New Furostanol Glycosides from the Roots of *Digitalis ciliata* TRAUTV.

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Three new furostanol glycosides, named ciliatasides A, B, and C (**1**–**3**, resp.), have been isolated from the roots of *Digitalis ciliata*, along with two known furostanol glycosides. The structures of the new compounds were identified as (2*α*,3*β*,5*α*,14*β*,25*R*)-26-(*β*-D-glucopyranosyloxy)-2-hydroxyfurost-20(22)-en-3-yl *β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranoside (**1**), (2*α*,3*β*,5*α*,14*β*,22*R*)-26-(*β*-D-glucopyranosyloxy)-2-hydroxy-22-methoxyfurost-25(27)-en-3-yl *β*-D-galactopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside (**2**), and (2*α*,3*β*,5*α*,14*β*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-2,22-dihydroxyfurostan-3-yl *β*-D-glucopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranoside (**3**).

Introduction. – Among cardiac glycosides, one of the most important class of natural biologically active compounds, foxglove (*Digitalis*) cardenolides, which have been successfully used in medicine for *ca*. 250 years, play a leading role.

In contrast to the 36 species of the genus *Digitalis* L. occurring in many countries, *Digitalis ciliata* TRAUTV. (Plantaginaceae) is a strictly endemic plant in the Caucasus. It is widespread in the Alpine part of the Main Caucasian Ridge, and its spurs at the height of 1200–1300 m above sea level. *D. ciliata* is a perennial herb which differs from other *Digitalis* species by more densely leafy stems, lanceolate, dentate leaves, loose trusses and a vigorous rootage [1].

Already in the past, *D. ciliata* appeared to be a high-grade medical raw material due to the presence of *ca.* 50 cardenolides with total product yield up to 4–4.5% [2].

Twenty-four cardenolides, namely digitoxigenin-D-glucoside, digitoxigeninbidigitoxoside, digitoxin, acetyldigitoxin-*α*, acetylgitoxin-*α*, gitoxin, gitaloxin, digoxin, strospeside, glucogitoroside, lanatosides A–C, and E, desacetyllanatosides A–C, digitalinum verum, evatromonoside, glucoevatromonoside, odoroside H, and odorobioside G were isolated from *D. ciliata* leaves and characterized. In addition, we have established an autofermentation procedure and applied it to obtain the secondary glycosides digitoxin and acetildigitoxin-*α* from the major constituent lanatoside A out of the plant material without any chemical intervention [3].

Novel triterpene glycosides, digitalosides A and B, derivatives of oleanolic acid, were also isolated from the leaves of *D. ciliata*. [4]. Drugs from *D. ciliata*, *Digicilen* (ampoules) and *Digicil* (tablets), were used extensively for treatment of heart failure [2]. No less than 20 cardenolides were found in *D. ciliata* seeds, which are also a rich source of the steroid glycoside digitonin, the yield of which reached up to 2% [5]. More

recently, new furostanol and pregnane glycosides were isolated from the seeds of *D. ciliata* [6].

In contrast to the leaves and seeds, the roots of this plant have never been investigated before. Thus, the present work was focused on the phytochemical investigation of the roots of *D. ciliata*.

Results and Discussion. – The roots of *D. ciliata* (500 g) were extracted with 80% MeOH once at room temperature and twice at 60°. The MeOH extract was partitioned between AcOEt, BuOH, and H_2O. Part of the BuOH extract was passed through a porous-polymer polystyrene resin (*Diaion HP-20*) column, and the 80% MeOH-eluted fraction was subjected to silica-gel column chromatography (CC) to furnish compounds **1**–**5** (*Fig.*). Compounds **4** and **5** were identified as (2*α*,3*β*,5*α*,14*β*,25*R*)-26-(*β*-D-glucopyranosyloxy)-2-hydroxyfurost-20(22)-en-3-yl *β*-D-galactopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside [7] and (2*α*,3*β*,5*α*,14*β*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-2,22-dihydroxyfurostan-3-yl *β*-D-galactopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-galactopyranoside [8], respectively, by comparing their physical and spectroscopic data with those in the literature.

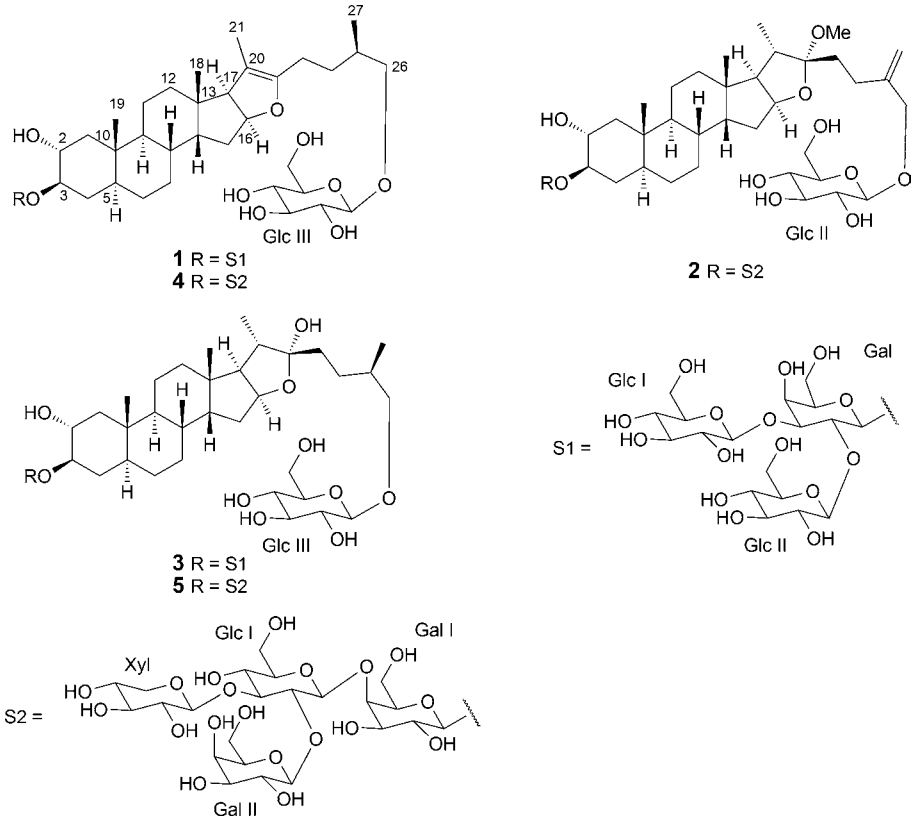

Figure. *Structures of compounds* **1**–**5** *isolated from* D. ciliata

The absolute configurations of the sugar units were assigned after acid hydrolysis of the BuOH extract and identification with authentic samples by means of TLC, followed by preparative separation of each sugar. The D-configuration of galactose, glucose, and xylose were established by comparison of their optical-rotation values with those reported in the literature [9][10].

The molecular formula of **1** was unequivocally established as $C_{51}H_{84}O_{24}$ by HR-TOF-MS (*m/z* 1081.5102 ($[M+H]^+$, $C_{51}H_{85}O_{24}^+$; calc. 1081.4989)). The positive-ion-mode ESI-MS of **1** showed the major ion peak at *m/z* 1081.5, which was attributed to $[M+H]^+$. The MS/MS of this ion showed peaks at *m/z* 919.5 ($[M+H-162]^+$), due to the loss of a sugar group, and 757.5 ($[M+H-162\times2]^+$), 595.5 ($[M+H-162\times3]^+$), and 433.5 ($[M+H-162\times4]^+$), corresponding to the loss of two, three, and four hexose units, respectively.

The ^{1}H-NMR spectrum of **1** showed signals of three tertiary Me groups (δ(H) 0.71 (*s,* Me(18)), 0.91 (*s*, Me(19)), and 1.62 (*s*, Me(21))), one secondary Me group (δ(H) 0.97 (*d, J* = 6.6, Me(27))), three CH groups δ(H) 3.50 (*m*, H–C(3)), 3.69 (*m*, H–C(2)), and 4.73 (*dd, J* = 14.5, 7.5, H–C(16))), indicative of CH–O moieties, two CH_2 groups (δ(H) 3.42 (*m*, H_a–C(26)) and 3.73 (*m*, H_b–C(26)), ascribable to a CH_2–O group, and four anomeric H-atoms (δ(H) 4.96 (*d*, *J* = 7.5, H–C(1″)), 4.68 (*d*, *J* = 7.5, H–C(1‴)), 4.47 (*d, J* = 7.5, H–C(1′)), and 4.27 (*d*, *J* = 7.5, H–C(1⁗))(*Tables 1* and *2*)). The ^{13}C-NMR spectrum displayed signals ascribable to a C(20)=C(22) bond (δ(C) 104.7 (C(20)) and 152.5 (C(22))), three secondary alcohol functions (δ(C) 71.5 (C(2)), 84.5 (C(3)), and 85.3 (C(16))), and one primary alcohol function (δ(C) 75.5 (C(26))), indicating the presence of a glycosidic furostanol skeleton. On the basis of the HSQC correlations and HMBCs and comparison with the literature data, the aglycon moiety of compound **1** was identified as (25*R*)-5α-furost-20(22)-ene-2α,3β,26-triol [7]. The configuration at C(25) was deduced to be (*R*) based on the difference of chemical shifts ($\Delta_{ab}=\delta_a-\delta_b$) of the geminal H-atoms of CH_2(26) (Δ_{ab} = 0.31 ppm). It has been observed that Δ_{ab} is usually >0.57 ppm in (25*S*)-compounds and <0.48 in (25*R*)-compounds [11]. The (*R*) configuration was also supported by the relative intensity of the IR bands ($918 < 898$ cm^{-1}) [12]. It was evident from the ^{1}H- and ^{13}C-NMR data that the glycosidic moiety of **1** consisted of four sugar units. The chemical shifts of all individual H-atoms of the four sugar units were ascertained by a combination of HSQC, HMBC, and COSY analysis, and the ^{13}C chemical shifts of the corresponding attached C-atoms were determined unambiguously by using the HSQC spectrum (*Table 2*). These data revealed the presence of three β-glucopyranosyl units (δ(H) 4.96 (H–C(1″)), 4.68 (H–C(1‴)) and 4.27 (H–C(1⁗))) and one β-galactopyranosyl unit (δ(H) 4.47 (H–C(1′))). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC spectrum, which showed key correlation peaks between the H-atom signal at δ(H) 4.47 (H–C(1′)) and the C-atom resonance at δ(C) 84.5 (C(3)); the H-atom signal at 4.96 (H–C(1″)) and the C-atom resonance at 84.8 (C(3′)); the H-atom signal at 4.68 (H–C(1‴)) and the C-atom resonance at 76.1 (C(2′)); and the H-atom signal at 4.27 (H–C(1⁗)) and the C-atom resonance at 75.5 (C(26)). Based on these data, the structure of compound **1** was established as (2α,3β,5α,14β,25*R*)-26-(β-D-glucopyranosyloxy)-2-hydroxyfurost-20(22)-en-3-yl β-D-glucopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-galactopyranoside and named ciliataside A.

Table 1. *^{1}H- and ^{13}C-NMR* (600 MHz, CD_3OD) *Data of the Aglycon Moieties of* **1**–**3**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	2.03–0.95 (*m*)	45.6	2.03–0.95 (*m*)	45.6	2.03–0.95 (*m*)	45.7
2	3.69 (*m*)	71.5	3.69 (*m*)	71.5	3.69 (*m*)	71.3
3	3.50 (*m*)	84.5	3.50 (*m*)	84.5	3.50 (*m*)	84.6
4	1.75–1.42 (*m*)	33.9	1.75–1.42 (*m*)	33.9	1.75–1.42 (*m*)	33.9
5	1.20 (*m*)	45.3	1.20 (*m*)	45.3	1.20 (*m*)	45.3
6	1.38 (*m*, 2 H)	29.2	1.38 (*m*, 2 H)	29.2	1.38 (*m*, 2 H)	29.2
7	1.75–0.97 (*m*)	33.1	1.75–0.97 (*m*)	33.1	1.75–0.97 (*m*)	33.1
8	1.59 (*m*)	35.5	1.59 (*m*)	35.5	1.59 (*m*)	35.5
9	0.78 (*m*)	55.2	0.78 (*m*)	55.2	0.78 (*m*)	55.2
10	–	38.0	–	38.0	–	38.0
11	1.59–1.42 (*m*)	21.9	1.59–1.42 (*m*)	21.9	1.59–1.42 (*m*)	21.9
12	1.81–1.27 (*m*)	40.6	1.81–1.27 (*m*)	40.6	1.88–1.20 (*m*)	40.6
13	–	44.1	–	41.6	–	41.6
14	1.08 (*m*)	55.6	1.08 (*m*)	57.0	1.11 (*m*)	57.0
15	2.17–1.40 (*m*)	34.8	1.73–1.26 (*m*)	32.5	1.73–1.26 (*m*)	32.5
16	4.73 (*dd*, J = 14.5, 7.5)	85.3	4.37 (*dd*, J = 14.5, 7.5)	82.2	4.37 (*dd*, J = 14.5, 7.5)	81.8
17	2.51 (*m*)	65.2	1.76 (*m*)	64.7	1.76 (*m*)	64.2
18	0.71 (*s*)	14.4	0.84 (*s*)	16.7	0.84 (*s*)	16.7
19	0.91 (*s*)	13.3	0.92 (*s*)	13.3	0.92 (*s*)	13.3
20	–	104.7	2.19 (*m*)	41.2	2.19 (*m*)	40.8
21	1.62 (*s*)	11.5	1.02 (*d*, J = 6.6)	15.6	1.02 (*d*, J = 6.6)	15.7
22	–	152.5	–	113.5	–	112.2
23	2.14 (*m*, 2 H)	23.8	1.74–1.72 (*m*)	32.0	1.82–1.64 (*m*)	31.1
24	1.64–1.28 (*m*)	31.5	2.55–2.17 (*m*)	28.2	1.61–1.20 (*m*)	28.9
25	1.78 (*m*)	33.9	–	146.0	1.76 (*m*)	34.8
26	3.73, 3.42 (2*m*)	75.5	4.32 (*d*, J = 12.1), 4.14 (*d*, J = 12.1)	72.0	3.75, 3.43 (2*m*)	75.3
27	0.97 (*d*, J = 6.6)	16.8	5.10, 4.95 (2 br. *s*)	112.4	0.98 (*d*, J = 6.6)	16.9
MeO			3.17 (*s*)	47.5		

The molecular formula of **2** was determined as $C_{57}H_{94}O_{29}$ (*m*/*z* 1243.4728 ($[M+H]^+$, $C_{57}H_{95}O_{29}^+$; calc. 1243.4720)). The positive-ion-mode ESI-MS of **2** showed the major ion peak at *m*/*z* 1243.5, which was assigned to $[M+H]^+$. The MS/MS of this ion showed peaks at *m*/*z* 1081.5 ($[M+H-162]^+$), due to the loss of a sugar group, and at *m*/*z* 949.5 ($[M+H-162-132]^+$), 787.5 ($[M+H-162\times2-132]^+$), 625.5 ($[M+H-162\times3-132]^+$), and 463.5 ($[M+H-162\times4-132]^+$), corresponding to the loss of one hexose and one deoxyhexose-pentose units, and then three and four hexose units, respectively.

The ^{1}H-NMR spectrum of **2** showed signals for two tertiary Me groups (δ(H) 0.84 (*s*, Me(18)), 0.92 (*s*, Me(19))), one secondary Me group (δ(H) 1.02 (*d*, J = 6.6, Me(21))), one MeO group at δ(H) 3.17 (*s*), three CH groups (δ(H) 3.50 (*m*, H–C(3)), 3.69 (*m*, H–C(2)), and 4.37 (*dd*, J = 14.5, 7.5, H–C(16))), indicative of secondary alcoholic functions, two CH_2 groups (δ(H) 4.14 (*d*, J = 12.1, H_a–C(26)) and 4.32 (*d*, J = 12.1, H_b–C(26)), ascribable to a primary alcohol function, and exocyclic methylidene H-atoms (δ(H) 4.95 (br. *s*, H_a–C(27)) and 5.10 (br. *s*, H_b–C(27)).

The ^{13}C-NMR spectrum displayed signals for the aglycon including those of an acetal function (δ(C) 113.5 (C(22))), three CH–O groups (δ(C) 71.5 (C(2)), 82.2 (C(16)), and 84.5 (C(3))), and one CH_2–O group (δ(C) 72.0 (C(26))), suggesting the occurrence of a furostanol skeleton [13]. On the basis of the HSQC correlations and HMBCs, the aglycon moiety of compound **2** was identified as 22-methoxy-5α-furost-25(27)-ene-2α,3β,26-triol. The configuration of the MeO group at C(22) (δ(H) 3.17, δ(C) 47.5), which could be resulted from the extraction with MeOH, was established from the ROESY correlations between δ(H) 2.19 (H–C(20)), and δ(H) 1.72 (H_a–C(23)) and 1.74 (H_b–C(23)). From the ^{1}H- and ^{13}C-NMR data, it was evident that the glycosidic moiety of **2** consisted of five sugar units. The chemical shifts of all individual H-atoms of the four sugar units were ascertained by the combination of 1D-TOCSY and DQFCOSY spectral analysis, and the ^{13}C chemical shifts of the corresponding attached C-atoms were determined unambiguously from the HSQC spectrum (*Table 2*). The ^{1}H-NMR spectrum of **2** exhibited signals of five anomeric H-atoms (δ(H) 4.92 (*d*, $J = 7.5$, H–C(1‴)), 4.65 (*d*, $J = 7.5$, H–C(1″)), 4.64 (*d*, $J = 7.5$, H–C(1⁗)), 4.41 (*d*, $J = 7.5$, H–C(1′)), and 4.27 (*d*, $J = 7.5$, H–C(1⁗′))), and the ^{13}C-NMR spectrum displayed signals ascribable to corresponding sugars (δ(C) 104.5 (C(1‴)), 104.1 (C(1″)), 104.2 (C(1⁗)), 102.2 (C(1′)), and 104.2 (C(1⁗′)), respectively. The HMBC spectrum showed key correlations between the H-atom signal at δ(H) 4.64 (H–C(1⁗)) and the C-atom resonance at δ(C) 87.2 (C(3″)); the H-atom signal at 4.65 (H–C(1″)) and the C-atom resonance at 79.9 (C(4′)); the H-atom signal at 4.92 (H–C(1‴)) and the C-atom resonance at 80.6 (C(2″)); the H-atom signal at 4.41 (H–C(1′)) and the C-atom resonance at 84.5 (C(3)), as well as the H-atom signal at 4.27 (H–C(1⁗′)) and the C-atom resonance at 72.0 (C(26)).

Therefore, the structure of **2** was established as (2α,3β,5α,14β,22*R*)-26-(β-D-glucopyranosyloxy)-2-hydroxy-22-methoxyfurost-25(27)-en-3-yl β-D-galactopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside named ciliataside B.

It should be noted that this compound might be an artefact resulting from the extraction procedure with MeOH.

The molecular formula of **3** was determined as $C_{51}H_{86}O_{25}$ (*m*/*z* 1099.2549 ($[M+H]^+$, $C_{51}H_{87}O_{25}^+$; calc. 1099.2541)). The positive-ion-mode ESI-MS of **3** showed the major ion peak at *m*/*z* 1099.5, which was assigned to $[M+H]^+$. The MS/MS of this ion showed peaks at *m*/*z* 937.5 ($[M+H-162]^+$), due to the loss of a sugar group, at *m*/*z* 919.5 ($[M+H-162-18]^+$), and at *m*/*z* 757.5 ($[M+H-162\times2-18]^+$), 595.5 ($[M+H-162\times3-18]^+$), 433.5 ($[M+H-162\times4-18]^+$), corresponding to the loss of H_2O, two, three, and four hexose units, respectively.

The ^{1}H-NMR spectrum of **3** showed signals for two tertiary Me groups (δ(H) 0.84 (*s*, Me(18)) and 0.92 (*s*, Me(19))), two secondary Me groups (δ(H) 0.98 (*d*, $J = 6.6$, Me(27)) and 1.02 (*d*, $J = 6.6$, Me(21))), three CH groups (δ(H) 3.50 (*m*, H–C(3)), 3.69 (*m*, H–C(2)), and 4.37 (*dd*, $J = 14.5, 7.5$, H–C(16))), indicative of secondary alcoholic functions, two CH_2 groups (δ(H) 3.43 (*m*, H_a–C(26)) and 3.75 (*m*, H_b–C(26))), ascribable to a primary alcoholic function, and four anomeric H-atoms (δ(H) 4.96 (*d*, $J = 7.5$, H–C(1″)), 4.68 (*d*, $J = 7.5$, H–C(1‴)), 4.47 (*d*, $J = 7.5$, H–C(1′)), and 4.27 (*d*, $J = 7.5$, H–C(1⁗))). The ^{13}C-NMR spectrum displayed signals typical of a hemiacetal function (δ(C) 112.2 (C(22))), three CH–O groups (δ(C) 71.3 (C(2)), 84.6 (C(3)), and

Table 2. *^{1}H- and ^{13}C-NMR* (600 MHz, CD_3OD) *Data of the Sugar Moieties of* **1**–**3**. δ in ppm, J in Hz.

Position	**1** and **3**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
	β-D-Gal		β-D-Gal I	
1′	4.47 (*d*, *J* = 7.5)	100.2	4.41 (*d*, *J* = 7.5)	102.2
2′	4.01 (*dd*, *J* = 9.0, 7.5)	76.1	3.84 (*dd*, *J* = 8.5, 7.5)	72.3
3′	3.82 (*dd*, *J* = 9.0, 9.0)	84.8	3.57 (*dd*, *J* = 8.5, 2.9)	75.3
4′	4.12 (*dd*, *J* = 9.0, 9.0)	69.8	4.07 (*dd*, *J* = 2.9, 12.0)	79.9
5′	3.54 (*ddd*, *J* = 9.0, 4.5, 2.0)	76.2	3.59 (*m*)	75.3
6′	3.96 (*dd*, *J* = 12.0, 2.0),	64.7	3.89 (*dd*, *J* = 12.0, 2.0),	61.0
	3.70 (*dd*, *J* = 12.0, 4.5)		3.69 (*dd*, *J* = 12.0, 4.5)	
	β-D-Glc I		β-D-Glc I	
1″	4.96 (*d*, *J* = 7.5)	103.4	4.65 (*d*, *J* = 7.5)	104.1
2″	3.13 (*dd*, *J* = 9.0, 7.5)	75.8	3.80 (*dd*, *J* = 9.0, 7.5)	80.6
3″	3.39 (*dd*, *J* = 9.0, 9.0)	77.9	3.75 (*dd*, *J* = 9.0, 9.0)	87.2
4″	3.16 (*dd*, *J* = 9.0, 9.0)	72.3	3.31 (*dd*, *J* = 9.0, 9.0)	70.0
5″	3.32 (*ddd*, *J* = 9.0, 4.5, 2.0)	77.7	3.33 (*m*)	78.0
6″	3.85 (*dd*, *J* = 12.0, 2.0),	63.4	3.93 (*dd*, *J* = 12.0, 2.5),	62.8
	3.65 (*dd*, *J* = 12.0, 4.5)		3.61 (*dd*, *J* = 12.0, 4.5)	
	β-D-Glc II		β-D-Gal II	
1‴	4.68 (*d*, *J* = 7.5)	104.3	4.92 (*d*, *J* = 7.5)	104.5
2‴	3.31 (*dd*, *J* = 9.0, 7.5)	75.0	3.59 (*dd*, *J* = 9.8, 7.5)	72.9
3‴	3.38 (*dd*, *J* = 9.0, 9.0)	77.9	3.51 (*dd*, *J* = 9.8, 3.4)	74.5
4‴	3.31 (*dd*, *J* = 9.0, 9.0)	71.4	3.88 (*dd*, *J* = 3.4, 1.1)	70.2
5‴	3.38 (*ddd*, *J* = 9.0, 4.5, 2.0)	77.8	3.61 (*m*)	77.0
6‴	3.87 (*dd*, *J* = 12.0, 2.0),	62.8	4.04 (*dd*, *J* = 11.8, 7,0),	62.4
	3.67 (*dd*, *J* = 12.0, 4.5)		3.74 (*dd*, *J* = 11.8, 5.9)	
	β-D-Glc III		β-D-Xyl	
1⁗	4.27 (*d*, *J* = 7.5)	104.2	4.64 (*d*, *J* = 7.9)	104.2
2⁗	3.22 (*dd*, *J* = 9.0, 7.5)	75.0	3.27 (*dd*, *J* = 9.0, 7.5)	75.0
3⁗	3.39 (*dd*, *J* = 9.0, 9.0)	77.9	3.37 (*dd*, *J* = 9.0, 9.0)	77.5
4⁗	3.31 (*dd*, *J* = 9.0, 9.0)	71.4	3.55 (*m*)	70.8
5⁗	3.28 (*ddd*, *J* = 9.0, 4.5, 2.0)	77.6	3.95 (*dd*, *J* = 10.5, 4.5)	67.0
6⁗	3.90 (*dd*, *J* = 12.0, 2.0),	62.6	3.29 (*t*, *J* = 10.5)	
	3.70 (*dd*, *J* = 12.0, 4.5)			
			β-D-Glc II	
1⁗′			4.27 (*d*, *J* = 7.5)	104.2
2⁗′			3.22 (*dd*, *J* = 9.0, 7.5)	75.0
3⁗′			3.39 (*dd*, *J* = 9.0, 9.0)	77.9
4⁗′			3.31 (*dd*, *J* = 9.0, 9.0)	71.4
5⁗′			3.28 (*ddd*, *J* = 9.0, 4.5, 2.0)	77.6
6⁗′			3.90 (*dd*, *J* = 12.0, 2.0),	62.6
			3.70 (*dd*, *J* = 12.0, 4.5)	

81.8 (C(16))), and one CH_2–O group (δ(C) 75.3 (C(26))), suggesting the occurrence of a glycosidic furostanol skeleton. On the basis of the HSQC and HMBC correlations, the aglycon moiety of compound **3** was identified as (25*R*)-5α-furostane-2α,3β,22α,26-

tetraol [12]. The C(25) configuration was deduced as (*R*) based on the difference of chemical shifts ($\Delta_{ab} = \delta_a - \delta_b$) of the geminal H-atoms at C(26) ($\Delta_{ab} = 0.32$ ppm). From the ^{1}H- and ^{13}C-NMR data, it was evident, that the glycosidic moiety of **3** consisted of four sugar units identical to those of compound **1**.

On the basis of all these data, the structure of the compound **3** was determined as (2*α*,3*β*,5*α*,14*β*,22*R*,25*R*)-26-(*β*-D-glucopyranosyloxy)-2,22-dihydroxyfurostan-3-yl *β*-D-glucopyranosyl-(1 → 2)-[*β*-D-glucopyranosyl-(1 → 3)]-*β*-D-galactopyranoside, named ciliataside C.

All three compounds, named ciliatasides A – C, have never been isolated or described before.

Conclusion. – *D. ciliata* roots can be considered as a rich source of steroidal glycosides which characteristically show *trans*-connected rings *A* and *B*, belonging to the 5*α*-series of furostanols.

Experimental Part

General. All solvents were of anal. grade and were purchased from *Merck* (DE-Darmstadt). TLC: percolated silica-gel *60 F254* plates (*Merck*); $CHCl_3$/MeOH/H_2O 26 : 14 : 3. Column chromatography (CC): *Diaion HP-20* (*Sigma–Aldrich*) and silica gel (0.040 – 0.063 mm; *Merck*). Optical rotations: *Perkin–Elmer 192* polarimeter. IR Spectra: *Perkin–Elmer 1600* spectrometer. NMR Spectra: *Avance II 600* MHz spectrometer (*BrukerBioSpinGmbH*, DE-Rheinstetten) equipped with a *Bruker TXI* probe-head at 300 K; δ in ppm rel. to Me_4Si as internal standard; *J* in Hz; all 1D- and 2D-NMR spectra were recorded in CD_3OD (99.95%, *Sigma–Aldrich*), and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC and HMBC spectra. The NMR data were processed using *MestRe-C* UXNMR software (ES-Santiago de Compostela). MS: *MicroOTOF-QII* (*Bruker*) mass spectrometer; in *m*/*z*; the samples were analyzed by high-resolution time-of-flight (HR-TOF) mass spectrometry in the pos.-ion electrospray ionization (ESI) mode; exact mass calibration was performed on the daily base with the ESI-L low concentration tuning mix from *Agilent* (Santa Clara, USA).

Plant Material. The roots of *Digitalis ciliata* were collected in September 2012 in the northwest Georgia (Svaneti region). The samples of *D. ciliata* were identified by Dr. *Jemal Aneli*, Department of Pharmacobotany, Institute of Pharmacochemistry, Tbilisi, Georgia. The herbarium specimen (No. 118) was deposited with this department.

Extraction and Isolation. Powdered underground parts (500 g) of *Digitalis ciliata* were extracted by shaking with MeOH 80% (2.5 l) for 1 h once at r.t. and twice at 60°. The collected extracts were dried under reduced pressure (70 g), and the concentrate was partitioned between AcOEt (4 g), BuOH (50 g), and H_2O (16 g). Part of the BuOH extract (10 g) was subjected to CC (*Diaion HP-20* (50 × 4 cm); H_2O/MeOH 10 : 0 to 0 : 10) to yield four fractions (500 ml each): 30% (0.64 g), 50% (5.2 g), 80% (3.3 g), and 100% (0.3 g). Part of 80% MeOH fraction (2.5 g) was then separated by CC (SiO_2; $CHCl_3$/MeOH/H_2O (26 : 14 : 3). This approach resulted in the isolation of five individual furostanol glycosides, *i.e.*, **1** (24 mg), **2** (16 mg), **3** (19 mg), **4** (32 mg), **5** (21 mg).

Ciliataside A (= *(2α,3β,5α,14β,25*R*)-26-(β-*D*-Glucopyranosyloxy)-2-hydroxyfurost-20(22)-en-3-yl β-*D*-Glucopyranosyl-(1 → 2)-[β-*D*-glucopyranosyl-(1 → 3)]-β-*D*-galactopyranoside*; **1**). Amorphous solid. $[\alpha]_D^{22} = -39.3$ ($c = 0.10$, MeOH). IR: 3403 (OH), 2929 (CH), 918 < 898 (25*R*). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-TOF-MS: 1081.5102 ($[M+H]^+$, $C_{51}H_{85}O_{24}^+$; calc. 1081.4989).

Ciliataside B (= *(2α,3β,5α,14β,22*R*)-26-(β-*D*-Glucopyranosyloxy)-2-hydroxy-22-methoxyfurost-25(27)-en-3-yl β-*D*-Galactopyranosyl-(1 → 2)-[β-*D*-xylopyranosyl-(1 → 3)]-β-*D*-glucopyranosyl-(1 → 4)-β-*D*-galactopyranoside*; **2**). Amorphous solid. $[\alpha]_D^{22} = -70.5$ ($c = 0.10$, MeOH). IR: 3405 (OH), 2925 (CH). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-TOF-MS:1243.4728 ($[M+H]^+$, $C_{57}H_{95}O_{29}^+$; calc. 1243.4720).

Ciliataside C (=*(2α,3β,5α,14β,22*R,*25*R*)-26-(β-*D*-Glucopyranosyloxy)-2,22-dihydroxyfurostan-3-yl β-*D*-Glucopyranosyl-(1→2)-[β-*D*-glucopyranosyl-(1→3)]-β-*D*-galactopyranoside*; **3**). Amorphous solid. $[\alpha]_D^{22} = -31.2$ ($c = 0.10$, MeOH). IR (film): 3406 (OH), 2927 (CH), 920 < 900 (25*R*). ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*. HR-TOF-MS: 1099.2549 ($[M+H]^+$, $C_{51}H_{87}O_{25}^+$; calc. 1099.2541).

Acid Hydrolysis. The BuOH extract (100 mg) was refluxed with 15 ml of 2N HCl for 4 h. The sapogenins were extracted with AcOEt (3 × 15 ml), and the org. layer was neutralized by washing with H_2O, and evaporated to dryness. The acid aq. layer was neutralized with 1N NaOH and freeze-dried. Three sugars were identified as glucose, galactose, and xylose by comparison with authentic samples (TLC; AcOEt/^{i}PrOH/acetone/H_2O 20:10:7:6). After prep. TLC of sugar mixture (50 mg) in this solvent system, the optical rotation of each purified sugar was measured. The D-configurations of galactose, glucose, and xylose were established by comparison of their optical rotation values with those reported in [9]. The optical rotations were determined after dissolving the sugars in H_2O and allowing them to settle for 24 h: D-glucose, $[\alpha]_D^{22} = +53.8$ ($c = 0.1$); D-galactose, $[\alpha]_D^{22} = +81.3$ ($c = 0.1$), D-xylose $[\alpha]_D^{22} = +12.7$ ($c = 0.1$).

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