
Substituent Effects on OH Bond Strength and Hyperfine Properties of Phenol, as Model for Modified Tyrosyl Radicals in Proteins

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ABSTRACT: Density functional theory is used to investigate the effects of a variety of substituents (CH₃, OH, OCH₃, SH, SCH₃, NH₂, NMe₂, NO₂, F, Cl, CN, and imidazole) on the phenol O—H bond dissociation energy (BDE) and phenoxyl radical hyperfine properties. Substitutions are made at the ortho position to model modified tyrosine residues found in enzymes. The calculations show that besides the electronic effects of the substituents, intramolecular hydrogen bonds between OH and the substituents will contribute considerably to stabilize the parent species. Substituent effects on anisole O—Me bond strengths can thus not correctly describe the effects on ortho-substituted phenol O—H bond strengths, as previously proposed. This fact is supported by a series of calculations on *o*-substituted anisoles. The odd-alternant spin pattern of the phenoxyl radical is conserved for most of the substitutions. In particular, it is predicted that the cysteine crosslink to tyrosine, present in the radical enzyme galactose oxidase, and the histidine crosslink, present in cytochrome-*c* oxidase, will only have minor effects on the BDE and the radical hyperfine coupling constants and spin distribution of the tyrosyl radical. © 2000 John Wiley & Sons, Inc. *Int J Quant Chem* 76: 714–723, 2000

Key words: phenol; substituent effects; radical; density functional theory; bond dissociation energy

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Introduction

An increasing number of enzymes are now known to utilize amino acid radical cofactors to facilitate catalysis [1]. Transient and stable amino acid radicals have been localized to tyrosine, tryptophan, glycine, and cysteine. For instance, tyrosine radicals have been found in ribonucleotide reductases [2], photosystem II [3], prostaglandin H synthase [4], galactose oxidase [5] and proposed in cytochrome-*c* oxidase [6]. Cytochrome-*c* oxidase and galactose oxidase share the interesting feature in having another amino acid covalently crosslinked to the ortho position to the phenolic oxygen of their active site tyrosines.

Galactose oxidase (GO) is a mononuclear copper enzyme that catalyzes the two-electron oxidation of a large number of primary alcohols to their corresponding aldehydes, coupled with the reduction of dioxygen to hydrogen peroxide. It was revealed in the crystal structure that the active site tyrosyl radical (Tyr272) is connected to a neighboring cysteine (Cys228) through a thioether bond [7]. It has been speculated that this thioether bond is in part responsible for the 0.5–0.6 V lowering of the oxidation potential of this species compared to normal tyrosyl [8].

Cytochrome-*c* oxidase (CcO), which is a key part of the respiratory chain, catalyzes the reduction of O₂ to water. The crystal structures showed that the active site tyrosine (Tyr244) and histidine (His240) residues are covalently linked to each other [9]. Based on that, and by analogy to photosystem II, Babcock and co-workers [6] recently suggested that the Tyr–His moiety acts as a hydrogen atom donor during the O—O bond cleavage.

Santos and Simões [10] recently reviewed all available experimental and theoretical information on the thermochemistry of the OH bond in phenolic compounds. The study included some 90 literature references, and the authors recommended a set of values for the OH bond dissociation energy for phenol and many substituted phenols. The recommended gas-phase OH bond strength was taken, as an average of seven gas-phase experiments that were considered accurate, to be 88.7 ± 0.6 kcal/mol.

Most of what is known about ortho-substituted phenols is derived from work on ortho-substituted anisoles. In their comprehensive work, Suryan,

Kafafi, and Stein [11] used the very low pressure pyrolysis (VLPP) technique to study the effects of a large number of substituents at different positions, on the anisole O—Me bond strength [12]. The authors argued that the substituent effects on the phenol OH bond strength are the same as for anisole O—Me bond strength, a fact that Santos and Simões referred to and used in their review. This seems to be valid for para- and meta-substitutions but, as will be shown below, the assumption cannot be transferred to the case of ortho-substitution. One reason is that in phenols, but not in anisoles, intramolecular hydrogen bonds can be formed between the OH and the substituent, thereby stabilizing the parent molecule.

There are only a few theoretical studies addressing substituent effects on phenol, especially those concerning ortho-substitution. Wu and Lai [12] used two density functional methods (LDA and BLYP) to study the effects of a number of substituents on O—H and O—CH₃ bonds in phenols and anisoles. This study covered only para-substitutions and they concluded that an electron-donating group at that position induces a destabilization in phenols and anisoles but a stabilization in the phenoxyl radical, resulting in a decreased O—R bond dissociation energy. For electron-withdrawing groups the effects were the opposite. Similarly, Brinck, Haeberlein, and Jonsson [13] used both *ab initio* methods (MP2 and MP4) and density functional methods (BLYP and B3LYP) together with large basis sets to study the effects of substitution of phenols, focusing on para-substitution. They found that MP2 and MP4 methods provide reasonable relative bond dissociation energies (BDEs), but the absolute values are overestimated. The density functional theory (DFT) methods were found to give relative BDEs within the experimental uncertainty with a tendency to underestimate the results for phenols with strong electron-donating substituents. Wright and co-workers [14] obtained excellent agreement with experimental BDEs using AM1 geometries for which the energies were calculated using B3LYP with a modified 6-31G(p) basis set. The substituents studied were methyl, methoxy, and amino groups, alone or together, at different positions in the ring. It was shown that an additivity scheme could be applied to the effects of the substituents on the OH bond strength.

In the present work, our interest in bioradicals [15] has led us to carry out a study of a series of 12

ortho-substituted phenols. The substitutions considered are: CH₃, OH, OCH₃, SH, SCH₃, NH₂, NMe₂, NO₂, F, Cl, CN, and imidazole. Our objective has been to study the effects of these substituents on the O—H bond dissociation energy and the hyperfine structure and spin population distribution of the phenoxyl radical. O—Me bond dissociation energies of ortho-substituted anisoles were also calculated in order to explain the large discrepancy between the theoretically calculated OH bond strengths of phenols and the experimentally determined O—Me strengths.

The theoretical method we have chosen to employ is the Hartree–Fock/density functional theory (HF/DFT) hybrid method B3LYP [16]. This method, developed by Becke, includes three parameters fitted to experimental atomization energies, ionization potentials, and electron affinities and has recently been proven to be very suitable for this kind of studies [17]. In particular, it has been shown that this method, together with the other DFT functional PWP68 [18], provides accurate hyperfine coupling constants of substituted benzyl and phenoxyl radicals [19].

Theoretical Methods

All calculations reported in the present study were carried out using the DFT functional B3LYP [16], as implemented in the Gaussian94 program package [20]. Geometries were optimized with the double-zeta plus polarization basis set 6-31G(d, p). Based on these geometries, more accurate energies were calculated using the large basis set, 6-311+G(2d, 2p). This is a triple-zeta basis set with one diffuse function and two polarization functions on each atom. Zero-point energies (ZPE) were calculated at the B3LYP/6-31G(d, p) level for the unsubstituted phenol and anisole. The ZPE corrections, 8.3 and 7.5 kcal/mol for phenol and anisole, respectively, were then added to the substituted systems. Primary tests showed that the zero-point correction is not affected by the different substitutions, a result also obtained by Wright et al. [14]. The spin densities reported are calculated using Mulliken population analysis.

Solvent effects were calculated using the self-consistent isodensity polarized continuum model (SCI-PCM) [21] implemented in the Gaussian program. The dielectric constant used to model the

protein environment was the standard $\epsilon = 4$. The effects are small and the results are therefore not sensitive to the choice of ϵ .

Results and Discussion

GEOMETRIES

As in the case of para-substitution [12–14], we found in the present study that the geometries of ortho-substituted phenols and phenoxyl radicals are virtually unaffected by the nature of the substituent. The critical C4–O distance, for instance, varies in the range of 1.35–1.37 Å for the different phenols, and 1.25–1.26 Å for the different phenoxyl radicals.

The fact that the substitution is made at the ortho position from the phenolic oxygen makes the two possibilities for the orientation of the OH bond (*toward* or *away* from the substituent) energetically distinct. The barrier for rotation between these two states in unsubstituted phenol was calculated to 3.2 kcal/mol (expt. 3.4 kcal/mol [22]).

For almost all substituted phenols examined in the present study, the isomer with the hydrogen pointing toward the substituent was found to be energetically favorable (exceptions are CH₃ and NH₂). The energy difference between the *toward* and the *away* conformations is a few kilocalories/mole (typically 2–4 kcal/mol; see Table I). In the extreme case of NO₂ substitution, the OH group forms a strong hydrogen bond to one of the nitro oxygens, increasing the energy difference to as much as 10.7 kcal/mol. In contrast, due to steric effects and lack of hydrogen bond acceptor, the *away* conformer is favored by 0.7 kcal/mol for *o*-methylphenol.

In the *toward* isomer of NH₂-substituted phenol, the amino group has to rotate to an unfavorable out-of-plane conformation in order to avoid H–H repulsion and to form a hydrogen bond to OH. The rotation of the amino group and formation of a hydrogen bond almost cancel energetically. Hence, the energy difference between the *away* and *toward* isomers is only 0.3 kcal/mol in favor of the *away* structure. Using NMe₂ as a substituent instead of NH₂ makes the *toward* isomer favored by 3.9 kcal/mol. This is because in both isomers of this species the substituent is rotated out of the plane due to repulsion between one of the methyl groups and the phenolic oxygen.

TABLE I
Calculated absolute and relative OH bond dissociation energies (kcal/mol) of *o*-substituted phenols.^a

Substituent	OH pointing	BDE	Δ BDE	Expt. ^b anisole Δ BDE
H		82.3	0.0	0.0
CH ₃	toward	79.4	-2.8	-2.6
	away	80.1	-2.2	
OH	toward	72.6	-9.7	-7.1
	away	68.8	-13.5	
OCH ₃	toward	80.4	-1.9	-4.2
	away	76.1	-6.2	
SH	toward	77.7	-4.6	—
	away	74.3	-7.9	
SCH ₃	toward	80.6	-1.7	—
	away	76.0	-6.3	
NH ₂	toward	70.4	-11.9	-7.4
	away	70.7	-11.6	
NMe ₂	toward	75.3	-7.0	—
	away	71.4	-10.9	
Imidazole	toward	81.3	-1.0	—
	away	79.1	-3.2	
F	toward	83.4	+1.1	-1.9
	away	80.6	-1.7	
Cl	toward	84.2	+2.0	-2.2
	away	81.0	-1.3	
CN	toward	86.5	+4.2	-0.2
	away	83.9	+1.6	
NO ₂	toward	95.5	+13.2	-1.3
	away	84.8	+2.5	

^aThe BDEs are corrected for a constant zero-point effect (8.3 kcal/mol). Experimental relative BDEs of substituted anisoles are included for comparison. **Boldface** indicates ground-state conformation.

^bFrom ref. [11].

Hydrogen bond in the *toward* structure will thus favor it to the *away*.

In both NH₂ and NMe₂ substitutions, creation of the radical will give rise to several resonance structures, as displayed in Figure 1. Apart from the normal phenoxyl radical resonances (structures A–D), there are new resonances of ionic character (structures E–H). These result in shorter N—C bond in the radical compared to the parent species (1.35 vs. 1.44 Å) and in the planarity of the amino group in the radical (pyramidal in parent molecule).

The SH and SCH₃ substitutions have the peculiarity that in the ground-state *toward* conformation, the S—R bond points perpendicular to the ring plane. No similar minima were found for OH and OCH₃ substitutions.

The imidazole ring, finally, was found to form a dihedral angle of 59° to the phenol ring in the nonradical molecule and 23° in the radical.

O—H BOND STRENGTHS

For unsubstituted phenol, the OH bond dissociation energy was calculated to 82.3 kcal/mol. The value includes a zero-point vibrational energy correction of 8.3 kcal/mol. This, of course, compares poorly with the recommended experimental value (88.7 kcal/mol). However, the method employed in the present work is known to systematically underestimate O—H bond energies. The BDE in H₂O, for example, is calculated to 114.7 kcal/mol compared to the experimentally determined BDE of 119 kcal/mol [23]. Hence, since in the present study we are interested primarily in relative BDEs, these energies should be much more accurate than the absolute ones, as also demonstrated by Brinck et al. [13].

Computed absolute and relative BDEs are presented in Table I. Experimentally measured anisole O—Me BDEs are also listed in the table. The

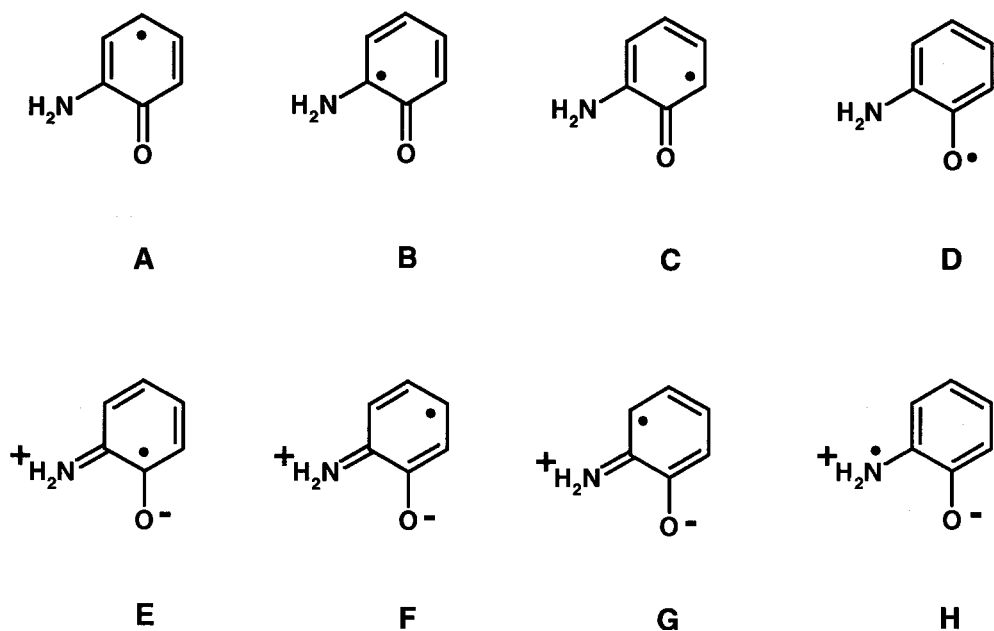


FIGURE 1. Resonance structures in *o*-aminophenoxy radical. Structures A–D are the same as the resonances present in unsubstituted phenoxy radical.

largest effect on the phenol OH bond strength is seen for substitution by NO_2 . Here, the parent molecule is stabilized due to a 10.7-kcal/mol strong intramolecular hydrogen bond between OH and one oxygen from the nitro group, giving an increase of the BDE by as much as 13.2 kcal/mol compared to unsubstituted phenol (Fig. 2).

The intramolecular hydrogen bonds formed between the OH and the other electron-withdrawing substituents considered in the present study are not as strong as in *o*-nitrophenol. The total effects on the BDE are hence much smaller: F, Cl, and CN substitutions only result in 1.1, 2.0, and 4.2 kcal/mol stronger OH bonds relative unsubstituted phenol, respectively.

In the case of NH_2 and NMe_2 substitutions, the additional resonance structures, shown in Figure 1 (structures E–H), contribute to stabilize the radical. The drop in BDE is calculated to 11.9 kcal/mol for NH_2 substitution and 7.0 kcal/mol for NMe_2 substitution.

The effects of hydroxy and methoxy substitutions differ quite significantly. The BDE reduction of 9.7 kcal/mol for OH-substituted phenol can be compared with the reduction of only 1.9 kcal/mol for methoxy substitution. The hydrogen bond formed in the radical of hydroxy-substituted phenol is 5.3 kcal/mol stronger than the hydrogen

bond in the parent molecule (see Fig. 2). Such a hydrogen bond is not possible to form in the OCH_3 substituted radical, explaining the large difference in ΔBDEs between these two substitutions.

Modeling the cysteine crosslink in galactose oxidase by a simple SH group will not correctly describe the effects of such a crosslink. Here, as in the case of OH substitution, the SH group will form a hydrogen bond to the phenolic oxygen in the radical species, giving a BDE that is 4.7 kcal/mol lower than in unsubstituted phenol. A more accurate model of cysteine is SCH_3 , for which the calculated effect on the OH-BDE is only -1.7 kcal/mol. We can thus conclude that a cysteine covalently crosslinked to the 3-position of tyrosine will not give a drastic decrease of the OH bond strength.

A histidine crosslink at the ortho position will not cause any substantial reduction of the OH bond strength either. A decrease of only 1.0 kcal/mol is calculated for *o*-imidazolphenol OH bond dissociation energy, compared to the unsubstituted molecule.

Finally, for SCH_3 and imidazole substitutions, we also performed calculations taking into account solvent effects, in a simple attempt to model the protein surrounding. The effects were found to be rather small. The BDE for SCH_3 -substituted phe-

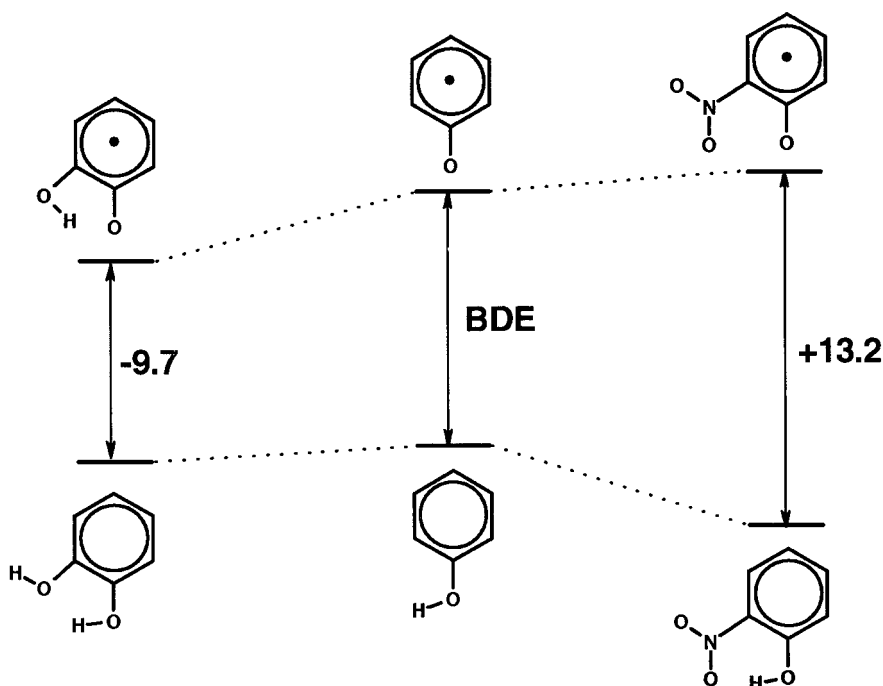


FIGURE 2. Schematic drawing of the effects of hydrogen bonding on the bond dissociation energy of substituted phenols. Two cases are represented. The hydrogen bond in hydroxy-substituted phenoxyl radical is 5.3 kcal/mol stronger than the hydrogen bond in the parent molecule, resulting in further decreased BDE (-9.7 kcal/mol) relative unsubstituted phenol. In nitro-substituted phenol, a 10.7 kcal/mol strong hydrogen bond results in increased BDE ($+13.2$ kcal/mol).

nol decreased another 1.6 kcal/mol giving a total Δ BDE of -3.3 kcal/mol, and BDE of imidazole-substituted phenol decreased 0.2 kcal/mol giving a total Δ BDE of -1.2 kcal/mol.

O—Me BOND STRENGTHS

As mentioned in the introduction, the gas-phase experiments performed by Suryan et al. [11] and cited by Santos and Simões [10] were made on substituted anisoles and not phenols. It was argued that the substituent effects are the same for both molecules.

Table I, however, shows that there is a large discrepancy between our calculated substituent effects on phenol and the measured substituent effects on anisole. Most striking is the effect of NO_2 , where the calculated Δ BDE for phenol is $+13.2$ kcal/mol, whereas the experimentally measured effect for anisole is -1.3 kcal/mol. Another example of the discrepancy is found for the halogen substituents. The calculations predict F and Cl to give positive contributions to the BDE ($+1.1$ and $+2.0$ kcal/mol, respectively), whereas experi-

mentally they were found to give negative contributions (-1.9 and -2.2 kcal/mol, respectively).

To address this discrepancy, we performed a series of calculations on substituted anisoles, using the same theoretical method as for substituted phenols. The results of these calculations along with the experimental data are displayed in Table II.

Consistent with the findings for the OH bond strength of unsubstituted phenol, the calculations underestimate the anisole O—Me bond strength by 8.5 kcal/mol (55.0 vs. 63.5 kcal/mol). For all the substituents tested, the methoxy tail of anisole was found to point *away* from the substituent in the ground state. In fact, in several of the systems, the methoxy group was forced out of the plane or even flipped to the other direction when optimization was started from a *toward* structure. The difference between *away* and *toward* conformations in the case of substituted anisoles is smaller than for the substituted phenols (less than 3 kcal/mol for all cases).

From Table II we notice that the anisole Δ BDEs are very similar to those obtained for the *away*

TABLE II
Calculated absolute and relative O—Me bond strengths (kcal/mol) of *o*-substituted anisoles^a.

Substituent	BDE	Δ BDE	Expt. Δ BDE ^b
H	55.0	0.0	0.0
CH ₃	52.7	-2.3	-2.6
OH	45.9	-9.1	-7.1
OCH ₃	48.9	-6.1	-4.2
SH	47.5	-7.5	—
SCH ₃	49.3	-5.7	—
NH ₂	43.6	-11.4	-7.4
F	53.6	-1.4	-1.9
Cl	53.9	-1.1	-2.2
CN	57.2	+2.1	-0.2
NO ₂	57.6	+2.6	-1.3

^aThe BDEs are corrected for a constant zero-point effect (7.5 kcal/mol). Experimental Δ BDEs are relative the gas-phase BDE of anisole (63.5 kcal/mol).

^bRef. [11].

conformation in phenol (Table I). This indicates that the electronic substituent effects on the O—H and O—Me bonds are very similar. The difference between phenol and anisole is rather that intramolecular hydrogen bonding might occur from phenolic OH group but is obviously lacking in anisole.

The calculated anisole Δ BDEs are generally in good agreement with the experimental results, indicating that the phenol results are reliable. It is, however, seen that the calculations have a tendency to somewhat exaggerate substituent effects in both directions, i.e., for electron-donating substituents, the effects are overestimated, whereas for electron-withdrawing substituents, the effects are underestimated.

o-Hydroxyanisole and *o*-aminoanisole have, for example, calculated Δ BDEs of -9.1 and -11.4 kcal/mol, respectively, compared to experimental results of -7.1 and -7.4 kcal/mol, respectively. CN and NO₂ give theoretically Δ BDEs of +2.1 and +2.6 kcal/mol, compared to -0.2 and -1.3 kcal/mol, for the experimentally determined values.

HYPERFINE COUPLINGS AND SPIN DISTRIBUTIONS

In Tables III and IV, calculated isotropic proton hyperfine couplings and the unpaired spin distri-

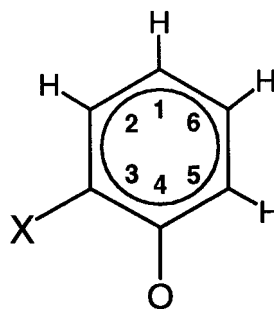


FIGURE 3. Numbering scheme of the phenol radical studied. Note that we here use the numbering commonly used for tyrosyl radicals.

butions for *o*-substituted phenoxy radicals are given. The numbering scheme used is given in Figure 3.

The phenoxy radical is known to have an odd-alternant spin population pattern, with large positive spin at the oxygen and at the ortho and para positions, and small negative spins at the rest. This pattern is nicely reproduced by the calculations. It is, however, known that DFT somewhat exaggerates these effects [24], i.e., positive spin populations get more positive and the negative ones more negative. Calculated hyperfine coupling constants are also in overall good agreement with available experimental data [25].

The largest effects on the spin densities and the hyperfine couplings are seen for the amino substituents, due to the resonance phenomena discussed above. For NH₂ and NMe₂ as substituents, the H1 coupling decreases from -9.0 to -6.3 G and -5.8 G, and the H5 coupling decreases from -6.9 to -3.7 G and -3.6 G, respectively. The spin patterns of these radicals are significantly different from the unsubstituted phenoxy. In accordance with resonance structures E-H in Figure 1, the Cl spin population drops from 0.40 to 0.25-0.27 and the negative spins at C4 and C6 almost vanish. Of the unpaired spin 0.17-0.20 will reside at the substituent nitrogen atom, inducing comparatively large hyperfine couplings (-4.7 and -4.6 G) on amino-protons of *o*-aminophenoxy. This will clearly change the character of the electron paramagnetic resonance (EPR) spectrum of this species.

Similar effects, although not as large, are observed for oxygen- and sulfur-substituted phenoxy radicals. The large H1 couplings, for instance, is

TABLE III
Calculated isotropic hyperfine coupling constants [gauss] of *o*-substituted phenoxyl radicals^a.

X	H1	H2	H5	H6	H _x
H	-9.0 (10.2)	2.8 (1.9)	-6.9 (6.6)	2.8 (1.9)	-6.9 (6.6)
CH ₃	-8.9 (11.5)	2.8 (1.9)	-6.6 (6.0)	2.5 (1.9)	11.0/11.0/0.2
OH	-7.4 (8.2)	2.1 (1.7)	-4.3 (4.0)	0.5 (0.2)	-2.4
OCH ₃	-6.8	2.2	-4.3	1.0	—
SH	-7.5	2.5	-4.8	1.4	-1.9
SCH ₃	-7.7	2.5	-5.3	1.7	—
NH ₂	-6.3	1.3	-3.7	-0.1	-4.7/ - 4.6
NMe ₂	-5.8	1.2	-3.6	0.2	—
Imidazole	-7.8	2.6	-5.3	1.9	—
F	-8.7 (10.0)	2.8 (1.4)	-6.2 (5.8)	2.8 (2.2)	—
Cl	-8.7 (9.8)	2.9 (2.0)	-6.4 (6.0)	2.5 (1.6)	—
CN	-8.8 (10.1)	2.7 (1.4)	-6.9 (7.1)	2.9 (2.1)	—
NO ₂	-9.0 (10.4)	2.4 (1.2)	-7.3 (7.5)	3.0 (2.2)	—

^aAvailable experimental values (absolute values) are given in parentheses. From Ref. [25] and references therein.

TABLE IV
Calculated spin population distributions of *o*-substituted phenoxyl radicals.

X	C1	C2	C3	C4	C5	C6	O	X ^a
H	0.40	-0.16	0.31	-0.08	0.31	-0.16	0.42	—
H ₃	0.39	-0.15	0.30	-0.07	0.29	-0.15	0.40	-0.02
OH	0.32	-0.15	0.29	-0.01	0.18	-0.06	0.35	0.09
OCH ₃	0.30	-0.14	0.30	-0.03	0.19	-0.07	0.38	0.10
SH	0.32	-0.15	0.27	-0.01	0.20	-0.09	0.33	0.16
SCH ₃	0.34	-0.15	0.25	-0.05	0.24	-0.11	0.34	0.16
NH ₂	0.27	-0.12	0.27	-0.00	0.16	-0.03	0.32	0.17
NMe ₂	0.25	-0.10	0.25	-0.03	0.16	-0.04	0.31	0.20
Imidazole	0.35	-0.16	0.34	-0.09	0.24	-0.12	0.37	0.03 ^b
F	0.38	-0.16	0.31	-0.07	0.27	-0.14	0.42	0.03
Cl	0.38	-0.17	0.31	-0.07	0.29	-0.15	0.40	0.04
CN	0.39	-0.15	0.29	-0.10	0.30	-0.16	0.40	-0.05
NO ₂	0.40	-0.13	0.30	-0.13	0.33	-0.17	0.42	-0.03

^aX denotes the atom of the substituent group, connecting to C3.

^bThe spin on the whole imidazole moiety is 0.09.

reduced from -9.0 to around -7 to -7.5 G, and the small H6 coupling is reduced from 2.8 to around 0.5 to 1.5 G. Although the unpaired spin on the substituent is not negligible (0.10 on oxygen and 0.16 on sulfur), the overall odd-alternant pattern of the phenoxyl is not significantly perturbed.

As the situation for the OH bond strength, imidazole substitution is predicted to cause very little change in the spin distribution. The spin density located on the whole imidazole ring was calculated to ca 0.09, with only 0.03 on the nitrogen

center connected to the phenoxyl. As a consequence, the hyperfine couplings also change very little compared to the unsubstituted case (Table III).

None of the electron-withdrawing substituents tested in the present study (F, Cl, CN, and NO₂) gave any substantial change in the hyperfine and spin properties. Solvent effects on SCH₃- and imidazole-substituted phenoxyl radicals gave virtually no change from the gas-phase results presented above.

Conclusions

We have in the present study investigated the effects of a number of substituents on the phenol OH bond strength and phenoxyl radical hyperfine properties, using density functional theory. We focused on ortho-substitution in order to model covalent crosslinks between tyrosines and neighboring amino acids, known to be present in at least two enzymes.

It was found that intramolecular hydrogen bonds between the OH and the substituents are of great importance to understand the energetics of the OH bond. This implies that, although electronic substituent effects are very similar, relative O—Me bond energies of ortho-substituted anisoles cannot properly describe the relative OH bond dissociation energies of ortho-substituted phenols. This conclusion was further supported by quite good agreement between calculated and measured anisole O—Me bond dissociation energies.

Both gas-phase and solvent model calculations show that cysteine and histidine substitutions will not significantly reduce the OH BDE. However, we think that special care needs to be exercised when these results are transferred to protein systems. In these systems, the crosslinked tyrosine residues are in general not isolated, but many times associated with transition metals, such as the copper centers in galactose oxidase and cytochrome-*c* oxidase. These will not only have a direct electronic effect on the O—H bond, but could also act as steric hindrance, favoring one or another direction of the OH bond and thereby affecting the bond strength. In this respect, the present model system results should not be over-interpreted. More detailed calculations, which include the specific interactions with neighbors, need to be performed to draw more detailed conclusions.

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