

ANTIRADICAL AND ANTIOXIDANT ACTIVITIES  
OF NEW NATURAL-LIKE HYDROXYLATED BIPHENYLS  
OF DEHYDROZINGERONE, ZINGERONE AND FERULIC  
ACID

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**Abstract**

Selected synthesized natural and natural-like hydroxylated biphenyls (dimers) and their corresponding monomers (dehydrozingerone, zingerone and ferulic acid) were tested experimentally for their capacity both as radical scavengers and chain-breaking antioxidants in individual and binary mixture with  $\alpha$ -tocopherol. Quantum-chemical calculations at the UB3LYP/6-13+G(d,p) level were used to get full geometry optimization of the compounds in neutral and radical forms. Good correlation between experimental data of radical scavenging and antioxidant activities and predicted activity was achieved.

**Key words:** antiradical activity, antioxidant activity, hydroxylated biphenyls

**Introduction.** Curcumin or diferuloylmethane derived from the rhizome of *Curcuma longa* manifests a wide spectrum of biological activities [1] and excel-

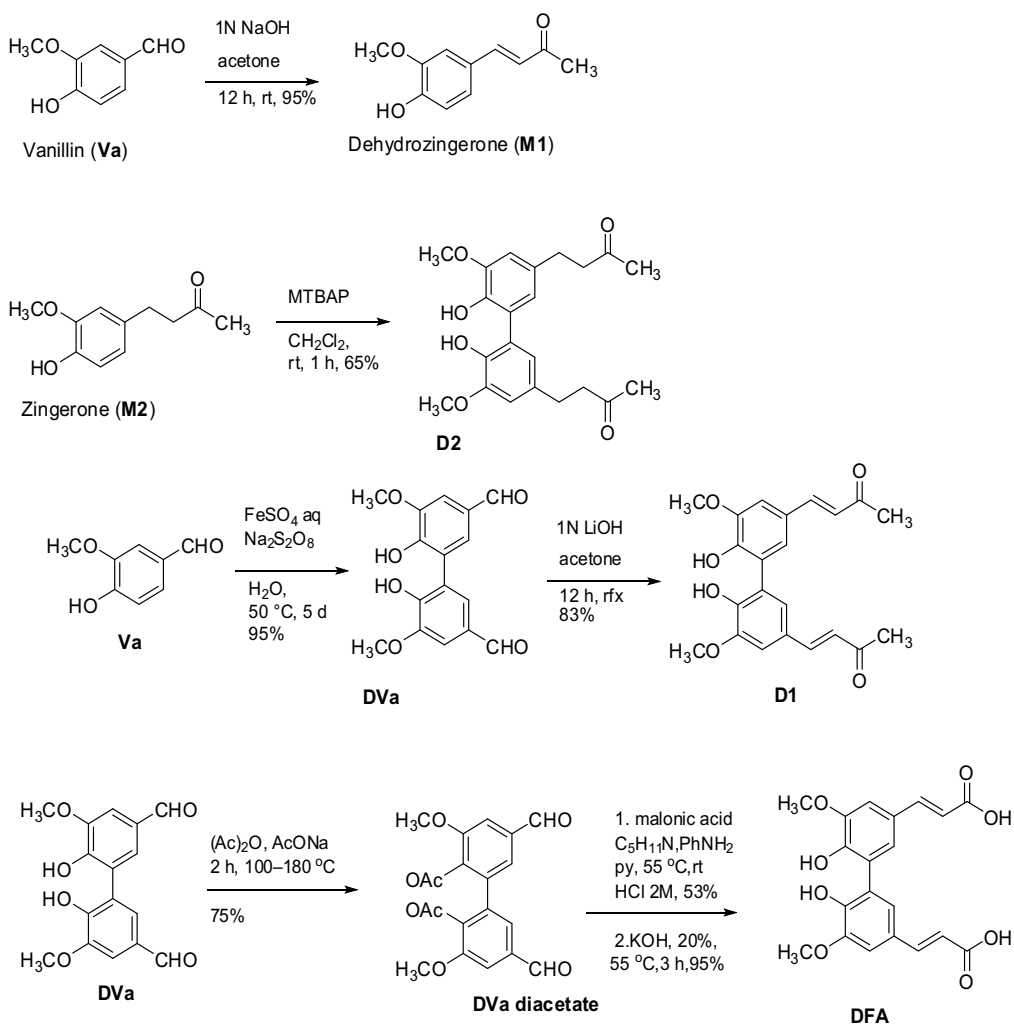
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lent free radical scavenging activity [2] due to the presence of two phenolic rings *ortho*-substituted by OMe group bonded in para position by  $\alpha, \beta$ -unsaturated 1,3-diketone chain which stabilizes the generated radicals [3]. Unfortunately, curcumin has low solubility in aqueous and physiological solutions where it undergoes rapid degradation into ferulic acid **FA**, vanillin **Va** and dehydrozingerone **M1** [4]. Curcumin metabolites are 4-substituted 2-methoxy phenols, a large class of naturally occurring phenols whose hydroxycinnamic acids, major components of the primary cell wall of cereals and plants, belong to [5]. The feruloyl residues, predominant species, can also be dimerized under an oxidative coupling mediated by peroxidases, forming cross-links, or dehydrodimers of ferulic acid (5,5'-disubstituted biphenols) [6]. The latter dimers manifest higher antioxidant activity and they are often less toxic than the corresponding phenolic monomer. Defence mechanisms developed by plants, animals and humans against the effects of excessive oxidations would be provided by the combined action of various antioxidants [7]. There is a growing interest to investigate the antioxidant activity of natural and natural-like polyphenols in combination with  $\alpha$ -tocopherol (**TOH**/vitamin E), one of the best antioxidants, for possible synergism between the components [8,9]. In the search of new bio-antioxidants, we have evaluated antiradical and antioxidant activities of hydroxylated biphenyls (**D1**, **D2**, **DFA**) and corresponding monomers (**M1**, **M2** and **FA**) (Scheme 1) with combination of spectral (DPPH absorbance), kinetic (lipid autoxidation) and theoretical (quantum-chemical calculations) methods.

**Materials and methods. Synthesis of the compounds.** Ferulic acid **FA** and zingerone **M2** were purchased (Sigma–Aldrich), dimers **D1** and **D2** were previously prepared by us following straightforward methods as described in Scheme 1. **D2** was prepared in 65% yield by C-C coupling of **M2** in the presence of MTBAP in dichloromethane at room temperature for 1 h. Unfortunately, the procedure resulted unsuccessfully in the preparation of **D1** which was obtained in 83% by Claisen–Schmidt condensation of vanillin dimer **DVa** [10] in the presence of large excess of LiOH in acetone at room temperature. With a slight modification of the above synthetic procedure, **M1** was achieved starting from **Va** in acetone using NaOH as base. **DFA** was prepared by Perkin reaction of diacetate of **DVa** in the presence of malonic acid and bases and further hydrolysis of the acetate groups. All compounds prepared were solid, air-stable and they were fully characterized. In the synthesis of unsaturated compounds **M1**, **D1**, **DFA** *trans* configuration was exclusively obtained.

**Screening for free radical scavengers by DPPH test. DPPH absorbance decrease – quantitative determination of radical scavenging activity as** %RSA =  $100[\text{Abs}_{(0)} - \text{Abs}_{(t)}] / \text{Abs}_{(0)}$ . The decrease in the absorption at 516–517 nm due to reaction of DPPH free radical with phenolic compound, AH ( $\text{AH} + \text{DPPH}^\bullet \rightarrow \text{DPPH} - \text{H} + \text{A}^\bullet$ ), was monitored by the by UV-Vis spectrophotometer, at room temperature (25–30 °C). We used acetone as a solvent



Scheme 1. Structures of compounds and synthesis

according to KANCHEVA et al. [11,12]. Kinetics of DPPH radical absorbance decrease was monitored for 20 min after mixing an excess of DPPH<sup>•</sup> with monomers and dimers (ratios [AH]/[DPPH<sup>•</sup>] = 0.25 and 0.40 mol/mol).

**Chain-breaking antioxidant activity. Lipid samples.** Triacylglycerols of commercially available sunflower oil (TGSO) were cleaned from pro- and antioxidants by adsorption chromatography and stored under nitrogen at  $-20\text{ }^\circ\text{C}$ . Fatty acid composition of the lipid substrate was determined by GC analysis of the methyl esters: 16:0–6.7%; 18:0–3.6%; 18:1–25.1%; 18:2–63.7%; 20:0–0.2%; 22:0–0.7%; the numbers “x:y” indicate, respectively, the number of carbon atoms and double bonds in the fatty acid. Lipid samples containing various inhibitors were prepared directly before use. Aliquots of the antioxidant solutions in purified

acetone were added to the lipid sample. Solvents were removed under a nitrogen flow. For more experimental details see reference [12].

**Lipid autoxidation.** The process was carried out in a thermostatic bath at  $80 \pm 0.2^\circ\text{C}$  by blowing air through the samples in special vessels. The oxidation process was monitored by withdrawing samples at measured time intervals and subjecting them to iodometric determination of the primary products (lipid hydroperoxides, LOOH) concentration, i.e. the peroxide value (PV). All kinetic data are expressed as the average of two independent measurements which were processed using the computer programmes Origin 6.1 and Microsoft Excel 97. For more experimental details see reference [12].

**Determination of the main kinetic parameters of the studied compounds** [13,14]. Protection factor (PF) is determined as the ratio between the induction period in the presence ( $\text{IP}_A$ ) and in the absence ( $\text{IP}_C$ ) of antioxidant, i.e.  $\text{PF} = \text{IP}_A/\text{IP}_C$ . It is a measure of antioxidant efficiency. Inhibition degree (ID) is a measure of the antioxidant reactivity, e.g. how many times the antioxidant shortens the oxidation chain length, i.e.  $\text{ID} = R_C/R_A$ . The initial oxidation rates  $R_C$  in the absence and  $R_A$  in the presence of antioxidant were found from the tangents at the initial phase of the kinetic curves of hydroperoxides accumulation.

**Quantum chemical calculations.** The homolytic bond dissociation enthalpy (BDE) is a broadly used descriptor of antiradical/antioxidant activity. B3LYP was used in this study because this functional provides reliable geometries, frequencies and bond energies [15]. The geometries of hydroxylated biphenyls (**D1**, **D2**, **DFA**), corresponding monomers (**M1**, **M2**, **FA**) and their radicals were optimized using unrestricted open-shell approach (UB3LYP) and 6-31+G(d,p) basis set [16–18] without symmetry constraints with the default convergence criteria. Frequency calculations at the same level of theory were carried out to confirm that the obtained structures corresponded to energy minima. Unscaled thermal corrections to enthalpy were added to the total energy values. The BDEs are calculated by the formula  $\text{BDE} = H_{298}(\text{A}^\bullet) + E_T(\text{H}^\bullet) - H_{298}(\text{AH})$  where  $H_{298}(\text{A}^\bullet)$  and  $H_{298}(\text{AH})$  are enthalpies calculated at 298 K for radical species  $\text{A}^\bullet$  and neutral molecule AH, respectively, and  $E_T(\text{H}^\bullet)$  (calculated total energy of  $\text{H}^\bullet$ ) is  $-313.93 \text{ kcal.mol}^{-1}$ . All quantum chemical calculations were carried out using GAUSSIAN 09 programme package [19].

**Results and discussion. Capacity as free radical scavengers by DPPH test.** Experimentally obtained results about %RSAmax are presented in Table 1. Biphenyls showed higher capacity to scavenge free radicals in comparison with the corresponding monomers. **D2** manifested strongest radical scavenging activity, **D1** and **DFA** and their corresponding monomers **M1** and **FA** showed similar activity.

**Chain-breaking antioxidant activity.** This model gives information about the potential of studied compounds to inhibit lipid oxidation process, i.e. to react as chain-breaking antioxidants. In contrast to DPPH, lipid autoxidation

Table 1

The main experimental (%RSA; PF, ID) and theoretical (BDE,  $\varphi$ ) parameters of studied compounds

AH	Radical scavenging activity (RSA) from absorbance decreases of DPPH		Chain-breaking antioxidant activity during TGSO autoxidation				Predicted activity from DFT calculations UB3LYP/6-31+(d,p)			
	[AH]/[DPPH]	RSAm <sub>max</sub> %	Activity	[AH] mM	PF	ID	Activity of AH	BDE kcal.mol <sup>-1</sup>	Dihedral angle $\varphi$ , °	Comparison of BDEs
M1	0.25	5.8	Weak	0.1	1.0	1.0	No activity	79.53	–	< FA < M2
D1	0.40	8.7	Moderate	1.0	3.5	6.3	Moderate			
	0.25	13.6	Moderate	0.1	2.6	3.1	Moderate	(1) 78.94 (2) 79.52	64.576 <sup>m</sup> 63.311 <sup>r</sup> 68.849 <sup>br</sup>	< M1 < DFA
M2	0.40	28.0	Strong	1.0	13.5	29.3	Strong			
	0.25	2.4	No activity	0.1	1.1	1.0	No activity	80.23	–	> M1 ≈ FA
D2	0.40	3.7	Weak	1.0	3.5	5.5	Moderate			
	0.25	31.4	Strong	0.1	1.6	1.6	Weak	(1) 79.60 (2) 80.44	62.231 <sup>m</sup> 59.741 <sup>r</sup> 69.640 <sup>br</sup>	< M2 ≈ DFA
FA	0.40	45.2	Strong	1.0	5.8	8.8	Moderate			
	0.25	4.7	Weak	0.1	1.5	1.6	Weak	80.09	–	> M1 < M2
DFA	0.40	7.7	Moderate	1.0	3.2	4.4	Moderate			
	0.25	14.8	Moderate	0.1	1.5	2.0	Weak	(1) 79.43 (2) 80.00	64.582 <sup>m</sup> 62.755 <sup>r</sup> 67.994 <sup>br</sup>	> D1 ≈ D2 < FA
0.40	18.5	Moderate	1.0	3.3	4.2	Moderate				

process involves a set of reactions, some of them are responsible for the inhibitory activity of the studied phenolic compounds, other – for the side reactions, which can reduce the antioxidant activity of them [11]. New order of antioxidant efficiency (as protection factor, PF) and antioxidant reactivity (as inhibition degree, ID) of the studied compounds was found:

0.1 mM; PF: **D1**(2.6) > **D2**(1.6) = **FA**(1.5) = **DFA**(1.5) > **M2**(1.1) ≥ **M1**(1.0)  
 ID: **D1**(3.1) > **DFA**(2.0) > **D2**(1.6) = **FA**(1.6) > **M1**(1.0) = **M2**(1.0)  
 1.0 mM; PF: **D1**(13.5) > **D2**(5.8) > **M1**(3.5) = **M2**(3.5) ≥ **DFA**(3.3) ≥ **FA**(3.2)  
 ID: **D1**(29.3) > **D2**(8.8) > **M1**(6.3) > **M2**(5.5) > **FA**(4.4) ≥ **DFA**(4.2)

**D1** manifested the highest antioxidant efficiency and reactivity in both concentrations, much higher than the corresponding monomer **M1**. **D2** and **DFA** in both concentrations showed slightly higher antioxidant efficiency and reactivity than the corresponding monomers but lower than **D1**. **D2** the strongest radical scavenger of free radicals is not able to inhibit effectively lipid oxidation due to the lack of  $\alpha, \beta$ -unsaturated ketone chain in *para* position and thus the stabilization of phenoxyl radicals formed. As a result, the level of side reaction of **D2** is higher than that of **D1**, which is of significance at higher concentration.

**Quantum chemical calculations.** For all studied compounds, similar BDE values (Table 1) are found (the biggest difference is 0.7 kcal.mol<sup>-1</sup>, i.e. lower than 1 kcal.mol<sup>-1</sup>). **M1** and **M2** manifest predicted antiradical activity very close to that of **FA**. Two BDE values are calculated for the biphenyls in accordance with the ability to form radical (r) and biradical (br) species. BDE (br) is equal or very close to the BDE value of the corresponding monomer, whereas BDE (r) < BDE (br). Therefore, a difference of about 0.6 kcal.mol<sup>-1</sup> calculated between BDE (r) dimers and BDE (r) monomers seems to anticipate a higher antioxidant activity and radical scavenging of dimers with respect to monomers. Compounds with  $\alpha, \beta$ -unsaturated ketone chain are characterized by lower BDE than the saturated ones. A carboxylic group at the end of the side chain for **FA** and **DFA** leads to higher BDE values in comparison with **M1** and **D1**. The changes in the dihedral angle between **D1**, **D2** and **DFA** are similar: the formation of the radical decreases (slightly) the angle, while the subsequent formation of the biradical increases the angle more noticeably. The values of dihedral angle of **D2** in radical and biradical species differ significantly, probably due to a more conformational flexibility of the structure in virtue of the presence of two chains with saturated bonds.

**Conclusions.** We report for the first time the experimentally obtained capacity of natural and natural-like hydroxylated biphenyls (dimers) and their monomers as radical scavengers and chain-breaking antioxidants whose activity is predicted by quantum-chemical calculations. Although small differences of BDE between monomers and dimers were achieved, the theoretical results were in reasonable accordance with experimental data. Dimers showed higher activity than

the corresponding monomers in all experiments. The presence of  $\alpha, \beta$ -unsaturated ketone chain seems to be a key factor in chain-breaking antioxidant activity of individual compounds due to a more resonance stabilization of the generated radical, more than the activity observed with compounds bearing  $\alpha, \beta$ -unsaturated acid chain.

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