

STEROIDAL GLYCOSIDES FROM *VERONICA CHAMAEDRYS L.* PLANTS. THE STRUCTURES OF CHAMAEDROSIDES A, B, C AND E.

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Abstract: Four steroidal glycosides, named by us chamaedrosides, where two are new steroidal glycosides, have been isolated from the plants of *Veronica chamaedrys L.* (*Scrophulariaceae*) for the first time and their structures have been elucidated. Complete assignments of the ¹H and ¹³C NMR chemical shifts for these glycosides were achieved by means of one- and two-dimensional NMR techniques, including 1H-1H COSY, HSQC, HMBC and ROESY spectra.

Keywords: steroidal glycoside, NMR analysis, *Veronica chamaedrys L.*

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]. 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 [3–5]. As it was reported earlier, *Veronica* species contain steroidal glycosides, phenylethanoid and iridoid glycosides [6–13].

In the course of our phytochemical studies on the plant *Veronica chamaedrys L.* we had previously reported phenylethanoid and iridoid glycosides [14]. This paper describes the structural elucidation of the steroidal glycosides isolated from *Veronica chamaedrys L.* plants on the basis of extensive spectral analysis, including 2D NMR spectral data and chemical evidences.

Results and Discussion

The water extract of plants of *Veronica chamaedrys L.* was successively subjected to Sephadex gel filtration and silica gel column chromatography to afford four steroidal glycosides, named by us chamaedroside A (**1**), chamaedroside B (**2**), chamaedroside C (**3**) and chamaedroside E (**4**). All isolated compounds gave positive Sannie test [15], and compound 2 and 4 – positive Ehrlich test [16]. All compounds have been isolated as amorphous powders. Their structures were determined by corresponding shifts of ¹H and ¹³C NMR spectral data.

The ¹H NMR spectrum of **1** displayed signals due to four steroidal methyl groups at δ 0.89 (3H, s, H–18), 1.1 (3H, s, H–19), 1.5 (3H, d, J=6.7 Hz, H–21), 1.19 (3H, d, J=6.7 Hz, H–27), two methine proton signals at δ 4.30 (1H, m, H-3) and 4.98 (1H, m, H-16) indicative of secondary alcoholic functions, two methylene proton signals at δ 4.128 (1H, m, H-26b) and 3.48 (1H, m, H-26a), ascribable to a primary alcoholic function, and signals for the one anomeric at δ 4.98 (1H, d, J=7.07 Hz). ¹³C-NMR signals due to a total of 27 carbon signals originating from the sapogenol were composed of four methyl groups at δ 16.5, 16.3, 23.9 and 17.3, one oxygen bearing methine carbon at δ 77.6 (C–3), three quaternary carbons at δ 35.9 (C–10), 110.5 (C–22) and 40.5 (C–13), six methine carbons at δ 35.4 (C–8), 36.6 (C–5), 40.4 (C–9), 56.3 (C–14), 81.1 (C–16), 63.7 (C–17), eleven methylene carbons at δ 21.3 (C–11), 30.5 (C–2), 37.0 (C–1), 32.4 (C–7), 27.1 (C–6), 34.9 (C–4), 40.2 (C–12), 32.3 (C–15), 36.6 (C–23), 28.3 (C–24), 65.4 (C–26). On the basis of the HSQC and HMBC correlations, the aglycone moiety of chamaedroside A was identified as (25S)-5 β -spirostan-3 β -ol (sarsasapogenin) [17]. The 25S stereochemistry was inferred by the resonance of H-27 methyl protons at δ 1.19, a few bigger than 1.00, and also by the resonance difference between equatorial proton signal (3.48, dd, J=6.5, 9.5 Hz) and axial proton signal (4.128, m) of H-26: 0.648 ppm, a few bigger than 0.57 ppm [18]. 5 β configuration was deduced by HMBC correlation between the methyl signal at δ 1.1 (Me-19) and carbon resonances at δ 40.4 (C-9), 36.6 (C-5) and 37.0 (C-1) [18]. Using a combination of 1D-TOCSY and DQF-COSY spectral analysis, the sugar chain has been identified to be composed of one unit of β -D-glucopyranoside. A glycosidation shift was observed for C-3_{Agl} (δ 77.6), a downfield shift from δ 66.9. The HMBC spectrum showed key correlation peaks between the proton signal at δ 4.98 (H-1Glc) and the carbon resonance at δ 77.6 (H-3 Agl). On the basis of the above results, the structure of chamaedroside A was deduced as 3-O- β -D-glucopyranoside-(25S)-5 β -spirostan-3 β -ol (Fig.1). Compound **1** was isolated from *Veronica chamaedrys L.* for the first time and has been previously reported in the literature [19].

The $^1\text{H-NMR}$ spectrum of **2** displayed signals for four steroidal methyl groups at δ 1.15 (3H, s, H-18), 0.89 (3H, s, H-19), 1.50 (3H, d, $J=6.0$ Hz, H-21) and 1.20 (3H, d, $J=6.9$ Hz, H-27), as well as signals for two anomeric protons at δ 4.98 (1H, d, $J=7.07$ Hz) and 4.84 (1H, d, $J=7.5$ Hz). The above $^1\text{H-NMR}$ data, an acetalic carbon signal at δ 110.7 in the $^{13}\text{C-NMR}$ spectrum, indicated chamaedroside B to be a furostanol saponin with two monosaccharides. The $^{13}\text{C-NMR}$ spectrum of **2** showed C-5 at δ 36.6, C-9 at δ 40.3, and C-19 at δ 23.9, characteristic of 5β -steroidal sapogenins. On the basis of the HSQC and HMBC correlations, the aglycone moiety of compound **2** was identified as (25S)- 5β -furostan-3 β , 22 α , 26-triol. One glycosyl unit was shown to be linked to the C-26 hydroxy group of the aglycone by an HMBC correlation of the anomeric proton at δ 4.84 with C-26 of the aglycone at δ 72.2. The HMBC spectrum also showed key correlation peaks between the proton signal at δ 4.98 (H-1Glc) and the carbon resonance at δ 77.8 (C-3 Agl). Thus, chamaedroside B was determined to be 3-O- β -D-glucopyranoside-(25S)- 5β -furostan-3 β , 22 α , 26-triol-26-O- β -D-glucopyranoside (fig. 1). Compound **2** was isolated from *Veronica chamaedrys* L. for the first time and has been previously reported in the literature [20].

Compound **3** was obtained as an amorphous powder. Its HR-ESI-MS showed a major ion peak at m/z 763.9176 ($\text{M}+\text{Na}$) $^+$, and significant fragments at m/z 601 ($\text{M}+\text{Na}-162$) $^+$, attributable to the loss of a hexose unit. The molecular formula of **3** was determined as $\text{C}_{39}\text{H}_{64}\text{O}_{13}$ by the HR-ESI-MS (m/z (M) $^+$). Furthermore, the prominent fragments were observed at m/z : 578.7($\text{M}-162$) $^+$, 416.6 ($\text{M}-162-162$) $^+$ attribute to the sequential loss of two hexose residues, respectively. The $^1\text{H-NMR}$ spectrum of chamaedroside C showed two singlet methyl signals at δ 0.90 (3H, s, Me-18) and 1.08 (3H, s, Me-19), and two doublet methyl signals at δ 1.27 (3H, d, $J=7.0$ Hz, Me-27), and 1.33 (3H, d, $J=7.2$ Hz, Me-21), which were recognized as typical spirostanol saponin methyls. 5β configuration was deduced by HMBC correlation between the methyl signal at δ 1.08 (Me-19) and carbon resonances at δ 40.2 (C-9), 36.8 (C-5) and 37.0 (C-1). Moreover, signals for two anomeric protons at δ 4.99 (1H, d, $J=7.4$ Hz) and 5.45 (1H, d, $J=7.4$ Hz) could be readily assigned. The J values (>7 Hz) of two anomeric protons indicated the β -orientation at the anomeric centre for the hexose. The $^{13}\text{C-NMR}$ spectrum of **3** showed two anomeric carbon signals at δ 101.9 and 105.8. The HMBC spectrum showed key correlation peaks between the proton signal at δ 4.99 (H-1 Glc) and the carbon resonance at δ 77.8 (C-3 of the aglycone), the proton signal at δ 5.45 (H-1 Glc') and the carbon resonance at δ 78.7 (C-4 Glc). On the basis of the above results, the structure of chamaedroside C was determined as 3-O- β -D-glucopyranosyl(1 \rightarrow 4)- β -D-glucopyranoside-(25S)- 5β -spirostan-3 β -ol. Chamaedroside C is a new compound earlier indescribable in the literature.

Compound **4** was obtained as a colorless powder. Its HR-ESI-MS showed a major ion peak at m/z 944.0735 ($\text{M}+\text{Na}$) $^+$ and significant fragments at m/z 782 ($\text{M}+\text{Na}-162$) $^+$, attributable to the loss of a hexose unit. The molecular formula of **4** was unequivocally established to be $\text{C}_{45}\text{H}_{76}\text{O}_{19}$ by HR-MALDIMS (m/z 942 [$\text{M}+\text{Na}$] $^+$). Positive coloration reactions were observed when **4** was subjected to Ehrlich and Sannie tests, which suggested that **4** have a steroidal saponin skeleton. The $^1\text{H-NMR}$ data of chamaedroside E contained two singlet methyl groups at δ 1.003 and 0.89 (each s), two doublet methyl groups at δ 1.22 (d, $J=7.0$ Hz) and 1.33 (d, $J=7.0$ Hz) and a methenyl proton at δ 4.98 (m), attributable to a steroidal aglycone moiety. Furthermore, the furostanol glycosidic nature of chamaedroside E suggested by the strong absorption bands at 3300 and 900 cm^{-1} in the IR spectrum, and a semiketal carbon signal at δ 110.63 in the $^{13}\text{C-NMR}$ spectrum.

The above $^1\text{H-NMR}$ spectral data and a comparison of the $^{13}\text{C-NMR}$ signals of the aglycone moiety of chamaedroside E with those described in the literature [18] showed the structure of the aglycone to be (3 β , 22 α , 25S)- 5β -furostan-3, 22, 26-triol. The α -configuration of C-22 hydroxyl group of the aglycone moiety was deduced from the semiketal carbon signal at δ 110.64, instead of δ 115.5 for β -configuration, and it was further confirmed by the ROESY correlation between H-20 (2.21, dq, $J=6.5$, 7.5 Hz) and H-23 (1.33, m). The 25S-stereochemistry was established by the resonance of H-27 methyl protons at δ 1.22, a few bigger than 1.00 [18], and also by the resonance difference between equatorial proton signal (3.48, m, $J=6.5$ and 9.5 Hz) and axial proton signal (4.13, m) of H-26: 0.64 ppm, a few bigger than 0.57 ppm [18]. 5β configuration was deduced by HMBC correlation between the methyl signal at δ 1.1 (Me-19) and carbon resonances at δ 40.4 (C-9), 36.7 (C-5) and 37.1 (C-1).

In the $^{13}\text{C-NMR}$ spectrum, among the 45 carbon signals, 27 signals were assignable to the aglycone, the remaining 18 signals were indicative of the presence of three glucose moieties, in good agreement with three anomeric proton signals appearing at δ 4.82 (d, $J=7.49$ Hz), 4.98 (d, $J=7.07$ Hz), 5.43 (d, $J=7.48$ Hz), and the corresponding anomeric carbon signals at δ 105.13, 101.9 and 105.9, respectively.

A sugar chain was determined to be attached to C-26 by an observation of glycosidation shift of C-26 signal from δ 66.0 to 71.4, and this linkage was further indicated by the cross peak between the ^{13}C signal at δ 71.4 and the anomeric proton signal at δ 4.82 of β -glucopyranosyl moiety in the HMBC spectrum of **4**.

Similarly, a cross peak between the C-3 signal of aglycone at δ 77.91 and the anomeric proton signal at δ 4.98 in the HMBC spectrum of **4**, indicated that another sugar chain was located at the C-3 position of aglycone. A 1 \rightarrow 4 linkage

of the one sugar moiety to another was revealed by a cross peak between the C-4 signal of the first glucose at δ 78.58 and the anomeric proton signal of the second glucose at δ 5.43 in the HMBC spectrum. The β -orientation of the anomeric centres of the sugar moieties were supported by the relatively large J values of their anomeric protons ($J=7.0-7.8$ Hz).

On the basis of all evidences, chamaedroside E was identified as 3-O- β -D-glucopyranosyl (1 \rightarrow 4)- β -D-glucopyranoside-(25S)-5 β -furostan-3 β , 22 α , 26-triol-26-O- β -D-glucopyranoside (fig. 1).

Chamaedroside E is a new steroidal glycoside isolated and reported for the first time.

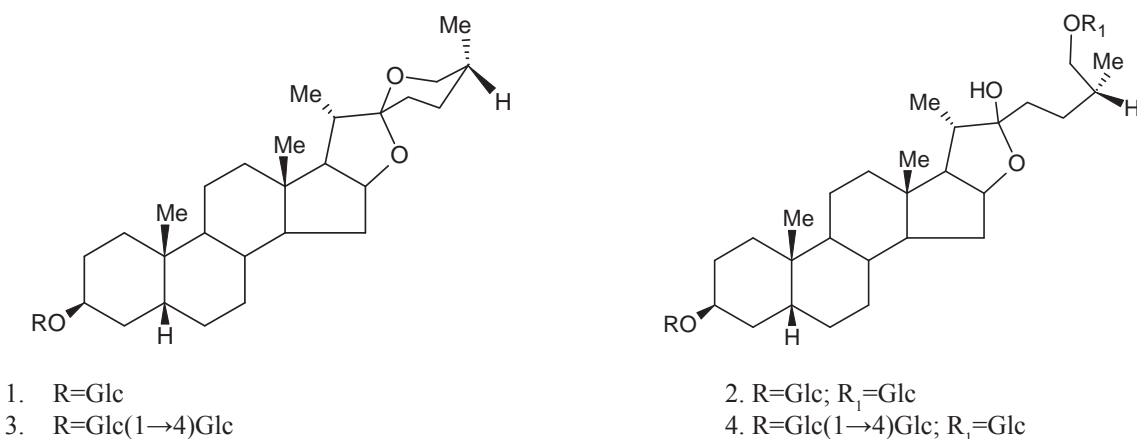


Fig. 1

Experimental

General experimental procedures

Melting points were measured on a Boetius table. Optical rotations were recorded with a Perkin-Elmer 243 spectropolarimeter. IR spectra were measured on a Bruker FT-IR IFS 66/s instrument as sample in pressed KBr disks. 1D and 2D NMR spectra were recorded on NMR Bruker Advance 600 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) at 300 K dissolving all the samples in C₅D₅N (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. The chemical shift values are reported as parts per million (ppm) units relative to tetramethylsilane (δ 0.00 ppm in both cases) for ¹H and ¹³C, and coupling constants are in hertz (in parentheses).

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. Samples were dissolved in MeOH (Baker) and infused in the ESI source by using a syringe pump. The capillary voltage was 43 V, the spray voltage 5 kV, and the tube lens offset 30 V. The capillary temperature was 280°C.

HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Waters XTerra Prep MSC₁₈ column (300 x 7.8 mm i.d.) and a Rheodyne injector.

TLC was performed on silica gel plates (Merck precoated silica gel 60 F₂₅₄). Gel filtration was performed on the Sephadex G-50 (Loba Feinchemie) and G-25 (Pharmacia, Fine Chemicals). Column chromatography was performed over Silica gel Merck 60 (70-230 mesh, Merck, Chemapol). Solvent systems: chloroform/methanol (4:1), chloroform-methanol-water (65:35:3). All solvents for chromatographic separation were of analytical grade. HPLC grade water (18 m Ω) was prepared using a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA).

Plant material has been collected in the scientific research field of the Institute of Genetics and Plant Physiology in May 2009 year. The voucher specimen has been deposited in the Laboratory of Genetics and Physiology of Plant Stability.

Extraction and separation

Fresh plant material of *Veronica chamaedrys* L. (1600 g) was extracted three times at 100°C with water for 4 hours. Water extracts were combined and extracted with n-butanol, after that n-butanol was evaporated under reduced pressure to give a mixture of saponins (10.3 g). This mixture was subjected to purification on Sephadex G-50 and G-25 and then has been chromatographed on silica gel column (70-230 mesh, Merck). The column was eluted with system chloroform-methanol-water (4:1:0-13:7:1). After monitoring by TLC [Si gel plates, chloroform-methanol (4:1)] fractions showing identical characteristics were combined to give A (105 mg) and B (120 mg).

Fractions A and B were submitted to HPLC on a Waters XTerra Prep MSC₁₈ column (300 x 7.8 mm i.d.), the flow rate was 1.5 μ L/min, using a MeOH:H₂O in the ratio 80:20 for A, and 85:15 for B (isocratic conditions). Pure **1** (20.2 mg) and **2** (26.6 mg) were obtained from A, **3** (40 mg) and **4** (52.2 mg) from B.

Table 1

¹³C-NMR spectral data (600 MHz, C₅D₅N, ppm) of glycosides (1–4)

	Aglycone					Monosaccharides			
	carbon 1	2	3	4		carbon 1	2	3	4
					Glc				
1	37.0	37.1	37.0	37.1	1	101.9	101.8	101.9	101.9
2	30.5	30.7	30.6	30.7	2	75.3	75.2	74.8	74.9
3	77.6	77.8	77.8	77.9	3	78.2	78.0	77.1	77.1
4	34.9	34.6	34.8	34.4	4	71.6	71.0	78.7	78.6
5	36.6	36.6	36.8	36.7	5	78.5	78.5	77.3	77.6
6	27.1	27.0	27.1	27.01	6	62.7	62.6	62.5	62.6
7	32.4	32.3	32.4	32.42	Glc'				
8	35.4	35.4	35.3	35.5	1'			105.8	105.9
9	40.4	40.3	40.2	40.4	2'			75.1	75.2
10	35.9	35.8	35.5	35.6	3'			77.7	77.9
11	21.3	21.2	21.2	21.1	4'			71.7	71.6
12	40.2	40.2	40.1	40.2	5'			78.6	78.6
13	40.5	40.6	40.5	40.6	6'			62.8	62.7
14	56.3	56.3	56.4	56.4	Glc''				
15	32.3	32.4	32.3	32.4	1''		105.2		105.1
16	81.1	81.3	81.2	81.2	2''		75.2		75.4
17	63.7	63.9	63.8	64.0	3''		78.0		78.2
18	16.5	16.5	16.7	16.7	4''		71.6		71.7
19	23.9	23.9	24.0	24.0	5''		78.3		78.5
20	41.0	41.1	41.1	41.2	6''		62.5		62.8
21	16.3	16.4	16.4	16.5					
22	110.5	110.7	110.6	110.6					
23	36.6	36.8	36.8	36.8					
24	28.3	28.2	28.3	28.3					
25	34.3	34.4	34.4	34.4					
26	65.4	72.2	65.6	71.4					
27	17.3	17.3	17.2	17.5					

1. Amorphous powder. M. p. 290–292°C, $[\alpha]_D^{20}$: –65 (CH₃OH, c 0.34). IR ν_{\max}^{KBr} cm⁻¹: 3402.5 (OH), 980, 910.5, 850, 757.3 (intensity 915>896, 25S-spiroketal). HR MS, m/z 578.7771 [calcd for C₃₃H₅₄O₈ (M)⁺]; 416.6 [M–162]⁺; ¹H NMR (aglycone) δ 4.98 (1H, m, H–16), 4.30 (1H, m, H–3), 1.1 (3H, s, Me–19), 0.89 (3H, s, Me–18), 1.5 (3H, d, H–21), 4.13 (1H, m, H–26a), 3.48 (1H, m, H–26b), 1.19 (3H, d, Me–27); (sugar) δ 4.98 (d, J=7.07 Hz, H–1 Glc), 4.06 (dd, J=7.5 and 9.0 Hz, H–4 Glc), 4.11 (dd, J=9.0 and 9.0 Hz, H–3 Glc), 4.22 (dd, J=9.0 and 9.0 Hz, H–4 Glc), 3.81 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc), 4.57 (dd, J=4.5 and 11.5 Hz, H–6a Glc), 4.35 (dd, J=2.5 and 11.5 Hz, H–6b Glc). For ¹³C NMR data see Table.

2. Amorphous powder. M. p. 183–184°C, $[\alpha]_D^{20}$: –107 (Py, c 0.34). IR ν_{\max}^{KBr} cm⁻¹: 3350 (OH), 900 (large band). HR MS, m/z 758.9329 [calcd for C₃₉H₆₇O₁₄ (M)⁺]; 596.7 [M–162]⁺; 434.6 [M–162–162]⁺; ¹H NMR (aglycone) δ 4.96 (1H, m, H–16), 4.29 (1H, m, H–3), 1.15 (3H, s, Me–19), 0.89 (3H, s, Me–18), 1.5 (3H, d, H–21), 4.132 (1H, m, H–26a), 3.5 (1H, m, H–26b), 1.20 (3H, d, Me–27); (sugars) δ 4.98 (d, J=7.07 Hz, H–1 Glc), 4.26 (dd, J=7.5 and 9.0 Hz, H–2 Glc), 4.13 (dd, J=9.0 and 9.0 Hz, H–3 Glc), 4.28 (dd, J=9.0 and 9.0 Hz, H–4 Glc), 3.85 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc), 4.52 (dd, J=4.5 and 11.5 Hz, H–6a Glc), 4.36 (dd, J=2.5 and 11.5 Hz, H–6b Glc), 4.84 (d, J=7.5 Hz, H–1 Glc''), 3.978 (dd, J=7.5 and 9.0 Hz, H–2 Glc''), 4.216 (dd, J=9.0 and 9.0 Hz, H–3 Glc''), 4.273 (dd, J=9.0 and 9.0 Hz, H–4 Glc''), 3.985 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc''), 4.559 (dd, J=4.5 and 11.5 Hz, H–6a Glc''), 4.339 (dd, J=2.5 and 11.5 Hz, H–6b Glc''). For ¹³C NMR data see Table.

3.

4. Amorphous powder. M. p. 292–294°C, $[\alpha]_D^{20}$: –58 (CH₃OH, c 0.34). IR ν_{\max}^{KBr} cm⁻¹: 3398 (OH), 980, 914, 892, 850 (intensity 915>896, 25S-spiroketal). HR MS, m/z 740.9176 [calcd for C₃₉H₆₄O₁₃ (M)⁺]; 578.7 [M–162]⁺; 416.6 [M–162–162]⁺; ¹H NMR (aglycone) δ 4.96 (1H, m, H–16), 4.32 (1H, m, H–3), 1.08 (3H, s, Me–19), 0.90 (3H, s, Me–18), 1.33 (3H, d, H–21), 4.17 (1H, m, H–26a), 3.5 (1H, m, H–26b), 1.27 (3H, d, Me–27); (sugars) δ 4.99 (d, J=7.4 Hz, H–1 Glc), 4.27 (dd, J=7.5 and 9.0 Hz, H–2 Glc), 4.12 (dd, J=9.0 and 9.0 Hz, H–3 Glc), 4.25 (dd, J=9.0 and 9.0 Hz, H–4 Glc), 4.27 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc), 4.56 (dd, J=4.5 and 11.5 Hz, H–6a Glc), 4.30 (dd, J=2.5 and 11.5 Hz, H–6b Glc), 5.45 (d, J=7.4 Hz, H–1 Glc'), 4.12 (dd, J=7.5 and 9.0 Hz, H–2 Glc'), 4.10 (dd, J=9.0 and 9.0 Hz, H–3 Glc'), 4.21 (dd, J=9.0 and 9.0 Hz, H–4 Glc'), 3.48 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc'), 4.56 (dd, J=4.5 and 11.5 Hz, H–6a Glc'), 4.28 (dd, J=2.5 and 11.5 Hz, H–6b Glc'). For ¹³C NMR data see Table.

5. Amorphous powder. M. p. 187–189°C, $[\alpha]_D^{20}$: –76 (H₂O, c 0.34). IR ν_{\max}^{KBr} cm⁻¹: 3300 (OH), 900 (large band). HR MS, m/z 921.0735 [calcd for C₄₅H₇₆O₁₉ (M)⁺]; 758.9 [M–162]⁺; 596.7 [M–162–162]⁺; ¹H NMR (aglycone) δ 4.98 (1H, m, H–16), 4.28 (1H, m, H–3), 1.003 (3H, s, Me–19), 0.89 (3H, s, Me–18), 1.33 (3H, d, H–21), 4.128 (1H, m, H–26a), 3.48 (1H, m, H–26b), 1.22 (3H, d, Me–27); (sugars) δ 4.98 (d, J=7.07 Hz, H–1 Glc), 4.26 (dd, J=7.5 and 9.0 Hz, H–2 Glc), 4.10 (dd, J=9.0 and 9.0 Hz, H–3 Glc), 4.24 (dd, J=9.0 and 9.0 Hz, H–4 Glc), 4.27 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc), 4.58 (dd, J=4.5 and 11.5 Hz, H–6a Glc), 4.34 (dd, J=2.5 and 11.5 Hz, H–6b Glc), 5.43 (d, J=7.48 Hz, H–1 Glc'), 4.12 (dd, J=7.5 and 9.0 Hz, H–2 Glc'), 4.10 (dd, J=9.0 and 9.0 Hz, H–3 Glc'), 4.22 (dd, J=9.0 and 9.0 Hz, H–4 Glc'), 3.50 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc'), 4.56 (dd, J=4.5 and 11.5 Hz, H–6a Glc'), 4.27 (dd, J=2.5 and 11.5 Hz, H–6b Glc'), 4.82 (d, J=7.49 Hz, H–1 Glc''), 3.98 (dd, J=7.5 and 9.0 Hz, H–2 Glc''), 4.22 (dd, J=9.0 and 9.0 Hz, H–3 Glc''), 4.26 (dd, J=9.0 and 9.0 Hz, H–4 Glc''), 3.98 (ddd, J=2.5, 4.5 and 9.0 Hz, H–5 Glc''), 4.53 (dd, J=4.5 and 11.5 Hz, H–6a Glc''), 4.34 (dd, J=2.5 and 11.5 Hz, H–6b Glc''). For ¹³C NMR data see Table.

Conclusion

Two steroidal glycosides of spirostane series chamaedroside A and chamaedroside C, and two steroidal glycosides of furostane series chamaedroside B and E have been isolated for the first time from the plants of *Veronica chamaedrys* L. During the investigation the structure of chamaedroside A was elucidated as 3-O- β -D-glucopyranoside-(25S)-5 β -spirostan-3 β -ol, chamaedroside B – 3-O- β -D-glucopyranoside-(25S)-5 β -furostan-3 β , 22 α , 26-triol-26-O- β -D-glucopyranoside, chamaedroside C – 3-O- β -D-glucopyranosyl(1 \rightarrow 4)- β -D-glucopyranoside-(25S)-5 β -spirostan-3 β -ol and chamaedroside E – 3-O- β -D-glucopyranosyl (1 \rightarrow 4)- β -D-glucopyranoside-(25S)-5 β -furostan-3 β , 22 α , 26-triol-26-O- β -D-glucopyranoside. Chamaedrosides C and E are new compounds, and chamaedrosides A and B are known steroidal glycosides identified in *Veronica chamaedrys* L. plants for the first time.

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