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5-*S*-Lipoylhydroxytyrosol, a Multidefense Antioxidant Featuring a Solvent-Tunable Peroxyl Radical-Scavenging 3-Thio-1,2-dihydroxybenzene Motif

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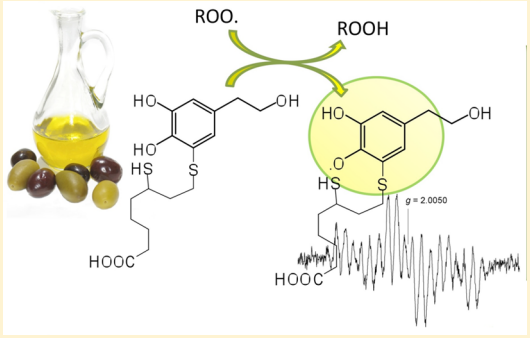
Supporting Information

ABSTRACT: 5-*S*-Lipoylhydroxytyrosol (**1**), the parent member of a novel group of bioinspired multidefense antioxidants, is shown herein to exhibit potent peroxyl radical scavenging properties that are controlled in a solvent-dependent manner by the sulfur center adjacent to the active *o*-diphenol moiety. With respect to the parent hydroxytyrosol (HTy), **1** proved to be a more potent inhibitor of model autoxidation processes in a polar solvent (acetonitrile), due to a lower susceptibility to the adverse effects of hydrogen bonding with the solvent. Determination of O−H bond dissociation enthalpies (BDE) in *t*-butanol by EPR radical equilibration technique consistently indicated a ca. 1.5 kcal/mol lower value for **1** relative to HTy. In good agreement, DFT calculations of the BDE_{OH} using an explicit methanol molecule to mimic solvent effects predicted a 1.2 kcal/mol lower value for **1** relative to HTy. Forcing the geometry of the -S-R group to coplanarity with the aromatic ring resulted in a dramatic decrease in the computed BDE_{OH} values suggesting a potentially higher activity than the reference antioxidant α-tocopherol, depending on geometrical constrains in microheterogeneous environments. These results point to sulfur substitution as an expedient tool to tailor the chain-breaking antioxidant properties of catechol derivatives in a rational and predictable fashion.



INTRODUCTION

The elucidation of the complex interplay of structural factors, electronic effects, and noncovalent (H-bonding) interactions that govern the activity of natural and synthetic phenolic antioxidants is an important goal toward the rational design of potent multidefense systems tailored for specific biomedical and technological applications, e.g., in food industry and functional packaging. Central issues of both theoretical and practical interest relate to the impact of intra- or intermolecular H-bonding on the H-atom donor properties of −OH moieties and the effects of ring substituents.[1] Considerable interest, in the latter context, has been raised by the contribution of organochalcogen substituents to the O−H bond dissociation enthalpy (BDE) and reactivity of phenolic antioxidants.[2−4] EPR, IR and computational investigations of *ortho*- and *para*-(S, Se, Te)-substituted phenols showed that the chalcogen lowers the O−H BDE by >3 kcal/mol in the *para* position, while the *ortho*-effect is modest because of hydrogen bonding (approximately 3 kcal/mol) to the O−H group.[4]

Recently, in the frame of a project directed to the synthesis of novel antioxidants inspired to bioactive thiol-conjugates of naturally occurring phenolic compounds,[5−8] we have focused our attention to hydroxytyrosol (2-(3,4-dihydroxyphenyl)-ethanol, HTy) as a most convenient and easily accessible catecholic platform for the synthesis and evaluation of a series of lipophilic bioinspired thiol conjugates. HTy, the most representative phenolic constituent of extra virgin olive oil, has been the focus of increasing interest over the past decades because of a range of biological properties purportedly implicated in the lower risk of cardiovascular diseases and malignant neoplasms commonly associated with the Mediterranean diet.[9,10] The beneficial role of HTy has been ascribed to its potent antioxidant and scavenging properties against reactive oxygen (ROS) and nitrogen (RNS) species[11−15] generated in oxidative stress-associated diseases,[16] and its reactivity with oxidizing systems of physiological relevance has been elucidated.[17,18] Several efforts have been directed toward the preparation of HTy derivatives and analogues with enhanced lipophilicity, antioxidant, and pharmacological activities.[19−23]

Inspired by the reported antioxidant effects of dihydrolipoic acid (DHLA)[24] and the promising properties of thiol-catechol conjugates, we were recently prompted to investigate a new set of HTy-dihydrolipoic acid *S*-conjugates, namely, 5-*S*-lipoylhydroxytyrosol (**1**) and its disulfide **2**, trisulfide **3**, and tetrasulfide **4**, as promising lead structures for the development of innovative, multidefense antioxidants mimicking natural prod-

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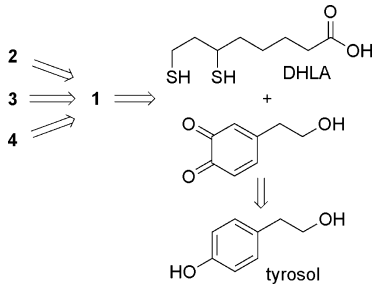
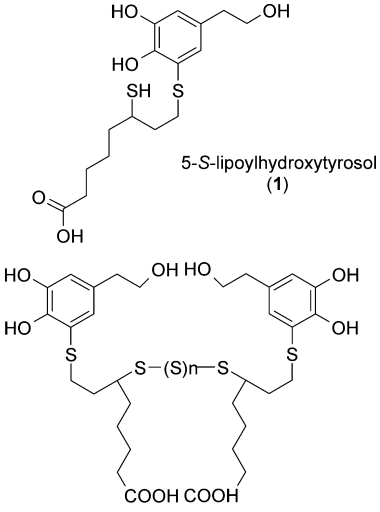
ucts.[25] Adduct **1** is formed by facile regioselective reaction of DHLA with HTy *o*-quinone generated by oxidation of tyrosol, and it spontaneously oxidizes to dimeric **2** upon standing in mildly alkaline (pH 7.4) aqueous solution in the presence of oxygen, as illustrated in Scheme 1.[25] Conversion into

Scheme 1. Preparation of Adducts 1−4



polysulfides **3** and **4** instead requires addition of sulfur.[25] Compounds **1−4** are stable in the dry form in refrigerated conditions and fairly stable under normal handling in organic solution.



2: n=0; **3**: n=1; **4**: n=2

Determination of the antioxidant activities of **1−4** in several assays revealed markedly potent effects compared to the parent HTy. Compounds **1−4** also exerted potent protective effects against ROS generation and oxidative cell damage in human liver HepG2 cells.[25]

These observations stimulated further investigations aimed at assessing the actual scope of sulfur-substituted HTy derivatives as antioxidants and at elucidating the structural factors underlying the enhanced antioxidant capacity of the 3-alkylthio-1,2-dihydroxybenzene motif. Herein, we disclose the efficient peroxyl radical scavenging properties of compound **1** and its congeners using an integrated kinetic, EPR, and computational approach. The main aim of the study was to assess the influence of the 3-alkylthio-substituent on the bond dissociation enthalpy (BDE) of the *o*-diphenol moiety, and the elucidation of the underlying stereoelectronic effects, in the prospects of a rational exploitation of chalcogen substitution as an expedient tool to tailor the antioxidant and chain breaking properties of HTy derivatives.

RESULTS AND DISCUSSION

Kinetic Measurements with Peroxyl Radicals. Kinetic measurements with peroxyl radicals were performed by studying the inhibited autoxidation of styrene or cumene in chlorobenzene or acetonitrile (50% vol/vol) at 303 K, initiated by AIBN (0.05 M), in the presence of variable amounts (2−10 × 10^{-6} M) of HTy and compounds **1−4** (indicated as ArOH in eqs 1−6):[26]

$$\text{initiator} + \text{RH} \xrightarrow{R_i} \text{R}^{\cdot} \quad (1)$$

$$\text{R}^{\cdot} + \text{O}_2 \rightarrow \text{ROO}^{\cdot} \quad (2)$$

$$\text{ROO}^{\cdot} + \text{RH} \xrightarrow{k_p} \text{ROOH} + \text{R}^{\cdot} \quad (3)$$

$$\text{ROO}^{\cdot} + \text{ROO}^{\cdot} \xrightarrow{2k_t} \text{nonradical products} \quad (4)$$

$$\text{ROO}^{\cdot} + \text{ArOH} \xrightarrow{k_{inh}} \text{ROOH} + \text{ArO}^{\cdot} \quad (5)$$

$$\text{ROO}^{\cdot} + \text{ArO}^{\cdot} \rightarrow \text{nonradical products} \quad (6)$$

The autoxidation was followed by monitoring the oxygen consumption in an oxygen uptake apparatus based on a differential pressure transducer (Figure 1).[27] The slope of the oxygen consumption trace during the inhibited period afforded k_{inh} values, while its length allowed the determination of the stoichiometric coefficient *n*, i.e., the number of peroxyl radicals entrapped by each antioxidant molecule (Table 1). For comparison, kinetic measurements under identical settings were extended to other well established catechol-type

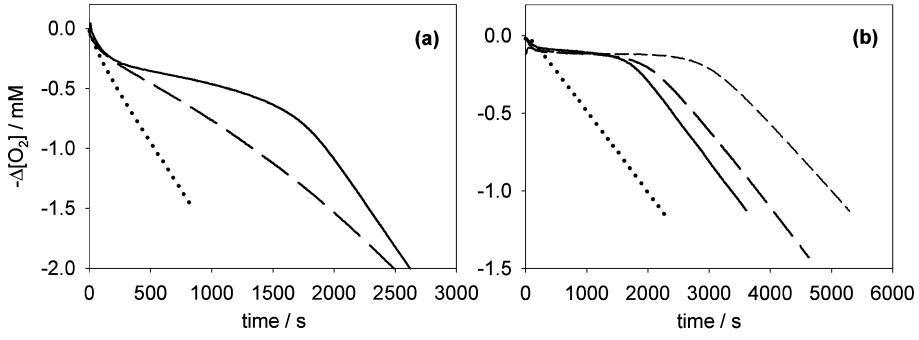


Figure 1. Oxygen consumption during the autoxidation of styrene in chlorobenzene (a) and of cumene in acetonitrile (b) (both 50% v/v) initiated by AIBN (0.05 M) at 303 K in the absence of inhibitors (dotted line) or in the presence of HTy (continuous), **1** (long dash), or **2** (short dash). All antioxidants are 6.3 μM.

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Table 1. Rate Constants k_{inh} for the Reaction of Compounds 1−4 with Peroxyl Radicals at 303 K and (in Brackets) Number of Radical Trapped by Each Antioxidant Molecule

compound[a]	k_{inh} (chlorobenzene) (M^{-1} s^{-1})[b]	k_{inh} (acetonitrile) (M^{-1} s^{-1})[b]
HTy	$(8 \pm 1) \times 10^5$ (1.9)	$(3.0 \pm 0.5) \times 10^4$ (1.9)
1	$(2.0 \pm 0.8) \times 10^5$ (2.2)	$(5.0 \pm 0.5) \times 10^4$ (2.0)
2	–	$(9.0 \pm 2.0) \times 10^4$ (3.5)
3	–	$(7.3 \pm 1.1) \times 10^4$ (3.2)
4	–	$(7.0 \pm 1.0) \times 10^4$ (3.0)
3,5-di-*tert*-butylcatechol	$(1.1 \pm 0.3) \times 10^6$ (2.0)	2.0×10^4 [c]
quercetin[d]	$(5.0 \pm 0.7) \times 10^5$ (2.1)	$(1.2 \pm 0.7) \times 10^4$ (~2)
epicatechin[e]	4.2×10^5	2.1×10^4 (in *t*-butanol)

[a]Data from reference antioxidants are also reported. [b]Mean of at least three measurements; errors correspond to ± SD. [c]From ref 29. [d]Literature k_{inh} values for quercetin at 50 °C are 4.3×10^5 and 2.1×10^4 M^{-1} s^{-1}, respectively, in chlorobenzene and *t*-butanol (ref 30). [e]Data at 50 °C from ref 30.

antioxidants both of natural (quercetin) and synthetic (3,5-di-*tert*-butylcatechol, DBC) origin. The synthetic analogue of α-tocopherol 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) was used as reference antioxidant.

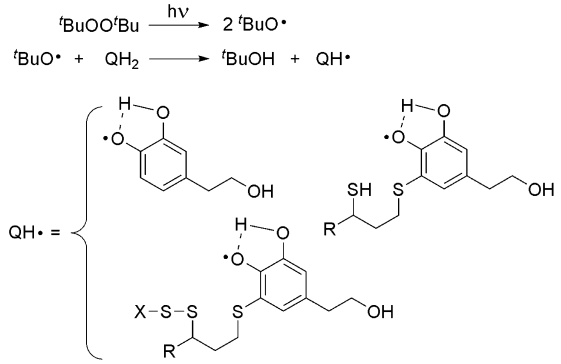
In chlorobenzene both HTy and **1** showed good antioxidant activity determined as the rate constant for trapping of peroxyl radicals, although HTy was significantly more reactive, outperforming other common catechol-type antioxidants such as natural quercetin and epicatechin (Table 1), paralleling the performance of industrial standard DBC. The reactivity of compounds **2**−**4** could not be investigated in this solvent because of the insufficient solubility.

In the more polar acetonitrile the reactivity of HTy was dramatically hampered because of the H-bonding of the reactive OH group with the solvent, a well-known phenomenon that has been recently reviewed.[28] Interestingly, the influence of the solvent was markedly less pronounced in the conjugate **1**, because of the neighboring sulfide moiety "protecting" the OH group from interaction with the solvent. Therefore, the reactivity ranking is reversed in polar solvents, and compound **1** behaves as a very effective antioxidant compared to common reference compounds, outperforming any of the tested benchmarks. The apparent higher reactivity recorded for the disulfide **2** (ca. twice as large as **1**) is simply due to statistical factors, related to the presence of two identical catechol rings. Indeed the stoichiometric factor (n = 3.5), which is nearly twice as large as that of **1**, suggests that both rings act as peroxyl radical trapping moieties in similar independent fashion (k_{inh} = 4.5×10^4 and n = 1.8 per aromatic ring). Parallel behavior was recorded for the tri- and tetrasulfide **3** and **4**, although the stoichiometric factor and the apparent reactivity were slightly decreasing with increasing the polysulfide chain.

Protection by the neighboring sulfide from negative kinetic solvent effect is clear on comparing the ratio of k_{inh} measured in the two solvents in the case of **1**, k_{inh}(ClPh)/k_{inh}(ACN) = 4 versus the corresponding ratio for HTy, 27, or quercetin, 42, or particularly DBC, 55.

EPR Studies. In order to rationalize the kinetic behavior recorded for compounds **1**−**4**, we performed a detailed EPR investigation. X-band EPR spectra were recorded by irradiating, with an unfiltered 500 W Hg-lamp, a solution of the desired compound in *t*-butanol containing 10% v/v di-*t*-butyl peroxide directly in the thermostatted cavity of the EPR spectrometer. Other solvents were tested (benzene, acetonitrile, propionitrile), but the solubility was generally insufficient. The solubility of polysulfides **2**−**4** was very limited even in *t*-butanol; therefore, spectra were recorded from a suspension in this solvent. Analysis of spectral data was aided by interactive computer simulations based on Monte Carlo method. Under the above conditions HTy afforded a noisy EPR signal centered at g = 2.0049 with well-defined hyperfine structure, which was attributed to the semiquinone radical illustrated in Scheme 2.

Scheme 2. Reactions Occurring in the EPR Spectrometer



Spectral parameters are in line with previous data on semiquinone radicals from hindered catechols[31] and hydroquinones[32] and are collected in Table 2.

Table 2. EPR Spectral Parameters (hfcc and *g*-factors) in *t*-Butanol at 298 K for Radicals Generated by OH Hydrogen Abstraction from HTy and Compounds 1 and 2 (See Scheme 2)

compound	hfcc (Gauss)	*g*-factor
HTy	a(pCH_2) 5.73; a(OH) 1.67; a(H_o) 2.19; a(H_m) 1.20; a(H_m) 0.90; a(2H) ~0.2	2.0049
1	a(pCH_2) 5.38; a(OH) 1.50; a(SCH_2) 1.84; a(H_m) 1.03; a(H_m) 0.86; a(2H) ~0.2; a(2H) ~0.1	2.0050
2	a(pCH_2) ~5.0; a(OH) ~1.6; a(SCH_2) ~2.0; a(H_m) ~1.0; a(H_m) ~0.9; a(2H) ~0.3	2.0050
2,4,6-tri-*t*-butylphenol (TBP) (reference)	a($2H_m$) 1.70; a(27H) ~0.2	2.0046

The conjugate **1** afforded a well resolved signal due to the semiquinone radical (Figure 2), with slightly higher *g*-factor (g = 2.0050), indicative of spin−orbit coupling with the sulfur atom.[4] Such an interaction is also apparent from the hyperfine coupling with $-CH_2-S-$ hydrogens (hfcc = 1.8 G, see Table 2) in line with previous observations with *ortho* RS- substituted phenoxyl radicals.[4] No signal attributable to the formation of thiyl radicals by H-atom abstraction at the S−H group of **1** was detected. This would rule out a significant participation of the thiol group in the antioxidant activity of **1**, which, hence, should be attributed primarily to the 3-alkylthio-1,2-dihydroxybenzene moiety. Because of its incomplete solubility, **2** afforded a much less resolved signal with a fine structure centered at g = 2.0050 in accordance with that of the corresponding semiquinone radical superimposed to a broad unresolved band (see Figure 2c). Compounds **3** and **4** in suspension yielded similar unresolved EPR signals (see Supporting Information).

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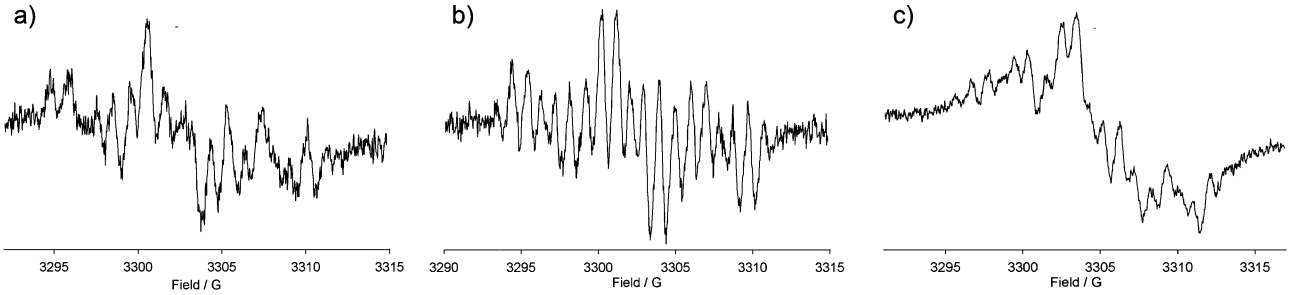

Figure 2. EPR signals of the semiquinone radicals obtained by irradiating a solution of HTy (a), or adduct **1** (b), or disulfide **2** (c) in *t*-butanol containing 10% v/v di-*t*-butyl peroxide at 298 K.

Measurement of O–H Bond Dissociation Enthalpy (BDE_{OH}). BDE_{OH} of **1** and HTy were determined using the radical equilibration EPR technique.[33−35] Solutions in *t*-butanol containing the test compound and 2,4,6-tri-*tert*-butylphenol (TBP) (BDE_{OH} = 80.1 kcal/mol)[35] as reference compound in variable ratio, in the presence of 10% v/v di-*t*-butylperoxide, were irradiated in the thermostatted cavity of the EPR spectrometer. Spectra of the equilibrating mixture, consisting in the superimposition of the semiquinone and phenoxyl radicals (see eq 7), were analyzed by interactive fitting of simulated to experimental spectra (Figure 3). From the measured value of the equilibrium constant K_{eq}, the BDE_{OH} of the unknown species could be determined according to eqs 8−10, under the assumption that the entropy change associated with the reaction is negligible.[34] Results are collected in Table 3.

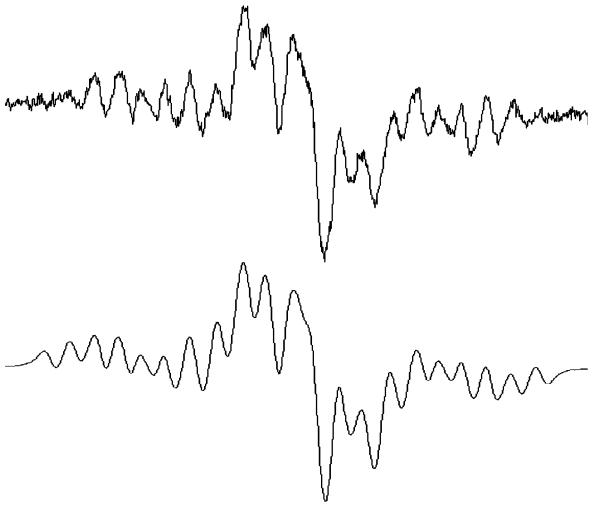

Figure 3. Experimental spectrum obtained by irradiating a mixture of **1** (6 mM) and TBP (12 mM) in *t*-butanol containing 10% di-*t*-butyl peroxide at 298 K (top) and its computer simulation for a ratio between the semiquinone and phenoxyl radical of 1:2 (bottom).

$$QH_2 + ArO\cdot \rightleftharpoons QH\cdot + ArOH \quad (7)$$

$$K_{eq} = \frac{[QH\cdot]}{[ArO\cdot]} \times \frac{[ArOH]}{[QH_2]} \quad (8)$$

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_{eq} \quad (9)$$

$$BDE_{OH(unknown)} = BDE_{OH(ref)} + \Delta H \quad (10)$$

Table 3. Equilibrium Constants for the Reaction of HTy and Compound 1 with 2,4,6-Tri-*t*-butylphenoxyl Radical and Corresponding BDE_{OH} Values in *t*-Butanol at 298 K

compound	K_{eq}[a]	BDE_{OH} (kcal/mol)[a]
HTy	0.30 ± 0.12	80.8 ± 0.4
1	3.60 ± 1.01	79.3 ± 0.3

[a]Mean of at least three measurements; errors correspond to ± SD.

The BDE_{OH} value determined for HTy in *t*-butanol was in very good agreement with the value of 79.6 previously reported for the structurally related 3,5-di-*t*-butylcatechol in the same solvent.[31,36] Indeed after subtraction of the additive contribution of the *ortho*-*t*-butyl group (ca. −1.7 kcal/mol),[34] the resulting BDE_{OH} estimated from that value for 4-alkylcatechol is ca. 81.3 kcal/mol in *t*-butanol, i.e., only 0.5 kcal/mol difference from our current measurement. Interestingly, in *t*-butanol the measured value for compound **1** was 1.5 kcal/mol lower than that observed for HTy, explaining the higher reactivity observed with peroxyl radicals in polar solvents (e.g., acetonitrile, see Table 1) as compared to HTy. Because of insufficient solubility in *t*-butanol (and in other tested solvents) the polysulfides **2**−**4** could not be subjected to radical equilibration EPR studies; however, the BDE_{OH} for each catechol ring is expected to be identical to that of **1** because of the identical substitution pattern and the similar spin distribution in the semiquinone radical indicated by the EPR spectra.

On the basis of kinetic (vide supra) and EPR data, it is clear that the nature of the alkyl chain in the RS-substituent has negligible influence of the homolytic reactivity of compound **1** in solution. Indeed, any alkylthio residue could have taken the place of lipoic acid with similar antioxidant performance. On the other hand, the amphiphilic nature of the lipoic acid residue might prove advantageous in biological environments, aiding the targeting of biomembranes.

DFT Calculations. To further rationalize the kinetic and thermodynamic properties displayed by HTy and compound **1**, we also performed DFT calculations on the thermodynamics of their formal H-atom abstraction.

The BDE_{OH} for the investigated compounds was calculated at the B3LYP/6-31+g(d,p) level from the difference between the enthalpies of the closed-shell molecules and the radicals,[37] by using the BDE_{OH} of catechol in isooctane (80.7 kcal/mol) as a reference.[35,36] To reduce the cost of computation, the alkyl chains of hydroxytyrosol and dihydrolipoic acid in the conjugates was truncated to a methyl group.[38]

In order to rationalize the role of the solvent in the thermodynamics of H-atom abstraction from the compounds under investigation, calculations were performed also in the

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presence of the solvent. While experimental data could not be reproduced by using a polarized continuum model, we included one explicit methanol molecule, used as a smaller analogue of *t*-butanol. Previous work in the field of kinetic solvent effects (KSE) on radical reactions of phenols showed that the main determinant of KSE is the 1:1 binding of a solvent molecule with the reactive phenolic OH.[28] Results are collected in Table 4, while the structures for the catechols and corresponding semiquinone radicals are displayed in Figures 4 and 5.

Table 4. Gas-phase Calculated BDE_{OH} at 298 K, for the Investigated Compounds in the Absence or Presence of Complexation with an Explicit Solvent Molecule[a]

compound	QH_2 BDE_{OH} (kcal/mol)	QH_2–CH_3OH BDE_{OH} (kcal/mol)
HTy	78.9[b]	80.6[c]
1	81.6[d]	79.4[e]

[a]See Figure 5. [b]Reaction A. [c]Reaction C. [d]Reaction B. [e]Reaction D.

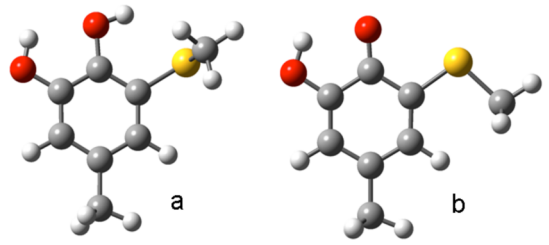

Figure 4. Optimized geometry for the analogue of **1** (a) and its semiquinone radical (b) at the B3LYP/6-31+g(d,p) level.

Computations show that in the absence of hydrogen-bonding solvents the calculated BDE_{OH} of **1** is higher (+2.7 kcal/mol) than HTy since the electron donating character of the RS-group in *ortho* (expected to decrease the BDE) is balanced by the occurrence of a strong intramolecular hydrogen bond between the catechol OH and the sulfide (see Figures 4 and 5B),[39] accounting for the significantly higher reactivity of HTy with peroxyl radicals in chlorobenzene (Table 1). On the other hand, in the presence of hydrogen-bonding solvents, different stabilization of the parent phenol and the corresponding phenoxyl radical in the case of HTy or **1** reverts the relative BDE_{OH} values, in agreement with the radical equilibration EPR studies in *tert*-butanol. In the case of HTy, we found that experimental data are well reproduced by considering that the binding occurs through hydrogen-bond donation from the phenolic O–H to methanol in the starting compound, while in the phenoxyl radical, the hydrogen-bond is reversed to achieve better stabilization, as depicted in Figure 5C. This result is also consistent with previous studies indicating that, when moving from benzene to *t*-butanol, the BDE_{OH} of 3,5-di-*tert*-butylcatechol is increased by 1.4 kcal/mol.[31] Conversely in the conjugate **1**, we found that the experimental results could be reproduced by allowing the solvent to interact with the two catecholic –OH groups, both in the starting molecule and the corresponding phenoxyl radical, as illustrated in Figure 5D. Using such models, calculated BDE_{OH} values (Table 4) differ by 0.2 kcal/mol or less from absolute experimental values, predicting a BDE difference of 1.2 kcal/mol between HTy and **1**, in excellent agreement with the experimental values of 1.5 kcal/mol.

An interesting aspect of the chemistry of the dihydrolipoic conjugates, which was evidenced when running the calculations, is the large geometric changes occurring about the alkylthio chain upon H-atom abstraction, as can be seen in Figure 4. In the parent phenol the dihedral angle between the aromatic plane and the $-SCH_3$ substituent is about 90°, to allow the formation of an intramolecular hydrogen-bond, while in the phenoxyl radical this angle is 0°, allowing for free radical delocalization on the sulfur center. This peculiar geometry in *ortho*-alkylthiophenols is due to the tendency of chalcogen atoms heavier than oxygen to have almost pure *p* orbitals in the outer electronic shell. It follows that hydrogen bonding of a lone pair with the O–H group forces the alkyl group to twist out of coplanarity with the aromatic ring by about 90°, causing the so-called "sigma hole" of sulfur[40] to conjugate with the aromatic π-system, thereby making the RS-moiety electron-withdrawing (σ = +0.14) in the phenol,[4] to become ED upon rotation in the phenoxyl radical. The O–H BDE values for the conjugates reported so far have been measured in homogeneous solutions, where these geometric changes can occur freely. However, in locally heterogeneous environments, such as in a protein or a membrane surface, free rotation around the Ar–S bond could be impaired, resulting in profound differences in the actual BDE_{OH} with respect to the value expected in solution. To explore these aspects on a quantitative ground, we estimated by DFT calculations the BDE_{OH} of **1** in which the Ar–S–CH_3 dihedral angle is fixed at different values. Results, shown in Figure 6, show that when the –S–CH_3 is forced to coplanarity with the aromatic ring, the BDE_{OH} decreases dramatically, becoming much lower than that of reference antioxidant α-tocopherol (77 kcal/mol).[41]

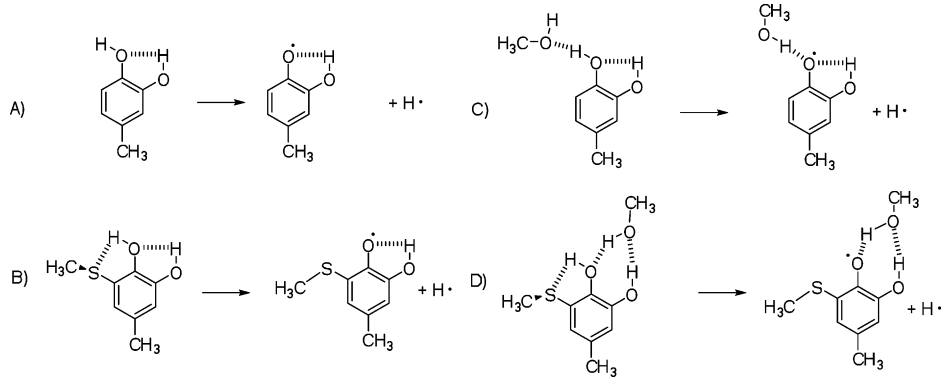

Figure 5. Relevant reactions and conformations used in the calculation of the BDE_{OH} of HTy and **1**.

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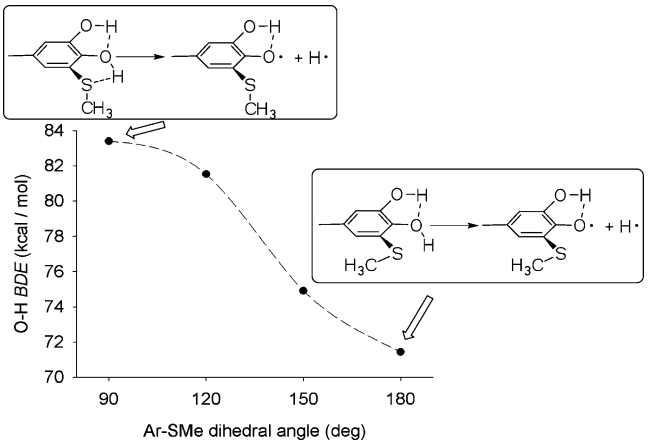

Figure 6. Variation of the calculated O−H BDE of the *S*-methyl analogue of compound **1** with Ar−S−CH_3 dihedral angle.

CONCLUSIONS

The results of this study provide for the first time a rational picture of the peroxyl radical scavenging properties of the 3-alkylthio-1,2-dihydroxybenzene system, the active core of the novel class of *S*-lipoylhydroxytyrosol antioxidants. Kinetic, thermodynamic, and computational data reported herein highlighted in particular the crucial influence of the sulfur center on the reactivity of the adjacent catechol system, including (a) a marked "protective" role against the deactivating effect of hydrogen bonding in polar solvents on the H-atom donor capacity; (b) a significant decrease of the BDE_{OH} in *t*-butanol, as opposed to HTy or other catechols; and (c) a dramatic conformation-dependent modulation of predicted BDE_{OH} attributable to both hydrogen-bonding interactions and the stereoelectronic features of the sulfide moiety.

Overall, these observations would point to the 3-alkylthio-1,2-dihydroxybenzene system as a most active peroxyl radical scavenging motif, which can respond dynamically to structural and environmental changes via competing inter- and intramolecular interactions at the sulfur center. The counterintuitive enhanced activity of **1** in more polar media (with respect to common catechol antioxidants) may ensure a more effective action against lipid peroxidation at cytoplasm/membrane interfaces compared to HTy and other more lipophilic chain-breaking antioxidants. From a practical perspective, the observed impact of geometrical constraint about the alkylthio group on the homolytic reactivity and, hence, antioxidant performance of the catechol moiety in **1** is remarkable and worthy of further pursuit for the design of innovative conformationally responsive antioxidants.

Although the biological relevance of the present findings has yet to be assessed, it is worth noting that *o*-thiosubstituted conjugates are major products of the oxidative metabolism of catechols in the presence of thiol compounds, typically glutathione or cysteine, and their role as antioxidants has so far been little investigated.

We believe that these results fill an important gap in the current knowledge of polyphenolic antioxidants and open new perspectives toward a rational exploitation of sulfur substitution as an expedient tool to tailor antioxidant and chain breaking properties in catechol derivatives.

EXPERIMENTAL SECTION

General. HRMS analyses were performed on an FT-ICR (ion cyclotron resonance) mass spectrometer equipped with an ESI source. ^{1}H NMR and ^{13}C NMR spectra were recorded in CD_3OD at 400 and 100 MHz, respectively.

Chemicals. Solvents were of the highest grade commercially available and were used as received. Quercetin (≥98%), 2,2,5,7,8-pentamethyl-6-chromanol (PMHC, 97%), and 3,5-di-*tert*-butylcatechol (98%) were commercially available and used without further purification. Commercially available 2,4,6-tri-*tert*-butylphenol (TBP, 98%) and 2,2′-azodiisobutyronitrile (AIBN ≥98%) were recrystallized from hexane; AIBN was stored at −20 °C. Cumene (98%) and styrene (≥99%) were distilled under reduced pressure and percolated twice through silica and alumina prior to use. All solutions were prepared fresh immediately prior to use.

3-Hydroxytyrosol (Hty), 5-*S*-lipoylhydroxytyrosol (**1**), and its disulfide **2**, trisulfide **3**, and tetrasulfide **4** were prepared as previously reported.[25] MS and NMR data are provided in the Supporting Information.

Autoxidation Studies. The chain-breaking antioxidant activity of the title compounds was evaluated by studying the inhibition of the thermally initiated autoxidation of either styrene or cumene (RH) in chlorobenzene or acetonitrile. Autoxidation experiments were performed in a oxygen-uptake apparatus already described elsewhere.[27] In a typical experiment, an air-saturated mixture of styrene or cumene in acetonitrile or chlorobenzene (50% v/v) containing AIBN (5×10^{-2} M) was equilibrated with the reference solution containing also an excess of PMHC (from 1×10^{-3} to 1×10^{-2} M) in the same solvent at 30 °C. After equilibration, a concentrated solution in the antioxidant (final concentration from 1×10^{-6} to 5×10^{-5} M) was injected into the sample flask, and the oxygen consumption in the sample was measured. From the slope of the oxygen consumption during the inhibited period (R_{inh}), k_{inh} values were obtained by using eq 11,[27c] where R_0 is the rate of oxygen consumption in the absence of antioxidants, R_i is the initiation rate, $2k_t$ is the bimolecular termination rate constant of styrene or cumene (4.2×10^7 and 4.6×10^4 M^{-1} s^{-1}, respectively),[26,27b] and n is the stoichiometric coefficient of the antioxidant. The n coefficient was determined experimentally from the length of the inhibited period (τ) by eq 12. Values of k_{inh} measured for reference PMHC in chlorobenzene and acetonitrile were 3.2×10^6 and 6.8×10^5 M^{-1} s^{-1}, respectively, in excellent agreement with literature.[26,29]

$$\frac{R_0}{R_{inh}} - \frac{R_{inh}}{R_0} = \frac{nk_{inh}[AH]}{\sqrt{2k_tR_i}} \quad (11)$$

$$n = \frac{R_i\tau}{[AH]} \quad (12)$$

Calculations. Geometry optimization and frequencies were computed in the gas phase at the B3LYP/6-31+g(d,p) level using Gaussian03,[42] and stationary points were confirmed by checking the absence of imaginary frequencies. Enthalpies at 298 K were computed using a scaling factor of 0.9806 to account for anharmonicity.[43] BDE values were obtained by using the isodesmic approach,[37] which consists of calculating the ΔBDE between the investigated compounds and catechol, and by adding this value to the known experimental BDE(OH) of catechol in isooctane (80.7 kcal/mol).[35,36]

EPR Spectroscopy. Spectra were recorded in 4 mm I.D. quartz tubes at 298 K on a X-band spectrometer equipped with a variable temperature unit. Spectral analysis was optimized by means of computer simulations and subjected to a least-squares fitting procedure based on the systematic application of the Monte Carlo method, available in WinESR Commander V.1.0 software, developed by Prof. Marco Lucarini (University of Bologna). Spectra were recorded in deoxygenated ^{t}BuOH solutions containing 10% (v/v) di-*tert*-butyl peroxide at 298 K, by irradiating the samples with a 500 W high-pressure Hg lamp using calibrated metal sectors to modulate the intensity of irradiation. Measured *g*-factors were corrected using those

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of the reference compound: 2,4,6-tri-*tert*-butylphenol (TBP) g = 2.0046 in ^{t}BuOH.

Thermodynamic Measurements. Deoxygenated ^{t}BuOH solutions containing HTy or **1** (5−50 mM), TBP as reference compound (5−50 mM,) and di-*tert*-butyl peroxide (10%, v/v) were sealed under nitrogen in a Suprasil quartz EPR tube placed inside the thermostatted (298 K) cavity the EPR spectrometer. Photolysis was carried out in the cavity. The temperature was monitored before and after each run with a copper-constantan thermocouple. The molar ratio of the two equilibrating radicals was obtained from the EPR spectra by computer simulation and interactive least-squares fitting. It was then used to determine the equilibrium constant, $K_{eq} = [QH_2]_0[Ref^\bullet]/[RefH]_0[QH^\bullet]$, where the subscript zero refers to the initial concentrations, chosen so to avoid significant reagent consumption during the experiment. This experimental approach allows determining real equilibrium concentration of transient radicals because they are under "radical buffer" conditions; hence, their equilibration with the parent phenol/catechol (eq 7) is faster than bimolecular self-decay.[34] To confirm that the two radicals were at their equilibrium under the experimental conditions, different initial absolute concentrations of the equilibrating species and different light intensities were investigated.[27,31−35]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MS and NMR data for **1**−**4**, EPR spectra and simulations, Cartesian coordinates for calculated minimized structures (11 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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