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Antioxidant Activity of Chalcones: The Chemiluminescence Determination of the Reactivity and the Quantum Chemical Calculation of the Energies and Structures of Reagents and Intermediates

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Abstract—Six antioxidants from the class of chalcones (ArOH), compounds from which flavonoids are obtained in nature, were studied. The antiradical activity of chalcones and a number of related compounds was determined by a chemiluminescence method using the scavenging of peroxide radicals $ROO^{\bullet} + ArOH \rightarrow ROOH + OAr^{\bullet}$ (with the rate constant k_7) in a model reaction of diphenylmethane (RH) oxidation. The structures and energies of the reagents and intermediates were determined by semiempirical quantum chemical (PM3, PM6) calculations. 3,4-Dihydroxychalcone and caffeic acid, which have a catechol structure, that is, two neighboring OH groups in phenyl ring B, exhibited high antioxidant activity ($k_7 \approx 10^7$ l mol^{-1} s^{-1}); this is consistent with the lowest bond strengths D(ArO–H) of 79.2 and 76.6 kcal/mol, respectively. The abstraction of a hydrogen atom by the $ROO^{\bullet}$ radical is the main reaction path of these compounds; however, the low stoichiometric coefficients of inhibition ($f = 0.3–0.7$) suggest a contribution of secondary and/or side reactions of ArOH and $OAr^{\bullet}$. In the other chalcones, the ArO–H bond is stronger (D(ArO–H) = 83–88 kcal/mol) and the antioxidant activity is lower ($k_7 = 10^4–10^5$ l mol^{-1} s^{-1}).

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INTRODUCTION

The oxidation of various organic materials (from the simplest individual substances to macromolecular compounds and complex biological systems in vivo) is often a detrimental, undesirable process. To protect the materials from oxidation, an antioxidant inhibitor is added to trap peroxide radicals—oxidation chain carriers. In naturally occurring compositions, protective antioxidants—bioantioxidants (for example, vitamin E and other tocopherols in plant oil)—were detected; the mechanism of formation of these bioantioxidants was developed in the course of evolution [1–3].

Substituted phenols (ArOH) [4], in particular, chalcones—natural phenolic compounds, which are considered the biological precursors of flavonoids [5, 6], are practically important antioxidants. All of the well-known chalcones possess biological and antioxidant activities, which manifest themselves to various extents depending on the conditions of oxidation either in a homogeneous anhydrous medium [7, 8] or in micellar systems and in the presence of water [9]. These properties and the nontoxicity of chalcones allow us to hope that they can be used as food stabilizers. However, systematic data on the antioxidant activity of these compounds are absent from the literature.

In this work, we studied six recently synthesized chalcones (Fig. 1) with different substituents in a ring usually referred to as ring B. The antioxidant activity of chalcones was determined by trapping peroxide radicals $ROO^{\bullet}$—chain carriers in the model oxidation reaction of a hydrocarbon (RH) initiated by the thermal decomposition of an initiator (Y) (see the scheme). The degree of quenching of chemiluminescence (CL), which was excited at the step of quadratic chain termination (VI) (e.g., see [10]), by antioxidants ArOH served as a measure of the antioxidant activity of ArOH:

$$\mathrm{Y}(+\mathrm{RH},\,\mathrm{O_2}) \rightarrow 2f_c\mathrm{r_YOO}^{\bullet} \rightarrow 2f_c\mathrm{ROO}^{\bullet} \qquad (0)$$
$$(\text{rate of initiation } W_i = 2f_c k_Y[\mathrm{Y}]),$$

$$\mathrm{R}^{\bullet} + \mathrm{O_2} \rightarrow \mathrm{ROO}^{\bullet} \qquad (\mathrm{I})$$
$$(\text{oxygen consumption, rate constant } k_1),$$

$$\mathrm{ROO}^{\bullet} + \mathrm{RH} \rightarrow \mathrm{R}^{\bullet} + \mathrm{ROOH} \qquad (\mathrm{II})$$
$$(\text{hydroperoxide formation, } k_2),$$

$$\mathrm{ROO}^{\bullet} + \mathrm{ROO}^{\bullet}$$
$$\rightarrow \text{ketone } \mathrm{R_{-H}{=}O} + \mathrm{O_2} + \text{alcohol ROH} + \mathrm{CL} \qquad (\mathrm{VI})$$
$$(\text{quadratic chain termination, } 2k_6),$$

$$\mathrm{ROO}^{\bullet} + \mathrm{ArOH} \rightarrow \mathrm{ROOH} + \mathrm{OAr}^{\bullet} \qquad (\mathrm{VII})$$
$$(\text{linear termination on an inhibitor, } k_7),$$

$$\mathrm{ROO}^{\bullet} + \mathrm{OAr}^{\bullet} \rightarrow \text{products} \qquad (\mathrm{VIII})$$
$$(\text{termination on an inhibitor radical, } k_8),$$

where r_Y is a primary radical, and f_c is the probability of the escape of radical r_Y from a cage.
(The conventional notation of rate constants is used.)

Scheme.

The molecular structures of chalcones and intermediate products were determined using semiempirical quantum chemical calculations, and the enthalpies of dissociation of ArO–H bonds were calculated; these enthalpies were compared with activity characteristics. The following related phenols were also studied: caffeic acid (CA), *p*-coumaric acid, ferulic acid, and chroman C_1 (2,2,5,7,8-pentamethylchroman-6-ol, CrC_1), which is a synthetic analog of α-tocopherol, as a model antioxidant.

EXPERIMENTAL

The chalcones were prepared by the Claisen–Schmidt reaction between acetophenone and a corresponding arylaldehyde [11]. The average product yield after purification by recrystallization was 80%, and the structures were confirmed by UV, IR, NMR, and mass spectra and elemental analysis. The ^{1}H NMR spectra indicated that the synthesis products were only *E*-isomers.

Caffeic acid from Merck and *p*-coumaric acid and ferulic acid from Fluka were used.

Chroman C_1 was kindly provided by Prof. V.A. Roginskii (Institute of Chemical Physics, Russian Academy of Sciences).

The efficiency of an antioxidant was studied using the inhibition of the liquid-phase oxidation of diphenylmethane (RH). Oxidation was initiated by the thermal decomposition of azobisisobutyronitrile (Y = AIBN); $w_i = 2f_c k_Y$ [Y], where $f_c = 0.60$, and the rate constant $k_Y = 1.58 \times 10^{15} \exp(-30800/RT)$ is almost independent of the nature of the solvent [12]. AIBN, the chlorobenzene solvent, and diphenylmethane were purified in accordance with standard procedures [10]. If diphenylmethane emitted CL with no addition of AIBN (this fact suggested the presence of a contaminating impurity of a peroxide initiator), the hydrocarbon was additionally purified by passing through an alumina column. Chalcones are sparingly soluble in nonpolar solvents. Therefore, they were initially dissolved in dimethyl sulfoxide; then, an aliquot of this solution was introduced into a reaction mixture. The concentration of dimethyl sulfoxide in the reaction vessel was insignificant (3×10^{-5}–6×10^{-4} mol/l), and the effect of polarity on the antioxidant activity could be ignored. The role of reaction products was minimized using a low rate of initiation ($w_i \approx 10^{-10}$ mol l^{-1} s^{-1}). This rate was measured from the kinetics of CL after adding a known amount of a standard inhibitor—CrC_1 (see Eq. (8) below; $f = 2.0$ and $w_i = 2[CrC_1]_0/\tau_{0.5}$ for CrC_1).

The oxidized mixture (5 ml) was placed in a thermostated (50°C) cell of a chemiluminometer. The mixture was saturated with oxygen by bubbling air. The weak primary luminescence (the triplet–singlet emission of the excited product—benzophenone) was enhanced by energy transfer to an efficient luminophor, the europium chelate Eu^{3+}-1,10-phenanthroline-tris(thenoyltrifluoroacetonate), which emits light as a narrow band at 612 nm. The chemiluminometer and the procedure of CL measurements were described elsewhere [10].

RESULTS AND DISCUSSION

Energetics and Structure of Chalcones

Because the antioxidant activity of phenols is associated with hydrogen atom abstraction from the phenolic OH group by a peroxide radical, it is reasonable to relate the reactivity of a phenol antioxidant to the strength *D*(ArO–H) of an OH bond, which is cleaved in reaction (VII) of an inhibitor with a peroxide radical

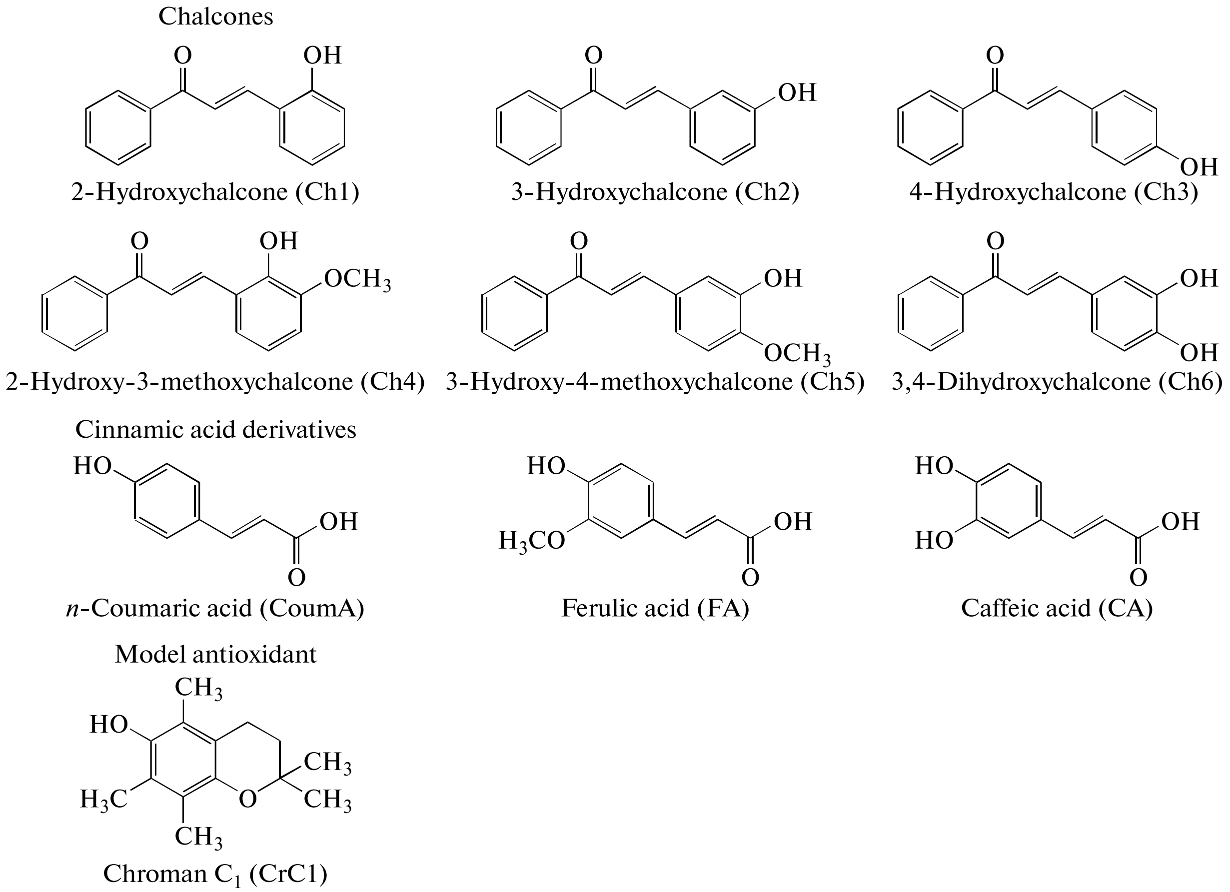

Fig. 1. Structures of the compounds examined.

(see the scheme). By definition, the strength (enthalpy or heat) of a bond is the difference of the heats of formation (ΔH_f^0) of bond cleavage products and the heat of formation of the reagent. In the case under consideration,

$$\mathrm{ArO{-}H} = \mathrm{OAr}^{\bullet} + \mathrm{H}^{\bullet};$$

consequently,

$$D(\mathrm{ArO{-}H}) = \Delta H_f^0(\mathrm{OAr}^{\bullet}) + \Delta H_f^0(\mathrm{H}^{\bullet}) - \Delta H_f^0(\mathrm{ArOH}).$$

The phenoxyl radical (the product of hydrogen abstraction from ArOH by the radical $\mathrm{ROO}^{\bullet}$) is often referred to as $\mathrm{ArO}^{\bullet}$; however, it has a quinoid structure and an unpaired electron is distributed over ring C atoms opposite to the C=O group resulting from O–H bond cleavage [13]. We reflect this structure peculiarity by denoting this radical as $\mathrm{OAr}^{\bullet}$ rather than $\mathrm{ArO}^{\bullet}$. It is well known that the heat of formation of the hydrogen atom is $\Delta H_f^0(\mathrm{H}^{\bullet}) = 52.10$ kcal/mol. Bakalbassis with coauthors (see [14, 15] and references therein) performed the most thorough ab initio (DFT/B3LYP) calculations of the energetics of phenol, its hydroxy- and *ortho*-methoxy-substituted derivatives, and corresponding phenoxyl radicals. In ten standard sets of basis functions, the values of *D*(ArO–H) gradually (but not monotonically) increased on going from a minimum (6-31G) to a maximum (6-311 + G(2d,2p)) basis set. However, they were found 3–5 kcal/mol lower than experimental values. Only nonstandard sets of basis functions (from 6–31G(,p) to 6-31 + G(,3pd)) made it possible

Table 1. Dissociation energy of the ArO–H bond in phenol and catechol calculated by ab initio and semiempirical methods

Calculation method	*D*(ArO–H), kcal/mol		Reference
	phenol	catechol	
6-31+G(,3pd)	88.5	81.7	[15]
CCSD/6-31G*	–	80.6	[16]
CCSD/6-31+G*	–	81.6	
CCSD/6-31++G**	–	81.5	
PM3	87.3	80.9	This work
PM6	–	81.0	
Experiment	87.0; 88.3; 88.7	81.6	[15]
		80.5	[17]

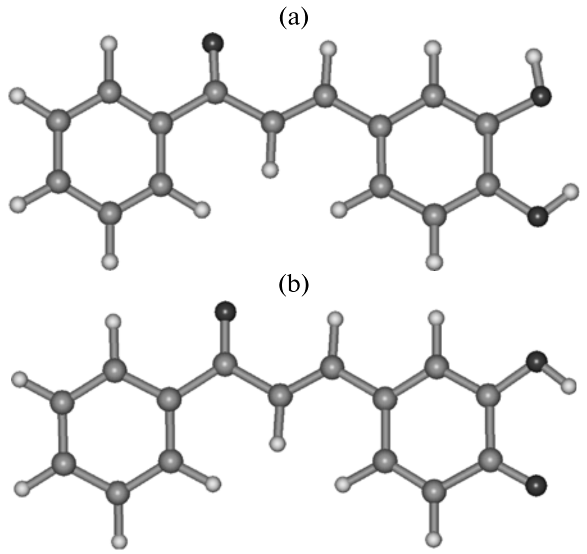

Fig. 2. Structures of (a) chalcone Ch6 and (b) its phenoxyl radical $\mathrm{OAr}^{\bullet}$.

to obtain the values of D(ArO–H) in good agreement with experimental data (Table 1). This example indicates that it is difficult to choose a reliable version of ab initio calculations, especially, for novel compounds whose energetics has not been studied previously. On the other hand, Table 1 indicates that the semiempirical PM3 and PM6 methods give an adequate accuracy (with consideration for experimental error). In this work, we used these methods to determine the structures of chalcones and related compounds and the ArO–H bond strength.

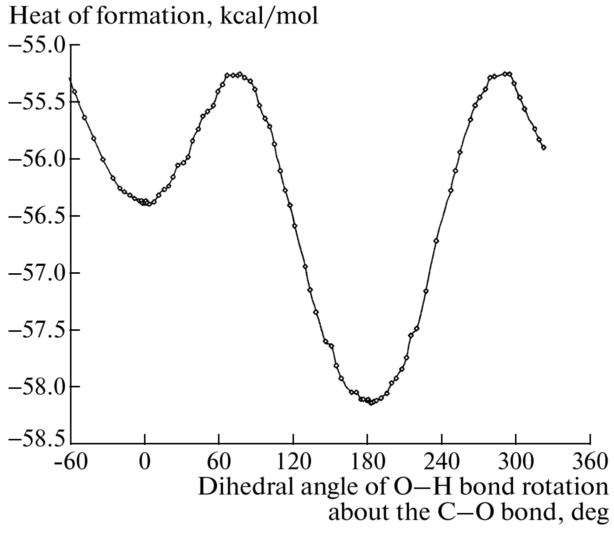

Fig. 3. Dependence of the heat of formation ($\Delta H_{\mathrm{f}}^{0}$) of Ch6 on the dihedral angle of O–H bond rotation about the C–O bond (PM3 calculation).

The main chalcone units (rings and C–C bonds) lie in a plane (Fig. 2); this fact is responsible for a high degree of their conjugation. Phenoxyl radicals also have a planar structure. They can occur in conformations ~180° different in the dihedral angle of the rotation of the O–H bond about the C–O bond and, correspondingly, in the heats of formation (Fig. 3). Analogous results were obtained for all of the compounds examined, for example, caffeic acid (Fig. 4).

In some ArOH conformations, hydrogen bonds are formed. Indeed, for example, the bond orders in the O–H–O chain are 0.93 (i.e., smaller than unity) and 0.004 (i.e., somewhat greater than zero) in the case of caffeic acid. The hydrogen bond was also retained in the O–H–O chain of a phenoxyl radical (bond orders of 0.920 and 0.009, respectively). As a result of an additional stabilization (by ~2 kcal/mol), these conformations became the participants of reaction (VII) (k_7), and the calculated values of $\Delta H_{\mathrm{f}}^{0}$ of the compounds examined pertain to them (see Table 5 below).

Two conformers with different rotation angles of the O–H bond about the O–C axis are also possible for chroman C_1; however, they are only slightly different (by 0.5 kcal/mol) in the heat of formation and, correspondingly, O–H bond strength.

Model Reaction of Hydrocarbon Oxidation and the Chemiluminescence Monitoring of Antioxidant Action

At moderate temperatures, hydroperoxide ROOH is the final product of the radical chain oxidation of hydrocarbon RH and the reaction is an unbranched chain reaction (see the scheme). In the exothermal act ($\Delta H \approx 100$ kcal/mol) of quadratic chain termination (VI), $\mathrm{R_{-H}{=}O}$ and $\mathrm{O_2}$ molecules become electronically excited and emitting light (CL) [10]. The main set of reactions given in the scheme (without considering

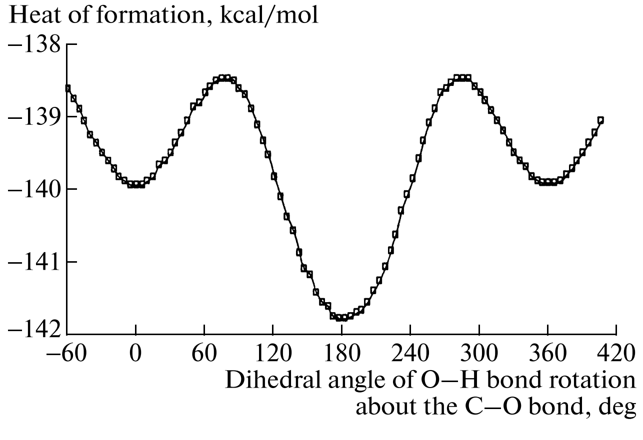

Fig. 4. Dependence of the heat of formation ($\Delta H_{\mathrm{f}}^{0}$) of CA on the dihedral angle of O–H bond rotation about the C–O bond (PM3 calculation).

possible additional and secondary processes) corresponds to the following set of differential equations:

$$\mathrm{d}[\mathrm{ROO}^\bullet]/\mathrm{d}t = w_\mathrm{i} - 2k_6[\mathrm{ROO}^\bullet]^2 - k_7[\mathrm{ArOH}][\mathrm{ROO}^\bullet] - k_8[\mathrm{ROO}^\bullet][\mathrm{OAr}^\bullet], \quad (1)$$

$$\mathrm{d}[\mathrm{OAr}^\bullet]/\mathrm{d}t = k_7[\mathrm{ArOH}][\mathrm{ROO}^\bullet] - k_8[\mathrm{ROO}^\bullet][\mathrm{OAr}^\bullet], \quad (2)$$

$$\mathrm{d}[\mathrm{ArOH}]/\mathrm{d}t = -k_7[\mathrm{ROO}^\bullet][\mathrm{ArOH}]. \quad (3)$$

Under steady-state conditions with respect to radicals ($\mathrm{d}[\mathrm{ROO}^\bullet]/\mathrm{d}t = \mathrm{d}[\mathrm{OAr}^\bullet]/\mathrm{d}t = 0$), Eq. (1) is transformed into a balance equation between chain initiation and two types of chain termination, quadratic and linear (with respect to the concentration of $\mathrm{ROO}^\bullet$ radicals):

$$w_\mathrm{i} = 2k_6[\mathrm{ROO}^\bullet]^2 + 2k_7[\mathrm{ArOH}][\mathrm{ROO}^\bullet]. \quad (4)$$

A particular manifestation of the effect of ArOH depends on the experimental procedure. If the consumption of O_2 or the buildup of a hydroperoxide is measured, a decrease in the reaction rate, which is proportional to $[\mathrm{ROO}^\bullet]$, is observed: $w = -\mathrm{d}[O_2]/\mathrm{d}t = \mathrm{d}[\mathrm{ROOH}]/\mathrm{d}t = k_2[\mathrm{ROO}^\bullet][\mathrm{RH}]$; now, it is a fraction $[\mathrm{ROO}^\bullet]/[\mathrm{ROO}^\bullet]_0 = r$ of the values of w_0 or $[\mathrm{ROO}^\bullet]_0$ for uninhibited reaction. If CL is measured, the luminescence intensity $I = \Phi_{\mathrm{CL}}k_6[\mathrm{ROO}^\bullet]^2$ (Φ_{CL} is the efficiency of CL), which is proportional to $[\mathrm{ROO}^\bullet]^2$, decreases; its fraction I/I_0 is denoted as i: $i = [\mathrm{ROO}^\bullet]^2/[\mathrm{ROO}^\bullet]_0{}^2 = r^2$. From Eq. (4) at $[\mathrm{ArOH}] = 0$, it follows that $[\mathrm{ROO}^\bullet]_0 = (w_\mathrm{i}/2k_6)^{1/2}$; $[\mathrm{ROO}^\bullet] = r[\mathrm{ROO}^\bullet]_0 = r(w_\mathrm{i}/2k_6)^{1/2}$; and Eq. (4) can be written in the form

$$1 - r^2 = r2k_7[\mathrm{ArOH}](2k_6w_\mathrm{i})^{-1/2}$$

$$\text{or}\quad 1 - i = i^{1/2}2k_7[\mathrm{ArOH}](2k_6w_\mathrm{i})^{-1/2} \quad (5)$$

$$\text{or}\quad 2k_7[\mathrm{ArOH}] = (2k_6w_\mathrm{i})^{1/2}(i^{-1/2} - i^{1/2}).$$

Equation (5) relates the current relative intensity of CL and the current concentration of ArOH.

The quenching effect of ArOH depends on the contribution of linear termination, that is, on the product $k_7[\mathrm{ArOH}]$ (see Eq. (4)). Initially, let us consider an ideal case of the model antioxidant chroman ($\mathrm{CrC_1}$; Fig. 5, curve *1*). The introduction of this antioxidant into the reaction mixture completely inhibited the reaction; that is, $k_6[\mathrm{ROO}^\bullet] \ll k_7[\mathrm{ArOH}]$. The CL intensities i and I, as well as the values of r and $[\mathrm{ROO}^\bullet]$, dramatically decreased to zero. Then, as ArOH, was consumed, the steady-state concentration of $\mathrm{ROO}^\bullet$ and, correspondingly, the initial rate of oxidation or CL intensity were restored. The S-shaped kinetic curve of CL is described by the equation (see [10])

$$F(t) = \ln(1 + i^{1/2}) - \ln(1 - i^{1/2}) - i^{-1/2} = (k_7/(2k_6)^{1/2})w_\mathrm{i}^{1/2} + \mathrm{const}, \quad (6)$$

and a maximum slope of the $i(t)$ curve (at an inflection point at $i = 0.535$) is

$$(\mathrm{d}i/\mathrm{d}t)_{\max} = 1/T = 0.237(k_7/(2k_6)^{1/2})w_\mathrm{i}^{1/2}. \quad (7)$$

The continuous recording of the value of i (photocurrent in arbitrary units), that is, an infinite number of taken samples, for the determination of the current values of i, r, $[\mathrm{ROO}^\bullet]$, and $[\mathrm{ArOH}]$ provides the high reliability and information content of CL monitoring. The area over the curve of $i(t)$, that is, the integral $\int_0^\infty (1 - i)\mathrm{d}t$, is the light sum of CL quenched by the antioxidant and its radical in equal fractions. That is, each molecule of ArOH eliminates two active $\mathrm{ROO}^\bullet$ radicals and the stoichiometric coefficient *f* is 2.0:

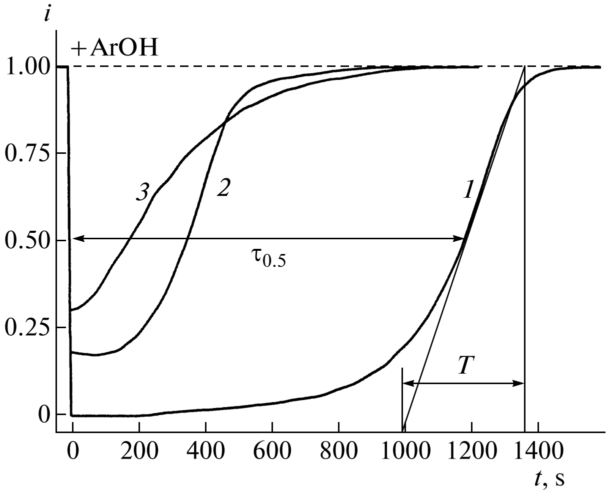

Fig. 5. Kinetics of CL on the oxidation of diphenylmethane (0.58 mol/l, 10 vol % in chlorobenzene; $[\mathrm{AIBN}] = 1.0 \times 10^{-4}$ mol/l; $w_\mathrm{i} = 3.3 \times 10^{-10}$ mol $\mathrm{l^{-1}\,s^{-1}}$; [Eu chelate] $= 2.0 \times 10^{-4}$ mol/l; 50°C) after the introduction of antioxidants: (*1*) $\mathrm{CrC_1}$, (*2*) Ch6, and (*3*) CA. $[\mathrm{ArOH}]_0 = 2.0 \times 10^{-7}$ mol/l. For curve *1*, the kinetic parameters are shown: the reciprocal slope *T* and the induction period $\tau_{0.5}$.

$$f[\mathrm{ArOH}]_0 = w_\mathrm{i}\int_0^\infty (1 - i)\mathrm{d}t = w_\mathrm{i}\tau_{0.5}. \quad (8)$$

From these equations and experimental data, $f[\mathrm{ArOH}]_0$ or the rate w_i (i.e., one of these quantities if the other is known), the rate constants ratio $k_7/(2k_6)^{1/2}$, and the constant k_7 (if $2k_6$ is known) are calculated. The use of the CL semirecovery time $\tau_{0.5}$ in place of the integral and Eq. (8) simplifies the procedure but somewhat overestimates the value of $f[\mathrm{ArOH}]_0$ (by no more than 2% [10]).

In Fig. 5, it can be seen that the effects of 3,4-dihydroxychalcone (Ch6) and CA (curves *2* and *3*, respectively; cf. the shape and slope of a CL recovery region) are comparable with the effect of $\mathrm{CrC_1}$ (curve *1*) at the same introduced concentration $[\mathrm{ArOH}]_0$. The maximum slopes $(\mathrm{d}i/\mathrm{d}t)_{\max}$ (i.e., $1/T$) are similar; consequently, the rate constants k_7 are also similar. However, the quenched light sum is much smaller, and this means that a considerable fraction of the antioxidant and/or its radical is consumed without participation in the scavenging of peroxide radicals. Therefore, the stoichiometric coefficient *f* should be calculated from

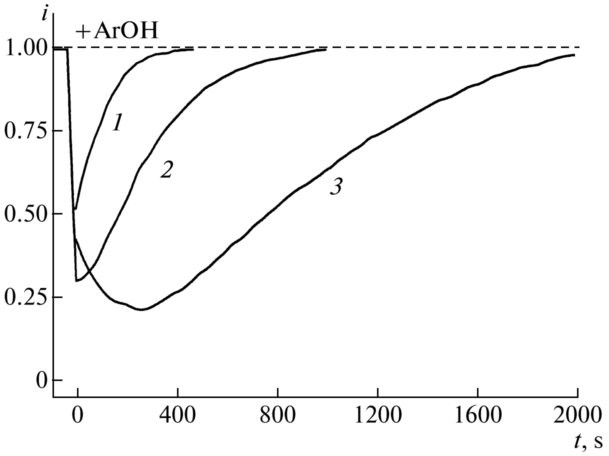

Fig. 6. Kinetics of CL on the oxidation of diphenylmethane after the addition of various concentrations of CA. $[\mathrm{ArOH}]_0 \times 10^7$, mol/l: (*1*) 0.4, (*2*) 2.0, and (*3*) 8.0. Experimental conditions are specified in Fig. 5.

the initial formula $f = w_\mathrm{i}\int_0^\infty (1-i)\mathrm{d}t/[\mathrm{ArOH}]_0$ (cf. Eq. (8)); it is 0.7–0.3 (see below).

At high initial concentrations of ArOH, CL was incompletely weakened (Figs. 6 and 7, curves *3*). The calculated rate constants also depend on $[\mathrm{ArOH}]_0$. In addition, after the complete consumption of the antioxidant, the rate of initiation was found higher (by 10–20%) than that at the onset of the experiment. Evidently, all of the above special features are related to processes that are not taken into account in the scheme. This is true in full measure for both chalcones and a number of structurally related phenols [18].

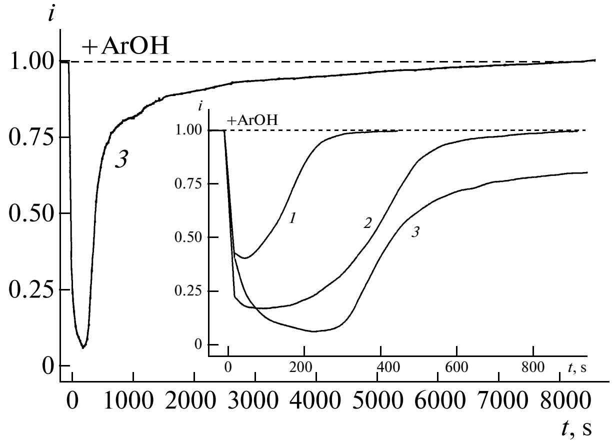

Fig. 7. Kinetics of CL on the oxidation of diphenylmethane after the addition of various concentrations of Ch6. $[\mathrm{ArOH}]_0 \times 10^7$, mol/l: (*1*) 0.4, (*2*) 2.0, and (*3*) 8.0. The insert shows the initial portions of kinetic curves *1–3*; *3* shows the result of a long (2-h) experiment. Experimental conditions are specified in Fig. 5.

Extended Scheme of Antioxidant Action, Participation of Oxygen

To interpret the experimental data, there were attempts to extend the kinetic scheme. For example, Belyakov et al. [18] formally introduced (without discussing the chemistry and structure of reactants) reaction (XVII) of phenoxyl radicals, which is additional to reaction (VIII):

$$\mathrm{OAr}^{\bullet} \rightarrow \text{product “r” } (+\mathrm{O_2}) \rightarrow \mathrm{rOO}^{\bullet} \qquad \text{(XVII)}$$

(in an excess of oxygen, k_{17} is the apparent rate constant of first-order reaction).

Loshadkin et al. [19] also formally introduced the bimolecular prooxidant reaction

$$\mathrm{OAr}^{\bullet} + \mathrm{O_2}\ (\mathrm{O_2}, \mathrm{RH}) \rightarrow \text{quinone}$$

$$(\text{like } \mathrm{O{=}Ar_{-H}{=}O}) + \mathrm{ROO}^{\bullet} + \mathrm{H_2O_2}.$$

Vasil'ev and Trofimov [20] discussed the addition of $\mathrm{O_2}$ to a phenoxyl radical $\mathrm{OAr}^{\bullet}$, that is, in fact, the reaction between two radical centers (triplet $\mathrm{O_2}$ can be considered as a biradical). The PM3 calculation for the case of ArOH = catechol demonstrated that, along with the expected peroxide radical $\mathrm{OArOO}^{\bullet}$, the C-centered radicals of 1,2-dioxetane can be formed (Fig. 8).

The reaction is weakly endothermic or weakly exothermic (Table 2); the activation energy was estimated at a few kcal/mol using the PM3 method (saddle energy, calculation with the keyword SADDLE). Dioxetane is a bicyclic compound; that is, its structure is more ordered than that of the starting pair $\mathrm{OAr}^{\bullet}$ + $\mathrm{O_2}$. Thus, the expected negative entropy change does not imply an equilibrium shift toward dioxetane, and

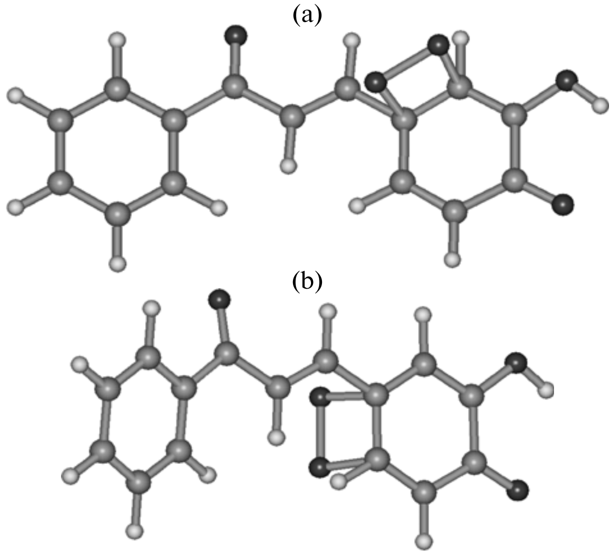

Fig. 8. Structure of the C-radicals of 1,2-dioxetane (a) $\mathrm{D}_1^{\bullet}$ and (b) $\mathrm{D}_2^{\bullet}$, products of the addition of $\mathrm{O_2}$ to the radical $\mathrm{OAr}^{\bullet}$ of chalcone Ch6; $\Delta H_\mathrm{f}^0(\mathrm{D}_1^{\bullet}) = -19.9$ kcal/mol; $\Delta H_\mathrm{f}^0(\mathrm{D}_2^{\bullet}) = -27.1$ kcal/mol.

Table 2. Heats of formation (ΔH_f^0) of dioxetane radical $D^\bullet$ (product of the addition of O_2 to $OAr^\bullet$) and the heats of the reaction $O_2 + OAr^\bullet \longrightarrow D^\bullet$ (ΔH) calculated by the PM3 method

ArOH	ΔH_f^0 ($D^\bullet$), kcal/mol	ΔH, kcal/mol	Reaction type: endo/exothermic
Ch6	−27.1	1.9	Endo
CA	−106.8	5.5	Endo
FA	−99.3	2.8	Endo
Ch4	−20.0	−2.4	Exo
Ch2	21.4	−0.3	Exo
Ch5	−20.2	−2.5	Exo
Catechol	−40.3	−4.1	Exo
	−32.4	3.8	Endo
Phenol	12.09	1.4	Endo
Guaiacol	−29.44	−4.0	Exo
	−24.34	−1.1	Exo

the rate constant of the reaction of $OAr^\bullet$ with O_2, of course, is several orders of magnitude lower than that of diffusion. However, we can expect that it can compete with the rapid reaction $OAr^\bullet + ROO^\bullet \rightarrow$ (see the scheme) because the concentration of O_2 in oxidized solution is higher by several orders of magnitude than the steady-state concentration of $ROO^\bullet$ radicals.

Note that even the complete removal of phenoxyl radicals from the antioxidant action would decrease the stoichiometric coefficient f only by a factor of 2—from 2 to 1. Indeed, f is much lower than unity (Table 3); this fact suggests that the scheme of inhibited oxidation should be seriously revised (complicated).

Nevertheless, even the formal introduction of only one side reaction, $OAr^\bullet + O_2$, allowed us to obtain k_7 rate constant values that are more realistic. Belyakov et al. [18] found by calculations that, in this case, Eq. (5) is replaced by the equation

$$2k_7[\mathrm{ArOH}] = (2k_6w_i)^{1/2}(i^{-1/2} - i^{1/2}) \times [1 + (k_{17}/k_8)(2k_6/w_i)^{1/2}i^{-1/2}], \quad (9)$$

and k_{17} is calculated from the dependence of i on [ArOH]:

$$k_{17} = k_8(2k_6w_i)^{-1/2}(2 - f)[\mathrm{ArOH}]/\int_0^\infty(i^{-1/2} - i^{1/2})dt.$$

Table 3 illustrates the degree of coincidence between calculated parameters upon the treatment of the results of measurements by various procedures. Note that the calculation of k_7 from the maximum slope of CL recovery, that is, by classical Eq. (7), resulted in values close to those obtained from modified refined Eq. (9); that is, the calculation was sufficiently reliable. Comparing the slopes at the end of an induction period for various antioxidants (Fig. 5), we can rapidly evaluate the rate constants k_7.

Unlike Ch6 and CA, the antioxidant effect of the other chalcones was weak. For example, at chalcone concentrations of 2×10^{-7} mol/l, CL was quenched by a negligible value of 1–2 to 5% (Table 4); thereafter, it remained constant. For these antioxidants, $k_7[\mathrm{ArOH}] \ll 2k_6[\mathrm{ROO}^\bullet]$, and the relationship between the intensity of CL and the concentration of ArOH is expressed by the equation

$$[\mathrm{ArOH}](i^{-1} - 1)^{-1} = (k_{17}k_6/k_7k_8) + (2k_6w_i)^{1/2}(2k_7)^{-1}i^{1/2}. \quad (10)$$

Measuring CL (i.e., i) upon sequentially adding antioxidant portions, we can estimate k_7 and k_{17}, for example, by determining the intercept and slope of a linear anamorphosis of Eq. (10) in the $[\mathrm{ArOH}](i^{-1} - 1)^{-1} - i^{1/2}$ coordinates.

On the Role of the Second O—H Bond in a Catechol-Type Phenol

The O—H bond order in the phenoxyl radicals of Ch6 chalcone and CA is 0.92, that is, smaller than unity, and we can expect that this bond is weakened. The

Table 3. Rate constants k_7 and k_{17} of the reactions of Ch6 and CA.* Estimation from a maximum slope of CL reduction: (A) from Eq. (7), (B) from classical Eq. (5), and (C) from modified Eq. (9)

[ArOH] × 10^7, mol/l	f	k_{17}, s^{-1}	k_7, 10^{-6} l mol^{-1} s^{-1}**		
			A	B	C
Ch6					
0.4	0.7	0.9	11.0	2.7	7.6
2.0	0.5	1.0	8.7	1.1	4.8
8.0	0.4	1.4	9.9	0.5	4.5
CA					
0.4	0.5	2.2	10.2	1.9	9.6
2.0	0.4	2.1	4.6	0.7	4.3
8.0	0.3	2.3	1.9	0.2	1.8

* Experimental conditions are specified in Fig. 5.

** $2k_6 = 1.5 \times 10^8$ l mol^{-1} s^{-1}; $k_8 = 5.0 \times 10^8$ l mol^{-1} s^{-1}.

Table 4. CL attenuation after the addition of an antioxidant

ArOH	CL intensity, % on an i_0 basis	
	[ArOH], mol/l	
	2.0×10^{-7}	8.0×10^{-7}
CrC_1	0	0
Ch6	18	6
CA	30	22
Ch1	96	85
Ch2	99	94
Ch3	92	76
Ch4	95	86
Ch5	98	93

Note: Experimental conditions are specified in Fig. 5.

bond cleavage product is *o*-quinone. In the case of Ch6 chalcone, the PM3 estimation gave ΔH_f^0(quinone) = –22.09 kcal/mol. Consequently, considering the hypothetical reaction of O–H bond cleavage in $OAr^•$

$$OAr^• = \text{quinone} + H,$$

we obtain $D(O{-}H) = \Delta H_f^0(\text{quinone}) + \Delta H_f^0(H) - \Delta H_f^0(OAr^•) = -22.09 + 52.10 + 29.03 = 59.04$ kcal/mol. An analogous evaluation for D(O–H) in CA phenoxyl (ΔH_f^0(quinone) = –105.1 kcal/mol; $\Delta H_f^0(OAr^•)$ = –112.3 kcal/mol) resulted in D(O–H) = 59.3 kcal/mol. That is, indeed, the bond is weak and, in principle, it can be cleaved in both the reaction of phenoxyl with a peroxide radical ($OAr^• + ROO^•$) and, likely, the reaction with O_2. As for the second O–H bond in Ch6 phenol, it is stronger than the former, and its participation in reaction with $ROO^•$ is less probable. However, the OH group plays the role of an *electron-donor* substituent, which weakens the neighboring ArO–H bond, as compared with the other chalcones (cf. the values of D(ArO–H) for various phenols in Table 5). It is interesting that the electron-withdrawing substituent CN increased D(ArO–H) by 2.1 kcal/mol and thus decreased the antioxidant activity of the model tocopherol [21].

CONCLUSIONS

Thus, we studied a number of chalcones, the biological precursors of flavonoids. We estimated the rate constants (k_7) of the main reaction path, hydrogen atom abstraction from ArOH by peroxide radical $ROO^•$ with the formation of aroxyl radical $OAr^•$ (Table 5), and the efficiency of inhibition (number *f* of $ROO^•$ radicals trapped by a molecule of ArOH).

In addition to the antioxidant effect, chalcones exert a prooxidant effect because of the participation of an oxygen molecule at the steps following the formation of radical $OAr^•$. The structure and energetics of possible products of the reaction of phenoxyl radicals $OAr^•$ with O_2 were calculated using the semiempirical PM3/PM6 method. It was found that adducts can be not only peroxide radicals $OArOO^•$ but also dioxetane radicals—the products of cyclization, that is, the addition of the terminal atom of $OArOO^•$ to carbon of the Ar ring. The reaction is almost thermally neutral; consequently, it is highly probable and can be a path involving O_2.

Table 5. Heats of formation (ΔH_f^0) of phenols and phenoxyl radicals calculated by the PM6 method, O–H bond energies D(O–H)*, and rate constants k_7^{**} and k_{17}

Phenols	ΔH_f^0 (ArOH)	ΔH_f^0 ($OAr^•$)	D(O–H)*	k_7^{**}, l mol^{-1} s^{-1}	k_{17}, s^{-1}
	kcal/mol				
Ch6	–54.8	–27.7	79.2	8.7×10^6	1.0
CA	–134.7	–110.2	76.6	4.6×10^6	2.1
CrC_1	–109.6	–83.7	78.0	7.6×10^6	–
FA	–134.7	–103.0	83.9	3.6×10^4	0.6
Ch5	–50.8	–18.3	84.5	1.2×10^4	0.1
Ch3	–14.4	21.4	87.9	1.1×10^5	2.5
Ch4	–57.1	–20.8	88.5	2.4×10^4	0.3
CoumA	–98.2	–61.6	88.7	2.2×10^4	0.1
Ch1	–13.8	22.8	88.7	3.9×10^4	1.9
Ch2	–13.5	23.5	89.1	1.1×10^4	1.7

* $D(ArO{-}H) = \Delta H_f^0(OAr^•) + \Delta H_f^0(H) - \Delta H_f^0(ArOH)$; $\Delta H_f^0(H^•) = 52.1$ kcal/mol.

** Estimated from experimental data for the first three substances at $[ArOH]_0 = 2.0 \times 10^{-7}$ mol/l using Eq. (7) and, for the other substances, from the dependence of *i* on [ArOH] using modified Eq. (10) at $2k_6 = 1.5 \times 10^8$ l mol^{-1} s^{-1} and $k_8 = 5.0 \times 10^8$ l mol^{-1} s^{-1}.

The compounds examined can be subdivided into two groups. 3,4-Dihydroxychalcone (Ch6), which has a catechol structure, that is, two neighboring OH groups in phenyl ring B, exhibits high antioxidant activity. This is consistent with the lowest bond strength D(O–H) = 79.2 kcal/mol. In this case, the second OH group does not react with the $ROO^{\bullet}$ radical, but it serves as an electron-donor substituent. For Ch6, the reaction of hydrogen abstraction by the $ROO^{\bullet}$ radical is the main reaction path, and the rate constant k_7 ($\sim 10^7$ l mol^{-1} s^{-1}) has the same order of magnitude as that in efficient antioxidants—chroman C_1 and caffeic acid. With consideration for similarity to a naturally occurring material and nontoxicity, this allows us to hope that chalcone Ch6 and/or other related compounds with a catechol structure can be used as stabilizers for food products and pharmaceuticals.

The other compounds are characterized by lower antioxidant activity and, correspondingly, stronger ArO–H bond: D(O–H) = 83–88 kcal/mol. The uncorrelated variation of k_7 from 10^4 to 10^5 l mol^{-1} s^{-1} and a low stoichiometric coefficient suggest that the reaction paths other than a classical path are considerable for these molecules; consequently, they hardly can serve as efficient antioxidants.

The correlation of antioxidant activity with the energy of the O–H bond was repeatedly discussed (see [14–16, 22–25] and references therein). In spite of the scatter of experimental data and theoretical evaluations, it is indubitable that the bond energy plays an important role in the reactivity of antioxidants, as evidenced, in particular, by the results of this work. In real systems, the correlation can be masked by other factors (the role of a medium, redox properties, structure peculiarities, side reactions, solubility, etc.).

The semiempirical calculation of the ArO–H bond strength is a rapid and convenient test for the applicability of one or another structure (at least among structurally similar compounds) as a potential antioxidant and, likely, as a test for the reasonability of the synthesis of the given molecule.

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