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TRITERPENE GLYCOSIDES FROM *Kalopanax septemlobum*. 1. GLYCOSIDES A, B, C, F, G_1, G_2, I_2, H, AND J FROM LEAVES OF *Kalopanax septemlobum* VAR. *maximowichii* INTRODUCED TO CRIMEA

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Eight known glycosides of hederagenin and the new triterpene glycoside 3-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-6-O-acetyl-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of hederagenin were isolated by chromatographic methods from leaves of Kalopanax septemlobum *var.* maximowichii *introduced to Crimea. The known 3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-O-6-O-acetyl-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester of hederagenin was observed for the first time in* Kalopanax septemlobum.

Key words: *Kalopanax septemlobum* var. *maximowichii*, Araliaceae, triterpene glycosides, hederagenin glycosides.

Representatives of the *Kalopanax* genus are distributed in southern Primorskii territory, southern Sakhalin, South Kuril islands, Japan, Korea, and northeastern and central China and have been studied in detail [1-7]. Nevertheless, we repeated a comparison of the glycoside composition of various organs of the two varieties *Kalopanax septemlobum* var. *typicum* (Nakai) Pojark and var. *maximowichii* (Van Houtte) Hara, which were introduced on the southern shore of Crimea (Nikitskii Botanical Garden) for a number of reasons. Thus, the *Kalopanax* genus is currently considered to be monotypic [8]. It includes only the species *K. septemlobum* (Thunb.) Koidz. {synonyms *K. pictum* (Thunb.) Nakai, *K. ricinifolium* Miq., *K. autumnalis* Koidz., *Panax ricinifolium* Sieb. et Zucc., *Brassaiopsis ricinifolia* Seem., *Acanthopanax ricinifolium* Seem., et al. [9]}. However, the broad synonymy in the names of this species and the existence of varieties that are often taken as separate species *K. septemlobum* and *K. pictum* (=*K. septemlobus* and *K. pictus*) make it difficult to understand with which plant material researchers worked since external morphological features are not indicated in the articles. The varieties *typicum* and *maximowichii* of *K. septemlobum* differ primarily in the shape of the leaves (slightly palmatilobate for the former and deeply palmatilobate with thorns on the petiole for the latter).

Preliminary two-dimensional TLC [10] of the leaf glycosides of both varieties showed fundamental differences between them. First of all it has been observed that hederagenin glycosides dominate substantially in the *maximowichii* variety (blue-violet chromatographic bands) whereas oleanolic acid glycosides (pink chromatographic bands) occur only in trace amounts. However, the fraction of oleanolic acid glycosides from the *typicum* variety consists of half or even more in addition to other hederagenin glycosides. It was even more unexpected that acylated glycosides occur in significant quantities in the *maximowichii* variety. The amount of these for different glycosides is half and more of their unacetylated analogs whereas only trace amounts of acylated glycosides are found in the *typicum* variety.

Several researchers studied previously triterpene glycosides from roots and stems of *Kalopanax*. The glycoside composition was given for leaves [4, 5]. Differences in the glycoside composition of leaves from plants from various habitats were noted [5]. It is possible that results from investigations of two *Kalopanax* varieties were given [4, 5]. However, they were not assigned to one variety or the other.

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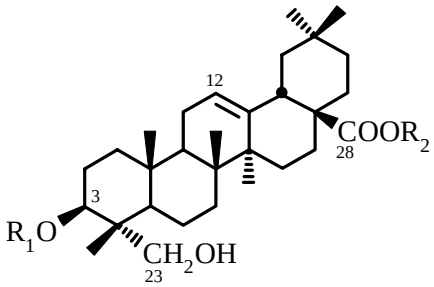
In the present article, we describe the isolation and structure determination for several glycosides from leaves of *Kalopanax septemlobum* var. *maximowichii*, which was introduced on the southern shore of Crimea. The total glycosides were separated by preparative chromatography over silica gel to afford glycoside fractions A-L.

Fractions A (**1**), B (**2**), C (**3**), F (**4**), H (**8**), and J (**9**) were chromatographically pure glycosides whereas fractions G and I were separated into glycosides with similar chromatographic mobilities G_1 (**5**), G_2 (**6**), I_1, and I_2 (**7**) by rechromatography of the fractions over the high-performance microsphere silica gel Silpearl.



1 - 9

	R_1	R_2
1:	α-L-Ara*p*$\rightarrow$	H
2:	α-L-Rha*p*$\rightarrow^2\alpha$-L-Ara*p*$\rightarrow$	H
3:	β-D-Xyl*p*$\rightarrow^3$*a*-L-Rha*p*$\rightarrow^2$*a*-L-Ara*p*$\rightarrow$	H
4:	α-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow$(β-D-Glc*p*$^6\leftarrow$OAc)$^4\leftarrow\alpha$-L-Rha*p*
5:	α-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow\beta$-D-Glc*p*$^4\leftarrow\alpha$-L-Rha*p*
6:	α-L-Rha*p*$\rightarrow^2\alpha$-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow$(β-D-Glc*p*$^6\leftarrow$OAc)$^4\leftarrow\alpha$-L-Rha*p*
7:	α-L-Rha*p*$\rightarrow^2\alpha$-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow\beta$-D-Glc*p*$^4\leftarrow\alpha$-L-Rha*p*
8:	β-D-Xyl*p*$\rightarrow^3$*a*-L-Rha*p*$\rightarrow^2$*a*-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow$(β-D-Glc*p*$^6\leftarrow$OAc)$^4\leftarrow\alpha$-L-Rha*p*
9:	β-D-Xyl*p*$\rightarrow^3$*a*-L-Rha*p*$\rightarrow^2$*a*-L-Ara*p*$\rightarrow$	$\leftarrow\beta$-D-Glc*p*$^6\leftarrow\beta$-D-Glc*p*$^4\leftarrow\alpha$-L-Rha*p*

Glycosides **1**, **2**, **3**, **5**, **7**, and **9** were identical by TLC with authentic samples of the hederagenin glycosides 3-O-α-L-arabinopyranoside, 3-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-O-α-L-arabinopyranoside, 3-O-β-D-xylopyranosyl-(1$\rightarrow$3)-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-O-α-L-arabinopyranoside, and their 28-O-α-L-rhamnopyranosyl-(1$\rightarrow$4)-O-β-D-glucopyranosyl-(1$\rightarrow$6)-O-β-D-glucopyranosyl esters, respectively, that were isolated previously from various *Hedera* species, for example, from *H. canariensis* [11] and *Acanthopanax sieboldianus* [12]. Furthermore, ^{13}C NMR spectra of **1**, **2**, **3**, **5**, **7**, and **9** were identical to those published earlier [11, 12]. Glycosides **1**-**3** and **5**-**7** were observed previously in leaves of *Kalopanax* [4, 5]; **9** was isolated only from *Kalopanax* stems [7].

Glycosides **4**, **6**, and **8** are converted by mild ammonolysis into the glycosides described above, **5**, **7**, and **9**, respectively. Obviously **4**, **6**, and **8** are acyl derivatives of **5**, **7**, and **9**, respectively. According to TLC, **4** coincided with an authentic sample of 3-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1$\rightarrow$4)-O-6-O-acetyl-β-D-gluccopyranosyl-(1$\rightarrow$6)-O-β-D-glucopyranosyl ester of hederagenin, which was isolated from leaves of *Hedera rhombea* [13]; **6**, with 3-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1$\rightarrow$4)-O-6-O-acetyl-β-D-glucopyranosyl-(1$\rightarrow$6)-O-β-D-glucopyranosyl ester of hederagenin that was isolated previously by us from leaves of *Hedera canariensis* [14]. The identity of these glycosides also follows from the complete coincidence of their ^{13}C NMR spectra (Tables 1 and 2) with those in the literature. Glycoside **4** was observed in *K. septemlobum* for the first time.

The ^{13}C NMR spectrum of **8** (Tables 1 and 2) shows two additional signals for an acyl fragment when compared with that of **9**. Considering the values of the chemical shifts, these signals (170.7 and 20.7 ppm) represent an acetate. This acyl fragment is located on the C-6 hydroxyl of the inner glucose in the triose fragment on the aglycon carboxyl. This is consistent with the shifts of the signals for the C atoms of this glucose unit, namely, C-6 from 61.3 to 63.7 ppm and C-5 from 77.1 to 73.8 ppm compared with **9**. Furthermore, after assigning signals of the aglycon, a disubstituted hederagenin, and the triose fragment on C-3 of the aglycon by comparison with **9**, the remaining signals of the acetylated triose fragment coincided with those of glycosides **4** and **6**. Thus, **8** is the 28-O-α-L-rhamnopyranosyl-(1$\rightarrow$4)-O-6-O-acetyl-β-D-glucopyranosyl-(1$\rightarrow$6)-O-β-D-glucopyranosyl ester of hederagenin 3-O-β-D-xylopyranosyl-(1$\rightarrow$3)-O-α-L-rhamnopyranosyl-(1$\rightarrow$2)-O-α-L-arabinopyranoside and is a new triterpene glycoside.

TABLE 1. ^{13}C Chemical Shifts for Aglycons of Glycosides **1-9**

C atom	**1-3**	**4-9**	C atom	**1-3**	**4-9**
1	39.0	39.0	16	23.7	23.4
2	26.2	26.2	17	46.7	47.0
3	81.0	81.1	18	42.0	41.7
4	43.5	43.5	19	46.4	46.2
5	47.8	47.7	20	30.9	30.8
6	18.2	18.2	21	34.3	34.0
7	32.9	32.8	22	33.2	32.6
8	39.8	39.9	23	63.8	63.9
9	48.2	48.2	24	13.8	13.9
10	36.9	36.9	25	16.0	16.2
11	23.8	23.9	26	17.5	17.6
12	122.6	122.9	27	26.1	26.1
13	144.7	144.1	28	180.1	176.5
14	42.1	42.2	29	33.2	33.1
15	28.3	28.3	30	23.8	23.8

Here and in Table 2, average chemical shifts are given for the compounds. The deviations for individual compounds are less than ±0.1 ppm.

TABLE 2. ^{13}C Chemical Shifts for Carbohydrates of Glycosides **1**-**9**

C atom	**1**, **4**, **5**	C atom	**2**, **6**, **7**	C atom	**3**, **8**, **9**	C atom	**5**, **7**, **9**	C atom	**4**, **6**, **8**
Ara-1	106.7	Ara-1	104.3	Ara-1	104.7	Glc-1	95.7	Glc-1	95.6
2	73.2	2	75.9	2	75.1	2	73.8	2	73.8
3	74.8	3	74.5	3	75.6	3	78.6	3	78.6
4	69.7	4	69.3	4	69.8	4	70.8	4	70.8
5	66.9	5	65.5	5	66.3	5	78.0	5	78.1
						6	69.4	6	69.4
		Rha-1	101.7	Rha-1	101.3	Glc-1	104.9	Glc-1	104.7
		2	72.3	2	72.0	2	75.3	2	75.1
		3	72.5	3	82.9	3	76.5	3	76.4
		4	74.2	4	73.0	4	78.4	4	79.3
		5	69.8	5	69.7	5	77.1	5	73.8
		6	18.6	6	18.6	6	61.3	6	63.7
								OAc	20.7
								OAc	170.7
				Xyl-1	107.5	Rha-1	102.8	Rha-1	102.9
				2	75.1	2	72.5	2	72.4
				3	78.3	3	72.7	3	72.6
				4	71.1	4	73.9	4	73.9
				5	67.4	5	70.4	5	70.7
						6	18.6	6	18.5

TABLE 3. ^{1}H Chemical Shifts for Carbohydrates of Glycoside **8**

H atom	Chemical shift	H atom	Chemical shift
Ara-1	5.05	Glc-1	6.19
2	4.58	2	4.09
3	4.03	3	4.18
4	4.13	4	4.21
5e	4.24	5	4.10
5a	3.66	6A	4.64
		6B	4.32
Rha-1	6.30	Glc-1	4.98
2	4.86	2	3.92
3	4.73	3	4.10
4	4.43	4	4.07
5	4.67	5	3.82
6	1.54	6A	4.62
		6B	4.52
		OAc	1.93
Xyl-1	5.30	Rha-1	5.50
2	4.02	2	4.55
3	4.12	3	4.47
4	4.13	4	4.28
5e	4.21	5	4.79
5a	3.63	6	1.68

The structures of the carbohydrate fragments of **8** were solved by an independent analysis of several two-dimensional (2D) NMR spectra: COSY, TOCSY, ROESY, HSQC, and HMBC. Based on an analysis of the COSY and TOCSY spectra, beginning with the easily interpreted signals for the anomeric protons, signals of the skeleton protons were completely assigned for each monosaccharide unit (Table 3). An analysis of the ROESY spectrum confirmed the types of glycoside bonds and the attachment sites of the carbohydrate chains to the aglycon since structurally informative interglycoside cross-peaks between signals of the anomeric protons and the skeletal protons of other monosaccharide units or the aglycon are easily followed in it. An analysis of the HSQC spectrum for glycosylation effects on C atoms compared with chemical shifts of unsubstituted carbohydrate units also confirmed the types of glycoside bonds. An analysis of the HMBC spectrum revealed the site of the acetyl from the cross-peak between the carbonyl C atom and the H-6 protons of the inner glucose unit.

We note that the variety *maximowichii* described by us, according to our data, contains a significant quantity of acetylated glycosides and is obviously identical to the *Kalopanax* variety growing in Japan that has been described [5]. However, it differs in glycoside composition from the variety growing in China that, according to the literature [4], lacks acetylated glycosides. It is curious that the Japanese variety is called [5] *K. pictus* whereas these same authors call the Chinese variety *K. septemlobus* [4] although they present no systematic basis for this (for example, external morphological features).

EXPERIMENTAL

NMR spectra were obtained on a Bruker DRX-500 instrument. Standard Bruker programs were used for 2D COSY, TOCSY, ROESY, HSQC, and HMBC experiments. The mixing time (spin-locking) in the 2D ROESY experiments was 200 ms. We used solutions of the glycosides in pyridine-d_5 with added TMS as internal standard.

TLC analysis and separation monitoring were performed on Silufol plates (Czech Rep.). Spots of triterpene glycosides and their aglycons were detected in the chromatograms by alcoholic (10%) phosphotungstic acid with added *p*-hydroxybenzaldehyde (2%) with subsequent heating at 100-120°C. Sugars were detected using alcoholic (10%) anilinium acid phthalate with subsequent heating at 100-150°C.

Two-dimensional TLC of extracts and pure fractions was performed on Silufol, Sorbosil, Polygram, and Merck plates using neutral solvents in one direction and alkaline solvents in the perpendicular direction. The neutral solvent system was a $CHCl_3$:CH_3OH:H_2O (100:40:7) mixture; alkaline, $CHCl_3$:CH_3OH:NH_4OH (25%) (100:40:10).

Total acid hydrolysis was carried out by treating glycoside (1 mg) with dioxane (0.1 mL) and aqueous CF_3CO_2H (0.1 mL, 2 N) and heating for 2 h at 100°C. The aglycon was extracted with benzene (0.5 mL). The resulting extract was analyzed by TLC using benzene:acetone (4:1) or $CHCl_3$:CH_3OH:NH_4OH (25%) (100:20:1) with authentic samples of aglycons. Sugar in the hydrolysate was identified by TLC using $CHCl_3$:CH_3OH:NH_4OH (100:40:10) with authentic samples of rhamnose, arabinose, glucose, galactose, xylose, and glucuronic acid.

Alkaline hydrolysis was performed by treating glycoside (2 mg) with KOH (0.2 mL, 10%) in H_2O:CH_3OH (1:1) and heating at 100°C for 2 h. The resulting solution was neutralized with aqueous H_2SO_4 (1 N) until slightly acidic. Progenins were extracted with butanol. The butanol extract was analyzed by TLC using $CHCl_3$:CH_3OH:H_2O (100:40:7).

Mild alkaline hydrolysis (ammonolysis) was carried out by dissolving glycoside (2 mg) in aqueous-alcoholic (1:1, 0.2 mL, 10%) ammonia and storing at 20°C for 2-3 h. The solution was neutralized with KU-2-8 cation-exchanger in the H^+-form. The filtrate was analyzed by TLC using $CHCl_3$:CH_3OH:H_2O (100:40:7).

Isolation of Glycosides. Leaves of *K. septemlobum* var. *maximowichii* that were collected in Nikitskii Botanical Garden in October during shedding (60 g, air-dried mass) were thoroughly ground and treated with benzene (3×300 mL). The defatted solid was extracted with isopropanol (80%, 4×400 mL). The combined extracts were evaporated to afford total extracted substances (20 g) that were dissolved in water-saturated butanol (700 mL) and washed with water (3×200 mL). Evaporation of butanol gave purified total glycosides (10 g).

Separation of Glycosides. Total glycosides (10 g) were chromatographed over silica gel L (1 kg, 40-100 μm) with gradient elution by $CHCl_3$:isopropanol (10:1→1:1) saturated with water to give fractions of glycosides A (50 mg), B (500 mg), C (800 mg), D (610 mg), E (300 mg), F (50 mg), G (490 mg), H (750 mg), I (880 mg), J (850 mg), K (860 mg), and L (350 mg).

Fractions A, B, C, F, H, and J gave pure glycosides **1** (20 mg), **2** (300 mg), **3** (650 mg), **4** (30 mg), **8** (300 mg), and **9** (350 mg) after additional chromatographic purification in water-saturated $CHCl_3$:isopropanol systems of the appropriate polarity. Fraction G (490 mg) was rechromatographed over silica gel Silpearl (250 g) with elution by water-saturated $CHCl_3$:isopropanol (3:1) to give **5** (50 mg) and **6** (300 mg). Fraction I (880 mg) was rechromatographed over Silpearl silica gel (500 g) with elution by water-saturated $CHCl_3$:isopropanol (2:1) to give I_1 (350 mg) and **7** (200 mg).

TLC identified hederagenin in the total acid hydrolysates of all glycosides and arabinose in **1**; rhamnose and arabinose in **2**; xylose, rhamnose, and arabinose in **3**; glucose, rhamnose, and arabinose in **4**-**7**; xylose, rhamnose, arabinose, and glucose in **8** and **9**. Alkaline hydrolysis of **4** and **5** gives **1**, **6**, and **7**; then **2**, **8**, and **9**; finally **3**. Ammonolysis of **4** gives **5**; of **6**, **7**; of **8**, **9**.

Tables 1 and 2 give the ^{13}C chemical shifts for **1**-**9**. Table 3 lists the chemical shifts for protons in the carbohydrate parts of **8**.

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