Structure-reactivity relationships of antioxidant flavonoids

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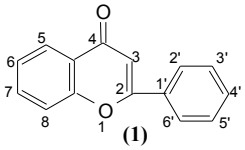
Abstract Extraction and hydrolysis conditions for flavonoid chromogene from *Hypericum Perforatum* have been presented. Structural determinations have been made by chromatographic and spectrometric methods and by reduction to flavylium salts. For antioxidant property estimation DPPH test has been applied. Antioxidant properties of hydroxy - flavones can be used in therapy of diseases generated or increased by radical mechanism oxidation and accumulation of radical O_2^- .

Keywords: flavonoid chromogene, hydroxy – flavones, antioxidant properties

1. Introduction

A high interest for pharmaceutical, medical and cosmetical applications or for food diet is extraction, stabilisation and structural characterisation of antioxidant vegetable dyes [1-5]. Some diseases can be induced by oxidation if biochemical control systems are overreached through radical superoxid $O_2\cdot^-$ accumulation. Lipid peroxides from biological membranes take part in chain radical reactions, with products involved in: ischemic cardiopathy, traumatic stroke, cataracts, retinopathies, silicosis, hepatotoxicity, cirrhosis, muscular dystrophies, atherosclerosis, malignant tumours etc. Superoxid dismutase (scavenger for $O_2\cdot^-$) allows antioxidant protection and inhibits lipid peroxidation. Aminoacids with thiol group, tocopherols, ascorbic acid, flavonoids, anthocyanins, carotenoids and some synthesis products also have antioxidant effects. Some flavonoids are anti-inflammatory, antiallergenic, antiviral, antibacterial, antitumorale or antioxidant activities [6-8].

Flavonoids are vegetable chromogenes, such as: flavone (2-phenil-benzo-γ-pyrone) (**1**), isoflavone (3-phenyl-benzo-γ-pyrone) (**2**), and flavonol (2-phenyl-3,5,7-oxy-benzo-γ-pyrone) (**3**), with hydroxyl groups, many of them being as glycosides.



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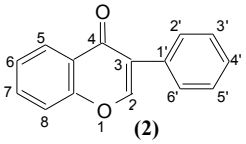

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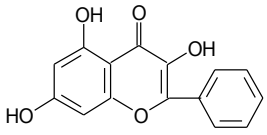

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2. Experimental

In order to characterise flavonoidic chromogene from *Hypericum Perforatum* has been applied following operations: extraction, purification of extract by column chromatography, hydrolysis for aglycone separation [8], identification of aglycone by thin layer chromatography and colour reactions. Reduction to flavylium salts, modifications depending of pH, and spectrometric methods confirm flavonoid structure. To identify chromatographic spots have been used: $AgNO_3$, $FeCl_3$, $AlCl_3$, $(CH_3COO)_2Pb$, $ZrOCl_2 \bullet 8H_2O$, H_3BO_3, Benedict Reagent etc. UV-vis absorption spectra have been registered in methanol solution, in quartz cuve, with a Specord UV-vis Carl-Zeiss Jena Spectrometer.

The following reagents: CH_3ONa, $AlCl_3$, $AlCl_3$-HCl, CH_3COONa and H_3BO_3 have been used in spectrometric characterisation of flavonoids.

Stable radical 2,2-diphenil-1-pycryl-hydrazil hydrate (DPPH) was used to estimation scavenging activity of flavonoide derivatives.

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Table 1

Spot	R_f				Identification				Structure
	BuOH:AcOH: H_2O 4:1:5	AcOH: H_2O 3:17	AcOH: H_2O 6:5	AcOEt with water	Colour	Fluorescence in UV	pH=8-9 vis	pH=8-9 UV	3-O-glycoside-flavonol
A	0,62	0,46	0,73	0,30	yellow	brown	yellow	yellow orange	

Table 2.

Reagent	Colour	Structure
$AgNO_3$+ NH_4OH	brown-black	glycoside and o-hydroxyl groups
$FeCl_3$	green	C_5-OH
$AlCl_3$	yellow, orange fluorescence	C_4-carbonyl, C_5-OH, orto-hydroxyl groups
Benedict Reagent	without UV-fluorescence	orto-hydroxyl groups
$(CH_3COO)_2Pb$	orange	C_5-OH in peri with C_4-carbonyl
$ZrOCl_2 \cdot 8H_2O$	yellow	C_5-OH and C_3-O- substituted

Table 3. UV-Vis absorption characteristics of flavonol-glycoside extract

Solvent and Reagents	λ, nm					
	Band II			Band I		
CH_3OH	255		265 sh	296		355
CH_3ONa	272			320		410
$AlCl_3$	273		300 sh	335 sh		427
$AlCl_3$ + HCl	265		297 sh	357		396
CH_3COONa		265			396	
CH_3COONa + H_3BO_3		258			366	

Table 4. UV-Vis absorption characteristics for flavonol aglycone

Solvent and Reagents	λ, nm					
	Band II			Band I		
CH_3OH	250		270 sh	310		365
CH_3ONa	280			325		420
$AlCl_3$	285		305 sh	340 sh		440
$AlCl_3$ + HCl	275		300 sh	360		410
CH_3COONa		278			400	
CH_3COONa + H_3BO_3		265			380	

3. Results and Discussions

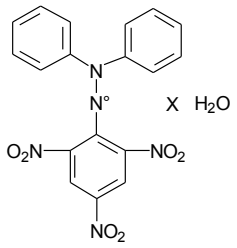
The separated flavonic chromogene has been identified by thin layer chromatography, UV-vis and IR spectrometry and chemical reactions.

In Table 1 the values from thin layer chromatography on silicagel for flavonic chromogenes and in Table 2 identification reactions are presented. Colour tests and R_f values present a 5-hydroxi-3-O-glycosid-flavonol structure with OH groups in A and B rings.

IR spectra, registered in KBr pellets, have following vibrations: 1670 cm^{-1}- $\nu_{C=O}$ from substitute chromone; 1230 cm^{-1} - ν_{C-OH} phenol; 3300 cm^{-1}, 2900 cm^{-1} - ν_{O-H} – intermolecular, respectively intramolecular hydrogen bonds; 1070 cm^{-1} - ν_{C-O-C} cyclic ether.

Characteristics of UV-VIS absorption spectra, registered in methanol solution of flavonol-glycoside extract and aglycone are presented in Tables 3 and 4. The structure has been confirmed by aglycone (**4**) reduction, with Zn and HCl, to flavylium chloride (**7**). In pH field 1-5 hydration, tautomerism and acid-basic equilibria are possible, between flavylium cation (**7**), quinone (**8**), carbinol

(**9**) and chalcone (**10**). Reduction product has an absorption maximum at 520 nm.



Low intensity band at 350 nm is due to chalcone, which coexists with flavylium cation. The antioxidant effect of seven flavonic derivatives, as model substances, and two *Hypericum Perforatum* extracts has been studied (Table 5). Scavenging activity for $O_2^{\cdot-}$ of flavonic derivatives has been evaluated through decreasing of 2,2-difenil-1-picril-hidrazil (DPPH) optical density at 517 nm [9].

Oxy-flavonoids have antioxidants properties show by: free radicals deactivation and, substitution position influencing biological effects. Flavonic compounds 5,7,3',4',5'-pentaoxylated have antioxidant effects. Substitution in 3',4',5' with hydroxyl groups is important for antioxidant effects (scavenger) (Table 5).

4. Conclusions

Extraction and hydrolysis conditions for 3,5,7,3',4'-pentaoxy flavonic chromogene from *Hypericum Perforatum* have been presented. Structural determinations for separated chromogene have been made by chromatographic and spectrometric methods and by reduction to flavylium salts. For antioxidant property estimation for model compounds and extraction products DPPH test has been applied. Antioxidant properties of hydroxy-flavones can be used in therapy of diseases generated or increased by radical mechanism oxidation and accumulation of radical $O_2^{\cdot-}$.

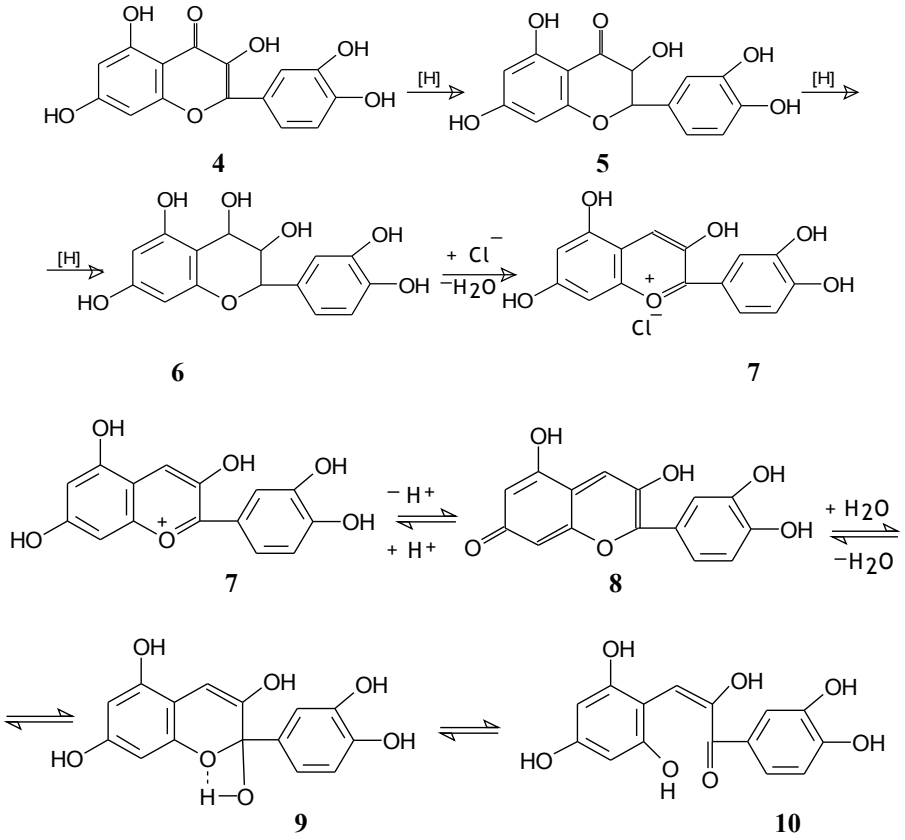
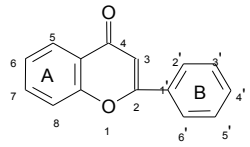


Table 5 Optical Density Decreasing of DPPH with Flavonoide (λ=517 nm)

Compound									Optical Density Decreasing at 517 nm
	3	5	6	7	2’	3’	4’	5’	
MODEL COMPOUNDS									
APYGENIN		OH		OH			OH		**0,085**
ACACETIN		OH		OH			OCH_3		**0,080**
LUTEOLIN		OH		OH		OH	OH		**0.235**
QUERCETIN	OH	OH		OH		OH	OH		**0,356**
MYRICETIN	OH	OH		OH		OH	OH	OH	**0,321**
MORYN	OH	OH		OH	OH		OH		**0,289**
FYSETIN	OH			OH		OH	OH		**0,287**
EXTRACT OF HYPERICUM PERFORATUM									
Crude extract									**0,137**
Hydrolysed Product	OH	OH		OH		OH	OH		**0,305**

5. References

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