QUERCETIN DIGLUCOSIDE FROM THE YELLOW SCABIOSA

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We have found four compounds of flavonoid character in the above-ground portion of pale yellow scabiosa (Scabiosa ochroleuca L. fam. Dipsacaceae). Three of them were identified by us earlier as luteolin-7-β-D-glucopyranoside, quercimeritrin (quercetin-7-β-D-glucopyranoside), and diosmin (diosmetin-7-rutinoside). The fourth glucoside was found to be a new diglucoside of quercetin, not previously described in the literature.

Spectral investigation of this material in UV light showed two absorption maxima characteristic for a quercetin glucoside (see Table 1). A significant bathochromic shift ($\Delta\lambda \pm 45$ mμ) of one band is observed upon the addition of aluminum chloride, and upon addition of sodium acetate to the formed complex, the absorption maximum of the first band of the initial material reappears (presence of a free hydroxyl group at carbon atom 5) [1]. The glucoside has free hydroxyl groups in the 3^1 and 4^1 positions (bathochromism in the presence of boric acid and sodium acetate) and does not have them at carbon atoms 7 (absence of bathochromism with sodium acetate) and 3 (Hörhammer test is negative [2, 3]).

Upon acid hydrolysis with a 5% solution of sulfuric acid, the glucoside is cleaved to an aglucon identical to quercetin, L-arabinose, and D-glucose. The presence of free hydroxyl groups at carbon atoms 7 and 3 (positive Hörhammer test and bathochromism in the UV spectrum in the presence of sodium acetate, see Table 1) in the aglucon indicates that the initial glucoside contains sugar residues in these positions.

Quantitative acid hydrolysis gave a 47.63% yield of aglucon, which is characteristic for diglucosides.

Upon gradual hydrolysis with a 15% solution of acetic acid, after 3 min a material (monoside A) appeared in the hydrolyzate which we identified as quercetin-7-β-D-glucopyranoside (quercimeritrin), in addition to L-arabinose. The UV spectral characteristics of this glucoside are given in Table 1.

Two materials, monoside A and monoside B, are formed upon acid hydrolysis with formic acid in cyclohexane. Monoside B is quercetin-3-β-L-arabofuranoside and differs from avikularin, isolated from Daursk rhododendron [4] by the presence of a β-glucoside bond. We have called it β-avikularin.

The diglucoside is cleaved by the enzyme Aspergillus niger with the formation of an aglucone and free sugars, which makes it possible to propose the presence of β-glucoside bonds [5].

Based on these data, we propose that the isolated compound is quercetin-7-β-D-glucopyranoside-3-O-β-L-arabofuranoside and have named it ochroside.

EXPERIMENTAL

Isolation of Ochroside. We processed 0.15 kg of air-dried, ground blooms of pale yellow scabiosa, gathered in the Kislovodsk region during the period of total bloom (July), with methanol in a Soxhlet apparatus. The methanol extracts were concentrated in vacuum. The formed precipitate was separated and a threefold amount of ether was added to the filtrate. The solid which precipitated was treated with boiling methanol three times, and the combined methanolic extracts were concentrated in vacuum and placed in the refrigerator.

After two days a yellow-colored solid precipitated. Crystals having mp 214-216°C were isolated after three recrystallizations from methanol. Yield 0.46 g.

The same compound can be obtained in higher yield by chromatography of the methanolic extract on a polyamide sorbent activated by a 0.5% solution of potassium hydroxide. Ochroside is easily eluted with

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TABLE 1. UV Spectral Characteristics of Ochroside and Its Cleavage Products

Compound	Absorption band	$2 \cdot 10^{-5}$ M solution in methanol λ_{max} (mμ)	$2 \cdot 10^{-5}$ M solution in methanol, sodium acetate λ_{max} (mμ)	sodium acetate Δλ (mμ)	H_3BO_3 + sodium acetate λ_{max} (mμ)	H_3BO_3 + sodium acetate Δλ (mμ)	$AlCl_3$ λ_{max} (mμ)	$AlCl_3$ Δλ (mμ)	$AlCl_3$ + sodium acetate λ_{max} (mμ)	$AlCl_3$ + sodium acetate Δλ (mμ)	Sodium ethoxide λ_{max} (mμ)	Sodium ethoxide Δλ (mμ)
Ochroside	1	360	360	0	380	+20	405	+45	365	+5	410	+50
	2	255	255	0	262	+7	264	+9	259	+4	275	+20
Monoside A	1	372	371	−1	396	+24	430	+58	—	—	370	−2
	2	257	258	+1	260	+3	262	+5	—	—	291	+34
Quercimeritrin	1	372	370	−2	392	+20	427	+55	—	—	371	−1
	2	256	257	+1	260	+4	260	+4	—	—	290	+34
Aglucon	1	370	380	+10	390	+20	430	+60	—	—	320	+50
	2	256	255	−1	260	+4	270	+14	—	—	276	+10
Quercetin	1	370	380	+10	390	+20	430	+60	—	—	320	+50
	2	270	270	0	272	+2	270	0	—	—	266	−4

Note. Spectra of quercimeritrin and quercetin isolated from the same plant are presented for comparison.

water and is very soluble in methanol, hot ethanol, and pyridine, and insoluble in chloroform, acetone, and petroleum ether. Found, %: C 42.69, 42.60; H 5.78, 5.72. $C_{26}H_{28}O_{16}$. Calculated, %: C 42.78; H 5.68. R_f 0.21 in a system of n-butanol–acetic acid–water(4:1:5), $[\alpha]^{20}_D$–250° (c 0.04, pyridine), $[M]^{20}_D$-1490°.

Acid Hydrolysis. We dissolved 0.05 g of the obtained material in 5 ml of a 5% solution of sulfuric acid. The mixture was boiled on an air bath for 4 h. The aglucon was filtered and recrystallized from 98% ethanol. We obtained needle-shaped crystals having mp 309-310°. It is identical with quercetin in R_f values in different systems in the presence of a "reference spot" and in elemental composition. A mixed sample with a sample of quercetin did not give a melting point depression. The UV spectral characteristic is given in Table 1. The following absorption bands are found in the IR spectrum: 1658 cm^{-1} (= C = O), 3340 cm^{-1} (-OH). After separation of the aglucon, the hydrolyzate was neutralized with barium carbonate. Paper chromatography detected D-glucose and L-arabinose (developed by aniline-phthalate and benzidine reagent). Quantitative acid hydrolysis was carried out under analogous conditions, collecting the aglucon in a weighed No. 4 glass filter.

Gradual Hydrolysis with a 15% Solution of Acetic Acid. We dissolved 0.06 g of glucoside in 10 ml of methanol and added 10 ml of a 30% solution of acetic acid. The mixture was heated on a boiling water bath for 2 h, following the hydrolysis process every 3 min in the first hour, and after every 7 min in the second hour by paper chromatography in a system of n-butanol–actic acid–water (4:1:5). A material (monoside A) having R_f 0.34 and L-arabinose was detected in the hydrolyzate after the third minute. The presence of quercetin and D-glucose in the hydrolyzate was established after 80 min.

Partial Hydrolysis with Formic Acid (6). We dissolved 0.06 g of ochroside in 5 ml of boiling cyclohexane, added 2 ml of 90% formic acid, and boiled the mixture for 10 h. After cooling, the hydrolyzate was neutralized with a 1% methanolic solution of potassium hydroxide. The hydrolysis products were separated by preparative paper chromatography in a system of n-butanol–acetic acid–water(4:1:5). Elution with methanol separated monoside A and monoside B.

Enzymatic Hydrolysis. We suspended 0.01-0.02 g of ochroside (or monoside A or monoside B, respectively) in 10 ml of hot water, cooled the mixture to 35°, and added an enzymatic preparation from Aspergillus niger (1 g of enzyme in 10 ml of water).

The obtained mixture was held at 37° for 24 h, then the enzyme was precipitated by boiling. The precipitate was separated and the filtrate was analyzed by paper chromatography. Quercetin, D-glucoside, and L-arabinose were detected.

Identification of Monoside A. The material was isolated by preparative paper chromatography after partial hydrolysis of ochroside. It consisted of yellow-green crystals having mp 245-248° (from acetone). Found, %: C 54.01, 54.18; H 4.28, 4.26. $C_{21}H_{20}O_{12}$. Calculated, %: C 54.31; H 4.31. After its hydrolysis with a 5% sulfuric acid solution for 4 h, quercetin and D-glucose were identified in the hydrolyzate. From UV spectroscopic data (see Table 1), the material is a 7-glucoside of quercetin and does not give a melting point depression with an authentic sample of quercimeritrin. $[\alpha]_D^{20}-62.5°$ (c 0.2, methanol), $[M]_D^{20}-287.6°$ Upon enzymatic hydrolysis as described above, quercetin and D-glucose were detected. The following absorption bands are found in the IR spectrum: 3420, 3380 (−OH), 1660 (= C = O), 1615 (=C = C =) and 820, 800, 735, 710 cm^{-1} (presence of substituents in the side phenyl group) [7].

Indentification of Monoside B. The material was isolated by preparative paper chromatography after hydrolysis of ochroside with formic acid. It consisted of yellow crystals which are sparingly soluble in water, and nicely soluble in methanol, ethanol, ethyl acetate, and basic solutions, and insoluble in chloroform, benzene, and petroleum ether. After acid hydrolysis, 1 mole of quercetin and 1 mole of L-arabinose (68.8% of aglucon, based on the weight of the glucoside) are formed. The rapidly occurring hydrolysis (3 min) indicates the presence of the furanose form of the sugar [8]. $[\alpha]_D^{20}-140°$ (c 0.05, pyridine), $[M]_D^{20}-439°$.

CONCLUSIONS

A new quercetin diglucoside was found in the above-ground portion of pale yellow scabiosa (Scabiosa ochroleuca L. Fam. Dipsacacea), which is quercetin-7-β-D-glucopyranosido-3-β-L-arbofuranoside, and was named ochroside.

LITERATURE CITED

1. L. Jurd and T. A. Geissmann, J. Org. Chem., 21, 1395 (1956).
2. L. Jurd, The Chemistry of Flavonoid Compounds, T. A. Geismann, New York (1962), p. 107.
3. L. Hörhammer and R. Hänsel, Arch. Pharm. (Weinheim), 286, 425 (1953).
4. É. T. Oganesyan, V. A. Bandyukova, and A. L. Shinkarenko, Khim. Prir. Soedin., No. 4, 279 (1967).
5. B. J. Harborne, Phytochemistry, 4, 107 (1965).
6. D. W. Fox, W. L. Sawage, and S. J. Wender, J. Amer. Chem. Soc., 75, 2504 (1953).
7. H. B. Wagner, Prigham Methods in Polyphenol Chemistry, New York (1964), p. 37.
8. A. B. Foster and W. G. Owerend, Chem. Ind. (London), 566 (1955).