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Reactions of α-hydroxyethyl radicals with flavonoids of various structures

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Abstract—It has been found that flavonoids (FL) are able to reduce, add or oxidize α-hydroxyethyl radicals (HER). The probability of these processes to occur depends on the structure of the FL under study. Namely, to cause reduction of HER, the presence of hydroxyl groups is necessary, and to effect oxidation or addition of HER, the presence of a carbonyl group at C_4 and a C_2–C_3 double bond in the C ring is required.

Flavonoids (FL) belong to a vast class of polyphenols of vegetal origin. The interest in studying properties of these compounds is determined by various kinds of pharmacological activity displayed by many of them.[1] It is believed that useful medicinal properties of FL result from their ability to inhibit oxidative processes in biosystems. One of the factors causing FL to display antioxidant activity is their high reactivity towards active oxygen species,[2] as well as their chelating properties, because formation of complexes with metal ions of variable valency leads to passivation of the latter. As to the possibility of regulating free-radical processes involving hydroxyl-containing carbon-centered radicals by means of FL, the reports available in the literature on this subject are not numerous.[3–6] At the same time, a serious motivation to obtain such data does exist.

First, a number of FL are used as hepatoprotectors, including in cases of liver diseases caused by alcohol abuse. Hydroxyethyl radicals (HER) formed in the course of biochemical and chemical transformations of ethanol can provoke reactions fraught with undesirable consequences.[7,8] Second, FL are also used for prevention and treatment of cardiovascular diseases, which, as a rule, are accompanied by hypoxia.[9] It has been shown in our studies that free-radical fragmentation of biologically important molecules involving hydroxyl-containing carbon-centered radicals takes place under these conditions.[10–12] The issue of how intermediates of such kind interact with FL has remained unexplored. In an attempt to bridge this gap to some extent, we studied effects of FL on product yields in reactions involving HER. To obtain alcohol radicals, generation methods using radiation were employed.

HER are known to be the major organic intermediates encountered in radiolysis of ethanol, and their formation occurs mainly according to the following scheme:[13]

$$CH_3CH_2OH \xrightarrow[-e^{\ominus}]{\text{irradiation}} CH_3CH_2OH^{+\bullet} \rightarrow \xrightarrow[-CH_3CH_2OH_2^{\oplus}]{CH_3CH_2OH} CH_3^{\bullet}CHOH \quad (1)$$

Subsequently, HER interact with one another to yield either acetaldehyde (AA) or 2,3-butanediol (BD):

$$2\ CH_3\dot{C}HOH \rightarrow CH_3CHO + C_2H_5OH \quad (2)$$

$$2\ CH_3\dot{C}HOH \rightarrow CH_3CH(OH)CH(OH)CH_3 \quad (3)$$

The reactivity of FL with respect to HER can be assessed by their influence on yields of AA and BD. It should be remembered that $CH_3^{\bullet}CHOH$ radicals are sole precursors of BD, while AA can be formed in the course of ethanol radiolysis not only by reaction 2 but also as a result of decomposition of excited molecules

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of the alcohol. AA yields can also be affected by reactions of AA with solvated electrons.[13] Besides yield values of AA and BD, some useful information about probability and mechanisms of HER reactions with FL can be obtained by assessing their yields of decomposition in ethanol.

Structural formulas of the compounds used in this work are shown in Figure 1. Flavonoids and caffeic acid (Aldrich) were used without further purification. Ethanol was purified using Wolfen Zeosorb LA ceolite and twice distilled. Prior to dissolving weighed samples, high purity argon was bubbled through ethanol solvent for 40 min. All further procedures, up to sealing ampoules filled with solutions, were carried out in argon atmosphere. Concentrations of additives were 1 mM. Free-radical processes were initiated using γ-radiation from a ^{137}Cs source, with a dose rate of 0.28 ± 0.01 Gy/s and within the absorbed dose interval 0.2–2.0 KGy. Concentrations of AA and BD were measured using a Shimadzu GC-17AAF/APC gas chromatograph. Analysis conditions: quartz capillary column RTX-Wax (l = 30 m; ID 0.32 mm; d_f 0.5 μm); starting temperature: 40 °C; rate of temperature rise up to 200 °C: 10 °C/min; isothermal period: 3 min; evaporator temperature: 220 °C; detector temperature: 220 °C; carrier gas: nitrogen; flow rate: 30 cm/s; detector: FID. FL concentrations were measured using HPLC. Analysis conditions: Column Nucleosil 120-5; eluent flow rate: 1 ml/min; eluents: (A) H_2O/AcOH (24:1, v/v), (B) MeOH/H_2O/AcOH (47:49:4, v/v), (C) MeOH; variable wavelength UV detector. Elution conditions and analytical wavelengths for each compound are given in Table 1. Radiation–chemical yields were determined from graph slopes of linear relationships 'concentration–dose.' The values given in Table 2 representing radiation–chemical formation yields of AA and BD, as well as FL decomposition yields have been obtained by averaging the results of at least three series of independent experiments.

The obtained data (Table 2) indicate that cathechins (X and XI) undergo virtually no destruction on irradiation of their solutions in ethanol. With all this, they belong to substances that decrease BD yields to a lesser degree, and consequently they are less reactive with respect to HER.

These facts give reasons for considering those reactions of X and XI with HER in which the starting compounds would be regenerated. Catechins are known to react with $ROO^{\bullet}$ radicals, reducing the latter 2.6 times more effectively than Trolox, a standard reducing agent of phenolic nature.[2] One can assume the following reactions to proceed on interaction of catechins with carbon-centered radicals:

$$FlOH + CH_3^{\bullet}CHOH \rightarrow FlO^{\bullet} + CH_3CH_2OH \quad (4)$$

$$FlO^{\bullet} + CH_3^{\bullet}CHOH \rightarrow FlOH + CH_3CHO \quad (5)$$

where FLOH are substances X and XI. reactions.

The effects observed on radiolysis of ethanolic solutions of compounds X and XI can be explained by realization of reactions 4 and 5. The same reactions appear to play an important role also in radiolysis of II, III, VIII and IX. This is evidenced by their decomposition yield values, which are substantially inferior to those found for flavone (V). Molecules of compound (V) do not contain hydroxyl groups, and therefore they cannot a priori react with $CH_3^{\bullet}CHOH$ radicals according to scheme 4. The presence of OH-groups in compounds II, III, VIII

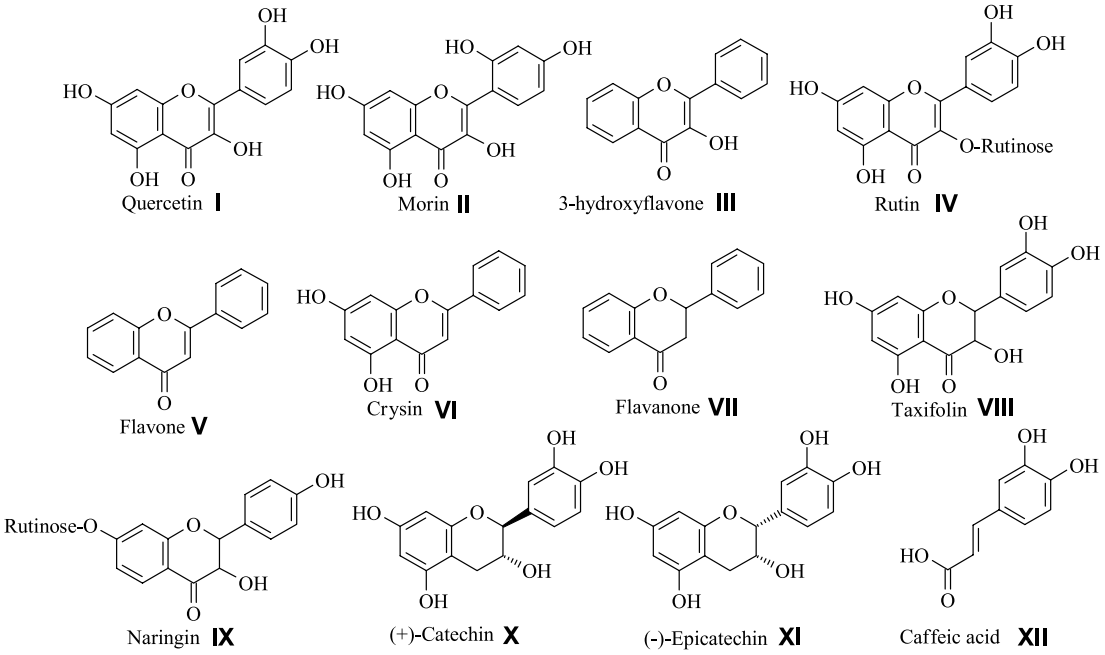

Figure 1. Structures of the compounds studied.

Table 1. Conditions of HPLC analysis of flavonoids

Compounds	λ (detect.), nm	Content of solvent A (%)					Stop (min)
		0 min	10 min	15 min	20 min	22 min	
Quercetin[a]	370	90	—	0	0	90	40
Morin[a]	358	90	—	0	0	90	40
3-Hydroxyflavone[a]	345	90	—	0	0	90	40
Rutin	360	50	0	—	0	50	40
Flavone[a]	296	90	—	0	0	90	40
Crysin[a]	315	90	—	0	0	90	40
Flavanone[a]	322	90	—	0	0	90	40
Taxifolin	290	80	0	0	80	—	30
Naringin	285	90	—	100	100	90	40
(+)-Catechin	280	100	50	100	—	—	30
(−)-Epicatechin	280	100	50	100	—	—	30
Caffeic acid	325	100	—	50	100	—	30

[a] Solvents A and C were used; solvents A and B were used in all other cases.

Table 2. Radiation–chemical formation yields of AA and BD, FL decomposition yields

Compounds	Compound class	*G* value (mol/100 eV)		
		AA	BD	FL
—	—	1.82 ± 0.15	1.49 ± 0.13	—
I	Flavonol	2.66 ± 0.17	0.28 ± 0.02	−1.45 ± 0.09
II	Flavonol	3.06 ± 0.12	0.12 ± 0.02	−0.58 ± 0.06
III	Flavonol	3.55 ± 0.32	0.08 ± 0.01	−0.79 ± 0.06
IV	Flavonol glycoside	2.98 ± 0.15	0.21 ± 0.02	−2.10 ± 0.16
V	Flavone	3.62 ± 0.23	0.08 ± 0.01	−4.09 ± 0.15
VI	Flavone	2.89 ± 0.18	0,13 ± 0.01	−2.46 ± 0.09
VII	Flavanone	2.39 ± 0.12	0.35 ± 0.03	−2.05 ± 0.06
VIII	Flavanonol	1.95 ± 0.07	0.98 ± 0.05	−0.98 ± 0.05
IX	Flavanonol	3.08 ± 0.17	0.86 ± 0.05	−0.81 ± 0.04
X	Flavanol	2.49 ± 0.15	0.99 ± 0.06	—
XI	Flavanol	2.46 ± 0.14	1.0 ± 0.07	−0.27 ± 0.04
XII	Phenyl propanoid	1.26 ± 0.09	0.09 ± 0.01	−5.43 ± 0.27

and IX, as well as X and XI, provides a possibility for reactions 4 and 5 to proceed, which enhances radiation stability of these FL.

Analysis of final products of radiation–chemical transformations of baicaline (a flavone) in ethanol has resulted in identification of the corresponding adducts,[3,6] allowing the authors to conclude that $CH_3^{\bullet}CHOH$ radicals can add to the C_2–C_3 double bond of FL. Taking this into account, realization of the addition reactions of HER should be expected to provoke a decrease in yields of products formed during ethanol radiolysis, and to cause destruction of the additives. In our case, the most marked effect is seen with caffeic acid (XII), which can be regarded as a model of FL structure. This process can be realized for FL with C_2–C_3 double bond as well, however, as judged by the yields of AA, its effect is masked by other.

High decomposition yields observed for flavone (V) indicate that compounds of such type are reactive towards HER. As a result of these reactions, a significant decrease in yields of BD and an increase in yields of AA are observed. These facts indicate that flavones and, to a substantial degree, other FL containing a carbonyl group at C_4 conjugating with the C_2–C_3 double bond can oxidize the alcohol radicals according to the following reaction:

$$Fl{=}O + CH_3^{\bullet}CHOH \rightarrow [FlOH]^{\bullet} + CH_3CHO \qquad (6)$$

where Fl═O are substances I, II, III and V.

Evidence for the possibility of the reaction 6 to proceed is provided by the well-known facts of oxidation of alcohol radicals by quinones and carbonyl compounds.[14,15] The obtained data indicate that the presence of a carbonyl group at C_4 in the C ring may be a determining factor relative to their ability to oxidize HER. To our knowledge, this capability of flavonoids has not been discussed in the literature thus far. The presence of such properties in FL makes them, as well as compounds of quinoid structure,[14] promising agents for regulation of free-radical fragmentation processes taking place in biologically important compounds.

The data we have obtained indicate that FL can reduce HER to the starting molecules, displaying a feature that is more characteristic of catechins. FL are capable of adding HER to carbon–carbon double bonds,[3,6] decreasing thereby the yields of the corresponding recombination and disproportionation products. The presence of a carbonyl group in the C ring of FL enables

them to oxidize alcohol radicals. This property is manifested even more markedly in cases of flavone and 3-hydroxyflavone as compared with other carbonyl-containing FL.

Interesting relationships can be revealed on comparing the obtained data with pharmacological activity of FL. Medications used in treatment of hepatic diseases,[9] such as Carsil and Cianidanol, contain derivatives of dihydroquercetin and catechins, that is, substances that, according to our data, manifest low activity towards HER and, consequently, do not form such toxic products as AA in high yields, which makes them advantageously different from flavones and flavonols. Flavone and its derivatives are components of drug products used for prevention and treatment of cardiovascular disorders.[9] These compounds effectively oxidize HER. This gives reasons to suppose that pharmacological activity of the named compounds may be associated with their capability of blocking fragmentation reactions taking place in biologically important substances. These processes occur under conditions of hypoxia via the stage of formation of α-hydroxyl-containing radicals, and lead to destruction of phospholipids and cerebrosides,[16,17] and formation of signaling molecules responsible for apoptosis.[18]

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