TRITERPENE GLYCOSIDES FROM *Kalopanax septemlobum*. VI. GLYCOSIDES FROM LEAVES OF *Kalopanax septemlobum* VAR. *typicum* INTRODUCED TO CRIMEA

D. A. Panov,[1] V. I. Grishkovets,[1] V. V. Kachala,[2] and A. S. Shashkov[2]

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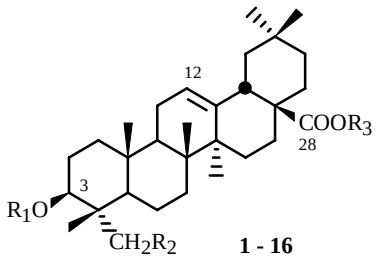
*Thirteen known glycosides of hederagenin and oleanolic acid and the three new triterpene glycosides of oleanolic acid - 28-*O-α-*L-rhamnopyranosyl-(1→4)-*β-*D-glucopyranosyl-(1→6)-*O-β-*D-glucopyranosyl ester 3-*O-β-*D-glucopyranosyl-(1→4)-*O-β-*D-xylopyranosyl-(1→3)-*O-α-*L-rhamnopyranosyl-(1→2)-*O-α-*L-arabinopyranoside of oleanolic acid and the 28-*O-α-*L-rhamnopyranosyl-(1→4)-*O-*6-*O-acetyl-β-*D-glucopyranosyl-(1→6)-*O-β-*D-glucopyranosyl esters 3-*O-β-*D-xylopyranosyl-(1→3)-*O-α-*L-rhamnopyranosyl-(1→2)-*O-α-*L-arabinopyranoside of oleanolic acid and 3-*O-β-*D-glucopyranosyl-(1→4)-*O-β-*D-xylopyranosyl-(1→3)-*O-α-*L-rhamnopyranosyl-(1→2)-*O-α-*L-arabinopyranoside of oleanolic acid were isolated from leaves of* Kalopanax septemlobum *var.* typicum *introduced to Crimea.*

Key words: *Kalopanax septemlobum* var. *typicum*, Araliaceae, triterpene glycosides, oleanolic acid and hederagenin glycosides.

We have previously reported results from a chromatographic analysis of the gylcoside composition of leaves of two varieties of *Kalopanax septemlobum*, var. *typicum* and *maximowiczii*, which were introduced to the southern shore of Crimea (Nikitskii Botanical Garden) [1]. The glycoside composition of leaves of the variety *maximowiczii* has been described in detail [1-3]. The goal of the present article was to isolate and establish the structures of triterpene glycosides from leaves of another variety, *K. septemlobum* var. *typicum* (Nakai) Pojark. (a variety with shallowly palmate leaves).



1 - 16

	R_1	R_2	R_3
1:	Rha→^{2}Ara→	H	H
2[a]**:**	Xyl→^{3}Rha→^{2}Ara→	H	H
3[a]**:**	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	H	H
4:	Rha→^{2}Ara→	OH	H
5:	Xyl→^{3}Rha→^{2}Ara→	OH	H
6:	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	OH	H
7[a]**:**	Rha→^{2}Ara→	H	←Glc^6 ←Glc^4←Rha
8[a]**:**	Xyl→^{3}Rha→^{2}Ara→	H	←Glc^6 ←Glc^4←Rha
9[b]**:**	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	H	←Glc^6 ←Glc^4←Rha
10:	Rha→^{2}Ara→	OH	←Glc^6 ←Glc^4←Rha
11:	Xyl→^{3}Rha→^{2}Ara→	OH	←Glc^6 ←Glc^4←Rha
12:	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	OH	←Glc^6 ←Glc^4←Rha
13[b]**:**	Xyl→^{3}Rha→^{2}Ara→	H	←Glc^6 ←(Glc^6←OAc)^4 ←Rha
14[b]**:**	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	H	←Glc^6 ←(Glc^6←OAc)^4 ←Rha
15:	Xyl→^{3}Rha→^{2}Ara→	OH	←Glc^6 ←(Glc^6←OAc)^4 ←Rha
16:	Glc→^{4}Xyl→^{3}Rha→^{2}Ara→	OH	←Glc^6 ←(Glc^6←OAc)^4 ←Rha

Rha – *α*-L-Rha*p*, Ara – *α*-L-Ara*p*, Xyl – *β*-D-Xyl*p*, Glc – *β*-D-Glc*p*

[a] - are new compounds, [b] - were founded in *Kalopanax* for the first time.

1) V. I. Vernadskii Tauric National University, 95007, Ukraine, Simferopol′, pr. Vernadskogo, 4, e-mail: vladgri@ukr.net; 2) N. D. Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, B-334, Leninskii pr., 47. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 40-43, January-February, 2006. Original article submitted November 1, 2005.

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TABLE 1. ^{13}C Chemical Shifts (δ, ppm, 0 = TMS, C_5D_5N) in Aglycons of **1-16**

C atom	**1-3**	**4-6**	**7-9**, **13**, **14**	**10-12**, **15**, **16**	C atom	**1-3**	**4-6**	**7-9**, **13**, **14**	**10-12**, **15**, **16**
1	38.8	39.0	39.0	39.1	16	23.8	23.7	23.4	23.4
2	26.5	26.2	26.7	26.3	17	46.7	46.6	47.1	47.1
3	88.7	81.1	88.8	81.2	18	42.0	42.0	41.8	41.7
4	39.5	43.6	39.6	43.6	19	46.6	46.4	46.3	46.3
5	56.0	47.7	56.1	47.7	20	30.9	30.9	30.8	30.8
6	18.5	18.2	18.6	18.3	21	34.3	34.2	34.1	34.1
7	33.3	32.8	33.2	32.8	22	33.3	33.2	32.6	32.7
8	39.8	39.8	40.0	40.0	23	28.2	64.0	28.3	64.0
9	48.0	48.2	48.2	48.3	24	17.0	14.0	17.2	14.1
10	37.0	36.9	37.1	37.0	25	15.5	16.1	15.8	16.3
11	23.8	23.8	23.9	23.9	26	17.4	17.5	17.6	17.6
12	122.5	122.6	122.9	123.0	27	26.3	26.2	26.2	26.2
13	144.8	144.8	144.2	144.2	28	180.1	180.2	176.7	176.6
14	42.2	42.2	42.2	42.2	29	33.3	33.2	33.2	33.2
15	28.3	28.3	28.4	28.4	30	23.8	23.8	23.8	23.8

TABLE 2. ^{13}C Chemical Shifts (δ, ppm, 0 = TMS, C_5D_5N) in Carbohydrates of **1-3**, **7-9**, **13**, and **14**

C atom	**1**,**7**	C atom	**2**, **8**, **13**	C atom	**3**, **9**, **14**	C atom	**7-9**	C atom	**13**, **14**
-Ara-		-Ara-		-Ara-		-Glc-		-Glc-	
1	104.9	1	105.2	1	105.2	1	95.7	1	95.7
2	76.0	2	75.3	2	75.5	2	73.9	2	73.9
3	73.8	3	74.6	3	74.4	3	78.7	3	78.6
4	68.7	4	69.3	4	69.2	4	70.8	4	70.8
5	64.7	5	65.6	5	65.4	5	78.1	5	78.1
						6	69.2	6	69.4
Rha-		-Rha-		-Rha-		-Glc-		-Glc-	
1	101.8	1	101.4	1	101.5	1	104.8	1	104.7
2	72.4	2	72.0	2	71.9	2	75.3	2	75.1
3	72.6	3	82.9	3	82.9	3	76.6	3	76.4
4	74.0	4	73.0	4	72.9	4	78.4	4	79.3
5	70.0	5	69.7	5	69.8	5	77.2	5	73.8
6	18.6	6	18.5	6	18.6	6	61.3	6	63.7
								-OAc	20.7
									170.7
		Xyl-		Xyl-		Rha-		Rha-	
		1	107.4	1	107.0	1	102.8	1	102.9
		2	75.6	2	75.3	2	72.6	2	72.5
		3	78.4	3	76.3	3	72.8	3	72.7
		4	71.1	4	77.9	4	74.0	4	74.0
		5	67.4	5	64.9	5	70.4	5	70.6
						6	18.6	6	18.6
				-Glc-					
				1	103.7				
				2	74.4				
				3	78.2				
				4	71.7				
				5	78.8				
				6	62.7				

TABLE 3. ^{13}C Chemical Shifts (δ, ppm, 0 = TMS, C_5D_5N) in Carbohydrates of **2-6**, **10-12**, **15**, and **16**

C atom	**4**, **10**	C atom	**5, 11, 15**	C atom	**6, 12, 16**	C atom	**10-12**	C atom	**15, 16**
-Ara-		-Ara-		-Ara-		-Glc-		-Glc-	
1	104.3	1	104.7	1	104.6	1	95.7	1	95.7
2	75.9	2	75.1	2	75.4	2	73.9	2	73.9
3	74.5	3	75.1	3	74.9	3	78.6	3	78.6
4	69.3	4	69.8	4	69.6	4	70.8	4	70.8
5	65.5	5	66.3	5	66.0	5	78.1	5	78.1
						6	69.3	6	69.4
Rha-		-Rha-		-Rha-		-Glc-		-Glc-	
1	101.7	1	101.3	1	101.4	1	104.8	1	104.7
2	72.3	2	72.0	2	71.9	2	75.3	2	75.1
3	72.5	3	82.9	3	82.9	3	76.5	3	76.4
4	74.2	4	73.0	4	72.9	4	78.4	4	79.4
5	69.8	5	69.7	5	69.7	5	77.2	5	73.8
6	18.6	6	18.6	6	18.6	6	61.3	6	63.8
								-OAc	20.8
									170.9
		Xyl-		Xyl-		Rha-		Rha-	
		1	107.5	1	107.0	1	102.8	1	103.0
		2	75.6	2	75.3	2	72.5	2	72.4
		3	78.3	3	76.2	3	72.7	3	72.6
		4	71.1	4	78.0	4	73.9	4	73.9
		5	67.4	5	64.9	5	70.4	5	70.8
						6	18.5	6	18.6
				-Glc-					
				1	103.6				
				2	74.4				
				3	78.1				
				4	71.7				
				5	78.8				
				6	62.7				

Total glycosides were isolated from the leaves of this variety by the usual method that includes grinding the dried and defatted plant material, extraction of glycosides by aqueous isopropyl alcohol (80%) followed by evaporation of the alcohol, dissolution in aqueous butanol, and washing with water to remove salts and other strongly polar compounds. After evaporation of the butanol layer, the purified total glycosides were separated by preparative chromatography over silica gel with gradient elution by water-saturated mixtures of $CHCl_3$:isopropyl alcohol (10:1→1:1) to produce glycoside fractions A-J. Fractions A, C, D, E, I, and J were chromatographically pure glycosides and were designated A (**1**), C (**3**), D (**5**), E (**6**), I (**9**), and J (**12**). Fractions B, F, G, and H were rechromatographed over high-efficiency silica gel "Silpearl" and separated into glycosides B_1 (**4**) and B_2 (**2**); F_1 (**13**), F_2 (**7**), and F_3 (**15**); G_1 (**10**), G_2 (**8**), and G_3 (**14**); and H_1 (**11**) and H_2 (**16**).

The chromatographic mobilities and chemical shifts of signals in the ^{13}C NMR spectra of **1**-**8**, **10**-**12**, **15**, and **16** were identical to those of previously described glycosides [1, 2, 4]. Glycosides **1**, **4**, **5**, **6**, **10**, **11**, **12**, **15**, and **16** were previously observed in leaves of *Kalopanax* species [1,2,5]; **2**, **3**, **7**, and **8**, for the first time in *K. septemlobum*.

We concluded from results of two-dimensional TLC [6] that **13** contained an acyl group. Deacylation of **13** by aqueous-alcoholic ammonia gave the aforementioned **8**. The nature and location of the acyl group in **13** was proved using chemical shifts and acylation effects in PMR and ^{13}C NMR spectra as compared with those of **8**, as we have described several times previously [1]. Thus, it was found that **13** is oleanolic acid 3-*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranoside 28-*O*-*α*-L-rhamnopyranosyl-(1→4)-*O*-6-*O*-acetyl-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl ester and is a new triterpene glycoside.

TABLE 4. ^{1}H Chemical Shifts (δ, ppm, 0 = TMS, C_5D_5N) in Carbohydrates of **9**, **13**, and **14**

H atom	**13**	H atom	**9**, **14**	H atom	**9**	H-atom	**13**, **14**
-Ara-		-Ara-		-Glc-		-Glc-	
1	4.82	1	4.80	1	6.18	1	6.19
2	4.54	2	4.48	2	4.09	2	4.09
3	4.22	3	4.21	3	4.18	3	4.18
4	4.23	4	4.23	4	4.27	4	4.21
5e	4.27	5e	4.26	5	4.05	5	4.10
5a	3.78	5a	3.76	6A	4.61	6A	4.64
				6B	4.28	6B	4.32
-Rha-		-Rha-		-Glc-		-Glc-	
1	6.20	1	6.11	1	4.94	1	4.98
2	4.87	2	4.79	2	3.89	2	3.92
3	4.70	3	4.60	3	4.09	3	4.10
4	4.44	4	4.40	4	4.33	4	4.07
5	4.59	5	4.54	5	3.60	5	3.82
6	1.51	6	1.49	6A	4.15	6A	4.62
				6B	4.04	6B	4.52
						-OAc	1.93
Xyl-		Xyl-		Rha-		Rha-	
1	5.30	1	5.19	1	5.77	1	5.50
2	4.03	2	4.00	2	4.62	2	4.55
3	4.12	3	4.11	3	4.50	3	4.47
4	4.15	4	4.21	4	4.27	4	4.28
5e	4.29	5e	4.33	5	4.88	5	4.79
5a	3.70	5a	3.56	6	1.62	6	1.68
		-Glc-					
		1	4.93				
		2	3.95				
		3	4.15				
		4	4.07				
		5	3.92				
		6A	4.47				
		6B	4.21				

Columns in Tables 1-4 give average chemical shifts.

The sugars glucose, xylose, rhamnose, arabinose, and the aglycon oleanolic acid were identified in the total acid hydrolysates of both **9** and **14**. Glycoside **3** did not change under alkaline hydrolysis conditions whereas alkaline hydrolysis of **9** and **14** gave **3**. Therefore, the aforementioned **3** is the progenin of **9** and **14**. Enzymatic hydrolysis of **9** by total glycosidases from hepatopancreatic juices of *Helix pomatia*, which cleave terminal glucoses, gave **8**, which partially determined the structure of **9**. The site of the terminal glucopyranose and the type and configuration of its glycoside bond were established as described previously [2]. The structure of the carbohydrate chain on C-3 of the aglycon in **9** was the same as for the analogous glycoside of hederagenin (**12**).

The PMR and ^{13}C NMR spectra showed that the trisaccharide fragment (←β-D-Glc*p*^{6}←β-D-Glc*p*^{4}←α-L-Rha*p*) bonded to the carboxylic acid of oleanolic acid is typical of glycosides from Araliaceae plants. Therefore, **9** is the new triterpene glycoside oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester.

Deacylation of **14** gave **9**. The NMR spectra showed that, like for **13**, the *O*-acetyl group was located on C-6 of the glucopyranose of the trisaccharide. Thus, **14** was the new glycoside oleanolic acid 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-6-*O*-acetyl-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester.

Comparison of the carbohydrate fragments in the glycosides of both varieties, *typicum* described in the present article and *maximowiczii* studied by us previously [1, 2], showed that their structures were completely identical. However, as we have already noted [1, 2], leaves of the variety *maximowiczii* contain mainly hederagenin glycosides and with oleanolic acid glycosides observed only in trace quantities whereas the variety *typicum* contains glycosides of both oleanolic acid and hederagenin although the content of the former is even slightly greater. Furthermore, leaves of the variety *typicum* contain acetylated glycosides in small quantities whereas they are one of the principal ones in the variety *maximowiczii*. These chemotaxonomic properties and considerable external morphological differences (leaf shape etc.) [7] suggest that the taxonomic rank of these varieties should be raised to independent species of the genus *Kalopanax* and the already existing synonymic names should be used: *K. pictum* (Thunb.) Nakai for the variety *maximowiczii* and *K. septemlobum* (Thunb.) Koidz. for the variety *typicum*.

EXPERIMENTAL

General comments [1] and the enzymatic hydrolysis method [2] have been published.

Isolation of Glycosides. Leaves of *K. septemlobum* var. *typicum* were collected in Nikitskii Botanical Garden during shedding in October (60 g air-dried mass), thoroughly ground, and treated with benzene (3 × 300 mL). The defatted solid was extracted with isopropyl alcohol (80%, 4 × 500 mL). The combined extracts were evaporated to afford total extracted substances (15 g), which were dissolved in water-saturated butanol (700 mL) and washed with water (3 × 200 mL). Evaporation of the butanol layer afforded purified total glycosides (6.4 g).

Separation of Glycosides. Total glycosides (6.4 g) were chromatographed over silica gel L (500 g, 40-100 μm) with gradient elution by water-saturated $CHCl_3$:$(CH_3)_2CHOH$ (10:1→1:1) to afford fractions of glycosides A (70 mg), B (210 mg), C (201 mg), D (45 mg), E (83 mg), F (170 mg), G (367 mg), H (275 mg), I (130 mg), and J (67 mg).

Additional chromatographic purification of fractions A, C, D, E, I, and J using water-saturated $CHCl_3$:$(CH_3)_2CHOH$ systems of the corresponding polarities afforded pure glycosides **1** (40 mg), **3** (100 mg), **5** (20 mg), **6** (50 mg), **9** (85 mg), and **12** (30 mg). Fraction B (210 mg) was rechromatographed over Silpearl silica gel (150 g) with elution by water-saturated $CHCl_3$:$(CH_3)_2CHOH$ (4:1) to afford **2** (80 mg) and **4** (80 mg). Fraction F (170 mg) was rechromatographed over Silpearl silica gel (100 g) with elution by water-saturated $CHCl_3$:$(CH_3)_2CHOH$ (3:1) to afford **7** (65 mg), **13** (17 mg), and **15** (14 mg). Fraction G (367 mg) was rechromatographed over Silpearl silica gel (200 g) with elution by water-saturated $CHCl_3$:$(CH_3)_2CHOH$ (3:1) to afford **8** (100 mg), **10** (62 mg), and **14** (20 mg). Fraction H (275 mg) was rechromatographed over Silpearl silica gel (150 g) with elution by water-saturated $CHCl_3$:$(CH_3)_2CHOH$ (2:1) to afford **11** (100 mg) and **16** (20 mg).

TLC of the total acid hydrolysates of **1**-**3**, **7**-**9**, **13**, and **14** identified oleanolic acid and the sugars rhamnose and arabinose in **1**; xylose, rhamnose, and arabinose in **2**; rhamnose, arabinose, and glucose in **7**; and glucose, xylose, rhamnose, and arabinose in **3**, **8**, **9**, **13**, and **14**. Alkaline hydrolysis of **7** gave **1**; of **8** and **13**, **2**; of **9** and **14**, **3**. Deacetylation of **13** gave **8**; of **14**, **9**.

TLC of the total acid hydrolysates of **4**-**6**, **10**-**12**, **15**, and **16** identified hederagenin and the sugars rhamnose and arabinose in **4**; xylose, rhamnose, and arabinose in **5**; rhamnose, arabinose, and glucose in **10**; and glucose, xylose, rhamnose, and arabinose in **6**, **11**, **12**, **15**, and **16**. Alkaline hydrolysis of **10** gave **4**; of **11** and **15**, **5**; of **12** and **16**, **6**. Deacetylation of **15** gave **11**; of **16**, **12**.

Table 1 lists chemical shifts of ^{13}C atoms in NMR spectra of the aglycons of **1**-**16**; Tables 2 and 3, of ^{13}C atoms in carbohydrates of **1**-**16**; Table 4, of ^{1}H atoms in carbohydrates of **9**, **13**, and **14**.

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