QUINQUELOSIDE – A NEW FLAVONOID GLYCOSIDE FROM LEONURIS QUINQUELOBATUS GILIB.

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Continuing an investigation of the components of Leonuris quinquelobatus Gilib. (quinquelobate motherwort) we have isolated the total flavonoid compounds [1], consisting of six substances. By separating the combined substances on polyamide columns [2] we have obtained a number of individual flavonoids.

The present paper gives the results of a chemical study of one of these substances that we have called quinqueloside. From a comparison of the properties of the substance isolated and the properties of terniflorin and its derivative cosmosiin [3] (Table 1) it can be assumed that they have similar chemical structures.

Table 1

Physicochemical Properties of Quinqueloside, Terniflorin, and Cosmosiin

Properties	Quinqueloside	Terniflorin	Cosmosiin	Arglycone of quinqueloside
Form of the crystals	Pale yellow needles	Yellow needles	Yellow needles	Pale yellow plates
Mp, °C	265—267	266–267	220—222	345—348
$[\alpha]_D^{20}$, degrees	−100.0 (*c* 1; Pyridine	—	−70.0 (*c* 0.5; Pyridine	—
Mol. wt.	578.0	596.0	432.0	270.0
Formula	$C_{30}H_{26}O_{12}$	$C_{30}H_{26}O_{12}\ H_2O$	$C_{21}H_{20}O_{10}$	$C_{15}H_{10}O_5$
R_f in systems*				
1	0.08	0.00	0.26	0.05
2	0.47	0.80**	0.64	0.45
3	0.44	—	0.10	0.92
Qualitative reactions (colorations)				
Cyanidin	Orange-red	Yellow	Orange	Orange
With ferric chloride	Dark green	Light blue	Green	Green
Caustic potash	Yellow	—	Yellow	Yellow

*1) 15% CH_3COOH; 2) 40% CH_3COOH; 3) Ethyl acetate – benzene – acetic acid (73.5 : 2).
**60% CH_3COOH.

To elucidate the nature of this flavonoid and the position of the free hydroxy groups in it, we have carried out a spectroscopic investigation of the UV spectrum using ionizing and complex-forming reagents [5], and of the IR spectrum, and also acidic, alkaline, and enzymatic hydrolysis with subsequent study of the hydrolysis products. From the results of these studies (Table 2) it may be concluded that quinqueloside is a glycoside containing a free hydroxy group in position 5 and D-glucose in position 7.

In the investigation of quinqueloside in the IR region (Figure) it was found that the spectrum of this compound contained, in addition to bands at 1656 and 830 cm^{-1} characteristic for flavonoids, a band at 1688 cm^{-1}, indicating the presence of an ester group in the molecule [4].

Acid hydrolysis gave an aglycone identified with apigenin, d-glucose, and an aromatic acid identified as p-coumaric acid. To establish the nature of the carbohydrate and the acid substituents, we carried out an analysis of the products of the acid, enzymatic, and alkaline hydrolyses of quinqueloside (Table 3).

The alkaline saponification of quinqueloside gave p-coumaric acid and substance D, which was decomposed by acid and enzymatic hydrolyses into apigenin and d-glucose. It was identified spectroscopically as apigenin 7-glucoside. The enzymatic decomposition of quinqueloside leads to d-glucose and substance E. The latter decomposes to apigenin and p-coumaric acid on alkaline saponification.

The material presented enables us to assume that d-glucose is present in the 7 position of apigenin. However, the p-coumaric acid is not attached to the glucose as in terniflorin [3]. This acid is perhaps attached to apigenin in position

4' as is confirmed by the UV spectrum, which shows a considerable hypsochromic displacement of the maximum of the long-wave band from 341 mμ in cosmosiin to 320 mμ in quinqueloside. This displacement can be explained only by the acylation of the 4'-hydroxy group [5]. However, the bathochromic displacement by 60 mμ observed in the spectrum of quinqueloside under the action of sodium ethoxide may lead to an erroneous conclusion as indicating the presence of a free 4'-hydroxy group. In this case, the bathochromism is probably connected with the ionization of the 4-hydroxy group in the p-coumaroyl substituent or its partial saponification in the alkaline medium. Moreover, the ease with which quinqueloside undergoes enzymatic hydrolysis indicates that the carbohydrate component is not acylated, while a 5-hydroxy group is shown by the qualitative reactions and by the results of spectroscopic analysis.

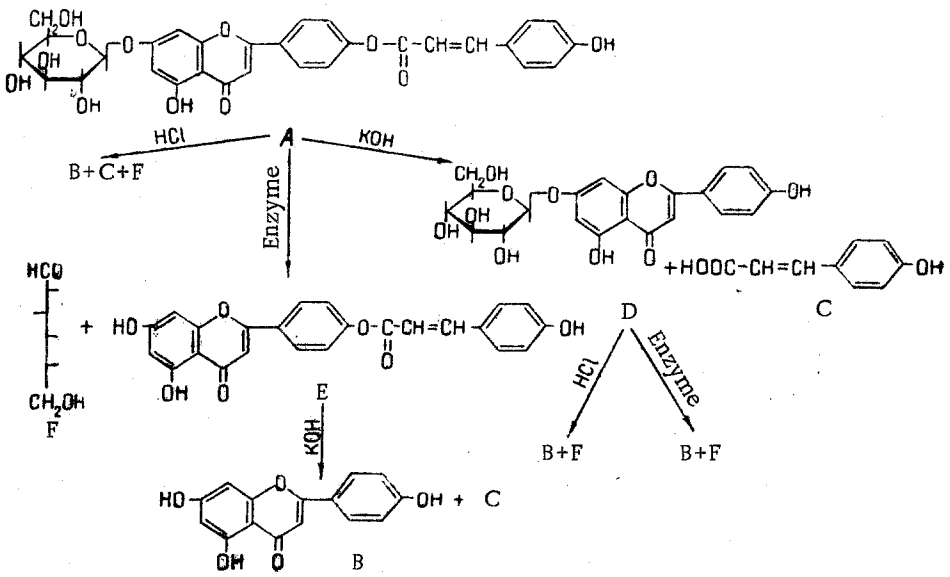
Table 2

Spectroscopic Characteristics of Quinqueloside and its Derivatives

Medium	Bands	Quinqueloside		Terniflorin		Cosmosiin		Apigenin	
		Absorption maxima, mμ							
		λ	Δλ	λ	Δλ	λ	Δλ	λ	Δλ
2×10^{-5} M solution in anhydrous ethanol	I	320	—	321	—	341	—	341	—
	II	270	—	270	—	269	—	270	—
The same + sodium acetate	I	320	0	—	—	341	0	380	39
	II	270	0	—	—	270	1	277	7
The same + sodium ethoxide	I	380	60	398	77	390	49	400	59
	II	270	0	270	0	265	—4	277	7
The same + aluminum chloride	I	380	60	380	59	385	44	382	41
	II	280	10	281	11	278	9	278	8

A comparison of the molecular rotations of quinqueloside and phenyl glycoside [6] has shown that the d-glucose is attached by a β-glycosidic bond and is present in the glycoside in the pyranose form. This is confirmed by enzymatic hydrolysis and the identification of the intermediate D with cosmosiin.

Thus, quinqueloside can be characterized as 4'-O-p-coumaroylapigenin-7-β-D-glucopyranoside, and its reactions can be represented by the following scheme:

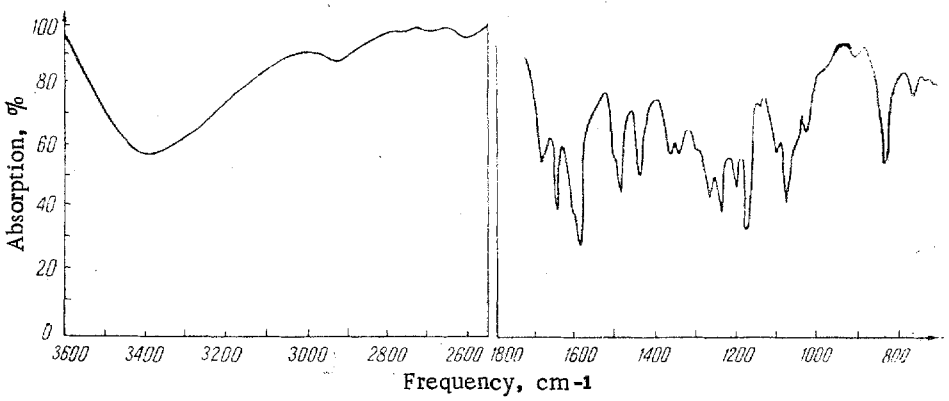


Experimental

Isolation of quinqueloside. 5 kg of the air-dried comminuted herb L. quinquelobatus collected in the flowering phase in the Dnepopetrovsk region was extracted three times (40 *l* each) with 70% ethanol. The alcoholic extracts were evaporated under vacuum until the alcohol had been completely eliminated. The aqueous solution was purified with

chloroform and the flavonoids were extracted with ethyl acetate. The ethyl acetate extracts were evaporated to dryness and the residue was dissolved in a small amount of 96% ethanol. On standing, the alcoholic solution deposited a yellow crystalline precipitate in which one substance of the flavonoid type was detected by chromatography. For further purification, the precipitate was dissolved in 96% ethanol and was passed through a column of polyamide sorbent activated with acid [7]. Elution was carried out with 96% ethanol and an eluate fraction having a bluish-green fluorescence in UV was isolated. The eluates were evaporated to small bulk and allowed to stand for crystallization. Small needle-like crystals formed. These were filtered off, recrystallized from 96% ethyl alcohol, and dried in a vacuum pistol. This gave a substance with mp 265-267°C sparingly soluble in ethyl and methyl alsochols, readily soluble in ethyl acetate, and insoluble in water and chloroform; with a solution of ferric chloride it gave a dark green coloration, with alkali a bright yellow coloration, and in the cyanidin reaction an oragne-red coloration.

Acid hydrolysis of quinqueloside. A solution of 0.01 g of the substance obtained in 2 ml of 50% methanol containing 5% of sulfuric acid was heated in a boiling water bath for 2 hr. The aglycone was filtered off and recrystallized from 96% ethanol. This gave pale yellow plate-like crystals with mp 345-348°C. The substance was chromatographically homogeneous and was identified by qualitative reactions, spectroscopic data, and a mixed-melting-point test as apigenin.



IR spectrum of quinqueloside (as tablet with potassium bromide).

The aqueous alcoholic part of the hydrolyzate was neutralized with KU-2 ion-exchange resin in the OH^- form and was evaporated to dryness. The residue was dissolved in a few drops of water and the sugars were analyzed in various solvent systems together with reference samples. The sugar component of quinqueloside was characterized as d-glucose.

The chromatographic analysis of the acid hydrolyzate showed the presence not only of the aglycone but also of a substance C, giving a purple spot on chromatograms when detected with alkali and a red spot when detected with diazotized sulfanilic acid and alkali. These results, and also those of the spectroscopic analysis in the IR region (see Figure) show the possible presence of an aromatic acid substituent in the molecule. To analyze the acid, reference samples of aromatic acids such as p-hydroxybenzoic acid, ferulic acid, cinnamic acid, p-coumaric acid, etc., were used, and, as shown in Table 3, the acid concerned was identified as p-coumaric acid.

Alkaline hydrolysis of quinqueloside. A solution of 0.01 g of quinqueloside in 2 ml of 1% aqueous KOH was heated on a boiling water bath for 20 minutes. The solution was acidified with hydrochloric acid to pH 4 and the saponification products were analyzed by paper chromatography (cf. Table 3).

Enzymatic hydrolysis of quinqueloside. 0.01 g of quinqueloside was dissolved with heating in 10 ml of water and the solution, cooled to 30°C, was treated with 10 ml of a solution of an enzyme preparation from the fungus Aspergillus oryzae (0.01 g) [8] and left overnight. After hydrolysis, the enzyme was precipitated by boiling, the precipitate was filtered off, and the filtrate was evaporated to 1-2 ml and was then treated with 96% ethanol (1 ml), heated, and analyzed chromatographically (see Table 3). A new substance E giving a blue-green coloration in UV light was found.

Enzymatic hydrolysis of substance D. In the alkaline saponification of quinqueloside, two new substances C and D were formed. Substance C was identified as p-coumaric acid and substance D is probably a glycoside of apigenin similar to cosmosiin [5]. To examine the properties of this substance, we isolated it from the products of alkaline hydrolysis by preparative paper chromatography. Substance D forms yellow needle-like crystals with mp 220-222°C (from alcohol). On enzymatic hydrolysis with a preparation from the fungus Aspergillus oryzae carried out as described for quinqueloside, substance D gave apigenin and d-glucose.

Alkaline hydrolysis of substance E. Assuming that the acid substituent was connected directly to the aglycone, we carried out the enzymatic decomposition of quinqueloside and isolated substance E by preparative paper chromatography. Substance E was saponified with 1% aqueous KOH as described for quinqueloside and the saponification products were

analyzed by paper chromatography (see Table 3). It was found that substance E decomposes into apigenin (substance B) and p-coumaric acid (substance C).

Table 3

Chromatographic Investigation of Quinqueloside and its Reaction Products

Products	Conditions of formation	R_f in system*			Spot color	
		1	2	3	c KOH **	With diazotized sulfanilic acid
Glycoside investigated	A	0.08	0.47	0.44	Light blue	
From acid hydrolysis	B	0.05	0.45	0.92	Yellow	
	C	0.58	0.79	0.80	Purple	
Apigenin	B	0.05	0.45	0.92	Yellow	
p-Coumaric acid	C	0.58	0.79	0.80	Purple	
From alkaline hydrolysis	C	0.58	0.79	0.80	"	
	D	0.26	0.64	0.10	Yellow	
Cosmosiin	D	0.26	0.64	0.10	"	
From the enzymatic hydrolysis of substance D with a fungus preparation	B	0.05	0.45	0.92	"	Red
From the enzymatic hydrolysis of substance A with a fungus preparation	E	0.12	0.49	0.23	Bluish-green	
From the alkaline hydrolysis of substance E	B	0.05	0.45	0.92	Yellow	
	C	0.58	0.79	0.80	Purple	
From the acid hydrolysis of substance D	C	0.05	0.45	0.92	Yellow	
From the hydrolysis with snail enzymes	D	0.26	0.64	0.10	"	
	C	0.58	0.79	0.80	Purple	

*Systems as in Table 1.

**In UV light.

Enzymatic hydrolysis of quinqueloside by a snail enzyme. 0.01 g of quinqueloside was dissolved in 10 ml of hot water and, after cooling to 30°C, the solution was mixed with a solution of the enzyme from the snail Helix plectotropis [9]. Hydrolysis was carried out at 30°C, being monitored by chromatography. In the first 5 hr p-coumaric acid was split off and then hydrolysis proceeded to completion with the liberation of apigenin and d-glucose.

The UV spectrum of quinqueloside was taken by I. P. Kovalev (KhNIKhFI [Kharkov Chemical and Pharmaceutical Scientific Research Institute]) on a UR-10 spectrometer.

V. I. Litvinenko (KhNIKhFI) acted as consultant for the investigation.

Summary

A new flavonoid called quinqueloside has been isolated from the herb Leonuris quinquelobatus Gilib. It is 4'-O-p-coumaroylapigenin-7-β-d-glucopyranoside.

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