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TRITERPENE GLYCOSIDES OF Hedera taurica.

III. STRUCTURES OF HEDEROSIDES A_3, B, E_2 AND F FROM THE BERRIES OF CRIMEAN IVY

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Four triterpene glycosides – hederoside A_3, B, E_2, and F – have been isolated from the berries of Hedera taurica Carr. (Crimean ivy), family Araliaceae. On the basis of the results of acid hydrolysis and physiochemical methods of investigation the following structures have been suggested: A_3 – 3-O-(α-L-arabinopyranosyl)hederagenin; B – 3-O-(β-D-glucopyranosyl)hederagenin; E_2 – 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oleanolic acid; and F – 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopuranosyl]hederagenin. Hederoside E_2 is a new glycoside of oleanolic aid.

Continuing investigations of the saponins of the berries of the Crimean species Hedera taurica Carr. [1, 2], we have analyzed the glycoside composition of the berries. TLC analysis showed the presence of six low-polarity components, which have been called, in order of increasing polarity, hederosides A, B, C, D, E, and F, in distinction to the taurosides from the leaves.

Preparative separation of the hederosides was effected by column chromatography on silica gel. The predominating components obtained – A, B, E, and F – were acetylated. TLC analysis showed that components A and E, unlike components B and F, were not individual compounds but consisted of mixtures of glycosides denoted in order of increasing polarity as hederosides A_1-A_3 and E_1 and E_2. The individual glycoside A_1-A_3, B, E_1, E_2, and F were isolated by the column chromatography of their acetates on silica gel. After deacetylation, the predominating hederosides A_3, B, E_2, and F were esterified with diazomethane and rechromatographed for additional purification.

The structures of the glycosides isolated were established with the use of the results of acid hydrolysis and their PMR and ^{13}C NMR spectra. The assignments of the signals in the PMR spectra were made with the aid of the procedure of ^1H_i–{^1H_j} selective homonuclear double resonance, and in the ^{13}C NMR spectra by the use of the method of $^{13}C_i$–{^1H_j} selective heteronuclear double resonance, by the observation of nuclear Overhauser effects (NOEs), by recording the J-modulated ^{13}C NMR spectra, and with the use of literature information on the chemical shifts of the C atoms of oleanolic acid, hederagenin, and unsubstituted methyl glycosides of sugars.

As a result of the acid hydrolysis of hederoside A_3, arabinose and hederagenin were detected, which permitted the assumption of the identity of hederoside A_3 and tauroside B [2].

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of Methyl Esters of Hederosides B (Ia), E_2 (IIa), and F (IIIa) (δ, ppm, 0 – TMS); C_5D_5N).

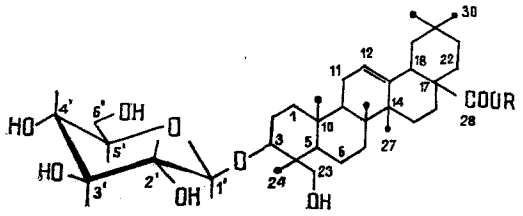
C-Atom	Chemical shift			C-Atom	Chemical shift			C-Atom	Chemical shift		
	Ia	IIa	IIIa		Ia	IIa	IIIa		Ia	IIa	IIIa
1	38,8	38,9	38,8	16	23,9	23,8	23,9	O–CH_3	51,7	51,6	51,6
2	26,0	26,7	25,9	17	46,2	46,2	46,2				
3	82,4	89,1	83,0	18	41,9	42,0	41,9	1′	105,9	105,0	103,8
4	43,5	39,6	43,5	19	47,1	47,1	47,0	2′	75,9	83,6	84,1
5	47,8	56,0	48,1	20	31,0	31,0	30,9	3′	78,7	78,0	78,0
6	18,3	18,6	18,4	21	34,1	34,1	34,1	4′	71,7	71,9	71,4
7	33,0	32,9	32,9	22	33,3	32,9	32,9	5′	78,3	78,0	78,1
8	39,9	39,8	39,8	23	64,9	28,3	65,4	6′	62,9	62,9	62,8
9	48,2	48,0	48,4	24	13,8	16,9	13,5				
10	37,1	37,1	37,0	25	16,3	15,6	16,1	1″		106,1	105,9
11	23,6	23,6	23,5	26	17,4	17,3	17,3	2″		77,0	76,7
12	123,0	122,9	122,9	27	26,3	26,2	26,2	3″		78,4	78,5
13	144,3	144,3	144,3	28	178,1	178,0	178,0	4″		71,7	71,4
14	42,1	42,1	42,1	29	33,3	33,2	33,2	5″		78,1	78,3
15	28,2	28,2	28,2	30	23,8	23,8	23,7	6″		62,9	62,6

Note. The assignment of the signals in Ia between the 11 and 16 and the 17 and 19 atoms; in IIa between the 7 and 22, the 11 and 16, and the 17 and 19 atoms; and in compound (IIIa) between the 11 and 16 and the 17 and 19 atoms have been made arbitrarily.

The values of the chromatographic mobilities and of the specific rotations, and the IR, PMR, and ^{13}C NMR spectra of hederoside A_3 and tauroside B were practically identical. Consequently, hederoside A_3 is hederagenin 3-O-α-L-arabinopyranoside, which has been isolated previously from a number of other plants [3].

On the acid hydrolysis of hederoside B, glucose and hederagenin were identified. From the intensities of the lines, the PMR spectra corresponded to a glucose:hederagenin (1:1) composition. The spin-spin coupling constants (SSCCs) agreed completely with the gluco configuration of the sugar residue. The $J_{1,2}$ values showed the β-configuration of the glycosidic center.

In the ^{13}C NMR spectrum of hederoside B (Table 1), the values of the chemical shifts of the signals of the carbon atoms of the hederogenin residue agree with those given in the literature [4]. The chemical shifts of the signals of the carbon atoms of the glucose residue agree with those for unsubstituted methyl β-D-glucopyranoside [5], confirming the structure of hederoside B as hederagenin 3-O-β-D-glucopyranoside:



I. R = H

Ia. R = CH_3

"Saponin IV" from Fatsia japonica [6], vitalboside B from Clematis vitalba [7], and saponin II from Hedera helix [8] have the same structure.

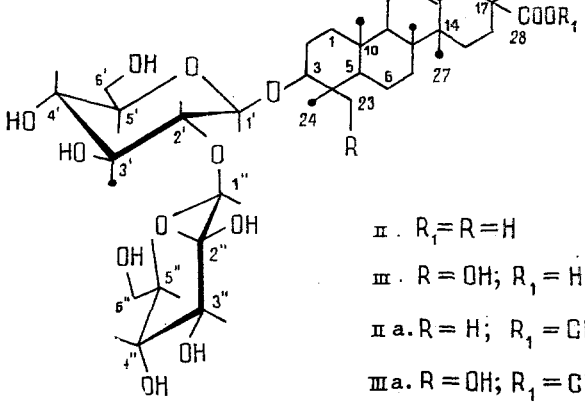
As a result of complete acid hydrolysis, only glucose was detected by paper chromatography in the composition of hederoside E_2 and F. With the aid of TLC, oleanolic acid was identified as the aglycon of glycoside E_2, and hederagenin as that of glucoside F.

From the ratio of the intensities of the signals, the PMR spectra of the two glycosides corresponded to the compositions glucose–oleanolic acid (2:1) for hederoside E_2 and glucose–hederagenin (2:1) for hederoside F. It was possible to determine the positions and nature of

the splitting of the signals of the majority of the skeletal protons of the monosaccharide residues for hederoside E_2. The SSCCs coincided completely with the gluco configuration of both saccharide residues. The SSCC value $J_{3,4}$ = 9.0 Hz for the nonterminal glucose residue was determined from the PMR spectrum of the acetate of hederoside E_2, since in the spectrum of the initial glycoside the positions of the signals of the H-3' and H-4' protons could not be interpreted unambiguously because of the closeness of the chemical shifts of the H-2' and H-3" signals.

For hederoside F we determined the position and nature of the splitting of the signals of all the skeletal protons of the monosaccharide residues. The values found for the SSCCs $J_{1,2}$ indicated the β-configuration of the glycosidic bonds of both monosaccharide residues in each of the glycosides.

The assignment of the signals in the ^{13}C NMR spectra of hederosides E_2 and F (Table 1) was made for the aglycons by comparison with literatures [4] for oleanolic acid and hederagenin. In the carbohydrate part of the ^{13}C NMR spectra of both glycosides the C-2' signals had undergone a considerable downfield shift (7.0-7.5 ppm); β-effects opposite in sign in comparison with unsubstituted methyl β-D-glucopyranoside [5] were observed on the C-1' and C-3' atoms. Thus, the carbohydrate moieties of hederosides E_2 and F are the same, consisting of β-1 → 2-bound glucopyranose residues, and the glycosides themselves have the structures of 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]oleanic acid (II) and 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]hederagenin (III):



II. $R_1 = R = H$

III. $R = OH$; $R_1 = H$

IIa. $R = H$; $R_1 = CH_3$

IIIa. $R = OH$; $R_1 = CH_3$

The structures of hederagenins E_2 and F were confirmed by NOE observations. Thus, when the H-1' of the nonterminal sugar residue was irradiated, positive NOEs were observed on H-5' (5% for E_2 and 7% for F) and on H-3 of the aglycon. When the H-1" proton of the terminal glucose residue was irradiated, in addition to an enhancement of the H-5" signals (5% for E_2 and F) and the H-2" signals (3% for both glycosides), considerable positive NOEs were also observed for the H-2' signals of the internal glucose residues (8% for E_2 and F). This showed the spatial propinquity of the H-1", H-2' protons, which is possible if there is a 1 → 2-bond between the monosaccharide residues. The PMR spectra of the complete acetates of hederosides E_2 and F likewise confirmed the presence of a 1 → 2-bond, since the H-2' signal was present in a considerably stronger field than any of the other signals of the skeletal protons apart from H-5'.

Analysis of literature figures in a review [9] showed coincidence of the ^{13}C subspectra of the carbohydrate moieties of hederoside E_2 and F and of the corresponding spectra of ginsenosides Ra_1–Ra_3, Rb_1–Rb_3, Rc, Rd, and Rf, each having a carbohydrate chain of the same structure.

Hederoside E_2 is a new glycoside of oleanolic acid, while hederoside F is similar to the "saponin V" isolated previously from Hedera helix [10].

EXPERIMENTAL

General Remarks. The total triterpene glycosides, their acetates, and their methyl esters were separated by column chromatography on silica gel L 40-100 μm (Czechoslovakia) using

the following solvent systems: 1) chloroform–methanol–water (80:20:1); 2) chloroform–methanol (20:1 → 4:1), saturated with water; 3) benzene–methyl ethyl ketone (10:1); 4) chloroform–methanol (10:1), saturated with water; and 5) chloroform–methanol (10:2), saturated with water.

TLC monitoring was conducted in Silufol plates (Czechoslovakia), using for detection a 10% aqueous solution of perchloric acid with subsequent heating of the chromatograms at 100-120°C.

NMR spectra were obtained on a Bruker WM-250 instrument. The replacement of the mobile hydrogen of the alcohol groups in the glycosides by deuterium, ^2H, atoms was achieved by keeping solutions of the glycosides in a mixture of methanol-d_4 and heavy water for a day, and evaporating to dryness. Specific rotations were measured on a SU-4 saccharimeter and a P-161 polarimeter at λ 589 nm.

Isolation of the Glycosides. The berries of Crimean ivy (2 kg) were comminuted and extracted with 80% isopropanol. The combined extracts were evaporated to 1/3 of their initial volume, diluted with two volumes of water, and extracted with chloroform. The chloroform extract, after evaporation to 1/5 of its volume, partially crystallized. When five volumes of benzene were added and the precipitate was separated off, 20 g of crude low-polarity triterpene glycosides (1% of the weight of the berries) was obtained. GLC analysis in solvent system 1 showed the presence of six glycosides (hederosides A, B, C, D, E, and F). They were separated by gradient elution from a column by solvent system 2. The amounts of the components A, B, C, D, E, and F were 13, 30, 3, 4, 14, and 36%, respectively.

Acetylation. Hederosides A, B, E, and F were acetylated with acetic anhydride in pyridine (1:1; 20°C, 12 h). The preparative separation of the acetates of components A and E and the purification of the acetates of B and F were carried out by elution from a column with solvent system 3. This gave: from 3 g of the acetate of component A, 0.3 g of the acetate of A_1, 0.1 g of the acetate of A_2, and 1.0 g of the acetate of A_3, mp 227-228°C (ethanol), $[\alpha]_D^{20}$ +68° (c 7.9; pyridine); and the acetate of B, mp 238-239°C (ethanol) $[\alpha]_D^{20}$ +44.5° (c 8.4; pyridine); and from 2 g of the acetate of component E were obtained 0.05 g of the acetate E_1; 1.0 g of the acetate E_2, amorphous, $[\alpha]_D^{20}$ +4.5° (c 7.8; pyridine); and the acetate of F, mp 179-180°C (ethanol), $[\alpha]_D^{20}$ +31° (c 6.3; pyridine).

Deacetylation. The acetates were treated with a 0.01 N solution of sodium methanolate in anhydrous methanol at 40-50°C for 3 h followed by neutralization with KU-2-8 cation-exchange resin in the H^+ form. This gave: hederoside A_3 $[\alpha]_D^{20}$ +64° (c 4.7; pyridine); hederoside B (I), $[\alpha]_D^{20}$ +41° (c 5.3; pyridine); hederoside E_2 (II), $[\alpha]_D^{20}$ +4° (c 2.2; pyridine); and hederoside F (III), $[\alpha]_D^{20}$ +22° (c 2.4; pyridine).

Esterification. Treatment of the glycosides with an ethereal solution of diazomethane gave C-28 methyl esters of hederosides A_3, B, (Ia), E_2 (IIa), and F (IIIa). Solvent system 4 was used for the chromatographic purification of the methyl ester of A_3 and (Ia), and system 5 for (IIa) and (IIIa).

Methyl ester of hederoside A_3: $[\alpha]_D^{25}$ +48° (c 3.0; pyridine).

PMR spectrum (250 MHz; C_5D_5N; δ, ppm; 0 – TMS): (Ia) – $[\alpha]_D^{25}$ +42° (c 7.9; pyridine). 5.04 (d, $J_{1,2}$ = 8.0 Hz, H-1'), 3.95 (t, $J_{2,3}$ = 8.0 Hz, H-2'), 4.09 (t, $J_{3,4}$ = 9.0 Hz, H-3'), 4.15 (dd, $J_{4,5}$ = 7.0 Hz, H-4'), 3.85 (ddd H-5'), 4.42 (dd, $J_{5,6A}$ = 2.6 Hz, H-6A'), 4.29 (dd, $J_{5,6B}$ = 5.0 Hz, $J_{6A,2B}$ = 12 Hz, H-6B'), 3.03 (dd, H-3), 5.33 (br.t, H-12), 3.67 (s, O–CH_3), 1.15, 0.92, 0.90, 0.88, 0.86, 0.81 (all s, 6 CH_3);

(IIa) – $[\alpha]_D^{25}$ + 15° (c 3.2; pyridine) 4.89 (d, $J_{1,2}$ = 7.5 Hz), 4.89 (d, $J_{1,2}$ = 7.5 Hz, H-1'), 4.20 (dd, $J_{2,3}$ = 9.0 Hz, H-2'), 4.28 (m, H-3', H-4'), 3.90 (m, H-5'), 4.42 (dd, $J_{5,6A}$ = 2.0 Hz, H-6A'), 4.32 (dd, $J_{5,6B}$ = 5.3 Hz, $J_{6A,6B}$ = 11.5 Hz, H-6B'), 5.34 (d, $J_{1,2}$ = 8.0 Hz, H-1"), 4.08 (br.t, $J_{2,3}$ = 9.0 Hz, H-2"), 4.21 (t, $J_{3,4}$ = 9.0 Hz, H-3"), 4.12 (t, $J_{4,5}$ = 95 Hz, H-4"), 3.90 (m, H-5"), 4.51 (dd, $J_{5,6A}$ = 2.5 Hz, H-6A"), 4.46 (dd, $J_{5,6B}$ = 3.0 Hz, $J_{6A,6B}$ = 12.0 Hz, H-6B"), 3.28 (dd, $J_{3,2e}$ = 4.5 Hz, $J_{3,2a}$ = 12.0 Hz, H-3), 5.36 (br.t, $J_{11,12}$ = 3.5 Hz, H-12), 3.69 (s, O–CH_3), 1.20, 1.14, 1.03, 0.90, 0.89, 0.83, 0.80 (all s, 7 CH_3);

(IIIa) – $[\alpha]_D^{25}$ +31° (c 3.0; pyridine) 5.03 (d, $J_{1,2}$ = 7.5 Hz, H-1'), 4.10 5.03 (d, $J_{1,2}$ = 7.5 Hz, H-1'), 4.10 (dd, $J_{2,3}$ = 8.5 Hz, H-2'), 4.18 (t, $J_{3,4}$ = 8.5 Hz, H-3'), 4.11 (t, $J_{4,5}$ = 8.5 Hz, H-4'), 3.77 (ddd, H-5'), 4.43 (dd, $J_{5,6A}$ = 2.5 Hz, H-6A'), 4.28 (dd, $J_{5,6B}$ = 5.0 Hz, $J_{6A,6B}$ = 11.5 Hz, H-6B'), 5.34 (d, $J_{1,2}$ = 8.0 Hz, H-1"), 4.07 (br.t, $J_{2,3}$ = 8.0 Hz, H-2"), 4.17 (t, $J_{3,4}$ = 8.8 Hz, H-3"), 4.24 (t, $J_{4,5}$ = 8.8 Hz H-4"), 3.87 (ddd, H-5"), 4.45 (dd, $J_{5,6A}$ = 3.0 Hz, H-6A"), 4.38 (dd, $J_{5,6B}$ = 4.0 Hz, $J_{6A,6B}$ = 11.5 Hz, H-6B"), 3.05 (dd, $J_{3,2e}$ = 4.5 Hz, $J_{3,2a}$ = 14.0 Hz, H-3), 5.35 (br.t, $J_{11,12}$ = 3.5 Hz, H-12), 4.33 (d, $J_{23A,23B}$ = 10.5 Hz, H-23A), 3.75 (d, H-23B), 3.69 (s, O–CH_3), 1.14, 0.92, 0.90, 0.88, 0.86, 0.80 (all s, 6 CH_3).

Acid hydrolysis and the identification of the reaction products were carried out as described in [2].

SUMMARY

The new triterpene glycoside hederoside E_2 – 3-O-[O-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranosyl)oleanolic acid, and the previously known hederagenin 3-O-α-L-arabinopyranoside, hederagenin 3-O-β-D-glucopyranoside, and 3-O-[O-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl]hederagenin have been isolated from the berries of Crimean ivy, Hedera taurica Carr.

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