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

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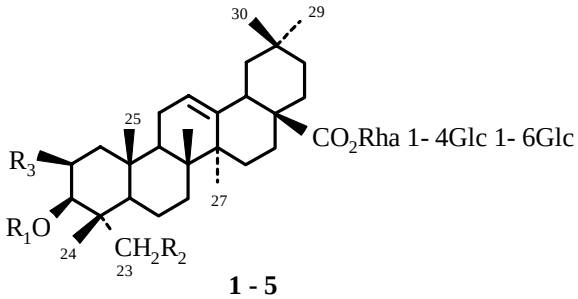
UDC 547.92:547.918

Five triterpenoid saponins were isolated from the stems of Hedera colchica *K. Koch (Araliaceae). Two of them are new natural substances. HCS-A (**1**): 3-O-α-L-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. A derivative of arjunolic acid is described for the first time in the Araliaceae family. The chemical structures of isolated compounds were established on the basis 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. The wide application of different parts of this plant in traditional medicine as a bronchospasmolitic, secrotolitic, and antiinflamatory remedy [1, 2] was the main reason of begining its deep phytochemical investigation. The chemical constitutents of triterpene saponins from the leaves and berries of *H.colchica* were 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**) are new compounds; a derivative of arjunolic acid (**1**) is 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**–**5** were identified as hederasaponin C [12], and hederacolchisides E and F [3, 4], respectively.



1 - 5

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

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 0009-3130/05/4101-0048

TABLE 1. ^{1}H and ^{13}C NMR Chemical Shifts (δ, ppm) of the Aglycon Parts of **1** and **2** in Pyridine-d_5, $SiMe_4$ as Internal Standard

Compounds											
1						**2**					
Atom	^{13}C	^{1}H	Atom	^{13}C	^{1}H	Atom	^{13}C	^{1}H	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, 2.01	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

Alkaline hydrolysis of compound **1** and **2** showed their bidesmosidic nature; the same monosaccharide constitutents, 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.

Acid hydrolysis of the obtained progenins yielded arabinose and an unidentified (by thin layer chromatography) 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 the *colchica* species of the 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).

The ^{13}C NMR resonance of C-3 (Table 1) at δ 88.6 for **1** and 83.3 for **2** and the resonance of C-28 (δ 176.8 and 176.2, respectively) show that both compounds are 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, we were able to elucidate the α- and β-positions of the protons in the aglycons. On the basis of an analysis of the 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, 105.3 for **1** and 106.8, 95.8, 104.9, 102.8 for **2**. On the basis of COSY, HSQC, and TOCSY experiments, α-arabinose, α-rhamnose, and two residues of β-glucose were identified in the case of **1**; β-xylose was the only difference (instead of arabinose) in the sugar chain of **2**. The position of the glycosyl attachments at the aglycones and their interglycosidic junctions were unambigously determined by the HMBC cross peaks.

Further analyses 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 results obtained the structures of HCS-A (**1**) and HCS-B (**2**) are 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-D-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** in Pyridine-d_5, $SiMe_4$ as Internal Standard (J/Hz)

Compounds							
1				**2**			
Atom		^{13}C	^{1}H	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 = 7.5)	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
Rha	1	103.0	5.75	Rha	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; Rha = α-L-rhamnopyranosyl.

EXPERIMENTAL

All specra were recorded in pyridine-d_5 at 313 K using a Varian UnityInova 600 MHz spectrometer with a 5 mm triple-resonance probe for the acqisition 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 the 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 "FLASH" column of Rp 18 (15–25 mm Lichroprep, Merck, air pressure 2B) 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 Hydrolyses. 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 saponin (5 mg) in 5% aqueous KOH (5 ml) was 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 system a).

ACKNOWLEDGMENT

We thank the Austrian Academy of Sciences and Karl-Franz University of Graz for financial and technical support.

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