

Antioxidant Activity of Hydroxystilbene Derivatives in Homogeneous Solution

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The antioxidant activity of the *cis* and *trans* isomers of several analogues of resveratrol and pterostilbene has been investigated, especially with regard to the effect of the stereochemistry about the olefinic double bond. The antioxidant power of these compounds was estimated by measuring the rate constants for their reactions with peroxy radicals and, with two of them, the bond dissociation enthalpy (BDE) of the phenolic O–H bond which is cleaved in the inhibition reaction. The present data show that in homogeneous solution the various hydroxystilbenes investigated behave as mild antioxidants with the notable exceptions of the *trans* isomer of **4** and **6**, whose activities are only slightly lower than that of α -tocopherol (vitamin E). The rate constants of the inhibition reaction show that the antioxidant activity of the *cis*-hydroxystilbene is in all the examined cases worse, by a factor ranging between 2 and 6, than that of the corresponding *trans* isomers. This lower reactivity depends on enthalpy factors as it can be inferred by the experimental values of the O–H bond dissociation enthalpy in the two geometric isomers of 3',5'-di-*tert*-butyl-4'-hydroxy-3,5-dimethoxystilbene showing that the strength of the O–H bond in the *cis* isomer is larger by 1.8 kcal/mol. DFT calculations provide a rationalization of this result, indicating that, although the *cis* geometry implies a destabilization with respect to the *trans* species of both phenoxyl radical and parent hydroxystilbene, the destabilization of the radical is larger because the folding of the structure strongly reduces the delocalization of the unpaired electron on the styryl group. A comparison of these results with previously reported data on the proapoptotic activity of these stilbenoids suggests that these two properties are not correlated.

Introduction

Many investigations have been recently reported on the antioxidant properties of *trans*-resveratrol (**1**), pterostilbene (**2**), and related derivatives. Some of these reports attribute to *trans*-resveratrol (3,4',5-trihydroxy-*trans*-stilbene) an antioxidant activity comparable to (or even higher than) α -tocopherol,¹ one of the most effective lipid-soluble chain-breaking antioxidants,² while others indicate that *trans*-resveratrol is the main contributor to the total antioxidant power of red wine despite the fact that it may not be the most abundant phenol in wine.³ Actually, some of us have recently demonstrated that resveratrol in homogeneous solution is not an outstanding antioxidant since it behaves only as a mild retarder of the thermally initiated autoxidation of styrene and

that other phenolic derivatives present in red wine might be more likely responsible for its antioxidant activity.⁴

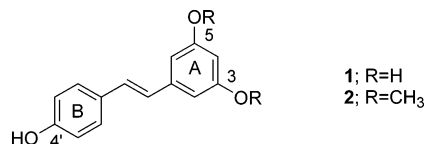
Resveratrol has also been suggested as a potential chemopreventive agent based on its striking inhibitory effect on cellular events associated with cancer initiation, promotion and progression.⁵ This phenolic stilbene has also displayed *in vitro* growth inhibition in a number of human cancer cell lines, and in this context, some of us have recently investigated a series of *cis*- and *trans*-resveratrol and pterostilbene derivatives with the aim of discovering new lead compounds with clinical potential. The synthesized derivatives were screened *in vitro* for cell growth inhibition and ability to induce apoptosis. The *cis* were generally more effective than their corresponding *trans* isomers, and some of them were found to have potent proapoptotic activity.⁶

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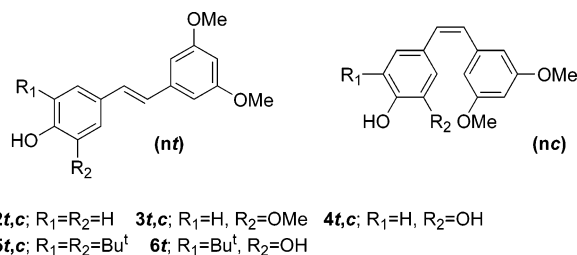
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To further explore the biological activity of these compounds, we thought it could be of interest to check

SCHEME 1



their antioxidant activity, especially with regard to the effect that the geometry about the olefinic double bond has on it and in view of the conflicting results about the antioxidant behavior of the *cis* and *trans* isomers of hydroxystilbenes. It has been reported that the experimental results show no important differences between the *E* and *Z* structures of these molecules,^{7a} while in the case of resveratrol a better activity is found for the *trans* isomer.⁷

Thus, stilbene derivatives bearing a 4'-hydroxyl function in both *cis* and *trans* geometry were selected and their antioxidant activity was evaluated quantitatively by measuring the two parameters which are generally believed to provide better quantitative estimates of the antioxidant power of a given compound, i.e., the rate constant for the reaction with peroxy radicals and the bond dissociation enthalpy (BDE) of the phenolic O–H bond. The former parameter was determined in hydrocarbon solution by measuring the rate constants for the inhibition of the thermally initiated autoxidation reaction of styrene (k_{inh})⁸ and the latter one by using the EPR radical equilibration technique.⁹

The investigated compounds (see Scheme 1) include the *cis* and *trans* isomers of 4'-hydroxy-3,5-dimethoxystilbene (**2c** and **2t**), 3'-methoxy-4'-hydroxy-3,5-dimethoxystilbene (**3c** and **3t**), and 3',4'-dihydroxy-3,5-dimethoxystilbene (**4c** and **4t**), prepared as described previously.⁵ Since the determination of the O–H BDE value by means of the EPR method requires phenols giving reasonably long-lived phenoxyl radicals, we also synthesized the *trans* and *cis* isomers of 3',5'-di-*tert*-butyl-4'-hydroxy-3,5-dimethoxystilbene (**5c** and **5t**) and the *trans* isomer 3',4'-dihydroxy-5'-*tert*-butyl-3,5-dimethoxystilbene (**6t**) which, due to the presence in the positions ortho to the OH group of bulky substituents, afford by irradiation phenoxyl radicals persistent enough to be studied by EPR spectroscopy.

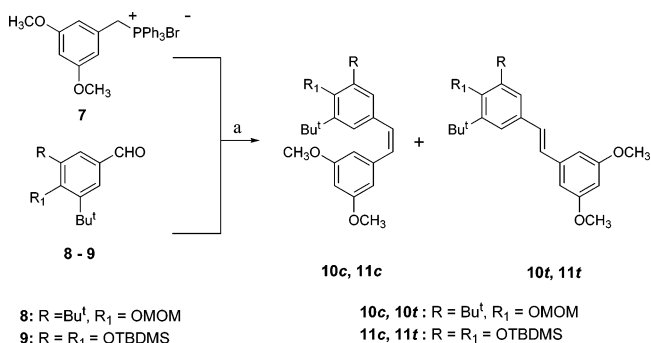
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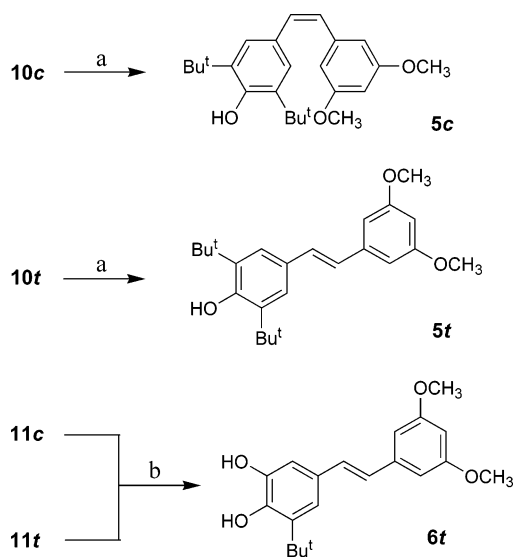
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SCHEME 2^a

^a Reagents and conditions: (a) *n*-BuLi, THF, –78 °C.

SCHEME 3^a

^a Reagents and conditions: (a) pyridinium *p*-toluenesulfonate, CH₃OH, reflux; (b) anhydrous KF, HBr 48%, DMF, rt.

Results and Discussion

Synthetic Procedures. The preparation of new derivatives **5c–t** and **6t** was accomplished by means of the Wittig reaction between the appropriate aromatic aldehydes **8** and **9** and the suitable aromatic ylide, in turn obtained from the phosphonium salt **7** (Scheme 2). Bond geometries of compounds were established by comparing the ¹HNMR of the isomeric pairs. *Z* isomers showed the olefinic protons at 0.3–0.4 ppm higher field compared with the *E* isomers. The coupling constants of the vinylic protons of the *E* isomers were about 16 Hz, whereas the *Z* isomers showed coupling constants of 12 Hz. Deprotection of the methoxymethoxy derivatives **10c–t**, performed with pyridinium *p*-toluenesulfonate (Scheme 3), gave the desired compounds **5t** and **5c**, whereas removal of the *tert*-butyldimethylsilyl (TBDMS) group from stilbene **11t** using potassium fluoride afforded compound **6t** in appreciable yield. Under the same conditions, the *cis* isomer **11c** isomerized to the more stable *trans* derivative **6t**.

Kinetic Measurements. The determination of the rate constants for the reaction with peroxy radicals of the above derivatives (k_{inh}) was made by studying the

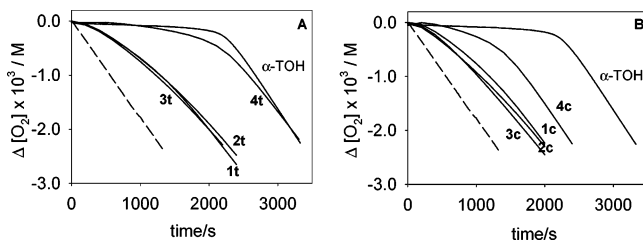
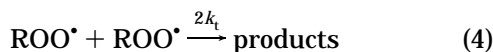
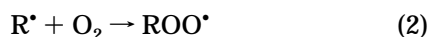


FIGURE 1. Oxygen consumption observed during the autoxidation of styrene (4.30 M) in chlorobenzene initiated by AMVN (5×10^{-3} M) at 30 °C in the absence of antioxidant (dashed line) and in the presence of α -tocopherol (α -TOH) and of one of the various trans (plot A) and cis (plot B) isomers of the hydroxystilbenes **2–4**, each 5.5×10^{-6} M.

inhibition of the thermally initiated autoxidation of styrene¹⁰ (eqs 1–6).



The reaction was followed by monitoring the oxygen consumption during the autoxidation with an automatic recording gas absorption apparatus, built in our laboratory and described previously,¹¹ which contains as detector a commercial differential pressure transducer. The reactions (initiated by the thermal decomposition of AMVN (2,2'-azobis(2,4-dimethylvaleronitrile)) were carried out at 30 °C under controlled conditions in an air-saturated solution of styrene, both in the absence and in the presence of various amounts of antioxidants. α -Tocopherol was used as reference chain breaking inhibitor.

Figure 1 shows that most of the investigated compounds do not give origin to a definite induction period (τ), but rather to a retarding of the autoxidation reaction. The inhibition rate constants k_{inh} (eq 5) of these compounds were determined by using a kinetic treatment¹¹ which consisted of measuring the initial rates of oxidation of styrene both in the presence ($-d[\text{O}_2]/dt = R_{\text{ox}}$) and in the absence ($(-d[\text{O}_2]/dt)_0 = R_{\text{ox},0}$) of antioxidant, AH, and calculating k_{inh} from these data by using eq 7. To use eq 7, we also need the termination constant $2k_t$ (eq 4), which for styrene is known from the literature¹⁰ as $4.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at 30 °C and the initiation rate R_i determined in preliminary experiments as described in the Experimental Section.

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$$\frac{R_{\text{ox},0}}{R_{\text{ox}}} - \frac{R_{\text{ox}}}{R_{\text{ox},0}} = \frac{nk_{\text{inh}}[\text{AH}]_0}{\sqrt{2k_t R_i}} \quad (7)$$

The term n represents the stoichiometric coefficient, i.e., the number of peroxy radicals trapped by each antioxidant molecule, and can be determined from eq 8 by measuring the length of the induction period τ .

$$n = \frac{R_i \tau}{[\text{AH}]} \quad (8)$$

In the present cases, the stoichiometric factor was determined experimentally for several compounds, as reported in Table 1, by studying the inhibited autoxidation of cumene which, due to the smaller k_p value, gives measurable inhibition periods more easily than styrene.¹²

An examination of the two graphs shows that all hydroxystilbenes behave as weak antioxidants with the exception of the derivative **4t**, where the good antioxidant activity is due to the presence of the catechol ring.¹³ When comparing the geometrical isomers of each compound, it appears that the trans isomers are characterized by inhibition rate constants approximately twice as large as those of the cis isomers. These data are consistent with a previous^{7b} rough determination of the relative antioxidant activity of *cis*- and *trans*-resveratrol **1**, which was found to be higher for the latter isomer. It was therefore important to confirm the above result by an independent experimental method.

EPR Spectra and BDE Values. Another experimental parameter closely related to the antioxidant activity of phenolic derivatives is the bond dissociation enthalpy (BDE) of the O–H bond. The method we have been extensively using during the last 10 years consists of the determination of the O–H BDE value of a given phenol, ArOH, by measuring, by means of EPR spectroscopy, the equilibrium constant, K_e , for its reaction with a phenoxy radical, ArO[•], whose heat of formation is known (eq 9) under the assumption that the entropic term can be neglected (eq 10).^{9a,13}



$$\text{BDE}(\text{ArO}-\text{H}) \cong \text{BDE}(\text{ArO}^\bullet-\text{H}) - RT \ln(K_e) \quad (10)$$

However, this technique requires that the investigated compounds give phenoxy radicals reasonably long-lived, while the hydroxystilbenes investigated so far (**1–4**) afford, by photolysis, transient species. We therefore synthesized the *cis* and *trans* isomers of two other derivatives, **5** and **6**, containing *tert*-butyl substituents ortho to the OH group, since this kind of substitution generally increases the persistency of the corresponding phenoxy radicals by preventing, for steric reasons, their decay by dimerization.

Actually, the phenoxy radical from the *trans* isomer of the di-*tert*-butyl-substituted derivative **5t**, obtained by photolyzing at room temperature a deoxygenated benzene

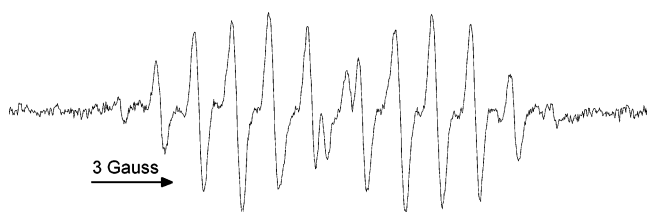
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TABLE 1. Rate Constants k_{inh} for the Reaction with Peroxyl Radicals in PhCl at 30 °C and OH Bond Dissociation Enthalpies (BDE) of Hydroxystilbenes

compd	n ^b	cis		trans	
		k_{inh}^a (M ⁻¹ s ⁻¹)	BDE (kcal mol ⁻¹)	k_{inh}^a (M ⁻¹ s ⁻¹)	BDE (kcal mol ⁻¹)
1		7.4×10^4		1.9	1.4×10^5 83.7 ^c
2	2.2	6.3×10^4		1.9	1.3×10^5 83.7 ^c
3	1.9	5.7×10^4		2.0	1.0×10^5 83.5 ^c
4	1.4	7.6×10^5		1.9	1.1×10^6 79.3 ^b
5		2.3×10^4	80.4 ^e	-	6.8×10^4 78.6 ^d (78.9 ^c)
6				1.6	2.4×10^6 77.7 ^d (77.6 ^c)

^a Inhibition rate constants are believed to be accurate within 10%. ^b Measured using cumene as the oxidizable substrate. ^c BDE value calculated using the additive rule.^{9b} The contribution for the (3,5-dimethoxyphenyl)ethenyl substituent was taken as -3.9 kcal/mol. ^d Value measured at room temperature by means of the EPR radical equilibration technique. ^e The value for **5c** was measured at -65 °C.

**FIGURE 2.** Room-temperature EPR spectrum of the phenoxyl radical obtained by photolyzing a deoxygenated solution of **5t** and di-*tert*-butyl peroxide in benzene.

solution of the precursor containing some di-*tert*-butyl peroxide, was reasonably persistent. The observed EPR spectrum (see Figure 2), indicating that the radical is formed by abstraction of the hydrogen atom from the OH group in position 4', was interpreted in terms of the following spectral parameters: $a_{1H} = 6.44$ G, $a_{1H} = 2.92$ G, $a_{2H} = 1.60$ G, $a_{3H} = 1.51$ G, $g = 2.0040$, where the first two splitting constants are assigned to the vinyl protons and the remaining ones to the couple of protons in positions 2', 6' of the B ring and to the three protons in positions 2, 4, and 6 of the A ring, respectively. Since the shape of the EPR spectrum remains unchanged with time, it seems reasonable to admit that this radical retains on formation the same *trans* geometry of the diamagnetic precursor and that it is characterized by a higher geometrical stability than the *cis* radical. This conclusion was confirmed by the study carried out on the *cis* isomer **5c** described below.

The measure of the BDE value of the O–H bond in **5t** was performed by studying the equilibrium constant for the hydrogen atom exchange reaction with the phenoxyl radical from 2,6-di-*tert*-butyl-4-methoxyphenol (BHA), whose BDE in benzene solution is 78.3 kcal/mol.^{9a} This phenol was chosen because its BDE value is expected to be similar to that of **5t** (vide infra) and because only a small portion of the EPR spectrum of the phenoxyl radical from **5t** overlaps with that of the radical from BHA. Due to this separation, the relative concentrations of the two radical species could be easily measured by double integration of the EPR spectra in order to obtain K_e . The BDE value for the O–H bond of **5t** was 78.6 ± 0.5 kcal/mol.

Attempts to record at room temperature the EPR spectrum of the corresponding *cis*-phenoxyl radical failed

since photolysis of a solution of benzene, di-*tert*-butyl peroxide and **5c** afforded a spectrum identical to that obtained from **5t** and assigned to the corresponding *trans* radical. This result is not unexpected since it is known that the radical anion and cation of stilbene undergo *cis*–*trans* interconversion much faster than the corresponding parent molecule due to the lower barrier to internal rotation.^{14–18}

On the basis of these results, it is conceivable that also the phenoxyl radicals from hydroxyl stilbenes are characterized by a rate of *cis* to *trans* conversion larger than that of the parent compounds, thus making the detection of the less stable *cis* radical difficult at room temperature. Experiments were therefore carried out at -65 °C in degassed toluene containing either **5t** or **5c** and 10% di-*tert*-butyl peroxide. In the case of **5t**, an EPR spectrum similar to that recorded at room temperature and interpreted in terms of the following hyperfine splitting constants, $a_{1H} = 6.51$ G, $a_{1H} = 2.67$ G, $a_{3H} = 1.65$ G, $a_{3H} = 1.45$ G, was observed. When the solution containing the *cis* isomer **5c** was photolyzed at -65 °C, a spectrum clearly consisting of the superimposition of two species was detected. One of them (ca. 50%) was easily identified as the *trans*-phenoxyl radical on the basis of its spectral parameters, while the other one was attributed to the *cis* radical having the following hyperfine splitting constants $a_{1H} = 8.36$ G, $a_{1H} = 3.22$ G, $a_{2H} = 1.62$ G, $a_{2H} = 1.86$ G, $a_{1H} = 0.91$ G. The EPR spectra changed gradually upon irradiation, with the amount of the *trans* species increasing and that of the *cis* isomer decreasing with time.

There are two reasons for the detection of such a large amount of the *trans* isomeric radical: one is that the synthesis of **5c** afforded a product containing **5t** as an impurity (although its percent was too small to be detected by NMR), the second one is that, since **5t** is characterized by an O–H BDE value lower than **5c** (vide infra), the hydrogen atom exchange reaction involving **5c**, **5t**, and the corresponding phenoxyl radicals is shifted toward the formation of the *trans* isomeric radical. Thus, even in the presence of a very low concentration of **5t**, a remarkable amount of the corresponding radical was observed.

To obtain the BDE value of the *cis* isomer we photolyzed at -65 °C a toluene and Bu^tOObu^t solution containing 95% **5c** and 5% **5t**. Under these conditions, the ratio between the *cis*- and *trans*-phenoxyl radicals dropped from ca. 1 to 0.2. From these data, we could obtain the percent of **5t** (1.3%) contained as an impurity in **5c**, as well as the equilibrium constant for the hydrogen atom exchange reaction (eq 9) involving **5c** and **5t** and the corresponding radicals ($K_e = 0.014$). Thus, from the difference between the BDEs of the *cis*- and *trans*-hydroxyl stilbenes (1.8 kcal/mol) the O–H BDE value of **5c** was obtained as 80.4 kcal/mol, consistent with the lower reactivity toward peroxy radicals of the *cis* isomers of pterostilbenes (see Table 1).

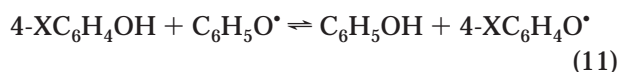
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The higher BDE value found in the *cis* isomers can be rationalized as follows. Being the O–H BDE given by the difference between the energies of the phenoxyl radical (plus that of the hydrogen atom) and of the starting phenol, the *cis* geometry for resveratrol and related compounds implies that the folding of the structure¹⁹ causes a greater destabilization of the phenoxyl radical than of the parent phenol. It will be shown in the next section that a density functional theory (DFT) study of the geometrical isomers of resveratrol correctly predicts these experimental results.

We also generated inside the EPR cavity the phenoxyl radical from **6t**, containing the catechol group, by photolyzing this compound in a 9:1 mixture of benzene/*di-tert*-butyl peroxide. The room-temperature EPR spectrum was interpreted in terms of the following spectroscopic parameters: $a_{1H} = 5.58$ G, $a_{1H} = 2.63$ G, $a_{1H} = 1.81$ G, $a_{1H} = 0.46$ G, $a_{1H}(\text{OH}) = 0.98$ G, $a_{2H} = 1.32$ G, $a_{1H} = 1.20$ G, $g = 2.0042$. The first two constants were assigned to the vinyl protons, the last two to the three protons of the A ring, and the 1.81 and 0.46 G splittings to the two protons of the B ring. The determination of the OH bond dissociation enthalpy of this catechol derivative was made by studying its equilibration with 2,6-*di-tert*-butyl-4-methoxyphenol (BHA) under continuous photolysis. A BDE value of 77.7 kcal/mol was obtained in benzene solution for **6t**.

With those hydroxystilbenes giving by photolysis scarcely persistent phenoxyl radicals, the O–H BDE values could not be determined experimentally but the O–H bond strength could be estimated using the additivity rule.^{9b} Actually, it is known that the change of the O–H bond strength due to a given substituent is approximately constant in the variously substituted phenols and that this contribution may be used to estimate the BDE of polysubstituted phenols. The contributions of *o-tert*-butyl, *o*-methoxyl, and *o*-hydroxyl substituents have been reported in previous papers,^{9b,13} while that due to the (3,5-dimethoxyphenyl)ethenyl substituent could be estimated from the experimental BDEs of **5t** (–4.2 kcal/mol) and **6t** (–3.8 kcal/mol). Since these values are quite close to that reported for the phenylethenyl substituent (–3.9 kcal/mol),^{9b} the two methoxy groups in the meta positions of the A phenyl ring have only a negligible effect on the BDE of hydroxystilbenes. It is therefore possible to estimate the O–H BDE value for the hydroxyl group in the 4' position of the B ring in all the investigated compounds, as shown in Table 1.

Computational Studies. The gas-phase bond dissociation enthalpies of the phenolic OH bond of both *trans* and *cis* isomers of 4'-hydroxy-3,5-dimethoxystilbene were also calculated by means of the DFT method at the B3LYP/6-31G* level using the isodesmic reaction showed in eq 11,²⁰ where X represents either the *cis*- or *trans*-3,5-dimethoxystyryl substituent.



This method yields reliable ΔBDE values (eq 12) for the various substituted phenols,²⁰ due to cancellation of

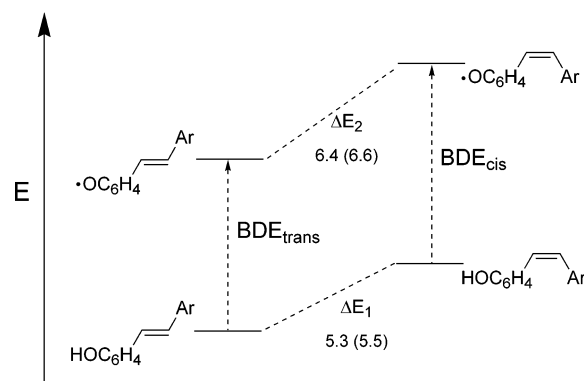


FIGURE 3. DFT-computed energy differences (kcal/mol) for the *cis* and *trans* isomers of **1** and of the corresponding phenoxyl radicals at the B3LYP/6-31G* level, in parentheses with ZPVE corrections.

errors. Absolute BDEs are obtained by adding to this term the experimental BDE value for phenol (eq 13).

$$\Delta\text{BDE}_{\text{calc}}(4\text{-XC}_6\text{H}_4\text{OH}) = [E(4\text{-XC}_6\text{H}_4\text{O}^\bullet) - E(4\text{-XC}_6\text{H}_4\text{OH})] - [E(\text{C}_6\text{H}_5\text{O}^\bullet) - E(\text{C}_6\text{H}_5\text{OH})] \quad (12)$$

$$\text{BDE}_{\text{calc}}(4\text{-XC}_6\text{H}_4\text{OH}) = \text{BDE}_{\text{exp}}(\text{C}_6\text{H}_5\text{OH}) + \Delta\text{BDE}_{\text{calc}}(4\text{-XC}_6\text{H}_4\text{OH}) \quad (13)$$

The DFT results indicate that substitution of the para hydrogen atom of phenol with either a *cis*- or a *trans*-3,5-dimethoxystyryl substituent causes a decrease of the O–H BDE value, since this reduction is somewhat higher for the *trans* (–5.4 kcal/mol)²¹ than for the *cis* isomer (–4.2 kcal/mol). These data should be compared to the corresponding experimental ΔBDE values, i.e., –4.2 and –2.4 kcal/mol for the *trans* and *cis* isomer, respectively. Although the computed ΔBDE are larger than the experimental ones, their differences are similar, being 1.2 kcal/mol for the calculated and 1.8 kcal/mol for the measured values.

To rule out any uncertainty about the effect of remote substituents in positions 3 and 5, similar calculations were carried out also on the *cis* and the *trans* isomers of resveratrol (**1**) and 4-hydroxystilbene. The results, reported in Table S1 (Supporting Information), clearly show that the influence of remote substituents is small on the calculated BDE values (<0.3 kcal/mol) as well as on the energy difference between the *cis* and the *trans* isomers (<0.3 kcal/mol).

The reason the *cis* isomer possesses a slightly lower ΔBDE is shown in Figure 3, which reports the differences between the energies of the isomeric phenols and those of the isomeric phenoxyl radicals. Actually, the folded and therefore strained structure of the *cis* isomer phenol (**2c**) is characterized by an energy value higher by 5.3 kcal/mol than that of the *trans* isomer (**2t**). On the other hand, in the corresponding phenoxyl radicals the difference between the energies of the two isomers increases to 6.4 kcal/mol, indicating that the destabilization associated

(21) The ΔBDE values obtained in this work for the *trans*-3,5-dimethoxystyryl substituent (–5.4 kcal/mol) is significantly lower than what obtained by Wright et al.²² for the *trans*-styryl (–8.5 kcal/mol) and the *trans*-3,5-dihydroxystyryl (–8.2 kcal/mol) substituent by using the locally dense basis sets (LDBS) method.

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with the adoption of the *cis* geometry is larger in the radical than in the phenol. Calculations also show that the spin densities on the A ring of **2c** are lower than those of **2t** while for the B ring the reverse is true (Figure 1S, Supporting Information). The above considerations are not modified by ZPVE corrections (see Figure 3 and Table S1, Supporting Information). In conclusion, the BDE difference between *cis* and *trans* isomers can be explained in terms of reduced conjugative interactions between the two rings of **2c**, this having a greater effect on the radical, where conjugation is more effective than in the phenol.

Conclusions

The kinetic investigation of the reaction between peroxy radicals and substituted hydroxystilbenes, here reported, has shown that the antioxidant activity of the *cis* geometrical isomers of these compounds is in all examined cases worse, by a factor of about 2, than that of the corresponding *trans* isomers. This lower reactivity is fully justified on a thermodynamic basis by the experimental determination of the O–H bond dissociation enthalpy (BDE) in the two geometric isomers of 3',5'-di-*tert*-butyl-4'-hydroxy-3,5-dimethoxystilbene (**5c** and **5t**). The BDE value for the *cis* isomer has been actually found to be larger by 1.8 kcal/mol than that for the *trans* isomer, this making the transfer of the hydroxyl hydrogen from the latter one to peroxy radicals easier. DFT calculations, besides predicting a difference (1.2 kcal/mol) between the strengths of the O–H bond in the two isomers of 4'-hydroxy-3,5-dimethoxystilbene (**2**) similar to the experimental value (1.8 kcal/mol) determined for the two isomers of **5**, provide an explanation of this result. In fact, the DFT data indicate that, although the *cis* geometry implies a destabilization of both phenoxyl radical and parent hydroxystilbene, destabilization of the phenoxyl radical is larger as a result of a reduced delocalization of the unpaired electron on the styryl group (see the Supporting Information).

The present data also corroborate our previous finding that in homogeneous solution resveratrol, despite its excellent reputation, is only a mild antioxidant.⁴ Between the various hydroxystilbenes presently investigated, a remarkable antioxidant efficacy is only exhibited by the *trans* isomers of **4** and **6**, which, however, should be attributed to the presence in these compounds of the catecholic function rather than of the arylvinyl group.

A comparison of the present results with previously reported data on the proapoptotic activity of these stilbenoids, for which the *cis* stereochemistry has been shown to be associated with a stronger activity than the *trans* geometry,⁶ suggests that the reverse is true for the antioxidant ability of these compounds. Thus, it seems that from the structural point of view there is no correlation between these two properties and that stilbenoids characterized by a better antioxidant behavior possess lower proapoptotic activities. To further explore this point, studies of structure–activity relationships (SAR) are under way.

Experimental Section

Materials and Methods. All solvents were redistilled prior to use. All reactions were carried out under an inert atmosphere. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate. Reactions were monitored by thin-

layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60 F₂₅₄). Flash column chromatographies were performed on silica gel (particle size 40–63 μm). Melting points were determined in open capillary tube and are uncorrected. ¹H NMR and ¹³C NMR spectra were recorded at 300 and 75 MHz, respectively, in CDCl₃ solution unless otherwise noted. Chemical shifts (δ_H) are reported relative to TMS as internal standard. IR–FT spectra were recorded in CCl₄ solution; ν_{max} is expressed in cm⁻¹.

Compound **8** was obtained from the commercially available 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde hemihydrate by protection of phenol with a methoxymethyl group (MOM) following standard procedures. Compound **9** was obtained from the known 3-*tert*-butyl-4,5-dihydroxybenzaldehyde²³ by protection of the hydroxyl groups with *tert*-butyldimethylsilyl groups (TBDMS) following standard procedures.

General Procedure for Preparing Stilbenes 10c–t and 11c–t. To a suspension of the phosphonium bromide salt **7** (1.0 equiv) in anhydrous tetrahydrofuran at –78 °C was added *n*-butyllithium (2.5 M in hexanes, 1.0 equiv), and the resulting red solution was stirred under nitrogen for 1 h. A solution of aldehydes **8** and **9** (1.0 equiv) in tetrahydrofuran was added dropwise over 30 min, and the reaction mixture was stirred for 2 h at room temperature. The resulting suspension was poured into water and extracted with CH₂Cl₂. The organic phase was washed with brine (2 × 15 mL) and dried over Na₂SO₄, and the solvent was removed in vacuo to afford a mixture of the *cis/trans*-stilbenes **10c–t** and **11c–t** that were separated by flash chromatography. The *cis*-stilbenes were eluted first, followed by the *trans* isomers.

3',5'-Di-*tert*-butyl-4'-methoxymethoxy-3,5-dimethoxystilbenes (10c–t). Reaction of phosphonium bromide salt **7** and 3,5-di-*tert*-butyl-4-methoxymethoxybenzaldehyde **8** (1.2 g, 4.31 mmol) gave a mixture of *cis*-stilbene **10c** and *trans*-isomer **10t**. Flash chromatography: petroleum ether/ethyl acetate 9.98:0.02.

10c: 0.85 g (yield 50%); red oil; ¹H NMR δ 1.43 (s, 18H), 3.71 (s, 3H), 3.75 (s, 6H), 4.98 (s, 2H), 6.43–6.37 (m, 3H), 6.50 (m, 2H), 6.60 (m, 2H); ¹³C NMR δ 31.9, 35.6, 55.1, 57.2, 99.4, 100.4, 106.6, 127.5, 129.1, 130.9, 131.5, 139.6, 143.8, 153.5, 160.5.

10t: 0.65 g (yield 38%); white solid; mp 62 °C; ¹H NMR δ 1.55 (s, 18H), 3.71 (s, 3H), 3.88 (s, 6H), 4.98 (s, 2H), 6.43–6.45 (t, 1H, *J* = 2.2 Hz), 6.74–6.73 (m, 2H), 6.85 (d, 1H, *J* = 16 Hz), 7.13 (d, 1H, *J* = 16.2 Hz), 7.48 (m, 2H); ¹³C NMR δ 32.0, 35.8, 55.3, 57.3, 99.7, 100.5, 104.3, 124.8, 127.3, 129.4, 131.7, 139.5, 144.5, 154.3, 160.8.

3',4'-Di-(*tert*-butyldimethylsilyloxy)-5'-*tert*-butyl-3,5-dimethoxystilbenes (11c, 11t). Reaction of phosphonium bromide salt **7** and 3-*tert*-butyl-4,5-di-(*tert*-butyldimethylsilyloxy)benzaldehyde **9** (0.28 g, 0.66 mmol) gave a mixture of the *cis*-**11c** and *trans*-**11t** isomers. Flash chromatography: petroleum ether/ethyl acetate 9.5:0.5.

11c: 0.10 g (yield 27%); pale yellow oil; ¹H NMR δ 0.16 (s, 6H), 0.27 (s, 6H), 0.83 (s, 9H), 0.96 (s, 9H), 1.27 (s, 9H), 3.69 (s, 6H), 6.30–6.32 (t, 1H, *J* = 2.2 Hz), 6.44–6.46 (m, 4H), 6.70 (d, 1H, *J* = 2.2 Hz), 6.86 (d, 1H, *J* = 2.2 Hz); ¹³C NMR δ –2.7, –1.2, 19.2, 19.4, 26.6, 27.2, 30.5, 35.1, 55.2, 99.7, 106.5, 118.9, 121.4, 128.3, 130.8, 139.8, 140.6, 145.0, 146.6, 160.6.

11t: 0.11 g (yield 30%); pale yellow oil; ¹H NMR δ 0.32 (s, 6H), 0.35 (s, 6H), 0.95 (s, 9H), 1.00 (s, 9H), 1.44 (s, 9H), 3.86 (s, 6H), 6.30–6.33 (t, 1H, *J* = 2.2 Hz), 6.67 (m, 2H), 6.82 (d, 1H, *J* = 16.2 Hz), 6.98 (d, 1H, *J* = 16.2 Hz), 6.99 (m, 1H), 7.07 (m, 1H); ¹³C NMR δ –2.4, –1.2, 19.36, 19.38, 26.7, 27.2, 30.6, 35.3, 55.4, 99.5, 104.3, 116.0, 119.3, 126.2, 128.6, 129.7, 139.9, 141.3, 145.9, 147.2, 160.9.

General Procedure for the Cleavage of the Methoxymethyl Group of Compounds 10c–t. A solution of the

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methoxymethoxy-protected stilbenes **10c-t** (1 equiv) and pyridinium *p*-toluenesulfonate (10 equiv) in methanol (10 mL) was refluxed for 15 h. The reaction mixture was cooled to room temperature and the solvent removed under reduced pressure. The residue was triturated with diethyl ether, and the solids were filtered off and washed with ether. The organic phase was dried over Na₂SO₄ and concentrated under reduced pressure to give the crude product which was purified by chromatography (petroleum ether/ethyl acetate 97:3).

cis-3',5'-Di-tert-butyl-4'-hydroxy-3,5-dimethoxystilbene (5c). Compound **5c** was obtained from **10c** (0.425 g, 1.07 mmol): 0.13 g (yield 33%); yellow oil; ¹H NMR δ 1.36 (s, 18H), 3.71 (s, 6H), 5.22 (s, 1H), 6.36–6.33 (t, 1H, *J* = 2.2 Hz), 6.46 (d, 1H, *J* = 11.6 Hz), 6.47 (m, 2H), 6.55 (d, 1H, *J* = 12 Hz), 7.14 (m, 2H); ¹³C NMR δ 30.2, 34.2, 55.2, 99.4, 106.5, 126.1, 127.86, 127.93, 131.3, 135.4, 140.1, 153.1, 160.6; IR ν_{max} (CCl₄) cm⁻¹ 1015, 1069, 1098, 1155, 1206, 1260, 1437, 1458, 1595, 2958, 3642.

trans-3',5'-Di-tert-butyl-4'-hydroxy-3,5-dimethoxystilbene (5t). Compound **5t** was obtained from **10t** (0.43 g, 1.08 mmol): 0.26 g (yield 65%); yellow powder; mp 106 °C; ¹H NMR δ 1.51 (s, 18H) 3 0.86 (s, 6H), 5.31 (s, 1H), 6.41–6.38 (t, *J* = 2.2 Hz, 1H), 6.69 (m, 2H), 6.89 (d, 1H, *J* = 16 Hz), 7.08 (d, 1H, *J* = 16.2 Hz), 7.37 (m, 2H); ¹³C NMR δ 30.3, 34.4, 55.3, 99.5, 104.2, 123.4, 125.7, 127.6, 128.3, 130.0, 136.1, 139.9, 153.8, 160.8; IR ν_{max} (CCl₄) cm⁻¹ 958, 1063, 118, 1155, 1205, 1437, 1458, 1592, 1693, 2958, 3641. Anal. Calcd for C₂₄H₃₂O₃: C, 78.22; H, 8.75. Found: C, 78.25; H, 8.71.

trans-3',4'-Dihydroxy-5'-tert-butyl-3,5-dimethoxystilbene (6t). To the *trans*-silyloxy-protected stilbene derivative **11t** (0.11 g, 0.19 mmol) in dry DMF (3–6 mL for mmol of TBDMS group) were added anhydrous KF (0.04 g, 0.79 mmol) and a catalytic amount of 48% aqueous HBr (7 μL, 0.06 mmol). The reaction mixture was stirred under nitrogen at room temperature for 4 h. The mixture was poured into 2 N HCl (2 × 15 mL), and the aqueous layer was extracted with diethyl ether (3 × 15 mL). The combined organic layers were washed with brine (2 × 15 mL) and dried over Na₂SO₄, and the solvent was evaporated. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to give **6t** (0.026 g, yield 43%): reddish-orange solid; mp 48–52 °C; ¹H NMR δ 1.46 (s, 9H) 3.85 (s, 6H), 5.06 (br, 1H), 5.76 (s, 1H), 6.38–6.40 (t, 1H, *J* = 2.2 Hz), 6.65–6.66 (m, 2H), 6.83 (d, 1H, *J* = 16.6 Hz), 6.99 (d, *J* = 15.8 Hz, 1H), 7.03–7.01 (m, 2H); ¹³C NMR δ 29.4, 34.7, 55.4, 99.5, 104.3, 110.1, 118.8, 126.0, 128.3, 129.5, 136.4, 139.8, 143.1, 143.7, 160.9; IR ν_{max} (CCl₄) cm⁻¹ 1068, 1155, 1205, 1260, 1294, 1429, 1457, 1507, 1596, 2958, 3546, 3615. Anal. Calcd for C₂₀H₂₄O₄: C, 73.15; H, 7.37. Found: C, 73.18; H, 7.35.

Kinetic Measurements. The rate constants for the reaction of hydroxystilbenes with peroxy radicals have been measured by following the autoxidation of styrene (4.30 M) at 303 K using as initiator AMVN (5 × 10⁻³ M). The antioxidant concentration was kept constant for all measurements (5.5 × 10⁻⁶ M) in order to compare more easily their behavior. Since the analyzed compounds **4–6** showed a scarce solubility in chlorobenzene, they were initially dissolved in a small amount of methanol and then this solution was diluted in chlorobenzene.²⁴ Initiation rates, *R*_i, were determined for each condition in preliminary experiments by the inhibitor method using α-tocopherol as reference antioxidant: *R*_i = 2[α-TOH]/τ.⁸ Stoichiometric coefficients, *n*, were determined by using cumene (6.2 M) as the oxidizable substrate.

(24) The small amount of added methanol is expected to decrease the antioxidant activity of the investigated phenols except for those containing two *o*-*tert*-butyl substituents.²⁵ On the basis of the *α* and *β* values reported by Abraham,²⁶ the reduction of *k*_{inh} induced by the methanol present (0.12 M) can be estimated as 47% for the resveratrol derivatives **1** and **2**, 72% for the catechols **4** and **6**, and 7% in the case of the *o*-methoxy derivatives **3**. Since, however, it seems reasonable that both *cis* and *trans* isomers are equally solvated, their relative reactivity should not be affected by methanol.

Thermochemistry. To determine the BDE value of **5t**, solutions were prepared by using as solvent benzene in the presence of a 10% v/v of di-*tert*-butylperoxide and the two phenols in concentration ratios: [5t]/[BHA] = 1.16 and [5t]/[BHA] = 1.76. The solutions were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted in the thermostated cavity of an EPR spectrometer and photolyzed with the unfiltered light from a 500 W high-pressure mercury lamp. The temperature was controlled with a standard variable-temperature accessory and was monitored before and after each run with a copper–constantan thermocouple. Δ*G* values of –0.30 kcal/mol and –0.37 kcal/mol were found from the EPR spectra, corresponding to an average ΔBDE value of –0.34 kcal/mol and therefore to a BDE value for **5t** of 78.6 kcal/mol.

A difference reference compound has also been used: 3,5-di-*tert*-butyl-4-hydroxystilbene.^{9b} Since the two phenols give phenoxy radicals with very similar EPR spectra, their mixture provided only a qualitative estimate of the O–H BDE value of **5t** but could not be used for accurate measurements. To determine the BDE of **5c**, we used conditions similar to the ones described above, apart from the temperature that was kept at 209 K and checked before and after every measurement with a copper–constantan thermocouple. These measurements were made in toluene, and the *trans* derivative **5t** was used as reference compound at a 5% concentration.

The OH bond dissociation enthalpy of the catechol derivative **6t** was made by studying its equilibration with 2,6-di-*tert*-butyl-4-methoxyphenol (BHA) under continuous photolysis at room temperature.

Computational Details. BDE values and energy differences between *cis* and *trans* isomers of the various stilbene derivatives were computed following the isodesmic approach, shown in eqs 11–13, from the total energies obtained from DFT calculations with the B3LYP functional, using the Gaussian 98 system of programs.²⁷ The unrestricted wave function was used for radical species. Geometries and single point calculations were obtained at the B3LYP/6-31G* level of theory. Stationary points were confirmed by frequency calculations at the same level of theory. Zero-point vibrational energies corrections (ZPVE), obtained using a scale factor of 0.9806,²⁸ had negligible effects.

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Supporting Information Available: ¹H and ¹³C NMR spectral data for compounds **5c**, **5t**, and **6t**. Table S1, Figure S1: computed geometries and total energies of the *cis* and *trans* isomers of **1**, **2**, and 4-hydroxystilbene (**12**) and their corresponding phenoxy radicals. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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