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TRITERPENE GLYCOSIDES FROM *Kalopanax septemlobum*. II. GLYCOSIDES E, K, AND L FROM LEAVES OF *Kalopanax septemlobum* VAR. *maximowiczii* INTRODUCED TO CRIMEA

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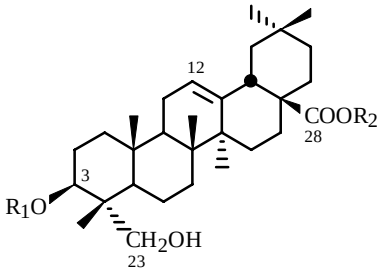
The known hederagenin 3-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside (sapindoside C) and its 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl and 28-O-α-L-rhamnopyranosyl-(1→4)-O-6-O-acetyl-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters, new triterpene glycosides, were isolated from leaves of Kalopanax septemlobum *var.* maximowiczii *introduced to Crimea. The structures of these compounds were established using chemical methods and two-dimensional NMR spectroscopy.*

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

We have previously reported the method for isolating glycosides E (**1**), K (**2**), and L (**3**) from leaves of *Kalopanax septemlobum* var. *maximowiczii* [1]. These glycosides were additionally purified of phenolic impurities by rechromatography over silica gel. Total acid hydrolysis of **1**-**3** showed that the carbohydrate fragments in all glycosides were glucose, xylose, rhamnose, and arabinose whereas the aglycon was hederagenin.

Glycoside **1** was insensitive to alkaline hydrolysis. However, it was methylated by diazomethane in ether. This defines it as a monodesmoside that lacks a carbohydrate chain on the hederagenin carboxyl. On the other hand, compounds **2** and **3** were bisdesmosides and underwent alkaline hydrolysis, as a result of which both **2** and **3** afforded **1**. Furthermore, **2** converted under mild alkaline hydrolysis into **3**, which indicates that it contains an additional acyl fragment. Treatment of **3** with an enzyme prepared from hepatopancreatic fluid of the grape snail (*Helix pomatia*), which cleaves terminal glucose units, produced from it the previously described 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)-*β*-D-glucopyranosyl-(1→6)-O-*β*-D-glucopyranosyl ester of hederagenin [1].



1 - 3

	R_1	R_2
1:	*β*-D-Clc*p*→^{4}*β*-D-Xyl*p*→^{3}*α*-L-Rha*p*→^{2}*α*-L-Ara*p*→	H
2:	*β*-D-Clc*p*→^{4}*β*-D-Xyl*p*→^{3}*α*-L-Rha*p*→^{2}*α*-L-Ara*p*→	←*β*-D-Clc*p*^{6}←(*β*-D-Clc*p*^{6} ←OAc)^4←*α*-L-Rha*p*
3:	*β*-D-Clc*p*→^{4}*β*-D-Xyl*p*→^{3}*α*-L-Rha*p*→^{2}*α*-L-Ara*p*→	←*β*-D-Clc*p*^{6}←*β*-D-Clc*p*^{4} ←*α*-L-Rha*p*

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TABLE 1. Chemical Shifts of ^{13}C Atoms of Aglycons of Glycosides **1-3**

C atom	**1**	**2**, **3***	C atom	**1**	**2**, **3***
1	39.0	39.1	16	23.7	23.4
2	26.2	26.4	17	46.6	47.1
3	81.1	81.2	18	42.0	41.7
4	43.6	43.6	19	46.4	46.3
5	47.7	47.7	20	30.9	30.8
6	18.2	18.3	21	34.2	34.1
7	32.8	32.8	22	33.2	32.7
8	39.8	40.0	23	64.0	64.0
9	48.2	48.3	24	14.2	14.2
10	36.9	37.0	25	16.1	16.3
11	23.8	23.9	26	17.4	17.6
12	122.6	123.0	27	26.3	26.2
13	144.8	144.2	28	180.2	176.7
14	42.2	42.2	29	33.2	33.2
15	28.3	28.4	30	23.8	23.8

*Here and in Table 2, average chemical shifts are given for these compounds. Deviations for individual compounds are less than ±0.1 ppm.

It is interesting to note that this same product was obtained by enzymatic degradation of **2** since the enzyme preparation contains glucosidase and esterase enzymes that cleave sterically unsubstituted acyl groups. Furthermore, it can be concluded that the additional terminal glucose unit is located in the carbohydrate chain on C-3 of hederagenin because enzymatic hydrolysis of **1** (and progenins of glycosides **2** and **3**) produced hederagenin 3-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside. NMR spectroscopy was used to determine the bonding site of the additional terminal glucose and to confirm conclusively the structures of **1-3**.

The nature of the aglycon in **1-3** was confirmed by analyzing the chemical shifts of C atoms in the one-dimensional ^{13}C NMR spectrum. The shifts are completely identical to those of 3-O-glycosylated hederagenin in **1** [2] and 3,2-di-O-glucosylated hederagenin in **2** and **3** [2]. Table 1 lists the chemical shifts of the ^{13}C atoms in NMR subspectra of the aglycons of **1-3**.

For **1** (and progenins of glycosides **2** and **3**), a combination of two-dimensional COSY, HSQC, and ROESY methods was used to assign completely and unambiguously the signals in the PMR and ^{13}C NMR spectra. Signals of skeletal protons of monosaccharides in the PMR spectrum (Table 2) were assigned based on the COSY and TOCSY spectra, starting with nonoverlapping signals in the low-field protion of the spectrum for anomeric H atoms and the methyl signals for rhamnose in the high-field portion. The multiplicity and magnitude of the spin—spin coupling constants for the skeletal protons of monosaccharides confirmed that they belonged to α-arabino-, α-rhamno-, β-xylo-, and β-glucopyranoses.

Signals for ^{13}C atoms of monosaccharide units were completely assigned using the two-dimensional HSQC method based on the magnitudes of the chemical shifts for skeletal protons. The magnitudes of the chemical shifts for C atoms compared with those reported for unsubstituted monosaccharide units [3] revealed significant positive α-effects from glycosylation for C-2 of arabinose, C-3 of rhamnose, and C-4 of xylose. Small negative β-effects on the corresponding neighboring C atoms were observed. Glycosylation did not affect the terminal glycopyranose unit. The bonding sequence of the monosaccharides was determined by enzymatic hydrolysis and ROESY spectra, in which structurally informative cross peaks were identified between rhamnose H-1 and arabinose H-2, xylose H-1 and rhamnose H-3, terminal glucose H-1 and xylose H-4, and arabinose H-1 and aglycon H-3.

The above data define unambiguously the structure of **1** (and progenins of **2** and **3**) as hederagenin 3-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopryanoside.

TABLE 2. Chemical Shifts of ^{1}H and ^{13}C Atoms of Carbohydrates of Glycosides **1-3**

^{13}C atom	**1-3***	^{1}H atom	**1-3***	^{13}C atom	**2**	^{1}H atom	**2**	^{13}C atom	**3**	^{1}H atom	**3**
Ara-1	104.6	Ara-1	5.06	Glc-1	90.7	Glc-1	6.19	Glc-1	95.7	Glc-1	6.18
2	75.4	2	4.53	2	73.9	2	4.09	2	73.9	2	4.10
3	74.9	3	4.05	3	78.6	3	4.18	3	78.6	3	4.20
4	69.6	4	4.15	4	70.8	4	4.21	4	70.8	4	4.28
5	66.0	5a	3.69	5	78.1	5	4.10	5	78.1	5	4.07
		5e	4.27	6	69.4	6A	4.64	6	69.3	6A	4.63
						6B	4.32			6B	4.30
Rha-1	101.4	Rha-1	6.22	Glc-1	104.7	Glc-1	4.98	Glc-1	104.8	Glc-1	4.97
2	71.9	2	4.83	2	75.1	2	3.92	2	75.3	2	3.90
3	82.9	3	4.65	3	76.4	3	4.10	3	76.5	3	4.10
4	72.9	4	4.42	4	79.4	4	4.07	4	78.5	4	4.32
5	69.7	5	4.66	5	73.8	5	3.82	5	77.2	5	3.63
6	18.6	6	1.56	6	63.8	6A	4.62	6	61.3	6A	4.18
				-OAc	20.9	6B	4.52			6B	4.05
				-OAc	171.0	-OAc	1.93				
Xyl-1	107.0	Xyl-1	5.22	Rha-1	103.0	Rha-1	5.50	Rha-1	102.8	Rha-1	5.76
2	75.3	2	4.02	2	72.4	2	4.55	2	72.5	2	4.64
3	76.2	3	4.12	3	72.6	3	4.47	3	72.7	3	4.50
4	78.0	4	4.25	4	73.9	4	4.28	4	73.9	4	4.28
5	64.9	5e	4.30	5	70.8	5	4.79	5	70.5	5	4.87
		5a	3.57	6	18.6	6	1.68	6	18.6	6	1.65
Glc-1	103.6	Glc-1	4.93								
2	74.4	2	3.96								
3	78.1	3	4.17								
4	71.7	4	4.10								
5	78.8	5	3.94								
6	62.7	6A	4.52								
		6B	4.24								

The ^{13}C NMR spectra of **2** and **3**, when compared with that of **1**, contained additional signals for C atoms of trisaccharide units bonded to the aglycon by an acylglycoside bond at C-28. Comparison of the chemical shifts, for example, with those previously reported [1], identified them unambiguously as 28-O-α-L-rhamnopyranosyl-(1→4)-O-6-O-acetyl-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl and 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl fragments, which are very characteristic of glycosides of Araliaceae plants [4]. The structures of these fragments were additionally confirmed using two-dimensional NMR methods, as described above for **1**. Table 2 gives the chemical shifts of ^{1}H and ^{13}C atoms of these fragments.

Thus, **2** and **3** are 3-O-β-D-glucopyranosyl-(1→4)-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-glucopryanosyl and 3-O-β-D-glucopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl esters of hederagenin and are new triterpene glycosides. The structure of **1** is identical to that of sapindoside C, which was isolated previously from *Sapindus mukorossi* [5]. The structure of this glycoside was established using only chemical methods and was not confirmed by NMR spectroscopy.

EXPERIMENTAL

General comments have been published [1]. Enzymatic hydrolysis was carried out by dissolving glycoside in a 100-fold excess of water, adding a 5-10-fold weight excess of the appropriate enzyme, and thoroughly stirring the mixture and holding it at 40-50°C from several hours to one day with TLC monitoring. After the process was complete, a 3-5-fold excess of CH_3OH was added. The mixture was heated to boiling. The denatured enzyme was separated by centrifugation. The supernatant was evaporated to dryness. The enzymolysis products were analyzed by TLC.

The method for isolating **1** (300 mg), **2** (860 mg), and **3** (350 mg) from leaves of *K. septemlobum* var. *maximowiczii* has been reported [1]. The crude glycosides were additionally purified by rechromatography over silica gel with elution by water-saturated $CHCl_3$:isopropanol (4:1) for glycoside E and water-saturated $CHCl_3$:isopropanol (1:1) for glycosides K and L. This produced pure **1** (150 mg), **2** (550 mg), and **3** (210 mg).

The total acid hydrolysates of **1**-**3** contained hederagenin and the sugars glucose, xylose, rhamnose, and arabinose. Alkaline hydrolysis of **2** and **3** afforded **1**. Mild alkaline hydrolysis of **2** afforded **3**. Enzymatic hydrolysis of **1** afforded hederagenin 3-O-β-D-glycopyranosyl-(1→4)-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranoside; of **2** and **3**, hederagenin 3-O-β-D-xylopyranosyl-(1→3)-O-α-L-rhamnopyranosyl-(1→2)-O-α-L-arabinopyranosyl-28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-O-β-D-glucopyranosyl ester.

Table 1 gives chemical shifts of ^{13}C atoms in the NMR of the aglycons of **1**-**3**; Table 2, of ^{1}H and ^{13}C in the carbohydrate parts of **1**-**3**.

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