Bond Dissociation Enthalpies of Polyphenols: The Importance of Cooperative Effects

Marco Lucarini,* Veronica Mugnaini, and Gian Franco Pedulli*

Dipartimento di Chimica Organica "A. Mangini", Università di Bologna, Via S. Donato 15, I-40127 Bologna, Italy

pedulli@alma.unibo.it

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The hydrogen−oxygen bond dissociation energies of 3,5-di-*tert*-butylcatechol, 2,5-di-*tert*-pentylhydroquinone, propyl gallate, and octyl gallate, which represent model compounds of three important classes of naturally occurring antioxidants, have been measured by an EPR equilibration technique, and the factors determining their values have been clarified. The excellent antioxidant activity of the these polyphenols is largely due to the stabilization of the aroxyl radical due to the formation of an intramolecular hydrogen bond.

Introduction

Most of the synthetic and naturally occurring antioxidants are phenolic compounds exerting their action via an initial transfer of a hydroxylic hydrogen to the chain carrying peroxyl radicals of the oxidizable substrate. This reaction takes place with rate constants depending on several factors, one of the more important being the strength of the phenolic O−H bond to be broken.[1−3] In the recent literature, there are several reports of the determination of the O−H bond dissociation enthalpies (BDE) of phenols based on a photoacoustic technique,[4] on the use of thermodynamic cycles by combining the heat of heterolysis of a given species and the redox potentials of the resulting ions,[5] and on the measure by EPR of the equilibrium constant between couples of phenols and of the corresponding phenoxyl radicals.[6] The relationships between phenolic structure and strength of the O−H bond have also been mostly clarified.[3]

On the other hand, very few quantitative studies have been reported for phenolic antioxidants containing two or more hydroxyl groups, such as hydroquinones, catechols, and pyrogallols, even though these units are contained in ubiquinols and in most flavonoid derivatives, a class of antioxidants almost ubiquitous in natural products. Actually, to our knowledge, no determination of the BDE values of catechols and pyrogallols has been reported, while the O−H bond strength has been recently measured for ubiquinol-0 by using the photoacustic calorimetric method.[7]

We wish to report here the determination, by means of the EPR equilibration technique, of the O−H bond dissociation energies in the commercially available 3,5-di-*tert*-butylcatechol (**1**), 2,5-di-*tert*-pentylhydroquinone (**2**), and the propyl (**3**) and octyl (**4**) esters of gallic acid.

Table 1. EPR Spectral Parameters of the Aroxyl Radicals 1a−4a

radical	solvent	hyperfine splittings (G)	*g*-value
1a	benzene	0.39 (9H), 1.45 (H_{OH}), 0.31 and 1.74 (H_4 and H_6)	2.0044
2a	benzene	5.18 (H_o), 0.93 (H_m), 1.38 (H_{OH})	2.0046
3a	*tert*-butanol	1.05 ($2H_m$), 1.74 (2H), 0.49 (2H)	2.0049
4a	*tert*-butanol	1.05 ($2H_m$), 1.74 (2H), 0.49 (2H)	2.0049

Results and Discussion

The phenoxyl radicals from **1** and **2** were generated by photolyzing, inside the EPR cavity, nitrogen saturated benzene solutions of each diol (0.1 M) containing di-*tert*-butylperoxide (10% v/v). The EPR spectral parameters of the observed radicals, reported in Table 1, were consistent with structures **1a** and **2a**. Actually, the spectrum of the former radical showed coupling of the unpaired electron with the two ring protons (1.74 and 0.31 G), with the remaining hydroxyl proton (1.45 G),[8] and with the nine equivalent protons of a *tert*-butyl group (presumably that one in the *para* position).

The formation of the other possible species, with the radical centered on the oxygen in position 1, can be discarded since larger splittings (ca., 10 and 5 G from the protons in 4 and 6, respectively) would be expected in this case. Of course, we cannot exclude the possibility that this species is initially formed, since it will immediately rearrange to the observed thermodynamically more stable radical. The EPR spectrum of radical **2a** (g = 2.0046) showed two doublet splittings from the ring protons *ortho* (5.18 G) and *meta* (0.93 G) to the radical center and a doublet splitting (1.38 G)[9] from the hydroxyl proton.

(1) Denisov, E. T.; Khudyakov, I. V. *Chem. Rev.* **1987**, *87*, 1313.
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(3) Borges dos Santos, R. M.; Martinho Simões, J. A. *J. Phys. Chem. Ref. Data* **1998**, *27*, 707.
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(5) (a) Bordwell, F. G.; Cheng, J.-P. *J. Am. Chem. Soc.* **1991**, *113*, 1736. (b) Bordwell F. G.; Zhang, X.-M. *J. Org. Chem* **1990**, *55*, 6078. (c) Bordwell, F. G.; Zhang, X.-M.; Satish, A. V. *J. Am. Chem. Soc.* **1994**, *116*, 6605. (d) Bordwell F. G.; Zhang, X.-M. *J. Phys. Org. Chem.* **1995**, *8*, 529.
(6) (a) Lucarini, M.; Pedulli, G. F.; Cipollone, M. *J. Org. Chem.* **1994**, *59*, 5063. (b) Lucarini, M.; Pedrielli, P.; Pedulli, G. F.; Cabiddu, S.; Fattuoni, C. *J. Org. Chem.* **1996**, *61*, 9259.
(7) (a) De Heer, M. I.; Korth H. G.; Mulder, P. *J. Org. Chem.* **1999**, *64*, 6969. (b) De Heer, M. I.; Mulder, P.; Korth, H. G.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **2000**, *122*, 2355.
(8) Addition of CH_3OD to the solution led to the replacement of the doublet by a 1:1:1 triplet separated by 0.23 G.
(9) Replaced by a 0.21 G triplet, after addition of CH_3OD.

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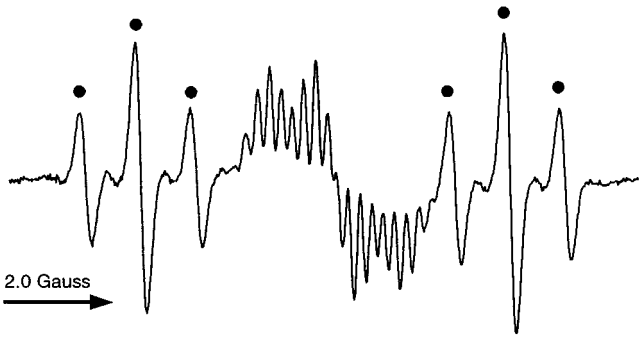


Figure 1. Room-temperature EPR spectrum observed under continuous irradiation of a benzene solution containing di-*tert*-butyl peroxide (10% v/v), BHT (0.28 M), and **1** (0.097 M). The six lines marked with a full circle are the central ones of the 12 lines spectrum of the aroxyl radical from BHT.

Table 2. Equilibrium Constants and Free Energy of Reaction 1

ArOH	solvent	K_1^{298}	ΔG°_{298} (kcal/mol)	BDE (ArO−H) (kcal/mol)
1	benzene	16.7 ± 4.0	−1.66 ± 0.13	79.3 ± 0.3
1	*tert*-butanol	1.75 ± 0.46	−0.34 ± 0.14	80.7 ± 0.3
2	benzene	1.51 ± 0.29	−0.24 ± 0.10	80.8 ± 0.2
3	*tert*-butanol	0.066 ± 0.014	+1.61 ± 0.15	82.6 ± 0.3
4	*tert*-butanol	0.081 ± 0.015	+1.49 ± 0.16	82.5 ± 0.3

The determination of the O−H bond dissociation enthalpies was done by measuring, by means of EPR spectroscopy, the equilibrium constant, K_1, for the hydrogen atom transfer reaction between two phenols and the corresponding phenoxyl radicals (eq 1) generated under continuous photolysis.

$$\mathrm{ArOH} + \mathrm{Ar'O\bullet} \rightleftharpoons \mathrm{ArO\bullet} + \mathrm{Ar'OH} \qquad (1)$$

The BDEs for the species ArOH were calculated, in the assumption that the entropic term can be neglected,[6] by means of eq 2 from K_1 and the known BDE value of a reference species Ar′OH, which in the present case was 2,6-di-*tert*-butyl-4-methylphenol (BHT) whose O−H BDE value in benzene is 81.0 kcal/mol.[6]

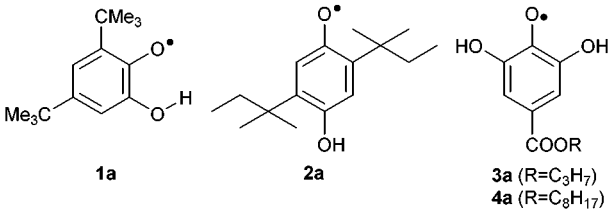
$$\mathrm{BDE(ArO\text{-}H)} = \mathrm{BDE(Ar'O\text{-}H)} - RT\ln(K_1) \qquad (2)$$

Figure 1 shows as an example the central part of the EPR spectrum obtained under irradiation of a benzene solution of 3,5-di-*tert*-butylcatechol (**1**) 0.097 M, BHT 0.28 M, and 10% (v/v) $(Me_3CO)_2$. Numerical treatment of the experimental data (reported in Table 2) provided the difference between the BDEs of BHT and of **1** as 1.7 kcal/mol, i.e., the O−H bond dissociation energy of the catechol **1** as 79.3 kcal/mol.

This value was checked by repeating the measurements under different experimental conditions, i.e., by using different concentrations of the reactants, and light intensity. Similarly, we could derive the BDE of the O−H bond of the hydroquinone **2** as 80.8 kcal/mol. It is remarkable that the O−H bond strength of catechol **1** in benzene is only slightly larger than that of α-tocopherol (78.2 kcal/mol),[6] this explaining the good antioxidant properties of natural compounds containing the catechol ring.

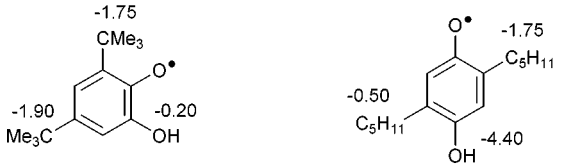
To check if in the two examined diols the magnitude of the BDE is only the result of electronic and steric effects of the various groups or is also due to additional interactions, we calculated the BDE of the O−H bond in **1** and **2** by using additive contributions for the various ring substituents. Actually, in ring substituted phenols it has been found that the change, with respect to PhOH, of the O−H bond strength due to a given group is roughly constant and that in polysubstituted phenols the contribution of the various substituents is additive.[4−6,10] Therefore, a large discrepancy between experimental and calculated BDE values for **1** and **2** will be indicative of some additional and specific interaction between the various substituents.

Scheme 1



1a **2a** **3a** (R=C_3H_7) **4a** (R=C_8H_{17})

Scheme 2



$BDE_{computed}$ = 87.6 - 3.9 = 83.7
$BDE_{experimental}$ = 79.4

$BDE_{computed}$ = 87.6 - 6.7 = 80.9
$BDE_{experimental}$ = 80.8

The additive contributions for a single *tert*-butyl group in the *para*, *meta*, and *ortho* position to the OH are −1.90, −0.50, and −1.75 kcal/mol,[6,11−12] respectively. The contribution due to a hydroxyl group was approximated as equivalent to that of a methoxy substituent, i.e., −4.4[6] and −0.2[7] kcal/mol for *para* and *ortho* substitution, respectively. By subtracting the appropriate substituent contributions (see Scheme 2) from the BDE of phenol (87.6 kcal/mol), the expected BDE values of the catechol **1** and of the hydroquinone **2** were calculated as 83.7 and 80.9 kcal/mol, respectively.[13]

A comparison of these BDE values with the experimental ones shows that the additive model predicts correctly the O−H bond strength for the hydroquinone while it overestimates by 4.4 kcal/mol the one for the substituted catechol. Since the O−H BDE is a measure of the difference between the enthalpies of the aryloxyl radical and of the parent phenol, this means that in benzene solution radical **1a** experiences an extra stabilization of 4.4 kcal/mol. Conceivably, this additional contribution arises from intramolecular hydrogen bonding involving the second hydroxyl group. Actually, we may represent the equilibration reaction as in eq 3 where

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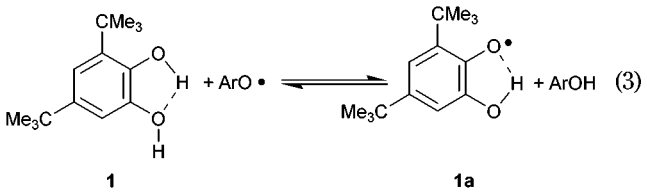
(12) The last value was assumed to be the same as that of a *ortho* methyl substituent[6] since the steric effect due to the repulsion of Me_3C with the OH group can be neglected when only one *tert*-butyl substituent is present.

(13) By repeating the calculations in the assumption that in **1** the hydrogen atom is abstracted from the hydroxyl group in position 1, a BDE value of 86.4 kcal/mol is obtained.



(3)

1 **1a**

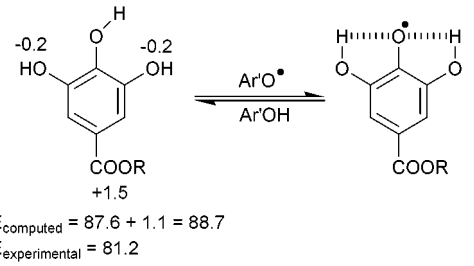
in the di-*tert*-butylcatechol the hydroxyl proton in position 1 is free, while that one in position 2 is intramolecularly hydrogen bonded.[14]

Since the contribution due the presence of one intramolecular hydrogen bond is included in the BDE calculation, we do not expect any extra term to be important in describing the enthalpy of the starting catechol. On the other hand, no contribution due to the possible formation of an intramolecular hydrogen bond in the semiquinone radical has been considered so far, for the obvious reason that these additive parameters have been obtained from the data of monophenols. It is therefore quite reasonable to attribute the large difference between the calculated and experimental BDE value of the 3,5-di-*tert*-butylcatechol to the presence, in the corresponding semiquinone radical, of an intramolecular hydrogen bond between the remaining hydroxyl proton and the oxygen radical center. The resulting radical stabilization energy, equal to 4.4 kcal/mol, is close to that one of intramolecular hydrogen bonds in *ortho* methoxy phenols.[7] Actually, intramolecular hydrogen bonding in the semiquinone radical from catechols, has been previously suggested to be responsible for the good antioxidant activity of catechols derivatives, although no quantification of this effect has been given.[10,15]

Cathecols have also been found to experience strong kinetic solvent effects on the hydrogen atom abstraction reaction by nitrogen-[16] and oxygen-[16−18] centered radicals. To check if also the O−H BDE values change with solvent, we measured in *tert*-butyl alcohol the equilibrium constant of reaction 1, with ArOH = **1** and Ar′OH = BHT. The latter was chosen since the steric protection of the hydroxyl group by the *ortho tert*-butyl substituents, prevents its solvation by hydrogen bond acceptor (HBA) solvents,[19] so that the O−H bond energy in BHT can be safely assumed to be the same as that one measured in hydrocarbons.[21]

The EPR equilibration experiments at room temperature provided the O−H BDE value of **1** in Me_3COH as

Scheme 3



$BDE_{computed}$ = 87.6 + 1.1 = 88.7
$BDE_{experimental}$ = 81.2

80.7 kcal/mol, i.e., 1.4 kcal/mol larger than in benzene. Thus, the substituted catechol **1** behaves similarly to other phenolic antioxidants[21] where hydrogen bonding by HBA solvents induces an increase of the BDE, through preferential stabilization of the starting phenol rather than of the phenoxyl radical. It should be emphasized that the solvated hydroxyl group of catechol **1** is not that one involved in the intramolecular hydrogen bonding, which is characterized by a lower BDE value,[13] but the free hydroxyl substituent in position 1. This was proved by determining the solvent effect on the O−H BDE of 2,4,6-trimethoxyphenol (**5**) not containing free hydroxyl groups. On passing from benzene to *tert*-butyl alcohol, the $\Delta G°$ for reaction 1 where ArOH = **5** and Ar′OH = 2,6-di-*tert*-butyl-4-methoxyphenol (BHA) shows a very small change (0.17 kcal/mol), this indicating that the intramolecularly hydrogen-bonded hydroxyl group of **5** is only weakly solvated by *tert*-butyl alcohol. This is in agreement with the small kinetic solvent effect on the hydrogen atom abstraction reaction from 2-methoxy phenols found by de Heer et al.[7b]

Another interesting example where hydrogen bonding is important in determining the antioxidant activity of polyphenols is that of pyrogallol derivatives. Because of the low solubility of pyrogallol itself or of gallic acid, we studied the propyl gallate (**3**) and the octyl gallate (**4**). However, even these two esters are sparingly soluble in benzene, so that measurements were carried out in *tert*-butyl alcohol due to the relatively large phenol concentrations requested for the EPR equilibration studies.

The EPR spectra (see Supporting Information) obtained under photolysis were characteristic of symmetric radicals where the unpaired electron is coupled with two equivalent ring protons and two equivalent hydroxyl protons (see Table 1). This means that the radical is centered on the oxygen atom in position 2. Equilibration with BHT as the reference phenol provided also the BDE in *tert*-butyl alcohol as 82.6 and 82.5 kcal/mol, respectively for **3** and **4**. By assuming that the solvent effect on the BDE is the same as in catechol **1** (1.4 kcal/mol), the bond strength in benzene can be estimated as ca. 81.2 kcal/mol.

To quantify the contributions of the various terms, the BDE value of the gallic acid ester was calculated by using the additivity rule. The contribution for each of the two hydrogen-bonded *ortho*-hydroxyl groups is −0.2 kcal/mol[6,11,12] and that one for the esteric group in *para* position[22] is +1.5 kcal/mol which gives 88.7 kcal/mol for the computed BDE (see Scheme 3). The difference between this value and the 81.2 kcal/mol obtained in benzene (7.5 kcal/mol) can be attributed to the contribu-

(14) The structure adopted by cathecol **1** in solution is demonstrated by the FT-IR spectrum recorded in CCl_4 at the same concentration (0.1 M) used in the EPR equilibration studies, which shows two sharp peaks of similar intensity centred at 3617 and 3555 cm^{-1}, characteristic of free and intramolecularly hydrogen bonded hydroxyl groups, respectively (see Supporting Information).

(15) (a) van Acker, S. A. B. E.; de Groot, M. J.; van der Berg, D. J.; Tromp, M. N. J. L.; den Kelder, G. D. O.; van der Vijgh, W. J. F.; Bast, A. *Chem. Res. Toxicol.* **1996**, *9*, 1305. (b) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. *Free Radical Biol. Med.* **1996**, *20*, 933. (c) Xi F.; Barclay, L. R. C. *Can. J. Chem.* **1998**, *76*, 171.

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(18) Pedrielli, P.; Pedulli, G. F.; Skibsted, L. H. *J. Agric. Food Chem.* **2001**, *49*, 3034.

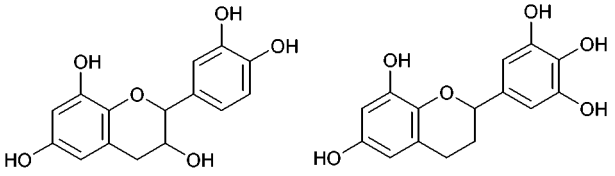
(19) The FT-IR spectrum of BHT shows in the OH region a sharp peak centred at 3643 cm^{-1}, characteristic of a free hydroxyl group, both in isooctane and in a strong HBA solvent such as γ-valerolactone. In addition, the reaction of the similar 2,6-di-*tert*-butylphenol with alkyl radicals shows a negligible kinetic solvent effect in γ-valerolactone and *tert*-butyl alcohol.[20]

(20) Franchi, P.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L.; Lunelli, B. *J. Am. Chem. Soc.* **1999**, *121*, 507.

(21) Pedrielli, P.; Pedulli, G. F. *Gazzetta* **1997**, *127*, 509.

(22) Brigati, G.; Lucarini M.; Mugnaini, V.; Pedulli, G. F. Results to be published.

Scheme 4



tion of the two hydrogen bonds formed by the radical oxygen and the adjacent hydroxyl groups (see Scheme 3).

This means that the strength of each H-bond in the aroxyl radical from **3** and **4** is 3.75 kcal/mol, a value slightly lower than that one (4.4 kcal/mol) found for the similar hydrogen bond in radical **1a**. This attenuation of the H-bond strength is likely due to the sharing of two hydrogen bonds by the central oxygen atom. It can be mentioned that in a recent DFT study[10] on similar polyphenols the stabilization of the corresponding radical was considerably overestimated, with respect to the present experimental results, since the contribution due to the formation of the intramolecular hydrogen bonds in the radical from catechols and pyrogallols was estimated to be 8 and 12 kcal/mol, respectively, which should be compared with the present values of 4.4 and 7.5 kcal/mol.

Conclusion

In conclusion, the present study provides a rationalization of the very good antioxidant properties of natural products containing catechol or pyrogallol moieties in terms of intramolecular hydrogen bonding in the aroxyl radical obtained in the reaction with the chain propagating peroxyl radicals and provide a quantification of this effect.

By using the additivity rules the BDE values of the more common polyphenolic antioxidants contained in natural products can be easily calculated. By choosing epicatechin and epigallocatechin (Scheme 4) as prototype derivatives of flavonoids contained in red wine and in black and green tea extracts, we can calculate in benzene solution an O−H BDE of 81.2 kcal/mol for epicatechin[23] and of 77.9 kcal/mol for epigallocatechin.[24]

Both values are very low (in the second case, even lower than that of α-tocopherol, 78.2 kcal/mol) and therefore characteristic of very good antioxidants. It is also remarkable that the antioxidant activity of these polyphenols is largely due to the stabilization of the aryloxyl radical due to the formation of intramolecular hydrogen bonding, and that this effect is obviously absent in other phenols such as vitamin E.

(23) The BDE value was calculated by adding the contribution of an *orto*-OH group (−4.6 kcal/mol) and a *para*-alkyl group (−1.8 kcal/mol).

(24) The BDE value was calculated by adding the contribution of two *ortho*-OH group (−7.9 kcal/mol) and a *para*-alkyl group (−1.8 kcal/mol).

Experimental Section

Materials. All materials were commercially available from Aldrich except compound **2** which was kindly supplied by Great Lakes Chemicals Italia srl (Milano, Italy).

Determination of the BDE Values. Deoxygenated benzene or *tert*-butyl alcohol solutions containing a polyphenol (0.1−0.5 M), a reference phenol (BHT or BHA), and di-*tert*-butyl peroxide (10% v/v) were sealed under nitrogen in a suprasil quartz EPR tube. The sample was inserted in the thermostated cavity of an EPR spectrometer and photolyzed with the unfiltered light from a 500 W high-pressure mercury lamp. The temperature was controlled with a standard variable temperature accessory and was monitored before and after each run with a copper−constantan thermocouple.

The EPR spectra were recorded on a Bruker ESP 300 spectrometer equipped with a Hewlett-Packard 5350B microwave frequency counter for the determination of the *g*-factors, which were corrected with respect to that of perylene radical cation in concentrated H_2SO_4 (g = 2.002 58).

The molar ratio of the two equilibrating radicals was obtained from the EPR spectra and used to determine the equilibrium constant, K_1, by introducing in the eq 1 the initial concentrations of the two reactants. Initial concentrations were high enough to avoid significative consumption during the course of the experiment.

Relative radical concentrations were determined by comparison of the digitized experimental spectra with computer simulated ones. In these cases an iterative least-squares fitting procedure based on the systematic application of the Monte Carlo method was performed in order to obtain the experimental spectral parameters of the two species including their relative intensities.

FT-IR Measurements. The FT-IR spectra were measured from 4000 to 3000 cm^{-1} using a Nicolet Protégé 460 spectrometer having a resolution 2 cm^{-1}. Tetrachloromethane solutions of catechol **1** (0.1 M) were examined in a sealed KBr cell with 0.1 mm optical path.

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Supporting Information Available: IR spectrum of catechol **1** and EPR spectra of the aryloxyl radicals **1a**−**3a**. This material is available free of charge via Internet at http://pubs.acs.org.

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