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TRITERPENE GLYCOSIDES OF *Hedera taurica*

XVI. STRUCTURES OF GLYCOSIDES St-A, St-B_1, St-B_2, St-C, St-D_1, St-D_2, St-E, St-F_1, AND St-F_2 FROM THE STEMS OF CRIMEAN IVY

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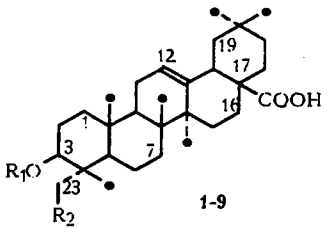
From the stems of Crimean ivy Hedera taurica *Carr. (fam. Araliaceae) we have isolated previously known glycosides of oleanolic acid — the 3-O-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside], the 3-O-β-D-glucopyranuronoside, and the 3-O-[O-β-D-galactopyranosyl-(1→2)-β-D-glucopyranuronoside]; known glycosides of hederagenin — the 3-O-α-L-arabinopyranoside, the 3-β-D-glucopyranoside, the 3-O-[O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside], and the 3-O-β-D-glucopyranuronoside; and also the new triterpene glycoside St-D_2, hederagenin 3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside].*

In the present paper we describe the determination of the structures of the weakly polar triterpene glycosides St-A — St-F from the stems of Crimean ivy. We have described the isolation of the total weakly polar glycosides previously [1]. These glycosides have now been separated by chromatography on silica gel L with gradient elution by a chloroform–ethanol–water solvent system. This led to the individual glycosides St-A, St-C, and St-E and also to the glycosidic fractions St-B, St-D, and St-F. TLC analysis of these fractions showed that each of them consisted of a pair of glycosides with close mobilities. They were separated into the individual glycosides St-B_1 and St-B_2, St-D_1 and St-D_2, and St-F_1 and St-F_2 by rechromatography on Silpearl silica gel.

Treatment of the weakly polar triterpene glycosides with an ethereal solution of diazomethane converted them into methyl esters, while alkaline hydrolysis caused no changed whatever, which showed the presence of a carbohydrate chain in these glycosides only at the C_3-OH group of the aglycon.

Glycosides St-A (**1**), -B_1 (**2**), -B_2 (**3**), -C (**4**), and -D_1 (**5**) were identified by TLC with weakly polar glycosides isolated previously from the leaves and fruit of Crimean ivy. They were, respectively, taurosides B and C [2] and hederosides B, C, and D_2 [3-5]. The results of complete acid hydrolysis confirmed the compositions of these glycosides. Their ^{13}C NMR spectra were identical with those published previously [2-5]. Thus, (**1-5**) were the previously known [6] hederagenin 3-O-α-*L*-arabinopyranoside (leontoside A), oleanolic acid 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside] (β-hederin), hederagenin 3-O-β-*D*-glucopyranoside (vitalboside B), hederagenin 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside] (α-hederin), and hederagenin 3-O-[O-β-*D*-glucopyranosyl-(1→2)-α-*L*-arabinopyranoside] (cauloside C), respectively.



1-9

	R_1	R_2
1	Ara'α	OH
2	Rha" →(α 2)→ Ara'α	H
3	Glc'β	OH
4	Rha" →(α 2)→ Ara'α	OH
5	Glc" →(β 2)→ Ara'α	OH
6	Rha" →(α 2)→ Glc'β	OH
7	GlcUA'β	H
8	GlcUA'β	OH
9	Gal" →(β 2)→ GlcUA'β	H

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of the Aglycon Moieties of the Methyl Esters of Glycosides St-D_2 (**6a**), St-E (**7a**), and St-F_1 (**8a**) (ppm, 0—TMS, C_5D_5N, 70°C)

C-Atom	6	7	8	C-Atom	6a	7a	8a
1	39.3	38.7	38.6	16	24.0	23.7	23.9
2	26.5	26.6	26.1	17	47.2	47.1	47.0
3	81.6	89.2	82.4	18	42.0	42.0	41.9
4	43.7	39.5	43.5	19	46.3	46.2	46.2
5	48.0	55.8	47.5	20	30.9	30.8	30.7
6	18.4	18.2	18.2	21	34.1	34.1	34.0
7	32.7	33.1	32.6	22	33.2	32.9	33.3
8	40.0	39.9	39.9	23	64.1	28.3	64.3
9	48.3	48.2	48.1	24	14.3	17.0	13.6
10	37.0	37.0	36.9	25	16.4	15.6	16.1
11	23.5	23.4	23.3	26	17.6	17.6	17.5
12	123.1	122.9	122.9	27	26.3	26.1	26.1
13	144.3	144.2	144.2	28	178.1	178.0	178.0
14	42.3	42.1	42.1	29	33.2	33.1	33.1
15	28.5	28.3	28.3	30	24.0	23.7	23.7
				$-O-CH_3$	51.7	51.6	51.6

In a complete acid hydrolysate of glycoside St-D_2 (**6**) we detected the sugars rhamnose and glucose and the aglycon hederagenin. Partial acid hydrolysis of (**6**) enabled us to establish the sequence of the monosaccharide residues, since it led to the splitting out of the rhamnose, and the resulting progenin was identified as hederagenin 3-O-β-*D*-glucopyranoside (**3**). According to TLC, (**6**) was identical with a progenin from glycoside St-I_2 [7] consisting of hederagenin 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside]. The type of bond and the localization of the carbohydrate moiety were confirmed by the ROESY spectrum of the full acetate (Fig. 1), in which we identified the structurally informative cross-peaks H-1″ — H-2′ and H-1′ — H-3 of the aglycon. The assignments of the signals in the ^{13}C NMR spectrum of (**6**) were made by comparison with the spectrum of glycoside St-I_2 [7] and also by correlation with literature figures for a 3-substituted hederagenin [3], for a 2-glycosylated β-*D*-glucose residue [3], and for terminal rhamnose in the disaccharide Rha$\xrightarrow{\alpha 2}$Ara- [8], and on the basis of glycosylation effects [9]. Thus, glycoside St-D_2 is hederagenin 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-β-*D*-glucopyranoside] and is a new triterpene glycoside.

According to the results of complete acid hydrolysis and enzymatic hydrolysis with β-glucuronidase (EC 3.2.1.31), glycosides St-E (**7**) and St-F_1 (**8**) each contained a β-*D*-glucuronic acid residue, and the aglycons oleanolic acid and hederagenin, respectively. According to TLC, they were identical with progenins of the glycosides St-J and St-K and were the 3-O-β-*D*-glucopyranuronosides of oleanolic acid (saponin F [6]) and of hederagenin (HN-saponin K [10]), respectively. These structures were additionally confirmed by ^{13}C NMR spectra, in which it was possible to assign the signals of the carbohydrate moieties by comparison with literature information for a β-*D*-glucopyranuronose residue and for the aglycons oleanolic acid and hederagenin [11]. In the Araliaceae family, oleanolic acid 3-O-β-*D*-glucopyranuronoside has been detected previously in *Aralia cordata* [12] and *Hedera nepalensis* [10], and hederagenin 3-O-β-*D*-glucopyranuronoside in *Hedera nepalensis* [10] and *Schefflera impresa* [13].

According to the results of acid hydrolysis, glycoside St-F_2 (**9**) consisted of residues of the monosaccharides galactose and glucuronic acid and of the aglycon oleanolic acid. The partial acid hydrolysis of (**9**) led to (**7**), which showed the sequence of the sugars. The type and configuration of the bond between the galactose and glucuronic acid residues were determined from an analysis of the chemical shifts and SSCCs of the signals of the skeletal protons in the PMR spectrum of the full acetate of glycoside St-F_2, as described previously [14].

Thus, (**9**) was oleanolic acid 3-O-[O-β-*D*-galactopyranosyl-(1→2)-O-β-*D*-glucopyranuronoside] (glycoside Rb-4), isolated previously from *Aralia cordata* [12] and *Tetrapanax papyriferum* [15].

EXPERIMENTAL

For general observations and the procedures for alkaline and acid hydrolysis and methylation, see [11, 14].

Separation of the Weakly Polar Glycoside Fraction. The weakly polar glycosides [1] (20 g) were separated on silica gel L by gradient elution with the water-saturated chloroform—ethanol (4:1 → 2.1) solvent system. This gave the indivi-

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Atoms of the Carbohydrate Components of the Methyl Esters of Glycosides St-D_2 (**6a**), St-E (**7a**), and St-F_1 (**8a**) (ppm, 0–TMS, C_5D_5N, 70°C)

C-Atom	6a	7a	8a	C-Atom	6a
	Glc	GlcUA	GlcUA		Rha
1′	104.7	107.3	106.4	1″	101.6
2′	79.8	75.4	75.4	2″	72.3
3′	77.7	78.0	77.8	3″	72.5
4′	72.1	73.1	73.1	4″	74.1
5′	78.0	77.2	77.3	5″	69.7
6′	62.8	170.9	170.9	6″	18.8
		52.0	52.1		
C_6 –O–CH_3					

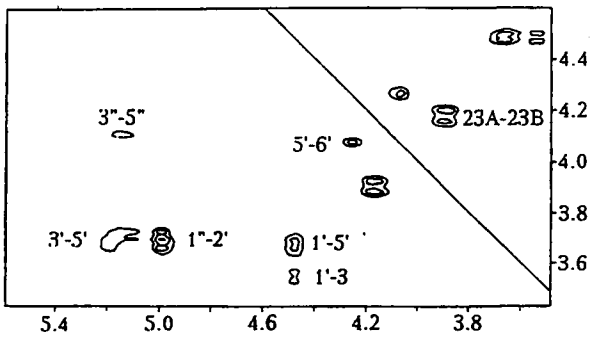

Fig. 1. Fragment of the two-dimensional ROESY spectrum of the full acetate of glycoside St-D_2.

dual glycosides (**1**) (0.8 g), (**4**) (3.3 g), and (**7**) (0.36 g) and the fractions St-B (0.4 g), St-D (5.0 g), and St-F (2.5 g). These fractions were separated into individual glycosides on Silpearl silica gel with elution by the solvent systems water-saturated chloroform-ethanol (4:1), (3:1), and (2:1), respectively. This gave glycosides (**2**) (0.12 g), (**3**) (0.18 g), (**5**) (3.0 g), (**6**) (1.5 g), (**8**) (2.2 g) and (**9**) (0.05 g).

Glycoside (1), $[\alpha]_D$ +69° (*c* 2.7; pyridine; lit.: $[\alpha]_D$ +64° (pyridine) [2]. According to TLC and its ^{13}C NMR spectrum, (**1**) was identical with an authentic specimen of hederagenin 3-O-α-*L*-arabinopyranoside [2].

Glycoside (2), $[\alpha]_D$ −2° (*c* 0.5; pyridine). According to TLC and its ^{13}C NMR spectrum, (**2**) was identical with an authentic specimen of oleanolic acid 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside] [2].

Glycoside (3), $[\alpha]_D$ +40° (*c* 2.0; pyridine); lit.: $[\alpha]_D$ +41° (pyridine) [3]. According to TLC and its ^{13}C NMR spectrum, (**3**) was identical with an authentic specimen of hederagenin 3-O-β-*D*-glucopyranoside [3].

Glycoside (4), $[\alpha]_D$ +9° (*c* 3.0; ethanol), lit.: $[\alpha]_D$ +7° (ethanol) [8]. According to TLC and its ^{13}C NMR spectrum, (**4**) was identical with an authentic specimen of hederagenin 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside] [8].

Glycoside (5), $[\alpha]_D$ +42° (*c* 1.0; pyridine); lit.: $[\alpha]_D$ +40° (pyridine) [4]. According to TLC and its ^{13}C NMR spectrum, (**3**) was identical with an authentic specimen of hederagenin 3-O-[O-β-*D*-glucopyranosyl-(1→2)-α-*L*-arabinopyranoside] [4].

Glycoside (6), $[\alpha]_D$ +11° (*c* 1.5; pyridine). In a complete acid hydrolysate of (**6**) we identified rhamnose, glucose, and hederagenin, while in a partial acid hydrolysate we detected rhamnose and (**3**). The methylation of (**6**) with diazomethane led to the 28-methyl ester (**6a**). The ^{13}C NMR spectrum of (**6a**) is given in Tables 1 and 2.

The acetylation of (**6a**) gave the full acetate (**6b**).

^1H NMR spectrum of (**6b**) (δ, ppm, 0–TMS, $CDCl_3$): 4.48 (d, H-1′, $J_{1,2}$ 8.0); 3.69 (t, H-2′, $J_{2,3}$ 8.5); 5.20 (t, H-3′, $J_{3,4}$ 9.0); 4.98 (t, H-4′, $J_{4,5}$ 10.0); 3.67 (m, H-5′); 4.10 (dd, H-6′A, $J_{5,6A}$ 2.5; $J_{6A,6B}$ 12.5); 4.24 (dd, H-6′B, $J_{5,6B}$ 5.0);

5.01 (d, H-1″, $J_{1,2}$ 1.5); 5.11 (dd, H-2″, $J_{2,3}$ 3.0); 5.16 (dd, H-3″, $J_{3,4}$ 10.0); 5.01 (t, H-4″, $J_{4,5}$ 10.0); 4.11 (dq, H-5″); 1.19 (d, H-6″, $J_{5,6}$ 6.0); 5.28 (bt, H-12, $J_{11,12}$ 3.5); 4.19 (d, H-23A, $J_{23A,23B}$ 12.0); 3.91 (d, H-23B) 2.77 (H-18); 3.76 (s, OCH_3).

Glycoside (7), $[\alpha]_D$ +18° (*c* 2.8, methanol); lit.: $[\alpha]_D$ +20.4° (methanol) [10]. The enzymatic hydrolysis of (**7**) with β-glucuronidase (EC 3.2.1.31) at pH 7, 37°C, for 10 h led to glucuronic and oleanolic acids. According to TLC, (**7**) was identical with an authentic specimen of oleanolic acid 3-O-β-*D*-glucopyranuronoside (a progenin of glycoside St-J) [11]. The methylation of (**7**) with diazomethane gave the 6′,28-dimethyl ester (**7a**). The ^{13}C NMR spectrum of (**7a**) is given in Tables 1 and 2.

Glycoside (8), $[\alpha]_D$ +20° (*c* 1.5; methanol), lit.: $[\alpha]_D$ +22.6° (methanol) [10]. The enzymatic hydrolysis of (**8**), carried out as for (**7**), gave glucuronic acid and hederagenin. According to TLC, (**8**) was identical with an authentic specimen of hederagenin 3-O-β-*D*-glucopyranuronoside (a progenin of St-K) [11]. The methylation of (**8**) with diazomethane led to the 6′,28-dimethyl ester (**8a**). The ^{13}C NMR spectrum of (**8a**) is given in Tables 1 and 2.

Glycoside (9), $[\alpha]_D$ −10° (*c* 0.5; pyridine). A complete acid hydrolysate of (**9**) contained galactose and glucuronic and oleanolic acids. Partial acid hydrolysis of (**9**) led to galactose and (**7**). According to TLC, (**9**) was identical with an authentic specimen of oleanolic acid 3-O-[O-galactopyranosyl-(1→2)-glucopyranuronoside] (a progenin of glycoside St-I_{4b}) [14]. The methylation of (**9**) with diazomethane, followed by acetylation, gave the full acetate of the methyl ester of (**9**), (**9a**).

^1H NMR spectrum of (**9a**) (δ, ppm, 0−TMS, $CDCl_3$): 4.51 (d, H-1′, $J_{1,2}$ 8.0); 3.87 (dd, H-2′, $J_{2,3}$ 9.0); 5.23 (t, H-3′, $J_{3,4}$ 9.0); 5.10 (t, H-4′, $J_{4,5}$ 10.0); 4.00 (d, H-5′); 4.66 (d, H-1″, $J_{1,2}$ 8.0); 5.11 (dd, H-2″, $J_{2,3}$ 10.5); 4.93 (dd, H-3″, $J_{3,4}$ 3.5); 5.34 (m, H-4″); 3.87 (m, H-5″); 4.10 (m, H-6″A, H-6″B); 5.33 (H-12); 3.11 (H-3); 2.82 (H-18); 1.25; 1.14; 1.05; 0.91; 0.91; 0.86; 0.74 (all s, 7 CH_3); 3.76 (s, OCH_3).

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