(M)^+$]; 620.34 $[M-136]^+$; 456.9 [M-136-164]. ^{1}H NMR and ^{13}C NMR see Table 1.

Chamaedroside (**3**): Amorphous powder, $[\alpha]^{22}_{D}$ −19.6° (MeOH). HRMS, *m/z* 595.29 [calcd for $C_{28}H_{34}O_{14}$ $(M)^+$]; 462.25 $[M-132]^+$; 316.15 [M-132-146]; 156.29 $[M-132-146-160]^+$. ^{1}H NMR and ^{13}C NMR see Table 1.

Conclusion

Four compounds **(1)**, **(2)**, **(3)** and **(4)** have been isolated from aerial parts of *Veronica chamaedrys L.* for the first time. The structures of **(1), (2)** and **(3)** were elucidated as acteoside (3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside), ehrenoside (3,4-dihydroxy-β-phenylethoxy-O-α-L-arabinopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-β-D-glucopyranoside), chamaedroside (3,4-dihydroxy-β-phenylethoxy-O-α-L-rhamnopyranosyl-(1→3)-4-O-caffeoyl-α-L-arabinopyranoside) and **(4)** aucuboside, respectively by means of physicochemical methods.

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