NEW FLAVONOID C-GLYCOSIDES OF GRATIOLA OFFICINALIS

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Continuing a study of the flavonoid compounds of Gratiola officinalis L. (drug hedgehyssop), we have found that this plant contains C-glycosides, as well as O-glycosides. We have obtained four individual compounds of flavonoid nature with the composition $C_{21}H_{20}O_{10}$ which we have named provisionally [from the Russian for hedgehyssop—avran] avroside (I) with R_f 0.30 (1) and 0.12 (2), isoavroside (II) with R_f 0.45 (1) and 0.20 (2), neoavroside (III) with R_f 0.14 (1) and 0.06 (2), and isoneoavroside (IV) with R_f 0.55 (1) and 0.16 (2).

Spectral studies in the UV region (Table 1) of all these compounds showed free 5-, 7-, and 4'-hydroxy groups. The approximately equal maxima of the first and second bands and also the distance separating them, between 50 and 60 mμ, permit these substances to be characterized as flavone derivatives [1].

Table 1. Spectral Characteristics of the Flavonoid C-glycosides from Drug Hedgehyssop

Substance	Absorption bands	Ethanolic solution		+ NaAc ethanol		+ EtONa ethanol		+ $Zr(NO_3)_2$ ethanol		$E^{1\%}_{1\,cm}$
		λ	lgε	λ	Δλ	λ	Δλ	λ	Δλ	
Avroside (I)	I	330	4.21	390	60	398	68	385 360	55	564
	II	272	4.19	282	10	288	16	312 290	40	—
Isoavroside (II)	I	330	4.21	384	54	396	66	390 350	60	565
	II	272	4.19	278	6	286	14	310 290	38	—
Neoavroside (III)	I	332	4.20	390	58	398	66	390 360	58	564
	II	270	4.18	282	12	286	16	310 290	40	—
Isoneoavroside (IV)	I	332	4.20	390	58	398	66	390 350	58	565
	II	272	4.18	282	10	288	16	310 290	38	—
Apigenin (V)	I	336	4.40	380	44	400	64	390 355	54	900
	II	270	4.38	274	4	275	5	305 285	35	—

Acid and enzymatic hydrolyses did not lead to the liberation of additional hydroxy groups. On acid hydrolysis by Kiliani's method [2] each of the glycosides gave apigenin and D-glucose together with some D-arabinose. Consequently it may be assumed that the substances considered are C-glycosides of apigenin.

Their C-glycosidic nature is confirmed by acid isomerization, as a result of which avroside is converted into isoavroside and neoavroside into isoneoavroside, and conversely, forming equilibrium mixtures of pairs of these glycosides. Rotational isomerism with the formation of only two isomers is characteristic of C-monoglycosides [1,3].

The monoglycosidic nature of the compounds is also shown by the magnitude of the specific absorption at 335 mμ in comparison with the aglycone (see Table 1). Consequently all four glycosides isolated are C-monoglucosides of apigenin, and the pairs avroside–isoavroside and neoavroside–isoneoavroside are rotation isomers, as is clearly illustrated by their chromatographic behavior. Chromatographic differences of isomers of C-glycosides have been reported by other authors [4] although such differences have been explained as 6–8-isomerization.

Until now, only two C-monoglycosides of apigenin were known, vitexin and saponaretin (isovitexin) [5]. The

results of a study of the structure of C-diglycosides containing sugars in positions 6 and 8 [3] enabled us to assume that plants must also contain 6-C-monoglycosides with properties similar to those of 8-C-monoglycosides, although they are not converted into one another under acid isomerization. The results of Bhatia and Seshadri's recent investigations [6] confirm this.

To prove the structure of the compounds isolated, we attempted to use the bathochromic shifts of the long-wave band in the UV spectra of the zirconyl complexes, taking into account the fact that in flavonoids with a substituent in position 6 the shift of the complex must be smaller or completely absent because of the steric influence of the substituent in position 6 [11].

On studying compounds with a substituent in position 6, it was found that it is impossible to detect a substituent in position 6 of apigenin and its derivatives by this method, since all these shifts are approximately equal, at 50–55 mμ, while in scutellarein derivatives they fall to 30–40 mμ (Table 2). However, in an analysis of the spectra of the zirconyl complexes of apigenin derivatives (see Table 2) an additional maximum in the 360 mμ region (B) and different intensities of the main (A) and subsidiary (B) maxima are observed.

Table 2. Spectral Characteristics of Zirconyl Complexes

Substance	λ_{max} in ethanol	λ_{max} $Zr(NO_3)_2$ + ethanol		Δλ	A/B · 100%
		A*	B**		
Apigenin	336	390	355	54	103.1
Cosmosiin	331	385	360	54	90.0
Apiin	337	394	355	57	98.6
Vitexin	335	390	352	55	88.0
Saponaretin	335	390	355	52	86.5
Avroside	330	385	360	55	52.0
Isoavroside	330	390	350	60	46.7
Neoavroside	332	390	360	58	44.8
Isoneoavroside	332	390	350	58	46.7

*Intensity of the main maximum.
**Intensity of the subsidiary maximum.

The ratio of the intensities A/B for apigenin and its O-glycosides, and also for vitexin and saponaretin is between 80 and 100%, while for the C-glycosides studied it is between 40 and 50%. The results obtained permit the assumption that in the C-monoglycosides of drug hedgehyssop the carbohydrate substituent is present in position C_6 (see Table 2).

If it is assumed that the compounds studied are 6-C-monoglycosides of apigenin, then by analogy with the 8-C-monoglycosides (saponaretin and vitexin) one pair of them must have the β-configuration of the bond. A comparison of the rates of isomerization and of the ratios of the isomers formed shows that avroside and isoavroside may form such a pair. Neoavroside and isoneoavroside isomerize under more sever conditions, which is apparently explained by a different position of the carbohydrate substituent. We assume that this pair is nothing other than rotation isomers of the 6-C-α-D-glycosides.

On comparing the differential spectra of saponaretin and isoneoavroside, it was found that the sugar in both C-glycosides has the pyranose form, but the first of them has the β- and the second the α-configuration of the glycosidic bond. To confirm these results, we obtained their acetyl derivatives and compared the molecular rotations of the initial glycosides and also of their acetates with phenyl C-glycosides and their acetates [1] (Table 3). It was established that isoneoavroside acetate possesses a high positive rotation, like the acetate of phenyl C-α-D-glucopyranoside.

Thus, four new C-monoglycosides have been isolated from drug hedgehyssop and it has been established that they consist of the syn- and anti- isomers of the corresponding 6-C-β- and 6-C-α-antipodes.

EXPERIMENTAL

Paper chromatography was carried out with the following systems of solvents: 1) 15% solution of acetic acid, 2) butan-1-ol–acetic acid–water (4 : 1 : 2) with chromatographic paper of type "Filtrak" F No. 11.

The UV spectra were recorded on an SF-4A spectrophotometer and the IR spectra on a UR-10 spectrophotometer; the optical activities were measured on a SPU-E spectropolarimeter. The melting points were determined on a Kofler block.

Table 3. Comparison of the Specific Rotations of the Flavonoid C-Glycoside of Drug Hedgehyssop

Substance	Position of the C-carbohydrate substituent	$[\alpha]_D^{20}$ of the glycoside, deg.	$[M]_D$	K_{ph}	$D \cdot K_{ph}$	$[\alpha]_D^{20}$ of the acetate, deg.	Configuration of the bond	Size of the rings
Isoneoavroside	6	+40.3	+172.8	0.54	+ 93.3	+163.4	α	Pyranose
Isovitexin	8	+26.7	+115.3	0.54	+ 62.8	− 32.0	β	
Phenyl C-glycoside	—	+23.2	+ 56.0	1.00	+ 56.0	− 16.0	β	
Phenyl C-glycoside	—	+90.0	+217.0	1.00	+217.0	+ 95.3	α	

Isolation of the glycosides I–IV. One kilogram of the air-dry comminuted raw material of Gratiola officinalis L. was extracted with 80% methanol (4 × 10 *l*). The methanolic extract was evaporated in vacuum until the organic solvent had been completely eliminated, and the aqueous extract was cooled to +4° C. The chlorophyll that deposited was filtered off, and the extract was purified with ether and then with chloroform. The aqueous extract was evaporated to 0.2 *l*, and 4 *l* of a mixture of ethanol and ether (1 : 1) was added to precipitate the saponins. The precipitate was filtered off, washed with the same mixture, and discarded. The filtrate was concentrated in vacuum to 0.2 *l* and was passed through a column (40 × 800 mm) filled with Kapron. The separation of the flavonoids was monitored by paper chromatography and the movement of the zones was observed in filtered UV light. The first portion of the aqueous eluate (0.6 *l*), containing no flavonoids, was discarded. The subsequent eluates yielded two fractions containing flavonoids I–III and II–IV. The second fraction consisted of the bulk of the flavonoids, and its rechromatography on polyamide sorbent gave the individual compounds II and IV in the form of pale yellow crystals. After recrystallization from aqueous methanol the latter had mp 244–246° C, $[\alpha]_D^{20}$ +40.3° (c 0.139; DMFA).

Found, %: C 58.27, 58.38; H 4.61, 4.65. Calculated for $C_{21}H_{20}O_{10}$, %: C 58.33; H 4.64.

Substances I and III were separated by preparative chromatography on cellulose in 15% acetic acid.

Enzymatic and acid hydrolysis. Exactly 0.01 g of each glycoside was dissolved in 1 ml of 50% ethanol and the solution was diluted with water to 10 ml; then to this solution was added 0.05 g of emulsin in 4 ml of water and the mixture was placed in a thermostat at 36° C for 48 hr. After this time, paper chromatography showed that the glycosides under study had remained unchanged.

The same amounts of glycosides were subjected to acid hydrolysis: avroside and isoavroside in 5% HCl in 50% methanol for 2 hr, and neoavroside and isoneoavroside in 10% HCl in 50% methanol for 4 hr. It was found that equilibrium systems of isomeric products, the pairs avroside–isoavroside (1 : 2) and neoavroside–isoneoavroside (1 : 10) were formed.

The isomeric products were separated by preparative chromatography in 15% acetic acid on cellulose, and each of them was hydrolyzed under the appropriate conditions. The same pairs of isomeric products in the same ratios were found.

Acid hydrolysis by Kiliani's method. In this experiment, 0.1 g of each of the glycosides studied was suspended in 4 ml of a mixture consisting of ten parts of conc HCl, 3.5 parts of glacial acetic acid and 5.5 parts of water. Hydrolysis was carried out in the boiling water bath for 20 hr. The mixture was diluted with water to 20 ml and the aglycones were separated on polyamide sorbent. All the glycosides gave one and the same aglycone with mp 346–348° C (from ethanol), shown to be identical by a mixed melting point and by its R_f values of 0.04 (1) and 0.92 (2) with apigenin.

The aqueous part of the hydrolysate was neutralized with AV-17 ion-exchange resin (OH^- form), evaporated to 0.5 ml, and chromatographed on paper, showing the presence of D-glucose together with some D-arabinose.

Acetylation of the glycosides. This was carried out with acetic anhydride in pyridine in the presence of H_2SO_4 at room temperature (0.5 hr). Colorless acicular crystals with mp 156–157° C (from alcohol), $[\alpha]_D^{20}$ +121.2° (c 0.1; DMFA) were obtained, and with ferric chloride these gave a lilac-violet coloration showing the presence of a free hydroxyl group in the C_5 position [8].

Found, %: C 57.82, 57.98; H 4.59, 4.63. Calculated for $C_{33}H_{32}O_{16}$, %: C 57.89; H 4.68.

The partial acetate obtained (0.1 g) was treated as described above for another 0.5 hr. Colorless acicular crystals precipitated with mp 159–160° C, $[\alpha]_D^{20}$ +163.4° (c 0.104; DMFA). The reaction with ferric chloride was negative [8].

Found, %: C 57.76, 57.80; H 4.58, 4.65. Calculated for $C_{35}H_{34}O_{17}$, %: C 57.84; H 4.68.

CONCLUSIONS

1. C-Glycosides have been isolated from a plant of the family Scrophulariaceae for the first time.

2. Four new C-monoglycosides of apigenin have been isolated from the herb Gratiola officinalis L.

3. On the basis of a chemical study it has been established that they are rotation isomers and they have been characterized as a syn-C-β-D-glucopyranoside (avroside), an anti-C-β-D-glucopyranoside (isoavroside), a syn-C-α-D-glucopyranoside (neoavroside), and an anti-C-α-D-glucopyranoside (isoneoavroside).

4. The presence of 6-C-monoglycosides in plants and their difference from 8-C-monoglycosides have been shown for the first time.

REFERENCES

1. N. P. Maksyutina and V. I. Litvinenko, in: Phenolic Compounds and their Biological Functions [in Russian], Moscow, 7, 1968.
2. V. I. Litvinenko, K. Amanmuradov, and N. K. Abubakirov, KhPS [Chemistry of Natural Compounds], 3, 159, 1967.
3. V. I. Litvinenko and V. M. Darmograi, DAN UkrSSR, no. 7, series B, 639, 1968.
4. M. Komatsu, T. Tomimori, Y. Makiguchi, and K. Asano, Yaugau Zasshi, 88, 832, 1968.
5. J. B. Harborne, Comparative Biochemistry of the Flavonoids, Academic Press, London-New York, 50, 1967.
6. V. Bhatia and T. R. Seshadri, Constitution of Orientien, epi-Orientin and their Methyl Ether, Phytochemistry, 6, 7, 1033, 1967.
7. G. E. Inglett, R. R. Miller, and J. P. Lodge, Microchemica Acta, 1, 95, 1959.
8. K. Venkataraman, Forscher Chem. org. Naturstoffe, vol. 17, p. 1, 1959.

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