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TRITERPENOID GLYCOSIDES OF *Fatsia japonica*. II. ISOLATION AND STRUCTURE OF GLYCOSIDES FROM THE LEAVES

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The previously known triterpenoid 3-O-α-L-arabinopyranosides of oleanolic and echinocystic acids and hederagenin, 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic acid and hederagenin, in addition to 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ethers of the 3-O-α-L-arabinopyranoside of hederagenin, and 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic acid and hederagenin, respectively, are isolated from leaves of Fatsia japonica *(Araliaceae). The structures of the glycosides are confirmed by chemical methods and ^{13}C NMR spectroscopy.*

Key words: *Fatsia japonica*, triterpenoid glycosides, NMR (^{13}C, COSY, ROESY).

Leaves of *Fatsia japonica* Decne. et Planch (Araliaceae Juss.) are used in eastern folk medicine [1]. The composition and structure of the triterpenoid glycosides of this plant have been previously studied by Japanese and Georgian scientists [2-6].

Preparative TLC of the alcohol extract of *Fatsia japonica* leaves revealed the presence of at least 14 triterpenoid glycosides. This is significantly greater than the number of glycosides isolated earlier from the leaves of this plant [2-6]. Furthermore, various types of bonds were proposed in the literature for a single glycoside and only methylation was used and not NMR spectroscopy, which enables the proposed structures to be determined unambiguously. We decided to study in detail the glycosides of the leaves and to elucidate the structures of the glycosides using various modern NMR techniques.

The triterpenoid glycosides from the leaves were isolated by extraction of the defatted and ground dry raw material with aqueous isopropanol followed by purification from strongly polar compounds (free sugars and salts) by dissolving the evaporated extract in aqueous butanol and washing with water. The purified total triterpenoid glycosides were separated by chromatography on silica gel using gradient elution by water-saturated $CHCl_3$—C_2H_5OH mixtures (10:1→1:1). This produced pure glycosides A and B; fractions C and D; glycosides E and F; fractions G and H; and glycosides I, J, K, L, and M. Fraction C was separated into pure glycosides C_1 and C_2 by rechromatography on silica gel with elution by $CHCl_3$—C_2H_5OH (4:1) saturated with aqueous ammonia.

According to TLC, glycoside A was identical to an authentic sample of β-sitosterol 3-O-β-D-glucopyranoside [7]. Glycosides B (**1**), C_1 (**2**), and C_2 (**3**) were identical by TLC with authentic samples of the 3-O-α-L-arabinopyranosides of oleanolic and echinocystic acids and hederagenin, respectively [8]. The carbohydrate and aglycone compositions of these glycosides were confirmed by TLC of the acid-hydrolysis products. The acid-hydrolysate of glycosides E (**4**) and F (**5**) contained glucose and arabinose in both glycosides and aglycones, oleanolic acid and hederagenin, respectively. Furthermore, glycoside **5** according to TLC was identical to an authentic sample of hederagenin 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside, which was isolated by us from stalks and fruits of Crimean ivy [9, 10]. The spectra of the carbohydrate parts of **4** and **5** are very similar. The chemical shifts correspond completely with the literature for the disaccharide β-D-Glc*p*-(1→2)-O-α-L-Ara*p* bound to oleanolic acid or hederagenin [9, 11, 12]. Chemical shifts in spectra of the aglycones of **4** and **5** also correspond with the literature for 3-O-substituted oleanolic acid and hederagenin [9, 11, 12]. Thus, glycosides E and F are

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TABLE 1. ^{13}C Chemical Shifts of Carbohydrate Parts of Glycosides C_2 (**3**), E (**4**), F (**5**), J (**6**), L (**7**), and M (**8**) (δ, ppm, 0 = TMS, C_5D_5N)

C-3 chain	Compound						C-28 chain	Compound		
	3	**4**	**5**	**6**	**7**	**8**		**6**	**7**	**8**
Ara							Glc			
1	106.3	104.9	104.0	106.3	104.6	104.7	1	95.6	95.6	95.7
2	72.9	80.8	80.4	73.0	80.5	81.0	2	73.8	73.8	73.7
3	74.5	73.5	73.6	74.6	73.4	73.6	3	78.5	78.8	78.7
4	69.4	68.4	68.4	69.4	68.1	68.3	4	70.8	71.0	70.9
5	66.7	65.1	64.5	66.6	64.7	64.9	5	77.9	77.9	78.0
							6	69.2	69.3	69.3
Glc							Glc			
1		105.9	105.3		105.5	105.7	1	104.6	104.6	104.7
2		76.4	76.1		76.1	76.1	2	75.2	75.2	75.3
3		78.2	78.3		78.1	78.3	3	76.5	76.5	76.6
4		71.6	71.5		71.4	71.8	4	78.5	78.5	78.6
5		78.2	78.1		77.9	78.4	5	77.0	76.9	77.0
6		62.6	62.6		62.7	62.7	6	61.4	61.5	61.5
							Rha			
							1	102.6	102.6	102.8
							2	72.4	72.4	72.5
							3	72.6	72.6	72.7
							4	73.8	73.8	73.9
							5	70.3	70.3	70.3
							6	18.4	18.4	18.5

3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic acid and hederagenin.

We note that structures with a 1→4-bond between the glucose and arabinose had been proposed in 1976 [2] for the two principal monodesmoside glycosides from leaves of *Fatsia japonica* on the basis of methylation and analysis of the specific rotation taking into account known rules of Klyne and Hudson. In 1979, Tomimori and Kizu [3] re-examined the structures of these glycosides. They proposed a 1→2 bond between the glucose and arabinose on the basis of methylation. However, ^{13}C NMR was still not used in these experiments.

Our analysis of ^{13}C NMR spectra of glycosides E and F compared with the literature on the chemical shifts in the arabinopyranosides of oleanolic acid and hederagenin [11, 13 and others] indicates that the C-1 signal of arabinose that is unambiguously identified in the spectrum is shifted by 1-1.5 ppm to strong field whereas the C-2 signal of arabinose undergoes a significant low-field shift of ~8 ppm. This is consistent with a 1→2 bond in the disaccharide part of glycosides E and F. Furthermore, the 1→2 bond in the disaccharide was also confirmed using COSY and ROESY for glycoside St-I_1 from stalks of *Hedera taurica* [14] and its total acetate.

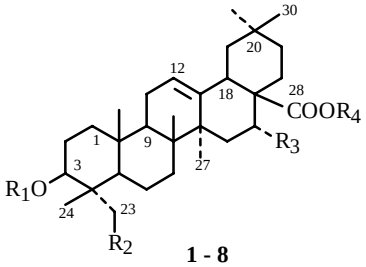
The progenins obtained by alkaline hydrolysis of glycosides J (**6**), L (**7**), and M (**8**) are identical according to TLC and ^{13}C NMR to the monodesmoside glycosides **3**, **4**, and **5**, which partially determines the structures of **6**-**8**. Comparision of the ^{13}C NMR spectra of **6**-**8** and **3**-**5** indicates that **6**-**8** also contain 18 signals for the same carbohydrate C atoms (including three signals of anomeric C atoms). Therefore, the same trisaccharide is bonded to the carboxylate of the aglycone in all bisdesmoside glycosides **6**-**8**. Comparison of the chemical shifts of these signals with several literature values for the trisaccharide α-L-Rha*p*-(1→4)-O-β-D-Glc*p*-(1→6)-O-β-D-Glc*p*- [11, 13] indicates that they are in complete agreement. Therefore, glycosides J, L, and M are 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ethers of the 3-O-α-L-arabinopyranoside of hederagenin and the 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranosides of oleanolic acid and hederagenin, respectively.

TABLE 2. ^{13}C Chemical Shifts of Aglycones of C_2 (**3**), E (**4**), F (**5**), J (**6**), L (**7**), and M (**8**) (δ, ppm, 0 = TMS,C_5D_5N)

C-atom	Compound					
	3	**4**	**5**	**6**	**7**	**8**
1	38.8	38.8	38.8	38.8	38.9	38.9
2	26.0	26.5	26.3	26.1	26.4	26.0
3	82.0	89.0	82.3	82.2	89.1	82.3
4	43.3	39.6	43.6	43.4	39.5	43.6
5	47.6	55.9	47.7	47.6	56.0	47.6
6	18.1	18.6	18.2	18.2	18.6	18.5
7	32.5	33.2	33.3	32.8	33.2	32.9
8	39.9	39.8	39.8	40.0	40.0	40.0
9	48.1	48.1	48.2	48.2	48.2	48.2
10	36.9	37.0	36.9	37.0	37.1	37.0
11	23.3	23.9	23.7	23.9	23.8	23.9
12	122.8	122.6	122.6	123.4	122.9	122.9
13	144.0	144.9	144.9	144.2	144.2	144.2
14	42.1	42.2	42.2	42.2	42.2	42.2
15	28.2	28.3	28.3	28.3	28.3	28.4
16	23.8	23.9	23.9	23.4	23.5	23.5
17	47.0	46.7	46.8	47.1	47.2	47.1
18	42.0	42.0	42.0	41.7	41.8	41.8
19	46.6	46.5	46.5	46.3	46.4	46.3
20	30.8	31.0	31.0	30.7	30.8	30.8
21	34.0	34.3	34.2	34.0	34.1	34.0
22	33.3	33.2	32.8	32.5	32.6	32.6
23	64.6	28.3	65.1	64.6	28.3	64.9
24	13.5	16.8	13.5	13.5	16.8	13.5
25	16.1	15.5	16.1	16.2	15.6	16.2
26	17.5	17.5	17.6	17.6	17.6	17.6
27	25.8	26.2	26.0	26.1	26.1	26.1
28	180.3	180.3	180.7	176.6	176.6	176.6
29	33.0	33.2	33.3	33.1	33.2	33.1
30	23.6	23.9	23.9	23.7	23.8	23.8

Georgian scientists proposed [5] the correct 1→2 bond in the disaccharide on the aglycone hydroxyl but the mistaken 1→4 bond between the glucose and trisaccharide on the aglycone carboxylate for the principal bisdesmoside glycosides from leaves of *Fatsia japonica*. The 1→6 bond between these glucoses is also unambiguously indicated by an analysis of the one-dimensional ^{13}C NMR spectrum, which contains only one glucose signal at 60-62 ppm from the trisaccharide (glycosides **7** and **8** have another signal for the terminal glucose of the disaccharide). However, the signal for C-6 of the other glucose of the trisaccharide is shifted to low field by ~8 ppm owing to glycosylation. Furthermore, the structure of this trisaccharide was completely confirmed using two-dimensional methods COSY and HETCOSY [15] in addition to COSY and ROESY [14].

The 3-O-α-L-arabinopyranoside of hederagenin and its 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ether were previously found in many plants of the Araliaceae family [16]. The 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside of oleanolic acid was previously also found in leaves of *Acanthopanax hypoleucus* [11] and several plants of other families [16]. The 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ether of the 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside of oleanolic acid was observed in leaves of *Acanthopanax senticosus* [13]. The 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside of hederagenin was found in leaves of *Polyscias dichroostachya* [17], fruits and stalks of *Hedera taurica* [9, 10], and in several plants of other families [16]. The 28-O-α-L-rhamnopyranosyl-(1→4)-O-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ether of 3-O-β-D-glucopyranosyl-(1→2)-O-α-L-arabinopyranoside of hederagenin was found in leaves of *Hedera helix* and *H. taurica* [18, 15] and in roots of *Caltha polypetala* [19].



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	R_1	R_2	R_3	R_4
1	Ara*p* α→	H	H	H
2	Ara*p* α→	H	OH	H
3	Ara*p* α→	OH	H	H
4	Glc*p*β-(1→2)-Ara*p* α→	H	H	H
5	Glc*p*β-(1→2)-Ara*p* α→	OH	H	H
6	Ara*p* α→	OH	H	←βGlc*p*-(6←1)-βGlc*p*-(4←1)-αRha*p*
7	Glc*p*β-(1→2)-Ara*p* α→	H	H	←βGlc*p*-(6←1)-βGlc*p*-(4←1)-αRha*p*
8	Glc*p*β-(1→2)-Ara*p* α→	OH	H	←βGlc*p*-(6←1)-βGlc*p*-(4←1)-αRha*p*

EXPERIMENTAL

NMR spectra were recorded on Bruker AM-300 and Varian VXR-300 (300 MHz for ^1H and 75 MHz for ^{13}C) instruments.

Total acid hydrolysis for sugar determination was performed by dissolving glycosides in dioxane—CF_3COOH (4 N, aqueous) (1:1) and 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 authentic samples. Aglycones were identified by total acid hydrolysis by dissolving glycosides in CH_3OH—H_2SO_4 (aqueous, 2 N) with heating for 2 h at 100°C with subsequent dilution of the hydrolysate with three times the volume of water, extraction of the aglycones with $CHCl_3$, and TLC compared with authentic samples in $CHCl_3$—CH_3OH—NH_4OH (25%) (100:20:3) or benzene—acetone (4:1).

Alkaline hydrolysis was carried out by dissolving glycosides in KOH (10%) in H_2O—CH_3OH (1:1), heating for 2 h at 100°C with subsequent dilution with water, neutralizing with aqueous H_2SO_4 until weakly acidic, extracting progenins with butanol and analyzing them by TLC in $CHCl_3$—CH_3OH—H_2O (100:30:5) or $CHCl_3$—CH_3OH—NH_4OH (100:30:6).

Isolation and Purification of Glycosides. Dried and finely ground leaves (30 g) of *Fatsia japonica* were defatted with benzene (3×150 ml) and extracted with aqueous isopropanol (3×300 ml). The alcohol extract was evaporated to give a solid (7.0 g) that was dissolved in water-saturated butanol (300 ml). The butanol layer was washed with water (3×100 ml) and evaporated to dryness. The purified glycosides (5.5 g), which were separated on silica gel using gradient elution by water-saturated $CHCl_3$—C_2H_5OH (10:1→1:1), consisted of A (30 mg), B (**1**, 50 mg), fractions C (110 mg), D (50 mg), glycosides E (**4**, 950 mg), F (**5**, 750 mg), fractions G (100 mg), H (200 mg), glycosides I (1.2 g), J (**6**, 200 mg), K (400 mg), L (**7**, 900 mg), M (**8**, 200 mg), and N (200 mg). Fraction C (110 mg) was separated into pure glycosides C_1 (**2**, 30 mg) and C_2 (**3**, 70 mg) by chromatography on silica gel with elution by $CHCl_3$—C_2H_5OH (4:1) saturated with aqueous ammonia (10%).

The acid hydrolysates of glycosides **1**-**3** contained arabinose and the aglycones, oleanolic and echinocystic acids and hederagenin, respectively. The acid hydrolysates of **4** and **5** contained glucose, arabinose and the aglycones, oleanolic acid and hederagenin, respectively. The acid hydrolysates of **6**-**8** contained arabinose, glucose, rhamnose and the aglycones, hederagenin in **6** and **8** and oleanolic acid in **7**. Progenins from **6**-**8**, which were obtained by alkaline hydrolysis, were identical to **3**-**5**, respectively, according to TLC.

^{13}C NMR spectra of **3**-**8** are listed in Tables 1 and 2.

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