Predicting the Activity of Phenolic Antioxidants: Theoretical Method, Analysis of Substituent Effects, and Application to Major Families of Antioxidants

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Abstract: A procedure based on density functional theory is used for the calculation of the gas-phase bond dissociation enthalpy (BDE) and ionization potential for molecules belonging to the class of phenolic antioxidants. We show that use of locally dense basis sets (LDBS) vs full basis sets gives very similar results for monosubstituted phenols, and that the LDBS procedure gives good agreement with the change in experimental BDE values for highly substituted phenols in benzene solvent. Procedures for estimating the O−H BDE based on group additivity rules are given and tested. Several interesting classes of phenolic antioxidants are studied with these methods, including commercial antioxidants used as food additives, compounds related to Vitamin E, flavonoids in tea, aminophenols, stilbenes related to resveratrol, and sterically hindered phenols. On the basis of these results we are able to interpret relative rates for the reaction of antioxidants with free radicals, including a comparison of both H-atom-transfer and single-electron-transfer mechanisms, and conclude that in most cases H-atom transfer will be dominant.

I. Introduction

Phenolic antioxidants form an important class of compounds which serve to inhibit the oxidation of materials of both commercial and biological importance. The nutritional and medical aspects of antioxidants in general have been the subject of numerous reviews[1,2] and an overview of the subject has been given by Halliwell and Gutteridge.[3] The function of antioxidants is to intercept and react with free radicals at a rate faster than the substrate, and since free radicals are able to attack a variety of targets including lipids, fats, and proteins, it is believed that they are implicated in a number of important degenerative diseases including aging itself.[4−7]

There are two pathways for oxidation in which antioxidants can play a preventive role. The first is H-atom transfer, illustrated below for the important case of lipid peroxidation:

$$\mathrm{RH} \rightarrow \mathrm{R}^{\bullet} \qquad \text{(initiation)} \tag{1}$$

$$\mathrm{R}^{\bullet} + \mathrm{O_2} \rightarrow \mathrm{RO_2}^{\bullet} \qquad \text{(addition of O}_2\text{)} \tag{2}$$

$$\mathrm{RO_2}^{\bullet} + \mathrm{RH} \rightarrow \mathrm{ROOH} + \mathrm{R}^{\bullet} \qquad \text{(H-atom exchange)} \tag{3}$$

Once a free radical R$^{\bullet}$ has been generated, then reactions 2 and 3 form a chain reaction. As the chain cycles through (2) and (3) many lipid molecules (R−H) are converted into lipid hydroperoxide (ROOH), resulting in oxidation and rancidity of fats. Reaction 2 is very fast, ca. 10^9 M^{-1} s^{-1}, whereas (3) is much slower, typically 10^1 M^{-1} s^{-1} .[8]

For the phenolic antioxidant we will use the generic term ArOH, since by definition it contains at least one hydroxy group attached to a benzene ring. The role of the antioxidant ArOH is to interrupt the chain reaction according to

$$\mathrm{RO_2}^{\bullet} + \mathrm{ArOH} \rightarrow \mathrm{ROOH} + \mathrm{ArO}^{\bullet} \tag{4}$$

To be effective ArO$^{\bullet}$ must be a relatively stable free radical, so that it reacts slowly with substrate RH but rapidly with $RO_2^{\bullet}$, hence the term "chain-breaking antioxidant". It is known that the most effective lipid-soluble chain-breaking antioxidant in human blood plasma is α-tocopherol (α-TOH), the most active component of Vitamin E.[9] In vivo, the α-tocopheroxyl radical (α-TO$^{\bullet}$) is regenerated by reaction with Vitamin C, so that the chain reaction causing lipid peroxidation is broken,[9,10] and a continuously regenerated source of α-TOH is available. α-TOH reacts with peroxyl radicals with a rate constant of about 10^6 M^{-1} s^{-1}, which is much faster than the reaction of peroxyl radicals with lipid RH.[9]

The rate of reaction of substrate RH with peroxyl radicals depends on the barrier height for transfer of an H-atom from RH (or ArOH in the case of an antioxidant). As the reaction with $RO_2^{\bullet}$ and ArOH becomes more exothermic the barrier should decrease, and the antioxidant will react faster with the peroxyl radical, thus preventing reaction with substrate. The same argument applies to other free radicals of interest, including

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alkoxyl, alkyl, and superoxide. From this discussion it is clear that the Bond Dissociation Enthalpy (BDE) in ArOH will be an important factor in determining the efficacy of an antioxidant, since the weaker the OH bond the faster will be the reaction with free radicals. An additional factor of selectivity will play a role, e.g. every phenolic antioxidant can react with hydroxy radical due to the very high BDE of the HO−H bond in water, 119 kcal/mol,[11] thereby making all reactions with ArOH very exothermic according to

$$HO^{\bullet} + ArOH \rightarrow HOH + ArO^{\bullet} \quad (5)$$

Other free radicals such as $RO_2^{\bullet}$ have a much lower BDE on formation of the parent ROOH, typically about 88 kcal/mol,[11] and as a result will react slowly in a thermoneutral reaction with phenol [BDE ≈ 88 kcal/mol] but rapidly in an exothermic reaction with α-tocopherol [BDE ≈ 77 kcal/mol, vide infra]. Thus Vitamin E is an effective chain-breaking antioxidant that prevents lipid peroxidation, but phenol is not.

Another possible mechanism by which an antioxidant can deactivate a free radical is electron transfer, in which the radical cation is first formed followed by rapid and reversible deprotonation in solution, according to

$$RO_2^{\bullet} + ArOH \rightarrow RO_2^{-} + ArOH^{+} \quad \text{(electron transfer)} \quad (6)$$

$$ArOH^{+} + H_2O \rightleftarrows ArO^{\bullet} + H_3O^{+} \quad \text{(deprotonation equilibrium)} \quad (7)$$

$$RO_2^{-} + H_3O^{+} \rightleftarrows ROOH + H_2O \quad \text{(hydroperoxide formation)} \quad (8)$$

The net result from above is $RO_2^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$, i.e., the same as in the atom-transfer mechanism. However, if the radical cation $ArOH^{+}$ has sufficient lifetime it can attack suitable substrates, e.g. radical cations derived from aminophenols have been shown to undergo substitution on DNA bases[12,13] and thereby exert mutagenic effects.

In addition to the two major mechanisms above, in some cases other factors may also play a role in determining what makes an effective antioxidant, including the presence of bulky groups near the OH group,[8] hydrogen bonding characteristics of the solvent[14,15] or in a biological context, solubility, and transport to specific tissues.[9] It is clear, however, that as far as specific molecular properties are concerned, the BDE and the Ionization Potential (IP) are of particular importance. Both the H-atom transfer (HAT) and the single-electron transfer (SET) mechanisms must always occur in parallel, but with different rates. One of the objectives of the present paper is to try to decide which mechanism will be most important (i.e. have the faster rate) in the reactions of phenolic antioxidants with free radicals. Since an electron-donating substituent on a phenol will usually lower both the BDE and the IP simultaneously, it is likely that the change in BDE and IP will be strongly correlated and indeed this correlation has been pointed out in the literature.[16,17] This aspect of strong correlation has led some authors to state that both mechanisms must be active, e.g., in the case of Vitamin E analogues,[17] but this begs the question as to which one is dominant or by how much. It is expected that the SET mechanism will be strongly solvent dependent due to solvent stabilization of the charged species, whereas HAT will be only weakly solvent dependent. Here we do not try to allow for the effects of solvent, but assume that IP values in solution will be highly correlated with IP values in gas and thus form a useful series, along which we try to establish reference points for reactivity.

To do a systematic study of antioxidants from a theoretical perspective, it is desirable to determine accurately both BDE and IP, the former relevant to the atom-transfer mechanism ($AOH \rightarrow AO^{\bullet}$) and the latter relevant to electron transfer ($AOH \rightarrow AOH^{+}$). A number of theoretical studies of varying levels of sophistication have addressed these points, many of which have simply been QSAR studies which attempt to correlate antioxidant activity with various molecular properties [see for example ref 18 and references therein]. Many such theoretical calculations have restricted the treatment to the AM1 semiempirical model.[19] More recently, density functional theory (DFT) has been used in studies of the BDE and the IP. One paper that is close in spirit to the present work is that of Fox and Kollmann,[20] who used DFT to try to determine whether a biochemical reaction mechanism proceeded via atom transfer or electron transfer. Our own previous work on substituent effects in phenolic antioxidants[21] and a general study of X−H bond energetics (X = C, N, O, F)[22] also provided an important foundation for the present paper.

II. Method of Calculation

The basic method of calculation has been described in a number of recent publications by DiLabio et al.[22−26] Here we review the essential methodology needed to obtain accurate BDE and IP values. All calculations refer to the gas phase.

Full Basis Calculation of BDE. The BDE is calculated as the enthalpy difference at 298 K for the reaction $ArOH \rightarrow ArO^{\bullet} + H^{\bullet}$, where ArOH is the parent phenol and $ArO^{\bullet}$ is the corresponding phenoxyl radical.[27] The full-basis calculations of the BDE are very similar to those described in ref 22, according to the lowest level model (LLM) procedure described in that paper. For the parent molecule, the geometry is optimized by using the AM1 method. Vibrational frequencies are determined by using AM1 and then scaled by a factor of 0.973 to obtain the (scaled) zero-point energy and the vibrational contribution to the enthalpy. The enthalpy of the parent molecule is then corrected for translational, rotational, vibrational, and PV-work terms within

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(27) The BDE of phenol was also calculated at 310 K (37 °C), a temperature that is more appropriate for biological systems. However, the BDE for phenol changed by less than 0.02 kcal/mol due to this temperature change, so all calculations were done for 298 K.

Gaussian-98[28] to obtain the thermal correction to the enthalpy, which includes the zero-point energy. Next, a preliminary single-point calculation with B3LYP/6-31G(d) is done at the AM1 optimum geometry; this intermediate step is used to speed convergence in the next step as well as for the calculation of IP. Orbitals from this step are used as input to the final B3LYP/6-311+G(2d,2p) calculation. The total enthalpy at 298 K is the sum of the thermal correction to the enthalpy and the B3LYP electronic energy from the final step.

For the radical, the calculation also uses AM1 for geometry and scaled frequencies, and a preliminary single-point calculation from ROB3LYP/6-31G(d), where the RO refers to a Restricted Open-Shell approach.[29] The orbitals from this step are used as input to the final calculation which is ROB3LYP/6-311+G(2d,2p). The enthalpy at 298 K is then the thermal correction to the enthalpy from the AM1 step plus the final ROB3LYP energy. The entire calculation can be labeled (RO)B3LYP/6311+G(2d,2p)//AM1/AM1 in the standard notation.

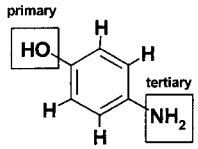
As discussed in ref 22, the electronic energy of the H-atom obtained with the B3LYP method with a 311G basis set is significantly too low at −0.50216 au and it is simply reset to its exact value, −0.50000 au. The enthalpy of the H-atom at 298 K, which includes translational and PV corrections, is then −0.49764 au and this is used in all calculations of the BDE.

A calculation of phenol with the above (full-basis) methodology gives a calculated BDE of 87.10 kcal/mol. There are several reviews in the literature which discuss the "best" gas-phase value derived from various sets of experimental data. Dos Santos and Martinho Simoes[30] obtained a value of 88.7 ± 0.5 kcal/mol after such a review. Pedulli et al.[8] used a value of 88.3 ± 0.8 kcal/mol based on a reference value of a substituted phenol obtained from calorimetric values.[31,32] Another recent estimate by Wayner et al. gives 87.0 ± 1 kcal/mol for the gas phase.[33] The BDE of phenol provides a reference value for all phenolic antioxidants, and it is satisfying to see that our calculated gas-phase BDE is essentially within experimental error. The contributions to obtaining this good absolute accuracy with the B3LYP method were discussed in ref 22, but several factors play a role, including use of the B3LYP functional, the H-atom correction (1.4 kcal/mol), the use of (RO)B3LYP instead of (U)B3LYP (ca. 1−3 kcal/mol), and the relatively large 6-311+G(2d,2p) basis set.

LDBS Calculation of BDE. There are a number of ways to extend the calculation of BDE values to large molecules. The ONIOM method of Morokuma and co-workers and its modifications has been shown to give BDE values of useful accuracy for a variety of molecules.[34] In our laboratory, we have found that the use of a methodology based on locally dense basis sets (LDBS) for BDE values has worked extremely well.[23,25] This has led to the possibility of studying most of the known antioxidants, including relatively large structures, with existing commercial software and without excessive use of CPU time or memory storage requirements. We have found the LDBS method to give excellent energetics for substituted benzenes, e.g. the BDE for phenol is 87.05 kcal/mol, vs the full basis result of 87.10 kcal/mol. We review here the use of the LDBS method as applied to p-aminophenol, which is an example of a phenol containing a substituent. To calculate the O−H BDE, the molecule is first partitioned according to criteria described in ref 25. The result of the partitioning is shown in Scheme 1.

Since the O−H bond is being broken this region is defined to be primary, and assigned the (large) 6-311+G(2d,2p) basis set, as in the full-basis calculation. In the phenoxyl radical the benzene ring is directly conjugated to the primary region and is therefore taken as secondary,

Scheme 1



LDBS Partition Scheme

along with the H-atoms attached to the benzene. The secondary basis set is (intermediate) 6-311+G(d), which reduces the number of polarization functions to a single d-function on each carbon. The amino group, which perturbs the electron distribution in the benzene ring, is taken to be tertiary and assigned the (small) 6-31G(d) basis. These primary, secondary, and tertiary basis sets are used throughout this paper. The same partitioning scheme must be strictly applied to the radical, which in *p*-aminophenol therefore has only the oxygen atom as primary and other regions are the same as in the parent.

In general, in the partitioning scheme in the parent molecule the OH is primary, the (attached) benzene and attached hydrogens are secondary, and all substituents including attached saturated rings are tertiary. The only time we depart from this procedure is when there are several OH groups which are hydrogen bonded as in catechol (1,2-dihydroxybenzene). In that case we examined the difference between a tertiary treatment for one OH group and primary for the other, vs primary for both. The differences are minor, but we have chosen to define OH groups adjacent to the OH group of interest to be primary, so as to be able to quickly alter data sets to examine multiple OH groups.

In practice the LDBS calculation is done as follows: The LDBS partitioning is done as described above and the appropriate basis set is assigned to each atom,[35] the geometry optimization and (scaled) frequency calculation is performed with AM1, followed by an intermediate (RO)B3LYP/6-31G(d) single point, and finally a (RO)-B3LYP/LDBS single point energy using starting orbitals from the previous step. This procedure worked very well for all molecules described in this paper, and convergence properties are better than those obtained with the full basis, which sometimes required several intermediate steps with increasing basis set size.

Calculation of IP Values. For reasons discussed previously,[24−26] LDBS methods are inappropriate for determination of IP values. This is because the IP is related to the structure of the HOMO which is a global molecular property, unlike the BDE which relates to the local properties of an O−H bond subject to only weak perturbations from the molecular environment. Therefore to calculate the IP a full-basis calculation must be used for both parent and cation. However, the basis set can be much reduced relative to the calculation of BDE, since we showed in a study of substituted benzene rings that systematic errors cancel out in a series of calculations, leading to accurate relative substituent effects.[20,24] In that case a systematic error in the IP of phenol (too low) remains through a series of substituted benzenes (all too low by the same amount) so that a suitable correction can be made. Our previous method calculated the adiabatic IP at 0 K, using AM1 geometries and AM1 frequencies scaled by 0.973. Single-point energies were calculated with (U)B3LYP/6-31G(d) or (U)B3LYP/6-311G(d), i.e., the unrestricted open-shell calculation was used for these calculations.[36] For monosubstituted benzenes this gave absolute deviations of 9.1 or 5.5 kcal/mol (compared to experiment) for the smaller and larger basis sets, respectively, but excellent relative deviations (ΔIP values). We consider these results sufficiently established in the previous two papers[24,26] that no further testing of the method is needed in the present application. To summarize the calculation of IP, then, we use the (U)-B3LYP/6-31G(d)//AM1/AM1 calculation to obtain the total electronic energy plus (scaled) zero-point energy. Both parent and cation are geometry-optimized, so the energy difference is the adiabatic ionization

(28) Gaussian 98, Revision A.7; Frisch, M. J. et al.; Gaussian, Inc.: Pittsburgh, PA, 1998.

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(36) Although the RO calculation has a smaller absolute error, the (U)-B3LYP calculation runs faster due to Gaussian 98's use of analytical gradients for U but not RO.

Table 1. Results for Monosubstituted Phenols, Comparing the Effect of the Substituent Calculated with Full Basis Set (FB) vs the Locally Dense Basis Set (LDBS) (all values in kcal/mol, relative to phenol)

	ortho		meta		para	
group	FB[a]	LDBS[b]	FB	LDBS	FB	LDBS
NH_2	−11.3	−11.5	−0.2	−0.2	−9.2	−9.4
OCH_3	−1.4	−1.4	−1.2	−1.2	−6.1	−6.1
OH[c]	−9.1	−9.2	−0.3	−0.2	−5.8	−5.9
$CHCH_2$	−3.9	−4.3	−0.3	−0.2	−4.4	−4.7
CH_3	−1.9	−1.8	−0.4	−0.4	−2.5	−2.5
tert-butyl	−3.3	−3.2	−0.6	−0.8	−2.0	−2.2
Cl	+1.3	+1.3	+1.2	+1.2	−1.5	−1.4
CN	+3.7	+3.6	+2.8	+2.7	+2.3	+2.2
CHO	+7.8	+7.9	+2.2	+2.2	+2.4	+2.4
COOH	+7.7	+8.1	+2.7	+2.5	+2.8	+2.6
CF_3	+3.5	+3.9	+2.1	+2.1	+3.3	+3.2
NO_2	+12.1	+12.3	+3.4	+3.4	+4.6	+4.6

[a] Relative to the full-basis value for phenol (87.10 kcal/mol) [b] Relative to the LDBS value for phenol (87.05 kcal/mol) [c] In this table the *o*-hydroxy group is treated as a tertiary substituent. In all other calculations to follow it is treated as primary. The difference in treatment is minor, as would be expected from the close agreement between the LDBS and the full calculation. See also ref 25.

potential at 0 K. Subtraction of the value for phenol then gives the relative values, or ΔIP values, for all the phenolic antioxidants reported in this paper.

III. Results and Discussion

A. Internal Consistency: Full Basis vs LDBS BDE Values for Ortho-, Meta-, and Para-Substituted Phenol. Phenolic antioxidants contain a number of frequently encountered functional groups which are electron-donating groups (EDG), including methyl, hydroxy, methoxy, and amino. Occasionally they also contain electron-withdrawing groups (EWG) such as formyl, acetyl carboxyl, and ester groups. To have confidence in the application of LDBS methods to an arbitrary phenolic antioxidant, it is important to examine the internal consistency between full-basis and LDBS calculations for a representative set of both electron-donating and electron-withdrawing groups. This was done previously by one of the present authors[25] for para-substituted phenols, although with a LDBS partitioning scheme which allowed the substituent to use both tertiary (6-31G(d)) and quaternary (STO-3G) basis sets. To allow for more possibilities, we enlarged upon the previous set somewhat to include in the EDG set amino, methoxy, hydroxy, vinyl, methyl, *tert*-butyl, and chloro and in the EWG set cyano, formyl, carboxyl, trifluoromethyl, and nitro. We also report calculations for ortho, meta, and para substituents. This set will allow a general treatment of substituent effects on aromatic rings derived from the results of these calculations, and will be applicable to BDE values of most of the known phenolic antioxidants. In all cases from this set the geometry is obvious, when H-bonding in parent and radical is maximized and nonbonded repulsions are minimized.

Table 1 shows the BDE for ortho, meta, and para substituents, relative to phenol, calculated with the full basis result (87.10 kcal/mol) or with the LDBS method (87.05 kcal/mol). For the LDBS partitioning the phenolic OH group being broken was taken to be primary, benzene carbon atoms and H-atoms attached to benzene secondary, and the substituent tertiary.[37] For a (brief) comparison to literature values from other theoretical calculations and from experiment, Table 2 shows results for the most extreme substituents in our set at opposite ends of the spectrum, i.e., *p*-aminophenol and *p*-nitrophenol. Our values are closer to the calculated values of Brinck et al.[38] and to our own previous DFT values[21,25] than to experiment.

Table 2. Comparison of Calculated Values for Δ*B*DE (full basis) for *p*-Nitrophenol and *p*-Aminophenol with Literature Values[a]

method	*p*-NO_2 (kcal/mol)	*p*-NH_2 (kcal/mol)	ref
B3LYP/6-311+G(2d,2p)// AM1/AM1	+4.6	−9.2	this work
B3LYP/6-311G(2d,p)// B3LYP/6-31G(d,p)	+4.2	−8.8	37
exptl	+6.4,[b] +4.4[c]	−12.7,[b] −12.6[c]	

[a] All theoretical values refer to phenol calculated with the same method. [b] Lind, J.; Shen, X.; Eriksen, T. E.; Merenyi, G. *J. Am. Chem. Soc.* **1990**, *112*, 479. [c] Bordwell, F. G.; Cheng, J.-P. *J. Am. Chem. Soc.* **1991**, *113*, 1736.

Examination of Table 1 shows that the LDBS calculation of ΔBDE generally agrees with the full-basis result to within 0.4 kcal/mol or better, with a mean absolute deviation of only 0.12 kcal/mol, for data which span a range from −11.5 kcal/mol (*o*-NH_2) to +12.3 kcal/mol (*o*-NO_2). Note that even for the groups which are strongly conjugated to benzene, e.g. $CHCH_2$, CHO, NO_2, CN, and COOH, tertiary treatment of the substituent is nevertheless adequate at the 0.4 kcal/mol level. This exercise shows that the LDBS approach is not only generally applicable to the set of substituents important to antioxidant activity, but also to a set of substituents of general importance in organic chemistry.

B. Comparison of BDE Calculation with Experimental Data. Pedulli and co-workers[8,39] have reported very precise BDE values for a family of phenolic antioxidants, obtained by measuring the equilibrium constant between a phenoxyl radical whose heat of formation is known and a given phenol. The reference standard for this work was 2,4,6-tri-*tert*-butylphenol, for which a BDE of 81.24 kcal/mol was obtained from calorimetric studies.[31] By obtaining the free energy change $\Delta G°$ from the equilibrium constant and assuming the entropy change to be negligible, the authors obtained a set of enthalpy and hence BDE values for a variety of phenolic species. The data were taken in (deoxygenated) benzene solution, but because of the small dielectric constant of benzene the values will be close to those expected in the gas phase.

In the present paper, BDE values were calculated with the LDBS approach described above. The data of Pedulli et al.[8,39] consist of phenols containing methyl, *tert*-butyl, and methoxy substituents located in ortho, meta, and para positions on the benzene ring, as well as α-tocopherol (α-TOH) and 6-hydroxy-2,2,5,7,8-pentamethylchroman (HPMC), a model compound similar to α-TOH but lacking the phytl ($C_{16}H_{33}$) tail. In most cases the ortho and meta compounds are disubstituted symmetrically with the same substituent. A subset of these data was taken, eliminating only α-TOH [see ref 23], and several of the compounds which contain two *tert*-butyl substituents ortho to the OH. In general, in all calculations of the BDE, we take the most stable conformer for parent and radical obtained with the AM1 geometry search.[40] One exception to this rule is the α-TOH model compound, i.e., HPMC. Here steric crowding about the

(37) The only exception to this statement occurs for *o*-hydroxyphenol, where we examined both a primary and a tertiary treatment of the substituent. The difference between a tertiary treatment (−9.18 kcal/mol) and treating both OH groups as primary (−8.91 kcal/mol) differs by less than 0.3 kcal/mol and both values are close to the full-basis result (−9.13 kcal/mol). This suggests that a tertiary treatment is perfectly adequate for the *ortho* substituent, even though is is hydrogen bonded to the primary O−H bond being broken, with a bond strength of ca.. 4 kcal/mol. See also ref 25.

(38) Brinck, T.; Haeberlein, M.; Jonsson, M. *J. Am. Chem. Soc.* **1997**, *119*, 4239.

(39) Lucarini, M.; Pudulli, G. F.; Cipollone, M. *J. Org. Chem.* **1994**, *59*, 5063.

Table 3. Bond Dissociation Enthalpies in Benzene (exptl) or Gas Phase (LDBS calculation)[a]

				BDE (kcal/mol)	
ArOH	R_{ortho}[ab]	R_{meta}[ab]	R_{para}	exptl	calcd[bc]
1	H	H	H	88.3 ± 0.8	87.05
1a	H	H	Me	86.2 ± 0.6	84.58
1b	H	H	CMe_3	85.3 ± 0.5	84.76
1c	H	H	OMe	82.81 ± 0.21	80.92
2a	Me	H	H	84.50 ± 0.38	82.88
2b	CMe_3	H	H	82.80 ± 0.21	76.51
2c	OMe	H	H	83.16 ± 0.15	82.44
3b	H	CMe_3	H	86.62 ± 0.26	85.68
3c	H	OMe	H	86.70 ± 0.3	86.00
4a	Me	H	Me	82.73 ± 0.18	80.58
4c	OMe	H	OMe	80.00 ± 0.12	78.07
5a	Me	H,Me	OMe	79.20 ± 0.15	76.69
5b	Me	Me	OMe	81.88 ± 0.20	79.04
6a	HPMC[d]			78.25 ± 0.18	75.78

[a] The numbering system and experimental data are from Pedulli et al.[39] [b] Unless otherwise indicated, "*ortho*" indicates two *ortho* groups, and "*meta*" indicates two *meta* groups. Compound **5a** is an exception, with only one *m*-methyl group. [c] These BDE values were calculated with a tight convergence criterion of 10^{-5} hartree in the B3LYP calculation, hence data are reported to ± 0.01 kcal/mol. [d] 6-hydroxy-2,2,5,7,8-pentamethylchroman.

OH due to two adjacent methyl groups caused the OH group to tilt out of plane by 30° in the AM1 calculation, but this is known to be planar by experiment.[9] Forcing the OH group to lie in plane and optimizing the geometry subject to this constraint actually gave a B3LYP/LDBS energy that was lower in the parent.

Table 3 shows the experimental BDE values (in benzene) according to the numbering scheme used by Pedulli,[39] along with their estimated error bars. It can be seen from the table that the experimental error estimates increase as the BDE becomes more different from the reference compound. By using 2,4,6-trimethylphenol as reference for their experimental scale Pedulli et al.[39] give a BDE for phenol of 88.3 ± 0.8 kcal/mol. Table 3 also shows our calculated values with use of the LDBS approach. With the exception of compound **2b**, the calculated values lie uniformly below the experimental value by a nearly constant amount, i.e., the values of ΔBDE match up well. The exception is **2b**, *o,o*-di-*tert*-butylphenol, the ΔBDE value of which differs dramatically from the experimental value for the BDE, with the B3LYP calculation being too low by ca. 5 kcal/mol.

We studied the origin of this error, and have satisfied ourselves that it does not arise from either the LDBS approach or the AM1 geometry optimization. In this sterically crowded compound the H-atom in the OH group comes very close to the methyl hydrogen on the *tert*-butyl group, i.e., within 1.98 Å (AM1 geometry). It appears that in this situation the B3LYP functional behaves incorrectly, causing excessive destabilization in the parent compound (the strain is relieved in the radical, when the phenolic H-atom has been removed). This is probably related to the documented problems when using DFT methods to treat dispersion forces.[41] Thus *o,o*-di-*tert*-butylphenol contains the only combination of functional groups we have not been able to treat accurately. For that reason other compounds containing this functionality described by Pedulli et al.[39] have been omitted from further study (Note, however, that the error is constant for a series of such compounds.) Omitting only **2b**, the mean absolute deviation between the ΔBDE values for calculated and experimental values is 0.38 kcal/mol, for data which span a range of about 10 kcal/mol. The calculated data lie on average about 1.5 kcal/mol below the experimental data, consistent with the fact that our value for phenol (87.05 kcal/mol) lies below Pedulli's value (88.3 kcal/mol). Recall, however, that the Pedulli data are all related through equilibrium contants to the calorimetrically measured value of 81.24 kcal/mol for 2,4,6-tri-*tert*-butylphenol, so any error in this measurement is reflected in a shift of their whole set of BDE values. In general, the agreement between calculated and experimental data is good except for the particular case of two *o*-*tert*-butyl substituents, as noted above.

Table 4. Recommended Additivity Values (ΔBDE values) on the OH BDE in Phenolic Compounds

Meta and Para Substituents

group	meta	para
NH_2	−0.2	−9.4
OMe	−0.6	−6.1
OH	−0.4	−5.9
$CHCH_2$	−0.2	−4.7
tert-butyl	−0.6	−2.5
CH_3	−0.4	−2.5
Cl	+1.2	−1.4
CN	+2.7	+2.2
CHO	+2.2	+2.4
COOH	+2.5	+2.6
CF_3	+2.1	+3.2
NO_2	+3.4	+4.6

(b) Ortho Substituents[a,b]

group	electronic effect	H-bond parent	H-bond radical	total
NH_2	−7.5	+4.0	−8.0	−11.5
OMe	−5.4	+4.0	0.0	−1.4
OH	−5.2	+4.0	−8.0	−9.2
$CHCH_2$	−4.0	+0.0	0.0	−4.0
tert-butyl	−2.2	+0.5	−1.0	−2.7
CH_3	−2.0	0.0	0.0	−2.0
Cl	−1.0	+2.0	0.0	+1.0
CN	+1.6	+2.0	0.0	+3.6
CHO	+2.0	+6.0	0.0	+8.0
COOH	+2.1	+12.0	−6.0	+8.1
CF_3	+2.0	+2.0	0.0	+4.0
NO_2	+4.0	+6.0	0.0	+10.0

[a] All values in kcal/mol relative to phenol. [b] Showing the contribution of electronic effect and H-bonding in the parent and the radical.

C. Additivity of Substituent Effects on BDE. On the basis of the above data, we can define a set of optimized ΔBDE values to allow prediction of BDE values based on group additivity. Of course other scales can be used for this purpose including the well-known σ^+ scale of Brown,[42] which has frequently been used to correlate both BDE data[39] and IP data.[43] However, our own ΔBDE values form a self-consistent set with a single calculation procedure, which we have found useful to apply to the phenolic antioxidants. We derived this set by performing LDBS calculations on many antioxidants and finding an approximate "best set", subject to some qualifications (see below). For meta and para substituents on phenol, the set is derived from Table 1 (and to a lesser extent from Table 3). The results are given in Table 4a.

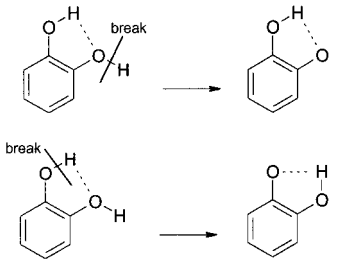
(40) It is usually possible to guess the most stable geometry through application of the following rules: (a) maximize hydrogen bonding in the parent and radical; (b) when no H-bonding is possible minimize nonbonded repulsions (e.g. by pointing a hydroxy group away from a methyl group in *o*-methylphenol). Structures are available on request from the authors: Address inquiries to jim_wright@carleton.ca.

(41) Pérez-Jordá, J. M.; Becke, A. D. *Chem. Phys. Lett.* **1995**, *233*, 134. Kristyá, S.; Pulay, P. *Chem. Phys. Lett.* 1994, *229*, 175.

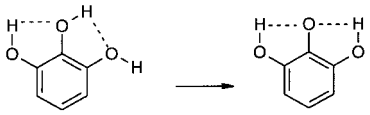
(42) Brown, H. C.; Okamoto, Y. *J. Am. Chem. Soc.* **1957**, *79*, 1913.

(43) Jovanovic, S. V.; Tosic, M.; Simic, M. G. *J. Phys. Chem.* **1991**, *95*, 10824.

Scheme 2



Scheme 3



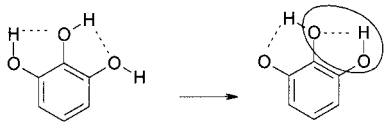
For ortho functional groups there is an additional interaction possible due to both steric effects and hydrogen bonding. Steric effects are not relevant in the case of a monosubstituted phenol (i.e. with one additional substituent) since the OH group can always point away from the substituent, but hydrogen bonding and conformational changes can be important. Consider for example the case of catechol forming the catechol radical, shown in Scheme 2.

In each case (parent and radical) we are assuming that the most stable conformer is the correct choice, i.e., after the bond has broken in the parent compound the radical is able to rearrange to the more stable conformation shown. The result is that regardless of which OH bond is broken in the parent, the radical is allowed to rearrange (lower diagram) and thus there is only one BDE value in catechol. The barrier height for this rotation in the radical is only ca. 4 kcal/mol (our calculation) so this rearrangement can occur at room temperature.

In the parent catechol (left), the *o*-OH group will have a slightly decreased electronic effect compared to the para group (−5.9). Following the Hammett parameters for which $\sigma^{+}_{\text{ortho}} < \sigma^{+}_{\text{para}}$,[44] we allow this ratio to float somewhat (again, to optimize the set of additivity calculations), and for the *o*-OH group we assign the value −5.2 kcal/mol. The parent is stabilized by a moderately strong H-bond of strength ca. 4 kcal/mol, which therefore increases the BDE. The radical has a much stronger H-bond of strength ca. 8 kcal/mol, which stabilizes the radical and decreases the BDE. The net result is that the BDE is 87.1 − 5.2 + 4−8 = 77.9, for a change in BDE of −9.2 kcal/mol. This result is consistent with the values reported in Table 1 (−9.2 by LDBS, −9.1 by full basis). By proceeding in this way we derive the data in Table 4b, which decompose the ΔBDE into an electronic contribution, a contribution from H-bonding in the parent phenol, and a contribution from H-bonding in the corresponding phenoxyl radical. In deriving Table 4b, H-bond strengths have been grouped according to similar types.

Finally, the trihydroxy functionality is encountered in many antioxidants, particularly the catechins, and it is necessary to consider additional factors for this case. In Scheme 3 the central O−H bond is weakest, due to the presence of two ortho groups. Again, one OH group in the radical (at the right) is assumed to rotate to the more stable conformer, which now contains two hydrogen bonds. The only difference here from the catechol compound (Scheme 2) is that there will be an attenuation of the H-bond strength in the radical due to the shared nature of the H-bonds. The radical is symmetric (shown in Scheme 3) and we find that an H-bond strength in the radical of 6.0 kcal/mol is optimum for a series of such compounds containing other functional groups as well. The ΔBDE of this compound is then 2(−5.2) + 2(+4) + 2(−6) = −14.4 kcal/mol, relative to phenol. This is close to our calculated value of −14.1 kcal/mol (next section, see compound **19**).

Scheme 4



It is also possible that the outer hydroxy groups will have some antioxidant activity, in addition to the central hydroxy group, e.g. as in **19**. Scheme 4 shows the situation where the outer OH bond is broken. The structure of the parent is identical with that in Scheme 3, and the radical arranges itself so as to maxmize hydrogen bonding. This gives a calculated ΔBDE = −7.6 kcal/mol, considerably less than the central OH bond but not altogether inactive. Due to the presence of the H-bond shown in the ellipse the other H-bond to the radical is attenuated somewhat. Assigning an attenuated H-bond contribution in the radical as −6 kcal/mol as in the example above, by additivity we obtain ΔBDE = −5.2 − 0.4 + 2(4) − 4 − 6 = −7.6 kcal/mol. This completes the discussion of our approach to calculating additivity contributions. Many examples are given in the next section, which makes a selection of important types of phenolic antioxidants to illustrate the calculation of BDE and IP and the determination of substituent effects.

Commercial Antioxidants Used as Food Additives. Four commercial antioxidants widely used as food additives to prevent the oxidation of fats are butylated hydroxyanisole (BHA, **7**, **8**), propyl gallate (**9**) and nordihydroguaiaretic acid (NDGA, **10**).[1] However, concerns about carcinogenic test results for BHA in high doses have led to reexamination of its use. BHA occurs as a mixture of two structural isomers, BHA-1 (**7**) and BHA-2 (**8**), whose structures are shown below. As described previously, the LDBS partition in each assigned the OH group as primary, and the substituents *tert*-butyl and methoxy as tertiary.

Table 5 shows that of the pair of compounds, BHA-1 is a significantly better antioxidant, with ΔBDE almost 3 kcal/mol larger than BHA-2, as expected from the location of the *tert*-butyl group ortho rather than meta to the OH. This suggests that purification of the mixture to eliminate BHA-2 would allow proportionately smaller doses of BHA-1, of potential importance if BHA is to be used as a food additive. The ΔIP values of **7** and **8** are −23.0 and −23.4 kcal/mol (relative to phenol), respectively, which are not very large relative to systems known to react by SET, so we expect that the mode of action of BHA is via HAT (see the discussion of phenolic amines).

Propyl gallate is assigned the LDBS partition where the trihydroxy group is primary and the gallate group is tertiary. It should be a more effective antioxidant than BHA due to a larger ΔBDE of −11.2 kcal/mol, making it comparable to Vitamin E (α-tocopherol component). Its ΔIP is only −7.8 kcal/mol, so this antioxidant will react by HAT. Note that in this case a large BDE lowering *does not* correlate with a large ΔIP; indeed one way we have observed to uncouple this correlation is to have large internal H-bonding effects. Another is to add substituents meta to the OH group, which have very small effects on the BDE but can have substantial effects on the IP.[45]

(44) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley & Sons: New York, 1979.

(45) DiLabio, G. A. To be submitted for publication.

Table 5. Change in BDE and IP Relative to Phenol (see text for the method of calculation)

name	compd	ΔBDE (calcd) (kcal/mol)	ΔIP (kcal/mol)
BHA-1	**7**	−8.9	−23.0
BHA-2	**8**	−6.1	−23.4
propyl gallate	**9**	−11.2	−7.8
nordihydroguaiaretic acid (NDGA) (model, in rectangle)	**10**	−11.1	−15.1
tocol (model)	**11**	−7.2	−27.4
δ-tocopherol (model)	**12**	−7.3	−30.5
β-tocopherol (model)	**13**	−9.4	−33.6
γ-tocopherol (model)	**14**	−8.9	−32.9
α-tocopherol (model)	**15**	−11.3[a]	−36.1
(−)-epigallocatechin-3-gallate	**16**	−16.1	−27.9
Tea Group 1	**17**	−2.3[b]	−18.6
Tea Group 2	**18**	−14.1	−10.9
Tea Group 3	**19**	−11.6	−7.2
p-aminophenol	**20**	−9.4	−29.1
N,*N*-dimethyl-*p*-aminophenol	**21**	−10.3	−37.5
6-hydroxy-5,7,8-trimethyl-1,2,3,4-tetrahydroquinoline	**22**	−14.6	−46.9
9-hydroxyjulolidine	**23**	−11.8	−48.7
p-butadienylphenol	**24**	−7.7	−27.8
p-vinylphenylphenol	**25**	−8.5	−32.5
resveratrol	**26**	−8.2	−33.1
piceatannol	**27**	−15.6	−35.2

[a] The OH group in the AM1 geometry optimization is twisted 28° out of plane. At this geometry Δ*B*DE = −12.0 kcal/mol, giving a BDE well below the experimental value. Constraining the OH to lie in-plane and reoptimizing AM1, followed by B3LYP gives our reported value of −11.3 kcal/mol, in much better agreement with the experimental value. [b] This refers to the OH group at the bottom of structure **17**. The other OH group has a similar value of ΔBDE = −1.3 kcal/mol.

NDGA (**10**) is a dimer of a catechol connected by a saturated hydrocarbon link; a useful model compound to estimate its BDE is therefore shown in the rectangle, i.e., 1,2-dihydroxy-4-methylbenzene. Here the dihydroxy grouping is taken to be primary and the methyl tertiary. For this compound also ΔBDE = −11.1 kcal/mol, whereas ΔIP = −15.1 kcal/mol. The full compound will have a very similar BDE and only a slightly lower IP, indicating a good antioxidant which reacts by HAT.

We can apply additivity rules to predict the ΔBDE for **7**−**10**. For **7**, use of Tables 4a and 4b gives ΔBDE = −2.7 − 6.1 = −8.8 (−8.9), where the B3LYP/LDBS calculated value is shown in parentheses. For **8**, we have −0.6 − 6.1 = −6.7 (−6.2). Compound **9** gives −14.4 + 2.6 = −11.8 (−11.2) by using the trihydroxy group value of −14.4 as discussed above, and the COOH group which should be close to the $COOC_3H_7$ substituent group value. Finally, for **10** we obtain −9.2 − 2.5 = −11.7 (−11.1) kcal/mol. Thus the values based on additivity are all well within 1 kcal/mol, which, as we shall see, is usually the case.

Tocopherols Present in Vitamin E. Compounds **12**−**15** (Figure 2) form the tocopherol group. These compounds, present in naturally occurring Vitamin E, are *δ*-tocopherol (**12**), *β*-tocopherol (**13**), *γ*-tocopherol (**14**), and α-tocopherol (**15**). The corresponding molecule which contains no *m*-methyl group is known as tocol (**11**) and does not occur naturally. In fact the tocopherols actually contain a $C_{16}H_{33}$ group ("phytyl tail") rather than the methyl group shown, but for purposes of calculation of BDE there is very little difference and the biological role of the tail is essentially to improve solubility in the lipid membrane[9] or in low-density lipoprotein. Burton, Ingold, and co-workers[9] have shown that α-tocopherol is the major lipid-soluble chain-breaking antioxidant in human blood plasma. The other tocopherols also possess some bioactivity. From this group, the antioxidant activity in vivo is in the order α > *β* > *γ* > *δ* by a ratio of 1.0:0.5:0.1:0.03.[9] One complication in interpreting this order is that transport to the cell and also solubility (i.e. bioavailability) can affect the in vivo activity. The same experiment was repeated by these authors in organic solvent,[9] which gives the order α > *β* ≈ *γ* > *δ*. We shall compare our data to the results of the experiment in organic solvent, which are useful at least as a first step in understanding the in vivo behavior.

The LDBS calculation of the tocopherol model compounds is straightforward, but the geometry optimization requires comment. The α-TOH geometry is crowded about the OH group and the AM1 optimization gives the OH group incorrectly out of plane, as discussed previously. In other cases the OH group points away from the adjacent methyl group to minimize nonbonded repulsions. Based on our BDE values from Table 5 we see that our calculated order of ΔBDE = −11.3, −9.4, −8.9, −7.3 kcal/mol for [α, *β*, *γ*, *δ*], so that the predicted order of antioxidant activity in nonpolar solvent is α > *β* ≈ *γ* > *δ*, precisely the result obtained by Burton et al.[46] This strongly suggests that a HAT mechanism is responsible for the observed rates with peroxyl radicals. However, SET rates also become higher as the IP drops. The corresponding values for ΔIP [α, *β*, *γ*, *δ*] are −36.1,−33.6, −32.9, and −30.5 kcal/mol, corresponding to a drop in IP of 3 kcal/mol per methyl group, regardless of position (tocol, with no methyl groups, has a ΔIP of −27.4 kcal/mol, in agrement with the above statement). This order of ΔIP leads to precisely the same prediction: α > *β* ≈ *γ* > *δ*.

Given the above results it is not surprising then that it is currently an open question as to whether the tocopherols act as HAT reagents or SET reagents. Burton, Ingold, and co-workers reported substantial deuterium isotope effects in the reaction of α-tocopherol with peroxyl radicals, which led them to state that HAT is the rate-controlling mechanism.[46] Pedulli[39] and co-workers have always assumed the HAT mechanism. Njus and Kelley have argued on thermodynamic grounds that both Vitamins C and E donate single hydrogen atoms in vivo.[47] Bisby and Parker[48] measured reaction rates of α-tocopherol in micelles and provided direct evidence in support of the arguments of Njus and Kelley. However, Mukai and co-workers[49] have argued in favor of the SET mechanism, or more recently in favor of a concerted charge-transfer proton-transfer mechanism.[50]

Our data do not unambiguously answer this question since clearly ΔBDE and ΔIP are strongly correlated, at least within a family of related structural types.[45] However, evidence we have been able to gather leads us to believe that up to about ΔIP = −36 kcal/mol and for values of ΔBDE ≈ −10 kcal/mol, the mechanism is dominated by atom transfer in aqueous solution, whereas for ΔIP above (i.e. greater than) about −45 kcal/mol the antioxidant mechanism is predominantly SET. A definitive way to resolve these questions is to substitute the hydroxy group with a methoxy group, where only SET is possible, and such experiments are currently under way in our laboratory.[51]

Catechins in Tea. Green tea and, to a lesser extent, black tea contain a number of bioflavanoids with significant antioxidant activity. The family of compounds known as flavanols is

(46) 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.
(47) Njus, D.; Kelley, P. M. *FEBS* **1991**, *284*, 147.
(48) Bisby, R. H.; Parker, A. W. *J. Am. Chem. Soc*. **1995**, *117*, 5664.
(49) Mukai, K.; Kageyama, Y.; Ishida, T.; Fukuda, K. *J. Org. Chem*. **1989**, *54*, 552.
(50) Nagaoka, S.; Kuranaka, A.; Tsuboi, H.; Nagashima, U.; Mukai, K. *J. Phys. Chem*. **1992**, *96*, 2754.
(51) Wright, J. S. To be submitted for publication.

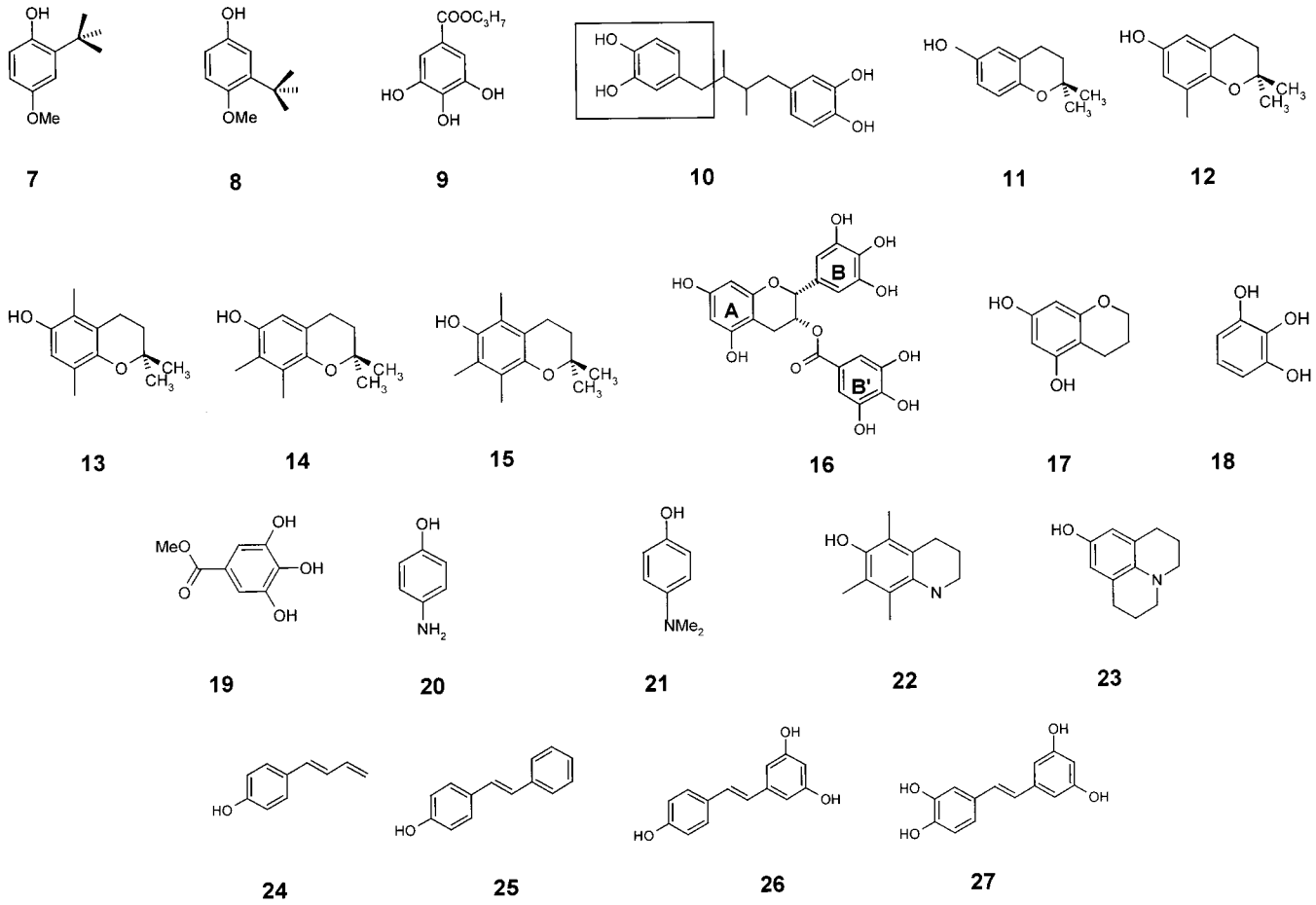


Figure 1. Structures of Phenolic Antioxidants used in Table 5.

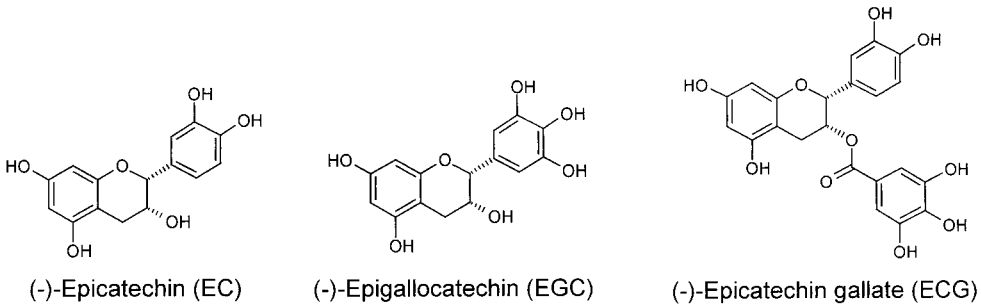


Figure 2. Structures of gallocatechins in tea.

represented by (−)-catechin, (−)-epigallocatechin, (−)epicatechin-3-gallate, and (−) epigallocatechin-3-gallate (**16**). Various structure−activity studies have been applied to this family to see what is responsible for the antioxidant activity.[52,53] Now it is generally agreed that the "B" ring in the flavanols is responible for most of the activity; this is the ring (or rings) containing the catechol or trihydroxy functionality.[18] Compound **16**, epigallocatechin gallate (ECGC), is one of the most active compounds in this family, and has shown cancer-preventive and antiviral activity in several clinical trials.[54]

The components of ECGC can be examined separately, and we looked at **17**, **18**, and **19** which we refer to as Tea Group 1 (containing A and C rings), Tea Group 2 (containing B ring), and Tea Group 3 (containing B′ ring). In **17** we expect a weak activity (small ΔBDE) and an approximately equal ΔBDE for the two OH bonds. In **18** and **19** the central OH group will have the greater ΔBDE due to the two remaining *o*-OH groups, so it was not necessary to examine the outer OH groups. The LDBS partitioning for the trihydroxy (or catechol) grouping makes this region primary. In the full compound ECGC, **16**, we were able to do the LDBS calculation by defining the trihydroxy region to be primary, the attached benzene secondary, and the remainder of the molecule tertiary. This compound is relatively large, with 54 atoms, so use of the LDBS methodology coupled with AM1 geometry optimization becomes important to make the calculation feasible.

As expected, Tea Group 1 (**17**) which has *m*-OH, *m*-OMe ,and *o*-(or *p*-)methyl had very small ΔBDE values of ca. −2.3 kcal/mol, indicating that this ring is not important in reacting as an antioxidant by HAT, e.g. with peroxyl free radicals. Note,

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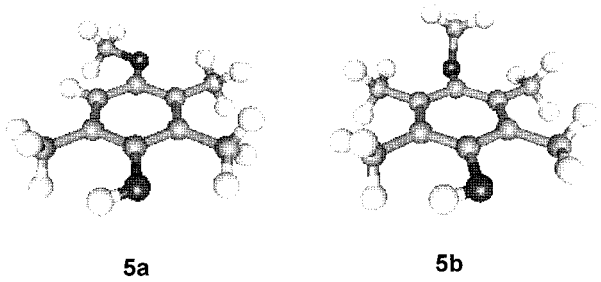

Figure 3. Three-dimensional structures of compounds **5a** and **5b** (Table 3).

however, that if the mechanism were indeed dominated by SET then the A ring would be an important contributor to the reactivity, since its IP (−18.6 kcal/mol) is considerably lower than the IP arising from the B and B′ rings. Tea Group 2, **18**, or 1,2,3-trihydroxybenzene, has a very large ΔBDE = −14.1 kcal/mol, so the BDE of **18** is below that of α-tocopherol. Compound **19** is similar to that of **18** except that it contains an electron-withdrawing ester group in the para position. Assuming this to behave like a carboxyl group and using additivity suggests a reduction by 2.6 kcal/mol (Table 4a, *p*-COOH) relative to **18** to give −11.5 kcal/mol, which is very close to the calculated value (−11.6 kcal/mol). The ΔBDE for the full compound **16**, which links the three Tea Groups together, is −16.1 kcal/mol. This is close to what would be expected by additivity for **18** substituted with a *p*-alkyl group, which gives −14.4 − 2.5 = −16.9 kcal/mol. The ΔIP values for **17**−**19** are not below −20 kcal/mol and even **16** is only −27.9 kcal/mol, so the flavanols are expected to react by HAT. It is clear that ECGC (**16**) is a superior antioxidant in this class of compounds, and it is also clear that the number of OH groups is largely irrelevant, it is the strategic placing of such groups that does matter. The B ring is indeed the most active, and the B′ ring will contribute to activity also. In the B or B′ ring the outer OH groups have ΔBDE values roughly half that of the central group, so these may make a nonnegligible contribution with the reaction rate with free radicals.

From the above discussion, we can also use additivity to predict the BDE of the other three important members of the catechin family, Epicatechin (EC), Epigallocatechin (EGC), and Epicatechin gallate (ECG). Structures for these three molecules are shown in Figure 2. For EC the BDE is essentially that of 1,2-dihydroxy-4-methylbenzene (i.e. the B-ring) with a *p*-alkyl group, which will have ΔBDE = −9.2 − 2.5 = −11.7 kcal/mol. Similarly for EGC the ΔBDE is essentially identical to EGCG derived above, which from additivity we estimate to be −16.6 kcal/mol. Finally, for ECG we have both the *p*-alkyl catechol group, at −11.7 kcal/mol, and the trihydroxybenzene with a *p*-carboxyl group, estimated at −11.5 kcal/mol (see discussion for **19**), thus both rings contribute approximately equally to the antioxidant activity in ECG. However, using this argument ECG may have a faster rate than EC since ECG has two antioxidant sites with OH BDE values of ca. −11.5 kcal/mol while EC has only one, and the individual rates are additive. Similarly, ECGC has some activity from the B′ ring, which has the trihydroxy functionality somewhat reduced in activity by the para electron-withdrawing group. Thus the order of antioxidant activity should be EC ≤ ECG < EGC ≤ EGCG, corresponding to the ordering of ΔBDE [−11.7, −11.7 (two sites), −16.6, −16.6 (two sites)]. The reaction rates of the catechins with superoxide radical were measured by Jovanovic et al. at pH 7.[55] They found that the order of reactivity was EC < ECG ≈ EGC < EGCG. The authors attribute the higher reactivity of EGCG as due to the presence of two antioxidant sites (two gallate moieties) in the epigallocatechin gallate molecule, and they believe that the mechanism involves SET rather than HAT. Note, however, that our predicted activity based on the BDE and their experiments on reaction with superoxide radical are nevertheless in substantial agreement.

Our calculated ΔIP values for the Tea Group compounds **16**−**19** cover the range from −7.2 to −27.9 kcal/mol. Extrapolating to the structures for epicatechin to epigallocatechin gallate would put these compounds in the approximate range from −18 to −30 kcal/mol. In their discussion of the antioxidant activity of the gallocatechins Jovanovic et al.[55] come to the conclusion that "because of their high solubility in water, gallocatechins are expected to act as antioxidants in polar aqueous phase, where one-electron transfer is likely to be the dominant reaction mechanism". However, our own data suggest that since they have substantial ΔBDE values and relatively small ΔIP values, it is probable that the rates of reaction of these compounds, even in aqueous solution, are dominated by HAT.

In medicinal applications of antioxidants it is important to note that when the BDE or IP become too low, the compound can act as a *pro-oxidant* rather than as an antioxidant. Methyl gallate, for example, has been reported to act as a pro-oxidant at pH 7.4, since in the presence of Fe(II) low levels of methyl gallate increase oxidative damage to deoxyribose.[2] Other compounds containing the pyrogallol group can lead to formation of superoxide anion, again with pro-oxidant acitvity.[18] It can also occur, however, that when the methyl gallate moiety is incorporated into larger phenolic structures such as tannins, then the molecule again reverts to antioxidant activity. In this respect Hagerman et al.[56] showed that polymeric polyphenols may be much more potent antioxidants than monomeric phenols, while showing no pro-oxidant behavior. This type of antioxidant activity clearly takes us beyond the reach of a prediction based on simple monomer characteristics such as BDE or IP, and it can become a question of sequestering metal ions or bioavailability. In the case of tannins, Hagerman et al.[56] discuss subtle issues such as the formation of complexes with protein which are resistant to digestion, as well as issues of retention in the digestive tract and transport to tissues.

Aminophenols. Compounds belonging to this class range from the simple *p*-aminophenol to more complex structures and are of great potential interest because of the large substituent effect associated with the amino group (ΔBDE = −9.4 kcal/mol, Table 5). One issue of interest in the aminophenols is which is the weaker bond, that in the OH group or that in the NH group. Our calculations have shown that it is usually the OH group which is most weakened, although Pedulli et al.,[8] Yamamura et al.,[57] and Nishiyama et al.[58] have pointed to the existence of very weak NH bonds in phenothiazine, for example, and this question must be kept in mind. The amino group also has a very substantial IP lowering effect on simple phenols, due to its electron-donating ability (HOMO is raised in the parent compound), and we have discussed this effect previously.[26] We have recently completed an extensive computational and experimental study of the amino phenols,[51] including a discussion of substituent effects for *N*-methylamino or *N*,*N*-

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dimethylamino groups, which also follow additivity rules. In the present paper we present some representative molecules for discussion.

Compounds **20**−**23** are *p*-aminophenol, *N*,*N*-dimethyl-*p*-aminophenol, 6-hydroxy-5,7,8-trimethyl-1,2,3,4-tetrahydroquinoline, and 9-hydroxyjulolidine. The electron-donating character increases when the amino group is methylated, and the ΔBDE also increases by ca. 1 kcal/mol. Note that the ΔIP increases by over 8 kcal/mol, however, to the point where *N*,*N*-dimethyl-*p*-aminophenol now has a lower IP than α-tocopherol (ΔIP of −37.5 vs −36.1 kcal/mol, respectively). Compounds **22** and **23** show very substantial ΔBDE values (−14.6 and −11.8 kcal/mol), even larger than for α-tocopherol, but their ΔIP values are now very large (−46.9 and −48.7 kcal/mol, respectively). It was found by Burton et al. that **22** was unstable in air even in crystalline form,[46] undergoing autoxidation. We also performed some experiments on 9-hydroxyjulolidine (**23**), which will be reported in a later publication,[51] but in that case we observed that when dissolved in benzene and exposed to oxygen the compound quickly turned red. By analogy to known aminophenol chemistry[12] we attribute this to formation of the radical cation of 9-hydroxyjulolidine, i.e., the system has apparently reacted directly with O_2 by SET, even in the (relatively) nonpolar solvent benzene. Since radical cation formation in aminophenols can lead to DNA adducts and genetic damage[13] this type of antioxidant pathway is most probably undesirable. This will happen when the IP drops too low, and (depending on the solvent) the SET mechanism becomes dominant. From these experiments on aminophenols and our calculations of ΔIP it appears likely that there is a mechanism change from HAT to SET that occurs around ΔIP = −40 kcal/mol.

The overall antioxidant activity must have a functional dependence on both BDE and IP, as stated in the Introduction. It would be very useful to define this dependence more carefully, and we are participating in experiments designed to do so. Of course it would be possible to create an antioxidant that incorporates amino groups into a phenol and then adds other electron-withdrawing groups at strategic locations to maintain a relatively low BDE and a relatively high IP. This type of molecular engineering of antioxidants can clearly be used to achieve desired characteristics of BDE and IP; the major problem in practice will be to decide what are the desired characteristics for a given application.

Stilbenes Related to Resveratrol. A major screening program to identify cancer-preventive compounds derived from plant extracts has been carried out recently by Pezzuto and coworkers.[59] These authors tested over 700 extracts for antioxidant potential, using a screening procedure. The extracts were first subjected to a battery of tests designed to assess their ability to scavenge free radicals, including reaction rate with the nitrogen radical diphenylpicrylhydrazyl (DPPH•), the ability to reduce TPA-induced free radical formation in human leukemia cells, and measurement of the inhibition response in a xanthine/xanthine oxidase assay. A small subset of compounds shown to be active was then tested for the ability to inhibit cancer formation in a mouse mammary culture model. Two of the compounds identified as most active were *trans*-resveratrol (**26**) and piceatannol (**27**). Resveratrol has received much publicity recently as a potent naturally occurring antioxidant which occurs in grapes and wine.[60] It belongs to the stilbene family, in which benzene rings are connected by an ethylene or larger conjugated linkage. Piceatannol also belongs to the stilbene family and, while closely related to resveratrol, also contains the catechol functionality. It was found to be even more potent than resveratrol in terms of its free radical scavenging activity and its cancer-preventive properties.[59]

The stilbene family was studied in our calculations, beginning with *p*-vinylphenol (Table 1) and continuing with *p*-butadienylphenol (**24**), *p*-hydroxystilbene (**25**), *trans*-resveratrol (**26**), and piceatannol (**27**). For the calculation of the BDE, since the conjugation is continuous across the substituent, it seems questionable whether the LDBS method could succeed in applying a tertiary basis to the substituent and a secondary basis to the benzene containing the (primary) hydroxy group. However, some experimentation with **25** convinced us that this was indeed the case, to within about 0.3 kcal/mol. There is a very substantial reduction in BDE in moving across the five members of this series, ranging over −4.7, −7.7, −8.5, −8.2, and −15.6 kcal/mol for ΔBDE on going from *p*-vinylphenol to **24**, **25**, **26**, and **27**, respectively. For piceatannol (**27**), for example, ΔBDE is larger than in α-tocopherol and it is clear that the extended conjugation is stabilizing the phenoxyl radical and lowering the BDE. Except for *p*-vinyl, these groups have not been considered in Tables 1 and 2, so we omit discussion of group additivity. Once again the trend in IP is roughly parallel to that in BDE, since by extending the conjugation from **24**−**27** and adding electron donors (*m*-hydroxy groups) the IP decreases, finally reaching a value for piceatannol (−35.2 kcal/mol) which is close to that in α-TOH (−36.1 kcal/mol in model compound **15**). Note, however, that the IP even of piceatannol is still higher than that in α-TOH, so that this family of stilbenes is expected to react by HAT.

Sterically Crowded Phenols. It was shown by Burton, Ingold, and co-workers[46] that stereoelectronic effects can be important in determining the effectiveness of a phenolic antioxidant. This point can be illustrated with respect to a methoxy substituent. When the O−C bond of the methoxy group lies coplanar with the benzene ring, overlap of the lone pair of π-symmetry is optimized and the maximum electron-donating effect occurs. As the methoxy group is twisted out of plane, its ED effect is reduced. Compare compounds **5a** and **5b** (below) whose rates of reaction with peroxyl free radicals were 13×10^5 and 3.9×10^5 M^{-1} s^{-1}, respectively.[46] Simple additivity rules suggest that the presence of an extra methyl group in **5b** should further reduce the BDE and enhance the rate, contrary to the observation. However, steric crowding in **5b** forces the dihedral angle between the methoxy group and the ring to ca. 90°, whereas the absence of crowding in **5a** leads to a dihedral angle of only 8°. The three-dimensional structure from the AM1 optimization confirms these results; these 3D structures are shown in Figure 3.

We calculated the rotational potential curve using the B3LYP/LDBS approach in *p*-methoxyphenol, so that additivity effects could be modified to allow for the stereoelectronic effects. The results are shown in Figure 4. It can be seen that ΔBDE is reduced from −6.13 to −2.58 kcal/mol on rotating from 0 to 90°, i.e., the rotational barrier height for the methoxy group is 3.55 kcal/mol. This agrees very well with the barrier measured experimentally by Burton, Ingold, and co-workers.[46] These authors also stated that when methoxy is rotated by 90° its substituent effect is comparable to that of a methyl group, again in close agreement with our own calculations.

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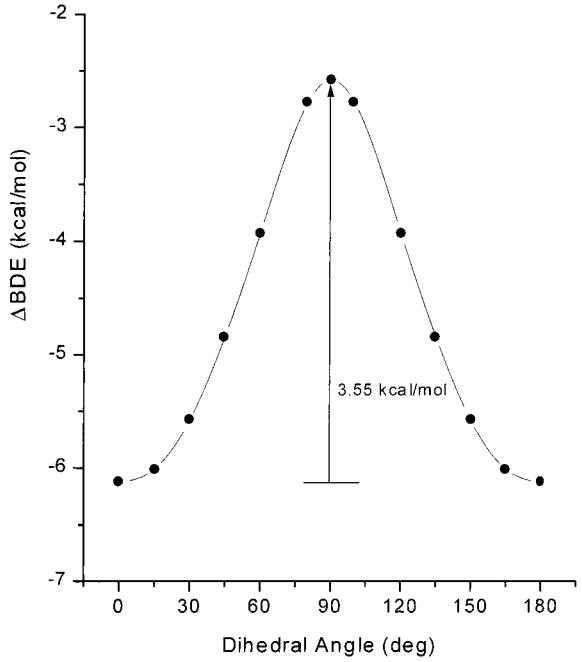

Figure 4. Rotational barrier for the methoxy group in *p*-methoxyphenol.

Let us now attempt to apply additivity corrections to **5b**, considering the result in Figure 4. Additivity values for substituents are −4.0 (two *o*-methyl), −0.8 (two *m*-methyl), and −2.6 (*p*-methoxy group rotated by 90°), for a total substituent effect of −7.4 kcal/mol, vs the calculated LDBS result (Table 3) of −8.0 kcal/mol. The agreement has become reasonable when the properties of the rotated methoxy group are taken into account. We have applied the same procedure to ubiquinol, another antioxidant that exhibits steric crowding due to the two methoxy groups being surrounded by adjacent methyl groups. Once again, when the deviation from planarity is considered the overall ΔBDE can be understood.

Conclusions

In this paper we have given a detailed account of how we calculate the gas-phase BDE and IP for phenolic antioxidants. These antioxidants act either by hydrogen atom transfer, for which the calculation of BDE is relevant, or by single-electron transfer, for which the calculation of IP is relevant. We showed that the LDBS method agrees well with the full-basis method, and by drastically reducing the basis set it is much more economical in practice. The LDBS results agreed well with accurate experimental data, where known, except for the case of di-*o*-*tert*-butyl substituents. A comprehensive set of optimized ΔBDE values was derived from these calculations and could be used to predict the effect on the BDE of many important electron-donating and electron-withdrawing substituents. These were shown to work well in practice when hydrogen bonding is taken into account. The BDE derived from additivity rules can be extended to include the case of steric crowding by considering the rotational potential of the affected group. In this way we can predict the BDE of a phenolic antioxidant with essentially any structure, to within ca. 1 kcal/mol.

The methods were applied to the calculation of the BDE and IP for several classes of phenolic antioxidants. These included commercial antioxidants used as food additives, compounds related to Vitamin E, flavonoids found in tea, aminophenols, and compounds containing a stilbene linkage related to resveratrol. This set was chosen somewhat arbitrarily from among hundreds of other examples of phenolic antioxidants, but they represent important chemical families and illustrate the approach. We discussed the relevance of the computed BDE values and IP values with respect to the mode of action of the antioxidant (H-atom vs electron transfer). Although all calculations were done in the gas phase it is nevertheless likely that these results are also relevant to reaction in solution, since solution-phase enthalpies of bond dissociation or electron transfer appear to follow the same trends which are apparent in the gas phase. In particular most of the antioxidants we studied are expected to react by H-atom transfer, except for the substituted aminophenols. To put these conclusions on a truly firm basis, however, would require introduction of a solvent model into the calculations, as well as consideration of the transition state(s) for the competing pathways.

Despite the above qualification, we now believe that the gas-phase BDE and IP are excellent primary indicators of antioxidant activity. It will be interesting and important in the future to test solvent models, particularly for the electron-transfer and acid−base reactions possible for antioxidants, but the gas-phase results correlate well with a variety of experimental results. The methods we have described in this paper are not limited in any way to studying phenols, since we have already shown that the DFT approach is equally accurate for X−H bonds, where X = C, N, O, S.[22] This means that, for example, we can treat antioxidants containing N−H bonds with equal ease, and such calculations are in progress.

The simple predictors described in this paper cannot be the whole story, and we have indicated some biological examples where other factors such as bioavailability must play a role. However, in attempting to design an optimum synthetic antioxidant, e.g. for a given biological role, it seems clear that one must first consider the BDE and the IP, and then attempt to "tune" the molecule to modify other factors such as solubility. Using the procedures outlined in this paper we are currently attempting to design synthetic lipid-soluble antioxidants more effective than Vitamin E. Results of this investigation will be reported in a future publication.

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