FLAVONOIDS FROM *Cephalaria gigantea* FLOWERS

I. S. Movsumov,[1] E. A. Garaev,[1] and M. I. Isaev[2]

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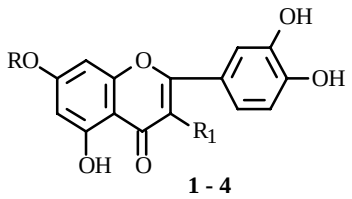
Luteolin, quercetin, cinaroside, quercimeritrin, and the new flavonol bioside gigantoside A were isolated from Cephalaria gigantea *(Ledeb.) Bobr. (Dipsacaceae) flowers. Spectral properties and chemical transformations established that gigantoside A had the structure quercetin-7-*O*-[α-L-arabinopyranosyl(1→6)]-β-D-glucopyranoside.*

Key words: *Cephalaria gigantea* (Ledeb.) Bobr., Dipsacaceae, flavonoids, luteolin, quercetin, cinaroside, quercimeritrin, gigantoside A, ^{1}H and ^{13}C NMR spectra, DEPT, 2D NMR (COSY, HMQC, HMBC, NOESY).

In continuation of phytochemical research on plants of the genus *Cephalaria* (Schrad.) growing in the Republic of Azerbaidzhan [1-5], we studied *C. gigantea* (Ledeb.) Bobr. (Dipsacaceae). Luteolin (**1**), quercetin (**2**), cinaroside (**3**), quercimeritrin (**4**), and a new bioside of quercetin, which we named gigantoside A (**5**), were isolated and identified from flowers of this plant. Herein we report the structure of the last of these.



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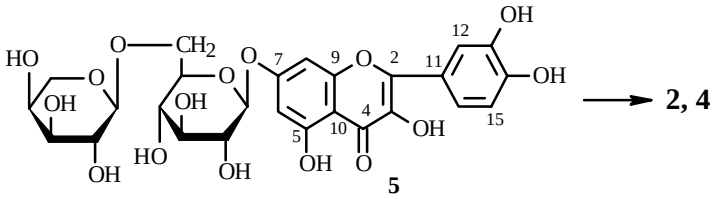

5 → **2, 4**

1: R = R_1 = H **3:** R = β-D-Glc*p*, R_1 = H
2: R = H, R_1 = OH **4:** R = β-D- Glc*p*, R_1 = OH

Acid hydrolysis of the new flavonoid glycoside **5** formed the genin, which was identified as quercetin (**2**). Paper chromatography (PC) of the carbohydrate part of the hydrolysate detected D-glucose and L-rhamnose.

According to the PMR and ^{13}C NMR spectra of **5** (Table 1), which contained a single set of H and C signals for the carbohydrate units, the new glycoside was a bioside of quercetin and contained D-glucose and L-arabinose in a 1:1 ratio.

The anomeric protons in the PMR spectrum of **5** in deuteropyridine resonated as doublets at δ 5.63 with SSCC J = 7.6 Hz (H-1 of D-glucose) and 4.90 with SSCC J = 6.4 Hz (H-1 of L-arabinose). These values indicated that the monosaccharide units had the pyranose form, the $^4C_1$-conformation, and therefore the β- and α-configuration, respectively [6, 7].

The chemical shifts of the anomeric C atoms of δ 101.92 (100.24) (values in DMSO-d_6 in parentheses) for β-D-glucopyranose and δ 104.91 (103.50) for α-L-arabinose indicated that **5** was a monodesmoside and the pentose was the terminal monosaccharide.

The HMBC spectrum of **5** (Table 1) contained a correlation peak between the signals of the anomeric proton of the β-D-glucopyranoside and C-7 of the genin. This unambiguously determined the attachment site at this position of quercetin. As expected, stepwise hydrolysis of **5** formed the progenin **4**, which was identical to quercimeritrin.

1) Azerbaidzhan Medical University, Pharmaceutical Department, Baku, ul. A. Bakikhanova, 23, e-mail: eldargar@mail.ru; 2) S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: m_isaev@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 552-554, November-December, 2006. Original article submitted June 3, 2006.

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TABLE 1. Chemical Shifts of H and C Atoms of **5**, DEPT Data, and Parameters of 2D NMR ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY (δ, ppm, J/Hz, C_5D_5N and DMSO-d_6, 0 = TMS)

C atom	DEPT	Data in Py-d_5			Data in DMSO-d_6			
		δ_C	δ_H (J/Hz)	HMBC (C atoms)	δ_C	δ_H (J/Hz)	HMBC (C atoms)	NOESY (H atoms)
2	C	148.50*	-		148.32	-		
3	C	137.84	-		136.49	-		
4	C	176.98	-		176.42	-		
5	C	161.54	-		160.78	-		
6	CH	99.57	6.77 d (2.1)	5, 7, 8, 10	99.27	6.44 d (1.9)	5, 7, 8, 10	
7	C	163.49	-		163.02	-		
8	CH	94.70	7.00 d (2.1)	6, 7, 9, 10	94.73	6.77 d (1.9)	7, 9, 10, Gl	Gl
9	C	156.50	-		156.19	-		
10	C	105.83	-		105.17	-		
11	C	123.30*	-		122.24	-		
12	CH	116.43	8.61 d (2.2)	2, 13, 14, 16	116.11	7.73 d (2)	2, 13, 14, 16	
13	C	146.69	-		145.44	-		
14	C	148.28	-		148.13	-		
15	CH	116.34	7.41 d (8.5)	11, 13, 14	115.80	6.92 d (8.5)	11, 13, 14	16
16	CH	121.22	8.15 dd (8.5, 2.2)	2, 12, 15	120.70	7.59 dd (8.5, 2)	2, 12, 14	15
				β-D-Glc*p* (G)				
1	CH	101.92	5.63 d (7.6)	7	100.24	5.08 d (7)	7	8
2	CH	74.37	4.16		73.49	3.30		
3	CH	77.96	4.28		76.50	3.25		
4	CH	70.86	4.21		69.78	3.20	G5	
5	CH	77.18	4.24		75.94	3.56		
6	CH_2	68.91	4.77** (9.5), 4.26		67.94	3.95 d (10.2), 3.56		
				α-L-Ara*p* (A)				
1	CH	104.91	4.90 d (6.4)	G6	103.50	4.17 d (6)	G6	
2	CH	71.98	4.49 dd (8, 6.5)	A1, A3	70.94	3.40	A1, A3	
3	CH	73.92	4.14		72.90	3.30		
4	CH	68.63	4.25		67.66	3.40		
5	CH_2	65.86	3.67** (11.3),	A1	65.16	3.25,		
			4.25	A1		3.62	A3	

*Position of signals found from HMBC spectrum.

**Broadened doublets. Chemical shifts of protons without multiplicities and SSCC were found from 2D spectra.

The attachment site of L-arabinose was also found using the HMBC spectrum and the ^{13}C NMR spectrum of the new flavonoid glycoside. Atom C-6 of D-glucose underwent a glycosylation effect in the ^{13}C HMR spectrum and was observed at δ 68.91 (67.94), indicating attachment of L-arabinose to this atom. We note that unglycosylated C-6 of D-glucose resonates around δ 62-63. The attachment site of L-arabinose was confirmed by the HMBC spectrum, where a cross-peak was observed between the signals of the anomeric proton of L-arabinose and C-6 of D-glucose.

Thus, the experimental results lead to the conclusion that gigantoside A has the structure quercetin-7-*O*-[*α*-L-arabinopyranosyl(1→6)]-*β*-D-glucopyranoside.

An analysis of the ^{13}C NMR spectra of quercetin [8], quercimeritrin [8], and gigantoside A indicated that C-7 had a small (up to -1.3 ppm) negative *α*-effect of glycosylation; C-9, an interesting *γ*-effect that was significant and also negative (up to -5 ppm).

EXPERIMENTAL

General Comments. Paper chromatography was performed on Filtrak No. 11 paper using *n*-butanol:acetic acid:water (4:1:5) and *n*-butanol:pyridine:water (6:4:3). Free monosaccharides were detected using anilinium phthalate. UV spectra were recorded on an SF-46 spectrophotometer. NMR (1D and 2D) spectra were obtained on a Bruker AM-300 spectrometer in deuteropyridine (Py-d_5) and DMSO-d_6 (δ, 0 = TMS). Water of crystallization was determined on a Q-1500D DTA (Paulik—Erdey—Paulik System).

Isolation of Flavonoids from *C. gigantea* Flowers. Air-dried flowers (1 kg) were collected during full flowering on July 10, 2005, in Rustam Aliev of Kedabek Region in the Republic of Azerbaidzhan and extracted twice with ethanol (80%) on a water bath for 3 h. The extracts were condensed to the watery residue and treated successively with $CHCl_3$ and ether.

Fractional crystallization of the ether extract from ethanol afforded pure luteolin (**1**) and quercetin (**2**).

The mother liquor was evaporated to half the volume and shaken with an equal volume of $CHCl_3$. Cinaroside (**3**) crystallized at the interface after one day.

Then the mother liquor was extracted with ethylacetate and *n*-butanol. The ethylacetate extract afforded quercimeritrin (**4**).

The butanol extract was evaporated to dryness and treated with hot ethanol to isolate gigantoside A (**5**).

A Bryant cyanidine test indicated that **1** and **2** were genins; **3**-**5**, glycosides.

Luteolin (1), $C_{15}H_{10}O_6$, mp 328-330°C (ethanol). UV spectrum (MeOH, λ_{max}, nm): 353, 265; (CH_3COONa, λ_{max}, nm): 373, 370.

Quercetin (2), $C_{15}H_{10}O_7$, yellow needles, mp 307-309°C (ethanol). UV spectrum (MeOH, λ_{max}, nm): 370, 256; (CH_3COONa, λ_{max}, nm): 380, 258.

Cinaroside (3), $C_{21}H_{20}O_{11}$, yellow crystals, mp 256-258°C (ethanol), $[\alpha]_D$ -52° (*c* 0.5, pyridine:methanol, 4:2). UV spectrum (MeOH, λ_{max}, nm): 352, 257 (264); (CH_3COONa, λ_{max}, nm): 350, 258. Quantitative hydrolysis (H_2SO_4, 5%) for 4 h of **3** formed luteolin (68.2%) and D-glucose.

Quercimeritrin (4), $C_{21}H_{20}O_{12}$, yellow crystals, mp 254-255°C (ethanol), $[\alpha]_D$ -56° (*c* 0.24, pyridine:methanol, 4:2). UV spectrum (MeOH, λ_{max}, nm): 372, 256; (CH_3COONa, λ_{max}, nm): 350, 256. Acid hydrolysis of **4** formed quercetin (68%) and D-glucose.

Compounds **3** and **4** were isolated previously from *C. Kotschyi* Boriss. et Hoh. [3]. These same glycosides were isolated from *C. gigantea* (Ledeb.) Bobr. growing in the northern Caucuses [9].

Gigantoside A (5), $C_{26}H_{28}O_{16}\cdot H_2O$, lemon-yellow crystals, soluble in water and ethanol, very soluble in aqueous ethanol, insoluble in ether, mp 220-222°C (ethanol), $[\alpha]_D$ -45.2° (*c* 0.52, DMF). UV spectrum (MeOH, λ_{max}, nm): 360, 260; (CH_3COONa, λ_{max}, nm): 358, 262; (CH_3COONa + H_3BO_3, λ_{max}, nm): 384, 266.

Table 1 gives the PMR and ^{13}C NMR spectra.

Thermal analysis established that **5** contained one water of crystallization. The determination of the water of crystallization was made at the Institute of Chemical Problems, National Academy of Sciences, Republic of Azerbaidzhan, by Candidate of Chemical Sciences N. S. Osmanov.

Quercetin (2) from 5. Gigantoside A (50 mg) was hydrolyzed by H_2SO_4 (20 mL, 5%) on a water bath for 4 h. The precipitate that formed on cooling was filtered off, washed with water until the washings were neutral, and chromatographed over a column of cellulose with elution by ethylacetate to isolate the aglycon (24 mg), which was identified as quercetin.

The carbohydrate part of the hydrolysate was neutralized by $BaCO_3$ and evaporated. PC of the solid using system 2 identified D-glucose and L-arabinose.

Stepwise Hydrolysis of 5. Compound **5** (100 mg) was dissolved in HCl (30 mL, 1%), heated on a water bath for about 1 h, cooled, and extracted with ethylacetate. After the usual work up, the ethylacetate extract was chromatographed over a column of polyamide with elution by water to isolate starting **5**.

Continued elution by ethanol (30%) afforded progenin **4**, mp 248-250°C (ethanol), $[\alpha]_D$ -50° (*c* 0.6, pyridine:ethanol, 3:2), which was identified as quercimeritrin.

The hydrolysate was neutralized and concentrated. PC using system 2 detected L-arabinose.

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