ARJUNOLIC ACID DERIVATIVE GLYCOSIDE FROM THE STEMS OF *Hedera colchica*

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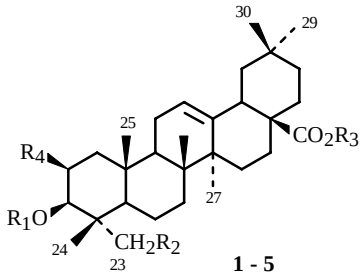
Five triterpenoid saponins were isolated from the stems of Hedera colchica K. Koch, *Araliaceae. Two of them are new natural substances. HCSt-A (**1**): 3-O-α-D-arabinopyranoside; 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-arjunolic acid. HCSt-B (**2**): 3-O-β-D-xylopyranoside; 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-hederagenin. The derivative of arjunolic acid is described for the first time in* Araliaceae *family. The chemical structures of isolated compounds were established on the base of chemical and 1D and 2D NMR experiments.*

Key words: *Hedera colchica,* Araliaceae, triterpenoid saponins, arjunolic acid, NMR.

Hedera colchica K. Koch, *Araliaceae*, is an endemic plant growing mainly in West Georgia. Wide application of different parts of this plant in traditional medicine as a bronchospasmolitic, secrotolitic, and antiinflammatory remedy [1, 2] was the main reason for the begining of its deep phytochemical investigation. The chemical constitutents of triterpene saponins from the leaves and berries of *H. colchica* was described previously [3–6].

The isolated triterpene glycosides have shown a wide spectrum of biological activities [7–11]. The aim of the present work was the investigation of triterpene saponins from the stems of *H. colchica* for the purpose of determining the chemical structure-biological activity relationship of glycosides isolated from the different parts of this plant. In this paper we describe the isolation and structure determination of five saponins from the stems of *H. colchica* (Fig. 1). Saponins HCS-A (**1**) and HCS-B (**2**) were new compounds, and the derivative of arjunolic acid (**1**) was described for the first time in the *Araliaceae* family. The *n*-butanolic extract of the stems containing crude saponins was subjected to repeated chromatography affording saponins **1**–**5** (see experimental part). The glycosides **3**, **4**, and **5** were identified as hederasaponin C [12], and hederacolchisides E and F [3, 4] respectively.



1 - 5

	R_1	R_2	R_3	R_4
1:	Ara	OH	Rha1-4Glc1-6Glc	OH
2:	Xyl	OH	Rha1-4Glc1-6Glc	H
3:	Rha 1- 2 Ara	OH	Rha1-4Glc1-6Glc	H
4:	Rha 1- 2 [Glc1-4] Ara	H	Rha1-4Glc1-6Glc	H
5:	Rha 1- 2 [Glc1-4] Ara	OH	Rha1-4Glc1-6Glc	H

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TABLE 1. ^{1}H and ^{13}C NMR Chemical Shifts (δ, ppm) of the Aglycon Parts of **1** and **2** (Py-d_5, O-TMS)

Compounds											
1						**2**					
C atom	^{13}C	^{1}H	C atom	^{13}C	^{1}H	C atom	^{13}C	^{1}H	C atom	^{13}C	^{1}H
1	47.4	1.26, 2.32	16	23.4	1.89, 2.02	1	39.0	1.09, 1.62	16	23.4	1.91, 2.03
2	67.1	4.18	17	n.d	-	2	26.1	2.23, 2.01	17	47.3	-
3	88.6	4.18	18	41.7	3.16	3	82.3	4.24	18	41.8	3.18
4	44.8	-	19	46.2	1.70, 1.22	4	43.6	-	19	46.4	1.72, 1.22
5	47.5	1.79	20	30.8	-	5	47.8	1.65	20	30.9	-
6	18.3	1.68, 1.36	21	34.0	1.31, 1.11	6	18.3	1.66, 1.37	21	34.1	1.33, 1.13
7	32.9	1.62, 1.32	22	32.6	1.73, 1.88	7	33.0	1.60, 1.31	22	32.7	1.77, 1.90
8	40.2	-	23	63.9	3.64, 4.44	8	40.4	-	23	64.9	3.70, 4.27
9	48.3	1.84	24	14.6	0.96	9	48.2	1.76	24	13.6	0.96
10	38.0	-	25	17.5	1.06	10	37.1	-	25	16.2	1.0
11	23.7	2.01, 1.01	26	17.6	1.09	11	23.4	1.95, 1.95	26	17.7	1.11
12	123.2	5.39	27	25.9	1.15	12	123.0	5.41	27	26.1	1.19
13	144.4	-	28	176.8	-	13	144.2	-	28	176.2	-
14	42.3	-	29	33.1	0.87	14	42.2	-	29	33.1	0.88
15	28.4	1.09, 2.25	30	23.7	0.90	15	28.4	1.10, 2.28	30	23.8	0.91

The alkaline hydrolysis of compound **1** and **2** showed their bidesmosidic nature; the same monosaccharide constituents, rhamnose and glucose, were detected in the acid hydrolysate of the oligosaccharide part connected to the C-28 position of the genin in both cases.

The acid hydrolysis of the obtained progenins yielded arabinose and an unidentified (by thin layer chromatography), unknown genin in the case of (**1**) and xylose and hederagenin for (**2**).

The identification of xylose in the carbohydrate part of HCS-B confirms once more [6] that this monosaccharide is characteristic only for *colchica* species of genus *Hedera*.

The final chemical structures of the isolated saponins were established by ^{1}H and ^{13}C NMR methods (1D and 2D experiments: COSY, HMBC, HSQC-TOCSY, ROESY).

From the ^{13}C NMR resonance of C-3 (Table 1) at δ 88.6 for **1** and 83.3 for **2**, and also the resonance of C-28 (δ 176.8 and 176.2, respectively), both compounds were bidesmosides. The downfield signal (δ 67.1) of C-2 of the genin of **1** and the proton chemical shift of H-2 (δ 4.18) confirm the substitution of the OH group at the C-2 position of the aglycon of **1**. On the basis of the ^{1}H, ^{1}H coupling constants and ROESY cross peaks the α- and β-positions of the protons in the aglycons were elucidated. On the basis of an analysis of COSY and HSQC experiments, the final structures of aglycon parts **1** and **2** have been established as arjunolic acid [13] and hederagenin, respectively.

The ^{13}C NMR spectrum exhibited four anomeric carbons (Table 2) located at δ 106.9, 96.0, 105.2, and 105.3 for **1** and 106.8, 95.8, 104.9, and 102.8 for **2**. On the basis of COSY and HSQC-TOCSY experiments, α-arabinose, α-rhamnose, and two residues of β-glucose were identified in **1**. In the sugar chain of **2**, the only difference was the presence of β-xylose instead of arabinose. The position of the glycosyl attachments at the aglycones and their interglycosidic junctions were unambigously determined by the HMBC cross peaks.

Further analysis of the ^{13}C NMR data showed that the sugar chain rhamnose 1–4 glucose 1–6 glucose, linked at C-28, was identical for saponins **1** and **2**. These results were in perfect agreement with the literature data for the sugar chain linked at C-28 [5].

On the basis of the obtained results the structures of HCS-A (**1**) and HCS-B (**2**) proposed as 3-O-α-L-arabinopyranoside; 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-arjunolic acid and 3-O-β-L-xylopyranoside; 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl-hederagenin respectively. Both are new natural compounds.

TABLE 2. ^{1}H and ^{13}C NMR Data of Carbohydrate Residues of **1** and **2** (Py-d_5, O-TMS, δ, J/Hz)

Compounds						
1				**2**		
C atom	^{13}C	^{1}H		C atom	^{13}C	^{1}H
Sugar on C-3						
Ara 1	106.9	4.94 (J = 7.6)	Xyl	1	106.8	5.01 (J = 7.3)
2	73.1	4.44		2	75.6	3.97
3	74.5	3.99		3	78.6	4.03
4	69.8	4.16		4	71.1	4.17
5	67.8	3.68, 4.20		5	67.2	3.63, 4.30
Sugar on C-28						
Glc 1	96.0	6.17 (J = 7.6)	Glc	1	95.8	6.19 (J = 7.8)
2	74.0	4.09		2	74.0	4.09
3	78.8	4.05		3	78.8	4.17
4	71.1	4.24		4	71.1	4.23
5	78.2	4.07		5	78.1	4.08
6	69.4	4.62, 4.29		6	69.5	4.64, 4.30
Glc 1	105.2	4.95 (J = 7.5)	Glc	1	104.9	4.96 (J = 7.4)
2	75.4	3.9		2	75.5	3.9
3	76.7	4.09		3	76.6	4.11
4	78.8	4.29		4	78.7	4.34
5	77.3	3.64		5	77.2	3.67
6	61.5	4.05, 4.18		6	61.6	4.07, 4.20
Rham 1	103.0	5.75	Rham	1	102.8	5.77
2	72.5	4.60		2	72.5	4.61
3	72.8	4.48		3	72.8	4.48
4	74.2	4.27		4	74.1	4.26
5	70.4	4.82		5	70.4	4.85
6	18.5	1.66		6	18.5	1.67

Ara - α-L-arabinopyranosyl; Xyl - β-D-Xylopyranosyl; Glc - β-D-glucopyranosyl; Rham - α-L-rhamnopyranosyl.

EXPERIMENTAL

All specra were recorded in pyridine-d_5 at 313 K using a Varian UnityInova 600 MHz with a 5 mm triple-resonance probe for the acquisition of proton detected spectra and UnityInova 400 NMR spectrometer with a 5 mm broadband probe for the acquisition of 1D ^{13}C spectra.

TMS was used as internal standard, and chemical shifts are given in ppm. Varian pulse sequences from the user library were used for the NMR experiments. 600 MHz ^{1}H, ^{1}H DQF-COSY spectra: acquisition time 0.20 s, relaxation delay 2.0 s, 8 transients per increment.

600 MHz ^{1}H,^{1}H ROESY spectra: spinlock 300 ms. 600/150 MHz gradient selected HSQC spectra with multiplicity editing: carrier at 5 ppm, acquisition time 0.2 s, relaxation delay 1.3 s. TLC analyses of saponins and sugars were performed on precoated silica gel plates (Kieselgel 60F254, Merck) using the following solvent systems: $CHCl_3$–MeOH–H_2O (a – 26:14:3; b – 55:40:10) for saponins, c – $CHCl_3$–MeOH (20:1) for genins, d – CH_2Cl_2–MeOH–H_2O (50:25:5) for sugars. Spots were detected by spraying the plates with phosphoric acid naphthoresorcinol for sugars and 25% H_2SO_4 in MeOH for saponins and genins followed by heating at 100°C.

Extraction and separation: Plant material was collected in the Bagdati region of Georgia (September 1996) and dried in the shade. A voucher speciman is kept in the Department of Pharmacobotany, Institute of pharmacochemistry, Tbilisi, Georgia (stems N80996).

Crushed stems (500 g), were extracted with MeOH–H_2O (80:20 v/v, 1 l three times). After evaporation of MeOH, the aqueous layer was treated with $CHCl_3$, and the water extract of saponins was dried. The 66 g of obtained saponin crude extract was subjected to column chromatography (CC) on Silica gel (0.04–0.06 mm, Merck) eluting with solvent system (a) to efford three different fractions. Further purification of the polar fraction, containing at least six compounds, by repeated CC eluting with system (b) and repurification of the obtained rich fractions on a "FLASH" column of Rp18 (15–25 μm Lichroprep, Merck, air pressure 2 bar), using solvent MeOH–H_2O (from 40:60 to 80:20), yielded **1** (20 mg), **2** (20 mg), **3** (300 mg), **4** (60 mg), and **5** (15 mg).

Acid hydrolysis: The saponins (3 mg) were heated with aqueous 10% HCl (3 ml) in a sealed tube at 100°C for 90 min. The sapogenin was extracted with Et_2O. Then the aqueous layer was neutralized with N,N-dioctylmethylamine (10% in $CHCl_3$) and dried. The sapogenin and sugars were identified by TLC analysis with authentic samples in systems (c) and (d), respectively.

Alkaline hydrolysis: The saponins (5 mg) in 5 % aqueous KOH (5 ml) were heated at 100°C in a sealed tube for 90 min. After neutralization with 10% HCl (pH 5) the prosapogenin was extracted with BuOH. TLC analysis was performed using systems (a).

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