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TRITERPENE GLYCOSIDES OF *Hedera canariensis* III. DETERMINATION OF THE STRUCTURES OF GLYCOSIDES L-F_1, L-F_2, AND L-I_2 FROM THE LEAVES OF ALGERIAN IVY

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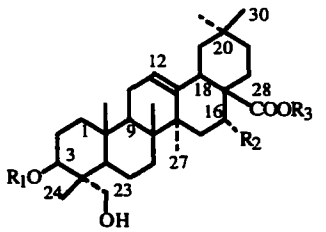
The leaves of Algerian ivy Hedera canariensis *Willd. (Araliaceae) have yielded two new triterpene glycosides — caulophyllogenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside (L-F_2) and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-gentiobiosyl ester (L-I_2) — and also the previously known hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-β-D-glucopyranoside (L-F_1). The structures of the glycosides were established on the basis of chemical transformations and ^1H and ^{13}C NMR spectroscopy.*

In continuation of a study of the triterpene glycosides of *Hedera canariensis* Willd. (Araliaceae), the present paper describes the determination of the structures of three saponins: L-F_1 (**1**), L-F_2 (**2**), and L-I_2 (**3**). We have described the isolation of the L-F fraction and of glycoside L-I_2 from the leaves of this plant previously [1].

Detailed TLC analysis of the L-F fraction showed the presence of three glycosides, designated as L-F_1, L-F_2, and L-F_3, which were obtained in the individual state by preparative separation on Silpearl microcrystalline silica gel (SiO_2) with elution by chloroform—ethanol—water solvent systems. The elimination of phenolic glycoside impurities from (**1**—**3**) was achieved by chromatography on silica gel with elution by a chloroform—ethanol—ammonia mixture.

According to the results of complete acid hydrolysis, the composition of (**1**) was represented by rhamnose, glucose and hederagenin. Partial acid hydrolysis of (**1**) gave rhamnose and hederagenin 3-O-β-*D*-glucopyranoside, which were identified by TLC with authentic specimens [2]. Glycoside (**1**) underwent no change under the conditions of alkaline hydrolysis, which showed the localization of the carbohydrate residue at the C-3 carbon atom of the aglycon. TLC and ^{13}C NMR spectroscopy showed that (**1**) was identical with tauroside St-D_2 from the stems of *Hedera taurica* [3], consisting of hederagenin 3-O-α-*L*-rhamnopyranosyl-(1→2)-O-β-*D*-glucopyranoside.



1. R_1 = α-*L*-Rhap″-(1→2)-β-*D*-Glcp′ → , R_2 = H, R_3 = H
2. R_1 = α-*L*-Rhap″-(1→2)-α-*L*-Arap′ → , R_2 = OH, R_3 = H
3. R_1 = α-*L*-Rhap″-(1→2)-α-*L*-Arap′ → , R_2 = OH,
   R_3 = ← β-*D*-Glcp‴-(6← 1)-β-*D*-Glcp⁗-(4← 1)-α-Rhap⁗⁗

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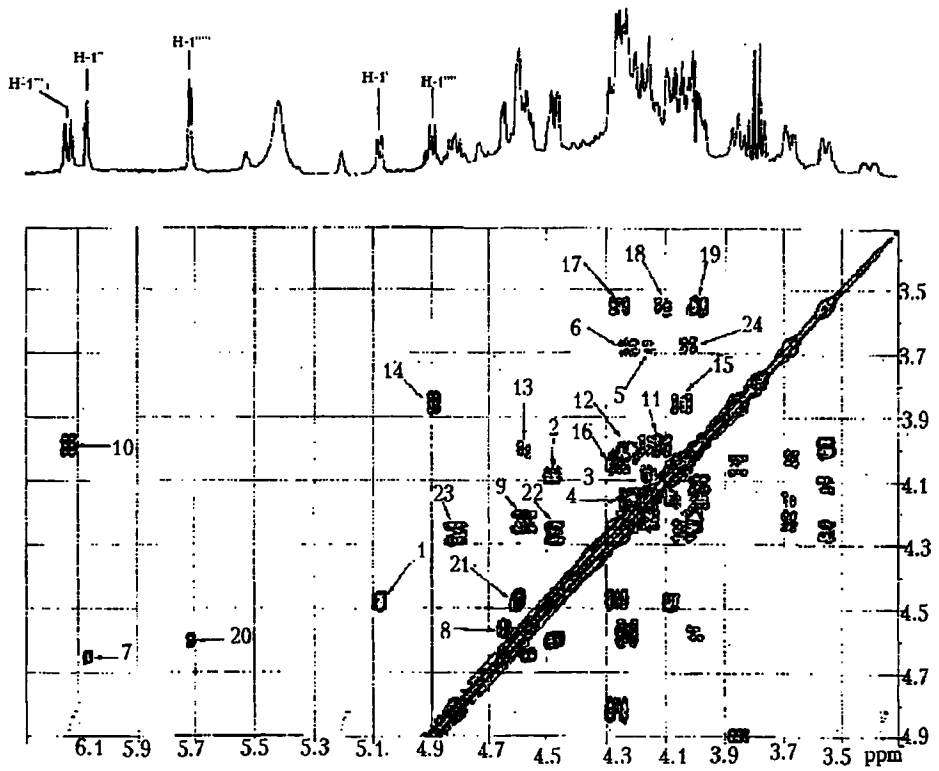

Fig. 1. Fragment of the COSY two-dimensional spectrum of glycoside L-I_2 (400 MHz). The numerals denote cross-peaks between the following protons: *1* - (1, 2)′; *2* - (2, 3)′; *3* - (3, 4)′; *4* - (4, 5e)′; *5* - (4, 5a)′; *6* - (5a, 5e)′; *7* - (1, 2)″; *8* - (2, 3)″; *9* - (3, 4; 4, 5)″; *10* - (1, 2)‴; *11* - (2, 3; 3, 4; 4, 5)‴; *12* - (5, 6B)‴; *13* - (5, 6A)‴; *14* - (1, 2)⁗; *15* - (2, 3)⁗; *16* - (3, 4) ⁗; *17* - (4, 5) ⁗; *18* - (5, 6A) ⁗; *19* - (5, 6B) ⁗; *20*-(1, 2) ⁗⁗; *21* - (2, 3) ⁗⁗; *22* - (3, 4) ⁗⁗; *23* - (4, 5) ⁗⁗; *24* - (23A, 23B).

In a complete acid hydrolysate of (2) we found the sugars rhamnose and arabinose and an unidentified aglycon. Glycoside (2) did not change under the conditions of alkaline hydrolysis but was methylated by an ethereal solution of diazomethane, which indicated the presence of a free carboxy group in its aglycon. The acid hydrolysis of (3) gave rhamnose, arabinose, and glucose and the same aglycon as glycoside (2). In contrast to glycoside (2), glycoside (3) was not methylated by diazomethane, while on alkaline hydrolysis it gave a progenin identical according to TLC in various solvent systems with (2). This showed the presence in (3), as compared with (2), of an additional carbohydrate component at the carboxy group of the aglycon.

The PMR spectrum of (3) revealed the signals of five anomeric protons and, with the aid of the COSY procedure (Fig. 1), the signals of the other skeletal protons corresponding to them, the nature of the splitting of which showed the presence of two rhamnopyranose residues, two β-glucopyranose residues, and one α-arabinopyranose residue, while the chemical shifts agreed with those for the fragments α-*L*-Rha*p*″-(1→2)-α-*L*-Ara*p*′→ and β-*D*-Glc*p*‴-(6←1)-β-*D*-Glc*p*⁗-(4←1)-α-*L*-Rha*p*⁗⁗ [4], which are found most frequently in glycosides from plants of the Araliaceae family. Analogously, in the PMR spectrum of (2) we found signals corresponding only to the disaccharide fragment α-*L*-Rha*p*″-(1→2)-α-*L*-Ara*p*′→. Of the other signals in the PMR spectra of (2) and (3), relating to the aglycon parts of the glycosides, in each case we found in the high-field region six signals of methyl groups at quaternary C atoms, and in the low-field region two pseudotriplet one-proton signals, one of which was assigned from its chemical shift and spin-spin-coupling constants (SSCCs) to the vinyl proton at C-12 [3, 4].

TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of the Aglycon Parts of Glycosides L-F_2 (2) and L-I_2 (3) (δ, ppm, 0 — TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound	
	2	3		2	3
1	39.3	39.4	16	74.6	74.3
2	26.5	26.6	17	49.3	49.5
3	81.4	81.4	18	41.7	41.6
4	43.7	43.8	19	47.6	47.4
5	47.5	47.6	20	31.1	31.0
6	18.6	18.6	21	36.4	36.2
7	33.4	33.4	22	32.9	32.4
8	40.3	40.4	23	64.2	64.2
9	48.0	48.1	24	14.2	14.2
10	37.2	37.2	25	16.5	16.6
11	24.1	24.1	26	17.9	17.9
12	122.7	123.0	27	27.4	27.5
13	145.0	144.9	28	180.5	176.6
14	42.3	42.4	29	33.4	33.4
15	36.1	36.2	30	25.0	25.0

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Atoms of the Carbohydrate Components of Glycosides L-F_2 (2) and L-I_2 (3) (δ, ppm, 0 — TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound
	2	3		3
Ara′			Glc‴	
1	104.5	104.4	1	96.0
2	76.1	76.2	2	74.0
3	74.1	74.1	3	78.7
4	69.2	69.2	4	70.6
5	65.4	65.5	5	78.2
			6	69.3
Rha″			Glc⁗	
1	101.9	101.9	1	104.9
2	72.3	72.4	2	75.4
3	72.6	72.6	3	76.6
4	74.4	74.4	4	78.6
5	70.1	70.1	5	77.2
6	18.7	18.8	6	61.4
			Rha⁗	
			1	102.9
			2	72.6
			3	72.8
			4	74.0
			5	70.6
			6	18.8

The definitive determination of the structure of the aglycon in (2) and (3) was achieved by an analysis of their ^{13}C NMR spectra. Here, in the low-field region of each spectrum (>80 ppm) we observed the signals of one C-atom of the carboxy group of the aglycon, two C-atoms of a trisubstituted double bond in the C-12 position [4], and of the C-3 atom, the chemical shift

of which permitted the assumption that a substituent was present at the C-23 atom, as in hederagenin [1]. At the same time, in the spectra of (2) and (3) we found with the aid of COSY in each case two doublet signals of protons, having a spin-spin coupling with one another, which, from the values of the SSCCs and the chemical shifts, corresponded to *AB* protons in a CH_2OH group at the C-23 atom [4].

A comparison of the NMR spectra of (2) and (3) with the spectrum of a 3-O-glycosylated hederagenin [1, 4] showed agreement of the chemical shifts of the signals for the C-atoms of rings *A*, *B*, and *C* and considerable differences for the C-atoms of rings *D* and *E* — namely, the absence of a signal from the C-16 atom in its usual region, considerable displacements in the positions of the C-15 and C-17 signals, and small effects on the other C-atoms. This permitted the assumption of an additional hydroxy group at C-16. Then the other pseudotriplet signal in each of the PMR spectra of (2) and (3) with δ 5.53 ppm undoubtedly belonged to the proton at C-16, while the nature of the splitting of this signal determined its equatorial position and, consequently, the axial (α) position of the hydroxy group, as in echinocystic acid (16α-hydroxyoleanolic acid). Then a comparison of the chemical shifts of the C-atoms for rings *D* and *E* with the chemical shifts of these atoms in echinocystic acid [1, 4] showed their agreement.

The signals of the C-atoms of the carbohydrate components of (2) and (3) were assigned unambiguously by comparison with the chemical shifts for the fragments α-*L*-Rha*p*″-(1→2)-α-*L*-Ara*p*′→and β-*D*-Glc*p*‴-(6←1)-β-*D*-Glc*p*⁗-(4←1)-α-*L*-Rha*p*⁗′ [1, 4].

The remaining signal in the region of the C-atoms of monosaccharide residues, with δ 74.3 ppm, undoubtedly belonged to the C-16 atom of the aglycon moiety. Thus, the aglycon of glycosides (2) and (3) is 3β,16α,23-trihydroxyolean-12-en-28-oic acid. An aglycon of such a structure, under the name caulophyllogenin, has been found previously in glycosides from *Caulophyllum robustum* [5], *Chrysanthemum procumbens* [6], and, under the name collinsogenin, in a glycoside from *Collinsonia canadensis* [7].

The glycosides L-F_2 and L-I_2 that we have isolated are new glycosides of caulophyllogenin and are caulophyllogenin 3-O-α-*L*-rhamnopyranosyl-(1→2)-O-α-*L*-arabinopyranoside and its 28-O-α-*L*-rhamnopyranosyl-(1→4)-O-β-D-gentiobiosyl ester, respectively.

EXPERIMENTAL

For general observations and the isolation of the L-F fraction, and the glycoside L-I_2, see [1].

PMR spectra were obtained on Bruker WM-250 and AM-400 instruments. Solutions of the glycosides in pyridine-d_5 and deuterochloroform were used.

Preparative separation of the L-F fraction on SiO_2 with elution by the solvent system chloroform—ethanol (2:1) saturated with water gave the individual glycosides (1) (15 mg), (2) (30 mg), and L-F_3 (10 mg). Additional purification of (1—3) was carried out by rechromatography on SiO_2 with elution by the solvent systems chloroform—ethanol (2:1) for (1) and (2), and chloroform—ethanol (1:1) for (3), saturated with 10% aqueous ammonia in each case.

In a complete acid hydrolysate of (1) we identified rhamnose, glucose, and hederagenin. Partial acid hydrolysis of (1) gave rhamnose and hederagenin 3-O-β-*D*-glucopyranoside, which was identified by TLC with authentic specimen of hederoside B [2]. According to TLC in various solvent systems, (1) was identical with an authentic specimen of tauroside St-D_2 [3].

In an acid hydrolysate of (2) we found the sugars rhamnose and arabinose and the aglycon caulophyllogenin, while in the case of (3) we found rhamnose, arabinose, glucose, and caulophyllogenin. The alkaline hydrolysis of (3) led to (2).

PMR spectrum of (2) (δ, ppm, 0 — TMS, C_5D_5N): 5.10 (d, H-1′, $J_{1,2}$=6.0 Hz), 4.50 (dd, H-2′, $J_{2,3}$=7.5 Hz), 4.10 (dd, H-3′, $J_{3,4}$=3.5 Hz), 4.18 (m, H-4′), 3.69 (dd, H-5a′, $J_{4,5a}$=3.5 Hz, J_{5a5e}=11.0 Hz), 4.25 (dd, H-5e′, $J_{4,5e}$=4.5 Hz), 6.10 (d, H-1″, $J_{1,2}$=1.5 Hz), 4.67 (dd, H-2″, $J_{2,3}$=3.5 Hz), 4.58 (dd, H-3″, $J_{3,4}$=9.5 Hz), 4.25 (t, H-4″, $J_{4,5}$=9.5 Hz), 4.61 (dq, H-5″), 1.60 (d, H-6″, $J_{5,6}$=6.5 Hz), 4.20 (H-3), 5.23 (br.t, H-12, $J_{11,12}$=3.5 Hz), 5.54 (br.t, H-16, $J_{15a,16}$= $J_{15e,16}$=3.5 Hz), 3.42 (dd, H-18, $J_{18,19e}$=4.5 Hz, $J_{18,19a}$=14.0 Hz), 4.04 (d, H-23A, $J_{23A,23B}$=11.0 Hz), 3.70 (d, H-23B), 1.70, 1.10, 1.01, 0.98, 0.95, 0.90 (all s, 6 CH_3).

PMR spectrum of (3) (δ, ppm, 0 — TMS, C_5D_5N): 5.08 (d, H-1′, $J_{1,2}$=6.0 Hz), 4.48 (dd, H-2′, $J_{2,3}$=7.5 Hz), 4.09 (dd, H-3′, $J_{3,4}$=4.0 Hz), 4.16 (m, H-4′), 3.68 (dd, H-5a′, $J_{5a,4}$=3.0 Hz), 4.22 (dd, H-5e′, $J_{5e,4}$=4.0 Hz, $J_{5a,5e}$=11.0 Hz), 6.07 (d, H-1″, $J_{1,2}$=1.5 Hz), 4.56 (dd, H-2″, $J_{2,3}$=3.5 Hz), 4.56 (d, H-3″, $J_{3,4}$=9.5 Hz), 4.22 (t, H-4″, $J_{4,5}$=9.5 Hz), 4.59 (dq, H-5″), 1.59 (d, H-6″, $J_{5,6}$=6.5 Hz), 6.13 (d, H-1‴, $J_{1.2}$=8.0 Hz), 3.99 (t, H-2‴, $J_{2,3}$=9.0 Hz), 4.02 (t, H-3‴, $J_{3,4}$=9.0 Hz), 4.18 (H-4‴,

$J_{4,5}$=9.0 Hz), 3.99 (H-5‴), 4.59 (H-6A‴), 4.22 (H-6B‴), 4.90 (t, H-1⁗, $J_{1,2}$=8.0 Hz), 3.86 (t, H-2⁗, $J_{2,3}$=8.5 Hz), 3.50 (t, H-3⁗, $J_{3,4}$=9.0 Hz), 4.26 (t, H-4 ⁗, $J_{4,5}$=9.0 Hz), 3.55 (m, H-5 ⁗), 4.11 (H-6A ⁗), 4.00 (H-6B ⁗), 5.72 (d, H-1 ⁗⁗, $J_{1,2}$=1.5 Hz), 4.60 (dd, H-2 ⁗⁗, $J_{2,3}$=4.0 Hz), 4.47 (dd, H-3 ⁗⁗, $J_{3,4}$=9.5 Hz), 4.27 (t, H-4 ⁗⁗, $J_{4,5}$=9.5 Hz), 4.82 (dq, H-5 ⁗⁗), 1.63 (d, H-6 ⁗⁗, $J_{5,6}$=6.5 Hz), 4.20 (H-3), 5.21 (br.t, H-12, $J_{11,12}$=3.5 Hz), 5.53 (br.t, H-16, $J_{15,16}$=3.5 Hz), 3.41 (dd, H-18, $J_{18,19e}$=4.5 Hz, $J_{18,19a}$=14.0 Hz), 3.68 (d, H-23B, $J_{23A,23B}$=11.0 Hz), 4.03 (d, H-23A), 1.68, 1.07, 0.98, 0.96, 0.92, 0.87 (all s, 6 CH_3).

The ^{13}C NMR spectra of (**2**) and (**3**) are given in Tables 1 and 2.

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