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TRITERPENE GLYCOSIDES OF *Fatsia japonica*. I. ISOLATION AND STRUCTURE OF GLYCOSIDES FROM *Fatsia japonica* SEEDS

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The known triterpene glycosides hederagenin 3-O-α-L-arabinopyranoside, hederagenin 3-O-β-D-glucopyranoside, oleanolic acid 3-O-β-sophoroside, hederagenin 3-O-β-sophoroside, and their 28-O-β-gentiobiosyl esters, respectively, in addition to the new triterpene glycoside 3-O-β-sophorosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl hederagenin are isolated from Fatsia japonica *(Araliaceae) seeds. The structures of these glycosides are established using chemical and spectral methods.*

Key words: *Fatsia japonica*, triterpene glycosides, 3-O-β-sophorosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl hederagenin, structure proof.

Fatsia japonica Decne. et Planch. (synonymous with *Aralia japonica* Thunb. and *A. sieboldii* Anon.) is a member of the Araliaceae Juss. family that grows wild on the Japanese islands and is widely cultivated as a decorative plant. It is cultivated on open land in Georgia and on the Black Sea coast of the Crimea and Caucuses [1].

The glycoside composition of various parts of *Fatsia japonica* was previously studied by Japanese and Georgian researchers. Thus, the isolation and structure of triterpene glycosides from the leaves [2, 3], flower buds [2, 4], and fruit [2, 5] of this plant have been reported.

We studied in detail the glycoside composition of *Fatsia japonica* fruit in order to compare it with those of fruits from other Araliaceae genera, in particular, the genus *Hedera*. In addition, the chemical methods that were used previously to establish the structure of glycosides in *Fatsia japonica* were unreliable.

TLC analysis of the pericarp (pulp) and seeds taken separately revealed significant qualitative differences in their glycoside composition. This made it necessary to study separately the glycosides of the pericarp and seeds.

The isolation of triterpene glycosides from *Fatsia japonica* seeds, the identification of known compounds, and the structure determination of one new glycoside are described in the present article. Ground seeds were defatted with benzene and exhaustively extracted with 80% isopropanol to isolate the triterpene glycosides. The solid after evaporation of the alcohol extract was dissolved in water-saturated butanol and washed with 5% aqueous ammonia to remove phenolic compounds, free sugars, and salts. Evaporation of the butanol layer gave the purified saponins, which were separated by preparative chromatography on silica gel with gradient elution by water-saturated $CHCl_3$-C_2H_5OH (10:1 → 1:1). The pure glycosides A-F, H-K, and a fraction G that was separated by rechromatography into fraction G_1 and a chromatographically pure glycoside G_2 were obtained.

According to TLC, glycosides A (**1**), B (**2**), E (**3**), F (**4**), G_2 (**5**), I (**7**), and J (**8**) were identical to hederagenin 3-O-α-L-arabinopyranoside [6], hederagenin 3-O-β-D-glucopyranoside [6], oleanolic acid 3-O-β-sophoroside [6], hederagenin 6-O-β-sophoroside [6], hederagenin 3-O-α-L-arabinopyranoside-28-O-β-gentiobiosyl ester [7], oleanolic acid 3-O-β-sophorosyl-28-O-β-gentiobiosyl ester [8], and hederagenin 3-O-β-sophorosyl-28-O-β-gentiobiosyl ester, respectively. These were isolated by us previously from various parts of Crimean ivy. The structures of these glycosides were confirmed by total acid hydrolysis with TLC analysis of the resulting sugars and aglycones. For glycosides **5**, **7**, and **8**, base hydrolysis with analysis of the resulting progenins, which are identical to **1**, **3**, and **4**, respectively, was also used. Furthermore, ^{13}C NMR and PMR spectra of **1-4**, **7**,

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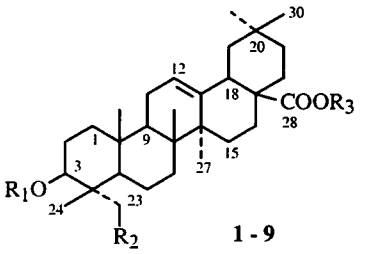
and **8** are identical to those previously published [6-8].

Glucose is the only sugar and hederagenin is the aglycone in glycoside H (**6**) according to total acid hydrolysis. Base hydrolysis of **6** gives glycoside **2**, i.e., progenin **6** is hederagenin 3-O-β-D-glucopyranoside. Analysis of the ^{13}C NMR spectrum of **6** indicates that three monosaccharide units (three signals of anomeric C atoms in the region 95-110 ppm) are present. The signals of one of these that is bound through a glycoside bond to the hydroxyl on C-3 of the aglycone are assigned based on the literature data for hederagenin 3-O-β-D-glucopyranoside [6]. Obviously the remaining signals belong to the two glucose units in the disaccharide that is bound to the aglycone through an acylglycoside bond.

Only two signals of the unsubstituted C-6 atoms of the glucose residues are found in the region 60-63 ppm. The signal of the third C-6 atom, which was found in the ^{13}C NMR spectrum with APT editing, occurs at 69.5 ppm, i.e., it experiences a large positive α-effect owing to glycosylation of the terminal glucose residue. Therefore, the disaccharide fragment on the carboxyl of the aglycone is a gentiobiose residue. The signals of the C atoms of this disaccharide were unambiguously assigned by comparing them with literature data for glycosides with β-gentiobiosyl fragments [7, 8]. Thus, **6** is hederagenin 3-O-β-D-glucopyranosyl-28-O-β-gentiobiosyl ester. A glycoside of analogous structure was previously found in *Lonicera nigra* [9].



1 - 9

	R_1	R_2	R_3
1	Arap*α*-	OH	H
2	Glc*p*β-	OH	H
3	Glc*p*β-(1→2)-Glc*p*β-	H	H
4	Glc*p*β-(1→2)-Glc*p*β-	OH	H
5	Arap*α*-	OH	-βGlc*p*-(6→1)-βGlc*p*
6	Glc*p*β-	OH	-βGlc*p*-(6→1)-βGlc*p*
7	Glc*p*β-(1→2)-Glc*p*β-	H	-βGlc*p*-(6→1)-βGlc*p*
8	Glc*p*β-(1→2)-Glc*p*β-	OH	-βGlc*p*-(6→1)-βGlc*p*
9	Glc*p*β-(1→2)-Glc*p*β-	OH	-βGlc*p*-(6→1)-βGlc*p*-(4→1)-αRha*p*

Total acid hydrolysis of glycoside K (**9**) revealed the presence of rhamnose, glucose, and the aglycone hederagenin. We proposed that **9** differs from **8** only by the presence of an additional rhamnose unit bound to the β-gentiobiose through a 1→4 glycoside bond, as occurs in most glycosides from plants of the Araliaceae family, because the chromatographic mobility of **9** is slower than that of **8**.

The final proof of structure of this glycoside came from an analysis of its ^{13}C NMR spectrum. Signals of all aglycone C atoms in 3,28-disubstituted hederagenin were identified in the ^{13}C NMR spectrum of **9** by comparison with the literature [8] (Table 2). The remaining signals belong to C atoms of the glycoside monosaccharide units (five unambiguously assigned signals of anomeric C atoms in the range 95-110 ppm). Other signals of carbohydrate fragments in **9** are assigned by comparison with literature data for the chemical shifts of C atoms in β-sophorose [6, 8] and for the trisaccharide chain rhamnopyranosyl-(1→4)-O-β-gentiobiose [8] (Table 1).

Thus, **9** is hederagenin 3-O-β-sophorosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-β-gentiobiosyl ester. It represents a new triterpene glycoside. It is noteworthy that hederagenin 3-O-α-L-arabinopyranoside and 3-O-β-D-glucopyranoside were previously isolated from *Fatsia japonica* fruit [2] and that glycosides **3-9** are found for the first time in the seeds of this plant.

TABLE 1. ^{13}C Chemical Shifts in Carbohydrates of Glycosides H (**6**) and K (**9**) (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound		C atom	Compound	
	6	9		6	9
	Glc′	Glc′		Glc″	Glc‴
1	105.6	103.7	1	95.7	95.7
2	75.8	83.6	2	73.9	73.9
3	77.6	77.8	3	78.0	78.9
4	71.8	71.5	4	71.1	71.1
5	77.2	77.9	5	77.8	78.1
6	62.9	62.7	6	69.5	69.4
		Glc″		Glc‴	Glc⁗
1		105.6	1	105.0	104.7
2		76.6	2	75.1	75.2
3		78.6	3	78.3	76.6
4		71.5	4	71.8	78.4
5		78.2	5	78.0	77.0
6		62.7	6	62.8	61.6
					Rha⁗′
			1		102.7
			2		72.4
			3		72.7
			4		73.9
			5		70.4
			6		18.4

TABLE 2. ^{13}C Chemical Shifts in Aglycones of Glycosides H (**6**) and K (**9**) (δ, ppm, 0 = TMS, C_5D_5N)

C atom	Compound		C atom	Compound	
	6	9		6	9
1	38.8	38.8	16	23.5	23.5
2	25.9	25.9	17	47.2	47.2
3	82.7	83.2	18	41.8	41.8
4	43.4	43.5	19	46.4	46.4
5	47.9	48.2	20	30.8	30.7
6	18.4	18.4	21	34.2	34.1
7	33.0	32.6	22	32.6	33.1
8	40.1	40.0	23	65.1	65.4
9	48.3	48.2	24	13.6	13.4
10	37.1	37.0	25	16.3	16.2
11	23.9	23.8	26	17.7	17.6
12	123.0	123.0	27	26.2	26.1
13	144.4	144.3	28	176.7	176.7
14	42.3	42.3	29	33.2	33.1
15	28.4	28.4	30	23.8	23.8

EXPERIMENTAL

NMR spectra were obtained on a Bruker AM-400 instrument (400 MHz for ^1H and 100 MHz for ^{13}C) in C_5D_5N. TLC monitoring was performed on Silufol plates using $CHCl_3$—CH_3OH—H_2O (100:40:7) and (100:30:5) and $CHCl_3$—CH_3OH—NH_4OH (25%) (100:40:10), (100:30:6), and (100:20:3). Glycosides and aglycones were detected with

phosphotungstatic acid in alcohol (10%); sugars, acidic aniline phthalate with heating of the chromatograms. Silica gel L (40-100 μm) was used for preparative separation of the glycosides.

Total Acid Hydrolysis. Sugars were determined by dissolving the glycosides in dioxane—CF_3COOH (4 N in H_2O) (1:1) with heating at 100°C for 2 h. Sugar in the hydrolysate was identified without preliminary treatment by TLC on Silufol plates using $CHCl_3$—CH_3OH—H_2O (100:40:7) or $CHCl_3$—CH_3OH—NH_4OH (25%) (100:40:10) and comparison with authentic samples. Total acid hydrolysis for identification of aglycones was carried out by dissolving glycosides in CH_3OH—H_2SO_4 (2 N) (1:1) and heating at 100°C for 2 h with subsequent dilution of the hydrolysate with three times the volume of water, extraction of the aglycones by $CHCl_3$, and TLC analysis using $CHCl_3$—CH_3OH—NH_4OH (25%) (100:20:3) or benzene—acetone (4:1) and comparison with authentic samples.

Base Hydrolysis. Glycosides were dissolved in 10% KOH in H_2O—CH_3OH (1:1), heated at 100°C for 2 h, diluted with water, and neutralized with aqueous H_2SO_4 until weakly acidic. The progenins were extracted by butanol and analyzed by TLC using $CHCl_3$—CH_3OH—H_2O (100:30:5) or $CHCl_3$—CH_3OH—NH_4OH (100:30:6).

Isolation and Purification of Glycosides. Seeds (15 g) from fruit of *Fatsia japonica* were collected on the southern shore of the Crimea, ground, defatted with benzene (3×150 ml), and extracted with 80% isopropanol (3×200 ml). The combined extracts were evaporated to give a dry solid (4.9 g) that was dissolved in water-saturated butanol (400 ml) and washed with 5% aqueous ammonia (2×100 ml). The butanol layer was evaporated to give 3.3 g of purified glycosides that were separated on silica gel using gradient elution with water-saturated $CHCl_3$—isopropanol (10:1 → 1:1) to give 11 fractions of triterpene glycosides: A (105 mg), B (820 mg), C (10 mg), D (160 mg), E (100 mg), F (750 mg), G (100 mg), H (100 mg), I (50 mg), J (25 mg), and K (35 mg). Fractions A, B, E, F, H, I, J, and K are pure glycosides **1-5** and **7-9**, respectively.

Fraction G was rechromatographed on silica gel with elution by water-saturated $CHCl_3$—isopropanol (2:1) to give fraction G_1 (30 mg) and glycoside G_2 (**6**, 60 mg).

Glycosides **1-4** are identical by TLC in various solvent systems and ^{13}C NMR spectra to known samples of hederagenin 3-O-α-L-arabinopyranoside [6], hederagenin 3-O-β-D-glucopyranoside [6], oleanolic acid 3-O-β-sophoroside [6], and hederagenin 3-O-β-sophoroside [6]. Glycosides **5**, **7**, and **8** are identical by TLC and ^1H and ^{13}C NMR spectra to known samples of 28-O-β-gentiobiosyl esters of hederagenin 3-O-α-L-arabinopyranoside [6] and oleanolic acid and hederagenin 3-O-β-sophorosides [8].

Glycoside H (6). The total acid hydrolysate of **6** contained glucose and hederagenin. The progenin from **6** that was obtained by base hydrolysis was identical to **1**. The ^{13}C NMR spectrum is given in Tables 1 and 2.

Glycoside K (9). The total acid hydrolysate of **9** contained glucose, rhamnose, and hederagenin. The progenin from **9** was identical to **4**. The ^{13}C NMR spectrum of **9** is given in Tables 1 and 2.

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