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Comparative Study of the Antioxidant Properties of Selected Flavonols and Flavanones

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Abstract—The antioxidant properties of morin, myricetin, fisetin, naringenin, and naringin were studied using the initiated oxidation of 1,4-dioxane as the model reaction. The antioxidant activity of these compounds was quantitatively characterized by the apparent rate constant of inhibition, fk_{in}. Morin, myricetin, and fisetin are comparable with ionol in antioxidant efficiency.

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Flavonoids find wide use in medical practice as physicologically active compounds with a wide range of pharmacological properties and a low toxicity level. They possess hepatoprotective, anti-inflammatory, antiallergenic, and anticancer properties [1–3]. The biological activity of flavonoids is manifested, e.g., as inhibition of enzymatic activity. Presently, the domestic and foreign literature contains numerous publications devoted to the antioxidant and antiradical properties of flavonoids of various classes in different model systems [3–9]. The antioxidant activity (AOA) of flavonoids was studied on lipid substrates: linoleic acid, ethyl linoleate, ethyl oleate, tallow, cottonseed oil, soybean oil, palm oil, and milk fat. The antioxidant properties of natural compounds are most frequently characterized by the concentration at which the rate of oxidation of the model substrate to the final products, such as malonaldehyde and diene conjugates, decreases by 50%. Other characteristics are the peroxide number and the percent efficiency of accepting of 2,2-diphenyl-1-picryhydrazyl radicals (DPPH$^{\bullet}$) and radical cations of 2,2-azobis(3-ethylbenzyazoline)-6-sulfonic acid (ABTS$^{\bullet+}$) and solvated electrons. Since the antioxidant effect of the compounds is intimately related to their reduction properties, it is essential to study the redox properties of flavonoids [4–13]. However, the thus-obtained information on the AOA of natural compounds of phenolic nature is mainly qualitative or semiquantitative, which does not allow one to predict their antioxidant efficiency under variable conditions. The most objective characteristic of the AOA of any compound is the rate constant of inhibition, which is determined by kinetic methods.

Here, we report a quantitative kinetic study of the antioxidant properties of selected flavones and flavanones.

EXPERIMENTAL

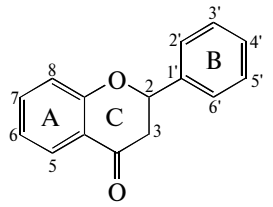
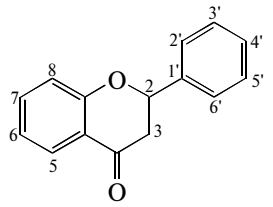
The objects of this study were the flavonoids listed in Table 1, which were purchased from Sigma (**I**, **II**, and **IV**) or Aldrich (**III** and **V**) and were used without further purification. Their purity exceeded 96%. The substrate of oxidation, 1,4-dioxane ($C_4H_8O_2$), was purified by a standard procedure [14]. The initiator was azobisisobutyronitrile (AIBN), which was twice recrystallized from freshly distilled ethanol and then dried in vacuo. Ionol (2,6-di-*tert*-butyl-4-methylphenol) was recrystallized from ethanol, and the crystals isolated were dried and sublimed in vacuo.

The antioxidant properties of flavonoids were studied under standard conditions using the chain radical oxidation of 1,4-dioxane at 348 K in the kinetically controlled regime (with AIBN as the initiator) as the model reaction [3, 15]. Measurements were carried out with a multipurpose differential manometric setup. The antioxidant efficiency of the compounds was estimated as the decrease in the initial rate of oxygen uptake. The apparent rate constant of oxidation chain termination (fk_{in}) was chosen to be the quantitative characteristic of the AOA of flavonoids, where f is the radical capacity of the antioxidant, equal to the number of peroxyl radicals of 1,4-dioxane decayed on one antioxidant molecule in a chain termination event [3, 15]. The reported fk_{in} value for ionol, which was determined under similar experimental conditions, is $(1.00 \pm 0.15) \times 10^4$ l mol^{-1} s^{-1} [16–18].

RESULTS AND DISCUSSION

The initiated oxidation of 1,4-dioxane under standard conditions occurred in the kinetically controlled regime via the radical chain mechanism with qua-

Table 1. Formulas of the compounds examined

General structural formula	Positions of substituents	Name of compound	Designation
	Flavonols		
	OH, 3,5,7,2',4'	Morin	**I**
	OH, 3,5,7,3,4',5'	Myricetin	**II**
	OH, 3,7,3',4'	Fisetin	**III**
	Flavanones		
	OH, 5,7,4'	Naringenin	**IV**
	Rhamnoside, 7; OH, 5,4'	Naringin	**V**

dratic-law chain termination. The mechanism of this reaction includes the following elementary steps[1] [3, 15]:

(i) $I \longrightarrow 2r^{\cdot}$ $r^{\cdot} + O_2 \longrightarrow rO_2^{\cdot}$, $rO_2^{\cdot} + RH \longrightarrow R^{\cdot} + rO_2H$,

(1) $R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot}$,

(2) $RO_2^{\cdot} + RH \longrightarrow ROOH + R^{\cdot}$,

(3) $ROOH \longrightarrow RO^{\cdot} + HO^{\cdot}$,

. . .

(6) $RO_2^{\cdot} + RO_2^{\cdot} \longrightarrow Pr_1$,

(7) $RO_2^{\cdot} + InH \longrightarrow ROOH + In^{\cdot}$,

(8) $RO_2^{\cdot} + In^{\cdot} \longrightarrow Pr_2$,

where RH is the oxidized substrate (1,4-dioxane), I is the initiator, Pr_1 and Pr_2 are the reaction products, and In is the inhibitor.

The introduction of the polyphenols into the model system decreases the oxidation rate of the model substrate due to chain termination on inhibitor molecules (step 7) [3]. The dependences of the initial rate of oxygen uptake (w_0) during 1,4-dioxane oxidation on the flavonoid concentration are shown in Fig. 1. It follows from Fig. 1 that compounds **I**–**V** exert a pronounced antioxidant effect.

To determine the apparent inhibition rate constants fk_{in}, the experimental data obtained under conditions of the chain oxidation of 1,4-dioxane were represented in the coordinates of the equation [15]

$$F = \frac{w_0^{O}}{w_0} - \frac{w_0}{w_0^{O}} = fk_{in}[AO]/\sqrt{2k_6 w_i}, \quad (1)$$

where w_0^{O} and w_0 are the initial rates of oxygen uptake in the oxidation of 1,4-dioxane in the absence and presence of the inhibitor, respectively; w_i is the initiation rate of the oxidation reaction; [AO] is the antioxidant concentration; k_{in} and $2k_6$ are the rate constant of chain termination on the antioxidant and the rate constant of quadratic-law chain termination on the peroxyl radicals of the substrate, respectively [3, 15].

It can be seen from the data in Table 2 that Eq. (1) is satisfactorily satisfied for all of the compounds examined (the correlation coefficient is larger than 0.95). The dependences of the inhibition parameter F calculated via Eq. (1) on the antioxidant concentration in the concentration interval examined are shown in Fig. 2.

The fk_{in} value for each flavonoid was derived from the slope of the corresponding F versus [AO] curve (Fig. 3). The quadratic-law chain termination rate constant known from the literature, $2k_6 = 6.67 \times 10^7$ l mol^{-1} s^{-1} [3], was used in the calculations. The results are presented in Table 2. The antioxidant efficiency of the compounds can be estimated from the ionol equivalent values (Table 2) calculated as

$$IE = fk_{in}/fk_{ionol}. \quad (2)$$

The plots of the oxidation rate of 1,4-dioxane versus the flavonoid concentration tend to a limit (Fig. 1): the introduction of the flavonoids at concentrations above 1.4×10^{-3} mol/l for morin, 4.5×10^{-4} mol/l for

[1] The elementary step numbering traditional for the oxidation mechanism is accepted.

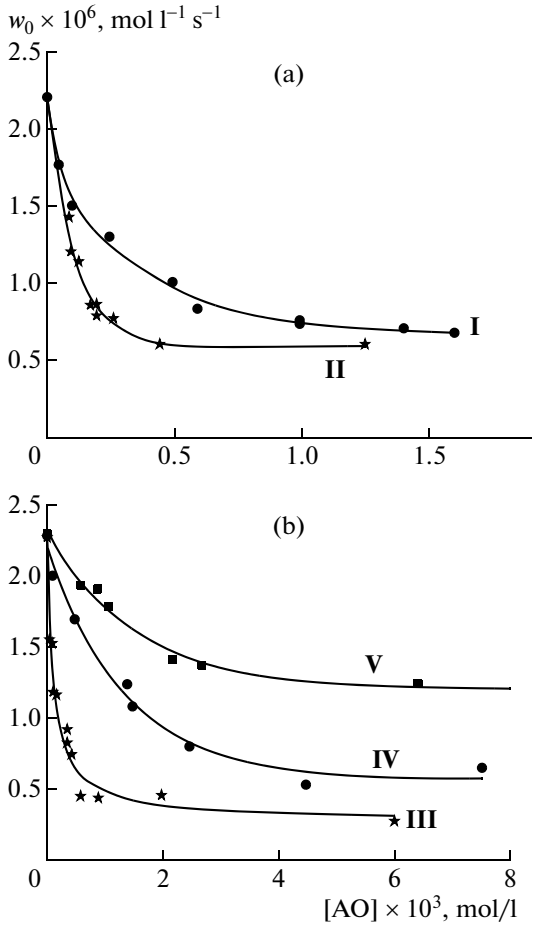


Fig. 1. Initial rate of oxygen uptake versus the flavonoid concentration for (a) **I** and **II** and (b) **III**–**V** in 1,4-dioxane oxidation. The reaction temperature is 348 K, and the initiation rate is $w_i = 1 \times 10^{-7}$ mol l^{-1} s^{-1}.

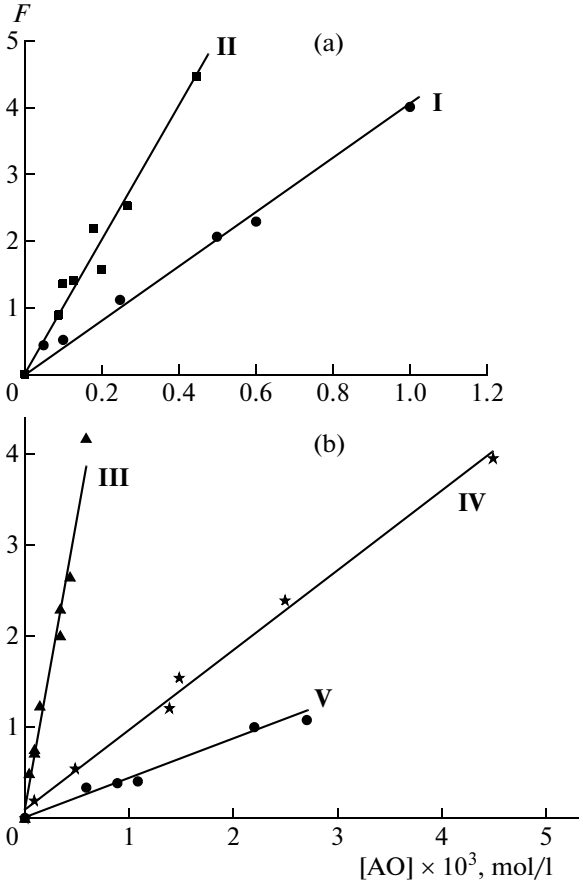


Fig. 2. Inhibition efficiency parameter versus the flavonoid concentration for (a) **I** and **II** and (b) **III**–**V**. The reaction temperature is 348 K, and $w_i = 1 \times 10^{-7}$ mol l^{-1} s^{-1}.

myricetin, 6.0×10^{-3} mol/l for fisetin, and 4.0×10^{-3} mol/l for naringin and naringenin causes no further decrease in the oxidation rate. When the antioxidant concentrations are higher than the limiting value, all $RO_2^{\bullet}$ radicals leading the oxidation chain decay on antioxidant molecules, which is confirmed by the lin-

Table 2. Apparent inhibition rate constants of initiated 1,4-dioxane oxidation by flavonoids at 348 K

Flavonoid	Position of OH groups in chromanone ring	$fk_{in} \times 10^{-4}$, l mol^{-1} s^{-1}		IE
		from Eq. (1)	from Eq. (2)	
Flavonols				
Myricetin	3,5,7,3',4',5'	2.6 ± 0.3	4.5 ± 0.6	2.6
Fisetin	3,7,3',4'	1.5 ± 0.2	2.0 ± 0.2	1.5
Morin	3,5,7,2',4'	1.0 ± 0.2	2.0 ± 0.2	1.0
Quercetin [17, 18]	3,5,7,3',4'	6.10 ± 0.2	–	2.2
Flavanones				
Naringenin	5,7,4'	0.2 ± 0.03	0.1 ± 0.02	0.2
Naringin	Rhamnoside, 7; OH, 5,4'	0.1 ± 0.02	0.1 ± 0.02	0.1
Dihydroquercetin [17, 18]	3,5,7,3',4'	17.0 ± 1.0		8.6
Ionol [17, 18]	–	1.0		

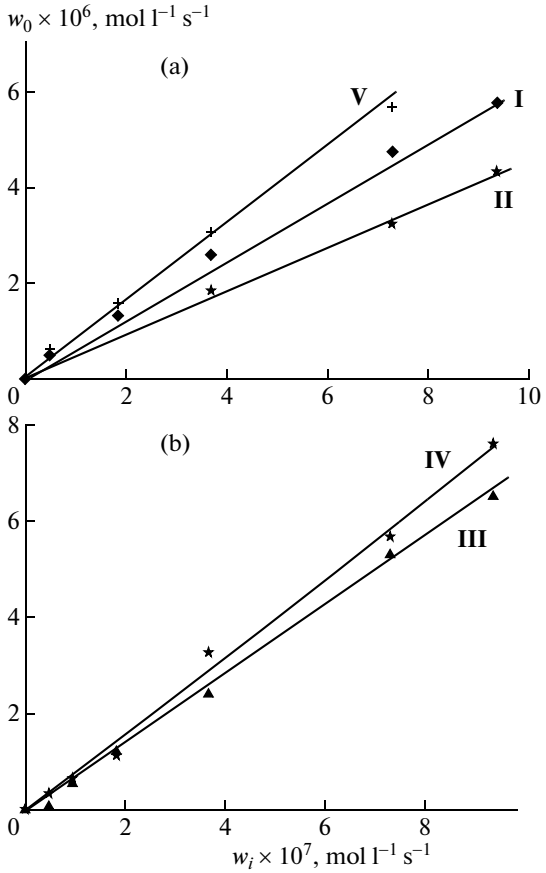


Fig. 3. 1,4-Dioxane oxidation rate versus the initiation rate in the presence of flavonoids: (a) **I**, **II**, and **V**; (b) **III** and **IV**. The flavonoid concentrations are [**I**] = 1.4×10^{-3}, [**II**] = 4.5×10^{-4}, [**III**] = 6×10^{-3}, [**IV**] = 7.5×10^{-3}, and [**V**] = 9×10^{-3} mol/l. The reaction temperature is 348 K.

ear dependence of the initial oxidation rate w_0 on the initiation rate w_i (Fig. 3) in the coordinates of Eq. (3) (correlation coefficient of 0.98) [15]:

$$w_0 = \frac{k_2[\mathrm{RH}]}{fk_{\mathrm{in}}[\mathrm{AO}]}w_i, \qquad (3)$$

where k_2 is the rate constant of step 2 (chain propagation), equal to 7.9 l mol^{-1} s^{-1}, [RH] = 11.75 mol/l, [**I**] = 1.4×10^{-3} mol/l, [**II**] = 4.5×10^{-4} mol/l, [**III**] = 6×10^{-3} mol/l, [**IV**] = 7.5×10^{-3} mol/l, and [**V**] = 9×10^{-3} mol/l.

At the same time, in the presence of the limiting concentrations of the antioxidants (Fig. 1), the chain regime is retained, the chain length being $\nu = 4$–10. The chain mechanism is probably conserved by the chain transfer to the inhibitor (step 10), resulting in chain reinitiation:

$$(10)\ \mathrm{In}^{\bullet} + \mathrm{RH} \longrightarrow \mathrm{InH} + \mathrm{R}^{\bullet}.$$

Note that a similar prooxidant effect observed upon the introduction of *ortho*-unsubstituted flavonoids was described in the literature [9, 12].

The fk_{in} values calculated from these data are listed in Table 2. As can be seen, the fk_{in} values determined by two different kinetic methods from the data presented in Figs. 1–3 are similar. They can be arranged in the following order according to the efficiency of their inhibition of 1,4-dioxane oxidation:

dihydroquercetin > quercetin > **II** > **III** > **I** = ionol > **IV** > **V**.

The above results suggest that the antioxidant efficiency of the compounds is intimately related to their structure and is defined both by the amount of hydroxyl groups and by their arrangement in rings A and B (Table 1). The following structural fragments are responsible for the high antioxidant efficiency of the flavonoids:

(1) the OH groups in the *ortho* position of ring B, i.e., the so-called *ortho*-catechol fragment,

(2) the OH groups in the 5- and 7-positions of ring A, and

(3) the C2–C3 double bond and the hydroxyl group at C3.

For instance, among the compounds studied by us, fisetin and quercitin, containing two OH groups in the *ortho* position, exhibit the highest AOA. Morin, in which two OH groups are in the *meta* position, is two time weaker than fisetin in this respect. The structures of both flavonoids contain the C2–C3 double bond and an electron-donating OH group in the C3 position. Their antioxidant efficiency is comparable with that of the standard inhibitor ionol. The presence of the OH group in the C3 position enhances the AOA, favoring the stabilization of the entire pyrone ring, and increases the stability of the phenoxyl radicals that are formed from molecules of these flavonoids via their interaction with the peroxyl radicals of 1,4-dioxane. At the same time, myricetin, which contains three OH groups in ring B, the C2–C3 double bond, and an OH group in the C3 position, is 1.5 times more efficient than fisetin, but three times weaker than quercetin. Similar regularities revealed by other authors in lipid systems were attributed to the prooxidant effect [9, 12]. Naringin, which contains one OH group in the 4'-position, shows the lowest AOA among aglycons. As can be seen from the experimental data, the OH group in the C5 position, which is present in all of the flavonoids but fisetin, exerts no effect on their AOA. This can be due to the formation of intramolecular hydrogen bonds between the OH group in the C5 position and the carbonyl group adjacent to ring C in the C3 position.

The regularities established by us agree with the experimental data obtained for other model systems (Table 3) [5–12, 19–23]. The reactivity of flavonoids as antioxidants depends on the nature of the attacking radical. Even the inversion of their AOAs is possible. Under the conditions of initiated oxidation of ethylbenzene at 333 K, of propan-2-ol at 348 K, and of 1,4-dioxane at 333 and 347 K, dihydroquercetin exhibits a

Table 3. Kinetic characteristics of flavonoids

Compound	Oxidized compound*	$fk_{in} \times 10^{-4}$, $l\ mol^{-1}\ s^{-1}$	Ferrylmyoglobin reduction rate of at 310 K, $\mu mol\ l^{-1}\ min^{-1}$	Rate constant of interaction with DPPH$^{\bullet}$ radicals, $l\ mol^{-1}\ s^{-1}$	Rate constant of interaction with galvinoxyl radical**, $l\ mol^{-1}\ s^{-1}$
Myricetin	–	–	52.8 ± 0.9	75.55***	1.44 ± 0.20
				130****	
Fisetin	–	–	–	–	0.16 ± 0.02
Morin	Ethylbenzene	1.0 ± 0.2	2.0 ± 0.2	–	1.01 ± 0.05
Naringin	–	–	0.1 ± 0.02	4.0***	–
Naringenin	Ethylbenzene	0.20 ± 0.03	2.9 ± 1.0	–	–
Quercetin	Ethylbenzene	2.95	40.8 ± 2.2	476***	0.24 ± 0.03
	1,4-Dioxane	3.6 ± 0.3		1890****	
	Propan-2-ol	2.5 ± 0.2			
Dihydroquercetin	Ethylbenzene	7.64	–	220***	0.033 ± 0.032
	1,4-Dioxane	5.4 ± 0.3			
	Propan-2-ol	11 ± 1.0			
Ionol (standard inhibitor)	Ethylbenzene	2.51	–	–	–
	1,4-Dioxane	0.7 ± 0.1			
	Propan-2-ol	1.0 ± 0.2			

* Ethylbenzene was oxidized at 331 K [19]; 1,4-dioxane, at 333 K [16, 20]; propan-2-ol, at 348 K [20].
** In an ethanolic solution at 293 K (EPR measurements) [8].
*** In a methanolic solution at 298 K [21].
**** in a 1 : 1 water-methanol solution at 298 K [21].

higher AOA than quercetin [8, 16, 20]. Conversely, in the reaction with the galvinoxyl radical, the strongest antioxidant effect is exerted by quercetin. Myricetin exhibits a higher antioxidant effect than quercetin or dihydroquercetin in the interaction with galvinoxyl radicals and in the enzymatic oxidation of ferrylmyoglobin. However, in the model systems of initiated oxidation of ethylbenzene and 1,4-dioxane and in the interaction with diphenylpicrylhydrazyl radicals, myricetin demonstrates a lower OAO. The antioxidant effects of morin and naringenin in the model systems are similar. In addition, the numerical values of the apparent inhibition rate constant fk_{in} for quercetin and dihydroquercetin in the initiated oxidation of 1,4-dioxane at 348 K are, respectively, 1.5 and 4.0 times higher than those for propan-2-ol oxidation in the presence of the same flavonoids. The observed decrease in the AOA of the phenolic compounds in polar media, including in the oxidation of secondary alcohols, is explained by hydrogen bonding between the antioxidant and solvent molecules [25].

Note that the antioxidant efficiency of quercetin isolated from Siberian larch wood depends substantially on its purity, particularly on the dihydroquercetin content. A mixture of these two flavonoids containing 22 wt % dihydroquercetin exhibits the maximum inhibition effect [26].

Thus, in the present work, the antioxidant properties of the following flavonoids were studied in initiated 1,4-dioxane oxidation as the model reaction: myricetin, fisetin, morin, naringenin, and naringin. It was found that, at 348 K, the apparent rate constants of the inhibition of the process by morin, myricetin, fisetin, naringenin, and naringin are fk_{in}, 1.0×10^{-4}, 2.6×10^{-4}, 1.5×10^{-4}, 0.2×10^{-4}, and $0.1 \times 10^{-4}\ l\ mol^{-1}\ s^{-1}$, respectively.

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