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TRITERPENE GLYCOSIDES FROM *Clematis*. I. GLYCOSIDES FROM THE ROOTS OF *Clematis vitalba*

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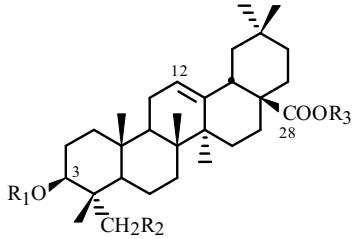
The genus *Clematis* is represented in the flora of Crimea by the four species *C. vitalba*, *C. flammula*, *C. integrifolia*, and *C. orientalis*. The most widely distributed is *C. vitalba*, which is used in folk medicine to treat various inflammatory diseases, rheumatism, prostatitis, gonorrhea, chronic skin diseases, and varicose veins. However, the chemical composition of this medicinal plant has until now been little studied.

Early investigations of glycosides from *Clematis* (several researchers reported that triterpene glycosides were present [1, 2] and described phenolic compounds [3]) were only preliminary in nature and used TLC and several analytical chemical methods. Therefore, we studied the composition of glycosides from *C. vitalba* roots using modern isolation and separation methods and determined their structures.

Total glycosides were isolated from *C. vitalba* roots using a procedure that included grinding dried and defatted plant material, extraction of glycosides by aqueous isopropanol (80%), evaporation of the alcohol extract, dissolution of the residue in aqueous BuOH, and washing with H_2O to remove salts and other highly polar compounds. The BuOH was evaporated. The purified total glycosides were separated by HPLC on an Agilent 1100 instrument using a Zorbax-SB-C18 column (150 × 2.1 mm, 3.5 μm) with gradient elution. Glycoside fractions A-D were obtained. Fraction D turned out to be a chromatographically pure glycoside (**7**). Fractions A, B, and C were rechromatographed over a Zorbax-Bonus-RP column (150 × 4.6 mm, 5 μm) with gradient elution by MeOH (65%), CH_3CN (5%), aqueous TFA (0.015%, 30%) up to MeOH (100%), over a Supelcosil C18 column (210 × 21 mm, 5 μm) with gradient elution from 50 to 100% MeOH, and over the same column using aqueous CH_3CN (75%) to separate glycosides A_1 (**1**) and A_2 (**4**), B_1 (**2**) and B_2 (**5**), and C_1 (**3**) and C_2 (**6**).



1 - 7

1 - 6: R_1 = Rib→^{3}Rha→^{2}Ara→; **7:** R_1 = Glc→^{4}Rib→^{3}Rha→^{2}Ara→
1 - 3: R_2 = H; **4 - 7:** R_2 = OH
1, 4: R_3 = H; **2, 5:** R_3 = ←Glc; **3, 6, 7:** R_3 = ←Glc^6←Glc^4←Rha
Rha = α-L-Rha*p*; Ara = α-L-Ara*p*; Rib = β-D-Rib*p*; Glc = β-D-Glc*p*

Total acid hydrolysates of **1**-**3** contained the sugars glucose, ribose, rhamnose, and arabinose and the aglycon oleanolic acid. Glycoside **1** did not change under alkaline hydrolysis conditions whereas alkaline hydrolysis converted **2** and **3** into **1**. Therefore, **1** was the progenin of **2** and **3**. Total acid hydrolysis of **4**–**6** gave analogous results. However, hederagenin was identified as the aglycon.

The chromatographic mobility and chemical shifts of resonances in PMR and ^{13}C NMR spectra for **1** and **4** were identical to known glycosides that were isolated earlier from *C. chinensis* [4, 5]. Thus, **1** and **4** were 3-*O*-β-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosides of oleanolic acid and hederagenin.

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TABLE 1. ^{13}C Chemical Shifts (C_5D_5N, 0 = TMS, δ, ppm) of the Aglycons of Glycosides **1**–**7**

C atom	**1**	**4**	**2, 3**	**5, 6, 7**	C atom	**1**	**4**	**2, 3**	**5, 6, 7**
1	39.8	39.0	39.0	38.9	16	23.7	23.7	23.4	23.2
2	26.7	26.4	26.7	26.2	17	46.7	46.7	47.1	46.8
3	88.8	81.1	88.8	80.9	18	42.0	42.0	41.7	41.4
4	39.6	43.6	39.7	46.0	19	46.5	46.4	46.3	46.0
5	56.1	47.8	56.1	47.5	20	31.0	30.9	30.8	30.5
6	18.5	18.1	18.6	18.3	21	34.3	34.2	34.1	33.8
7	33.2	33.2	33.2	32.5	22	33.3	32.9	32.6	32.4
8	38.9	39.8	40.0	39.7	23	28.3	64.0	28.3	63.7
9	48.1	48.2	48.2	48.0	24	17.2	14.1	17.3	13.9
10	37.1	36.9	37.1	36.7	25	15.6	16.1	15.7	16.0
11	23.7	23.8	23.9	23.6	26	17.4	17.5	17.5	17.3
12	122.6	122.6	122.9	122.6	27	26.2	26.2	26.1	25.8
13	144.8	144.8	144.2	143.9	28	180.2	180.2	176.6	176.3
14	42.2	42.2	42.2	41.9	29	33.2	33.2	33.2	32.9
15	28.3	28.3	28.4	28.1	30	23.8	23.8	23.8	23.5

TABLE 2. ^{13}C Chemical Shifts (C_5D_5N, 0 = TMS, δ, ppm) of the Carbohydrates of Glycosides **1**–**7**

C atom	**1**	**2, 3**	**4**	**5, 6**	**7**	C atom	**2**	**3**	**5**	**6,7**
	Ara	Ara	Ara	Ara	Ara		Glc	Glc	Glc	Glc
1	105.3	105.4	104.7	104.4	104.4	1	95.8	95.7	95.7	95.4
2	75.6	75.6	75.3	75.0	75.1	2	74.2	73.9	74.2	73.7
3	74.7	74.6	75.4	75.0	75.0	3	78.9	78.7	78.9	78.5
4	69.4	69.4	69.8	69.5	69.5	4	71.1	70.8	71.1	70.7
5	65.7	65.8	66.3	66.1	66.0	5	79.4	78.2	79.3	77.9
	Rha	Rha	Rha	Rha	Rha	6	62.3	69.2	62.2	69.1
1	101.5	101.5	101.4	101.1	101.2			Glc		Glc
2	72.1	72.2	72.0	71.8	71.7	1		104.8		104.7
3	81.3	81.3	81.3	81.0	81.6	2		75.3		75.1
4	72.9	72.8	72.9	72.6	72.6	3		76.5		76.3
5	69.9	69.9	69.8	69.6	69.5	4		78.4		78.1
6	18.5	18.6	18.5	18.2	18.2	5		77.2		77.0
	Rib	Rib	Rib	Rib	Rib	6		61.3		61.1
1	104.7	104.8	104.7	104.4	104.5			Rha		Rha
2	72.9	72.8	72.8	72.5	72.3	1		102.8		102.6
3	68.9	68.9	68.8	68.7	69.5	2		72.6		72.3
4	70.3	70.2	70.3	70.0	76.3	3		72.8		72.6
5	65.3	65.4	65.3	65.0	61.6	4		74.0		73.8
					Glc	5		70.4		70.1
1					103.3	6		18.6		18.3
2					74.5					
3					78.2					
4					71.3					
5					78.4					
6					62.4					

Glycosides **2** and **5** also contained a glucopyranose bonded to the aglycon C-28 atom according to NMR spectroscopy. Thus, **2** and **5** were 28-*O*-*β*-D-glucopyranosyl esters of oleanolic acid and hederagenin 3-*O*-*β*-D-ribopryanosyl-(1→3)-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*α*-L-arabinopyranosides. Glycosides of analogous structures were isolated earlier from representatives of the genus *Clematis* [5, 6].

Analysis of PMR and ^{13}C NMR spectra of **3** and **6** showed the aforementioned trisaccharide bonded to the C-3 atom of the aglycon in addition to the trisaccharide chain typical of glycosides from plants of the families Ranunculaceae and Araliaceae (←*β*-D-Glc$_p$^{6}←*β*-D-Glc$_p$^{4}←*α*-L-Rha$_p$) that was bonded to the aglycon carboxylic acid. Therefore, **3** and **6** were

28-*O*-α-L-rhamnopyranosyl-(1→4)- β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl esters of the 3-*O*-β-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosides of oleanolic acid and hederagenin. These glycosides were previously reported [6, 7].

Resonances in PMR and ^{13}C NMR spectra of **7** were assigned completely and unambiguously. The structure of **7** turned out to be the same as in an analogous glycoside of hederagenin that was isolated, for example, from *C. chinensis* [5]. Thus, **7** was the previously known glycoside 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ester of 3-*O*-β-D-glucopyranosyl-(1→4)-*O*-β-D-ribopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranoside of hederagenin.

All isolated and described glycosides were observed for the first time in *C. vitalba* roots.

Tables 1 and 2 present average values for chemical shifts for the compounds shown in the columns. Deviations for individual compounds were less than ±0.1 ppm.

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