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TRITERPENE GLYCOSIDES OF *Hedera canariensis* II. DETERMINATION OF THE STRUCTURES OF GLYCOSIDES L-E_2 AND L-H_3 FROM THE LEAVES OF ALGERIAN IVY

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The structures of two new triterpene glycosides, L-E_2 and L-H_3 from the leaves of Algerian ivy Hedera canariensis *Willd. (fam. Araliaceae), have been established on the basis of chemical and spectral characteristics: they are 30-norhederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside and the 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-gentiobiosyl ester of 30-norhederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside, respectively.*

The present paper describes the determination of the structures of glycosides L-E_2 (**1**) and L-H_3 (**2**) the isolation of which from the leaves of Algerian ivy *Hedera canariensis* Willd. (Araliaceae) we have described previously [1]. Additional purification of glycosides (**1**) and (**2**) to eliminate contaminating phenolic compounds and glycosides L-H_3 in (**1**) and L-E_2 in (**2**) was achieved by chromatography on silica gel (SiO_2) with elution by the chloroform—ethanol—ammonia solvent system.

Complete acid hydrolysis of (**1**) showed the presence in it of rhamnose and arabinose residues and an unidentified aglycon close in chromatographic mobility to hederagenin (the aglycon of glycosides L-E_1 and L-H_2) [1]. In addition, (**1**) underwent no change under the conditions of alkaline hydrolysis (cleavage of acylglycosidic bonds) but was methylated by an ethereal solution of diazomethane, which showed both the presence of a free carboxy group in the aglycon and the usual type of glycosidic bond of the carbohydrate moiety with the aglycon.

In the PMR spectrum of (**1**), the doublet signals of the two anomeric protons of the rhamnose and arabinose residues were readily identified. The signals corresponding to the remaining skeletal protons of the monosaccharide residues were found with the aid of COSY two-dimensional homonuclear correlation spectroscopy. The nature of the splitting of these signals and the values of the spin-spin coupling constants (SSCCs) corresponded to α-rhamnopyranose and α-arabinopyranose residues.

In an analysis of the ^{13}C NMR spectrum of (**1**), three signals were found in the 90—110 ppm region, two of which, after the editing of the spectrum using a J-modulated spin echo in the procedure with the decoupler turned on at $\tau = 1/J$ [2], proved to be C atoms in CH groups and were assigned to the anomeric C atoms of the rhamnose and arabinose residues through comparison with literature information [3]. We determined the direct $^1J_{CH}$ SSCCs for these signals from the ^{13}C NMR spectrum without suppression of spin-spin coupling with protons; they were 160 and 171 Hz for C-1 of rhamnose and C-1 of arabinose, respectively. Such values of the SSCCs unambiguously determined the axial position of the oxygen atom at C-1 of rhamnose and the equatorial position at C-1 of arabinose, and, consequently, the α-configuration of the glycosidic bond of the rhamnose residue ($^1C_4$ conformation for the *L*- series) and the α-configuration of the glycosidic bond of the arabinose residue ($^4C_1$ conformation for the *L*- series) [4]. The types of conformations of the monosaccharide residues were confirmed by the SSCC values in the PMR spectrum. The signals of the other C-atoms of the carbohydrate part of (**1**) (Table 1) were present in the region of 60—80 ppm and their chemical shifts agreed totally with literature results for the disaccharide fragment α-*L*-Rha*p*-(1→2)-α-*L*-Ara*p* [3] that is found most frequently in glycosides from plants of the Araliaceae family.

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of the Carbohydrate Moieties of Glycosides L-E_2 (1) and L-H_3 (2) (δ, ppm, 0 — TMS, C_5D_5N)

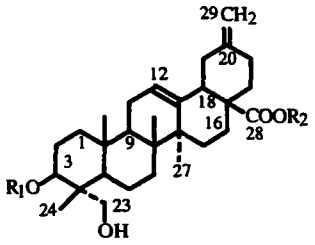
C-atom	Compound		C-atom	Compound
	1	2		2
Ara′			Glc‴	
1	104.5	104.5	1	96.0
2	76.1	76.1	2	74.0
3	74.1	74.1	3	78.6
4	69.4	69.3	4	70.7
5	65.4	65.5	5	78.1
			6	69.3
Rha″			Glc⁗	
1	101.9	101.9	1	104.9
2	72.4	72.4	2	75.3
3	72.6	72.6	3	76.6
4	74.4	74.4	4	78.6
5	70.1	70.0	5	77.2
6	18.8	18.7	6	61.1
			Rha⁗′	
			1	102.9
			2	72.6
			3	72.8
			4	74.0
			5	70.6
			6	18.7

A comparison of the chemical shifts of the remaining signals in the ^{13}C NMR spectrum of (1) corresponding to the aglycon moiety of the glycoside with the chemical shifts of 3-O-glycosylated oleanolic acid [1] revealed considerable differences between them; however, a comparison of the spectral characteristics with the chemical shifts of 3-O-glycosylated hederagenin [1] showed agreement for the C-atoms in rings *A*—*D* but substantial differences for the atoms of ring *E*. It is obvious that the third signal in the 90—110 region relates to one of the atoms of ring *E* in a CH_2 group, as follows from a comparison of the J-modulated ^{13}C NMR spectrum at $\tau = 1/J$ and from the subspectrum of quaternary C-atoms obtained by an analogous method with decoupler turned on at $\tau = 1/2J$ [2, 5]. In the ^{13}C NMR spectrum of (1) without spin decoupling from protons, a triplet with the SSCC $J_{CH} = 152$ Hz relating to a sp^2-hybrid carbon atom corresponds to this C-atom, and, in the light of the chemical shift, this is an olefinic C atom in a $=CH_2$ group. Taking this into account, the two broadened singlet signals ($W_{1/2} = 7$ Hz) in the region of 4.7 ppm of the PMR spectrum, which have a cross-peak only with one another in the COSY spectrum undoubtedly belong to the two chemically nonequivalent *AB* protons of this terminal double bond. Then the form of the peaks of these signals, representing unresolved complex multiplets with a small geminal SSCC $^2J_{AB}$ of a few Hertz and, in addition, a small long-range constants ^4J with the protons at C-19 and C-21 becomes understandable. The remaining unassigned signal with δ 149.3 ppm in the ^{13}C NMR spectrum of (1) undoubtedly relates to the other disubstituted C-atom of this double bond, which is present in ring *E*. At the same time, an exocyclic type of double bond is possible only when one of the methyl groups at C-20 is absent (30-nortriterpenoids), as is confirmed by the absence of one of the signals of methyl groups in the PMR spectrum of (1) as compared with hederagenin glycosides. A comparison of the chemical shifts of the remaining C-atoms of ring *E* (C-17—C-22) with literature figures for these atoms in 30-noroleanolic acid and its glycosides [6] enabled their assignment to be made unambiguously (Table 2).

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Atoms of the Aglycon Moieties of Glycosides L-E_2 (1) and L-H_3 (2) (δ, ppm, O— TMS, C_5D_5N)

C-atom	Compound		C-atom	Compound	
	1	2		1	2
1	39.2	39.3	16	24.0	24.1
2	26.7	26.4	17	47.3	47.3
3	81.3	81.3	18	48.1	47.8
4	43.7	43.7	19	42.2	41.9
5	47.8	47.6	20	149.3	148.6
6	18.4	18.4	21	30.2	30.3
7	33.0	33.4	22	38.6	37.8
8	40.0	40.1	23	64.1	64.1
9	48.3	48.4	24	14.2	14.2
10	37.1	37.1	25	16.3	16.4
11	24.0	24.1	26	17.7	17.8
12	123.3	123.1	27	26.5	26.3
13	144.4	143.8	28	179.9	176.3
14	42.4	42.4	29	107.5	107.7
15	28.5	28.5			

Thus, the aglycon of glycoside (1) is 30-norhederagenin (3β,23-dihydroxy-30-noroleane-12,20(29)-dien-28-oic acid), while glycoside L-E_2 itself is 30-norhederagenin 3-O-α-*L*-rhamnopyranosyl-(1→2)-O-α-*L*-arabinopyranoside and is a new triterpenoid glycoside. Glycosides of 30-norhederagenin have been found previously in *Akebia quinata* (Lardizabaceae) [7].



1. R_1 = α-*L*-Rha*p*''- (1→2) -α-*L*-Ara*p*' →, R_2 = H
2. R_1 = α-*L*-Rha*p*''- (1→2) -α-*L*-Ara*p*' →,
R_2 = ← β-*D*-Glc*p*'''- (6← 1) -β-*D*-Glc*p*'''' (4← 1) -α- Rha*p*''''

According to the results of TLC analysis of the products of complete acid hydrolysis, the aglycon of glycoside (2) was identical with the aglycon from (1), while the sugar components were rhamnose, arabinose, and glucose. The alkaline hydrolysis of (2) gave a progenin shown by TLC to be identical with (1), which partially determines the structure of (2). Unlike (1), compound (2) was not methylated by an ethereal solution of diazomethane, which showed the presence of an additional carbohydrate fragment linked with the aglycon by an acylglycosidic bond.

In the PMR spectrum of (2) we readily identified the signals of five anomeric protons, of which two were analogous to those of (1), while the other three corresponded to a trisaccharide fragment at the carboxy group of the aglycon. Complete assignments of the signals of the skeletal protons of the monosaccharide residues were made with the use of COSY spectroscopy. The nature of the splitting of these signals corresponded to two rhamnopyranose residues, two β-glucopyranose residues, and one α-arabinose residue, while the chemical shifts agreed with those given in the literature for the fragments α-*L*-Rha*p*-(1→2)-O-α-*L*-Ara*p* and α-*L*-Rha*p*-(1→4)-β-*D*-Glc*p*-(1→6)-O-β-*D*-Glc*p*- [8].

In the ^{13}C NMR spectrum of (2) the signals of the C-atoms of the aglycon part were practically identical with those for (1), with the exception of small effects of the glycosylation of the carboxy group on the C-16, C-17, C-18, and C-22 atoms. This additionally confirmed the structure of the aglycon of glycoside (2) as 30-norhederagenin. The signals of the ^{13}C atoms

of the disaccharide fragment substituting the hydroxy group at the C-3 atom of the aglycon were found by comparison with the spectrum of (1). The chemical shifts of other signals, belonging to the trisaccharide fragment at the carboxy group of the aglycon, agreed completely with literature figures for the fragment α-*L*-Rha*p*-(1→4)-O-β-*D*-Glc*p*-(1→6)-O-β-*D*-Glc*p*- [8]. Thus, (2) is the 28-O-α-*L*-rhamnopyranosyl-(1→4)-O-β-*D*-gentiobiosyl ester of 30-norhederagenin 3-O-α-*L*-rhamnopyranosyl-(1→2)-O-α-*L*-arabinopyranoside, and is a new triterpene glycoside.

EXPERIMENTAL

For general observations and the isolation of glycosides (1) and (2), see [1]. NMR spectra were obtained on Bruker WM-250 and AM-400 instruments. Solutions of the glycosides in pyridine-d_5 and deuterochloroform were used.

Purification of glycosides (1) and (2) was achieved on SiO_2 with elution by the solvent systems chloroform—ethanol (2:1) for (1) and (1:1) for (2), saturated with 10% aqueous ammonia. In the acid hydrolysate of (1) by TLC we identified rhamnose, arabinose, and 30-norhederagenin (3), and in (2) rhamnose, arabinose, glucose, and (3). The alkaline hydrolysis of (2) gave a progenin identical in TLC with (1). Unlike (2), compound (1) was esterified by an ethereal solution of diazomethane at the carboxy group of the aglycon (monitoring by TLC).

PMR spectrum of (1) (δ, ppm, 0 — TMS, C_5D_5N): 5.07 (d, H-1′, $J_{1,2}$=6.0 Hz), 4.48 (dd, H-2′, $J_{2,3}$=8.0 Hz), 4.09 (dd, H-3′, $J_{3,4}$=3.5 Hz), 4.16 (m, H-4′), 3.68 (dd, H-5a′, $J_{4,5a'}$=2.5 Hz, $J_{5a'5e'}$=12.0 Hz), 4.22 (dd, H-5e ′, $J_{4,5e'}$ =3.5 Hz), 6.09 (d, H-1″, $J_{1,2}$=1.5 Hz), 4.67 (dd, H-2 ″, $J_{2,3}$=3.5 Hz), 4.57 (dd, H-3″, $J_{3,4}$=9.5 Hz), 4.24 (t, H-4″, $J_{4,5}$=9.5 Hz), 4.59 (dq, H-5″), 1.56 (d, H-6″, $J_{5,6}$=6.5 Hz), 4.20 (dd, H-3), 5.39 (br.t, H-12, $J_{11,12}$=3.0 Hz), 3.10 (dd, H-18, $J_{18,19e}$=6.0 Hz, $J_{18,19a}$=14.0 Hz), 2.50 (t, H-19a, $J_{19a,19e}$=14.0 Hz), 2.05 (H-19e), 4.05 (d, H-23A, $J_{23A,23B}$=11.0 Hz), 3.68 (d, H-23B), 4.71 (m, H-29A), 4.67 (m, H-29B), 1.13, 0.95, 0.90, 0.82 (all s, 4 CH_3).

The ^{13}C NMR spectrum of (1) is given in Tables 1 and 2.

PMR spectrum of (2) (δ, ppm, 0 — TMS, C_5D_5N): 5.11 (d, H-1′, $J_{1,2}$=6.0 Hz), 4.52 (dd, H-2′, $J_{2,3}$=7.0 Hz), 4.11 (dd, H-3′, $J_{3,4}$=3.5 Hz), 4.19 (m, H-4′), 3.71 (dd, H-5a′, $J_{5a,4}$=2.0 Hz), 4.27 (dd, H-5e′, $J_{4,5e}$=4.0 Hz, $J_{5a,5e}$=10.0 Hz), 6.13 (d, H-1″, $J_{1,2}$=1.5 Hz), 4.69 (dd, H-2″, $J_{2,3}$=3.5 Hz), 4.61 (dd, H-3″, $J_{3,4}$=9.5 Hz), 4.27 (d, H-4″, $J_{4,5}$=9.5 Hz), 4.62 (dq, H-5″), 1.60 (d, H-6″, $J_{5,6}$=6.5 Hz), 6.12 (d, H-1‴, $J_{1,2}$=8.5 Hz), 4.08 (t, H-2‴, $J_{2,3}$=9.0 Hz), 4.1-4.3 (m, H-3‴, H-4‴), 4.01 (m, H-5‴), 4.62 (H-6A‴), 4.28 (H-6B‴), 4.92 (d, H-1⁗, $J_{1,2}$=8.0 Hz), 3.90 (dd, H-2⁗, $J_{2,3}$=9.0 Hz), 4.08 (t, H-3⁗, $J_{3,4}$=9.0 Hz), 4.30 (t, H-4⁗, $J_{4,5}$=9.0 Hz), 3.58 (m, H-5⁗), 4.15 (H-6A⁗), 4.01 (H-6B⁗), 5.76 (d, H-1⁗⁗, $J_{1,2}$=1.5 Hz), 4.64 (dd, H-2⁗⁗, $J_{2,3}$=3.5 Hz), 4.51 (dd, H-3⁗⁗, $J_{3,4}$=9.5 Hz), 4.30 (t, H-4⁗⁗, $J_{4,5}$=9.5 Hz), 4.87 (dq, H-5⁗⁗), 1.64 (d, H-6⁗⁗, $J_{5,6}$=6.5 Hz), 5.38 (br.t, H-12, $J_{11,12}$=3.5 Hz), 3.03 (dd, H-18, $J_{18,19e}$=5.0 Hz, $J_{18,19a}$=13.5 Hz), 2.47 (t, H-19a, $J_{19a,19e}$=14.0 Hz), 3.71 (d, H-23B, $J_{23A,23B}$=11.0 Hz), 4.06 (d, H-23A), 4.70 (m, H-29A), 4.64 (m, H-29B), 1.11, 1.00, 0.90, 0.84 (all s, 6 CH_3).

The ^{13}C NMR spectrum of (2) is given in Tables 1 and 2.

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