Chain-breaking antioxidant activity of two new chalcones from propolis of El Salvador in homogeneous and micellar media

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Dedicated to Academician Ivan Juchnovski on the occasion of his 70th birthday

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The chain-breaking antioxidant activities of two new chalcones from propolis of El Salvador 2',3'-dihydroxy-4,4'-dimethoxy chalcone (AH 2) and 2',3',4-trihydroxy-4'-methoxy chalcone (BH 2) were determined by applying three oxidation kinetic models in homogeneous and micellar systems: model 1 - chemiluminescence of cumene in homogeneous solution; model 2 - oxidation of methyl linoleate and ethylbenzene in homogeneous solution; and model 3 - oxidation of methyl linoleate in sodium dodecyl sulfate (SDS) micelles. The oxidation was carried out at a constant and well-controlled rate of radical generation. The results demonstrated that AH 2 and BH 2 showed moderate chain-breaking activities, higher than naringenin, but lower that caffeic acid, 2,6-di-tert-butyl-4-methylphenol (BHT) and DL-α-tocopherol in peroxidation in SDS micellar solution. The moderate chain-breaking activity of the chalcones obtained in homogeneous solution, was similar to that for BHT, but lower than those of caffeic and sinapic acids, and higher than p-coumaric and ferulic acids. Full geometric optimization of AH 2 and BH 2 and three-dimensional structures filed as Z matrices were obtained with Chem 3D MOPAC energy minimization at the AM1/RHF level. The determination of the hyperfine coupling constants was carried out with density-functional theory (DFT) calculations, using the B3LYP hybrid function, the CPCM option for considering the solvent effect for the mostly dissociated aroxyl radicals and the basis sets 6-311 G* and 6-311G** of the Gaussian 98 program.

Key words: Antioxidants, propolis, kinetics, methyl linoleate, chalcones.

INTRODUCTION

Chalcones are natural precursors of flavones and flavanones. These polyphenolics are multifunctional and can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers [1–4] (Fig. 1). It is amazing that propolis samples of different geographic and plant origin and with different chemical composition possess pronounced antioxidant properties. The beneficial effects of antioxidant activity of propolis against oxidation stress and cancer, i.e. the important role of propolis on human health, have been proven by experimental, chemical and epidemiological data [3–5]. Two new chalcones (AH 2 and BH 2, Fig. 2), isolated from El Salvador propolis [6], display pronounced biological activity, particularly significant antibacterial and antifungal activity and moderate toxicity to Artemia salina nauplii. This observation, as well as the fact that these chalcones are rather accessible substances of low-toxicity, makes the idea intriguing to apply them as stabilizers with antioxidative action for food, cosmetics and other lipid-containing products.

The chain-breaking antioxidative abilities of chalcones were determined by applying three kinetic models:

Model 1: chemiluminescence of cumene in homogeneous solution;

Model 2: oxidation of methyl linoleate (ML) and ethylbenzene (EB) in homogeneous solution;

Model 3: oxidation of ML in sodium dodecyl sulfate (SDS) micelles.

The oxidation was carried out at a constant and well-controlled rate of free radical generation in all models by using free-radical initiators: lipid-soluble azo compound 2,2'-azobisisobutyronitrile (AIBN) for models 1 and 2 and the water-soluble 2,2'-azo-bis(4-carboxyisovalero)-nitrile (ACVN) for micellar model 3 (this model imitated peroxidation in heterogeneous lipid systems and under in vivo conditions).

A antioxidative activity of new chalcones AH 2 and BH 2 studied are compared at the same molar concentration with the flavonoid naringenin (Ng) possessing a similar structure and with the following reference compounds: 2,6-di-tert-butyl-4-methyl-
phenol (BHT), DL-α-Tocopherol (TOH), caffeic (CA), ferulic (FA), sinapic (SA) and p-coumaric (p-CA) acids.

AH₂ and BH₂ have quite different structures in comparison with previous chalcones from the propolis reported. There are no literature data (for comparison) concerning their full geometry optimization as well as hyperfine coupling constants for such a kind of chalcones (neither theoretical, nor experimental). This study illustrates the possibility of using two new chalcones from propolis of El Salvador as effective chain-breaking antioxidants during lipid peroxidation in homogeneous and micellar systems.

Fig. 1. Structure of chalcones and flavones.

Fig. 2. Structure of chalcones and all possible aroxyl-anion radicals.
EXPERIMENTAL

2',3'-dihydroxy-4,4'-dimethoxychalcone (AH₂) and 2',3',4-trihydroxy-4'-methoxy-chalcone (BH₂) were isolated from propolis of El Salvador and characterized by mass spectra, UV, ¹H- and ¹³C-NMR spectra. The isolation procedure was described in Popova et al. [6]. All chemicals were of highest quality and were purchased from Merck (Germany): anhydrous sodium phosphates, Na₂HPO₄ and Na₂PO₄, the initiator AIBN, the chemiluminescence activator 9,10-dibromantracene, cumene, ethylbenzene and chlorobenzene were purified by standard methods. Methyl linoleate, Stock solutions, depending on solubility, in dimethyl sulfoxide, chlorobenzene, water, or in mixture of these solvents. The kinetics of oxygen consumption was studied with a Yellow Springs Instrument Co. (Yellow Springs, OH) Model 5300 Biological Oxygen Monitor with a Clark electrode as sensor. Similar to the study of the oxidation of homogeneous solutions of ML, the run started with the determination of R₀ and Rᵢ, then, aliquots of a stock solution of chalcones were added without suspending [O₂] monitoring by using microsyringe with long needle.

Quantum chemical calculations: All aryl radicals (at 2', 3' and 4 positions) formed from AH₂ and BH₂ and three-dimensional structures filed as Z-matrices were obtained with Chem 3D MOPAC energy minimization at the AM1/RHF level (CambridgeSoft, Cambridge, MA). The determination of the coupling constants was carried out with density-functional theory calculations, using the B3LYP hybrid function, the CPCPM option for considering the solvent effect for the mostly dissociated aroxyl radicals and the basis sets 6-311G* and 6-311G** of the Gaussian98 program [13].

RESULTS AND DISCUSSION

Kinetic study

Determination of the chain-breaking antioxidant activity of chalcones during the oxidation of cumene in homogeneous (chlorobenzene) solution (Model 1). It is known that during the recombination of peroxy radicals in an oxidation system, electronically excited states of carbonyl compounds are generated with a certain probability [7-9]. As a result of their relaxation to the ground state, light emission is observed (chemiluminescence, CL) that can be recorded using very sensitive photomultipliers [14, 15]. The mechanism of oxidation of cumene (RH), initiated by a free radical initiator, AIBN, in the presence of an antioxidant, A H₂, may be described by the following kinetic Scheme 1:

\[
\begin{align*}
\text{Scheme 1.} \\
\text{Chain generation (i): } & \quad \text{AIBN} + (O_2, RH) \rightarrow \text{RO}_2^* + \text{RH}^+ \quad \text{Chain termination (t): } \quad \text{RO}_2^* + \text{RO}_2^* \rightarrow \text{ROO}^\cdot + \text{RH}^+ \\
\text{Chain propagation (p): } & \quad \text{RO}_2^* + RH \rightarrow \text{ROOH} + \text{R}^* ( O_2, \text{RH}) \quad \text{k}_0 \quad \text{Inhibition 1 (A): } \quad \text{RO}_2^* + A H_2 \rightarrow \text{ROO}^\cdot + \text{AH} \quad k_A \\
\text{Disproportionation (A''':) } & \quad \text{AH} + \text{AH} \rightarrow \text{AH}_2 + \text{A} \quad k_A \\
\text{Inhibition 2 (A'): } & \quad \text{RH}^+ + \text{AH} \rightarrow \text{ROOH} + \text{AH} \quad k_A \\
\end{align*}
\]

where \(\text{RO}_2^*\) is the cumene peroxy radical, \(\text{P}^*\) - the
molecule of the oxidation product in excited state, A\(H\) is an aroxyl radical, and A – a quinone.

The CL intensity \((I)\) is proportional to the chain termination rate, i.e. to the square of the peroxyl radicals’ concentration in the system \([7-9]\):

\[ I = j \left(2k_{\text{RO}_2^*}\right)^2 \]  

(1)

where \(j\) is a proportional coefficient which depends on chemiluminescence parameters of the system oxidized and on the parameters of photometer device.

The determination of \(k_A\) actually involves the evaluation of the competition between reaction of chain termination \((k_t)\) and inhibition \((k_{inhib})\) (see Scheme 1). The antiradical activity of an inhibitor is characterized by the stoichiometric coefficient \((n)\) that is equal to the number of free radicals scavenged by one inhibitor’s molecule. The key rate constant of inhibited oxidation \((k_{inhib})\) may be calculated from the kinetic curves of CL of the inhibited oxidation from the value of the minimum intensity of CL \((I_{\text{min}})\) in the presence of the inhibitor, A\(H_2\),

\[ k_{\text{inhib}}(\text{min}) = \left(1 - I_{\text{min}}\right)/(2R_i k_t)_{0.5}/\left\{n[AH_2]I_{\text{min}}^{0.5}\right\}, \]  

(2)

and the stoichiometric coefficient of inhibition, \(n\), is determined according to \([8-10]\):

\[ n = R_i IP/[A H_2]_0 \]  

(3)

In real experiments, IP is equal to the time from the moment of the inhibitors’ injection to the moment of 50% restoration of the CL intensity. It is known that \(n\) for chroman C1, is equal to 2 \([7-9]\). Thus, the CL method allows the direct determination both of the rate constant of inhibition, \(k_t\), and the stoichiometric coefficient of inhibition, \(n\), from one and the same CL curve.

The kinetic parameters, obtained during the CL oxidation of cumene, in the presence of different concentrations of both chalcones, are presented in Table 1. It could be seen that the rate constants for inhibiting \((k_A)\) for both chalcones are almost the same, but the stoichiometric coefficients are quite different. It means that the mechanism of action of the chalcones differs from BH\(_2\) to A\(H_2\).

Table 1. Kinetic parameters characterizing the chain-breaking activity of chalcones A\(H_2\) and BH\(_2\) at various concentrations during the oxidation of cumene at 60ºC.

<table>
<thead>
<tr>
<th>Chalcone type</th>
<th>Chalcone concentration, M</th>
<th>(R_i), M/s</th>
<th>(k_A), (M/s(^{-1}))</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(H_2)</td>
<td>2.08\times10^{-6}</td>
<td>2.45\times10^{-8}</td>
<td>1.20\times10^{4}</td>
<td>0.3</td>
</tr>
<tr>
<td>A(H_2)</td>
<td>4.95\times10^{-6}</td>
<td>2.33\times10^{-8}</td>
<td>1.27\times10^{4}</td>
<td>0.5</td>
</tr>
<tr>
<td>BH(_2)</td>
<td>4.50\times10^{-6}</td>
<td>3.23\times10^{-8}</td>
<td>1.46\times10^{4}</td>
<td>1.9</td>
</tr>
<tr>
<td>BH(_2)</td>
<td>8.70\times10^{-6}</td>
<td>3.12\times10^{-8}</td>
<td>1.04\times10^{4}</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The moderate chain-breaking antioxidant activity and the decrease of the stoichiometric coefficient \(n\) are due to the increased contribution of the following side reactions \([8, 11, 12]\):

\[
\begin{align*}
AH + ROOH & \rightarrow AH_2 + RO_2^* \\
AH + O_2 & \rightarrow A + HO_2^* \\
AH + RH + O_2 & \rightarrow AH_2 + RO_2^* \\
AH - product & "r"(\cdot O_2) - rO_2^*
\end{align*}
\]

\(r\) denotes undefined structure derived from the original aroxyl radical. Belyakov et al. \([8]\) suggested that reaction (7) of the monomolecular transformation of A\(H\) (dissociation or isomerization) seems to be the most probable reason for the lower values of the stoichiometric coefficient \(n\) of some polyphenols.

The comparison of the chain-breaking activity of the two studied chalcones with known and standard antioxidants at the same experimental conditions and antioxidant’s concentrations is presented in Table 2. The results obtained demonstrated that the chain-breaking antioxidant activity of A\(H_2\) and BH\(_2\) in reaction with peroxide radicals, \(k_A\), in a homogeneous solution is similar to those obtained for BHT, but lower than those of caffeic (CA) and sinapic (SA) acids, and higher than p-coumaric (p-CA) and ferulic (FA) acids. It could be seen that \(k_A\) decreases in the following sequence:

Cr\(_{C1}\) \((1.2\times10^5) > CA \ (8.3\times10^4) > SA \ (2.5\times10^4) > BH\(_2\) \( (1.5\times10^4)^2 \ BHT \ (1.4\times10^4)^2 \ A\(H_2\) \ (1.3\times10^4)^2 > FA \ (0.8\times10^4) > p-CA \ (0.7\times10^4)\).

Table 2. The values of the rate constant \((k_A)\) and the stoichiometric coefficient \((n)\) obtained from the kinetics of cumene oxidation in presence of A\(IBN\), 60ºC, for known antioxidants (p-CA, FA, SA, CA, BHT and Croman C\(_{1}\)) as well as for the tested chalcones A\(H_2\) and BH\(_2\).

<table>
<thead>
<tr>
<th>Phenolic antioxidant</th>
<th>(k_{inhib}) (M/s(^{0.5}))</th>
<th>(n)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Coumaric acid (p-CA)</td>
<td>0.7\times10^4</td>
<td>1.9</td>
<td>[9]</td>
</tr>
<tr>
<td>Ferulic acid (FA)</td>
<td>0.8\times10^4</td>
<td>3.5</td>
<td>[9]</td>
</tr>
<tr>
<td>Sinapic acid (SA)</td>
<td>2.5\times10^4</td>
<td>2.6</td>
<td>[9]</td>
</tr>
<tr>
<td>Caffeic acid (CA)</td>
<td>8.3\times10^4</td>
<td>2.5</td>
<td>[9]</td>
</tr>
<tr>
<td>Butylated hydroxyltoluene (BHT)</td>
<td>1.4\times10^4</td>
<td>2.0</td>
<td>[9]</td>
</tr>
<tr>
<td>Chroman C(<em>{1}) (Cr(</em>{C1}))</td>
<td>11.6\times10^4</td>
<td>2.0</td>
<td>[9]</td>
</tr>
<tr>
<td>Chalcone BH(_2)</td>
<td>1.46\times10^4</td>
<td>1.9</td>
<td>This work</td>
</tr>
<tr>
<td>Chalcone A(H_2)</td>
<td>1.27\times10^4</td>
<td>0.5</td>
<td>This work</td>
</tr>
</tbody>
</table>

Kinetics of oxygen consumption during oxidation of ML and EB in homogeneous (chlorobenzene) solutions and in the presence of chalcones (Model 2). The basic kinetic scheme of lipid (LH) oxidation under conditions of a constant rate of free-radical
generation due to the thermo-decompositions of a free-radical initiator, \( Y \), in the presence of an antioxidant \( (A_H^2) \) is presented in Scheme 2 [12]:

\[
\begin{align*}
(i) \ Y (+LH,+O_2) & \rightarrow LO_2^- R_i \\
(p) \ LO_2^- + LH (+O_2) & \rightarrow LOOH + LO_2^- \text{ Chain generation} \\
(t) \ LO_2^- + LO_2^- & \rightarrow \text{products} \text{ Chain propagation} \\
(A) \ LO_2^- + AH & \rightarrow LOOH + AH \text{ Inhibition 1} \\
(A') \ AH + LO_2^- & \rightarrow AH-OOL \text{ Inhibition 2} \\
(A'') \ AH + AH & \rightarrow AH_2 + A \text{ Disproportionation}
\end{align*}
\]

Scheme 2.

where \( LO_2^- \) are the lipid peroxide radicals, \( LOOH \) – lipid hydroperoxides. In the absence of antioxidants, the rate of chain oxidation is as follows:

\[
R_0 = \frac{d[O_2]}{dt} = \frac{d[LOOH]}{dt} = k_i[AH_2] \left( R_i/k_i \right)^{1/2} \tag{8}
\]

In the presence of an antioxidant when \( k_a[PhOH] \) is high enough so that \( R \ll R_0 \), the system is in the induction period. The duration of the induction period, \( IP \), and the rate of chain inhibited oxidation \( R_A \) are given by:

\[
\begin{align*}
IP &= n[AH_2]_0/R_i \\
R_A &= k_p[R][LH]/n \cdot k_A[AH_2]_0 \tag{10}
\end{align*}
\]

where \( [AH_2]_0 \) is the starting concentration of inhibitor. In the classic variant under consideration, a value of \( n \) for phenolic antioxidants containing only one active \(-OH\) group is generally equal to two without reference to whether \( AH \) decays in the reaction of chain inhibition 1 and 2. When \( AH \) decays by reaction of chain inhibition 2, Eqn. (10) remains valid, but \( n \) changes from 2 to 1 [11, 12]. In conclusion, for a classic phenol \( IP \) increases directly with \( [AH_2]_0 \) and \( R_A \) decreases inversely with \( [AH_2]_0 \).

Eqs. (9) and (10) have been confirmed experimentally many times for a phenol with two \(-OH\) groups, as well as for many flavonoids and other catechol derivatives.

Table 3 presents the experimental conditions and the main kinetic parameters obtained during lipid (ML) and model hydrocarbon (EB) oxidation in the presence of BH\(_2\). It could be seen that chalcone BH\(_2\) demonstrates also moderate chain-breaking activity, which is almost the same in the both oxidizing systems (ML and EB). It is also evident that the value of \( k_a \) of BH\(_2\) in ML is lower than in EB. Roginsky [16] reported that \( k_a \) has a lower value in ML than in hydrocarbons (cumene, ethylbenzene, etc.) and explained this phenomena with the possible H-bond formation between carbonyl group (\( C=O \)) of ML and \(-OH\)-group of the phenol. The maximal effect of the \( k_a \) decrease in ML oxidation was obtained for 4-methoxyphenol (a phenol without substitutions in ortho- position). Under these conditions, the chain length (\( v \)) of ML and EB oxidation process was found to be 72 for ML and 8 for EB, respectively.

**Table 3** Kinetic parameters, characterizing the chain-breaking activity of chalcone BH\(_2\) obtained from the kinetics of oxygen absorption in methyl linolate and ethyl benzene in presence of an initiator AIBN, 60ºC.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>[BH(_2)]</th>
<th>( R_i )</th>
<th>( R_0/R_A )</th>
<th>( k_a ) (( M ))</th>
<th>( v )</th>
<th>( R_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl linolate</td>
<td>0.1</td>
<td>5.0 ( \times 10^{-4} )</td>
<td>4.5</td>
<td>1.5 ( \times 10^4 )</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Ethyl benzene</td>
<td>0.1</td>
<td>1.0 ( \times 10^{-7} )</td>
<td>4.0</td>
<td>2.0 ( \times 10^4 )</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Determination of the inhibiting activities of chalcones during the oxidation of ML in SDS micelles (Model 3). This model imitates peroxidation in heterogeneous lipid systems and under in vivo conditions. With this model, it is possible to determine an inhibiting activity of both lipid- and water-soluble chain-breaking antioxidants. In contrast to ML oxidation in homogeneous (chlorobenzene) solution, where \( R \) is proportional to \( (R_i)^{1/2} \), reflecting the bimolecular chain termination \( (LO_2^- + LO_2^- - \text{ products}) \), in SDS micellar solutions, \( R \) is nearly proportional to \( R_i \) [12]. The latter means that chain termination occurs by first-order reaction with respect to \( [LO_2^-] \) [12]:

\[
LO_2^- - \text{ products} \quad k_t \quad (\cdot)^t
\]

For this reason, the kinetic scheme under consideration includes reactions (i), (p), (\( \cdot \)), (A), (A'). The following system of differential equations corresponds to this scheme:

\[
\begin{align*}
d[LO_2^-]/dt &= R_i - k_a[LO_2^-][AH_2] - k_a[LO_2^-][A_H] - k_a[LO_2^-][AH_2] \\
-d[AH_2]/dt &= k_a[LO_2^-][AH_2] \quad (11) \\
\end{align*}
\]

The rate of non-inhibited oxidation equals

\[
R_0 = k_p[LH]/R_i/k_t \tag{14}
\]

The rate of inhibited oxidation and the relative rate of oxidation are:

\[
R = k_p[LO_2^-][LH] \tag{15}
\]

\[
R/R_0 = k_t[LO_2^-]/R_i \tag{16}
\]
The solution is given for quasi-stationary approximation for all free radicals. Combining Eqns. (14), (15) and (16) results in:

$$R_i - k_t[LO_2^•] - 2k_A[LO_2^•][AH_2] = 0 \quad (17)$$

and the $k_A/k_p$ ratio, which determines actually the inhibiting ability of phenols during the oxidation of ML, may be calculated from the kinetics of oxygen consumption with the help of Eqn. (18):

$$F = R_0/R - 1 = 2k_A R_0[AH_2]/k_p R_i[LH] + \text{const} \quad (18)$$

The tested chalcones $AH_2$ and $BH_2$ displayed an ability to depress ML chain oxidation in the system under consideration. The values of $k_A/k_p$ calculated from the slope of the line by using Eqn. (18) (the plot of $F$ vs $[AH_2]$, resp. $[BH_2]$ - see Fig. 3) are listed in Table 4. Structure-activity relationship was studied by using comparable kinetic analysis with other antioxidants, studied at the same experimental conditions. For that reason, the chain-breaking antioxidant activity of the studied chalcones ($AH_2$ and $BH_2$) is compared at the same molar concentration with the flavonoid naringenin ($Ng$) of similar structure and with the reference compounds: 2,6-di-tert-butyl-4-methylphenol ($BHT$), DL-$\alpha$-Tocopherol ($TOH$). The data shown in Fig. 3 and Table 4 demonstrate the moderate chain breaking antioxidant activity of $AH_2$ (2.3) and $BH_2$ (7.7), which is higher than naringenin (0.5), but lower than CA (23.0), $BHT$ (240.0) and $TOH$ (290.0).

The antioxidant activity of chalcones is based mainly on the chalcone-chalcone-quinone redox system (Fig. 4). Chalcones ($AH_2$ and $BH_2$) may act as radical scavengers and produce chalcone-semiquinone radicals ($AH$, $BH$). The bimolecular recombination with disproportionation reaction of these two chalcone-semiquinone radicals ($AH$, $BH$, Fig. 4) may form a molecule of chalcone-quinone ($A$, $B$) and regenerated molecule of chalcone ($AH_2$, $BH_2$). Figures 4a and 4b present the reactivity, transformation and degradation products of $AH_2$ and $BH_2$ during the oxidation process.

![Fig. 3. Kinetics of oxygen consumption during the oxidation of 0.02 M methyl linoleate in 0.2 M SDS micellar solution in 0.05 M Na phosphate buffer pH = 7.40 at 37°C ($R_i = 1.65 \times 10^{-9}$ M/s) in the presence of $AH_2$ and $BH_2$.](image)

**Table 4.** Kinetic parameters characterizing the chain breaking activity of chalcones $AH_2$ and $BH_2$ and known antioxidants during the methyl linoleate oxidation in SDS micellar solution, 37°C.

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>$k_A/k_p$</th>
<th>Ref.</th>
<th>Antioxidant</th>
<th>$k_A/k_p$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naringenin</td>
<td>0.5</td>
<td>[12]</td>
<td>Cafeic acid</td>
<td>23</td>
<td>[12]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AH_2$</td>
<td>2.3</td>
<td>This work</td>
<td>Tocopherol</td>
<td>290</td>
<td>[12]</td>
</tr>
</tbody>
</table>

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Fig. 4a. Reactivity, transformation and degradation products for chalcone AH\textsubscript{2}.

Fig. 4b. Reactivity, transformation and degradation products for chalcone BH\textsubscript{2}.
Table 5. Theoretical coupling constants of aroxyl radicals from new chalcones of El Salvador propolis.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Hx</th>
<th>Hy</th>
<th>Hx'</th>
<th>Hy'</th>
<th>Hα</th>
<th>Hβ</th>
<th>H2</th>
<th>H3</th>
<th>H5</th>
<th>H6</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BH2 rad2'</td>
<td>CA</td>
<td>4.6903</td>
<td>1.2380</td>
<td>0.2464</td>
<td>0.0180</td>
<td>0.0065</td>
<td>0.0029</td>
<td>0.0020</td>
<td>0.0074</td>
<td>CPCM mode, 6-311G*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>4.5682</td>
<td>1.2143</td>
<td>0.2474</td>
<td>0.0185</td>
<td>0.0066</td>
<td>0.0029</td>
<td>0.0020</td>
<td>0.0076</td>
<td>CPCM mode, 6-311G**</td>
<td></td>
</tr>
<tr>
<td>BH2 rad3'</td>
<td>CA</td>
<td>4.7419</td>
<td>1.2047</td>
<td>0.2662</td>
<td>0.0100</td>
<td>0.0031</td>
<td>0.0031</td>
<td>0.0007</td>
<td>0.0029</td>
<td>CPCM mode, 6-311G*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>4.6192</td>
<td>1.1817</td>
<td>0.2675</td>
<td>0.0106</td>
<td>0.0033</td>
<td>0.0032</td>
<td>0.0007</td>
<td>0.0030</td>
<td>CPCM mode, 6-311G**</td>
<td></td>
</tr>
<tr>
<td>BH2 rad4</td>
<td>CA</td>
<td>0.8403</td>
<td>0.0031</td>
<td>3.4360</td>
<td>7.4023</td>
<td>1.8274</td>
<td>5.2172</td>
<td>4.7962</td>
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Quantum chemical calculations

Calculated coupling constants for all possible aroxyl radical-anions formed from AH2 and BH2 (see Fig. 2) are shown in Table 5. It is no doubt that the chalcones AH2 will produce aroxyl radicals only in ring a. The calculated hyperfine coupling constants for aroxyl radicals in positions 2' and 3' for chalcone BH2 showed no differences. Therefore, it is impossible to predict which kind of aroxyl radical (in which position of the same ring) will be formed on the base of DFT calculations. From the point of view of theory of phenol antioxidant's efficiency, it seems to be more easily the formation of aroxyl radical AH in position 3' than in position 2'. It is well-known, that the addition of electron-donating constituents on ortho-position increases the hydrogen abstraction (respectively to the radical formation) of the phenol group. In our case, the hydrogen abstraction in position 3' (ring a) is much possible than in position 2', due to the double substituents –OCH3 and –OH existing in ortho-position of 3' phenol group. It must be noted that the reactivity of OH groups in ring a is expected to be much lower than of OH group in ring b [1, 8, 11, 12].

From the calculated hyperfine coupling constants of AH radical in position 4 (ring b), it is evident that they are drastically different in comparison with those of aroxyl radicals formed in ring a (see the data in Table 5). It is clear that the formation of an aroxyl radical in ring b for the chalcone BH2 demonstrated a stronger effect on the calculated hyperfine coupling constants of α and β hydrogen atoms than the effect observed in the case of aroxyl radicals (2' or 3') formed in ring a. No differences were observed for the both chalcones AH2 and BH2 in the coupling constants of hydrogen atoms near to the radicals formed in ring a. The differences for the calculated hyperfine coupling constants of aroxyl radicals formed in ring a and b (in the case of chalcone BH2) are significant.

AM1/MOPAC full geometry optimization of the geometry of these chalcones and the corresponding aroxyl radicals is shown in Figure 5. A formation of hydrogen bonding between H and OH group in position 2' and oxygen from the neighbouring C=O group (close distance is about 1.42 Å) for the chalcone BH2 is observed. In contrast, in case of AH2, the two rings are turned on about 30º and there is no possible formation of hydrogen bond (close distance in this case is almost 2.52 Å).
Fig. 5b. Equilibrium structural parameters for the chalcones A H2 and B H2.

CONCLUSIONS

New data demonstrate a moderate chain-breaking antioxidant activity of the new chalcones A H2 and B H2 isolated from propolis of El Salvador in homogeneous and in SDS micellar systems. At the present time it is difficult to suggest either mechanism as the only reason for the moderate antioxidative capability of both chalcones. Most likely, all arguments presented are valid, but their real contributions still have not be determined. The calculations of the theoretical coupling constants of aroxyl-anion radicals by density-functional theory, allow to show the absolutely different type of aroxyl radical-anions, formed from the tested chalcones. Further run of EPR experiments will allow also permitting which type of these aroxyl radicals are indeed formed from the two chalcones. These new data about the chain-breaking antioxidant activity for both chalcones are important, especially from the point of view of their possible application as bio-antioxidants for stabilization of lipids and lipid-containing products.

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Посветена на акад. Иван Юхновски по повод на 70-та му годишнина

Постъпила на 31 януари 2008 г.; Преработена на 11 февруари 2008 г.

(Резюме)

Прекъсващата вегиетата антиоксидантна активност на два нови халкони от прополис от Ел Салвадор в хомогенна и мицеларна среда

В. Д. Къчева, В. С. Банкова

Прекъсващата веригите антиоксидантна активност на два нови халкони от прополис на Ел Салвадор представлява жълт бял или бял хомогенен разтвор, който се получава чрез прилагане на три кинетични модела на окисление в хомогена и мицеларна среда: модел 1 - хемилуминисценция на халкони в хомогениран разтвор, модел 2 - окисление на метиллинове в мицела на натриев додецилсулфат (SDS). Окислениеят е проведено при постоянна и добре контролирана скорост на инхибитър, т.е. на зараждане на радикали. Резултатите показват, че при окисление на SDS и SDS халконите A2H2 и B2H2 проявяват умерена антиоксидантна активност, прекъсващата веригите, по-висока от тази на нарингенина, но по-ниска от тази на кафеената киселина и по-висока от тази на 2,6-ди-тетротил-4-метиленол (BHT) и DL-α-токоферола. Умерена прекъсващата окислителната верига активност е наблюдавана също така за халконите в хомогениран разтвор, подобно на тази за BHT, но по-ниска от тази на кафената киселина, както и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-висока от тази на кафената киселина и по-в