Triterpenoid Saponins from the Leaves of Hedera helix

R. Elias, A. M. Diaz Lanza, E. Vidal-Ollivier,
G. Balansard, R. Faure, and A. Babadjamian

J. Nat. Prod., **1991**, 54 (1), 98-103• DOI:
10.1021/np50073a006 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 1, pp. 98-103, Jan-Feb 1991

TRITERPENOID SAPONINS FROM THE LEAVES OF *HEDERA HELIX*

R. ELIAS,* A.M. DIAZ LANZA, E. VIDAL-OLLIVIER, G. BALANSARD,

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 27, Boulevard Jean Moulin, 13385 Marseille Cédex 5, France

R. FAURE, and A. BABADJAMIAN

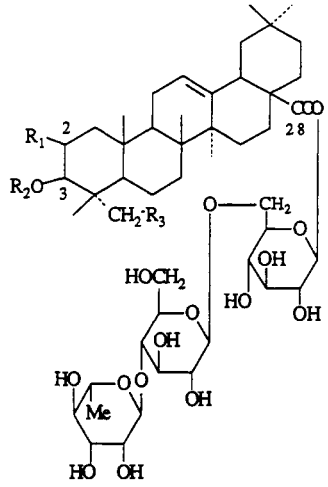
UA 126, CNRS, EIPSOI, Université d'Aix-Marseille III, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cédex 13, France

ABSTRACT.—From the leaves of *Hedera helix* four new triterpenoid saponins, hederasaponins E [**1**], F [**2**], H [**7**], and I [**8**], and one known saponin (cauloside F) were isolated and characterized by chemical and spectroscopic methods.

Saponins from the leaves of *Hedera helix* L. (Araliaceae) have antifungal, anthelmintic, molluscicidal, antileishmanial, and antimutagenic activities (1–5). Major saponins related to hederagenin and oleanolic acid have been found (6–8).

As a part of our investigations of *H. helix*, we have previously reported the isolation and identification of hederasaponins B [**6**], C [**4**], and D (saponin K 10) [**5**] (9).

In the present paper we describe the isolation and structural elucidation of five triterpenoid saponins. One of these, hederasaponin G, is cauloside F [**3**] and four are new saponins which we named hederasaponin E [**1**], hederasaponin F [**2**], hederasaponin H [**7**], and hederasaponin I [**8**].



R_1	R_2	R_3
OH	ara-1↦	OH
H	$SO_3^- H^+$	H
H	glc-(1↦2)-ara-1↦	OH
H	rha-(2↦2)-ara-1↦	OH
H	ara-1↦	OH
H	rha-(1↦2)-ara-1↦	H
H	gal-(1↦4)-glu-1↦	H
H	glu-1↦	OH

ara = α-L-arabinopyranosyl; glc = β-D-glucopyranosyl; rha = α-L-rhamnopyranosyl; gal = β-D-galactopyranosyl; glu = β-D-glucuronopyranosyl.

RESULTS AND DISCUSSION

The *n*-BuOH extract of the leaves containing crude saponins was subjected to repeated cc on reversed-phase RP 18 and Si gel (see Experimental) affording saponins **1**–**8**. Three compounds, **4**, **5**, and **6**, were identified to be, respectively, hederasaponin C, hederasaponin D (saponin K 10), and hederasaponin B, as previously established by using ^{1}H and ^{13}C 2D nmr spectroscopy studies (9).

Saponins **1**, **2**, **3**, **7**, and **8** were submitted to alkaline and acid hydrolysis, fabms, and ^{13}C nmr. On alkaline hydrolysis the five saponins yielded glucose and rhamnose; on the other hand the fabms of these saponins showed the loss of the same fragment at *m/z* 470 corresponding to two hexose and one rhamnose moieties (see Experimental). On the basis of ^{13}C-nmr spectral data (Table 1), signals due to the C-28 of aglycone moieties at 178 ppm indicated the esterification of the carboxyl groups with a sugar chain. From the ^{13}C-nmr spectra of sugar moieties (Table 2) and by comparison with literature data (9,10), it can be concluded that the sugar chain linked at C-28 was identical for all these saponins and elucidated as 28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside.

TABLE 1. ^{13}C-nmr Chemical Shifts of Aglycone Moieties in CD_3OD.[a]

Carbon	Compound			
	1[b]	**2**	**7**	**8**
C-2	70.1	25.3	26.9	26.4
C-3	88.8	87.8	90.8	82.3
C-12	123.6	123.8	124.0	123.9
C-13	n.o.[c]	144.9	145.1	144.9
C-23	64.0	28.9	28.6	65.2
C-28	n.o.	178.2	178.3	178.3

[a] In ppm, TMS as internal reference.
[b]DEPT-45 ^{13}C-nmr data.
[c]n.o. = not observed.

Acid hydrolysis of **1** afforded glucose, rhamnose, and arabinose as sugar components and bayogenin as the aglycone as identified by tlc comparisons. The fabms exhibited a quasi molecular peak at *m/z* 1089 $[M-H]^-$ and a major fragment at *m/z* 619 $[M-H-470]^-$, corresponding to the loss of two hexose and one rhamnose moieties. From the DEPT-45 ^{13}C-nmr spectra, signals attributed to C-1, C-2, C-3, C-12 and C-23 (47.2, 70.1, 88.8, 123.6, and 64.0 ppm, respectively) of the aglycone moiety were compared with published data (11) to confirm that the aglycone of **1** is bayogenin (Table 1). The important downfield shift for C-3 of the aglycone indicated that a sugar, which was identified as α-L-arabinopyranosyl (Table 2), was linked at this position. From these results the structure of **1**, which we named hederasaponin E, was elucidated as 3-*O*-α-L-arabinopyranosylbayogenin-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside.

Saponin **2** afforded oleanolic acid, glucose, and rhamnose on acid hydrolysis. Alkaline hydrolysis gave a compound that furnished a sulfate group after acid hydrolysis (by pc). The microanalysis of this saponin showed 3% of S. The fabms exhibited a molecular ion at *m/z* 1005 $[M-H]^-$ and a fragment ion at *m/z* 535 $[M-H-470]^-$, which indicated the loss of two hexose and one rhamnose moieties and the attachment of a sulfate group on oleanolic acid. The ^{13}C-nmr spectra (Tables 1 and 2) of compound **2**

TABLE 2. ^{13}C-nmr Chemical Shifts of Sugar Moieties in CD_3OD (in ppm, TMS as internal reference).[a]

Compound							
1		**2**		**7**		**8**	
3-*O*-ara-1	106.3			3-*O*-glu-1	105.3	3-*O*-glu-1	104.9
2	72.9			2	76.3	2	76.5
3	74.7			3	78.2	3	78.3
4	67.8			4	83.2	4	73.8
5	67.9			5	74.1	5	75.2
				6	176.8	6	176.9
				gal-1	106.2		
				2	73.7		
				3	74.9		
				4	69.9		
				5	76.8		
				6	61.9		
28-*O*-glc-1	95.8	28-*O*-glc-1	95.8	28-*O*-glc-1	95.9	28-*O*-glc-1	95.9
2	73.8[b]	2	73.9[b]	2	73.9[b]	2	73.9[b]
3	79.6	3	79.9	3	79.9	3	79.9
4	70.9	4	71.3	4	71.2	4	71.2
5	78.1	5	78.1	5	78.1	5	78.1
6	69.4	6	69.7	6	69.7	6	69.7
glc-1	104.3	glc-1	104.4	glc-1	104.4	glc-1	104.4
2	75.3	2	75.3	2	75.3	2	75.4
3	76.8	3	76.8	3	76.8	3	76.9
4	78.2	4	78.3	4	78.4	4	78.3
5	76.7	5	76.8	5	76.8	5	76.9
6	61.8	6	62.1	6	62.1	6	62.0
rha-1	102.9	rha-1	102.9	rha-1	103.0	rha-1	103.0
2	73.2	2	72.4	2	72.4	2	72.4
3	72.4	3	72.4	3	72.5	3	72.5
4	73.7[b]	4	73.8[b]	4	73.9[b]	4	73.9[b]
5	70.6	5	70.7	5	70.8	5	70.8
6	17.9	6	17.8	6	17.8	6	17.8

[a]ara = α-L-arabinopyranosyl; glc = β-D-glucopyranosyl; glu = β-D-glucuronopyranosyl; gal = β-D-galactopyranosyl; rha = α-L-rhamnopyranosyl.

[b]Assignments in the same column may be interchanged.

and comparison with those of compound **7** and sulfated saponins in the literature data (12) established the location of the sulfate group at C-3 and of the sugar chains at C-28. Based on the above evidence, the structure of **2** was formulated as 3-*O*-β-sulfate-oleanolic acid-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside, which we named hederasaponin F.

On acid hydrolysis saponin **3** yielded hederagenin as the aglycone and glucose, rhamnose, and arabinose as sugar components. The fabms showed a quasi molecular peak *m/z* 1235 and a signal at *m/z* 765 resulting from the loss of sugar chains linked at C-28. From the ^{13}C-nmr spectra, it can be concluded that the structure of **3**, named hederasaponin G, was 3-*O*-[β-D-glucopyranosyl-(1↦2)-α-L-arabinopyranosyl]-hederagenin-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside, which was identical to cauloside F, previously isolated from *Caulophyllum robustum* (13) and *Pulsatilla campanella* (14).

Saponin **7** was hydrolyzed with mineral acid to give oleanolic acid and glucose, rhamnose, arabinose, galactose, and glucuronic acid as sugar components. The fabms spectrum gave a molecular ion at *m/z* 1263 $[M-H]^-$ and a fragment ion at *m/z* 793

$[M-H-470]^-$ (two hexose and one rhamnose moieties). The ^{13}C-nmr spectra (Tables 1 and 2) showed the presence of five monosaccharide units. The glycosylation shifts observed for C-2, C-3, C-23, and C-28 of the aglycone moiety indicated that **7** was a bidesmoside of oleanolic acid with glycosyl linkages at both the 3-hydroxyl and 28-carboxyl groups. The sugar chain at C-3 was identified as containing a glucuronic acid moiety and a galactose moiety; the β-D-configuration of the sugars was deduced from ^{13}C-nmr spectra (Table 2) by comparison with literature data (15–19). The downfield shifts for C-4 of the glucuronic acid moiety indicated that the galactose moiety is attached to glucuronic acid at this position. These results demonstrated that saponin **7** was 3-*O*-[β-D-galactopyranosyl-(1↦4)-β-D-glucuronopyranosyl]-oleanolic acid-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside and named hederasaponin H.

Acid hydrolysis of **8** afforded hederagenin, glucose, rhamnose, and glucuronic acid. The fabms exhibited a quasi molecular peak at *m/z* 1117 $[M-H]^-$ and signals at *m/z* 972 $[M-H-145]^-$ and 647 $[M-H-470]^-$, corresponding, respectively, to the loss of one rhamnose moiety and two hexose and one rhamnose moieties. In addition, the fabms MIKE of the peak at *m/z* 647 showed an ion at *m/z* 471 $[M-H-470-176]^-$ resulting from the loss of one glucuronic acid moiety, and this suggested that saponin **8** should be a bidesmoside of hederagenin, containing one unit of glucuronic acid at C-3. From the ^{13}C-nmr spectra (Tables 1 and 2) and comparison of literature data (15–18), it can be concluded that the structure of **8** was 3-*O*-β-D-glucuronopyranosyl-hederagenin-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside, which we named hederasaponin I.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on Bruker AM-200 and AMX-500 multinuclear spectrometers; the chemical shifts were measured in CD_3OD with TMS as an internal standard; carbon multiplicities were determined by the DEPT or DEPT-45 pulse sequence. Fab mass spectra were obtained on a VG Micromass ZAB-HF mass spectrometer in the negative ion mode in a thioglycerol matrix. RP 18 reversed-phase (15–25 μm, Merck) was used for low pressure chromatography (lpc) on a Jobin Yvon Chromatospac prep 10. Si gel (Kieselgel 60, 230–400 mesh, Merck) and Sephadex LH-20 (Pharmacia) were used for cc. Hplc was carried out on a Waters model 6000 A pump with a U6K injector and a UV Model 490 detector; the column used was μBondapak C 18 (10 μm, 7.8 × 300 mm, Waters); MeCN-H_2O (35:65) was the mobile phase with 2 ml/min as flow rate.

PLANT MATERIAL.—The leaves of *H. helix* were collected around Marseille, France, in January 1988. A voucher specimen is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Marseille.

EXTRACTION AND ISOLATION.—The dried leaves (500 g) were extracted with MeOH-H_2O (60:40), concentrated in vacuo to an H_2O layer, and freeze-dried. MeOH was added to the freeze-dried residue, and the MeOH extract was shaken with Et_2O to give a precipitate which was partitioned between H_2O and *n*-BuOH. Saponins were isolated from the *n*-BuOH extract (42 g). The preparative lpc of this extract (2.5 g), on RP 18 with a gradient of MeOH in H_2O, yielded two pure saponins **4** (1 g) and **6** (70 mg), and four fractions (F_1–F_4) which were further subjected to reversed-phase hplc or cc on Si gel to afford four pure compounds, **1** (10 mg), **2** (40 mg), **3** (15 mg), and **5** (45 mg). The *n*-BuOH extract (10 g) chromatographed over a Si gel column and eluted with $CHCl_3$-MeOH-H_2O (55:37:7) furnished a fraction F_5 (600 mg), which was submitted to lpc on RP 18 and eluted with a gradient of MeOH in H_2O, followed by cc on Sephadex LH-20 for fractions F_6 and F_7 to give, respectively, pure **7** (24 mg) and **8** (21 mg).

ANALYTICAL TLC.—Tlc analyses of saponins were performed on precoated Si gel plates (Kieselgel 60 F254, 0.25 mm, Merck) using the following solvent systems: (a) *n*-BuOH–HOAc–H_2O (4:1:5); (b) $CHCl_3$-MeOH-H_2O (64:50:10); (c) $CHCl_3$-MeOH-H_2O (60:40:10); (d) $CHCl_3$-MeOH-H_2O (55:37:7); (e) C_7H_8-MeOH (50:6). The plates were visualized by spraying with 50% methanolic H_2SO_4, followed by heating at 110°. For sugars the solvent systems used were (f) CH_2Cl_2-MeOH-H_2O (50:25:5) and (g) EtOAc-H_2O-MeOH-HOAc (65:15:15:25); plates were developed with aniline hydrogen phthalate by heating at 110°.

ALKALINE HYDROLYSIS.—Saponins (2 mg) in KOH 10% (2 ml) were heated at 100° in a sealed tube for 75 min. After acidification with HCl (pH 5), the monodesmoside was extracted with *n*-BuOH.

ACID HYDROLYSIS.—Each saponin (2 mg) was heated with HCl 10% (2 ml) in a sealed tube at 100° for 4 h. Sapogenin was extracted with Et_2O; then the aqueous layer was neutralized with *N,N*-dioctylamine (10% in $CHCl_3$) and lyophilized. Sapogenin was detected in the organic layer, while sugars were identified in the aqueous layer by tlc analysis with authentic samples.

DETECTION OF SULFATE GROUP.—After acid hydrolysis the aqueous layer was subjected to pc with EtOH-H_2O (7:3), and the sulfate groups were identified by spraying a solution of $BaCl_2$ followed with a solution of sodium rhodizonate (12).

Compounds **4**, **5**, and **6** are, respectively, hederasaponin C, hederasaponin D (saponin K 10), and hederasaponin B, as previously described (9).

COMPOUND **1**.—Hederasaponin E, $C_{53}H_{86}O_{23}$, 3-*O*-α-L-arabinopyranosylbayogenin-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside: fabms *m/z* $[M-H]^-$ 1089, $[M-H-470]^-$ 619; ^{13}C nmr see Tables 1 and 2. Compound **1** afforded a monodesmoside on alkaline hydrolysis and bayogenin, D-glucose, L-rhamnose, and L-arabinose on acid hydrolysis.

COMPOUND **2**.—Hederasaponin F, $C_{48}H_{78}O_{20}S$, 3-*O*-β-sulfate-oleanolic acid-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside: fabms *m/z* $[M-H]^-$ 1005, $[M-H-470]^-$ 535; ^{13}C nmr see Tables 1 and 2. Compound **2** afforded oleanolic acid, D-glucose and, L-rhamnose on acid hydrolysis. Alkaline hydrolysis gave a monodesmoside which furnished a sulfate group after acid hydrolysis. On the other hand, microanalysis of **2** showed 3% of S.

COMPOUND **3**.—Hederasaponin G (cauloside F), $C_{59}H_{96}O_{27}$, 3-*O*-[β-D-glucopyranosyl-(1↦2)-α-L-arabinopyranosyl]-hederagenin-28-*O*-[α]-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside: fabms *m/z* $[M-H]^-$ 1235, $[M-H-470]^-$ 765. Alkaline hydrolysis of **3** gave a monodesmoside, and acid hydrolysis afforded hederagenin, D-glucose, L-rhamnose, and L-arabinose.

COMPOUND **7**.—Hederasaponin H, $C_{60}H_{96}O_{28}$, 3-*O*-[β-D-galactopyranosyl-(1↦4)-β-D-glucuronopyranosyl]-oleanolic acid-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside: fabms *m/z* $[M-H]^-$ 1263, $[M-H-470]^-$ 793; ^{13}C nmr see Tables 1 and 2. Compound **7** furnished a monodesmoside on alkaline hydrolysis and oleanolic acid, D-glucose, L-rhamnose, D-galactose, and D-glucuronic acid on acid hydrolysis.

COMPOUND **8**.—Hederasaponin I, $C_{54}H_{86}O_{24}$, 3-*O*-β-D-glucuronopyranosylhederagenin-28-*O*-[α-L-rhamnopyranosyl-(1↦4)-β-D-glucopyranosyl-(1↦6)]-β-D-glucopyranoside: fabms MIKE *m/z* $[M-H]^-$ 1117, $[M-H-145]^-$ 972, $[M-H-470]^-$ 647, $[M-H-470-176]^-$ 471; ^{13}C nmr see Tables 1 and 2. Alkaline hydrolysis of **8** showed a monodesmoside; acid hydrolysis exhibited hederagenin, D-glucose, L-rhamnose, and D-glucuronic acid.

LITERATURE CITED

1. P. Timon-David, J. Julien, M. Gasquet, G. Balansard, and P. Bernard, *Ann. Pharm. Fr.*, **6**, 545 (1980).
2. J. Julien, M. Gasquet, C. Maillard, G. Balansard, and P. Timon-David, *Planta Med.*, **3**, 205 (1985).
3. K. Hostettmann, *Helv. Chim. Acta*, **63**, 606 (1980).
4. B. Majester-Savornin, R. Elias, A.M. Diaz-Lanza, G. Balansard, M. Gasquet, and F. Delmas, *Planta Med.*, in press.
5. R. Elias, M. De Meo, E. Vidal-Ollivier, M. Laget, G. Balansard, and G. Dumenil, *Mutagenesis*, **5**, 327 (1990).
6. R. Tschesche, W. Schmidt, and G. Wulff, *Z. Naturforsch.*, **20b**, 708 (1965).
7. G.H. Mahran, S.H. Hilal, and T.S. El-Alfy, *Egypt. J. Pharm. Sci.*, **15**, 149 (1974).
8. H. Wagner and H. Reger, *Dtsch. Apoth. Ztg.*, **126**, 2613 (1986).
9. A. Babadjamian, R. Elias, R. Faure, E. Vidal-Ollivier, and G. Balansard, *Spectrosc. Lett.*, **21**, 565 (1988).
10. H. Kizu, S. Kitayama, F. Nakatani, T. Tomimori, and T. Namba, *Chem. Pharm. Bull.*, **33**, 3324 (1985).
11. B. Domon and K. Hostettmann, *Helv. Chim. Acta*, **67**, 1310 (1984).
12. E. Akai, T. Takeda, Y. Kobayashi, and Y. Ogihara, *Chem. Pharm. Bull.*, **33**, 3715 (1985).
13. R.S. Chandel and R.P. Rastogi, *Phytochemistry*, **19**, 1889 (1980).
14. Y.G. Li, D.Z. Wang, S.G. Wu, and C.R. Yeng, *Phytochemistry*, **29**, 595 (1990).

15. E. Vidal-Ollivier, G. Balansard, R. Faure, and A. Babadjamian, *J. Nat. Prod.*, **52**, 1156 (1989).
16. C. Pizza, Z. Zhong-Liang, and N. de Tommasi, *J. Nat. Prod.*, **50**, 927 (1987).
17. P. Colson and R.R. King, *Carbohydr. Res.*, **47**, 1 (1976).
18. B. Domonand K. Hostettmann, *Helv. Chim. Acta*, **66**, 422 (1983).
19. S.B. Mahato, S.K. Sarkar, and G. Poddar, *Phytochemistry*, **27**, 3037 (1988).

Received 16 April 1990

American Society of Pharmacognosy Research Achievement Award

The American Society of Pharmacognosy Research Achievement Award is awarded annually if there are candidates of sufficient merit. Candidates must be members of the American Society of Pharmacognosy and must have made distinguished contributions to natural product research. The award consists of a special honorarium of $2,500 and travel expenses to present the award lecture at an annual meeting of the society.

Nominations of candidates for this award are now being received. A nomination may be made by any member of the ASP and must include a curriculum vitae of the nominee, a list of publications, and a statement emphasizing the nominee's accomplishments and their significance. Attachment of representative reprints may be helpful.

Nominations and inquiries should be received no later than 1 April 1991, and be directed to:

John P.N. Rosazza
Research Achievement Award Committee
College of Pharmacy, University of Iowa
Iowa City, Iowa 52242
(319)-335-8842