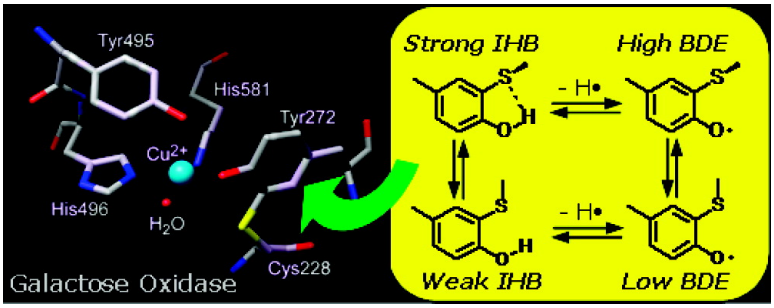
Article

Effect of *ortho*-SR Groups on O–H Bond Strength and H-Atom Donating Ability of Phenols: A Possible Role for the Tyr-Cys Link in Galactose Oxidase Active Site?

Riccardo Amorati, Francesca Catarzi, Stefano Menichetti, Gian Franco Pedulli, and Caterina Viglianisi

J. Am. Chem. Soc., **2008**, 130 (1), 237-244 • DOI: 10.1021/ja075554h



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 12/12/2007

Effect of *ortho*-SR Groups on O−H Bond Strength and H-Atom Donating Ability of Phenols: A Possible Role for the Tyr-Cys Link in Galactose Oxidase Active Site?

Riccardo Amorati,*,† Francesca Catarzi,‡ Stefano Menichetti,*,‡ Gian Franco Pedulli,*,† and Caterina Viglianisi‡

Dipartimento di Chimica Organica "A. Mangini", Università di Bologna, Via S. Donato 11, 40126 Bologna, Italy, and Dipartimento di Chimica Organica, e Laboratorio di Progettazione Sintesi e Studio di Eterocicli Bioattivi (HeteroBioLab), Polo Scientifico e Tecnologico Università di Firenze, Via della Lastruccia 13, 50019 Sesto Fiorentino (FI), Italy

Received July 25, 2007; E-mail: gianfranco.pedulli@unibo.it

Abstract: Rotation about the Ar−S bond in *ortho*-(alkylthio)phenols strongly affects the bond dissociation enthalpy (BDE) and the reactivity of the OH group. Newly synthesized sulfur containing heterocycles **3** and **4**, where the −SR group is almost coplanar with the phenolic ring, are characterized by unusually low BDE(O−H) values (79.6 and 79.2 kcal/mol, respectively) and by much higher reactivities toward peroxyl radicals than the *ortho*-methylthio derivative **1** (82.0 kcal/mol). The importance of the intramolecular hydrogen bond (IHB) in determining the BDE(O−H) was demonstrated by FT-IR experiments, which showed that in heterocycles **3** and **4** the IHB between the phenolic OH group and the S atom is much weaker than that present in **1**. Since the IHB can be formed only if the −SR group adopts an out-of-plane geometry, this interaction is possible only in the methylthio derivative **1** and not in **3** and **4**. The additive contribution to the phenolic BDE(O−H) of the −SR substituent therefore varies from −3.1 to +2.8 kcal/mol for the in-plane and out-of-plane conformations, respectively. These results may be relevant to understanding the role of the tyrosine−cysteine link in the active site of galactose oxidase, an important enzyme that catalyzes the two-electron aerobic oxidation of primary alcohols to aldehydes. The switching of the *ortho* −SR substituent between perpendicular and planar conformations may account for the catalytic efficiency of this enzyme.

Introduction

Knowledge of the O−H bond dissociation enthalpy (BDE) of phenols[1] is essential for understanding a wide range of redox processes involving molecules containing the phenolic moiety, such as the redox cycle of enzymes containing tyrosyl radicals[2] and the activity of phenolic antioxidants.[3] The BDE(O−H) values of phenols have been demonstrated to depend on the nature of the ring substituents[4] and, to a remarkable extent, on the presence of intramolecular hydrogen bonds formed by the reactive center with nearby functional groups such as in the case of *ortho*-methoxy[5] and *ortho*-hydroxy[6] phenols and as in bisphenols.[7] In all these cases, the overall effect on the BDE has been explained in terms of differential stabilization of the couple phenol/phenoxyl radical.

Among phenols that can give rise to the formation of an intramolecular H-bond, *ortho*-thio substituted phenols are important because this basic structure is present in the active site of galactose oxidase (GOase), a copper metalloenzyme that catalyzes the two-electron oxidation of primary alcohols to aldehydes (eq 1).[8]

$$RCH_2OH + O_2 \rightarrow RCHO + H_2O_2 \qquad (1)$$

The alcohol oxidation is achieved due to the presence in the active site of two distinct one-electron acceptors, a Cu(II) metal center and a stable protein free radical.[8] The latter one is located on a modified tyrosine, resulting from a covalent linkage between the carbon *ortho* to the phenolic OH and the S atom of a cysteine, as shown in Figure 1.[9] Since GOase can exploit its catalytic activity in water using atmospheric oxygen as oxidant, it represents an attractive target for the development of green catalysts for oxidation of alcohols under mild conditions. For this reason, GOase has been the object of intense research aimed to clarify the catalytic mechanism[8,10] and to obtain synthetic functional analogues.[11]

† Università di Bologna.
‡ Polo Scientifico e Tecnologico Università di Firenze.

(1) Borges dos Santos, R. M.; Martino, Simoes, J. A. *J. Phys. Chem. Ref. Data* **1998**, *27*, 707−739.
(2) Stubbe, J.; Donk, W. A. *Chem. Rev.* **1998**, *98*, 705−762.
(3) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065.
(4) Brigati, G.; Lucarini, M.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2002**, *67*, 4828−4832.
(5) de Heer, M. I.; Korth, H. G.; Mulder, P. *J. Org. Chem.* **1999**, *64*, 6969−6975.
(6) Lucarini, M.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2002**, *67*, 928−931.
(7) Amorati, R.; Lucarini, M.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2003**, *68*, 5198−5204.
(8) Whittaker, J. W. *Chem. Rev.* **2003**, *103*, 2347−2363.
(9) Ito, N.; Phillips, S. E. V.; Stevens, C.; Ogel, Z. B.; McPherson, M. J.; Keen, J. N.; Yadav, K. D. S.; Knowles, P. F. *Nature* **1991**, *350*, 87−90.

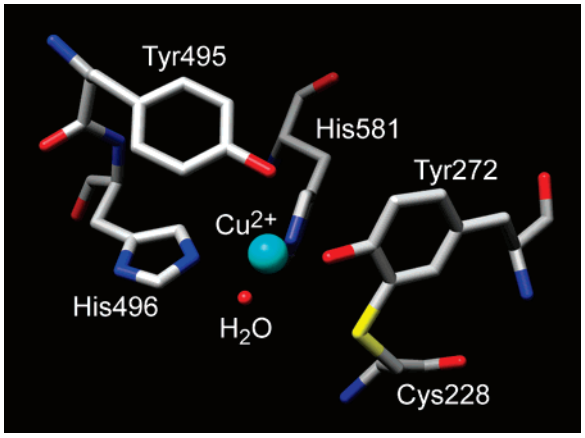


Figure 1. X-ray crystal structure of the active site of GOase[9] where the oxidizable substrate is replaced by a water molecule.

Despite the GOase catalytic cycle being thoroughly investigated, the role of the Tyr-Cys linkage remains a controversial point. Although the thioether bond seems essential for the function of GOase,[12] studies on model compounds[13] and theoretical simulations of the catalytic cycle[10a] show no major effects of an *ortho* thioether substituent on the redox behavior of tyrosine. Therefore, it has been suggested that the role of the Tyr-Cys cross-link is basically structural; that is, it contributes to maintaining the three-dimensional structure of the enzyme.[10a]

Clearly, the knowledge of the contribution of the *ortho* thiosubstituents to the phenolic BDE(O−H) is an important prerequisite for understanding the radical reactions involving the tyrosine OH group.

In a recent work, the BDE(O−H) of *ortho* and *para* alkylthio substituted phenols have been measured.[14] The additive contributions of *ortho* and *para* $-SCH_3$ groups to the phenolic BDE(O−H) were obtained as −0.8 and −3.5 kcal/mol, respectively, by studying compounds **1** and **2** (see Scheme 1). The large difference between these two values has been explained as being due to the formation of an Intramolecular Hydrogen Bond (IHB) that preferentially stabilizes the parent phenol of **1** with respect to the phenoxyl radical **1′** (Scheme 1).[14] These results are similar to those previously found in the case of *ortho* methoxyphenols, where the relatively strong IHB raises the BDE(O−H) to about 3.7 kcal/mol.[5,6]

However, it should be pointed out that a striking difference between *ortho* −OR and −SR groups in the formation of IHB with the phenolic OH resides in the directionality of such an interaction: 2-methoxyphenol adopts a planar geometry,[5] while in 2-(methylthio)phenol the $S-CH_3$ bond points perpendicular to the ring plane, as shown in Scheme 1 for compound **1**.[15] Therefore, in systems where the *ortho* alkylthio substituent cannot assume this geometry, the strength of the hydrogen bonding interaction may differ significantly and the contribution to the phenolic BDE(O−H) of an *ortho* −SR group may be quite far from the value of −0.8 kcal/mol found in **1**.

This peculiarity may be important for understanding the role of the −SR linkage between the Tyrosine 272 and Cysteine 228 in GOase as, in the enzyme crystallized without the substrate, the S−R bond assumes a conformation nearly coplanar (∼7°) with the aromatic ring (Figure 1).[9] Actually, the reactivity and the BDE of the phenolic OH of the modified tyrosine of GOase has been estimated using 2-(methylthio)phenols,[13] which seem to be poor models since they do not allow us to take into account the coplanarity with the aromatic ring of the −SR substituent.

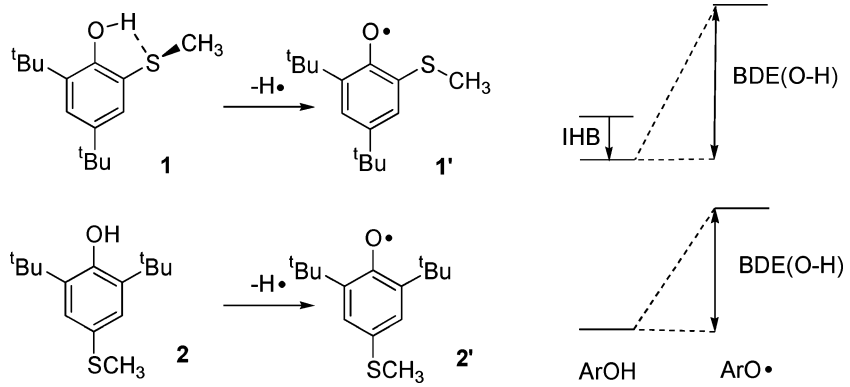
In order to study the effect of the conformation of *ortho*-SR groups on the phenolic BDE(O−H) and on the reactivity of the hydroxyl group toward free radicals, we synthesized compounds **3** and **4**, in which the critical substituent was expected to be in a nearly planar geometry (see later). Compounds **5** and **6**, both accessible through the same synthetic pathway, were prepared to have a reference compound to probe the *ortho vs para* electronic effects of the −SR group (**5**) and to compare the effects of S and O atoms on the BDE(O−H) of *ortho* substituted phenols (**6**).

Results and Discussion

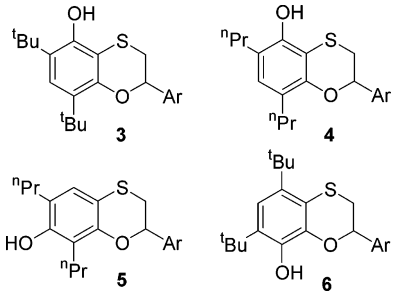
Synthesis. Heterocycles **3**−**6** (Scheme 2) were prepared by the inverse electron demand hetero Diels−Alder reaction of an electron-poor dienic *o*-thioquinone with 4-methoxystyrene used as an electron-rich dienophile. Following our original synthetic strategy,[16] transient *o*-thioquinones have been obtained from the corresponding *o*-hydroxy-*N*-thiophthalimides which, in turn, are the product of the reaction of phthalimidesulfenyl chloride (PhtNSCl, Pht = Phthaloyl) with the required phenol. Derivatives **3**−**6** were prepared using as starting materials commercially available 4,6-di-*tert*-butylresorcinol (**3a**), 3,5-di-*tert*-butylcatechol (**6a**), and 4,6-di-*n*-propyl- and 2,4-di-*n*-propylresorcinol (**4a** and **5a**, respectively) which were prepared from *O*,*O*′-diallyl resorcinol *via* a double Claisen rearrangement followed by separation and hydrogenation as previously reported (see Supporting Information).[17] Remarkably, for phenols **3a**, **4a**, and **6a** the sulfenylation and the cycloaddition reactions can be carried out without protection of the extra phenolic OH not involved in the formation of *o*-thioquinone. For **5a** the protection

(10) (a) Himo, F.; Eriksson, L. A.; Maseras, F.; Siegbahn, P. E. M. *J. Am. Chem. Soc.* **2000**, *122*, 8031−8036. (b) Rothlisberger, U.; Carloni, P.; Doclo, K.; Parrinello M. *J. Biol. Inorg. Chem.* **2000**, *5*, 236−250. (c) Rokhsana, D.; Dooley, D. M.; Szilagyi, R. K. *J. Am. Chem. Soc.* **2006**, *128*, 15550−15551.
(11) (a) Wang, Y.; DuBois, J. L.; Hedman, B.; Hodgson, K. O.; Stack, T. P. D. *Science* **1998**, *279*, 537. (b) Itoh, S.; Taki, M.; Takayama, S.; Nagatomo, S.; Kitagawa, T.; Sakurada, N.; Arakawa, R.; Fukuzumi, S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2774−2776. (c) Guidoni, L.; Spiegel, K.; Zumstein, M.; Rothlisberger, U. *Angew. Chem., Int. Ed.* **2004**, *43*, 3286−3289. (d) Pratt, R. C.; Stack, T. D. P. *J. Am. Chem. Soc.* **2003**, *125*, 8716−8717. (e) Thomas, F.; Gellon, G.; Gautier-Luneau, I.; Saint-Aman, E.; Pierre, J.-L. *Angew. Chem., Int. Ed.* **2002**, *41*, 3047−3050.
(12) Baron, A. J.; Stevens, C.; Wilmot, C. M.; Seneviratne, K. D.; Blakeley, V.; Dooley, D. M.; Phillips, S. E. V.; Knowles, P. F.; McPherson, M. J. *J. Biol. Chem.* **1994**, *269*, 25095−25105.
(13) Itoh, S.; Taki, M.; Fukuzumi, S. *Coord. Chem. Rev.* **2000**, *198*, 3−20.
(14) Amorati, R.; Fumo, M. G.; Menichetti, S.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2006**, *71*, 6325−6332.
(15) (a) Schaefer, T.; Wildman, T. A.; Salman, R. S. *J. Am. Chem. Soc.* **1980**, *102*, 107−110. (b) Schaefer, T.; Salman, R. S.; Wildman, T. A.; Clark, P. D. *Can. J. Chem.* **1982**, *60*, 342−348. (c) Schaefer, T.; McKinnon, D. M.; Sebastian, R.; Peeling, J.; Penner, G. H.; Veregin, R. P. *Can. J. Chem.* **1987**, *65*, 908−914. (d) Himo, F.; Eriksson, L. A.; Blomberg, M. R. A.; Siegbahn, P. E. M. *Int. J. Quantum Chem.* **2000**, *76*, 714−723. (e) Schaefer, T.; Penner, G. H. *Can. J. Chem.* **1988**, *66*, 1229−1238.
(16) (a) Capozzi, G.; Falciani, C.; Menichetti, S.; Nativi, C. *J. Org. Chem.* **1997**, *62*, 2611−2615. (b) Capozzi, G.; Lo Nostro, P.; Menichetti, S.; Nativi, C.; Sarri, P. *Chem. Commun.* **2001**, 551−552. (c) Menichetti, S.; Aversa, M. C.; Cimino, F.; Contini, A.; Tomaino, A.; Viglianisi, C. *Org. Biomol. Chem.* **2005**, *3*, 3066−3072. (d) Amorati, R.; Fumo, M. G.; Pedulli, G. F., Menichetti, S.; Pagliuca, C.; Viglianisi, C. *Helv. Chim. Acta* **2006**, *89*, 2462−2472; e) Amorati, R.; Cavalli, A.; Fumo M. G:, Masetti, M.; Menichetti, S.; Pagliuca, C.; Pedulli, G. F.; Viglianisi, C. *Chem.—Eur. J.* **2007**, *13*, 8223−8230.
(17) Liu, K.; Xu, L.; Berger, J. P.; MacNaul, K. L.; Zhou, G.; Doebber, T. W.; Forrest, M. J.; Moller, D. E.; Jones, A. B. *J. Med. Chem.* **2005**, *48*, 2262−2265.

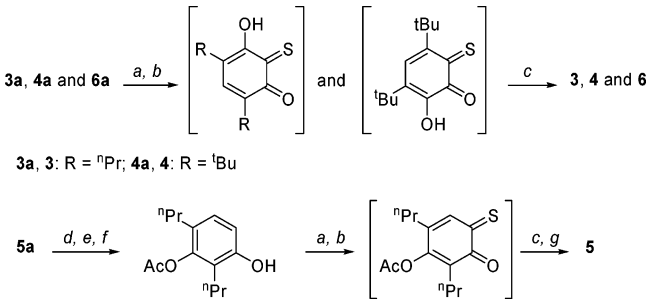
Scheme 1. Effect of Intramolecular Hydrogen Bond (IHB) on the BDE(O−H) of **1**



Scheme 2. Ar = 4-Methoxybenzene

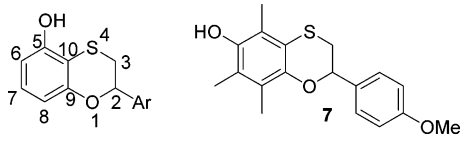


Scheme 3 [a]



[a] Reagents and conditions: (a) PhtNSCl, $CHCl_3$; (b) Et_3N, $CHCl_3$, 60 °C; (c) 4-methoxystyrene; (d) $^tBuMe_2SiCl$, IMI, DMF; (e) Ac_2O, Py; (f) TBAF, AcOH;[19] (g) $LiAlH_4$, THF, −78 °C.

Scheme 4



of the more hindered[18] C3−OH was required to obtain good yields in the cycloaddition step as reported in Scheme 3.

Crystallographic Studies. We have recently observed that, at least in the solid state, 2-aryl-benzoxathiins, like **7** (Scheme 4), adopt a peculiar conformation with C-3 being almost on the aromatic plane.[16e] In agreement with this result, the conformation of compound **4**, determined by X-ray crystallography (see Figure 2 and Supporting Information), showed the dihedral angles between atoms 9-10-4-3 and 10-9-1-2 as −8° and −26°, respectively (see Scheme 4). This indicates that the position of the alkylthio group in **4**, and reasonably in **3**, is very similar to

Table 1. EPR Spectral Parameters of the Aryloxyl Radicals from **1**−**6**

phenol	hyperfine splittings (Gauss)	*g*-value
1[a]	1.29 (1H), 1.59 (1H), 1.89 (3H), 0.34 (9H)	2.0053
2[a]	1.30 (2H), 2.24 (3H)	2.0055
3	1.33 (1H), 2.72 (1H), 0.09[b] (9H), 0.15[b] (9H)	2.0050
4	1.38 (1H), 2.39 (1H), 4.28 (2H), 6.67 (2H)	2.0049
5	1.14 (1H), 1.45 (1H), 3.83 (1H), 2.81 (2H), 4.00 (2H)	2.0055
6	1.17 (1H), 2.31 (1H), 0.09[b] (9H), 0.17[b] (9H)	2.0047

[a] From ref 14. [b] From computer simulation.

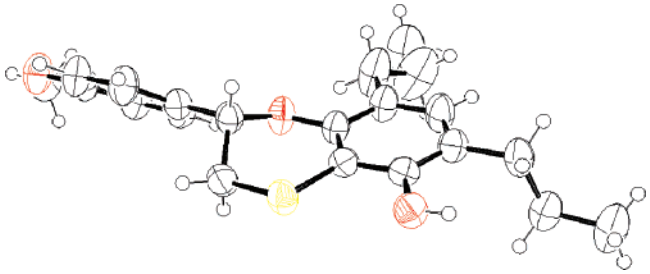


Figure 2. Structure of compound **4** obtained by crystallographic studies.

that observed in the case of the Tyr-Cys linkage in GOase (7°).[9] As additional, yet important, information obtained from the X-ray structure, the electron density on the phenolic oxygen indicates that, in the crystal, the orientation of the hydroxyl H atom is toward the *n*-propyl group and thus opposite to the sulfur atom (Figure 2).

EPR Spectra and Determination of BDE(O−H) Values. When photolyzing, inside the EPR cavity, oxygen-free solutions of **3**−**6** in benzene containing di-*tert*-butylperoxide (10% v/v), intense EPR spectra characterized by *g*-factors typical of aryloxyl radicals were observed.[20] The spectra showed, in all cases, coupling of the unpaired electron with the hydrogen of the aromatic ring and with one of the three protons of the heterocyclic condensed ring, being consistent with the aryloxyl radical structures resulting from abstraction of the hydroxyl hydrogen atom of the investigated compounds (see Table 1 and Supporting Information). Additional splitting from the *tert*-butyl protons in the radicals from **3** and with the CH_2 protons of the *n*-propyl groups in the radicals from **4** and **5** were detected.

The determination of the O−H bond dissociation enthalpies was done by measuring, by means of EPR spectroscopy, the equilibrium constant, K_1, for the hydrogen atom transfer reaction

(18) Yoshioka, T.; Fujita, T.; Kanai, T.; Aizawa, Y.; Kurumada, T.; Hasegawa, K.; Horikoshi, H. *J. Med. Chem.* **1989**, *32*, 421−428.
(19) Feldman, K. S.; Ensel, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 1162−1170.
(20) Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Cabiddu, S.; Fattuoni, C. *J. Org. Chem.* **1996**, *61*, 9259−9263.

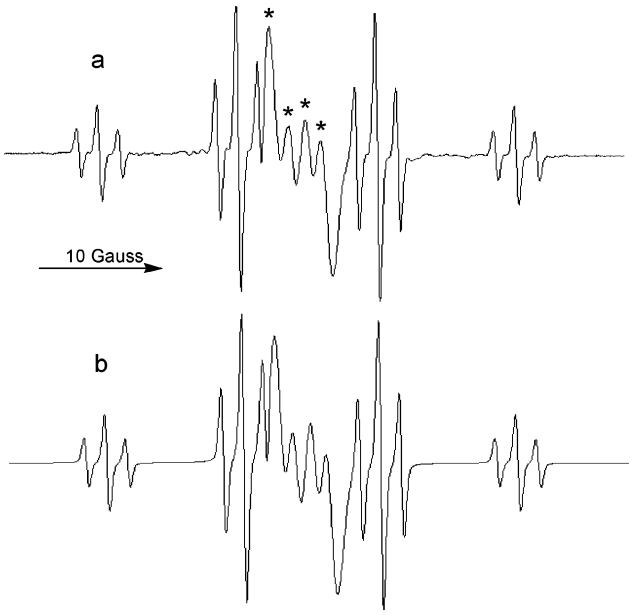


Figure 3. Experimental (a) and computer simulated (b) EPR spectrum observed at room temperature when photolyzing a deoxygenated benzene solution of **3** and BHT in a concentration ratio of 2.9:1. Lines of the aryloxyl radical from **3** are marked by asterisks.

between two phenols and the corresponding phenoxyl radicals (eq 2) generated under continuous photolysis.

$$\mathrm{ArOH} + \mathrm{Ar'O}\bullet \overset{K_1}{\rightleftharpoons} \mathrm{ArO}\bullet + \mathrm{Ar'OH} \qquad (2)$$

The BDEs for the species ArOH were calculated, with the assumption that the entropic term can be neglected,[20] by means of eq 3 from K_1 and the known BDE value of a reference species Ar′OH, which in the present case was 2,6-di-*tert*-butyl-4-methylphenol (BHT) whose recently revised BDE(O−H) value is 79.9 kcal/mol.[20,21]

$$BDE(\mathrm{ArO{-}H}) = BDE(\mathrm{Ar'O{-}H}) - RT\ln(K_1) \qquad (3)$$

Figure 3 shows, as an example, the spectrum obtained under irradiation of a 2.9:1 mixture of **3** and BHT. From the concentration ratio of the two radicals, determined by computer simulation of the experimental EPR spectrum, an equilibrium constant K_1 of 1.60 was obtained. Thus, the difference between the BDEs of BHT and of **3** was calculated as −0.3 kcal/mol, and the BDE(O−H) of the thiaflavane **3**, as 79.6 kcal/mol.

The BDE(O−H) values of the other thiaflavanes were determined similarly. Only with compound **6** this could not be done due to the fast formation of secondary radicals when irradiating mixtures of **6** and several reference phenols. Both radicals were detected only in the case of the mixture of **6** and BHT; however, the amount of BHT requested was so small that it was consumed quickly during irradiation. We could only estimate the lower limit of the O−H bond dissociation enthalpy of **6** as 82.5 kcal/mol.

The BDE values of Table 2 and the known additive contributions of the various substituents[22] allowed us to calculate the additive contribution of the condensed −SR group to the BDE(O−H) of **3** and **4,** as −2.8 and −3.4 kcal/mol, respectively. Thus, *ortho*-SR substituents incorporated in a six-membered condensed ring induce a BDE decrease at the phenolic O−H bond by about 3.1 ± 0.3 kcal/mol, a value surprisingly large with respect to that of an *ortho*-SCH_3 substituent (−0.8 kcal/mol).

Table 2. Phenolic Bond Dissociation Enthalpies, Rate Constants for the Reaction with Peroxyl Radicals (k_{inh}), and Stoichiometric Coefficients (n) of Phenols **1**−**6**

compound	BDE(O–H) kcal/mol	$k_{ROO\bullet}$ $M^{-1}s^{-1}$	n
1	82.0[a]	3.9×10^3[a]	1.6[a]
2	78.1[a]	3.0×10^4[a]	1.7[a]
3	79.6 ± 0.2	$(3.1 \pm 0.2) \times 10^5$	1.6 ± 0.1
4	79.2 ± 0.2	$(4.6 \pm 0.3) \times 10^5$	1.8 ± 0.1
5	79.4 ± 0.2	$(5.8 \pm 0.2) \times 10^5$	1.4 ± 0.1
6	>82.5[b]	$(5.3 \pm 0.3) \times 10^3$	2.1 ± 0.1

[a] From ref 14 where the BDE values of **1** and **2** were erroneously reported as 82.2 and 78.3 kcal/mol. [b]Lower limit; see text.

It is worth pointing out that *para*-alkylthio substituents show practically the same efficiency in decreasing the BDE(O−H) (−3.6 and −3.2 kcal/mol in **2** and **5**, respectively) independently of the fact that the SR group experiences free rotation or is part of a cyclic condensed structure.

Autoxidation Experiments. In order to evaluate the reactivity of the investigated phenols with peroxyl radicals, the inhibited autoxidation of a suitable substrate (styrene or cumene), initiated by AIBN at 30 °C, was studied in the presence of small amounts of compounds **3**−**6** (eqs 4−9). The reaction was followed by monitoring the oxygen consumption with an automatic recording gas absorption apparatus previously described.[23]

$$\mathrm{Initiator} \xrightarrow{R_i} \mathrm{R}\bullet \qquad (4)$$

$$\mathrm{R}\bullet + \mathrm{O_2} \rightarrow \mathrm{ROO}\bullet \qquad (5)$$

$$\mathrm{ROO}\bullet + \mathrm{RH} \xrightarrow{k_p} \mathrm{ROOH} + \mathrm{R}\bullet \qquad (6)$$

$$\mathrm{ROO}\bullet + \mathrm{ROO}\bullet \xrightarrow{2k_t} \mathrm{Products} \qquad (7)$$

$$\mathrm{ROO}\bullet + \mathrm{ArOH} \xrightarrow{k_{inh}} \mathrm{ROOH} + \mathrm{ArO}\bullet \qquad (8)$$

$$\mathrm{ROO}\bullet + \mathrm{ArO}\bullet \rightarrow \mathrm{Products} \qquad (9)$$

During the inhibition period, the oxygen consumption is given by eq 10, where k_p is the propagation rate constant of the oxidizable substrate (41 M^{-1} s^{-1} for styrene[24] and 0.32 M^{-1} s^{-1} for cumene[14]) and τ is the length of the induction period. Cumene was used for measuring the k_{inh} value of weak inhibitors such as **6**. The number of peroxyl radicals trapped by each antioxidant molecule, n, was experimentally determined from eq 11, where R_i is the rate of free radical initiation obtained using 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) as the reference antioxidant for which $n = 2$.[3]

(21) Mulder, P.; Korth, H.-G.; Pratt, D. A.; DiLabio, G. A.; Valgimigli, L.; Pedulli, G. F.; Ingold, K. U. *J. Phys. Chem. A* **2005**, *109*, 2647−2655.

(22) Substituent contributions in kcal/mol to the O-H BDE are as follows: −1.75 for an *ortho* alkyl group; −1.7 and −1.9 for *para* *n*-propyl and *tert*-butyl groups; −0.45 for a *meta* methoxy group. These values should be added to 86.5 kcal/mol to get the BDE of a given substituted phenol (refs 4, 6, 20, 21).

(23) Amorati, R.; Pedulli, G. F.; Valgimigli, L.; Attanasi, O. A.; Filippone, P.; Fiorucci, C.; Saladino, R. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2142−2146.

(24) Howard, J. A. In *Free Radicals*; Kochi, J. K., Ed.; Wiley-Interscience: New York, 1975; Vol. 2, Chapter 12.

$$-\Delta[O_2]_t = \frac{k_p[\text{styrene}]}{k_{\text{inh}}} \ln(1 - t/\tau) \quad (10)$$

$$R_i = \frac{n[\text{ArOH}]}{\tau} \quad (11)$$

The experimental results, shown in Table 2 and Figure 4, indicate that compounds **3** and **4** possess a reactivity about 2 orders of magnitude greater than that of **1** and **6**, despite the similar steric crowding around the reactive OH, this being consistent with the BDE(O−H) values that in **3** and **4** are smaller than those in **1** and **6**. The lower reactivity of **2** with respect to compounds **3**−**5** is due to the presence of the two bulky *tert*-butyl groups *ortho* to the phenolic OH.

FT-IR Spectra. To assess the role of the IHB on the kinetics of the hydrogen atom transfer to peroxyl radicals, the IR spectra of phenols **3** and **4** were recorded in diluted CCl_4 solutions as shown in Figure 5. The spectra were different from those previously reported for **1** which showed a single peak in the O−H stretching absorption region, centered at 3375 cm^{-1}.[14] This peak is characteristic of an intramolecularly hydrogen-bonded species and indicates that the adopted conformation by the phenolic OH is the one pointing toward the S atom (see Scheme 1). On the other hand, in the IR spectra of **3** and **4**, two different absorptions were observed in the OH stretching region: a broad band at about 3500 cm^{-1} attributed to an intramolecularly H-bonded species (*syn* conformation with respect to the sulfur atom)[25] and a sharp peak typical of the "free" OH (*anti* conformation), at about 3620 cm^{-1}. The presence of a relatively intense peak due to the *anti* phenolic OH and the shift at higher frequencies of the absorption due to the H-bonded species clearly indicate that in compounds **3** and **4** the IHB is much weaker than that in **1**.

In contrast with the spectra of **3** and **4**, the IR spectrum of compound **6** shows a single peak at 3523 cm^{-1} (i.e., at a frequency slightly lower than that of 2-methoxyphenol, 3557.6 cm^{-1}),[26] indicating that **6** exists only in the *syn* conformation as the result of a strong IHB of the OH-proton with the heterocyclic oxygen atom.

From the IR spectra it was also possible to roughly estimate the molar fractions of the phenols in the *anti* conformation by comparing the area of the peak of the free OH with that measured in structurally related phenols, i.e., BHT for **3** and 2,4,6-tri-methylphenol for **4**, under the same conditions and at the same concentration.[27] Because of the unavoidable structural differences between **3**−**4** and the reference phenols, the uncertainty of this procedure is expected to be ca. 30%. From the equilibrium constants for the *syn* to *anti* isomerization obtained in this way (see Table 3) the free energy differences between the two conformations were calculated as 1.5 and 0.3 kcal/mol for **3** and **4**, respectively.

The strength of the IHB of the phenolic OH group with sulfur could be obtained by comparing the free energy required to force the OH to point toward the *tert*-butyl group in **3** (1.5 ± 0.2 kcal/mol) with that measured by Ingold et al. for the *syn*−*anti* isomerization in 2-*tert*-butylphenol (1.6 kcal/mol).[27] These almost coincident values indicate that the OH····S IHB strength in **3** is negligible.

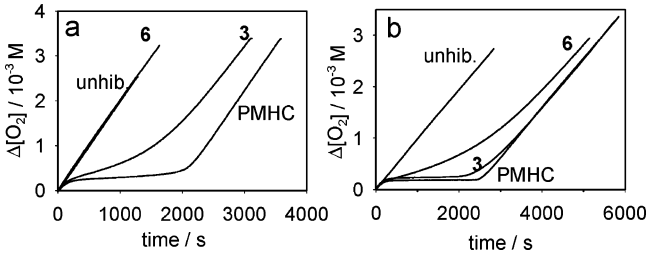


Figure 4. Oxygen consumption traces observed during the AIBN (0.05 M) initiated autoxidation of styrene (chart a) or cumene (chart b) in chlorobenzene solution at 30 °C in the presence of 2,2,5,7,8-pentamethyl-6-chromanol (PMHC) or of the investigated compounds (5×10^{-6} M).

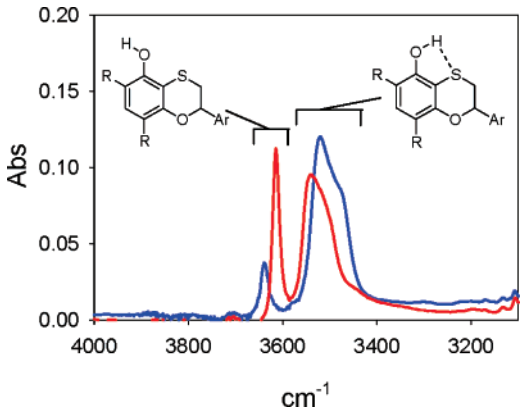


Figure 5. FT-IR spectra of 0.05 M of **3** (R = *t*-Bu, blue) and **4** (R = *n*-Pr, red) in CCl_4 solution in the O−H stretching region. The two conformations "*anti*" (left) and "*syn*" (right) are shown.

Table 3. IR Stretching Frequencies of the Phenolic O−H Groups in the *anti* and *syn* Conformations (See Figure 5), Molar Fraction of the *anti* Conformer (X_{anti}), and Free Energy Variation ($\Delta G°$) for the *syn* to *anti* Isomerization

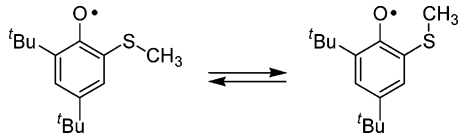
phenol	OH_{anti}/cm^{-1}	OH_{syn}/cm^{-1}	X_{anti}	$\Delta G°$/kcal mol^{-1}
1	-	3375[a]	0	-
3	3638	3520; 3478	0.07 ± 0.02	1.5 ± 0.2
4	3613	3538; 3509	0.4 ± 0.1	0.3 ± 0.2
6	-	3523	0	-

[a] From ref 14.

Intramolecular H-Bond and O−H BDE Values in *ortho* Alkythiophenols. The present results demonstrate that, in substituted phenols, *ortho* alkylthio groups show a peculiar behavior with respect to other substituents. A strong intramolecular hydrogen bond can only be formed when the molecule adopts the conformation where the S−R bond is oriented perpendicularly to the aromatic plane.[15] This geometry is preferred in *ortho* alkylthio phenols where the substituent is free to rotate about the aryl−sulfur bond. This behavior has been described previously by Schaefer et al.[15a] and has been explained by considering the poor tendency of alkylthio substituents to conjugate with the aromatic ring and their preference to form a stereospecific IHB using a 3p lone pair on sulfur.[15a] Barriers to rotation in 4-X-thioanisoles were found to be in the range from 1.5 (X = H) to 0.18 kcal/mol (X = OCH_3), suggesting that in **1** the rotation of the $-SCH_3$ group around the aryl−sulfur bond is essentially free.[15e] The IHB strength in 2-(methylthio)phenol was reported as 2.6 kcal/mol from ^{1}H NMR studies of the hydroxyl proton chemical shift.[15e] Schaefer et al. also pointed out that, in the case of 8-hydroxythiochroman,

(25) This band derives from the superimposition of two peaks, which presumably reflect two slightly different arrangements of the OH group.
(26) Foti, M. C.; Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **2002**, *124*, 12881−12888.
(27) Ingold, K. U.; Taylor, D. R. *Can. J. Chem.* **1961**, *39*, 471−480.

Scheme 5. Possible Conformations for the Radical **1′**



where the *ortho* alkylthio substituent is part of a condensed ring, the IHB with the phenolic OH is much weaker.[15c]

From the present FT-IR studies, the strength of the intramolecular OH····S bond in the planar conformation is estimated to be practically zero. This conclusion was reached on the basis of the shift to higher frequencies (ca. 3500 cm^{-1}) with respect to **1** (3375 cm^{-1}) of the O−H stretching vibration in **3** and **4** of the hydrogen-bonded species, and by crystallographic data suggesting that in compound **4** the phenolic OH points toward the *n*-propyl chain instead of sulfur. Therefore, in the cyclic derivatives **3** and **4** the additive contribution (−3.1 kcal/mol) to the BDE(O−H) of an *ortho* sulfur substituent is entirely due to electronic effects. Since in **1** this contribution (−0.8 kcal/mol) depends instead on both electronic and H-bonding effects, it is possible to obtain the strength of the OH···S bond in the latter phenol as the difference between these two values: −0.8 − (−3.1) = 2.3 kcal/mol.

The IHB strength calculated in this way is in good agreement, although not identical, with the literature value (2.6 kcal/mol).[15e] The small difference between these values may be due to experimental errors, or it may derive from the fact that the conformation of the −SR group in radicals **1′** and **3′** or **4′** is not the same. Actually, the phenoxyl radical **1′** can exist in two distinct conformations, with the $-SCH_3$ group pointing toward the phenoxyl oxygen or away from it (Scheme 5). The presence of such a conformational equilibrium has been emphasized by deHeer et al. in the 2-methoxyphenoxyl radical, where DFT calculations suggest that the toward conformation is favored by 1.6 kcal/mol.[5] From our experimental results, the preference, if any, of the radical **1′** for the toward conformation should not exceed 0.3 kcal/mol.

The kinetic data for the reactions of phenols with peroxyl radicals can be compared to the dissociation energies of the O−H bond by applying the linear relationship between log(k_{inh}) vs BDE(O−H), which holds for phenols with *ortho* substituents[20] characterized by similar steric crowding around the OH group. Since in Figure 6 compounds **3**, **4**, and **5** are along the same plot line of 2,6-dimethylphenols, this means that in these thiaflavanes the steric hindrance to the approach of ROO• radicals is similar to that due to two *ortho* methyl groups. In the case of **5**, this is in agreement with a previous report that two *ortho*-ethyl and *ortho*-propyl groups appear to have about the same steric effect as two *ortho*-methyl groups.[28] In the case of **3** and **4**, these data can be explained considering that the van der Waals radii of sulfur and of a methyl group are almost coincident (2.0 and 1.8 Å, respectively).[29]

The fact that compound **1** is not along the same line in Figure 6 indicates instead that the *ortho*-SCH_3 group behaves quite differently from the thiachromane fused ring. A reasonable explanation can be given by considering the different time scales of H-atom abstraction and $-SCH_3$ group rotation. Since the

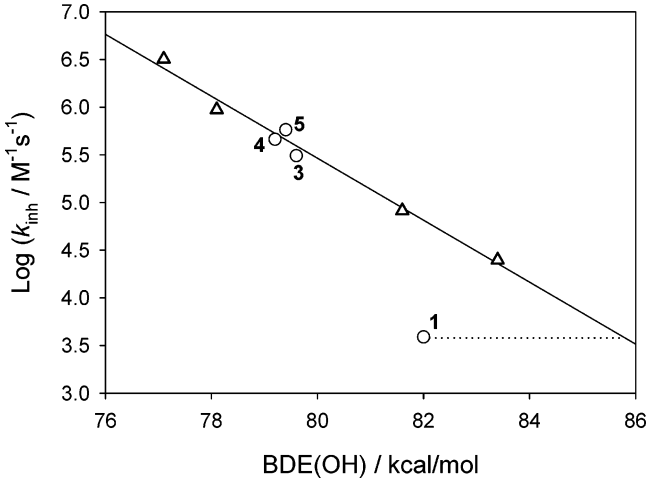
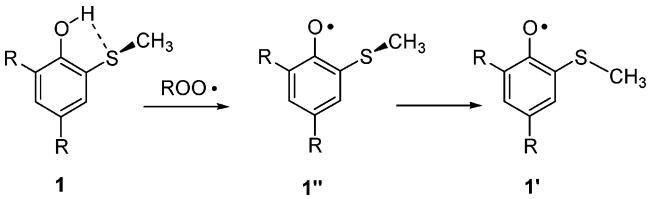


Figure 6. Logarithm of the rate constant for the reaction with peroxyl radicals against the BDE(OH) of the investigated compounds (○) and of other 4-X-2,6-dimethylphenols (△). On the basis of its k_{inh} value (log k_{inh} = −0.325*BDE*(O−H) + 31.45), the BDE(O−H) of compound **1** would be expected to be much larger (85.7 kcal/mol) than experimentally found.

Scheme 6. Relationship between Conformational Modifications and H-Atom Transfer in the Substituted Phenol **1**



former process must occur during the time of a stretching vibration, its time scale is about 3 orders of magnitude shorter than that of an intramolecular rotation.[30] Being the reacting species (Scheme 6) the phenol with the *ortho*-SCH_3 group in the out-of-plane conformation, H-atom transfer gives rise to a phenoxyl radical (**1″**) having the same geometry of the phenol, which only afterward relaxes to the planar conformation (**1′**). Therefore, the k_{inh} value reflects the energy difference between **1** and **1″** rather than that between **1** and **1′**, which is measured by EPR.

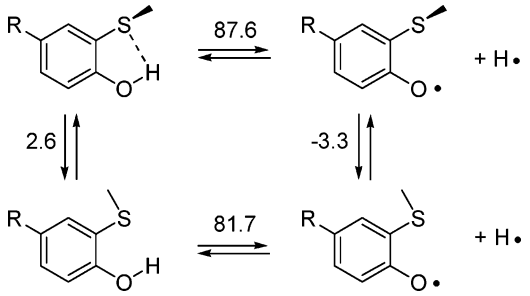
From the plot of Figure 6, the energy difference between **1** and **1″** can be estimated as 85.7 kcal/mol using the k_{inh} value of **1**. By applying the additive rules, this value provides a contribution to the BDE(O−H) of an out-of-plane *ortho*-SCH_3 group of +2.8 kcal/mol, a value similar to the strength of the IHB in **1** (2.6 kcal/mol)[15e] indicating that the perpendicular $-SCH_3$ group possesses no radical stabilizing effect.

Importance of the Tyr-Cys Link in Galactose Oxidase Active Site. Growing evidence supports the view that enzymatic activity results from a subtle interplay between chemical kinetics and molecular motions.[31] By means of NMR relaxation methods, conformational fluctuations of the active site that occur on the same time scale of the substrate turnover were found in the case of the enzyme cyclophilin A.[32]

On the basis of the information obtained in the present work we propose that GOase may take advantage of the peculiar

(28) a) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1963**, *41*, 2800−2806; b) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1963**, *41*, 1744−1751.
(29) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441−451.
(30) Banwell, C. N. *Fundamentals of Molecular Spectroscopy*; McGraw-Hill: London, 1966; Chapter 3.
(31) Benkovic, S. J.; Hammes-Schiffer, S. *Science* **2003**, *301*, 1196−1202.
(32) Eisenmesser, E. Z.; Bosco, D. A.; Akke, M.; Kern, D. *Science* **2002**, *295*, 1520−1523.

Scheme 7. Energy Diagram (kcal/mol) for the H-Atom Transfer in *ortho*-(Methylthio)tyrosine (R = $CH_2CH(NH_2)COOH$) as Function of the −SMe Group Rotation, Computed Using the Additive Contributions of the Various Substituents[a]



[a] C_6H_5OH = 86.5; R = −1.7; SMe(out-of-plane) = +2.8; SMe(in-plane) = −3.1 kcal/mol.

characteristic of the Tyr-Cys residue by undergoing conformational modifications in the presence of the substrate.[33] Actually, the Tyr-Cys residue can be present in two BDE(O−H) states, separated by 5.9 kcal/mol, depending on the −SR group conformation as shown in Scheme 7.

The catalytic cycle of GOase, depicted in Scheme 8, consists of two half-reactions, namely substrate oxidation and oxygen reduction.[8] The oxidative half-reaction (**A** → **D**) comprises the following: substrate deprotonation by the tyrosinate ligand Tyr495 (**A** → **B**); hydrogen atom transfer from the substrate to the *ortho*-thiotyrosyl radical (**B** → **C**); and electron transfer from the ketyl radical to the Cu^{II} ion (**C** → **D**). The replacement of the newly formed aldehyde by O_2 initiates the reductive half-reaction (**D** → **A**), including electron transfer from Cu^{I} to O_2 and hydrogen transfer to re-establish a tyrosyl radical on Tyr272 (**E** → **F**). Then, after deprotonation of Tyr495, hydrogen peroxide is removed and a new molecule of alcoholic substrate is coordinated by the active site (**F** → **A**).[8] Studies of kinetic isotope effect, performed by using deuterated substrates, have shown that the transition states for both half-reactions possess substantial hydrogen atom transfer character.[8]

It is suggested that the Tyr-Cys residue with the −SR link in the out-of-plane conformation will promote the oxidative half-reaction by increasing the BDE(O−H) of Tyr272 and making the H-atom transfer from the substrate more exothermic. On the contrary, the reductive half-reaction will be favored by coplanarity with the aromatic plane of the *ortho* −SR group (see Scheme 8) that implies a lower BDE value of the phenolic O−H. Therefore, perpendicular and coplanar conformations of the −SR group of Cys228 will make easier the oxidative and the reductive half-reactions, respectively, in the GOase ping-pong kinetic process.[34]

The switching of the *ortho* −SR substituent between the two conformational states might be triggered by the presence of the substrate in the enzyme active site.[31−33] This is not in contrast with the available crystallographic structures of GOase (see Figure 1) obtained in the absence of the substrate in the catalytic site.[9]

The dynamic role of the thioether link here envisaged may indeed contribute to explaining why synthetic analogues of GOase possess a catalytic efficiency roughly 4 orders of magnitude smaller than that for the natural enzyme.[11c] In fact, efficient oxidation of alcohols can only occur if two conflicting requirements are achieved: a high phenolic BDE(O−H) of the catalyst in its oxidized form to abstract a H-atom from the substrate, and a low BDE(O−H) in its reduced form to easily donate an H-atom to O_2. In the natural enzyme this can be achieved by a simple rotation around the −SR bond of Cys228, but it can be hardly realized in a synthetic model.

Conclusions

The present study demonstrates that the rotation around the Ar−S bond in *ortho*-(alkylthio)phenols strongly affects the bond dissociation enthalpy and thus the reactivity of the O−H group. Sulfur containing heterocycles such as **3** and **4**, where the −SR group is coplanar with the phenolic ring, are characterized by lower BDE(O−H) values and by higher reactivity toward peroxyl radicals than the *ortho*-methylthio derivative **1**. This difference has been rationalized by considering that hydrogen bonding between the phenolic OH and the S atom requires an out-of-plane orientation of the −SR substituent. Thus, when this conformation is accessible as in the *ortho*-SCH_3 derivative, the strong intramolecular hydrogen bond present in the phenol raises the BDE(O−H) and reduces the reactivity toward ROO• radicals, while, in heterocycles **3** and **4**, where the intramolecular hydrogen bond is weaker for structural reasons, the OH group is characterized by a low BDE and high reactivity.

An additional problem with phenols bearing an *ortho*-SR substituent which may exist in two conformations is that, due to the different time scales of H-abstraction and internal rotation of the SR −group, the BDE(O−H) measured by the EPR radical equilibration technique reflects only partially the reactivity of the OH group toward ROO• radicals. Indeed, the BDE(O−H) value represents the energy difference between the phenol and phenoxyl radical in their minimum energy conformations (coplanar for the radical to maximize the conjugation of the −SR substituent with the aromatic system and perpendicular to the ring for the phenol to optimize the H-bonding interaction between sulfur and the OH group), while the rate constant for H-atom transfer is related to the energy difference between the two species in the "frozen" out-of-plane conformation.

Although in the present study the influence of the coordinated Cu^{II} atom could not be taken into account, these results may be relevant to understand the role of the tyrosine−cysteine link in the active site of galactose oxidase. We suggest that this peculiar amino acidic residue was selected by nature not only for structural requirements but also because the switching of the *ortho* −SR substituent between the perpendicular and planar conformation ensures the catalytic efficiency of the enzyme.

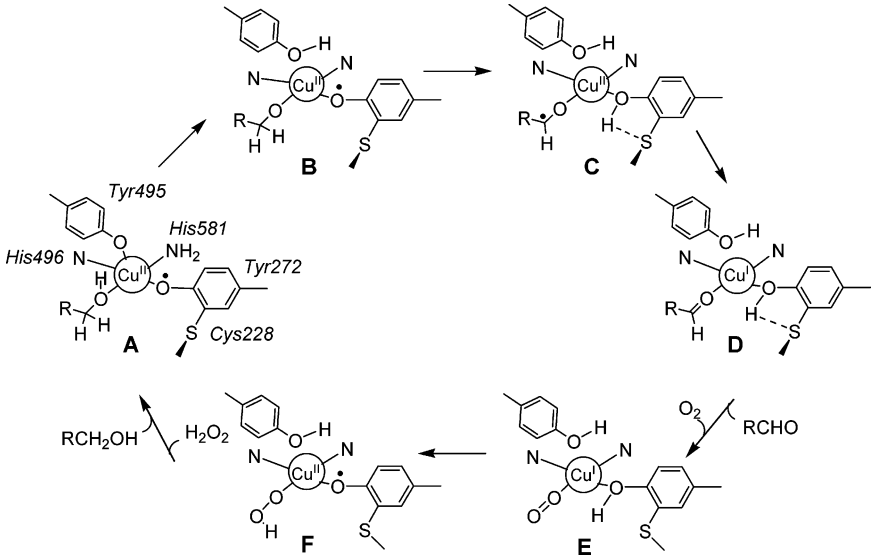
Experimental Section

Materials. Compounds **3**−**6** were synthesized by following our previously reported procedure.[16] Experimental data are available as Supporting Information. All other compounds used in the present investigation were commercially available. Styrene was percolated on alumina before each experiment to remove traces of inhibitor, and cumene was distilled under reduced pressure.

EPR and Thermochemical Measurements. Deoxygenated benzene solutions containing compounds **3**−**6** (0.1 M) and di-*tert*-butyl peroxide (10% v/v) were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted in the cavity of an EPR spectrometer and photolyzed with the unfiltered light from a 500 W high-pressure

(33) Koshland, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 98−104.
(34) (a) Whittaker, M. M.; Whittaker, J. W. *J. Biol. Chem.* **1988**, *263*, 6074−6080. (b) Whittaker, M. M.; Ballou, D. P.; Whittaker, J. W. *Biochemistry* **1998**, *37*, 8426−8436.

Scheme 8. Catalytic Cycle of Galactose Oxidase[8]



mercury lamp at room temperature. The EPR spectra were recorded on a spectrometer equipped with a microwave frequency counter for the determination of the *g*-factors, which were corrected with respect to that of the perylene radical cation in concentrated H_2SO_4 (g = 2.00258). EPR spectra of phenoxyl radicals from **3**−**6** are reported in the Supporting Information. The BDE values were determined by photolyzing concentrated solutions of BHT and **3**−**6** in the presence of di-*tert*-butyl peroxide (10% v/v). The molar ratio of the two equilibrating radicals, obtained from the EPR spectra, was used to calculate the equilibrium constant, K_1. Different concentration ratios of starting phenols were used in order to check if the equilibrium was reached. Spectra were recorded a few seconds after starting to irradiate in order to avoid significant consumption of the phenols during the course of the experiment. Relative radical concentrations were determined by comparison of the digitized experimental spectra with computer simulated ones as previously described.[20]

Autoxidation Experiments. The rate constants for the reaction of the title compounds with peroxyl radicals were measured by following the autoxidation of either styrene (4.3 M) or cumene (7.1 M) in chlorobenzene at 30 °C using as initiator AIBN (0.05 M). The reaction was performed in an oxygen uptake apparatus built in our laboratory and based on a differential pressure transducer. The entire apparatus was immersed in a thermostatted bath which ensured a constant temperature within ±0.1 °C. In a typical experiment, an air-saturated solution of styrene or cumene in chlorobenzene containing AIBN was equilibrated with a reference solution of the same composition also containing an excess of PMHC (1×10^{-4} M). When constant oxygen consumption was reached, a small amount of the antioxidant in chlorobenzene was added to the sample and the oxygen consumption was measured from the differential pressure between the two channels recorded as function of time. This instrumental setting allowed us to have the N_2 production and the oxygen consumption derived from the azo-initiator decomposition already subtracted from the measured reaction rates. Initiation rates, R_i, were determined for each condition in preliminary experiments using PMHC as reference antioxidant.

IR Measurements. FT-IR spectra of compounds **3**−**5**, BHT, and 2,4,6-trimethylphenol were measured in diluted tetrachloromethane solutions (0.01−0.05 M) in a sealed KBr cell with a 0.5 mm optical path.

Acknowledgment. Financial support from MIUR (Research projects "Radicals and Radical Ions: Basic Aspects and Role in Chemistry, Biology, and Material and Environmental Sciences", Contract 2006033539, and "Stereoselezione in Sintesi Organica. Metodologie ed Applicazioni" Contract 2005035330) is gratefully acknowledged by G.F.P. and S.M. We also wish to thank Dr. Elisabetta Mileo for technical assistance and Dr. Cristina Faggi for X-ray crystallographic analysis.

Supporting Information Available: Synthesis of compounds **3**−**6**; EPR spectra of phenoxyl radicals from **3**−**6**; crystallographic data for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA075554H