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TRITERPENE GLYCOSIDES OF *Hedera taurica*
XIV. STRUCTURES OF GLYCOSIDES St-G_{0-2}, St-J, AND St-K FROM THE STEMS OF CRIMEAN IVY

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The structures of new triterpene glycosides isolated from the stems of Crimean ivy Hedera taurica *Carr. (fam. Araliaceae) — St-J, St-K, and St-G_{0-2} — have been established as the 3-O-α-L-glucopyranuronoside 28-O-[O-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside]s of oleanolic acid and of hederagenin and the 3-O-(6'-O-ethyl-β-D-glucopyranuronoside) 28-O-[O-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-glucopyranoside of hederagenin, respectively.*

In the present paper we describe the determination of the structures of the glycosides St-J (**1**), St-K (**2**), and St-G_{0-2} (**3**). We have reported the isolation of glycosides St-J and St-K from the stems of Crimean ivy previously [1]. For additional purification, (**1**) and (**2**) were converted into the methyl esters (**1a**) and (**2a**) by treatment with an ethereal solution of diazomethane and chromatography on silica gel (SiO_2).

In complete acid hydrolysates of (**1a**) and (**2a**) we identified the monosaccharides glucose, rhamnose, and glucuronic acid, and the aglycons oleanolic acid, in (**1a**), and hederagenin, in (**2a**). By alkaline hydrolysis of (**1a**) and (**2a**) we obtained the corresponding progenins (**4**) and (**5**). In acid hydrolysates of the latter we identified glucuronic acid and, in (**4**), oleanolic acid, and, in (**5**), hederagenin. Progenin (**4**) was additionally identified by TLC with an authentic specimen of oleanolic acid 3-O-β-*D*-glucopyranuronoside (saponin F) from the flowers of *Calendula officinalis* [2]. Consequently, the carbohydrate constituent at the C-3 hydroxy group of the aglycon in both glycosides (**1**) and (**2**) consisted of a glucuronic acid residue, and the glucose and rhamnose were components of the carbohydrate chain at the carboxy group of each aglycon.

In the PMR spectra of the full acetates of the methyl esters (**1b**) and (**2b**), the subspectra of the carbohydrate moieties were practically identical. Then, with the aid of the procedure of a one-dimensional HOHAHA experiment [3] it was possible to make an unambiguous assignment of the signals of the skeletal protons of the two β-glucopyranoses and the β-glucopyranuronose by exciting the H-1 anomeric protons having δ 5.54, 4.50, and 4.58 ppm with the aid of DANTE pulses [4]. The remaining signals were readily assigned to the rhamnopyranose residue in the light of the specific, easily recognized, nature of their splitting. The high-field position of the signals of the H-6‴ and H-4‴ skeletal protons of the glucopyranose residues in the spectra of the full acetates of (**1b**) and (**2b**) witnessed the participation of the corresponding OH groups in the formation of hydrogen bonds (see, for example, [1]) and thus determined the 1→6 and 1→4 types of glycosidic bonds in the trisaccharide fragment. The signal of an anomeric proton with δ 5.54 ppm corresponded to the Glc″ residue substituted at C-6, its rather low-field position being due to its bond with the carboxy group of the aglycon. Since, according to the position of the signal, the rhamnose residue is unsubstituted, the other glucose residue, Glc‴, was attached to C-6 of Glc″ and was substituted by the rhamnose residue at C-4. Consequently, the carbohydrate chain linked by an acyl glycosidic bond with the aglycon was the trisaccharide $Rha \xrightarrow{\alpha 4} Glc \xrightarrow{\beta 6} Glc^{\beta}$, which is typical for glycosides from plants of the Araliaceae family [5].

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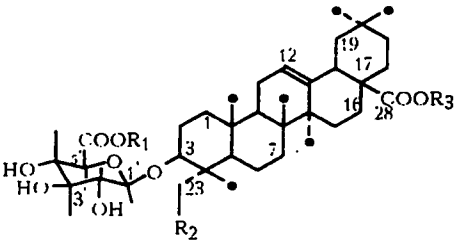

1-5

	R_1	R_2	R_3
1	H	H	βGlc″ ←6β— Glc‴ ←4α— Rha ⁗
1a	CH_3	H	βGlc″ ←6β— Glc‴ ←4α— Rha ⁗
2	H	OH	βGlc″ ←6β— Glc‴ ←4α— Rha ⁗
2a	CH_3	OH	βGlc″ ←6β— Glc‴ ←4α— Rha ⁗
3	C_2H_5	OH	βGlc″ ←6β— Glc‴ ←4α— Rha ⁗
4	H	H	H
5	H	OH	H

In the ^{13}C spectra of (**1a**) and (**2a**), the signals of the carbohydrate components were assigned by comparison with literature information for a *β-D*-glucopyranose residue [6] and for the trisaccharide fragment Rha$\xrightarrow{\alpha 4}$Glc$\xrightarrow{\beta 6}$Glc$^{\beta}$ [7], and those of the aglycon moieties by comparison with that for 3,28-diglycosides of oleanolic acid and of hederagenin [7], which unambiguously confirmed the structures of (**1**) and (**2**) as the 3-O-α-*L*-glucopyranuronside 28-O-[O-*L*-rhamnopyranosyl-(1→4)-O-*β-D*-glucopyranosyl-(1→6)-*β-D*-glucopyranoside]s of oleanolic acid and of hederagenin, respectively. Taurosides St-J and St-K are new triterpene glycosides.

We have described the preparation of the full acetate of glycoside St-$G_{0\text{-}2}$ (**3a**) previously [8]. The deacetylation of (**3a**) with an excess of sodium methanolate in methanol gave a product identical in chromatographic mobility with the glycoside St-K. According to the results of complete acid hydrolysis, the compositions of these glycosides and their aglycons were identical. However, the full acetates of $StG_{0\text{-}2}$ and of St-K differed both in TLC behavior and with respect to their PMR spectra.

In the PMR spectrum of (**3a**) we identified the signals of the skeletal protons of the *β-D*-glucopyranuronose residue and of the trisaccharide fragment Rha$\xrightarrow{\alpha 4}$Glc $\xrightarrow{\beta 6}$Glc$^{\beta}$, their positions and the nature of their splittings coinciding completely with those for glycoside St-K, while the types and orders of the bonds were additionally confirmed by the ROESY spectrum (Fig. 1) in which we observed cross-peaks of the signals of the following protons: H-1 GlcUA — H-3 of the aglycon, H-1 Rha‴ — H-4 Glc‴, and the others, the assignments of which are given in Fig. 1. However, in the contour representation of the ROESY spectrum we found no informative cross-peak for the H-1 proton of the Glc‴ residues with H-6 of Glc″ in view of the small magnitude of the negative Overhauser effect. It was possible to detect the H-1‴ signal in a one-dimensional section of the ROESY spectrum from the chemical shift of H-6″ with δ 3.87 ppm. The PMR spectrum of (**3a**), as compared with that of (**2b**), lacked a signal with δ 3.74 ppm of the protons of a methoxyl group (methyl ester of a glucuronic acid residue). On the other hand, two triplet signals were observed, with δ 4.20 and 1.26 ppm, having a ratio of their integral intensities of 2:3 and a common spin-spin coupling constant J = 7 Hz. Double homonuclear resonance experiments showed a link of these two protons only with one another. Since (**3a**) was not methylated by an ethereal solution of diazomethane, the two-carbon fragment formed an ester at the carboxy group of the glucuronic acid residue. A detailed analysis of the 4.20 ppm region (Fig. 2) revealed a number of weak peaks belonging to this multiplet, which enabled it to be interpreted as the *AB* part of an ABX_3 spin system, where *AB* are the protons of an $-O-CH_2-$ group, and the signal with δ 1.26 ppm is a true triplet of a CH_3 group forming the X_3 part of this spin system. Thus, the glucuronic acid residue was esterified with ethanol. In this case, the methylene protons of the $-O-CH_2-CH_3$ group are not equivalent because of the presence of the very close asymmetric C-5 carbon atom of the glucuronic acid residue.

Thus, glycoside St-$G_{0\text{-}2}$ is the ethyl ester of glycoside St-K and is a new compound. No formation of St-$G_{0\text{-}2}$ from pure St-K on direct prolonged treatment with 70% aqueous ethanol (extraction conditions) was observed, which permits the conclusion that this glycoside was of natural origin.

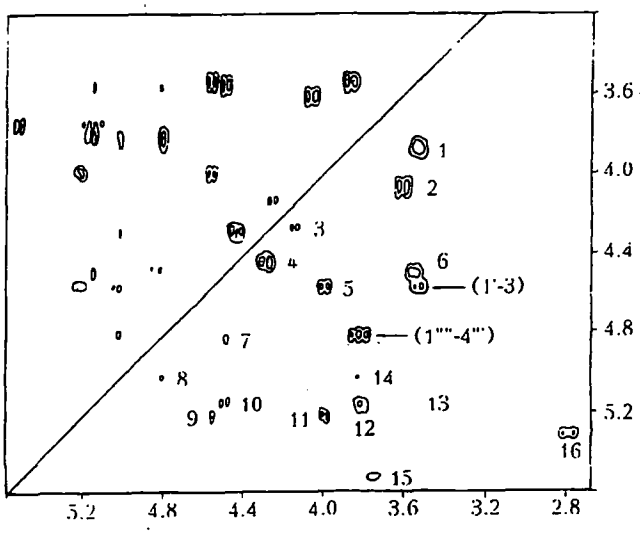

Fig. 1. Fragment of the two-dimensional ROESY spectrum of the full acetate of St-G_{0-2}. The numerals indicate cross-peaks between the signals of the following protons: 1) (6A−6B)″; 2) (23A−23B); 3) (H_A-H_B) in $O-C\underline{H}_2-CH_3$; 4) (6A−6B)‴; 5) (1−5)′; 6) (1−5)‴; 7) (1−2)‴: 8) (1−2)‴; 9) (1−3)′; 10) (1−3)‴; 11) (4−5)′; 12) (3−5)‴; 13) (3−5)‴ 14) (4−5)‴; 15) (1−5)″; 16) (12−18).

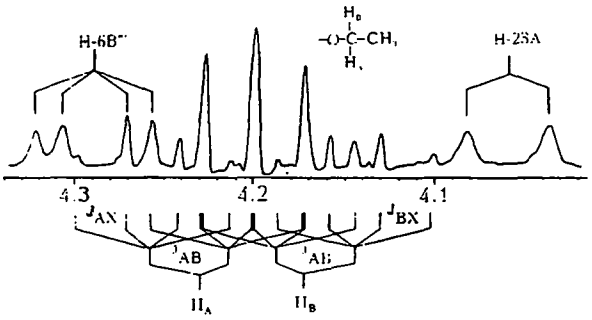

Fig. 2. Fragment of the ^1H NMR spectrum of the acetate of glycoside St-G_{0-2}.

EXPERIMENTAL

For general observations and the procedures for acetylation, deacetylation, and acid and alkaline hydrolysis, see [9].

Methyl Ester of Glycoside St-J (1a). An excess of an ethereal solution of diazomethane was added to a solution of 0.5 g of (**1**) in 50 ml of 90% aqueous methanol [10]. Evaporation and chromatographic purification on SiO_2 with elution by the solvent system water-saturated chloroform−ethanol (1:1) gave 0.4 g of pure (**1a**), $[\alpha]_D$ −5° (*c* 2.3; pyridine). In an acid hydrolysate of (**1a**) we identified rhamnose, glucose, and glucuronic and oleanolic acids. The alkaline hydrolysis of (**1a**) gave, after purification on SiO_2 in the water-saturated chloroform−ethanol (2:1) system, the progenin (**4**), mp 260-265°C, $[\alpha]_D$ +16° (*c* 0.5, methanol); lit.: mp 264-267°C, $[\alpha]_D$ +20.4° (methanol) [6]. Glucuronic and oleanolic acids were identified in an acid hydrolysate of (**4**).

Full Acetate of the Methyl Ester of Glycoside St-J (1b). The acetylation of 100 mg of (**1a**) gave (**1b**), and, after chromatographic purification on SiO_2 with elution by the chloroform−benzene (10:1) system, 90 mg of pure (**1b**) was obtained, with $[\alpha]_D$ −6° (*c* 2.0; chloroform).

^1H NMR spectrum of (**1b**) (δ, ppm, 0 — TMS, $CDCl_3$): 4.58 (d, H-1′, $J_{1,2}$ 8.0); 5.04 (t, H-2′, $J_{2,3}$ 9.0); 5.21 (t, H-3′, $J_{3,4}$ 9.5); 5.18 (t, H-4′, $J_{4,5}$ 9.5); 4.00 (d, H-5′); 5.54 (d, H-1″, $J_{1,2}$ 8.0); 5.11 (t, H-2″, $J_{2,3}$ 9.5); 5.21 (t, H-3″, $J_{3,4}$ 9.5); 4.98 (d.d, H-4″, $J_{4,5}$ 10.0); 3.73 (m, H-5″); 3.85 (d.d, H-6″ A, $J_{5,6A}$ 3.0; $J_{6A,6B}$ 12.0); 3.54 (d.d, H-6″B, $J_{5,6B}$ 5.0);

TABLE 1. Chemical Shifts of the ^{13}C Atoms of the Aglycon Moieties of the Methyl Esters of Glycosides St-J (**1a**) and St-K (**2a**) (δ, ppm, 0 — TMS, C_5D_5N, 70°C)

C-Atom	1	2	C-Atom	1	2
1	38.7	38.8	16	23.4	23.9
2	26.7	26.2	17	47.1	47.1
3	89.3	82.4	18	41.7	41.7
4	39.6	43.5	19	46.3	46.3
5	55.8	47.6	20	30.8	30.8
6	18.2	18.3	21	34.1	34.1
7	33.2	32.6	22	32.6	33.0
8	40.0	40.0	23	28.4	64.4
9	48.2	48.2	24	16.2	13.7
10	37.0	37.0	25	15.6	16.3
11	23.7	23.4	26	17.6	17.6
12	123.0	123.0	27	26.1	26.3
13	144.3	144.3	28	176.6	176.6
14	42.2	42.2	29	33.2	33.2
15	28.4	28.4	30	23.7	24.0

TABLE 2. Chemical Shifts of the ^{13}C Atoms of the Carbohydrate Moieties of the Methyl Esters of Glycosides St-J (**1a**) and St-K (**2a**) (δ, ppm, 0 — TMS, C_5D_5N, 70°C)

C-Atom	1	2	C-Atom	1	2
GlcUA			Glc		
1′	107.4	106.4	1‴	104.9	104.9
2′	75.3	75.4	2‴	75.4	75.3
3′	78.0	77.8	3‴	76.6	76.5
4′	73.2	73.2	4‴	78.4	78.4
5′	77.3	77.3	5‴	77.2	77.2
6′	171.0	170.9	6‴	61.3	61.3
Glc			Rha		
1″	95.7	95.7	1⁗	102.8	102.8
2″	73.9	73.8	2⁗	72.6	72.5
3″	78.7	78.7	3⁗	72.8	72.7
4″	70.9	70.8	4⁗	74.0	74.0
5″	78.0	78.1	5⁗	70.4	70.4
6″	69.5	69.2	6⁗	18.6	18.6
O-CH_3	52.1	52.1			

4.50 (d, H-1‴, $J_{1,2}$ 8.0); 4.81 (t, H-2‴, $J_{2,3}$ 9.0); 5.14 (t, H-3‴, $J_{3,4}$ 9.0); 3.81 (t, H-4‴, $J_{4,5}$ 9.5); 3.55 (m, H-5‴); 4.43 (d.d, H-6‴A, $J_{5,6A}$ 2.5, $J_{6A,6B}$ 13.0); 4.28 (d.d, H-6‴B, $J_{5,6B}$ 4.0); 4.80 (d, H-1⁗, $J_{1,2}$ 2.0); 5.02 (d.d, H-2⁗, $J_{2,3}$ 3.0); 5.17 (d.d, H-3⁗, $J_{3,4}$ 10.0); 5.02 (t, H-4⁗, $J_{4,5}$ 10.0); 3.82 (dq, H-5⁗); 1.13 (d, H-6⁗, $J_{5,6}$ 6.0); 5.30 (bt, H-12, $J_{11,12}$ 3.5); 3.11 (d.d, H-3, $J_{2e,3}$ 5.0, $J_{2a,3}$ 12.0); 2.80 (d.d, H-18, $J_{18,19e}$ 4.0; $J_{18,19a}$ 13.0); 3.75 (s, OCH_3); 1.25; 1.01; 0.91; 0.90; 0.90; 0.73; 0.73 (all s, 7 CH_3); 1.95-2.15 (all s, 12$OCOCH_3$).

Methyl Ester of Glycoside St-K (2a). Compound (**2**) (1.0 g) was esterified with diazomethane and the product was purified chromatographically as in the case of compound (**1a**). This gave 0.8 g of pure (**2a**), $[\alpha]_D$ −7° (*c* 3.3; pyridine). Rhamnose, glucose, glucuronic acid, and hederagenin were identified in an acid hydrolysate of (**2a**). The alkaline hydrolysis of (**2a**) gave progenin (**5**), which, after purification as for (**4**), had mp 220-225°C, $[\alpha]_D$ +24° (*c* 2.0; methanol); lit: 224-227°C, $[\alpha]_D$ +22.6 (methanol) [6]. Glucuronic acid and hederagenin were identified in an acid hydrolysate of (**5**).

Full Acetate of the Methyl Ester of Glycoside St-K (2b). The acetylation of 100 mg of (**2a**) and purification as for (**1b**) gave 80 mg of pure (**2b**), $[\alpha]_D$ −2° (*c* 1.1; chloroform).

^1H NMR spectrum of (**2b**) (δ, ppm, 0 — TMS, $CDCl_3$): 4.56 (d, H-1′, $J_{1,2}$ 7.5); 5.01 (d.d, H-2′, $J_{2,3}$ 9.5); 5.24 (t, H-3′, $J_{3,4}$ 9.0); 5.20 (t, H-4′, $J_{4,5}$ 9.5); 3.99 (d, H-5′); 5.52 (d, H-1″, $J_{1,2}$ 8.0); 5.11 (t, H-2″, $J_{2,3}$ 10.0); 5.21 (t, H-3″, $J_{3,4}$ 9.5); 4.98 (d.d, H-4″, $J_{4,5}$ 10.0); 3.73 (m, H-5″); 3.87 (dd, H-6″A, $J_{5,6A}$ 2.5; $J_{6A,6B}$ 11.5); 3.53 (d.d, H-6″B, $J_{5,6B}$ 5.0); 4.49 (d, H-1‴, $J_{1,2}$ 7.5); 4.82 (d.d, H-2‴, $J_{2,3}$ 9.5); 5.14 (t, H-3‴, $J_{3,4}$ 8.0); 3.82 (t, H-4‴, $J_{4,5}$ 9.5); 3.53 (m, H-5‴); 4.43 (d.d, H-6‴A, $J_{5,6A}$ 2.0, $J_{6A,6B}$ 12.0); 4.29 (d.d, H-6‴, B, $J_{5,6B}$ 3.5); 4.81 (d, H-1⁗, $J_{1,2}$ 2.0); 5.01 (d.d, H-2⁗, $J_{2,3}$ 3.0); 5.17 (d.d, H-3⁗, $J_{3,4}$ 10.0); 5.02 (t, H-4⁗, $J_{4,5}$ 10.0); 3.81 (dq, H-5⁗); 1.13 (d, H-6⁗, $J_{5,6}$ 6.0); 5.30 (bt, H-12, $J_{11,12}$ 3.5); 3.53 (d.d, H-3, $J_{2e,3}$ 4.0, $J_{2a,3}$ 13.0); 4.06 (d, H-23A, $J_{23A,23B}$ 11.5); 3.60 (d, H-23B); 3.74 (s, O-CH_3); 1.08; 0.95; 0.89; 0.89; 0.73; 0.71 (all s, 6 CH_3); 1.95-2.15 (all s, 12$OCOCH_3$).

Full Acetate of Glycoside St-G_{0-2} (3a). The chromatography of 40 mg of crude (**3a**) [8] on SiO_2 with elution by the solvent system chloroform–benzene (8:2) gave 36 mg of pure (**3a**), $[\alpha]_D$ −3° (*c* 1.7; chloroform).

^1H NMR spectrum of (**3a**) (δ, ppm, 0–TMS, $CDCl_3$): 1.26 (t, $-O-CH_2-C\underline{H}_3$, ^3J 7.0); 4.20 (m, $-O-C\underline{H}_2-CH_3$); there was no signal at 3.74 ppm ($O-CH_3$). The chemical shifts and SSCCs of the skeletal protons of the monosaccharide residues and of the aglycon were identical with those for (**2b**).

The deacetylation of (**3a**) with a catalytic amount of sodium methanolate in anhydrous methanol gave a product identical with (**2a**) according to TLC, while treatment with an excess of sodium methanolate in methanol (40°C, 10 h) gave a product identical on TLC with (**2**). The progenin obtained from (**3a**) by alkaline hydrolysis was identical with (**5**).

Glycoside St-G_{0-2} (3). The deacetylation of 30 mg of (**3a**) by a catalytic amount of sodium ethanolate in anhydrous ethanol gave 20 mg of (**3**), $[\alpha]_D$ −5° (*c* 0.5, pyridine).

^{13}C NMR spectrum of (**3**) (δ, ppm, 0 — TMS, C_5D_5N); 61.7 ($O-\underline{C}H_2CH_3$); 9.3 ($O-CH_2\underline{C}H_3$); there was no signal at 52.1 ($O-CH_3$); the other signals were completely identical with those for (**2a**) (Tables 1 and 2).

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