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TRITERPENE GLYCOSIDES OF *Hedera helix* III. STRUCTURE OF THE TRITERPENE SULFATES AND THEIR GLYCOSIDES

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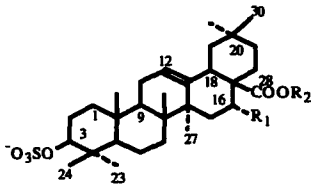
From the leaves of English ivy Hedera helix *L. we have isolated the known 3-sulfates of oleanolic and echinocystic acids and their 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters and the new 3-sulfate of 28-O-β-gentiobiosyl oleanolate — helicoside L-8a. The structures of the compounds isolated were deduced from the results of chemical transformations and NMR spectroscopy.*

On TLC analysis, the triterpenoid fraction L-5 that we had obtained previously in the separation of the total glycosides from the leaves of English ivy [1] proved to consist of two components, designated as L-5a (**1**) and L-5b (**2**). They were separated preparatively on silica gel (SiO_2) with subsequent additional purification by esterification with diazomethane and chromatography on SiO_2. In acid hydrolysates of (**1**) and (**2**) we identified oleanolic and echinocystic acids, respectively, but did not detect any sugars, and in both cases found the sulfate anion. Obviously, (**1**) and (**2**) were sulfated oleanolic and echinocystic acids. In the PMR spectra of (**1**) and (**2**) the rather low-field position of the H-3 signal (4.45 and 4.54 ppm) witnessed the presence of the sulfate group at the C-3 atom of the triterpenoid [2]. The assignment of the signals in the PMR spectra of (**1**) and (**2**) was made by a comparison with the literature [2, 3] and on the basis of COSY spectra.

The ^{13}C NMR spectra of (**1**) and (**2**) and of the 3-sulfates of oleanolic and echinocystic acids [4] were practically identical, which confirmed the structures of compounds L-5a and L-5b. Analogous sulfates have been obtained previously as progenins of glycosides from *Bupleurum rotundifolium* [4], and we have also isolated them from the leaves of Crimean ivy [3, 5].



1. R_1=H; R_2=H
2. R_1=OH; R_2=H
3. R_1=H; R_2= ← β-*D*-Glc p6' ← β-*D*-Glc p
4. R_1=H; R_2= ← β-*D*-Glc p6' ← β-*D*-Glc p4'' ← α-*L*-Rha p'''
5. R_1=OH; R_2= ← β-*D*-Glc p6' ← β-*D*-Glc p4'' ← α-*L*-Rha p'''

According to TLC, the fraction L-8 obtained previously [1] consisted of three glycosides, designated as L-8a (**3**), L-8b (**4**), and L-8c (**5**), and these were separated by chromatography on SiO_2.

In acid hydrolysates of (**4**) and (**5**) we identified oleanolic and echinocystic acids as the respective aglycons, together with the same sugars — glucose and rhamnose. The progenins from (**4**) and (**5**), obtained by alkaline hydrolysis, were identical with the above-mentioned compounds (**1**) and (**2**), respectively, while glycosides (**4**) and (**5**) themselves were identical, according to TLC, with taurosides I and J [3]. Their ^{13}C NMR spectra also coincided completely, which confirmed the structures of (**4**) and (**5**) as the 3-sulfates of the 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of oleanolic and echinocystic acids, respectively.

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TABLE 1. Chemical Shifts of the Signals of the ^{13}C Atoms of the Aglycon Moiety of Glycoside L-8a (3) (δ, ppm, 0 — TMS, C_5D_5N)

C atom	Chem. shift	C atom	Chem. shift	C atom	Chem. shift
1	38.9	11	23.5	21	34.1
2	25.1	12	123.0	22	32.5
3	85.2	13	144.5	23	29.0
4	39.1	14	42.2	24	17.4
5	56.5	15	28.3	25	15.5
6	18.5	16	23.8	26	17.1
7	33.3	17	47.2	27	26.2
8	40.0	18	41.8	28	176.8
9	48.1	19	46.4	29	33.3
10	37.3	20	30.9	30	23.9

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Atoms of the Carbohydrate Fragment of Glycoside L-8a (3) (δ, ppm, 0 — TMS, C_5D_5N)

C atom	Chem. shift	C atom	Chem. shift
1'	98.8	1"	105.3
2'	74.0	2"	75.3
3'	78.8	3"	78.3
4'	71.4	4"	71.7
5'	77.9	5"	78.4
6'	69.9	6"	63.0

Complete acid hydrolysis showed that compound (3) contained oleanolic acid, glucose, and a sulfate group. A progenin from (3) obtained by alkaline hydrolysis was identical with (1). In the ^{13}C NMR spectrum of (3) the assignments of the signals of the aglycon part was made by comparison with the literature [3—5]. The remaining signals, belonging to the carbohydrate fragment were identical, in terms of chemical shifts, with those of β-gentiobiose bound to an aglycon by an acylglycosidic bond [6]. For an additional confirmation of its structure, glycoside (3) was obtained from (4) by splitting out the terminal rhamnose residue under mild acid hydrolysis.

Thus, helicoside L-8a (3) consists of the 3-sulfate of the 28-O-β-gentiobiosyl ester of oleanolic acid and is a new glycoside.

We did not detect in the leaves of *Hedera helix* the glycosides of oleanolic acid with glucuronic acid residues — hederasaponin I and hederasaponin H [8] with the structures of the 28-O-β-*D*-rhamnopyranosyl-(1→4)-O-β-*D*-glucopyranosyl-(1→6)-O-β-*D*-glucopyranosyl ester of 3-O-β-D-glucuronopyranosyloleanolic acid and oleanolic acid 3-O-β-*D*-galactopyranosyl-(1→4)-3-O-β *D*-glucuronopyranoside, respectively — that have been found previously in the leaves of this plant.

The glycoside compositions of the leaves of *Hedera helix* L. and of the close species *Hedera taurica* Carr. that we have studied previously [3, 5, 7, 9] are qualitatively similar, but a higher level of the 3-sulfates of oleanolic and echinocystic acids and their 28-glycosides was observed in the leaves of *Hedera taurica* than of *Hedera helix*.

Conversely, there was a somewhat higher level of neutral oleanolic acid glycosides in the leaves of *Hedera helix* than in those of *Hedera taurica*.

EXPERIMENTAL

For general observations, see [1].

Separation of Fraction L-5. Fraction L-5 [1] (100 mg) was separated on SiO_2 with elution by the solvent system

chloroform—ethanol (100:30) saturated with 30% NH_3. This gave 70 mg of (1) and 15 mg of (2). In complete acid hydrolysates of (1) and (2) we identified the sulfate anion and also oleanolic and echinocystic acids, respectively.

Methyl Ester of L-5a (1a). Compound (1) (60 mg) was esterified with an ethereal solution of CH_2N_2, and the (1a) obtained was chromatographed on SiO_2 with elution by the water-saturated chloroform-ethanol (100:20) system. This gave 45 mg of pure (1a), $[\alpha]_D$ +40° (*c* 1.0; methanol; lit. $[\alpha]_D$ +42° (methanol) [5].

PMR spectrum of (1a) (δ, ppm, 0-TMS, C_5D_5N): 5.24 (t, $J_{11,12}$=3.0 Hz, H-12); 4.45 (dd, $J_{2a,3}$=12.0 Hz, $J_{2e,3}$=4.4 Hz, H-3); 3.59 (s, O-CH_3): 2.97 (dd, $J_{18,19a}$=12.8 Hz, $J_{18,19e}$=4.8 Hz, H-18); 2.64 (dd, $J_{19a,19e}$=14.5 Hz, H-19e); 1.31, 1.07, 0.90, 0.82, 0.81, 0.74, 0.69, (all s, 7CH_3); 0.7-2.2 (skeletal CH, CH_2).

Methyl Ester of L-5b (2a). Compound (2) (12 mg) was esterified with CH_2N_2, and the product was purified in a similar way to (1a), giving 7 mg of pure (2a), $[\alpha]_D$ +15° (*c* 0.5; methanol), lit. $[\alpha]_D$ +21° (methanol) [3].

PMR spectrum of (2a) (δ, ppm, 0-TMS, C_5D_5N): 5.49 (t, $J_{11,12}$=3.5 Hz, H-12); 5.01 (qt, $J_{15,16}$=3.0 Hz, H-16); 4.54 (dd, $J_{2e,3}$=4.4 Hz, H-3); 3.66 (s, O-CH_3); 3.36 (dd, $J_{18,19a}$=14.0 Hz, $J_{18,19e}$=4.0 Hz, H-18); 2.70 (dd, $J_{19a,19e}$=14.0 Hz, H-19e); 1.30 (m, H-19a); 2.72, 1.90 (mm, H-2a, H-2e); 2.00 (H-15e); 1.62 (H-15a); 1.72; 1.36; 1.07; 0.99; 0.98; 0.86; 0.82 (all s, 7CH_3); 0.7-2.5 (skeletal CH, CH_2).

Separation of Fraction L-8. Fraction L-8 [1] (140 mg) was separated on SiO_2 with elution by the water-saturated chloroform—ethanol (100:40) system. This gave 15 mg of (3), 17 mg of (4), and 15 mg of (5). Sulfate anion was identified in acid hydrolysates of (3—5), oleanolic acid in (3) and (4), and echinocystic acid in (5), and the sugars glucose in (3) and rhamnose and glucose in (4) and (5).

Glycoside L-8a (3). Compound (3) (12 mg) was purified on SiO_2 with elution by the chloroform—ethanol (100:40) solvent system saturated with 10% aqueous NH_3. This yielded 10 mg of pure (3). The alkaline hydrolysis of (3) gave (1).

Glycoside L-8b (4). The purification of (4) was similar to that of (3), and 60 mg of pure (4) was obtained, with $[\alpha]_D$ -8° (*c* 3.0; methanol), lit. $[\alpha]_D$ -6° (methanol) [3]. The alkaline hydrolysis of (4) yielded (1). According to TLC and its ^{13}C NMR spectrum, (4) was identical with tauroside I [3].

Glycoside L-8c (5). The purification of (4) was similar to that of (3), yielding 5 mg of pure (5), $[\alpha]_D$ -11° (*c* 0.4; methanol), lit. $[\alpha]_D$ -15° (methanol) [3]. The alkaline hydrolysis of (5) gave (2).

According to TLC and its PMR spectrum, (5) was identical with tauroside J [3].

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