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Antioxidative effect of some hydroxy substituted aromatic bisimines

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Abstract. This work deals with antioxidative properties of some derivatives 4,4'-*bis*(dihydroxybenzylanilidenamino)diphenylmethanes and diphenylethers, which structure is similar to resveratrol. Four derivatives of above-mentioned compounds were synthesized with hydroxyl (OH) groups in various positions. It was found that derivatives with two OH groups in 2 and 5 positions were very good scavengers of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals in both methanol and chloroform. On the other hand derivatives with OH groups in positions 2 and 4 did not scavenge DPPH radicals. The calculation of free enthalpies (by quantum chemical method AM1) necessary for the formations of anions or radicals support above mentioned findings because the enthalpies for effective derivatives were lesser, than for ineffective ones. Also, it was found that studied compounds did not scavenge hydroxyl radicals whereas resveratrol did it.

Key words: Antioxidant activity — Resveratrol analogues — Structural aspects

Introduction

The molecular structure of hydroxyl-substituted aromatic imines, which are the part of studied *bis*(dihydroxybenzylideneamino)diphenylmethanes and ethers is similar to resveratrol molecule. Resveratrol (*trans*-3,5,4'-trihydroxystilbene) (I) is phytoalexin, which is produced by several plants (e.g. grape, eucalyptus, spruce, peanut, mulberry, lily, etc.). The highest amount of resveratrol was found in red grape skin. Recently, it has been published that resveratrol exhibits various biological, such as: anti-cancer, anti-viral, anti-inflammatory, sugar-lowering and anti-oxidative activities. It is known that resveratrol prolongs the life, and has a beneficial effect on liver and cardiovascular system (Celotti et al. 1996; Soleas et al. 1997; Gu et al. 2000; Kaeberlein et al. 2005). Therefore, resveratrol analogues are highly valuable target for synthesis and study of their physical, chemical and biological properties.

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Imines are very common organic compounds used often as building blocks in organic synthesis (Layer 1963). Due to their properties they are widely used as ligands for metal complexation, liquid crystalline materials, and in analytical and medicinal chemistry (Alexander 1995). The traditional method of imine synthesis is based on heating of the mixture of an aldehyde with an amine in organic solvents (typically benzene or toluene), usually accompanied by an azeotropic removal of water (Layer 1963; Castellano et al. 1968; Taguchi and Westheimer 1971). Synthetic procedures replacing the organic solvent by water (Tanaka and Shiraishi 2000) or recyclable reaction medium such as poly(propyleneglycol) (van den Ancker et al. 2006) were described as a "green" alternatives to this process. However, the most effective and environmentally benign approach, which is used in this work, is to perform the reaction under solvent-less condition (Schmeyers et al. 1998; Tanaka and Toda 2000). In such a case the only by-product of the reaction is water and complete conversion alongside with high purity of product can be achieved.

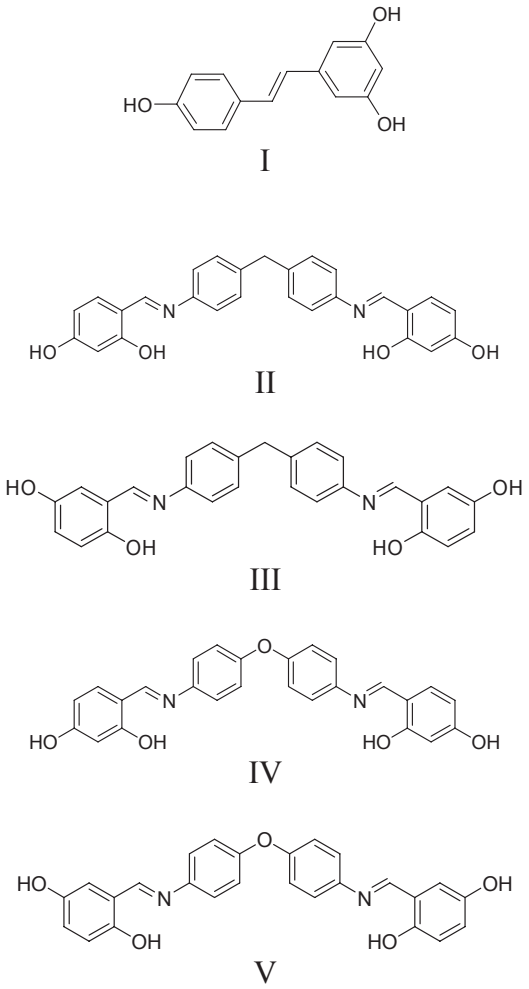
The aim of this work is to investigate anti-oxidative activity of new bisimines, with molecular structure (Scheme 1) similar to that of resveratrol:

4,4'-bis(2,4-dihydroxybenzylideneamino)diphenylmethane (II),

4,4'-bis(2,5-dihydroxybenzylideneamino)diphenylmethane (III),

4,4'-bis(2,4-dihydroxybenzylideneamino)diphenyleter (IV),

4,4'-bis(2,5-dihydroxybenzylideneamino)diphenyleter (V).



Scheme 1

Materials and Methods

Methanol, p.a., and chloroform, p.a., were purchased from Centralchem (Slovakia). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 30% H_2O_2 from Sigma (Germany). Aldehydes and diamines for the synthesis were purchased from Acros (Belgium) and $CDCl_3$ and dimethyl-d_6-sulfoxide (DMSO-D6) from Merck (Slovakia). UV–VIS spectra were measured using spectrophotometer Genesys 6 (Thermo-Scientific, USA). The spectra of electron paramagnetic resonance (EPR) were recorded by the equipment ERS 230 (ZWG Akad. Wiss., Berlin, Germany), which operates in X-band (~9.3 GHz) at modulation amplitude 0.1 mT and microwave power 5 mW. Nuclear magnetic resonance (NMR) spectra were measured by the spectrometer Varian Mercury Plus (400 MHz). All experiments were carried out at 25°C.

The resveratrol analogues were prepared under solvent-less condition by grinding the mixture of 2 mmol of the diamine and 4 mmol of aldehyde in a mortar for 10 min. Then the mixture was allowed to stand at room temperature for 48 h in order to achieve complete conversion. The yields were quantitative and the purity >90%. For further purification of the product and elimination of water resulting from the condensation reaction, crystallization from methanol or simple trituration in methanol (for compounds insoluble in methanol) was applied. The identity of synthesized compounds was confirmed by their ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ or DMSO-D_6. The parameters of NMR spectra are presented below:

4,4'-bis(2,4-dihydroxybenzylideneamino)diphenylmethane (II)

^{1}H NMR (DMSO-D_6, 400 MHz) d 3.97 (s, 2H), 6.29 (d, *J* = 2.1 Hz, 2H), 6.40 (dd, *J* = 8.5, 2.1 Hz, 2H), 7.28 (s, 8H), 7.40 (d, *J* = 8.5 Hz, 2H), 8.77 (s, 2H), 10.25 (s, 2H), 13.61 (s, 2H).

^{13}C NMR (DMSO-D_6, 100.6 MHz) d 39.99, 102.31, 107.74, 111.99, 121.04, 129.59, 134.26, 139.34, 146.00, 162.00, 162.29, 162.97.

4,4'-bis(2,5-dihydroxybenzylideneamino)diphenylmethane (III)

^{1}H NMR (DMSO-D_6, 400 MHz) d 3.97 (s, 2H), 6.30 (s, 2H), 6.40 (d, *J* = 8.5 Hz, 2H), 7.28 (s, 8H), 7.41 (d, *J* = 8.5 Hz, 2H), 8.77 (s, 2H), 10.25 (s, 2H), 13.62 (s, 2H).

^{13}C NMR (DMSO-D_6, 100.6 MHz) d 39.97, 102.30, 107.72, 111.97, 121.02, 129.57, 134.24, 139.32, 146.00, 161.99, 162.27, 162.95.

4,4'-bis(2,4-dihydroxybenzylideneamino)diphenylether (IV)

^{1}H NMR (DMSO-D_6, 400 MHz) d 6.30 (d, *J* = 1.6 Hz, 1H), 6.40 (dd, *J* = 2.1, 8.5 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 2H), 7.40 (d, *J* = 7.7 Hz, 2H), 7.42 (d, *J* = 8.2 Hz, 1H), 8.79 (s, 1H), 10.23 (s, 1H), 13.52 (s, 1H).

^{13}C NMR (DMSO-D_6, 100.6 MHz) d 102.28, 107.74, 112.00, 119.35, 122.56, 134.22, 143.68, 154.99, 161.91, 162.24, 162.74.

4,4'-bis(2,5-dihydroxybenzylideneamino)diphenylether (V)

^{1}H NMR (DMSO-D_6, 400 MHz) d 6.79 (d, *J* = 8.8 Hz, 1H), 6.86 (dd, *J* = 2.9, 8.8 Hz, 1H), 7.04 (d, *J* = 2.8 Hz, 1H), 7.12 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.8 Hz, 2H), 8.86 (s, 1H), 9.10 (s, 1H), 12.27 (s, 1H).

^{13}C NMR (DMSO-D_6, 100.6 MHz) d 116.79, 117.05, 119.23, 119.38, 120.86, 122.98, 143.90, 149.53, 152.91, 155.40, 162.32.

The anti-oxidative activity was tested by two methods:

i) The scavenging of DPPH radicals was carried out in the methanol and chloroform solutions according to Nagy et al. (2006). Briefly: Various amounts of studied compounds were added to DPPH solution, so that the final DPPH concentration was always 7×10^{-5} mol/dm^3. A decrease of the intensity of DPPH absorption band at 517 nm was measured 30 min after addition of an antioxidant. The evaluation of the anti-oxidative efficiency was carried out from the linear part of the plot of absorbance vs. anti-oxidant concentration and is expressed as a SC_{50} values i.e. the concentration which caused 50% decrease of absorbance at 517 nm.

ii) The scavenging of hydroxyl radicals ($HO^\bullet$) was carried out according to Valentová et al. (2005). Briefly: Fenton reaction of H_2O_2 with $FeSO_4$ and the spontaneous decomposition of H_2O_2 were used as sources of $HO^\bullet$. The scavenging activity of the tested samples was determined using EPR spectroscopy. Because $HO^\bullet$ are very unstable and cannot be detected readily by the continuous wave EPR spectrometer, a spin trap of DMPO was used to capture them, and the EPR spectra of this spin adduct were subsequently recorded. The reaction solution contained 5×10^{-4} mol/dm^3 of $FeSO_4$, 0.025 mol/dm^3 of DMPO, the tested substances (5 mg/cm^3), and 0.05 mol/dm^3 H_2O_2 in a phosphate buffer (0.19 mol/dm^3 Na_2HPO_4, pH = 7.4). The EPR spectra of these solutions were registered 20 min after the addition of H_2O_2. Due to low solubility of studied compounds, the efficiency of the $HO^\bullet$ scavenge were determined only for their saturated solutions.

Results and Discussion

It was found that some derivatives of studied bisimines compounds are able to scavenge DPPH radicals in methanol (Table 1). From this table, it is evident that some bisimine derivatives (II, IV) were ineffective. On the other hand, the other bisimine derivatives (III, V) exhibited very good anti-oxidative activity.

From Table 1 is evident that bisimine derivatives, which have hydroxyl (OH) groups in 2 and 5 positions, are able to scavenge DPPH radicals 2–12 times (6–8 in methanol, 2–12 in cloroform) more effectively than resveratrol. On the other hand derivatives with OH groups in the position 2 and 4 do not scavenge DPPH radicals.

It is known, that the DPPH scavenging can be realized by several mechanism. The first mechanism goes through hydrogen radical ($H^\bullet$) abstraction from an anti-oxidant molecule (ArOH) according to the scheme: $R^\bullet + ArOH \rightarrow RH + ArO^\bullet$ (Yokozawa et al. 1998), where $R^\bullet$ is a radical and RH is its reduced form. This mechanism takes place in non-polar solvents, such as chloroform. The second mechanism runs by H^+ abstraction, which takes place in polar solvents such as methanol or ethanol (Foti et al. 2004). The third possibility of reaction DPPH with antioxidant molecule is a combination of both above-mentioned processes (Litwinienko and Ingold 2007). The other mechanism is realized trough the one-electron transfer from antioxidant to the radical ($R^\bullet + ArOH \rightarrow R^- + ArOH^{\bullet+}$) (Cheng et al. 2002; Leopoldini et al. 2004). Properties of antioxidant and solvent determine which mechanism takes place in the reaction of DPPH with an anti-oxidant.

The fact, that several studied bisimines scavenge DPPH radicals and other do not, can be explained by computations of free enthalpies for the formations of anions or radicals from antioxidant molecules. These computations were carried out by the quantum-chemical method AM1 (AMPAC 6. 7. 2001 Semichem, 7128 Summit, Shawnee, KS 66216) (Dewar et al. 1985). In Table 2 are presented values of free enthalpies for anion formation, after H^+ dissociation (ΔE_A)

Table 1. SC_{50} values of the DPPH scavenging in methanol and chloroform

Compound	I	II	III	IV	V	Solvent
SC_{50} (μmol/dm^3)	26.37	ineffective	4.11	ineffective	3.25	methanol
r^2	0.998		0.992		0.998	
SC_{50} (μmol/dm^3)	120.83	ineffective	62.90	ineffective	10.17	chloroform
r^2	0.986		0.974		0.997	

SC_{50}, concentration which caused 50% decrease in DPPH absorbance at 517 nm; r^2, the average square deviation.

Table 2. Free enthalpies for anion or radical formations of studied imines

Compound	ΔE_A (kJ/mol)	ΔE_R (kJ/mol)	IP (kJ/mol)	IP (eV)
II	158.10	166.56	838.70	8.69
III	121.15	140.49	831.82	8.62
IV	164.48	166.62	824.76	8.55
V	130.01	139.79	826.14	8.56

ΔE_i, energy differences among ground and anionic or radical states of studied molecules; IP, ionization potentials of studied imines.

and radical formation after $H^{\bullet}$ abstraction (ΔE_R). From data presented in Table 2 is evident that the bisimine derivatives with OH groups in 2,5 positions need less energy for the formations of anions or radicals as those with OH groups in 2,4 positions. Also the fact, that $\Delta E_A < \Delta E_R$ for equivalent derivatives support our finding that studied bisimines exhibit higher DPPH scavenging in methanol then in chloroform. On the base of these findings we suggest that the scavenging DPPH radicals take place through H^+ dissociation. This assumption support also the literature data (http:/www.zirchrom.com/organic.htm, 2008) of pKa values for 1,4-dihydroxybenzene (10.35) and for 1,3-dihydroxybenzene (9.81), which are a part of compounds under study. Also these findings support the assumption that studied bismines with OH groups in 2,5 positions will be easier release H^+ as those with OH groups in 2,4 positions. So as we are found, whether DPPH scavenging take place through the one-electron transfer mechanism, ionization potentials (IP) of studied bisimines were calculated (Table 2, columns 4 and 5). From these data is evident that correlation among IPs and DPPH scavenging is not fully good. All above-mentioned calculations support our results that derivatives with OH groups in 2,5 positions are better antioxidants as ones with OH groups in 2,4 positions.

As the scavenging of DPPH takes place also in aprotic solution (in chloroform), we suppose the mechanism involves $H^{\bullet}$ abstraction from bisimine molecule, according to mechanism, which was suggested by Foti et al. (2004). Therefore, we can predict that the compounds under study would be able to scavenge also $HO^{\bullet}$. The experiments with scavenging of $HO^{\bullet}$ show that lower amount of $DMPOOH^{\bullet}$ adduct was registered in the presence of studied compounds if $HO^{\bullet}$ were generated by Fenton reaction. On the other hand, the unchanged amount of $DMPOOH^{\bullet}$ was registered if $HO^{\bullet}$ were generated by the spontaneous H_2O_2 decomposition. On the base of these findings we assume that studied bisimine derivatives do not scavenge $HO^{\bullet}$. The lower amount of $DMPOOH^{\bullet}$ registered in Fenton reaction is caused by lower amount of generated $HO^{\bullet}$ due to the complex formation of bisimine molecules with Fe^{2+}.

Conclusion

It was found that some of the newly synthesized resveratrol analogues are very good scavengers of DPPH radicals, even better (compounds III and V) than natural resveratrol. On the other hand the compounds II and IV do not scavenge the DPPH radicals. The factor determining efficiency of the scavenging of DPPH radicals are the positions of the OH groups in the antioxidant molecule. Compounds bearing two hydroxyl groups in the *para* position to each other are very effective scavengers, because their oxidized form can be stabilized through isomerization to a quinoid structure.

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