Triterpenoid Saponins from Berries of *Hedera colchica*

Vakhtang MSHVILDADZE,[a] Riad ELIAS,*[,b] Robert FAURE,[c] Laurent DEBRAUWER,[d] Genri DEKANOSIDZE,[a] Etheri KEMERTELIDZE,[a] and Guy BALANSARD[b]

Institute of Pharmacochemistry, Academy of Sciences of Georgia,[a] 36, St. P. Sarajishvili, 380059 Tbilisi, Georgia, Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université de la Méditeranée,[b] 27 Boulevard Jean Moulin, 13385 Marseille, cedex 5, France, ESA 6009, Université d'Aix-Marseille III,[c] Avenue Escadrille Normandie Niemen, 13397 Marseille, cedex 20, France, and INRA Centre de Recherche de Toulouse, Laboratoire des Xénobiotiques,[d] 180, Chemin de Tournefeuille, BP 3, 31931 Toulouse, France. Received October 16, 2000; accepted February 10, 2001

Fifteen triterpenoid saponins were isolated from the berries of *Hedera colchica* and their structures established on the basis of chemical and spectroscopic evidence. Among them, two are new compounds: colchiside A (3) and colchiside B (15) and four are described for the first time in the berries of *Hedera colchica* (compounds 1, 8, 9 and 11).

Key words *Hedera colchica*; Araliaceae; triterpenoid saponin; NMR; MS; colchiside

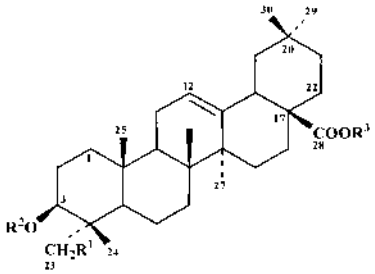
Hedera colchica K. KOCH. (Araliaceae), a plant mainly growing in West Georgia, was used in traditional medicine as a bronchospasmolytic, secretolytic and antiinflammatory remedy.[1,2] The occurrence of triterpenoid saponins in the leaves of this plant has been previously reported.[3a,b,4] In this paper, we describe the isolation and structure elucidation of fifteen saponins from the berries of *Hedera colchica* (Fig. 1). Saponins **3** and **15**, respectively named colchisides A and B were new compounds, while saponins **1**, **8**, **9** and **11** were isolated for the first time from the berries of this plant. The *n*-butanolic extract of the berries containing crude saponins was subjected to repeated chromatography affording saponins **1**—**15** (see experimental part).

Compounds **8** and **11**, which were obtained for the first time in the genus Hedera, were identified as staunoside A[5] and scheffleraside II[6] respectively, while triterpenoid saponins **1** and **9**, identified as saponin 1[7] and saponin I,[8] were isolated for the first time from the berries of *Hedera colchica.* The other saponins **2**, **4**, **5**, **6**, **7**, **10**, **12**, **13** and **14** were identified as saponin B,[8] saponin 2,[7] α-hederin,[9] saponin 4,[7] heteroside E2,[10] heteroside I,[11] hederasaponin D,[12] hederasaponin B[12,13] and hederasaponin C,[13,14] respectively.

Colchiside A (**3**) was assigned the molecular formula $C_{35}H_{56}O_8$ (FAB-MS (*m*/*z*): 603 $[M-H]^-$). Acid hydrolysis of **3** yielded xylose as the sugar and hederagenin as the aglycone. The ^{13}C-NMR spectrum exhibited one anomeric carbon at δ 106. The resonances of C-3 at δ 83 and C-28 at δ 182 reflected the fact that the sugar chain was linked to C-3. Moreover, the ^{13}C chemical shifts of the xylose moiety were indicative of a β-D-xylopyranosyl.[15,16] Thus **3** was a monodesmoside and its structure was elucidated as 3-*O*-(β-D-xylopyranosyl)-hederagenin.

The FAB-MS of colchiside B (**15**) gave the molecular ion at *m*/*z* 1247 $[M-H]^-$ in agreement with a molecular formula of $C_{60}H_{96}O_{27}$. TLC analysis of the acid hydrolysis of **15** yielded the same sugars (glucose, rhamnose, glucuronic acid) and aglycone (oleanolic acid) as **11**. The ^{13}C-NMR spectrum of **15** exhibited five anomeric carbons (Table 1) located at δ 95.76, 102.72, 102.94, 104.27 and 106.83 ppm. Moreover, the ^{13}C-NMR signal due to the C-28 genin moiety indicated esterification of the carboxyl group with a sugar, while the deshielded position of C-3 reflected a substitution by a sugar chain. Saponin **15** was consequently a bidesmoside. On alkaline hydrolysis **15** gave a prosapogenin. Acid hydrolysis of this prosapogenin yielded glucuronic acid and rhamnose as the sugars. Further analysis of the ^{13}C-NMR data showed that the sugar chain linked at C-28 was identical for saponins **11** and **15**. Moreover, the downfield shift (83.6 ppm) of C-3 in the glucuronic acid moiety was indicative of a substitution at C-3 by a rhamnose unit. Indeed, these ^{13}C-NMR results were in perfect agreement with the literature data for the sugar chain linked at C-28[13] and for the sugar arrangement at C-3[14] in which C-3 of glucuronic acid was found at δ 83.

Moreover, the values for the carboxylic group of the glucuronic part were found for compounds **11** and **15** at δ 176. In previously reported data,[14] the carboxylic signals res-



	R^1	R^2	R^3
1	H	Ara	H
2	OH	Ara	H
3	OH	Xyl	H
4	OH	Glc	H
5	OH	Ara^2—^{1}Rha	H
6	OH	Glc^2—^{1}Glc	H
7	H	Glc^2—^{1}Glc	H
8	OH	Glc	Glc^6—^{1}Glc
9	H	Glc A	H
10	OH	Glc^2—^{1}Glc	Glc^6—^{1}Glc
11	H	Glc A	Glc^4—^{1}Glc^1—^{2}Rha
12	OH	Ara	Glc^6 ^{1}Glc^1 ^{2}Rha
13	H	Ara^2—^{1}Rha	Glc^6—^{1}Glc^1—^{4}Rha
14	OH	Ara^2 ^{1}Rha	Glc^6—^{1}Glc^1 ^{4}Rha
15	H	Glc A^3—^{1}Rha	Glc^6 ^{1}Glc^1—^{4}Rha

Ara : α-L-arabinopyranosyl
Rha : α-L-rhamnopyranosyl
Xyl : β-D-xylopyranosyl
Glc : β-D-glucopyranosyl
Glc A : β-D-glucuronopyranosyl

Fig. 1. List of Triterpenoid Glycosides Isolated from the Berries of *Hedera colchica*

* To whom correspondence should be addressed. e-mail: Riad.Elias@pharmacie.univ-mrs.fr

Table 1. ^{13}C-NMR Data for Sugar Moieties of Saponins **1**, **3**, **8**, **9**, **11** and **15** (CD_3OD)

1		**3**		**8**		**9**		**11**		**15**	
Sugar on C-3[a]											
ara 1	107.08	xyl 1	106.30	glc 1	105.72	glc A1	106.97	glc A1	106.96	glc A1	106.83
2	72.84	2	75.55	2	75.62	2	76.52	2	76.55	2	75.37
3	74.34	3	78.14	3	78.33	3	77.70	3	77.72	3	83.59
4	69.46	4	71.26	4	71.53	4	73.22	4	73.26	4	71.85
5	66.29	5	66.84	5	77.72	5	75.32	5	75.32	5	76.71
				6	62.72	6	172.91	6	175.97	6	176.06
										rham 1	102.72
										2	72.26
										3	72.36
										4	74.04
										5	69.94
										6	17.87
Sugar on C-28[a]											
				glc 1	95.75			glc 1	95.77	glc 1	95.76
				2	73.83			2	73.86	2	73.84
				3	79.79			3	79.59	3	79.52
				4	70.94			4	70.99	4	70.95
				5	78.16[b]			5	78.06	5	78.06
				6	69.49			6	69.42	6	69.41
				glc 1	104.63			glc 1	104.28	glc 1	104.27
				2	75.12			2	75.32	2	75.31
				3	77.99[b]			3	76.84	3	76.84
				4	71.53			4	78.21	4	78.18
				5	77.81			5	76.74	5	76.71
				6	62.72			6	61.88	6	61.87
								rham 1	102.96	rham 1	102.94
								2	72.24	2	72.23
								3	72.44	3	72.43
								4	73.78	4	73.76
								5	70.67	5	70.65
								6	17.85	6	17.87

a) ara=α-L-arabinopyranosyl; xyl=β-D-xylopyranosyl; glc=β-D-glucopyranosyl; glc A=β-D-glucuronopyranosyl; rham=α-L-rhamnopyranosyl. *b*) Assignments may be reversed.

onated between 172 and 176 ppm depending on the pH of the saponin solutions. It can be concluded that **15** was 3-*O*-[-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyl]-28-*O*-[-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl]-oleanolate.

Experimental

FAB-MS were obtained from a Nermag R-10-10H mass spectrometer in the negative ion mode. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard. Melting points were determined on an Electrothermal IA 9300 apparatus. Optical rotations $[\alpha]_D^{25}$ were measured on a Perkin-Elmer model 341 Orot polarimeter. TLC analyses of saponins and sugars were performed on precoated silica gel plates (Kiesegel 60F254, Merck) using the following solvent systems: $CHCl_3$–MeOH–H_2O (26 : 14 : 3) [system 1]; *n*-BuOH–HOAc–H_2O (4 : 1 : 5) [system 2]; $CHCl_3$–MeOH (20 : 1) [system 3]; CH_2Cl_2–MeOH–H_2O (50 : 25 : 5) [system 4]. Spots were detected by spraying the plates with phosphoric acid naphthoresorcinol for sugars and H_2SO_4 for saponines and genins followed by heating at 110 °C.

Extraction and Separation Plant material was collected in the Bagdathi region of Georgia (January 1996) and dried in the shade. A voucher specimen is kept in the Department of Pharmacobotanic, Institute of Pharmacochemistry, Tbilisi, Georgia (berries No. 80197). Crushed berries (720 g) were extracted with MeOH–H_2O (80 : 20). After concentration, the aqueous layer was treated with $CHCl_3$, then with BuOH to obtain a crude extract of saponins. The BuOH extract (146 g) was subjected to colum chromatography (CC) on silica gel (0.04—0.063 mm, Merck) eluting with $CHCl_3$–MeOH–H_2O (26 : 14 : 3) to afford 3 fractions. Fraction 1 subjected to repeated CC, eluting with $CHCl_3$–MeOH (from 9 : 1 to 4 : 1) yielded **1** (11 mg), **2** (80 mg), **3** (51 mg), **4** (600 mg), **5** (1.0 g) and a mixture of **6** and **7** with other unidentified minor compounds. Saponins **6** and **7** were purified by LPC on RP18 (15—25 μm, Merck), eluting with 80% MeOH to give **6** (45 mg) and **7** (1.0 g). Fraction 2 submitted to silica gel CC afforded saponin **14** (600 mg) and a mixture which treated by repeated CC on polyamide (SC6 0.07, Macherey-Najel) eluting with a gradient of MeOH in H_2O (from 10 to 65%) yielded **8** (75 mg), **9** (22 mg), **10** (57 mg), **12** (300 mg) and **13** (110 mg). Fraction 3 was chromatographied on a silica gel column eluting with $CHCl_3$–MeOH–H_2O (55 : 40 : 10), then subjected to repeated purification on a polyamide column (MeOH from 30 to 50%) to give **11** (35 mg) and colchiside B (**15**) (100 mg).

Acid Hydrolysis of 3 and 15 The saponin (3 mg) was heated with aqueous 10% HCl (3 ml) in a sealed tube at 100 °C for 4 h. 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 3 and 4, respectively.

Alkaline Hydrolysis of 3 and 15 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 systems 1 and 2.

Colchiside A (**3**): White powder; *Rf*=0.87 (in system 1). $[\alpha]_D^{25}=+12.6°$ (MeOH). Neg. FAB-MS (*m*/*z*): 603 $[M-H]^-$ (Calcd for $C_{35}H_{56}O_8$). ^{13}C-NMR data for aglycone part (CD_3OD): 39.52 (C-1), 26.31 (C-2), 83.45 (C-3), 43.90 (C-4), 49.03 (C-5), 18.95 (C-6), 33.80 (C-7), 40.60 (C-8), 48.24 (C-9), 37.73 (C-10), 24.15 (C-11), 123.70 (C-12), 145.28 (C-13), 43.00 (C-14), 28.85 (C-15), 24.67 (C-16), 47.70 (C-17), 42.80 (C-18), 47.35 (C-19), 31.60 (C-20), 34.98 (C-21), 33.50 (C-22), 65.02 (C-23), 13.51 (C-24), 16.49 (C-25), 17.87 (C-26), 26.57 (C-27), 182.15 (C-28), 33.63 (C-29), 24.07 (C-30). For sugars see Table 1.

Colchiside B (**15**): White powder; *Rf*=0.12 (in system 1); mp 180 °C; $[\alpha]_D^{25}=+15°$ (MeOH). Neg. FAB-MS (*m*/*z*): 1247 $[M-H]^-$ (Calcd for $C_{60}H_{96}O_{27}$). 1101 $[(M-H)-146]^-$; 777 $[(M-H)-146-2\times162]^-$; ^{13}C-NMR data for aglycone part (CD_3OD): 39.8 (C-1), 26.9 (C-2), 91.2 (C-3), 40.73 (C-4), 57.02 (C-5), 17.9 (C-6), 33.28 (C-7), 40.19 (C-8), 48.81 (C-9),

37.92 (C-10), 24.59 (C-11), 123.85 (C-12), 144.85 (C-13), 42.94 (C-14), 28.95 (C-15), 24.04 (C-16), 48.07 (C-17), 42.55 (C-18), 47.25 (C-19), 31.57 (C-20), 34.91 (C-21), 33.95 (C-22), 28.54 (C-23), 17.05 (C-24), 16.13 (C-25), 17.87 (C-26), 26.34 (C-27), 178.10 (C-28), 33.52 (C-29), 24.15 (C-30). For sugars see Table 1.

Acknowledgements This work was supported by the INTAS program (project No. 97-0491). We wish to thank Mr Gilbert BOUDON for his technical assistance.

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