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Pharmacochemistry

Furostanol Glycosides from the Roots of *Tribulus terrestris* L.

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ABSTRACT. A new furostanol glycoside was isolated from the roots of *Tribulus terrestris* growing in Georgia, together with four known furostanols. Their structures were elucidated by classical physical and chemical methods as well as by modern spectral analysis of mono- and bidimensional NMR (^{1}H, ^{13}C, HSQC, HMBC, COSY) and mass-spectroscopy (ESI/MS) data. The chemical structures of new compound was established as: 26-O-β-D-glucopyranosyl (25*R*), 5α-furost-20(22)-en-3β,26-diol-12-one 3-O-β-D-xylopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-O-β-D-galactopyranoside. The chemical composition of the roots of *T. terrestris* was studied and the presence of steroidal glycosydes was determined. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: *Tribulus terrestris* L. Zygophyllaceae, steroidal glycosides, furostanols

The usage of *Tribulus terrestris* L. has the history of a few centuries. The plant was used in Asian traditional medicine against many diseases, especially for increasing the hormonal activity. *T. terrestris* is recognized as a natural source as stimulant of human generative function. The chemical composition and biological activities of the plant were widely studied. Its diverse influence on almost every vital function of the body is already determined. Ten of steroidal glycosides derived from different aglycones and the metabolites of other classes are isolated, especially from the leaves and fruits, but the roots was never was investigated.

During the last 15-20 years, intensive studies of the chemical composition and pharmacological efficiency of *Tribulus terrestris* L. started in many countries around the world [1-4]. Different compositions of *T. terrestris* growing in diverse geographical zones were shown.

The presence of steroidal compounds in *T. terrestris* was determined at the institute of pharmacochemistry. Based on them, a preparation „Tribusponin" was developed and it was successfully used in the medical practice for treatment and prevention of atherosclerosis. It is also used as a stimulant of human generative function, a source for

Table 1

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

C	δ(C)	δ(H)	C	δ(C)	δ(H)
1	37.6	1.59, m, 1.00, m	15	34.3	2.29, m, 1.61, m
2	30.1	1.88, m, 1.54, m	16	83.8	4.66, m
3	78.9	3.66, m	17	56.8	3.20, d, (10.0)
4	35.1	1.75, m, 1.33, m	18	14.4	0.94, s
5	45.6	1.13, m	19	12.5	0.85, s
6	29.4	1.39 (2H), m	20	103.9	-
7	32.0	1.80, m, 1.24, m	21	11.4	1.57, s
8	35.4	1.96, m	22	153.5	-
9	57.0	1.12, m	23	23.9	2.11 (2H), m
10	37.3	-	24	31.6	1.61 (2H), m
11	38.7	2.50, m, 2.17, m	25	33.9	1.75, m
12	215.9	-	26	75.6	3.70, dd, (10.5, 6.0), 3.38, m
13	58.4	-	27	17.2	0.95, d, (6.6)
14	55.6	1.37, m			

Table 2

^{1}H- and ^{13}C-NMR (600 MHz, CD_3OD) Data of the Sugar Moiety of 1. (*J* in Hz, d in ppm)

Gal	δ(C)	δ(H)	Glc I	δ(C)	δ(H)	Glc II	δ(C)	δ(H)
1"	102.4	4.52, d, (7.7)	1'''	104.3	4.58, d, (7.7)	1''''	104.0	4.91, d, (8.0)
2"	72.8	3.60	2'''	80.8	3.74	2''''	75.3	3.18
3"	75.3	3.50	3'''	87.6	3.69	3''''	77.8	3.33
4"	79.9	4.01	4'''	70.4	3.26	4''''	71.3	3.31
5"	75.2	3.49	5'''	77.6	3.28	5''''	77.7	3.27
6"	60.8	3.89, 3.60	6'''	62.9	3.88, 3.56	6''''	62.4	3.87, 3.81

Xyl	δ(C)	δ(H)	Glc	δ(C)	δ(H)
1'''''	104.7	4.60, d, (7.8)	1'	104.3	4.22, d, (7.9)
2'''''	75.0	3.24	2'	75.2	3.17
3'''''	77.8	3.32	3'	78.1	3.34
4'''''	70.7	3.51	4'	71.7	3.27
5'''''	67.0	3.90, 3.25	5'	77.9	3.25
			6'	62.9	3.84, 3.66

increasing the working capacity of sportsmen and rehabilitation of sick people. Due to its chemical composition and pharmacological efficiency, Tribusponin is prioritized among other preparations of *T. terrestris* [5].

In *T. terrestris* growing in Georgia there were found the new flavonoids with rare monosaccharide - apiose, named apiotribosides A-D and the compounds of the new class for genus *Tribulus* - nucleosides: adenosine, guanosine, cytidine, uridine and adenine. The discovery of new data of chemical composition of *T. terrestris* can explain the mechanism of multilateral action of the plant.

The further study of the chemical composition and pharmacological efficiency of *T. terrestris* of Georgian flora is giving a hope for getting new components and revealing interesting effects. The present work belongs to the phytochemical researches of *T. terrestris* roots, especially on the steroidal glycosides. 500 g of powdered roots were extracted by shaking with 2.5 lof 80%MeOH once an hour at room temperature and then twice at 60 °C. The collected extracts were dried under reduced pressure and the concentrate was partitioned between $CHCl_3$ and H_2O. The H_2O extract was subjected to *Diaion HP-20* column chromatography (50 x 4 cm) and eluted with a

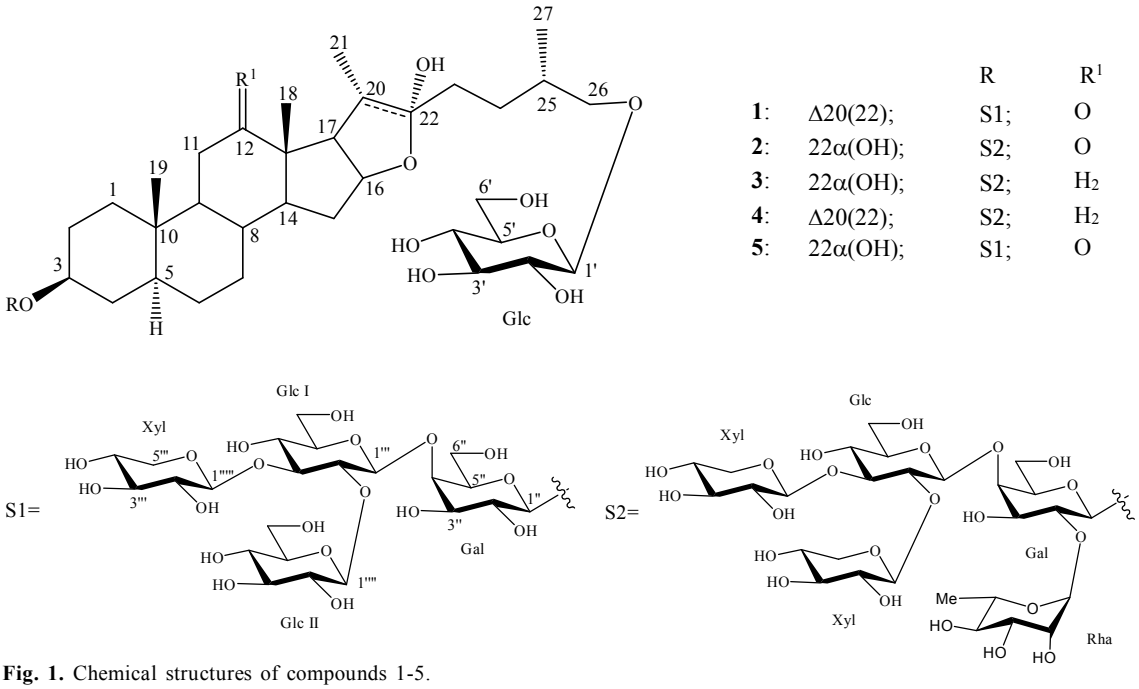

Fig. 1. Chemical structures of compounds 1-5.

gradient system of H_2O-MeOH 10:0 to 0:10 to yield two fractions (500 ml each) - 35% MeOH, 80% MeOH. A part of the 80% MeOH fraction (3 g) was separated on *Sephadex LH-20* (100 g), eluted with MeOH, enriched fractions were then separated by CC (SiO_2; 100 g, 500 x 25 mm), eluted with $CHCl_3$/MeOH/H_2O 26:14:3, to afford five furostanol glycosides: compound **1** (2.3 mg), chloromaloside E (**2**;2.1 mg) [7], terestrinin B (**3**;2.2 mg) [7], terestroside A (**4**;2.4 mg) [7] and polianthoside D (**5**;2.6 mg) [8] (Fig. 1).

The molecular formula of **1** was unequivocally established to be $C_{56}H_{91}O_{28}$ by HRTOFMS (*m/z* 1211.2540 $[M+H]^+$). The positive ESIMS mass spectrum of **1** showed the major ion peak at *m/z* 1211 which was assigned to $[M+H]^+$. The MS/MS of this ion showed peaks at *m/z* 1049 $[M+H-162]^+$ due to the loss of a sugar group, and at *m/z* 917 $[M+H-162-132]^+$, *m/z* 775 $[M+H-162x2-132]^+$, *m/z* 593 $[M+H-162x3-132]^+$ and *m/z* 431 $[M+H-162x4-132]^+$, corresponding to the loss of pentose, one, two and three hexose units, respectively.

The ^{1}H-NMR spectrum of compound **1** showed signals for three tertiary methyl groups at δ(H) 0.85 (*s,* Me(19)), 0.94 (*s*, Me(18)) and 1.57 (*s*, Me(21)), a secondary methyl group at δ(H) 0.95 (*d, J*=6.6 Hz, Me(27)), two methine proton signals at δ(H) 3.66 (*m*, H-C(3)) and 4.66 (*m*, H-C(16)), two methylene proton signals at δ(H) 3.70 (*dd*, *J*=10.5, 6.0 Hz, H_a-C(26)) and 3.38 (*m*, H_b-C(26)) ascribable to a primary alcoholic function. The ^{13}C-NMR spectrum displayed, for the aglycon moiety, signals ascribable to a keto group at *d*(C) 215.9, a double bond in position C-20(22) at δ(C) 103.9 (C(20)) and 153.5 (C(22)), two secondary alcoholic functions at δ(C) 78.9 (C(3)) and 83.8 (C(16)), and one primary alcoholic function at δ(C) 75.6 (C(26)), suggesting the occurrence of a glycosidic furostanol skeleton characterized by the occurrence of a keto group (Table 1). The C(25) configuration was deduced to be *R* based on the difference of chemical shifts of the geminal protons at $H_{a,b}$-C(26) (”ab=0.32 ppm). Resonances in ^{1}H- and ^{13}C-NMR spectra with *d*(H) 1.13 (*m*, H-C(5)) and *d*(C) 45.6 (C(5)) were indicative of a 5α-series steroid [9]. The aglycon of **1** was identified as (25*R*), 5α-furost-20(22)-en-3β,26-diol 12-one – pseudohecogenin [10]. The HSQC spectrum of **1** displayed five cross peaks of δ(H)/δ(C) 4.91 (*d*, *J*=8.0 Hz)/104.0, 4.60 (*d*, *J*=7.8 Hz)/104.7, 4.58 (*d*, *J*=7.7 Hz)/104.3, 4.52 (*d*, *J*=7.7 Hz)/102.4, and 4.22 (*d*, *J*=7.9 Hz)/

104.3 indicating the presence of five sugar moieties. Units of three β-D-glucopyranosyl (Glc I, Glc II, and Glc III), one β-D-galactopyranosyl (Gal) and one β-D-xylopyranosyl (Xyl) unit were identified by extensive 2D NMR spectral analysis (Table 2). The Gal and Glc I residues were shown to be attached at C(3) and C(26) of the aglycone by an observed HMBC correlations of δ(H)/*r*(C) 4.52 (H-C(1'))/78.9 (C(3)) and 4.22 (H-C(1'))/78.9 (C(26)), respectively. The HMBC cross-peaks of δ(H) 4.58 (H-C(1''))/δ(C) 79.9 (C(4'')), δ(H) 4.91 (H-C(1'''))/δ(C) 80.8 (C(2''')) and δ(H) 4.60 (H-C(1''''))/δ(C) 87.6 (C(4''')), allowed the sequence Xyl-(1→3)-[Glc III-(1→2)]-Glc II- to be linked at C(4'') of Gal. On the basis of the above results, the structure of compound **1** was elucidated as 26-O-β-D-glucopyranosyl (25*R*), 5α-furost-20(22)-en-3β,26-diol-12-one 3-O-β-D-xylopyranosyl-(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-O-β-D-galactopyranoside.

It gives attention that the roots of *T. terrestris*, as the leaves, contain steroidal glycosides, especially furostanol type.

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ფარმაკოქიმია

Tribulus terrestris L. კუროსთავის ფესვების ფუროსტანოლური გლიკოზიდები

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საქართველოში მოზარდი კუროსთავის *Tribulus terrestris* ფესვებიდან იზოლირებულია ერთი ახალი და ოთხი ცნობილი ფუროსტანოლური გლიკოზიდი. ნივთიერებათა სტრუქტურები დადგენილია კლასიკური ფიზიკურ-ქიმიური მეთოდებით და თანამედროვე სპექტრული ანალიზების ერთ- და ორგანზომილებიანი ბმრ (^{1}H, ^{13}C, HSQC, HMBC, COSY) და მას-სპექტროსკოპიის (ESI/MS) გამოყენებით. ახალი ნივთიერების ქიმიური სტრუქტურა დახასიათებულია როგორც: 26-O-β-D-გლუკოპირანოზილ(25R), 5α-ფუროსტ-20(22)-ენ-3β,26-დიოლ-12-ონ 3-O-β-D-ქსილოპირანოზილ-(1→2)-O-[β-D-გლუკოპირანოზილ-(1→3)]-O-β-D-გლუკოპირანოზილ-(1→4)-O-β-D-გალაქტოპირანოზიდი. *T.terrestris* ფესვების ქიმიური შედგენილობა არის შესწავლილი და დადგენილია მასში სტეროიდული გლიკოზიდების არსებობა.

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