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TRITERPENE GLYCOSIDES OF Hedera taurica.

V. STRUCTURE OF HEDEROSIDES C AND E_1 FROM CRIMEAN IVY BERRIES

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A description is given of the isolation from the berries of Crimean ivy Hedera taurica Carr. (family Araliaceae) and the determination of their structures on the basis of chemical transformations and spectral characteristics of two triterpene glycosides – the known 3-O-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin and the new hederoside E_1 which is 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]erythrodiol.

Continuing a study of the saponins from the berries of Crimean ivy Hedera taurica Carr. (family Araliaceae) we have established the structures of hederosides C and E_1.

The isolation of hederosides C and E_1 has been described previously [1]. The acid hydrolysis of hederoside C showed the presence in its molecule of residues of hederagenin, arabinose, and rhamnose. Tauroside E, isolated from ivy leaves, has a similar chemical composition [2]. The physical constants and chromatographic mobilities of hederoside C and tauroside E coincided completely. The structure of hederoside C was confirmed by NMR spectroscopy. The ^{13}C NMR spectra of hederoside C and tauroside E were practically identical. Consequently, hederoside C is 3-O-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]hederagenin.

In the products of the acid hydrolysis of hederoside E_1, obtained by the deacetylation of the acetate of E_1 [1], glucose was identified, and an aglycon not agreeing in chromatiographic mobility with hederagenin or oleanolic acid – the predominating aglycons of the triterpene glycosides of Crimean ivy – was detected.

The IR spectrum of hederoside E_1 contained the absorption bands ν_{OH}, ν_{CH}, $\nu_{C=C}$, δ_{CH_3}, and ν_{C-O}, but lacked absorption band $\nu_{C=O}$ that is characteristic for aglycons with a carboxy group.

In an analysis of the PMR spectrum of the acetate of hederoside E_1, doublet signals with δ 4.71 and 4.46 ppm belonging to anomeric protons showed the presence of two sugar residues. In the light of the results of the acid hydrolysis, hederoside E_1 undoubtedly contains two glucose residues. It follows from the values $J_{1,2}$ = 7.7 and 8.0 Hz that both monosaccharide residues have the β-configuration of the anomeric center. By using the method of selective

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Carbon Atoms of Hederosides C (I) and E_1 (II) (δ, ppm; 0 – TMS; C_5D_5N)

C-atom	Chemical shift		C-atom	Chemical shift		C-atom	Chemical shift	
	I	II		I	II		I	II
1	39,0	39,5	16	23,9	22,9	1′	104,9	105,1
2	26,2	26,6	17	46,5	37,6	2′	76,0	83,5
3	81,2	88,9	18	42,0	42,7	3′	74,1	77,9
4	43,5	38,9	19	46,7	47,1	4′	69,8	71,6
5	47,8	55,7	20	31,0	31,2	5′	65,1	78,0
6	18,2	18,5	21	34,3	34,7	6′		62,8
7	33,0	32,9	22	33,3	31,8			
8	39,9	40,1	23	64,4	28,2			
9	48,2	47,9	24	13,9	16,8	1″	101,6	106,1
10	37,0	36,8	25	16,1	15,7	2″	72,2	77,1
11	23,9	23,9	26	17,5	16,8	3″	72,5	78,3
12	122,7	122,5	27	26,2	26,1	4″	74,1	71,6
13	144,9	145,1	28	179,0	68,6	5″	69,7	78,2
14	42,3	42,0	29	33,3	33,4	6″	18,5	62,7
15	28,4	26,1	30	23,9	23,9			

In compound (I) the assignment of the signals between atoms 17 and 19 was made arbitrarily.

homonuclear resonance $\{^1H_i - ^1H_j\}$ it was possible to determine the positions of the signals of the majority of skeletal protons (see the Experimental part). The spin-spin coupling constants found confirmed the gluco- configuration of the sugar residues. Analysis of the positions of the signals from the skeletal protons showed that the signal from one of the H-2 protons was present in a considerably higher field (3.81 ppm) than the other (4.92 ppm) and than the remaining skeletal protons (with the exception of H-5). It is obvious that the glycosidic bond between the glucose residues involves the hydroxy group at C-2', and the signal at 3.81 ppm relates to H-2' of the internal glucose residue. Such a structure of the carbohydrate moiety of glycoside E_1 can be represented by 1→2-bound β-D-glucopyranose residues.

A signal with δ 3.11 ppm in the PMR spectrum of the acetate of E_1 having a doublet-doublet structure obviously related to H-3 of the aglycon. The presence of eight singlet signals of acetate groups (2.0-2.1 ppm) indicated that one free hydroxy group was present in the aglycon of the glycoside, since the signals of seven acetyl groups belonged to the glucose residues. Then the presence of two doublet signals with δ 3.72 and 4.03 ppm having J = 11 Hz became understandable as relating to the AB protons of an aglycon CH_2OH group.

In the ^{13}C NMR spectra of hederoside E_1 the majority of signals in the low-field region belonged to the carbohydrate moiety. It was possible to assign them unambiguously by comparison with literature figures to the disaccharide fragment O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl [1, 3].

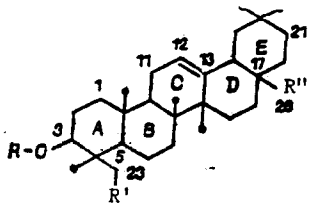
The ^{13}C NMR spectrum of the aglycon moiety of glycoside E_1 was largely analogous to the spectrum of oleanolic acid [1, 4] but it lacked the signals at 178 and 46.2 ppm corresponding to the C atom of the carbonyl group and the C-17 atom, but did contain signals with δ 68.5 and 37.6 ppm. It is obvious that the chemical shift of 37.6 ppm corresponds to the C-17 atom with a changed function which, in the light of the chemical shift of 68.5 ppm and the characteristics of the IR and PMR spectra is a CH_2OH group.

The chemical-ionization mass spectrum of hederoside E_1 (with ammonia as the reagent gas) showed a molecular cluster ion with m/z 784 $[M + NH_4]^+$, the molecular ion $[M + H]^+$ with m/z 767, and fragmentary ions with m/z 622 $[M + NH_4 - 162]^+$, 605 $[M + H - 162]^+$, 460 $[M + NH_4 - 162 - 162]^+$, and 443 $[M + H - 162 - 162]^+$, corresponding to the loss of one or two glucose residues (162 c.u.), and also ions with m/z 180 ($Glc\text{-}\overset{+}{N}H_3$) and 342 ($Glc\text{-}Glc\text{-}\overset{+}{N}H_3$). Consequently, the mass of the aglycon, M, was 442 c.u.

The electron-ionization mass spectrum of the aglycon of glycoside E_1 contained the molecular ion with m/z 442 (M^+) and fragmentary ions with m/z 234 (fragment a), 203 (fragment c – ejection of the substituent at C-17 from fragment a), and 133 (fragment f) arising as a consequence of retrodiene breakdown in ring C [5]. These results showed that the mass of the substituent at C-17 was 31 c.u., which corresponds to a CH_2OH group.

On comparing the facts given above, we came to the conclusion that the aglycon of hederoside E_1 was olean-12-ene-3β,28-diol, or erythrodiol. The chromatographic mobilities of the aglycon of glycoside E_1 and of erythrodiol obtained by the synthetic reduction of methyl oleanolate with lithium tetrahydroaluminate coincided completely.

It followed from the chemical shifts of C-28 (68.5 ppm) and C-3 (88.9 ppm) that the carbohydrate chain was attached at the C-3 atom of the aglycon. Thus, hederoside E_1 is 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]erythrodiol (II).



	R	R′	R″
I.	α-*L*-Rha$_p$-(1→2)-O-α-*L*-Ara$_p$	—OH	—COOH
II.	β-*D*-Glc$_p$-(1→2)-O-β-*D*-Glc$_p$	—H	—CH_2OH
III.	—	—H	—CH_2OH

At the present time, natural glycosides of erythrodiol have been isolated from Lemaireocereus griseus (family Cactaceae) [6] and Acacia myrtifolia (family Mimosaceae) [7], but their structures have not been established. Erythrodiol glycosides have also been obtained by the lithium tetrahydroaluminate cleavage of the acyl glycosidic bond in bisdesmosides of oleanolic acid. Thus, the chemical shifts of the signals of the C-atoms of the erythrodiol residue in the cleaved glycoside from Zexmenia buphthalmiflora [8] coincide completely with those for the aglycon residue in the spectrum of glycoside E_1, additionally confirming its structure. Hederoside E_1 is the first natural glycoside of erythrodiol for which the structure has been established.

EXPERIMENTAL

IR spectra were obtained on a Specord 75-IR spectrophotometer, electron-ionization (70 eV) and chemical-ionization (reagent gas ammonia) mass spectra on a Varian-MAT 44S instrument, and NMR spectra on a Bruker WM-250 instrument with a working frequency of 250 MHz for ^1H and 62.9 MHz for ^{13}C at 40°C in pyridine-d_5. The replacement of the hydrogen atoms of the alcoholic groups of the glycosides by deuterium, ^2H, atoms was effected by keeping solutions of the glycosides in a mixture of methanol-d_4 and heavy water for a day. Specific rotations were measured on a SU-4 saccharimeter at λ = 589 nm.

TLC monitoring was carried out on Silufol plates in the following solvent systems: 1) chloroform–methanol (8:2) for glycosides; 2) benzene–acetone (8:2) for the acetates of glycosides and aglycons; and 3) chloroform–methanol–ammonia (7:3:2) for sugars. Aniline phthalate was used to detect sugars, and a 10% solution of perchloric acid followed by heating of the chromatograms to 100-120°C for glycosides and aglycons.

Complete acid hydrolysis was carried out with 2 N trifluoroacetic acid in dioxane–water (1:1) at 100°C for 2 h. Deacetylation was performed in anhydrous methanol with a catalytic amount of sodium methanolate.

Hederoside C (I) [1] was additionally purified by recrystallization from ethanol, mp 244-250°C, $[\alpha]_D^{20}$ + 9° (c 1.2; ethanol). According to the literature: mp 235-240°C, $[\alpha]_D^{20}$ + 7° (ethanol) [2]; mp 248-250°C, $[\alpha]_D^{20}$ + 19° (pyridine) [9]; $[\alpha]_D^{20}$ + 8° (ethanol) [10]. Rhamnose, arabinose, and hederagenin were identified in an acid hydrolyzate of (I).

The acetate of hederoside E_1 [1], after chromatographic purification on silica gel (solvent system 2), $[\alpha]_D^{20}$ + 9° (c 0.9, chloroform). PMR spectrum (250 MHz, C_5D_5N, δ, ppm; 0 – TMS): 4.46 (d, $J_{1',2'}$ = 7.7 Hz, H-1'), 3.81 (dd, $J_{2',3'}$ = 9.5 Hz, H-2'), 5.17 (t, $J_{3',4'}$ = 9.5 Hz, H-3'), 4.92 (t, $J_{4',5'}$ = 9.5 Hz, H-4'), 3.62-3.71 (m, H-5', H-5"), 4.00-4.11 (m, H-6B', H-6B"), 4.22-4.32 (m, H-6A', H-6A"), 4.71 (d, $J_{1'',2''}$ = 8.0 Hz, H-1"), 4.92 (dd, $J_{2'',3''}$ = 9.7 Hz, H-2"), 5.18-5.30 (m, H-3", H-4"), 3.11 (dd, $J_{3,2e}$ = 5.3 Hz, $J_{3,2a}$ = 11.5 Hz, H-3), 5.21 (br. t, H-12), 3.72 (d, J_{AB} = 11.0 Hz, H-28B), 4.03 (d, H-28A), 2.0-2.1 (8 s, CO–CH_3).

Hederoside E_1 (II) was obtained by the deacetylation of the acetate. After chromatographic purification on silica gel (solvent system 1) $[\alpha]_D^{20}$ +5° (c 0.5; pyridine). IR spectrum of (II) (thin film): 3400 (ν_{OH}); 2925, 2870 (ν_{CH}); 1645 ($\nu_{C=C}$); 1540; 1455 ($\delta_{CH_3}^{as}$); 1365 ($\delta_{CH_3}^{s}$); 1300; 1160; 1070; 1025 (ν_{C-O}); 890; 820.

Chemical-ionization mass spectrum of (II): ($C_{42}H_{70}O_{12}$): m/z 784 $[M + NH_4]^+$, 767 $[M + H]^+$, 622 $[M + NH_4 - 162]^+$, 605 $[M + H - 162]^+$, 460 $[M + NH_4 - 162 - 162]^+$, 443 $[M + H - 162 - 162]^+$, 342 (Glc-Glc-$\overset{+}{N}H_3$), 180 (Glc-$\overset{+}{N}H_3$).

In an acid hydrolyzate of (II) glucose and erythrodiol (III), mp 234-235°C (ethanol), were identified. According to the literature: mp 235-237°C [11]. Electron-ionization mass spectrum of (III) ($C_{30}H_{50}O_2$): m/z 442 (M^+), 234 (a), 203 (c), 133 (f).

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A STUDY BY THE METHOD OF CIRCULAR DICHROISM OF THE INFLUENCE OF VARIOUS DENATURING AGENTS ON COTTONSEED GOSSYPULIN (11S)

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The structure of the reserve plant globulins is the main factor determining the functional properties of food proteins obtained from them. Under the action of various denaturing agents on cottonseed gossypulin, the main reserve protein, conformational changes take place which, in a number of cases affect both the secondary and the tertiary structures. Ten samples of gossypulin obtained under the action of acid, alkali, heat, and urea on the native molecule have been studied by the method of CD spectroscopy. The new protein products are systems relatively ordered with respect to their secondary structure but with a modified tertiary structure.

At the present time, the seed proteins of various plants are being widely studied [1-3]. Interest in them is due, on the one hand, to the uniqueness of their structure and their significance in the formation and breakdown of the seed protein bodies [4] and, on the other hand, to the ever-increasing role of plant proteins satisfying the demands of the world population for protein foodstuffs. Pathways for the rational utilization of plant protein lie through the processing of natural sources into protein forms acceptable for foodstuffs. The processing of a natural raw material into a foodstuff includes obtaining biologically valuable protein substances (in the form of flour, concentrates, isolates) and the creation from them of combined and artificial food products.

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