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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 linoneate and ethylbenzene in homogeneous solution; and model 3 - oxidation of methyl linoneate 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 signifycant 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 linoneate (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). Antioxidant 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-

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phenol (BHT), DL-α-Tocopherol (TOH), caffeic (CA), ferulic (FA), sinapic (SA) and *p*-coumaric (*p*-CA) acids.

AH_2 and BH_2 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.

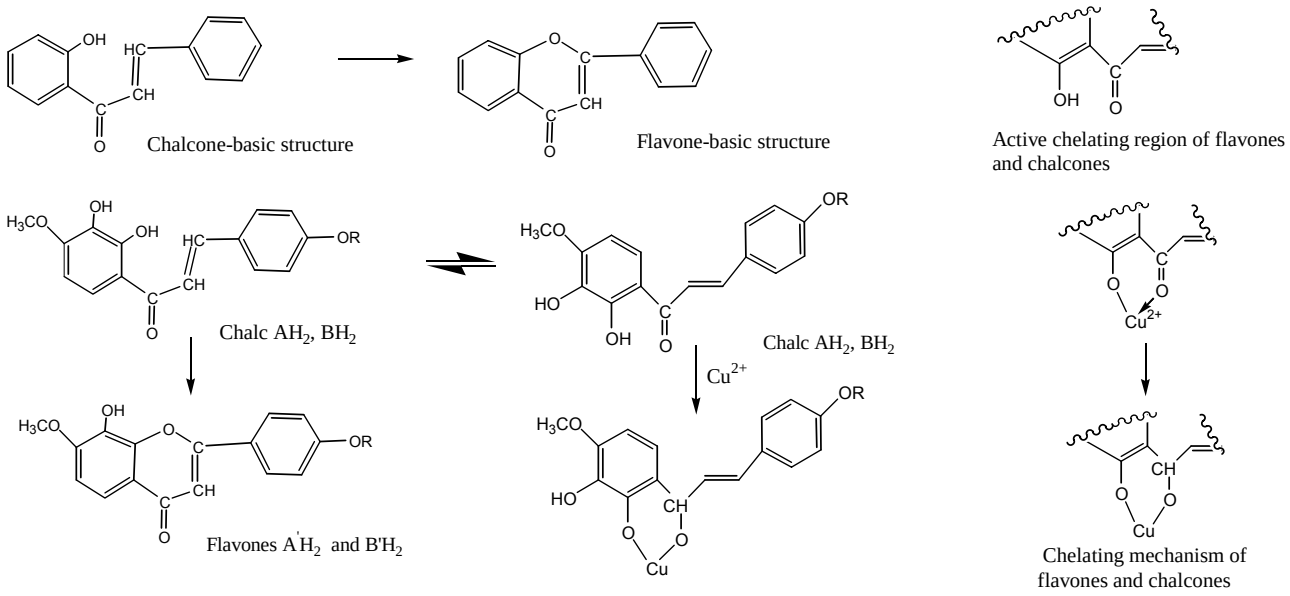


Fig.1. Structure of chalcones and flavones.

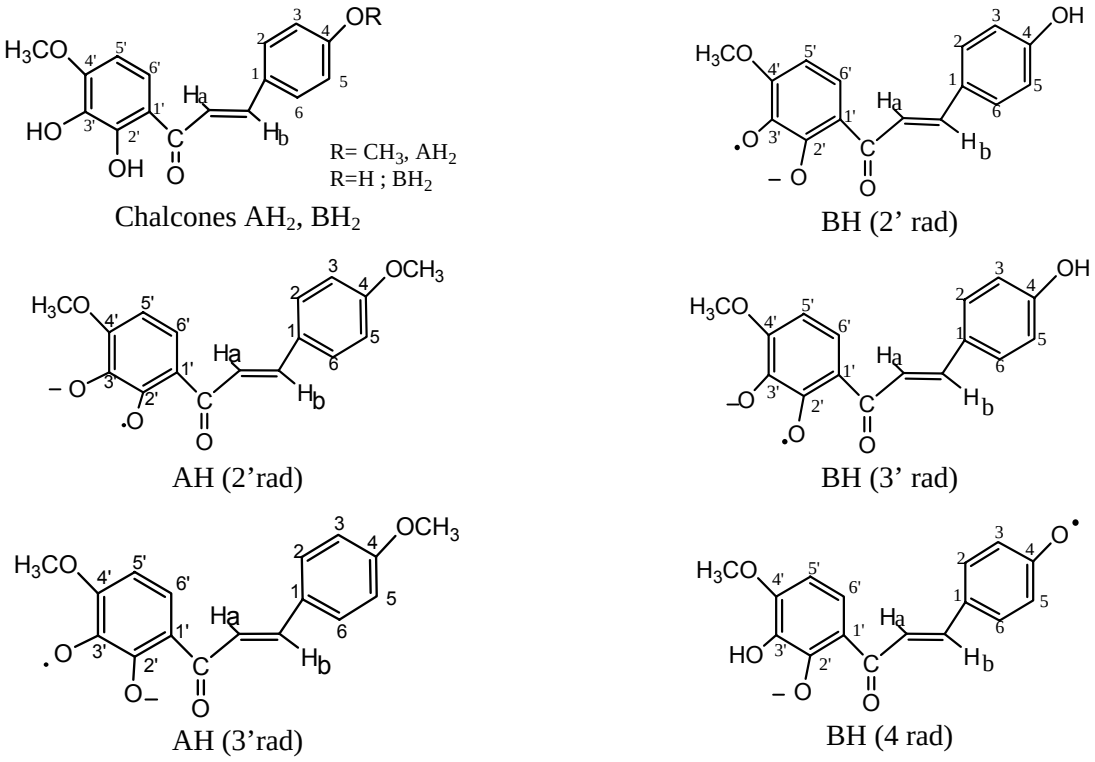


Fig. 2. Structure of chalcones and all possible aroxyl-anion radicals.

EXPERIMENTAL

2',3'-dihydroxy-4,4'-dimethoxychalcone (AH_2) and 2',3',4-trihydroxy-4'-methoxy-chalcone (BH_2) were isolated from propolis of El Salvador and characterized by mass spectra, UV, ^{1}H- and ^{13}C-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, Na_2HPO_4 and Na_2PO_4, the initiator AIBN, the chemiluminescence activator 9,10-dibromantracene, cumene, ethylbenzene and chlorobenzene were purified by standard methods. Methyl linoleate (Sigma, Germany) and ACVN (Deisenhofen, Germany) were used as received.

Model 1: The inhibited cumene oxidation was studied by means of a chemiluminescence method (CL) that is known to be one of the most sensitive and informative methods to explore the reactivity of chain-breaking antioxidants [7–9]. The oxidation of cumene (3.6 M) was carried out in chlorobenzene as a solvent in 1:1 ratio, at 60ºC, under air atmosphere. AIBN was used as a free radical initiator. The rate of free radical generation (R_i) was determined in every experiment from the induction period, IP, caused by adding the strong inhibitor 2,2,5,7,8-pentamethyl-6-chromanol (chroman C1, a synthetic analogue of TOH) at known concentration [7–9].

Model 2: The kinetics of oxygen consumption during the oxidation of chlorobenzene solutions of methyl linoleate, ML, and ethylbenzene, EB, (60 ± 0.1ºC, air, 10 mM AIBN as a free-radical initiator, volume of sample tested of 2.0 mL) was studied by using a homemade glass-capillary micro-volumometer of high sensitivity with a cell construction that allowed addition of the required components without opening the cell [10, 11]. A kinetic run was started by measuring the rate of oxidation in the absence of antioxidants (non-inhibited oxidation, R_0) and rate of initiation, R_i. After the determination of R_0 and R_i, chalcones or other phenols at various concentrations were added. Oxygen consumption monitoring started 3–5 min after adding the antioxidant.

Model 3: The oxidation of 0.02 M ML in 0.2 M SDS micelles was studied in 0.05 M Na phosphate buffer, pH 7.40 ± 0.02, at 37.0 ± 0.2ºC in the presence of 50 mM ACVN as an initiator [12]. Under these conditions, ML peroxidation was found to be a chain process with a kinetic chain length of about 40. Chalcones AH_2 and BH_2 were used as 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 R_0 and R_i, then, aliquots of a stock solution of chalcones were added without suspending [O_2] monitoring by using microsyringe with long needle.

Quantum chemical calculations: All aroxyl radicals (at 2', 3' and 4 positions) formed from 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 (CambridgeSoft, Cambridge, MA). The determination of the coupling constants was carried out with density-functional theory calculations, using the B3LYP hybrid function, the CPCM option for considering the solvent effect for the mostly dissociated aroxyl radicals and the basis sets 6-311G* 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 peroxyl 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 registered 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, AH_2, may be described by the following kinetic Scheme 1:

Chain generation (i)

$$\text{AIBN} + (O_2, \text{RH}) \rightarrow RO_2^{\bullet} \qquad R_i$$

Chain propagation (p)

$$RO_2^{\bullet} + \text{RH} \rightarrow \text{ROOH} + R^{\bullet}\ (+O_2 \rightarrow RO_2^{\bullet}) \qquad k_p$$

Chain termination (t)

$$RO_2^{\bullet} + RO_2^{\bullet} \rightarrow \text{P*} \rightarrow \text{P} + h\nu \qquad k_t$$

Inhibition 1 (A)

$$RO_2^{\bullet} + AH_2 \rightarrow \text{ROOH} + \text{AH} \qquad k_A$$

Inhibition 2 (A')

$$RO_2^{\bullet} + \text{AH} \rightarrow \text{products} \qquad k_{kA'}$$

Disproportionation (A'')

$$\text{AH} + \text{AH} \rightarrow AH_2 + \text{A} \qquad k_{A''}$$

Scheme 1.

where $RO_2^{\bullet}$ is the cumene peroxyl radical, P* - the

molecule of the oxidation product in excited state, AH 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]:

$$I = \phi\ (2k_t\ [RO_2^\bullet]^2) \qquad (1)$$

where φ 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 1 (k_A) (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_A) may be calculated from the kinetic curves of CL of the inhibited oxidation from the value of the minimum intensity of CL (I_{min}) in the presence of the inhibitor, AH_2,

$$k_{A(min)} = (1 - I_{min})\ (2R_i\ k_t)^{0.5}/\{\boldsymbol{n}[AH_2]\ (I_{min}^{0.5})\}, \qquad (2)$$

and the stoichiometric coefficient of inhibition, ***n***, is determined according to [8–10]:

$$\boldsymbol{n} = R_i\ IP/[AH_2]_0 \qquad (3)$$

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_A, 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 AH_2.

Table 1. Kinetic parameters characterizing the chain-breaking activity of chalcones AH_2 and BH_2 at various concentrations during the oxidation of cumene at 60ºC.

Chalcone type	Chalcone concentration, M	R_i, M/s	k_A, $(M{\cdot}s)^{-1}$	***n***
AH_2	2.08×10^{-6}	2.45×10^{-8}	1.20×10^{4}	0.3
AH_2	4.95×10^{-6}	2.33×10^{-8}	1.27×10^{4}	0.5
BH_2	4.50×10^{-6}	3.23×10^{-8}	1.46×10^{4}	1.9
BH_2	8.70×10^{-6}	3.12×10^{-8}	1.04×10^{4}	2.0

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]:

$$AH + ROOH \rightarrow AH_2 + RO_2^\bullet \qquad (4)$$

$$AH + O_2 \rightarrow A + HO_2^\bullet \qquad (5)$$

$$AH + RH + O_2 \rightarrow AH_2 + RO_2^\bullet \qquad (6)$$

$$AH \rightarrow \text{product "r"}(+O_2) \rightarrow rO_2^\bullet \qquad (7)$$

"r" denotes undefined structure derived from the original aroxyl radical. Belyakov *et al*. [8] suggested that reaction (7) of the monomolecular transformation of AH (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 AH_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:
CrC_1 (1.2×10^5) > CA (8.3×10^4) > SA (2.5×10^4) > > BH_2 (1.5×10^4) ≥ BHT (1.4×10^4) ≥ AH_2 (1.3×10^4) > FA (0.8×10^4) > *p*-CA (0.7×10^4).

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 AIBN, 60ºC, for known antioxidants (*p*-CA, FA, SA, CA, BHT and Croman C_1) as well as for the tested chalcones AH_2 and BH_2.

Phenolic antioxidant	k_{inh} $(Ms)^{0.5}$	***n***	Refs.
p-Coumaric acid (*p*-CA)	0.7×10^4	1.9	[9]
Ferulic acid (FA)	0.8×10^4	3.5	[9]
Sinapic acid (SA)	2.5×10^4	2.6	[9]
Caffeic acid (CA)	8.3×10^4	2.5	[9]
Butylated hydroxyl toluene (BHT)	1.4×10^4	2.0	[9]
Chroman C_1 (CrC_1)	11.6×10^4	2.0	[9]
Chalcone BH_2	1.46×10^4	1.9	This work
Chalcone AH_2	1.27×10^4	0.5	This work

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 (AH_2) is presented in Scheme 2 [12]:

(i) Y (+LH,+O_2) → $LO_2^{\bullet}$ R_i
Chain generation

(p) $LO_2^{\bullet}$ +LH (+O_2) → LOOH + $LO_2^{\bullet}$ k_p
Chain propagation

(t) $LO_2^{\bullet}$ + $LO_2^{\bullet}$ → products k_t
Chain termination

(A) $LO_2^{\bullet}$ + AH_2 → LOOH + AH k_A
Inhibition 1

(A') AH+ $LO_2^{\bullet}$ → AH-OOL $k_{A'}$
Inhibition 2

(A") AH+ AH → AH_2 + A $k_{A"}$
Disproportionation

Scheme 2.

where $LO_2^{\bullet}$ are the lipid peroxide radicals, LOOH – lipid hydroperoxides. In the absence of antioxidants, the rate of chain oxidation is as follows:

$$R_0 = d[O_2]/dt = d[LOOH]/dt = k_p[LH](R_i/k_t)^{1/2} \quad (8)$$

In the presence of an antioxidant when k_A[PhOH] is high enough so that $R << R_0$, 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:

$$IP = \boldsymbol{n}[AH_2]_0/R_i \quad (9)$$

$$R_A = k_pR_i[LH]/\boldsymbol{n}.k_A[AH_2]_0 \quad (10)$$

where $[AH_2]_0$ is the starting concentration of inhibitor. In the classic variant under consideration, a value of $\boldsymbol{n}$ 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 $[AH_2]_0$. Eqns. (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 (ν) 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 linoleate and ethyl benzene in presence of an initiator AIBN, 60°C.

Substrate	$[BH_2]$, M	R_i, M/s	R_0/R_{A-}	k_A $(Ms)^{0.5}$	$\nu = R_0/Ri$
Methyl linoleate	0.1	$5.0\ 10^{-8}$	4.5	1.5×10^4	72
Ethylbenzene	0.1	$1.0\ 10^{-7}$	4.0	2.0×10^4	8

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 ($LO_2^{\bullet}$ + $LO_2^{\bullet}$ → 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 $[LO_2^{\bullet}]$ [12]:

$LO_2^{\bullet}$ → products $k_{t'}$ (t').

For this reason, the kinetic scheme under consideration includes reactions (i), (p), (t'), (A), (A'). The following system of differential equations corresponds to this scheme:

$$d[LO_2^{\bullet}]/dt = R_i - k_{t'}[LO_2^{\bullet}] - k_A[LO_2^{\bullet}][AH_2] - -k_{A'}[LO_2^{\bullet}][AH] \quad (11)$$

$$d[AH]/dt = k_A[LO_2^{\bullet}][\ AH_2] - k_{A'}[LO_2^{\bullet}][AH] \quad (12)$$

$$-d[AH_2]/dt = k_A[LO_2^{\bullet}][AH_2] \quad (13)$$

The rate of non-inhibited oxidation equals

$$R_0 = k_p[LH]R_i/k_{t'} \quad (14)$$

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

$$R = k_p[LO_2^{\bullet}][LH] \quad (15)$$

$$R/R_0 = k_{t'}[LO_2^{\bullet}]/R_i \quad (16)$$

The solution is given for quasi-stationary approximation for all free radicals. Combining Eqns. (14), (15) and (16) results in:

$$R_i - k_{t'}[LO_2^{\bullet}] - 2k_A[LO_2^{\bullet}][AH_2] = 0 \qquad (17)$$

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):

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

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-α-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.

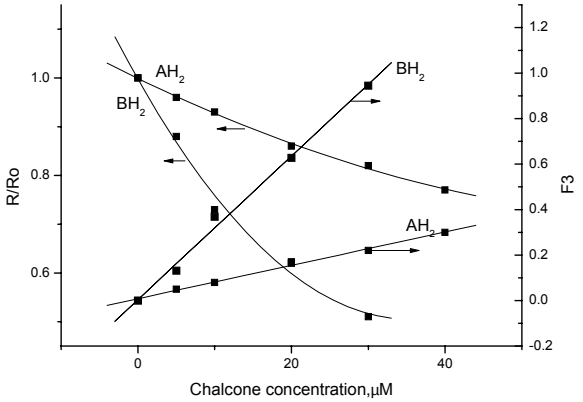
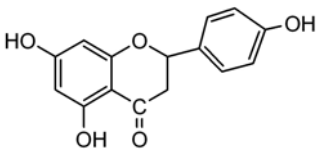
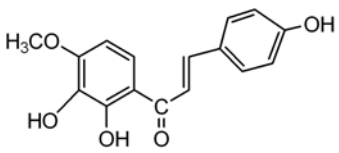
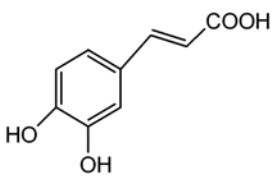
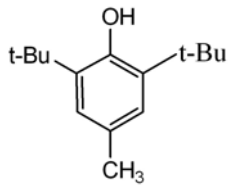
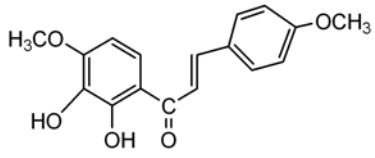
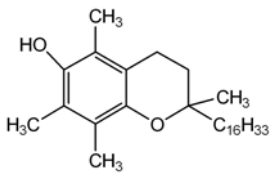


Fig. 3. Kinetics of oxygen consumption during the oxidation of 0.02 M methyl linoleate in 0.2 M SDS micellar solution in 0.05 M Na phosphate buffer pH = 7.40 at 37ºC ($R_i = 1.65 \cdot 10^{-9}$ M/s) in the presence of AH_2 and BH_2.

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, 37ºC.

Antioxidant	k_A/k_p	Ref.	Antioxidant	k_A/k_p	Ref.
 Naringenin	0.5	[12]	 BH_2	7.7	This work
 Cafeic acid	23	[12]	 BHT	240	[12]
 AH_2	2.3	This work	 Tocopherol	290	[12]

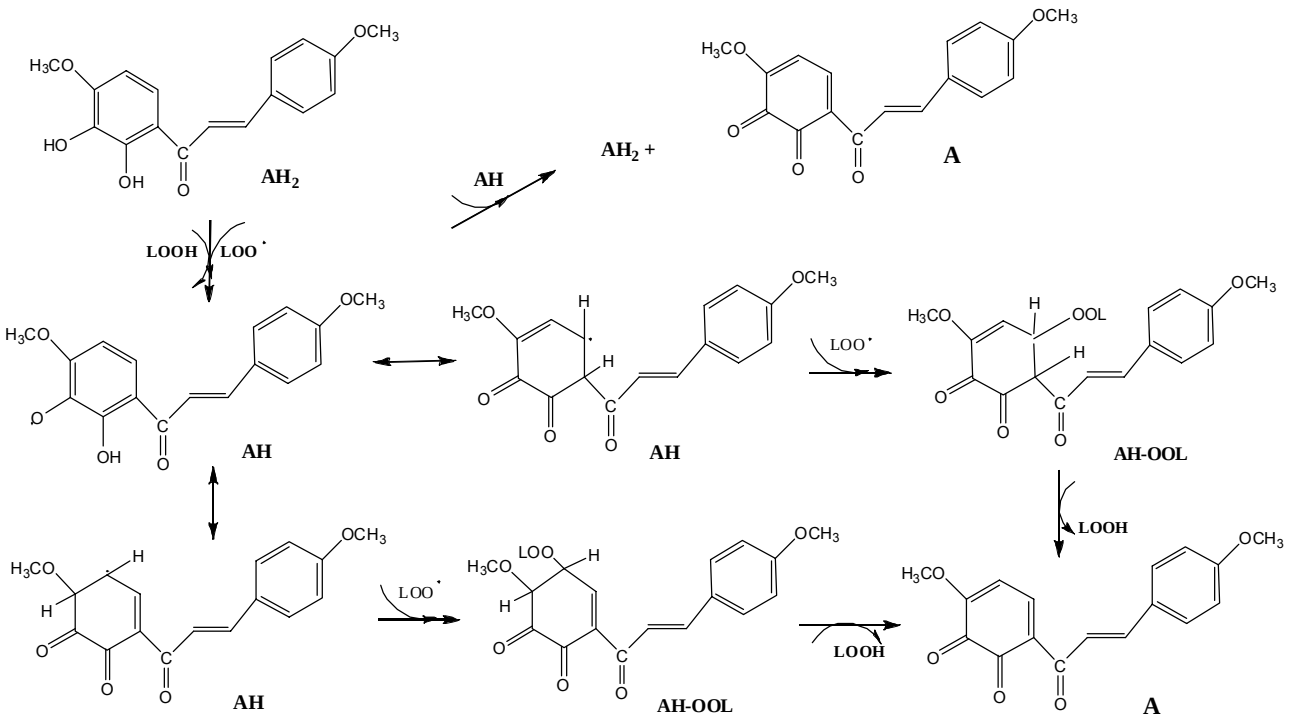


Fig. 4a. Reactivity, transformation and degradation products for chalcone AH_2.

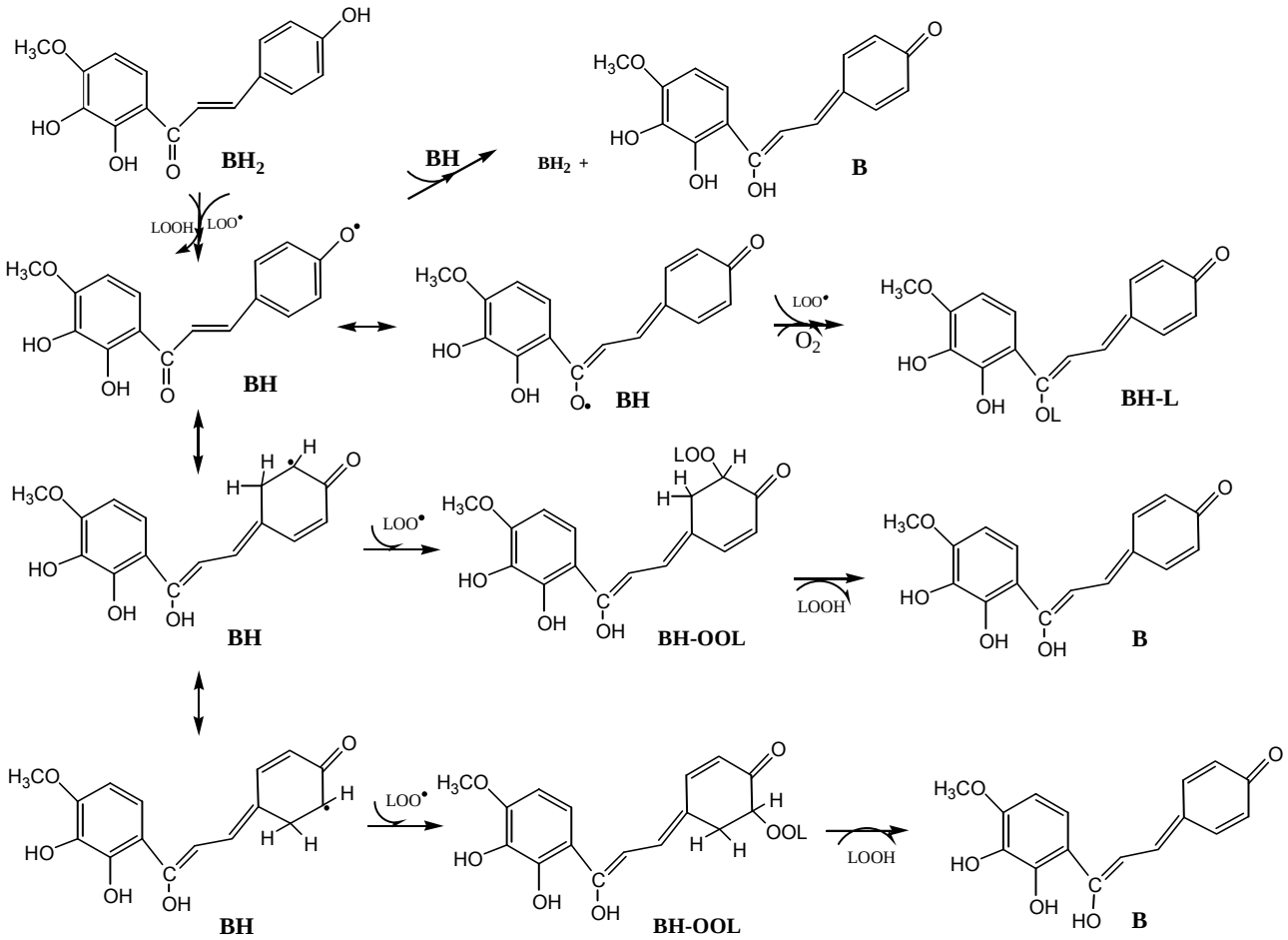


Fig. 4b. Reactivity, transformation and degradation products for chalcone BH_2.

Table 5. Theoretical coupling constants of aroxyl radicals from new chalcones of El Salvador propolis.

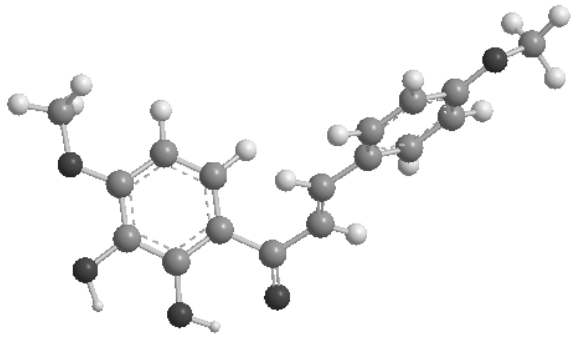
Substance		$H_{6'}$	$H_{1'}$	H_α	H_β	H_2	H_3	H_5	H_6	Comments
BH_2 rad2'	CA	4.6903	1.2380	0.2464	0.0180	0.0065	0.0029	0.0020	0.0074	CPCM mode, 6-311G*
	CB	4.5682	1.2143	0.2474	0.0185	0.0066	0.0029	0.0020	0.0076	CPCM mode, 6-311G**
BH_2 rad3'	CA	4.7419	1.2047	0.2662	0.0100	0.0031	0.0031	0.0007	0.0029	CPCM mode, 6-311G*
	CB	4.6192	1.1817	0.2675	0.0106	0.0033	0.0032	0.0007	0.0030	CPCM mode, 6-311G**
BH_2 rad4	CA	0.8403	0.0031	3.4360	7.4023	1.8274	5.2172	4.7962	1.7516	CPCM mode, 6-311G*
	CB	0.8556	0.0022	3.3627	7.2074	1.7743	5.1104	4.6988	1.7012	CPCM mode, 6-311G**
	PA	0.8615	0.0022	3.4246	7.3813	1.8212	5.2190	4.7943	1.7516	PCM mode, 6-311G*
AH_2 rad2'	CA	4.7017	1.2324	0.2457	0.0181	0.0069	0.0026	0.0024	0.0071	CPCM mode, 6-311G*
	CB	4.5788	1.2091	0.2466	0.0187	0.0070	0.0026	0.0025	0.0073	CPCM mode, 6-311G**
AH_2 rad3'	CA	4.7390	1.2022	0.2652	0.0127	0.0038	0.0034	0.0010	0.0031	CPCM mode, 6-311G*
	CB	4.6161	1.1796	0.2664	0.0133	0.0040	0.0034	0.0010	0.0032	CPCM mode, 6-311G**

Quantum chemical calculations

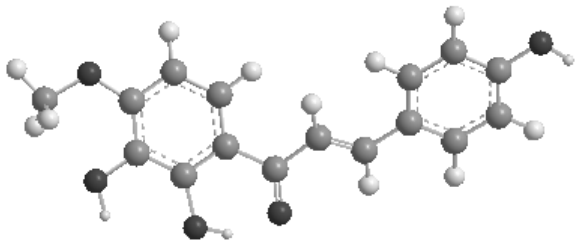
Calculated coupling constants for all possible aroxyl radical-anions formed from AH_2 and BH_2 (see Fig. 2) are shown in Table 5. It is no doubt that the chalcones AH_2 will produce aroxyl radicals only in ring a. The calculated hyperfine coupling constants for aroxyl radicals in positions 2' and 3' for chalcone BH_2 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 $–OCH_3$ 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 that 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 BH_2 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 AH_2 and BH_2 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 BH_2) 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 BH_2 is observed. In contrast, in case of AH_2, the two rings are turned on about 30° and there is no possible formation of hydrogen bond (close distance in this case is almost 2.52 Å).

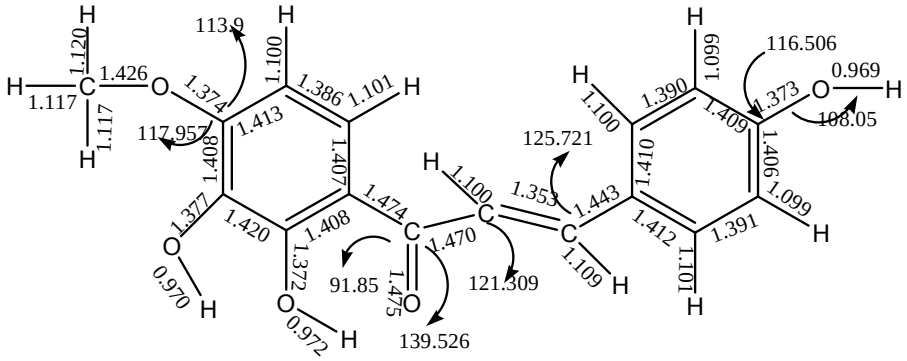


Chalcone AH_2



Chalcone-BH_2

Fig. 5a. Optimized 3D structures of the studied chalcones.



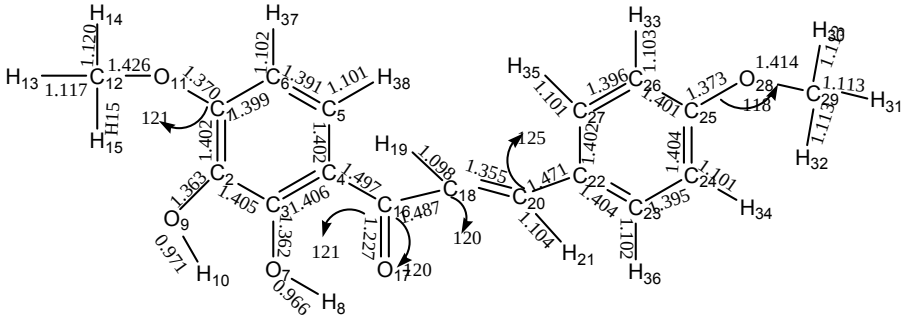


Fig. 5b. Equilibrium structural parameters for the chalcones AH_2 and BH_2.

CONCLUSIONS

New data demonstrate a moderate chain-breaking antioxidant activity of the new chalcones AH_2 and BH_2 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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ПРЕКЪСВАЩА ВЕРИГИТЕ АНТИОКСИДАНТНА АКТИВНОСТ НА НОВИ ХАЛКОНИ ОТ ПРОПОЛИС ОТ ЕЛ САЛВАДОР В ХОМОГЕННА И МИЦЕЛАРНА СРЕДА

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(Резюме)

Прекъсващата веригите антиоксидантна активност на два нови халкона от прополис на Ел Салвадор 2',3'-дихидрокси-4,4'-диметокси халкон (AH2) и 2',3',4-трихидрокси-4'-метокси халкон (BH2) е определена чрез прилагане на три кинетични модела на окисление в хомогенна и мицеларна среда: модел 1 – хеми-луминисценция на кумол в хомогенен разтвор, модел 2 – окисление на метиллинолеат и етилбензол в хомогенен разтвор, и модел 3 – окисление на метиллинолеат в мицели на натриев додецилсулфат (SDS). Окислението е проведено при постоянна и добре контролирана скорост на инициране, т.е. на зараждане на радикали. Резултатите показват, че при окислението в разтвор на SDS мицели AH_2 и BH_2 проявяват умерена антиоксидантна активност, прекъсваща веригите, по-висока от тази на нарингенина, но по-ниска от тази на кафеената киселина и по-висока от тази на 2,6-ди-*трет*-бутил-4-метилфенол (BHT) и DL-α-токоферола. Умерена прекъсваща окислителните вериги активност е наблюдавана също така за халконите в хомогенни разтвори, подобно на тази за BHT, но по-ниска от тази на кафеена и синапова киселини, и по-висока от тази на р-кумарова и ферулова киселини. Чрез прилагане на Chem 3D MOPAC минимална енергия при ниво AM1/RHF е получена пълната оптимизация на геометрията на AH_2 and BH_2 и три-дименсионалните им структури като Z матрици. Определянето на свръхфините константи на взаимодействие са получени като са използвани изчисления с плътностно-фунционалната теория (DFT) и е приложена B3LYP хибридна функция и CPCM опция за отчитане на ефекта на разтворителя за дисоциираните ароксилни радикали и базовите мрежи 6-311 G* и 6-311G** на програма Gaussian 98.