Chemistry of Natural Compounds, Vol. 30, No. 6, 1994

TRITERPENE GLYCOSIDES OF *Hedera helix*

I. THE STRUCTURES OF GLYCOSIDES L-1, L-2a, L-2b, L-3, L-4a, L-4b, L-6a, L-6b, L-6c, L-7a, AND L7-b FROM THE LEAVES OF COMMON IVY

V. I. Grishkovets, A. E. Kondratenko, N. V. Tolkacheva, A. S. Shashkov, and V. Ya Chirva

UDC 547.918:543.422

The leaves of common ivy have yielded 11 triterpene glycosides: the 3-O-α-L-pyranosides of oleanolic acid (1), of echinocystic acid (2), and of hederagenin; the 3-O-[O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside]s of oleanolic acid (4), of echinocystic acid (5), and of hederagenin (6); the O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of hederagenin 3-O-α-L-pyranoside (7); the O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of hederagenin 3-O-[O-α-L-pyranosyl-(1→2)-α-L-arabinopyranoside] (9); and the O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of oleanolic acid, echinocystic acid, and hederagenin 3-O-[O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside]s (8), (10), and (11), respectively. This is the first time that compounds (1), (2), (5), (7), (9), and (10) have been found in this plant.

Common ivy *Hedera helix* L. (*Hedera lobata* Gilib., *Hedera communis* S. F. Gray), fam. Araliaceae [1], growing in most of the territory of Europe and in the western regions of the Ukraine, has long been used in folk medicine and officinal medicine [2].

The study of the glycoside composition of this plant began a comparatively long time ago, and before the present investigations the structures had been established of five hederagenin and oleanolic acid glycosides from the leaves [3-6] and of three hederagenin glycosides from the fruit [7].

The aim of the present series of investigations is a systematic study of the glycoside compositions of various organs of common ivy. The TLC analysis of an alcoholic extract of leaf blades of *Hedera helix* showed the presence of eight groups of glycosides, which we have designated as L-1—L-8 in order of increasing polarity.

The dried and comminuted plant material was extracted successively with hexane and chloroform to eliminate substances of low polarity, and the glycosides were then extracted with water-saturated butanol. The preparative separation of the butanolic extract into fractions L-1—L-8 was conducted on silica gel with gradient elution by water-saturated mixtures of chloroform and ethanol.

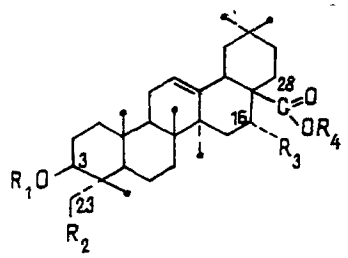
According to the results of TLC in various solvent systems, L-1 (**1**) was an individual glycoside. The rechromatography of L-2 on silica gel showed that it consisted of two glycosides — a minor one, L-2a (**2**) and a predominating, more polar, glycoside, L-2b (**3**). The further purification of (**1-3**), as of all the glycosides described below, was carried out in the chloroform—ethanol—ammonia system.

Acid hydrolysis of (**1-3**) showed that they all contained the same monosaccharide — arabinose — and that their aglycons were oleanolic acid, echinocystic acid, and hederagenin, respectively. The treatment of these glycosides with an ethereal solution of diazomethane converted them into methyl esters, while attempted alkaline hydrolysis caused no changes whatever. Consequently, in (**1-3**) the arabinose residues were attached to the hydroxy group at the C-3 atom of each aglycon. According to their chromatographic mobilities in various solvent systems and their specific rotations, compounds (**1-3**) were identical with authentic specimens of the 3-O-α-*L*-arabinopyranosides of oleanolic and echinocystic acids and of hederagenin.

Simferopol' State University. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 742-746, November-December, 1994. Original article submitted November 24, 1993.

0009-3130/94/3006-0689$12.50

Hederagenin 3-O-α-*L*-arabinopyranoside has been isolated previously from common ivy fruit [7], while this is the first time that the minor 3-O-α-*L*-arabinopyranosides of oleanolic and echinocystic acids have been detected in common ivy. Of other plants of the Araliaceae family, they have been found in Japan fatsia, *Fatsia japonica* (Thunb.) Decne et Planch. [8-10].



	R_1	R_2	R_3	R_4
1	*L*-Arap (α)	H	H	H
2	*L*-Arap (α)	H	OH	H
3	*L*-Arap (α)	OH	H	H
4	*L*-Rhap (α2) → *L*-Arap (α)	H	H	H
5	*L*-Rhap (α2) → *L*-Arap (α)	H	OH	H
6	*L*-Rhap (α2) → *L*-Arap (α)	OH	H	H
7	*L*-Arap (α)	OH	H	←β *D*-Glcp (6β→) *D*-Glcp (4α→) *L*-Rhap
8	*L*-Rhap (α2) → *L*-Arap (α)	H	H	←β *D*-Glcp (6β→) *D*-Glcp (4α→) *L*-Rhap
9	*L*-Rhap (α2) → *L*-Arap (α)	OH	H	←β *D*-Glcp (6β→) *D*-Glcp
10	*L*-Rhap (α2) → *L*-Arap (α)	H	OH	←β *D*-Glcp (6β→) *D*-Glcp (4α→) *L*-Rhap
11	*L*-Rhap (α2) → *L*-Arap (α)	OH	H	←β *D*-Glcp (6β→) *D*-Glcp (4α→) *L*-Rhap

As a result of TLC analysis it was established that the L-3 fraction was an individual glycoside, (**4**), while fraction L-4 was a mixture of two glycosides, L-4a (**5**) and L-4b (**6**). The separation of this pair was achieved by rechromatography on silica gel in the chloroform—ethanol—water system. Acid hydrolysis of compounds (**4-6**) showed that their molecules contained the sugars rhamnose and arabinose and the aglycons oleanolic and echinocystic acids and hederagenin, respectively. According to the results of attempted alkaline hydrolysis and methylation with diazomethane, compounds (**4-6**) contained carbohydrate chains only at the C_3-OH groups of the aglycons, and they were identical with authentic specimens of the 3-O-[O-α-*L*-rhamnopyranosyl-(1→2)-α-*L*-arabinopyranoside]s of oleanolic and echinocystic acids and of hederagenin, which we had isolated previously from Crimean ivy, *Hedera taurica* Carr. [11-13]. This is the first time that compound (**5**) has been isolated from *Hedera helix*, while (**4**) and (**6**) have been found previously in the fruit of this plant [3, 4, 6].

The TLC analysis of fraction L-6 in the chloroform—methanol—ammonia system showed that it consisted of four glycosides: L-6a (**7**), L-6b (**8**), L-6c (**9**), and L-6d, with (**8**) predominating. The individual glycosides were obtained by the preparative separation of this fraction on silica gel in the chloroform—ethanol—ammonia system. The carbohydrate compositions of (**7-9**) consisted of rhamnose, arabinose, and glucose, while the aglycons were hederagenin in (**7**) and (**9**), and oleanolic acid in (**8**). Progenins of these glycosides were identical with the compounds (**3**), (**4**), and (**6**) described above, while glycosides (**7-9**) were identical with taurosides G_1—G_3 from Crimean ivy leaves [14]. From its structure, compound (**8**) was identical with hederasaponin B, isolated previously from common ivy [4, 5], while (**7**) and (**9**) have not previously been isolated from *Hedera helix* L.

The rechromatography of fraction L-7 permitted it to be separated into the two glycosides L-7a (**10**) and L-7b (**11**). The carbohydrate compositions of (**10**) and (**11**) were the same, consisting of rhamnose, arabinose, and glucose, while the aglycons were echinocystic acid and hederagenin, respectively. Progenins from (**10**) and (**11**) were identical with glycosides (**5**) and (**6**), while (**10**) and (**11**), themselves, were identical with taurosides H_1 and H_2 from Crimean ivy leaves [14]; this is the first time that (**10**) has been detected in the leavs of *Hedera helix* L, while (**11**) was identical with the previously isolated hederasaponin C [4, 5].

The ^{13}C NMR spectra of compounds (**3-6**), (**8**), (**10**) and (**11**) were identical with those of the corresponding glycosides isolated by us previously from Crimean ivy [11-14].

EXPERIMENTAL

NMR spectra were taken on a Bruker WM-250 instrument (62.9 MHz for ^{13}C atoms) in deuteropyridine. Specific rotations were measured on a SU-4 saccharimeter at λ 589 nm.

TLC monitoring was conducted on Silufol plates in the following solvent systems: chloroform—methanol—water (100:40:7) and (100:30:5); and chloroform—methanol—25% ammonia (100:50:15), (100:40:10), and (100:30:6). The glycosides and aglycons were detected with a 10% alcoholic solution of tungstophosphoric acid, and sugars with acid aniline phthalate followed by the heating of the chromatograms. Preparative separation was carried out on silica gel L (40-100 μm) and Silpearl (Czechoslovakia).

Complete acid hydrolysis was effected with a 2 N solution of CF_3COOH in water—methanol (1:1) at 100°C for 2 h. Partial acid hydrolysis was conducted with a 1 N solution of CF_3COOH in water—dioxane (1:1) at 100°C for 30 min.

Alkaline hydrolysis was performed with 10% KOH in water—methanol (1:1) at 100°C for 2 h, followed by neutralization with a 1 N aqueous solution of H_2SO_4 to a weak acid reaction and extraction of the progenin with butanol.

After separation from petioles, *Hedera helix* leaves gathered in the environs of L'vov in an amount of 2.5 kg (dry weight 0.5 kg) were comminuted and treated successively with hexane and chloroform (3 times with 5 liters of each solvent) and the glycosides were then extracted with water-saturated butanol (4 × 5 liters). The evaporated butanolic extract (60 g) was separated on 4 kg of silica gel with gradient elution by water-saturated chloroform—ethanol (10:1→1:1). This led to fractions L-1—L-8. Fractions L-2, L-4, and L-7 were rechromatographed with elution by water-saturated chloroform—ethanol systems (10:1, 7:3, 1:1), and fraction L-6 with elution by the chloroform—ethanol (7:3) system saturated with 10% aqueous ammonia.

The individual glycosides obtained were additionally purified by chromatography on silica gel in the following solvent systems: chloroform—ethanol (10:1) saturated with 10% NH_3 for (**1-3**); chloroform—ethanol (7:3) saturated with 10% NH_3 for (**4-6**); and chloroform—ethanol (1:1) saturated with 10% NH_3 for (**7-11**). As a result, 25 mg of (**1**), 75 mg of (**2**), 120 mg of (**3**), 625 mg of (**4**), 500 mg of (**5**), 7.5 g of (**6**), 100 mg of fraction L-5, 50 mg of (**7**), 1.0 g of (**8**), 100 mg of (**9**), 125 mg of L-6d, 1.25 g of (**10**), 6.25 g of (**11**), and 140 mg of fraction L-8 were obtained.

Glycoside L-1 (1), $[\alpha]_D$ +45° (c 0.3; methanol); lit.: $[\alpha]_D$ +53.1° (methanol) [9]. In an acid hydrolysate of (**1**) arabinose and oleanolic acid were identified. According to TLC, (**1**) was identical with the oleanolic acid 3-O-α-*L*-arabinopyranoside obtained by the partial acid hydrolysis of tauroside C [11].

Glycoside L-2a (2), $[\alpha]_D$ +39° (c 0.4, methanol); lit.: $[\alpha]_D$ +42.1° (methanol) [9]. An acid hydrolysate of (**2**) contained arabinose and echinocystic acid. According to TLC, (**2**) was identical with the echinocystic acid 3-O-α-*L*-arabinopyranoside obtained by partial acid hydrolysis of tauroside D [12].

Glycoside L-2b (3), $[\alpha]_D$ +72° (c 1.3, pyridine); lit.: $[\alpha]_D$ +82.1° (pyridine). An acid hydrolysate of (**3**) contained arabinose and hederagenin. According to TLC, (**3**) was identical with tauroside B [11].

Glycoside L-3 (4), $[\alpha]_D$ +8° (c 0.9, methanol); lit.: $[\alpha]_D$ +10.9° (methanol) [15]. An acid hydrolysate of (**4**) contained rhamnose, arabinose, and oleanolic acid. According to TLC, (**4**) was identical with tauroside C [11].

Glycoside L-4a (5), $[\alpha]_D$ −26° (c 1.1; pyridine); lit.: $[\alpha]_D$ −29.9° (pyridine) [12]. An acid hydrolysate of (**5**) contained rhamnose, arabinose, and echinocystic acid. According to TLC, (**5**) was identical with tauroside D [12].

Glycoside L-4b (6), $[\alpha]_D$ +8° (c 0.4; ethanol); lit.: $[\alpha]_D$ +7° (ethanol) [13]. An acid hydrolysate of (**6**) contained rhamnose, arabinose, and hederagenin. According to TLC, (**6**) was identical with tauroside E [13].

Glycoside L-6a (7), $[\alpha]_D$ +6° (c 0.5; pyridine); lit.: $[\alpha]_D$ +3.6° (pyridine) [14]. An acid hydrolysate of (**7**) contained rhamnose, arabinose, glucose, and hederagenin. According to TLC a progenin from (**7**) was identical with (**3**), and (**7**) itself with tauroside G_1 [14].

Glycoside L-6b (8), $[\alpha]_D$ −25° (c 2.2; methanol); lit.: $[\alpha]_D$ −28.8° (methanol) [14]. An acid hydrolysate of (**8**) contained rhamnose, arabinose, glucose, and oleanolic acid. Acording to TLC a progenin from (**8**) was identical with (**4**), and **8** itself with tauroside G_2 [14].

Glycoside L-6c (9), $[\alpha]_D$ −10° (c 0.6; pyridine); lit.: $[\alpha]_D$ −8.2° (pyridine) [14]. An acid hydrolysate of (**9**) contained rhamnose, arabinose, glucose, and hederagenin. A progenin from (**9**) was identical with (**6**), and (**9**) itself with tauroside G_3 [14].

Glycoside L-7a (10), $[\alpha]_D$ −37° (c 0.5; pyridine); lit.: $[\alpha]_D$ −34.9° (pyridine) [14]. An acid hydrolysate of (**10**) contained rhamnose, arabinose, glucose, and echinocystic acid. A progenin from (**10**) was identical with (**5**), and (**10**) itself with tauroside H_1 [14].

Glycoside L-7b (11), $[\alpha]_D$ −11° (c 2.5; pyridine); lit.: $[\alpha]_D$ − 14.9° (pyridine) [14]. An acid hydrolysate of (**11**) contained rhamnose, arabinose, glucose, and hederagenin. A progenin from (**11**) was identical with (**6**), and (**11**) itself with tauroside H_2 [14].

REFERENCES

1. Flora of the USSR [in Russian], Izd-vo AN SSSR, Moscow—Leningrad, Vol. XVI (1950).
2. Plant Resources of the USSR. Flowering Plants, their Chemical Composition and Use. The Families Rutaceaey—Elaeagnaceae [in Russian], Nauka, Leningrad (1988).
3. J. J. Scheidegger and E. Cherbuliez, Helv. Chim. Acta, **38**, 547 (1955)
4. R. Tschetsche, W. Schmidt, and G. Z. Wulff, Z. Naturforsch, **290**, 708 (1965).
5. G. H. Mahran, S. H. Hilal, and T. S. El-Alfy, Egypt. J. Pharm. Sci., **15**, 149 (1974).
6. B. Pasich and K. Terminska, Pharm. Pol., **39**, 657 (1983).
7. K. Hostettmann, Helv. Chim. Acta, **63**, 606 (1980).
8. T. Aoki, Y. Tanio, and T. Suga, Phytochemistry, **15**, 781 (1976).
9. T. Aoki and T. Suga, Phytochemistry, **17**, 771 (1978).
10. Z. S. Kemoklidze, G. E. Dekanosidze, O. D. Dzhikiya, M. M. Vugal'ter, and É. P Kemertelidze, Khim. Prir. Soedin., 788 (1982).
11. A. A. Loloiko, V. I. Grishkovets, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin., 379 (1988).
12. V. I. Grishkovets, N. V. Tolkacheva, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin., 686 (1991).
13. A. S. Shashkov, V. I. Grishkovets, A. A. Loloiko, and V. Ya. Chirva, Khim. Prir. Soedin., 363 (1987)
14. V. I. Grishkovets, N. V. Tolkacheva, A. S. Shashkov, and V. Ya. Chirva, Khim. Prir. Soedin., 522 (1992).
15. C.-J. Shao, R. Kasai, J.-D. Xu, and O. Tanaka, Chem. Pharm. Bull., **36**, 601 (1988).