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Pyrroles As Antioxidants: Solvent Effects and the Nature of the Attacking Radical on Antioxidant Activities and Mechanisms of Pyrroles, Dipyrrinones, and Bile Pigments

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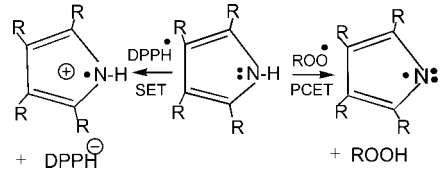

The absolute rate constants, k_{inh}, and stoichiometric factors, n, of pyrroles, 2-methyl-3-ethylcarboxy-4,5-di-*p*-methoxyphenylpyrrole, **6**, 2,3,4,5-tetraphenylpyrrole,**7**, and 2,3,4,5-tetra-*p*-methoxyphenylpyrrole, **8**, compared to the phenolic antioxidant, di-*tert*-butylhydroxyanisole, **DBHA**, during inhibited oxidation of cumene initiated by AIBN at 30 °C gave the relative antioxidant activities (k_{inh}) **DBHA** > **8** > **7** > **6** and $n = 2$, whereas in styrene, **8** > **DBHA**. These results are explained by hydrogen atom transfer, HAT, from the N−H of pyrroles to **ROO•** radicals. The k_{inh} values in styrene of dimethyl esters of the bile pigments of bilirubin ester (**BRDE**), of biliverdin ester (**BVDE**), and of a model compound (dipyrrinone, **1**) gave k_{inh} in the order pentamethylhydroxychroman (**PMHC**) ≫ **BRDE** > **1** > **BVDE**. These antioxidant activities for **BVDE** and the model compound, **1**, and **PMHC** dropped dramatically in the presence of methanol due to hydrogen bonding at the pyrrolic N−H group. In contrast the k_{inh} of **BRDE** *increased* in methanol. We now show that pyrrolic compounds may react by HAT, proton-coupled electron transfer, PCET, or single electron transfer, SET, depending on their structure, the nature of the solvent, and the attacking radical. Compounds **BVDE** and **1** react by the HAT or PCET pathway (HAT/PCET) in styrene/chlorobenzene with **ROO•** and with the **DPPH•** radical in chlorobenzene according to N−H/N−D k_H/k_D of 1.6, whereas the DKIE with **BRDE** was only 1.2 with **ROO•**. The antioxidant properties of polypyrroles of the **BVDE** class and model compounds (e.g., **1**) are controlled by intramolecular H bonding which stabilizes an intermediate pyrrolic radical in HAT/PCET. According to kinetic polar solvent effects on the monopyrrole, **8**, and **BRDE**, which gave increased rates in methanol, some pyrrolic structures are also susceptible to SET reactions. This conclusion is supported by some calculated ionization potentials. The antioxidant mechanism for **BRDE** with peroxyl radicals is described by the PCET reaction. Experiments using the 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl radical (**DBMP•**) showed this to be a better radical to monitor HAT activities in stopped-flow kinetics compared to the use of the more popular **DPPH•** radical.

Introduction

Aerobic organisms are continuously exposed to reactive oxygen species (ROS), and when an imbalance occurs between ROS and protective defense mechanisms, *oxidative stress* occurs.[1a] The potential damage is commonly initiated by oxygen-centered radicals resulting in free radical chain *autoxidation*, which is implicated in a number of degenerative diseases in humans.[1b] As a result, there is a continuing interest by scientists and the public on the control of oxidative stress by antioxidants. Phenolic antioxidants, such as vitamin E in our diet, deactivate oxygen radicals by reductive processes, and also

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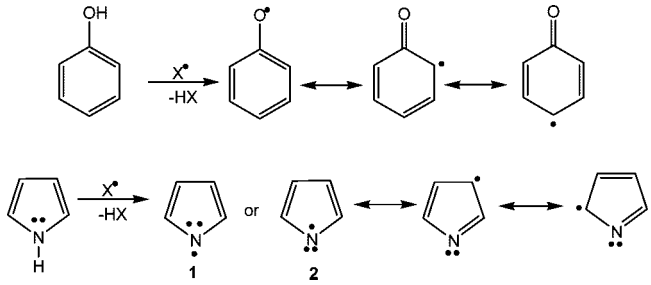
SCHEME 1



there is a continuing interest in synthetic antioxidants of high antioxidant activity.[2] In contrast, pyrrole-containing molecules provide a possible *in vivo* protection against free radical damage, and the possible beneficial effects of the bile pigment bilirubin in this regard was pointed out in 1990.[3a] Such natural pyrroles are of particular interest because they are formed continuously during the catabolism of hemoglobin, and more recently, bilirubin was discovered to contribute to the total antioxidant capacity of human blood plasma.[3b] We now present results on the relative antioxidant activities and antioxidant mechanisms of some synthetic pyrrole-containing molecules and the natural pigments compared to some conventional phenolic antioxidants.

Phenolic antioxidants deactivate oxygen radicals by a process that generally involves transfer of hydrogen atoms, resulting in a stabilized phenolic radical which does not continue the oxidative chain process (Scheme 1). Pyrroles also contain an active hydrogen atom (N−H), and by analogy, one might expect that they would also act as hydrogen atom transfer (HAT) agents and possess antioxidant activity, especially in compounds having electron-supplying groups positioned to stabilize intermediate radicals as is known for active phenolic antioxidants (Scheme 1).

The obvious structural differences between phenols and pyrroles, where the former have the active phenolic O−H donor group attached to the aromatic ring in contrast to pyrroles where the N−H group is imbedded as part of the aromatic system, are expected to result in very significant differences when considering their antioxidant properties. In addition, one of the very surprising observations is the resulting electronic structure of the pyrrole radical arising from hydrogen atom transfer.[4] The radical has been observed to possess the electronic structure **2** in its lower energy state rather than the "expected" **1**, as indicated by both experimental and theoretical data.[5] This is one of the factors that determines the antioxidant mechanism and activity of pyrroles.

The structural factors controlling the mechanisms and antioxidant activities of phenols are very well understood.[6] In contrast, the antioxidant mechanisms and activities of pyrroles and pyrrolic derivatives are still open to question, although the naturally occurring tetrapyrrolic bilirubin was discovered to possess antioxidant activity 50 years ago.[7] More recently, biliverdin and bilirubin have been studied quite extensively for their antioxidant properties, and several different antioxidant mechanisms have been advanced to account for these properties.[8]

Previously, we have discussed the antioxidant capabilities of polypyrroles, bilirubin, biliverdin, and some model compounds in organic solvents,[8f] micelles, and lipid bilayers.[8e] Herein we report significant advancements in understanding the antioxidant properties/mechanisms of the bile pigments bilirubin and biliverdin. Indeed, using pyrroles, dipyrrins, the methyl esters of bilirubin and biliverdin, and a dipyrrinone (see Figure 1 for the structures of all molecules used), we have (i) established the importance of internal hydrogen bonding for antioxidant ability; (ii) used *N*−H DKIE measurements to investigate the role of hydrogen atom transfer (HAT) and proton-coupled electron transfer mechanism (PCET) transfer; (iii) calculated ionization potentials (IPs) as an indication of the tendency for single electron transfer (SET) reactions; (iv) demonstrated the unreliability of using **DPPH•** in stopped-flow kinetics typically used to determine hydrogen atom donating ability of antioxidants; and (v) demonstrated, for the first time, the superiority of using **DPMB•** to this effect. Specifically, the experimental investigations reported herein include (A) determination of the absolute rate constants, k_{inh}, with peroxyl radicals and the number of peroxyl radicals trapped, *n*, per molecule of the pyrrolic compounds using inhibition of oxygen uptake kinetics; (B) kinetic deuterium isotope effects of *N*−D versus *N*−H for reactions of the bile pigments and a model dipyrrinone with peroxyl radicals and with the nitrogen-centered radical, diphenylpicrylhydrazyl (**DPPH•**) to elucidate reaction mechanisms; (C) determination of the effects of variation of the solvent polarity on these antioxidant properties; and (D) determination of the rate constants of the antioxidants with **DPPH•** in a nonpolar and polar solvent using stopped-flow kinetics, as well as an evaluation of the use of the synthetic 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl, **DBMP•**, radical compared to **DPPH•** in stopped-flow kinetics. Calculated IPs are reported as an aid in evaluating the role of HAT or SET reactions as contributing mechanisms of antioxidant activity.

Results

I. Antioxidant Activities, k_{inh}, and Stoichiometric Factors, *n*, of Pyrroles during Inhibited Peroxidation of Cumene and Styrene. Autoxidation and its inhibition by antioxidants are reviewed in various articles.[6b–d] The process commonly involves peroxyl radicals in initiation, propagation, and termination steps. For experiments conducted in vitro, this process is usually initiated by azo-initiators such as AIBN. The mechanism of uninhibited autoxidation of typical substrates, cumene (a) and styrene (b), is outlined as follows:

(1) (a) *Oxidative Stress: Oxidants and Antioxidants*; Sies, H., Ed.; Academic Press: London, 1991. (b) Halliwell, B.; Gutteridge, J. M. C.; *Free Radicals in Biology and Medicine*, 3rd ed.; Oxford University Press: New York, 1999.

(2) (a) Foti, M.; Johnson, E. R.; Vinquist, M. R.; Wright, J. S.; Barclay, L. R. C; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190−5196; Foti, M. C. Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **2002**, *124*, 12881−12888. (b) Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc.* **2001**, *123*, 4625–4626. (c) Barclay, L. R. C.; Vinqvist, M. R.; Mukai, K.; Itoh, K.; Morimoto, H. *J. Org. Chem.* **1993**, *58*, 7416–7420.

(3) (a) McDonagh, A. F. *Clinics Perinatol.* **1990**, *17*, 359–369. (b) MacLean, P. D.; Drake, E. C.; Barclay, L. R. C. *Free Radical Biol. Med.* **2007**, *43*, 600–609.

(4) It would be reasonable to expect that the loss of a hydrogen atom from pyrrole would result in a nitrogen radical with the unpaired electron in a σ orbital (structure 1, Scheme 1) leaving six π electrons in the aromatic pyrrole ring.

(5) (a) Bacskay, G. B.; Martoprawiro, M.; MacKie, J. C *Chem. Phys. Lett.* **1998**, *290*, 391–398. (b) Gianola, A. J.; Ichino, T.; Hoenigman, R. L.; Kato, S.; Bierbaum, V. M.; Lineberger, W. C. *J. Phys. Chem. A* **2004**, *108*, 10326–10335.

(6) (a) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053–7065. (b) Barclay, L. R. C.; Vinqvist, M. R. *The Chemistry of Phenols*; John Wiley and Sons, Ltd.: Chichester, England, 2003. (c) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201. (d) Ingold, K. U. *Acc. Chem. Res.* **1969**, *2*, 1–9. (e) Ingold, K. U. *Chem. Rev.* **1961**, *61*, 563–589.

(7) Bernhard, K.; Ritzel, G.; Steiner, K. U. *Helv. Chim. Acta* **1954**, *3*, 306–313.

Initiation:

$$[(CH_3)_2C(CN)N{=}]_2 \xrightarrow{\Delta} \xrightarrow{O_2} R\text{-}OO^{\bullet}(\text{rate} = R_1) \quad (1)$$

Chain Propagation:

$$R\text{-}OO^{\bullet} + (CH_3)_2C\mathbf{H}Ph \xrightarrow{k_p} R\text{-}OO\text{-}\mathbf{H} + (CH_3)_2\text{-}C^{\bullet}Ph \quad (2a)$$

$$(CH_3)_2\text{-}C^{\bullet}Ph + O_2 \rightarrow (CH_3)_2\text{-}C(OO^{\bullet})Ph(=R'OO^{\bullet}) \quad (3a)$$

$$R - OO^{\bullet} + CH_2{=}CHPh \rightarrow R\text{-}OOCH_2C^{\bullet}HPh \quad (2b)$$

$$R\text{-}OOCH_2C^{\bullet}HPh + O_2 \rightarrow R\text{-}OOCH_2CH(OO^{\bullet})Ph(=R'OO^{\bullet}) \quad (3b)$$

$$R'\text{-}OO^{\bullet} + (CH_3)_2C\mathbf{H}Ph \rightarrow R'\text{-}OO\text{-}\mathbf{H} + (CH_3)_2\text{-}C^{\bullet}Ph \quad (4a)$$

$$R'\text{-}OO^{\bullet} + CH_2{=}CHPh \rightarrow R'\text{-}OOCH_2C^{\bullet}HPh \quad (4b)$$

Chain Termination:

$$2R'\text{-}OO^{\bullet} \xrightarrow{2k_t} \text{nonradical products} + O_2 \quad (5)$$

Equation 6, where R_sH = the substrate, represents the kinetic expression which generally applies to such uninhibited autoxidations.

$$\frac{-d[O_2]}{dt} = \frac{k_p}{(2k_t)^{1/2}} \times [R_sH] \times R_i^{1/2} \quad (6)$$

In general, an antioxidant molecule (typically a phenolic antioxidant, ArOH) "traps" peroxy radicals by H atom donation with a rate constant of k_{inh} forming a relatively stable radical (see eqs 7 and 8).

$$R'OO^{\bullet} + ArOH \xrightarrow{k_{inh}} R'OOH + ArO^{\bullet} \quad (7)$$

$$R'OO^{\bullet} + ArO^{\bullet} \xrightarrow{\text{fast}} \text{nonradical products} \quad (8)$$

The oxygen uptake is suppressed for a length of time, τ, during an induction or inhibition period, and is given by

$$\tau = \frac{n \times [ArOH]}{R_i} \quad (9)$$

where n, the stoichiometric factor, is the number of peroxyl radicals trapped per molecule of antioxidant. In general, the n for phenols is 2.0.[6]

During the induction period, the rate of oxygen uptake is given by eq 10:[6]

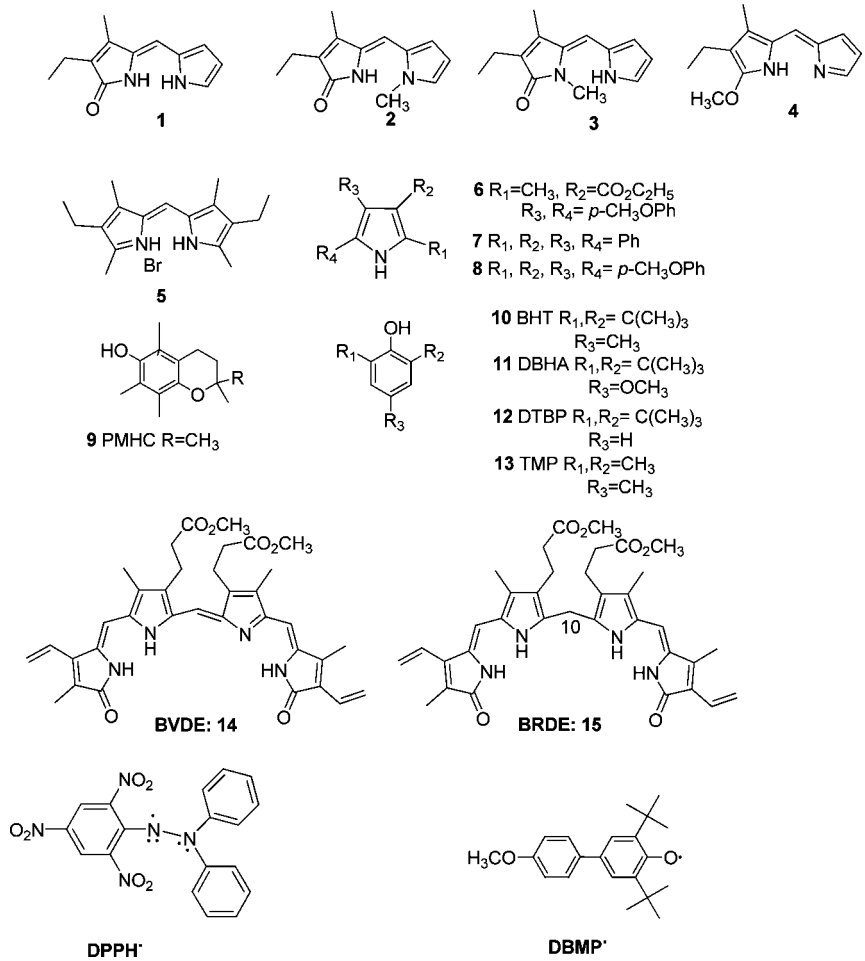


FIGURE 1. Pyrroles, phenols,and radicals used in this study.

$$\frac{-\mathrm{d}[O_2]}{\mathrm{d}t_{inh}} = \frac{k_p}{k_{inh}} \times [R_sH] \times \frac{R_i}{n[\mathrm{ArOH}]} \quad (10)$$

Integrating eq 10 gives the incremental oxygen uptake, as shown in eq 11:

$$-\Delta[O_2]t = \frac{k_p[\mathrm{RH}]}{k_{inh}} \ln(1 - t/\tau) \quad (11)$$

A linear plot of $-\Delta[O_2]_t$ versus $\ln(1 - t/\tau)$ will give a slope of k_p [RH]/k_{inh} from which the absolute rate constant for inhibition, k_{inh}, is obtained using a known value for the substrate k_p. Alternately, where there is sufficient oxygen uptake at the beginning of the induction period, the initial, $-\mathrm{d}[O_2]/\mathrm{d}t_{inh}$ can be measured and k_{inh} calculated using eq 10. In order for a substance to suppress effectively the oxidation of a substrate, the k_{inh} must be substantially greater than the rate constant of propagation, k_p of the substrate (eq 10). Application of these kinetic methods for rate constant calculations requires significant kinetic chain lengths (e.g., $\upsilon > 5$) throughout the induction period to provide measurable oxygen uptake.

In order to quantify the antioxidant capability of the pyrrolic and phenolic molecules shown in Figure 1, cumene and styrene were chosen as oxidizable substrates based on the following: (i) cumene has a low k_p of 0.18 M^{-1} s^{-1} at 30 °C,[9a] and therefore, relatively weak antioxidants give well-defined induction periods for measurements;[9b] (ii) styrene has a k_p of 41 M^{-1} s^{-1}at 30 °C,[6a] allowing k_{inh} values for more efficient antioxidants to be measured; and (iii) styrene forms a polyperoxide in an irreversible reaction as it does not contain a readily abstractable hydrogen atom, and therefore, it does not undergo complicating side reactions.[10] Solutions of cumene or styrene of convenient concentrations in an inert solvent (e.g., chlorobenzene) were used to permit measurements of oxygen uptake during inhibition periods.

Quantitative kinetic studies of cumene and styrene autoxidation were carried out to determine the antioxidant activity of our pyrroles. To our knowledge, there are no reported quantitative determinations of antioxidant activities of simple monopyrroles, and we found 2,4-dimethylpyrrole not to possess antioxidant activity.[8f] However, the stoichiometric factor of 2,3,4,5-tetraphenylpyrrole, **7**, in cumene was reported 50 years ago by Hammond et al.[11] Consequently, we started our studies with a comparison of the antioxidant activities of **7** and the tetramethoxy derivative, **8**, with the commercial antioxidant, **DBHA** (**11**), in cumene. The profiles for the inhibition of oxygen uptake by pyrroles **7** and **8** show that **8** is a superior antioxidant compared to **7** but clearly less active than **DBHA** (see Figure 2). Under these conditions, **DBHA** reduced the oxygen uptake almost completely; therefore, its k_{inh} was not calculated, but it gave reliable determinations of the rate of chain initiation, R_i.

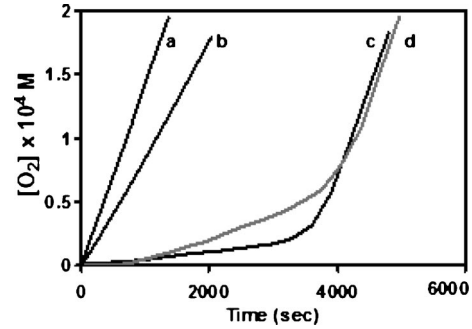


FIGURE 2. Oxygen uptake profile of the oxidation of cumene, 1.77 M, in chlorobenzene initiated with AIBN, 21 mM, at 30 °C: (a) uninhibited; (b) inhibited by **7**, 4.00 μM; (c) inhibited by **DBHA**, 4.06 μM; (d) inhibited by **8**, 3.97 μM.

TABLE 1. Antioxidant Activities, k_{inh}, and Stoichiometric Factors, n, During Peroxidation (a) of Cumene, Inhibited by BHT, 6, 7, and 8; (b) of Styrene Inhibited by DBHA or 8

substrate[a]	inhibitor, M × 10^6	k_{inh} M^{-1} s^{-1} × 10^{-3}[b]	n[c]
(a) cumene, 1.64−1.68 M	**BHT**	5.2[d]	2*
	6 4.99	(0.93)	2.0
	7 4.00−6.34	(1.30)	1.8
	8 3.94, 3.97	10.8	2.2
(b) styrene, 0.87 M	**DBHA**	161[d]	2*
	8 2.63−2.67	254	3.2

[a] Reactions were initiated by AIBN (21.0−22.5 mM) in C_6H_5Cl at 30 °C. [b] Rate constants were calculated from plots of $-\Delta[O_2]_t$ versus $\ln(1 - t/\tau)$ using a k_p = 0.18 M^{-1} s^{-1} for cumene and 41 M^{-1} s^{-1} for styrene, except for **6**, calculated from the initial inhibited oxygen uptake using eq 10. Results are from at least three determinations with error limits less than 12%, except for **8** in (a). [c] Stoichiometric factors were calculated from $n = R_i \times \tau$/[antioxidant], where $R_i = 2[\mathrm{ArOH}]/\tau$ and ArOH = **DBHA** or **PMHC**, and n* for **DBHA** and **BHT** was taken to be 2[6]. The induction period for **7** was estimated from the intersection of initial rate and the final rate, not shown in Figure 2. [d] Values of k_{inh} and n are taken from ref 8f.

Our kinetic methods now provide an actual antioxidant activity of **7** along with two other pyrroles, **6** and **8**, in cumene as shown in Table 1(a). The absolute rate constants, k_{inh}, and stoichiometric factors, n, were compared with those for the commercial antioxidants **BHT**. Compound **8** is the most effective monopyrrole antioxidant studied in cumene, and the order of antioxidant activity found is **8** > **BHT** > **7** > **6**. Stoichiometric factors of all three monopyrroles were approximately 2, indicating that these pyrroles trap two peroxyl radicals per molecule. The k_{inh} values for **6** and **7**, shown in parentheses in Table 1(a) are overestimated due to the combination of reactions by peroxyl radicals and by the antioxidant (eqs 5 and 7) because **6** and **7** are relatively weak antioxidants (see note/reference).[12]

The relative antioxidant profiles in styrene of **8** and **DBHA** along with **PMHC** are shown in Figure 3. The inhibited oxygen uptake profiles in this substrate show that the relative inhibition properties of **8** and **DBHA** are switched compared to those in cumene (see Figure 2). The antioxidant activities, k_{inh}, values given in Table 1(b) indicate **8** to be 1.7 times more reactive than **DBHA** in styrene. The dependence of the relative inhibition

(8) (a) Neužil, J.; Stocker, R. *J. Biol. Chem.* **1994**, *24*, 16712–16719. (b) Stocker, R.; Glazer, A. N.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5918–5922. (c) Stocker, R.; Yamamoto, Y.; McDonagh, A. F.; Glazer, A. N.; Ames, B. N. *Science* **1987**, *235*, 1043–1046. (d) Stocker, R.; Peterhans, E. *Biochim. Biophys. Acta* **1989**, *1002*, 238–244. (e) Hatfield, G. L.; Barclay, L. R. C. *Org. Lett.* **2004**, *6*, 1539–1542. (f) Chepelev, L. L.; Beshara, C. S.; MacLean, P. D.; Hatfield, G. L.; Rand, A. A.; Thompson, A.; Wright, J. S.; Barclay, L. R. C. *J. Org. Chem.* **2006**, *71*, 22–30. (g) Dudnik, L. B.; Khrapova, N. G. *Membr. Cell. Biol.* **1998**, *12*, 233–240. (h) Adhikari, S. K.; Guha, S. N.; Gopinathan, C. *Int. J. Chem. Kinetics* **1994**, *26*, 903–912. (i) Ihara, H.; Aoki, Y.; Hashizume, N; Aoki, T.; Yoshida, M.; Osawa, S *Clin. Chem. Enzymol. Commun.* **1998**, *8*, 31–36. (j) Barañano, D. E.; Rao, M.; Ferris, C. D.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *99*, 16093–16098. (k) Tomaro, M. L.; del Battle, A. M. C. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 216–220.

(9) (a) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1965**, *43*, 2729–2736. (b) Horswell, E. C.; Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1966**, *44*, 985–991.

(10) Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6472–6477.

(11) The stoichiometric value for **7** was reported to be 1.6: Hammond, G. S.; Boozer, C. E.; Hamilton, C. E.; Sen, J. N. *J. Am. Chem. Soc.* **1955**, *77*, 3238−3244, and also 3.3: Boozer, C. E.; Hammond, G. S.; Hamilton, C. E.; Sen, J. N. *J. Am. Chem. Soc.* **1955**, *77*, 3233−3237. The latter value was determined to be the same as that of BHT, known to be 2,[10] so this value for **7** should also be taken as 2.

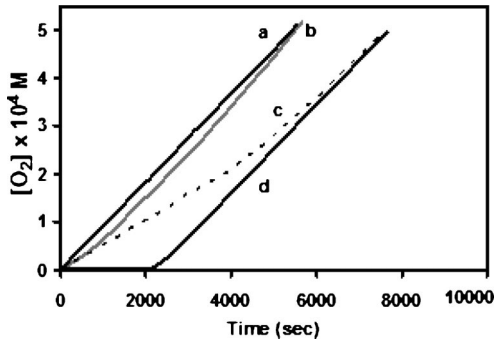


FIGURE 3. Oxygen uptake profile of the oxidation of styrene, 0.87 M, in chlorobenzene initiated with AIBN, 21 mM, at 30 °C: (a) uninhibited; (b) inhibited by **DBHA**, 2.69 μM; (c) inhibited by **8**, 2.64 μM; (d) inhibited by PMHC, 2.52 μM.

efficiencies of phenolic antioxidants on the substrate used was pointed out decades ago,[13a] and the results herein indicate again the importance of the substrate when determining antioxidant properties. In addition, the relative reactivity of a pyrrole may also depend on the structure of the peroxyl radical. Tertiary peroxyl radicals (e.g., from cumene, eq 3a) are generally known to exhibit lower reactivity compared to that of secondary peroxyl radicals[13b] (e.g., from styrene, eq 3b), and so relative antioxidant properties must be reported with reference to a common substrate. The remaining experiments with peroxyl radicals were carried out using styrene as the substrate. These results show that an activated pyrrole (e.g., **8**) possesses HAT activity of the N−H group at least as great as that of a typical phenolic antioxidant like **DBHA**. Such results provide a useful comparison with the antioxidant properties of the dipyrrinones and the bile pigments.

II. Antioxidant Activities and Mechanisms of Dipyrrinones and Bile Pigments (a) Applications Using Synthetic Model Compounds. Model compounds of the bile pigments, such as the dipyrrinones **1**−**3** (Figure 1), contain important structural features present in both biliverdin and bilirubin and are expected to mimic some of the important physical−organic properties of these bile pigments without exhibiting their marked sensitivity to laboratory light. Earlier we found that **1** has antioxidant properties comparable to those of **BVDE** and proposed that free N−H groups are required in both rings of a dipyrrinone for significant antioxidant activity since a dipyrrinone with both nitrogen atoms protected with methyl groups lacked such activity.[8f] To test this more specifically, we have now synthesized two other dipyrrinones containing single N−CH_3 groups: **2**, in which the pyrrolic ring contains the N−CH_3, and **3**, where the lactam ring contains a N−CH_3 group. We now find that neither **2** nor **3** exhibits significant antioxidant activity under conditions used for **1**.[8f]

(12) Methods of estimating the k_{inh} of weak antioxidants have analyzed the data by relating the ratio of initial rates of oxidation of a substrate both in the absence and the presence of antioxidant in terms of the composite rate constants for inhibition, k_{inh}, and termination by peroxyl radicals by *a* relationship, $nk_{inh}[AH]_0/(R_i 2k_t)^{1/2}$, assuming $n = 2$. (See, for example: Amorati, R.; Pedulli, G. F.; Valgimigli, L.; Attanasi, O. A.; Filippone, C.; Fiorucci, C.; Saladino, R. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2142–2146. Using this relationship and a reported $2k_t$ of 0.035×10^6 M^{-1} s^{-1} in cumene at 30 °C (Howard, J. A. *Adv. Free Radical Chem.* **1972**, *4*, 49–73.), we find that the k_{inh} of **6** given in Table 1 is overestimated by about 30% and that of **7** is overestimated by 6%. Since these estimates depend on the $2k_t$ used in the calculations, the values used in Table 1 or calculated values are not claimed to be reliable but the relative order of antioxidant activities shown in the text are correct.

(13) (a) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1964**, *42*, 2324–2333. (b) Korcek, S.; Chenier, J. H. B.; Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1972**, *50*, 2285–2297.

Two other compounds, **4** and **5**, were synthesized and tested to further establish the relevance of the dipyrrinone model, **1**. The dipyrrin, **4**, lacks a second N−H group but possesses an electron-donating methoxy group in the pyrrolic ring. It did not exhibit antioxidant activity. While compound **5** contains two N−H groups, it was not an antioxidant under the conditions used. The lack of HAT activities for compounds **2**−**5** is not unexpected, as these results support the earlier conclusion regarding the importance of internal hydrogen bonding to stabilize the pyrrolic radical in order for a dipyrrinone to possess significant antioxidant activity[8f] in a HAT reaction (see Discussion).

(b) Deuterium Kinetic Isotope Effects on Dipyrrinone, 1, and Dimethyl Esters of Bilrubin and Biliverdin with Peroxyl Radicals and DPPH•. The study of antioxidant mechanisms of phenols using deuterium kinetic isotope effects (DKIE) was begun in the 1960s by Howard and Ingold,[14] and their experiments provided the first clear evidence for the HAT antioxidant mechanism for phenolic antioxidants. In our study reported herein, we employed DKIE with peroxyl and **DPPH•** radicals in an attempt to provide insight into the antioxidant mechanism of mono- and polypyrrolic molecules.

A. Deuterium Kinetic Isotope Effect with Peroxyl Radicals. Kinetic studies of H atom, k_H, and D atom, k_D, abstractions by peroxyl radicals under aerobic conditions were determined using values for the inhibited oxidation of styrene initiated by AIBN in chlorobenzene. The rate constants, k_H and k_D, and the DKIE (k_H/k_D) of **1**, **BVDE**, and **BRDE** are reported in Table 2. Both compounds **1** and **BVDE** exhibited a DKIE of 1.6. The DKIE for **BRDE** was only 1.2.

TABLE 2. Rate Constants[a], k_H and k_D (M^{-1} s^{-1} × 10^{-4}) (N−H/N−D of Pyrrolic Compounds), for Hydrogen Atom and Deuterium Atom Abstraction during the Inhibited Peroxidation of Styrene[b]

compound[c]	k_H	k_D	k_H/k_D
1	13	8	1.6
BVDE	16	10	1.6
BRDE	26.6	22.5	1.2

[a] Rate constants are an average of at least three determinations, and error limits were less than 25%. [b] Oxidation of styrene (0.87 M) in C_6H_5Cl was initiated by AIBN (21.4−22.4 mM) at 30 °C in C_6H_5Cl containing trace amounts of H_2O or D_2O to ensure N−D groups do not undergo back exchange. [c] Compound concentrations ranged from 4.21 to 11.5 μM.

B. Deuterium Kinetic Isotope Effect with DPPH• Radicals. Kinetic studies of H atom and D atom abstractions by **DPPH•** in chlorobenzene under anaerobic conditions were monitored by following the pseudo-first-order decay of the 526 nm absorption of **DPPH•** radicals, k_{obs}. Using pseudo-first-order kinetics, the concentration of the antioxidant [A-H] was between 8 and 2200 times larger than that of **DPPH•**. At least five different concentrations of antioxidant were employed, and at least three separate measurements of k_{obs} were made at each concentration. The second-order rate constants, k_2, were determined by plots of the k_{obs} versus [A-H] from the following equation:

$$k_{obs} = k_0 + k_2[\text{A-H}] \qquad (12)$$

Least-squares fitting of the plots gave R^2 values >0.98 for all compounds except for **DBHA**, which gave $R^2 = 0.90$.

Rate constants of H atom, k_H, and D atom, k_D, and DKIE (k_H/k_D) of model compound **1**, **DBHA**, and **BRDE** in chlo-

TABLE 3. Rate Constants[a], k_H and k_D(M^{-1} s^{-1}) (N−H/N−D), for Hydrogen and Deuterium Atom Abstraction by DPPH• (1.4−1.5 × 10^{-4} M) in C_6H_5Cl[b]

compound[c]	k_H	k_D	k_H/k_D
1	2.6	1.6	1.6
BRDE	153	141	(1.1)[d]
DBHA	3.63[e]	0.41	9

[a] Rate constants at 30 °C are an average of at least two experimental runs, excluding **DBHA** (one experiment), using pseudo-first-order stopped-flow methods with an R^2 value greater than or equal to 0.98. [b] C_6H_5Cl contained trace amounts of H_2O or D_2O (see Table 2 footnote b). [c] Compound concentrations ranged from 0.31 to 0.0011 M and **DPPH•** 1.4−1.5 × 10^{-4} M giving a [**DPPH•**] to [A-H] ratio of 1:8 to 1:2200. [d] This value is uncertain due to the typical experimental variations in rate constants. [e] In hexane, we obtained the value k_H = 22.4 M^{-1} s^{-1}, in agreement with the reported value, 22.6 M^{-1} s^{-1}.[17e]

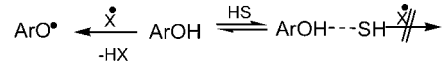
robenzene are reported in Table 3. Compound **1**, a dipyrrinone which mimics the terminal pyrroles of biliverdin and bilirubin, exhibited a DKIE of 1.6. The hindered phenol, **DBHA**, showed a high DKIE of 9 as previously reported for other phenols.[14–16] While the plots according to eq 12 for deuterated (N−D) **BRDE** were distinguished from those of the N−H compound (see Supporting Information), the calculated DKIE (Table 3) was not significantly different from experimental variations. The reaction of **BVDE** with **DPPH•** by stopped-flow studies could not be quantified using pseudo-first-order kinetics as the UV spectrum of **BVDE** interfered with the absorbance of **DPPH•** used in the measurements.

The DKIE of the selected model dipyrrinone, **1**, was remarkably consistent, 1.6, with that of **BVDE**, showing that **1** is an appropriate model for these free radical reactions and indicates participation of the pyrrolic N−H group in the mechanism (see Discussion). The lower DKIE result with **BRDE** (1.1) makes an interpretation uncertain and, while the value with peroxyl radicals (1.2) appears to distinguish **BRDE** from **1** and **BVDE**, the latter two giving DKIE of 1.6, there is no obvious explanation for such a difference in DKIE. Consequently, we decided to study solvent effects on these compounds to determine if their kinetic behavior and mechanisms could be differentiated. Compound **8** was included in some of these experiments as a representative monopyrrole.

III. Kinetic Solvent Effects on 8, Dipyrrinone, 1, Bilrubin and Biliverdin Dimethyl Esters with Peroxyl Radicals and with DPPH•. Pyrrolic molecules, such as the bile pigments, have important antioxidant properties in polar aqueous systems.[3b] Consequently, it was important to determine the effects of a polar solvent on representative pyrroles under controlled conditions. The importance of hydrogen bonding between the hydroxyl group of phenols and protic solvents on the antioxidant activities of phenols was recognized decades ago.[17a] Such solvent effects have been extensively investigated more recently and arranged on a systematic basis for phenolic antioxidants by Ingold et al.[17b–j] based on the assumption that hydrogen bond accepting solvents (HBAs) prevent HAT from the bonded OH group so that HAT is limited to the "free" (non-H-bonded) OH group (see Scheme 2).

Such systematic quantitative studies of free radical reactions on pyrrolic molecules are lacking. However, it was desirable to investigate the effect of a polar solvent on the antioxidant

SCHEME 2



activities of available active antioxidants used in this investigation, with the object of determining if HBA solvents affect the antioxidant activities and mechanisms of these compounds especially in view of the fact that the two esters, **BVDE** and **BRDE**, of the bile pigments gave quite different results using kinetic isotope effects (Table 2).

A. Kinetic Solvent Effects (KSE) with Peroxyl Radicals. Quantitative kinetic studies of styrene autoxidation with and without the presence of methanol were carried out to obtain the KSE of a HBA solvent on the antioxidant activity of monopyrrole **8**, dipyrrinone **1**, **BVDE**, and **BRDE** compared to that of **PMHC** (Table 4). In the presence of a HBA solvent, the k_{inh} of **PMHC** dropped 7-fold, a common trend with phenolic antioxidants in HBA solvents.[17] In contrast, the effect of a HBA/ionizing solvent on the antioxidant activity of the mono- and polypyrroles varied considerably. While the antioxidant activity of **8** was only slightly suppressed by methanol from 25.4 × 10^4 to 17.5 × 10^4 M^{-1} s^{-1}(Table 4), the antioxidant activities of both **1** and **BVDE** were completely suppressed in the presence of methanol and, surprisingly, the antioxidant activity of **BRDE** activity *increased* from 22.5 × 10^4 to 37.9 × 10^4 M^{-1} s^{-1} (see ref 18).

TABLE 4. Antioxidant Activities, k_{inh}, and Stoichiometric Factors, *n*, of PMHC, BRDE, BVDE, 1, and 8 during the Inhibited Peroxidation in C_6H_5Cl, of (a) Styrene, (b) Styrene with MeOH, Initiated by AIBN

substrate	inhibitor, M × 10^6	k_{inh} M^{-1} s^{-1} × 10^{-4}[a]	n[b]
(a) styrene, 0.87 M	**PMHC**[c]	380	2*
	1[c]	12.4	1.8
	BVDE[c]	10.2	2.7
	BRDE[c]	22.5	2.0
	8	25.4	
(b) styrene, 1.74 M, MeOH, 11.1 M	**PMHC** 2.23−9.24	57.3	2*
	8 9.06−9.86	17.5	2.1
	1	not measurable	−
	BVDE	not measurable	−
	BRDE 9.14− 13.0	37.9	1.8

[a] Rate constants for reactions with peroxyl radicals initiated by AIBN (21.6−22.5 mM) at 30 °C calculated from plots of eq 11, using a k_p = 41 M^{-1} s^{-1} for styrene. Results are from at least three measurements with error limits less than 10% for **PMHC** and **BRDE**, and less than 20% for **8**. The k_{inh} for **8** in styrene is taken from Table 1. [b] The stoichiometric factor of **PMHC** from ref 6. The stoichiometric factors were calculated from $n = R_i \times \tau$/[antioxidant], where R_i = 2[ArOH]/τ and ArOH= **PMHC**. [c] Values of k_{inh} and n were previously reported.[6a,8e,f]

(14) Howard, J. A.; Ingold, K. U. *Can. J.Chem.* **1962**, *40*, 1851–1864.
(15) de Heer, M. Doctoral Dissertation, de Universiteit Leiden, May 2002.
(16) Mayer, J. M. *Annu. Rev. Phys. Chem.* **2004**, *55*, 363–390.
(17) (a) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1964**, *42*, 1044–1056. (b) Litwinienko, G.; Ingold, K. U. *Acc. Chem. Res.* **2007**, *40*, 222–230. (c) Litwinienko, G.; Ingold, K. U. *J. Org. Chem.* **2005**, *70*, 8982–8991. (d) Litwinienko, G.; Ingold, K. U. *J. Org. Chem.* **2004**, *69*, 5888–5897. (e) Litwinienko, G.; Ingold, K. U. *J. Org. Chem.* **2003**, *68*, 3433–3438. (f) Snelgrove, D. W.; Lusztyk, J.; Banks, J. T.; Mulder, P.; Ingold, K. U. *J. Am. Chem. Soc.* **2001**, *123*, 469–477. (g) Valgimigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. *J. Org. Chem.* **1999**, *64*, 3381–3383. (h) Valgimigli, L.; Ingold, K. U.; Lusztyk, J. *J. Org. Chem.* **1996**, *61*, 7947–7950. (i) MacFaul, P. A.; Ingold, K. U.; Lusztyk, J. *J. Org. Chem.* **1996**, *61*, 1316–1321. (j) Valgimigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1995**, *117*, 6226–6231. (k) Musialik, M.; Litwinienko, G. *Org. Lett.* **2005**, *7*, 4951–4955. (l) Foti, M. C.; Daquino, C.; Geraci, C. *J. Org. Chem.* **2003**, *69*, 2309–2314. (m) Foti, M. C.; Johnson, E. R.; Vinqvist, M. R.; Wright, J. S.; Barclay, L. R. C.; Ingold, K. U. *J. Org. Chem.* **2002**, *67*, 5190–5196. (n) Barclay, L. R. C.; Edwards, C. E.; Vinqvist, M. R. *J. Am. Chem. Soc.* **1999**, *121*, 6226–6231.

B. Kinetic Solvent Effects (KSE) with DPPH• Radicals. Initial KSE studies on pyrroles **7** and **8** with the DPPH• radical were carried out in *tert*-butylbenzene, methanol, and methanol containing acetic acid. *tert*-Butylbenzene was used as a nonpolar solvent, and the initial rates method was used[17l,19] because of limited solubility of these compounds in alkanes.

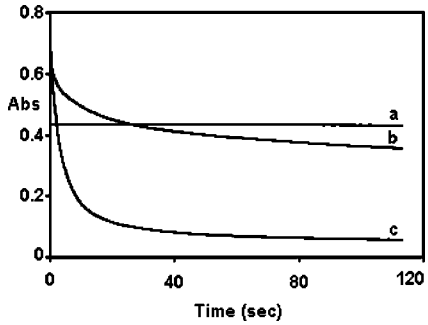


FIGURE 4. Absorption decay by time for the reaction of **DPPH•**, with **8** in (a) *tert*-butylbenzene, **DPPH•**, 99.7 μM; **8**, 108 μM; (b) MeOH, **DPPH•**, 105 μM; **8**, 89.9 μM; and (c) MeOH containing 100 mM acetic acid, **DPPH•**, 105 μM; **8**, 89.9 μM. The concentrations are before mixing in the stopped-flow wherein the concentrations will be one-half.

Qualitatively, **7** was not an active hydrogen atom donor in the three solvents, and similarly, **8** exhibited no activity in the nonpolar solvent, *tert*-butylbenzene (see Figure 4a). However, in methanol, **8** was surprisingly active, and its activity increased in methanol in the presence of acetic acid (see Figure 4b,c). The decay of **DPPH•** in the presence of acetic acid was too fast to measure for calculations of the initial rates by this method.

Quantitative studies on **BRDE** and **I** were carried out by monitoring the second-order decay of the absorption of the **DPPH•** radicals, and the second-order rate constants, k_2, were calculated from the initial rates (IR) of the **DPPH•** decay as outlined below:

$$k_2 = \mathrm{IR}/(\mathrm{Abs}_0) \times [\text{A-H}]_0) \qquad (13)$$

where (Abs_0) is the initial absorbance and $[\text{A-H}]_0$ is the initial concentration of the antioxidant. The IR of the decay was calculated by fitting the initial decay to the parabola equation $y = ax^2 + bx + c$. R^2 values for the parabola equation were >0.98. The IR at t_0 is given by the following equation:

(18) A reviewer questions the calculations where we used the $k_p = 41$ M^{-1} s^{-1} for styrene containing methanol (Table 4b). Based on a reported k_p of 0.3 M^{-1} s^{-1} for the reaction of peroxyl radicals with methanol (Denisov, E. T.; Denisova, T. G. *Handbook of Antioxidants*, 2nd ed.; CRC Press: Boca Raton, FL, 2000),this reviewer suggests that the use of the k_p for styrene is "certainly wrong". However, some control experiments showed that this is definitely *not* the case. For example, experiments with the concentrations of AIBN and methanol shown in Table 4b indicated *no oxygen uptake until styrene was added.* In addition, in the styrene/methanol mixture used, the rate of oxidation depended only on the styrene concentration. The rate at a styrene concentration of 0.87 M was 0.154×10^{-6} M s^{-1} and at 1.74 M styrene, the rate $= 0.326 \times 10^{-6}$ M s^{-1}, a 2-fold increase, as expected. The R_i was determined for these experiments and the oxidizability, $(k_p/(2k_t)^{1/2}$, eq 6) of styrene determined to be 4.49×10^{-2} $M^{-1/2}$ $s^{-1/2}$. This value is consistent with literature values $(3.4-4.6 \times 10^{-2}$ $M^{-1/2}$ $s^{-1/2}$ for the oxidizability of styrene in polar solvents (Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1964**, *42*, 1044–1056). Quantitative kinetic studies between peroxyl redicals and alkenes have been carried out by others in methanol solvent, even with very reactive perhaloperoxyl radicals without interfering reactions by methanol (Shoute, L. C.; Alfassi, Z. R.; Neta, P.; Huie, R. E. *J. Phys. Chem.* **1994**, *98*, 5701–5704.). The lack of interference by methanol was attributed to the lower reactivity of the $HOCH_2O_2^{\bullet}$ radical. Further examination of this question of the reactivity of peroxyl radicals with methanol is beyond the scope of this work.

(19) Espenson, J. H. *Chemical Kinetics and Reaction Mechanisms*, 2nd ed.; McGraw-Hill, Inc.: New York, 1995.

TABLE 5. Rate Constants,[a] k_2 (M^{-1} s^{-1}), for the Reaction of DPPH•[b] with BRDE and 1 in *tert*-Butylbenzene, Methanol, and Methanol Containing Acetic Acid

	solvent		
compound[c]	*t*-butyl C_6H_5	MeOH	MeOH + Acetic Acid[d]
BRDE[e]	77	1100	4075
1	5.1	3.3	18

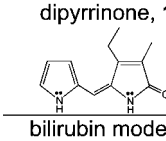
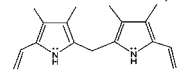
[a] Rate constants at 30 °C calculated from an average of at least three determinations using second-order stopped-flow methods. Individual rates were determined from plots of the initial decay of **DPPH•** absorption and k_2 calculated from eq 13. [b] **DPPH•** concentrations ranged from 137 to154 μM. [c] Compound concentrations ranged from 96 to 174 μM. [d] The acetic acid concentration was 100 mM. [e] Results from at least three determinations with error limits less than 10% for **BRDE** and less than 30% for **1**.

$$\mathrm{IR} = 2a \times t_0 + b \qquad (14)$$

Therefore, since the Abs_0, $[\text{A-H}]_0$, and t_0 are known, the second-order rate constant can be calculated. Rate constants for **DBHA**, **BRDE**, and **1** are given in Table 5. Interestingly, the dipyrrinone, **1**, and the tetrapyrrole, **BRDE**, exhibited quite different behaviors in a polar solvent. The k_2 of **BRDE** increased by 14-fold, whereas the k_2 of **1** slightly decreased in methanol. Surprisingly, both second-order rate constants of **BRDE** and **1** increased with the addition of acetic acid to methanol. The other dipyrrinones **2**−**4**, and dipyrrin, **5** (Figure 1), did not have measurable rate constants with **DPPH•**.

IV. Ionization Potentials (IPs) of Monopyrroles, Derivatives and Model: Compounds. It was thought that consideration of the ionization potentials of the two pyrroles, **7** and **8**, compared to those of pyrrole, dimethylpyrrole, and some model compounds representative of the bile pigments would help clarify the possible reaction mechanisms involved in the inhibition experiments. Calculated IPs for pyrrole and 2,4-dimethylpyrrole from the literature are compared with our calculations for tetraphenyl- and tetra-4-methoxyphenylpyrrole in Table 6. As would be expected, electron-supplying groups

TABLE 6. Ionization Potentials (IPs) of Pyrroles and Derivatives

Compound	IP, kcal/mol	Source
pyrrole	188[b]	ref. 20
2,4-dimethylpyrrole	169	ref. 8f
2,3,4,5-tetraphenylpyrrole, **7**	143[a]	this work
2,3,4,5-tetra-4-(methoxy-phenylpyrrole, **8**	100[a]	this work
dipyrrinone, **1** 	221	ref. 8f
bilirubin model, 	153	ref. 8f

[a] Values are calculated. [b] This compares with an experimental value of 189 kcal /mol.[20]

lower the IP values as the resulting cation would be more stabilized. Methyl groups at positions 2 and 4 lower the IP, and the presence of aryl groups, especially those bearing *para*-methoxy groups, provided marked lowering of the IP values. Such substituent effects are probably most significant on the

kinetics in polar solvents and could account for the observed variations of antioxidant activities and even mechanism shifts, for example, from hydrogen atom abstraction to single electron transfer (see Discussion).

The IP of the dipyrrinone, **1**, is the highest among the calculated values, and this is probably due to the strong electron-attracting carbonyl group conjugated with the system. This contrasts with the bilirubin model compound where the IP drops by nearly 70 kcal/mol compared to that of **1** (Table 6). It is thought that this "theoretical" structure would mimic the behavior of the two central pyrrole rings of derivatives of bilirubin (e.g., **BRDE**, Figure 1) because, in the latter, the two carbonyl groups of the dipyrrinones should give equal and opposite electron-attracting effects.

V. Reactions of Phenols with DPPH• and an Oxygen-Centered Radical. While **DPPH•** has been the most popular radical with which to monitor hydrogen atom transfer activities (HAT) of phenols, other stable oxygen-centered radicals have been used in stopped-flow kinetics, such as galvinoxyl (**G•**)[21] and the 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl, **DBMP•**, radical which has been shown to follow the same relative trend in rate constants as peroxyl radicals upon reaction with vitamin E type phenols.[22] We carried out a few exploratory experiments for the purpose of showing the trend of solvent effects with **DPPH•** compared to the **DBMP•** radical using the pseudo-first-order methods. The results for HAT reactions with the nitrogen-centered radical, **DPPH•**, in heptane (Table 7) agree with literature values.[17e] With acetic acid present, the rate decreased substantially, as expected.[17e]

TABLE 7. Trends in Rate Constants, k (M^{-1} s^{-1}) of 2,4,6-Trimethyl- (13) and 2,6-Di-*tert*-butylphenol (12) with DPPH• ($1-2 \times 10^{-4}$ M) and DBMP• ($6.0-8.5 \times 10^{-5}$ M) in Heptane, Methanol, and Methanol−Acetic Acid (AA) (10 mM)

compound[a]	solvent	DPPH•	DBMP•
13	heptane	47.7	96.4
	MeOH	179[b]	13.3
	MeOH +AA	0.948	
12	heptane	0.132	22.2
	MeOH	17.1[b]	1.02
	MeOH +AA	NA[c]	

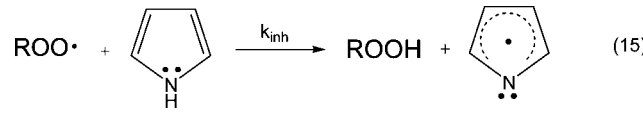
[a] Compound concentrations ranged from 0.41 to 0.37 mM. [b] The values in methanol are higher than reported, probably due to partial ionization of the phenols and incursion of the SPLET reaction.[17e] [c] The rate was too low to measure (<0.03 M^{-1} s^{-1}).

However, with the oxygen-centered radical, **DBMP•**, a different trend was observed. With the latter as the monitoring radical, the HAT activities of trimethylphenol, **13**, and that of the hindered phenol, **12**, both dropped significantly in methanol compared to that in heptane. As expected, methanol suppresses the HAT of the phenols by hydrogen bonding at the phenolic OH group, so that the **DBMP•** radical reacts with the "free" ArOH (Scheme 2). This implies that this radical is superior to **DPPH•** to monitor the HAT activities of H atom donors in a solvent that supports ionization.

(20) von Niessen, W.; Cederbaum, L. S.; Diercksen, G. H. F. *J. Am. Chem. Soc.* **1976**, *98*, 2066–2073.
(21) Shi, H.; Noguchi, N.; Niki, E. *Methods Enzymol.* **2001**, *335*, 157–166.
(22) Mukai, K.; Fukyda, K.; Ishizu, K. *Chem. Phys. Lipids* **1988**, *46*, 31–36.

Discussion

I. Antioxidant Activities and Mechanisms of Pyrroles in a Nonpolar Solvent. As proposed in the Introduction, pyrroles might be expected to act as hydrogen atom donors from the N−H group to provide antioxidant activity, much like the well-known HAT mechanism from the O−H group of phenolic antioxidants. Assuming that the resulting pyrrolyl radical adopts the 5π-electron system, the effect of strong electron-supplying or -attracting groups present in **6**, **7**, and especially in **8** should control the relative stabilities of the incipient radicals in an analogous fashion as recognized[6] for *ortho* and *para* substituents in phenoxyl radicals. Therefore, the observed relative HAT antioxidant activities of **8** > **7** > **6** during the inhibition of peroxyl radical reactions on a substrate in a nonpolar solvent (Table 1) is as expected since electron-supplying aryl groups, especially in **7** and **8**, accelerate the rate-determining antioxidant step (eq 15).



(15)

The superior antioxidant activity of **8** is attributed to the electron-supplying *para*-methoxyphenyl groups. The four aryl groups in **7** and **8** cause steric crowding especially at positions 3 and 4, and as a result, these aryl groups are almost orthogonal to the plane of the pyrrole ring in **8**, while those at positions 2 and 5 were less twisted from this plane in the minimum energy conformation. (See Supporting Information for a minimized structure of the parent, **8**, the derived radical, and calculated twist angles.) The twist angles at positions 2 and 5 decrease in the radical by 12−16° compared to the parent, while those at positions 3 and 4 change by only 2−3°. Consequently, most of the stabilization of the radical is expected to come from the groups at positions 2 and 5, where the stabilizing effects of the methoxy groups can occur through the aromatic systems by direct resonance (see Figure 5).

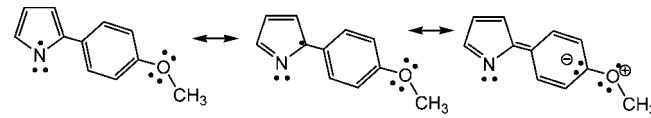


FIGURE 5. Resonance structures of compound **8** radical.

Compound **7** lacks the methoxy groups of **8**, so it is less active in the HAT reaction, and compound **6**, which bears a strong electron-attracting ester carbonyl group at position 3, exhibits very low antioxidant activity as a result. 2,4-Dimethylpyrrole did not exhibit antioxidant activity in styrene.[8f] The inductive effects of two methyl groups do not provide sufficient stability of the pyrrolyl radical, and in this regard, the result is similar to that found for dimethylphenol which possesses only very weak antioxidant activity in styrene.[6a]

II. Antioxidant Mechanisms of Dipyrrinones, the Derivatives BVDE and BRDE of Bile Pigments: Deuterium Kinetic Isotope Effects (DKIE) N−H/N−D. The rationalization of antioxidant activity of the dipyrrinones, **1**−**4**, and the dipyrrin, **5**, and application to biliverdin and bilirubin are more complex issues. Earlier results using the dipyrrinones showed that only systems bearing free N−H groups in *both* rings (e.g., compound **1**) exhibited significant antioxidant activity. This was accounted for by calculations showing strong hydrogen bonding in the

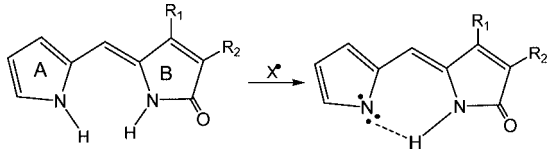


FIGURE 6. Hydrogen bonding in the intermediate radical of a dipyrrinone.

intermediate radical (Figure 6).[8f] The results herein on the isomeric dipyrrinones, **2** and **3** (Figure 1), provide important support for this proposal. An *N*-methyl group on either ring would block such H bonding in the intermediate pyrrolyl radical. In these compounds, **2** lacks the pyrrolic N−H for HAT while **3** does not possess a donor H bond to stabilize the radical, and both of these compounds are inactive as HAT antioxidants. Even a strong electron-supplying methoxy group (compound **4**) is not sufficient to impart significant HAT activity, without internal H bonding. In addition, in **4**, the N−H of the pyrrolic ring is probably H bonded to the N of the other ring to contribute to the low reactivity of **4**. The dipyrrin **5** has NH groups in both rings but also proved to be inactive in a HAT reaction, even in cumene. In solution **5** exists as a delocalized cation, as indicated by the ^{1}H NMR spectrum, where the N−H signals and alkyl groups in the two pyrrolic rings exhibited identical resonances.[23] The protonated dipyrrin cation need not exclusively maintain the natural *Z*-configuration at the connecting CH. As this configuration was shown to be necessary for stabilization of the intermediate pyrrolyl radical by internal H bonding,[8f] HAT activity is lost by **5**. There is some precedent for the role that internal H bonding can play in controlling the antioxidant activity of other polyfunctional compounds, such as catechols and 1,8-naphthalene diols.[2a] Such intramolecular interaction could also be a factor in controlling the antioxidant activity of the more complex bile pigments. However, it does not differentiate between the various possible pathways for the reaction. Some of these proposed earlier include HAT from the connecting CH_2 group in the case of bilirubin,[8c] radical addition to the pyrrolic rings,[8g] HAT from free N−H groups,[8f] and SET in polar media.[8e]

To the best of our knowledge, there are no reports regarding recent applications of DKIE for radical reactions relevant to the antioxidant ability of pyrrolic systems. We recently reported some DKIE on H atom transfer from other heterocyclic compounds, including a dihydropyridine derivative and dihydroacridane. The k_H/k_D values ranged from 1.3 to 2.1 for reactions with peroxyl radicals and 1.8−2.4 with **DPPH**$^\bullet$.[24] Our observed DKIE ratios of about 1.6 for compounds **1** and **BVDE** (Tables 2 and 3) suggest a relatively unsymmetrical transition state for these reactions. It was thought that an estimate of reaction enthalpies for the HAT reactions with **ROO**$^\bullet$ and with **DPPH**$^\bullet$ with the N−H group of **1** (eqs 16 and 17) might support this explanation for a low k_H/k_D. Using the calculated BDE, 78.3 kcal/mol, of the N−H bond of the pyrrole ring A of **1**[8f] (see Figure 6) and the experimental O−H BDE, 85.0 kcal/mol, of *tert*-butylhydroperoxide,[25] and 78.9 kcal/mol recently determined for the BDE of the **DPPH**−H bond,[26] the enthalpy change indicated by eq 16 is −6.7 kcal/mol and for eq 17 only −0.6. kcal/mol. It is improbable that the thermochemistry alone accounts for the low DKIE for these reactions.

$$\mathbf{1}(\text{N}-\text{H})+\text{ROO}^\bullet \rightarrow (\text{N}^\bullet)+\text{ROO}-\text{H} \qquad (16)$$

$$\mathbf{1}(\text{N}-\text{H})+\text{DPPH}^\bullet \rightarrow (\text{N}^\bullet)+\text{DPPH}-\text{H} \qquad (17)$$

The low but consistent k_H/k_D of 1.6 observed for compound **1** and **BVDE** with **ROO**$^\bullet$ radicals and **1** with **DPPH**$^\bullet$ as well as **BRDE** with these radicals (Tables 2 and 3) is indicative of a transition state involving both transfer of the N−H hydrogen and electron. Bearing in mind the tendency for a pyrrole to transfer a π-electron from the ring, the proton-coupled electron transfer (PCET) mechanism[17b,27] should prevail. Nevertheless, the different observed kinetic solvent effects exhibited by these compounds showed unexpected trends for such a common mechanism and require separate interpretations especially in view of the expectation that the HAT and PCET mechanisms are expected to show similar kinetic solvent effects from analogy with such effects on phenols.[17b]

III. Kinetic Solvent Effects on 8, Dipyrrinone, 1, BRDE, and BVDE on Reactions with Peroxyl Radicals and with DPPH$^\bullet$**.** The antioxidant activities and mechanisms of these pyrrolic compounds are controlled by several factors: (i) the molecular structures, (ii) the nature of the attacking radical, and (iii) the solvent used. A change in any one of these factors may switch the mechanism or change the activity. In some cases, even competing reactions may occur. The effects of a hydrogen bond accepting (HBA) or ionizing solvent for reactions with peroxyl radicals will be reviewed followed by those with **DPPH**$^\bullet$, and finally, the two processes will be compared by a quantitative treatment. A HBA solvent, such as methanol, is expected to reduce HAT antioxidant activity by hydrogen bonding of the solvent (N−H−$OHCH_3$). In contrast, if the antioxidant activity is dependent on a SET mechanism, the ionizing solvent will stabilize the polar transition state involved and the antioxidant activity will increase. To simplify rather complex solvent effects on these structures, the effects of methanol could depend on the predominance of HAT or SET within the HAT/PCET pathways and on the electron deficiency of the attacking radical.

The small effect of methanol on the reaction of the pyrrole, **8**, with peroxyl radicals (a decrease of only 32% in k_{inh}, Tables 1 and 4) contrasts with the typical large effects observed on the k_{inh} of methanol for phenolic antioxidants and for this solvent effect on **1** and on **BVDE** (see below). The different solvent effects of alcohols on **8** compared to those on phenols can be accounted for by two factors: (i) pyrrole **8** involves a N−H−$OHCH_3$ interaction, whereas phenols have a OH−$OHCH_3$ interaction, and these presumably have different H bond strengths,[28] and (ii) the net effect will depend on the predominance of HAT or SET within the HAT/PCET pathways.

As noted in our previous article,[8f] the antioxidant activities of dipyrrinone, **1**, and **BVDE** are due to the very strong

(23) Wood, T. E.; Berno, B.; Beshara, C. S.; Thompson, A. *J. Org. Chem.* **2006**, *71*, 2964–2971.

(24) Mulder, P.; Litwinienko, G.; Lin, S.; MacLean, P. D.; Barclay, L. R. C.; Ingold, K. U. *Chem. Res. Toxicol.* **2006**, *19*, 79–85.

(25) Clifford, E. P.; Wenthold, P. G.; Garejev, R.; Lineberger, W. C.; DePuy, C. H.; Bierbaum, V. M.; Ellison, G. B. *J. Chem. Phys.* **1998**, *109*, 10293–10310.

(26) Foti, M. C.; Daquino, C. *Chem.Commun.* **2006**, 3252–3254.

(27) (a) Hodgkiss, M. J.; Damrauer, N. H.; Pressé, S.; Rosenthal, J.; Nocera, D. G. *J. Phys. Chem. B* **2006**, *110*, 18853–18858. (b) Pressé, S.; Silby, R. *J. Chem. Phys.* **2006**, *124*, 164504(1−7). (c) Hammes-Schieffer, S. *Acc. Chem. Res.* **2001**, *34*, 273–281. (d) Decornez, H.; Hammes-Schieffer, S. *J. Phys. Chem. A* **2000**, *104*, 9370–9384. (e) Cukier, R. I. *J. Phys. Chem.* **1996**, *100*, 15428–15443. (f) Reece, S. Y.; Hodgkiss, J. M.; Stubbe, J. A.; Nocera, D. G. *Philos. Trans. R. Soc. London, Ser. B* **2006**, *361*, 1351–1364.

(28) The fact that the hydrogen bond donating ability of α-tocopherol (and presumably PMHC) as measured by the α^{H}_2 of 0.370[17f] is less than that of pyrrole, 0.408,[32a] indicates that different H-bonding effects are NOT the factor causing the greater decrease in antioxidant activity of PMHC compared to **8**.

intramolecular hydrogen bond (9.0 kcal/mol) from the N−H of the adjacent lactam ring as calculated for the intermediate radical. This strong intramolecular H bond formed in the nitrogen radicals derived from **1** and **BVDE** could dominate their antioxidant properties and may determine the different effect of methanol on their HAT activities compared to that on **BRDE**, which forms strong *intermolecular* H bonds giving dimers in solution.[29] Therefore, the loss in activities of **1** and **BVDE** observed with peroxyl radicals in the presence of methanol (Table 4) can be attributed to hydrogen bonding in two ways: (i) intermolecular hydrogen bonding between the solvent and the antioxidant active pyrrole ring prevents HAT by the radical and/or (ii) an intermolecular hydrogen bond formed between the adjacent pyrrole ring and solvent blocks the intramolecular hydrogen bond (see Figure 6). Surprisingly, the antioxidant activity of **BRDE** *increased* somewhat (by 69%, Table 4) in the presence of a polar solvent, which suggests SET may be predominant in the HAT/PCET antioxidant mechanism.

The kinetic solvent effect of radical reactions of **DPPH•** is an important issue because this radical is very frequently used to monitor the "so-called antioxidant activities" by stopped-flow kinetics, and because of the convenience of this method, it has had many applications to natural products.[30] This radical is highly electron-deficient, especially at the reaction center, due to contributing polar resonance structures, such as B and C in Figure 7.

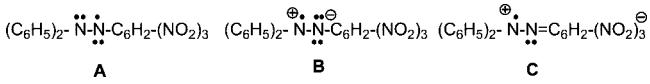


FIGURE 7. Resonance structures of **DPPH•**.

The dipyrrinone, **1**, and **BRDE** exhibit remarkably different effects in the presence of methanol. The rate constant of **1** with **DPPH•** in methanol is only slightly lower than that in a hydrocarbon solvent, whereas **BRDE** exhibits a large increase (by 14-fold, Table 5) on changing from the hydrocarbon solvent to methanol. Such contrasting kinetic solvent effects on compounds with similar structures as **1** and **BRDE** can be accounted for by a different predominance in the HAT/PCET reactions. In methanol, electron transfer may predominate on a PCET reaction on **BRDE**. The slight effect of methanol on the rate constant of **1** with **DPPH•** can be accounted for by competing SET and HAT reactions. That is, methanol reduces the classical HAT reaction due to interfering H bonding at the pyrrolic N−H group and simultaneously increases the rate through a SET reaction. It is interesting to note that, under similar conditions, phenols were reported to react with **DPPH•** by a combination of HAT and SPLET mechanisms.[16] The HAT/PCET reaction with **1** occurs with **DPPH•** but apparently not with peroxyl radicals and illustrates the major effect that the electron-deficient **DPPH•** has on both the kinetics and the mechanism of the reactions of antioxidants.

The acceleration effects of acetic acid on the reactions of **8**, **BRDE**, and **1** (Figure 4 and Table 5) were completely unexpected. The resulting enhanced rate constants cannot be attributed to the SPLET reaction, where acetic acid decreases the rates, at least for phenols by suppressing their ionization.[16,31]

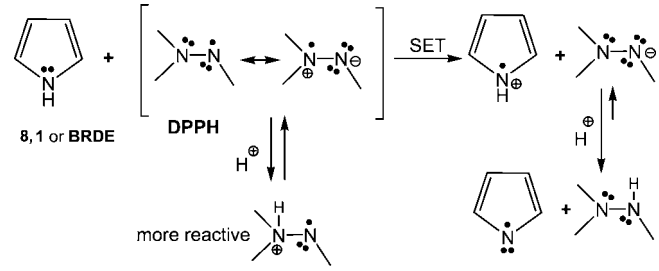


FIGURE 8. The effect of acetic acid on **DPPH•**/ pyrrole reactions.

The effect of acetic acid on the **DPPH•** reactions with these pyrroles may be accounted for by one or both of two factors: (i) the addition of acid to the solvent system may cause protonation of **DPPH•**, resulting in increased reactivity of the radical (see Figure 8), and (ii) the presence of acid may displace the equilibrium toward the **DPPH**−H product. This latter effect is quite clear in Figure 4 since the reaction does not proceed to completion in the presence of methanol alone; the addition of acetic acid is required. Consequently, the latter effect is the more probable explanation for the accelerating effect of acetic acid.

Overall, these results demonstrate that SET is an important pathway in the reactions of **8** and **BRDE**, especially with **DPPH•** in an ionizing solvent.

Snelgrove et al. developed a relationship to predict rate constants in a polar hydrogen bond accepting solvent from the rate constant in a nonpolar solvent and a knowledge of the linear free energy relationships of Abraham et al.,[32a] where α^{H}_2 is the hydrogen bond donating ability of the substrate (e.g., antioxidant), and β^{H}_2 is the hydrogen bond accepting ability of the solvent. Their derived equation made it possible to calculate rate constants in a hydrogen bond accepting solvent from the measured rate constant in a nonpolar solvent, as shown below (eq 18).[17f]

$$\log(k^s/\mathrm{M}^{-1}\,\mathrm{s}^{-1}) = \log(k^0/\mathrm{M}^{-1}\,\mathrm{s}^{-1}) - 8.3\alpha_2{}^{\mathrm{H}}\beta_2{}^{\mathrm{H}} \quad (18)$$

It is of interest to determine if such a quantitative treatment can be applied to the solvent effect results of our pyrrolic compounds, in particular, to compare the data with the peroxyl and **DPPH•** radicals. We can predict the HAT rate constants (k^0) with peroxyl radicals of our compounds in methanol with the following assumptions: (i) we can use $\alpha_2{}^{\mathrm{H}} = 0.370$ from α-tocopherol for **PMHC**;[17f] (ii) that the k^0 in styrene can be represented as that in a saturated hydrocarbon; (iii) a correction for the concentration of methanol ($\beta_2{}^{\mathrm{H}} = 0.41$[32a]) can be made for those experiments in styrene/methanol since the theory is for a neat solvent; and (iv) we can use $\alpha_2{}^{\mathrm{H}} = 0.408$ from pyrrole[32a] for our pyrrolic compounds. First, it is useful to test the calculation in styrene for a known antioxidant, **PMHC**. The results with peroxyl radicals in styrene with **PMHC** (Table 8(a)) show that the experimental rate in methanol $57 \times 10^4\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ is very similar to the calculated HAT value, $47 \times 10^4\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. Calculations for compound **1** and **BVDE** give very low values of 1.1 and 0.93 for k^s but are in general agreement with our experimental results (Table 4(b)) where the rates were too small

(29) Lightner, D. A.; Trull, F. R. *Spectrosc. Lett.* **1983**, *16*, 785–803.

(30) A recent search of SciFinder Scholar using the words 'DPPH radical and antioxidants' responded with at least 3500 examples. Many of these employed **DPPH•** to measure the antioxidant properties of plant extracts from aqueous mixtures.

(31) The very low acidity of pyrrole, (pK_{HA}= 23; Bordwell, F. G.; Zhang, X.; Chen, J.-P. *J. Org. Chem.* **1991**, *56*, 3216–3219.) rules out the ionization of the N−H group of pyrroles so that the SPLET mechanism observed for phenols in ionizing solvents[17b] would not occur for these pyrroles.

(32) (a) Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Duce, P. P.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 699–711. (b) Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1990**, 521–529.

TABLE 8. Experimental Rate Constants in a Nonpolar Solvent, k^0, in Methanol, k^{exp}, and Calculated in Methanol, k^s

radical	compound	k^{0a} (M^{-1} s^{-1} × 10^{-4})	k^{sb} (M^{-1} s^{-1} × 10^{-4})	$k^{exp a}$ (M^{-1} s^{-1} × 10^{-4})
(a) ROO•	**PMHC**	380	47	57
	8	25	2.3	18
	1	12	1.1	
	BVDE	10	0.93	
	BRDE	22	2	38
(b) DPPH•	**1**	5.1	0.21	3.3
	BRDE	77	3.2	1100

[a] Results taken from Tables 1, 4, and 5. [b] Calculated using eq 18.

to measure. In contrast, our experimental results for **8** and **BRDE** are not consistent with the predicted rates calculated from eq 18. In this case, the experimental values are 8 and 19 times larger, respectively, than the calculated values. It is thus concluded that **8** and **BRDE**, unlike **PMHC**, do not react via a simple HAT mechanism, but may undergo a HAT/PCET reaction in a polar solvent or possibly a combination of reaction pathways where SET becomes more prominent.

Using the same method, HAT rate constants, k^s, of compounds **1** and **BRDE** with **DPPH•** in methanol are calculated. Experimental rates in methanol with compound **1** and **BRDE** were 15 and nearly 350 times higher, respectively, than those calculated assuming the HAT mechanism, supporting the importance of a SET mechanism in the reaction of **DPPH•** with these compounds. The calculated k^s values herein for the pyrrolic compounds cannot be claimed to be highly reliable because of the assumptions involved. However, they do indicate that great care must be shown when determining antioxidant activities in polar solvents, especially when **DPPH•** is used as the monitoring radical when reported "antioxidant activities" are of questionable significance.

IV. Ionization Potentials (IPs) and Electron Transfer Reactions of Pyrroles. A few selected examples of chemical and physical evidence from the literature indicate that substituted pyrroles are susceptible to both HAT and SET reactions. The oxidation of the pyrrole **7** is known to form the tetraphenylpyrrolyl radical which is stable in solution.[33] 2,5-Di-*tert*-butyl- and 2,5-diphenylpyrrole form long-lasting radical cations by electron transfer in ESR observations.[34] Gas phase ionization of monosubstituted pyrroles showed that their IPs gave good linear correlations with σ^+_p constants,[35] where the $\rho = -18.2$ was indicative of high sensitivity of the ionization to substituent effects. The self-initiated autoxidation of 1,2,3-trimethylpyrrole in chlorobenzene at 131 °C was interpreted as a SET reaction to oxygen.[36]

We are well aware of the limitations when comparing gas phase IP calculations to experimental results in polar solvents. However, as indicated in our Results, ionization potentials of the substituted pyrroles compared to pyrrole, and of a bilirubin model compound compared to a typical dipyrrinone (see Table 6), might provide some insight into the antioxidant activities and mechanisms of these compounds. In particular, large decreases in IPs such as those of the tetraarylpyrroles **7** and **8** compared to pyrrole might be indicative of a propensity toward single electron transfer (SET) rather than hydrogen atom transfer (HAT).[37]

Actual comparisons to our kinetic data become complicated by the fact that competitions between HAT or SET pathways are also expected to be affected by the ionization capacity of the solvent used and the nature of the attacking radical, **ROO•** or **DPPH•**. Nevertheless, some clear comparisons between the kinetic data and IPs can be made. For example, in the data for reactions of **BVDE** and its model compound **1** with **ROO•** (Table 4), the effects of methanol indicate an ordinary solvent effect that retards HAT-type reactions by hydrogen bonding; the high IP of **1** supports this conclusion, and the SET reaction is retarded. In contrast the effect of methanol on the rate constants of **8** and **BRDE** with peroxyl radicals in styrene (Tables 1 and 4), a small decrease and a small increase, respectively, appear ambiguous. However, it would appear that either HAT or SET reactions could predominate within the HAT/PCET reactions wherein the relatively very low IP of **8** indicates susceptibility toward a SET partway. So the SET pathway must predominate in the faster reactions of **8** and **BRDE** with the more electron-deficient **DPPH•** radical in methanol (Figure 4 and Table 5). Finally, the relatively low IP of the bilirubin model compound (Table 7) indicates that the central pyrrole rings of **BRDE** could be susceptible to facile electron transfer reactions promoting the PCET process.

V. Reactions of Phenols with DPPH• and the 2,6-Di-*tert*-butyl-4-(4′-methoxy)phenoxyl, DBMP•, Radical. The use of **DPPH•** as the monitoring radical under anaerobic conditions is very convenient[30] but susceptible to complex kinetic solvent effects as indicated in the Results. We carried out a few experiments to compare results with this radical and the oxygen-centered radical, **DBMP•**. The results in Table 7 illustrate the complexity of results using **DPPH•** where a polar solvent results in a marked acceleration in reaction rate most likely due to initial partial ionization of the phenol, followed by rapid electron transfer (SPLET).[17b] Consequently incorrect conclusions on "antioxidant activities" will result when using this radical in common alcoholic solvents. Indeed, such results should NOT be referred to as "antioxidant" experiments. We suggest that the use of an oxygen-centered radical, such as **DBMP•**, of much lower electron affinity than **DPPH•**, is much superior for such monitoring of HAT activities by antioxidants using stopped-flow kinetics because it does exhibit "normal" solvent effects.[38] The phenolic precursor for the radical is available from a convenient one-pot aryl coupling synthesis.[39]

(33) (a) Kuhn, R.; Kainer, H. *Biochim. Biophys. Acta* **1953**, *12*, 325–328. (b) Blinder, S. M.; Peller, M. L.; Lord, N. W.; Aamodt, L. C.; Ivanchukov, N. S. *J. Chem. Phys.* **1962**, *36*, 540–544.

(34) Avila, D. A.; Davies, A. G. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1111–1118.

(35) Linda, P.; Marino, G.; Pignataro, S. *J. Chem. Soc. (B)* **1971**, 1585–1587.

(36) Beaver, B.; Teng, Y.; Guiree, P.; Hapiot, P.; Neta, P *J. Phys. Chem. A* **1998**, *102*, 6121–6128.

(37) It has been suggested that, in the case of phenolic antioxidants, a lower IP of about 45 kcal/mol compared to phenol indicates that the SET antioxidant mechanism predominates. Wright, J. S.; Johnson, E. R.; DiLabio, G. A *J. Am. Chem. Soc.* **2001**, *123*, 1173–1183. However, such an estimate would be subject to a large uncertainty by solvent effects.

(38) In hindsight, there is some evidence that the oxygen-centered radical, DBMP•, is a useful monitor to determine HAT activities in different solvents. Earlier we reported the rate constants of several phenolic antioxidants using DBMP• in several solvents.[17n] For example, from the value of PMHC in hexane of 93.9×10^3 M^{-1} s^{-1} as k^0 and the $\alpha^H_2 = 0.370$ (found for α-tocopherol),[17c] the calculated k^s value in 1-propanol ($\beta^H_2 = 0.45$)[32a] of 3.90×10^3 M^{-1} s^{-1} and in acetone ($\beta^H_2 = 0.50$)[32a] of 2.74×10^3 M^{-1} s^{-1}, employing eq 18, are in reasonable agreement with the reported values[17n] of 4.40×10^3 M^{-1} s^{-1} (1-propanol) and 3.28×10^3 M^{-1} s^{-1} (acetone).

(39) Kawamura, Y.; Satoh, T.; Miura, M.; Nomura, M. *Chem. Lett.* **1998**, *9*, 931–932.

Conclusions

The polypyrroles in bile pigments exhibit antioxidant properties in various heterogeneous aqueous/lipid dispersions, including blood plasma.[3b] This work provides new evidence toward the *antioxidant activities* and *mechanisms* of pyrrolic compounds in solution. A simple monopyrrole requires strong electron-donating groups (e.g., *p*-methoxyphenyl) as in tetra-(4′-methoxyphenyl)pyrrole, **8**, for effective HAT antioxidant ability as large as those of polyalkyl phenolic antioxidants with peroxyl radicals in chlorobenzene.

The antioxidant mechanisms and activities of the dipyrrinones and related bile pigment derivatives, **BVDE** and **BRDE**, are strongly influenced by various factors including the structure of the pyrrolic moiety, the nature of the attacking radical, and the ionizing capacity of the solvent such that a single comprehensive antioxidant reaction mechanism cannot be applicable for these pyrroles. Only dipyrrinones that possess two "free" N−H groups are active antioxidants, confirming the proposal that strong internal H bonding from an adjacent ring (e.g., as in **1**) is required to provide HAT activity in the HAT/PCET reactions. The HAT activity for **1** is also supported by N−D/N−H DKIE results, the retarding effect of a HBA solvent, methanol, on the k_{inh} due to external H bonding, and the high ionization potential of **1**. The bile pigment of biliverdin (**BVDE**) showed similar DKIE and solvent effects to **1** on reaction with peroxyl radicals and therefore reacted by a HAT/PCET path. Pyrroles readily undergo electron transfer reactions, probably due to the facile formation of a 5π-electron ring system. In methanol containing **DPPH•**, this is accompanied by proton transfer. In the case of the readily ionizable **8**, the proton transfer might *follow* SET in methanol (Figure 8). Compound **BRDE** may follow this route on reaction with DPPH• in the presence of both methanol and acetic acid. This is in sharp contrast to the reactions of phenols where claims for this mechanism are judged to be "without merit".[17b]

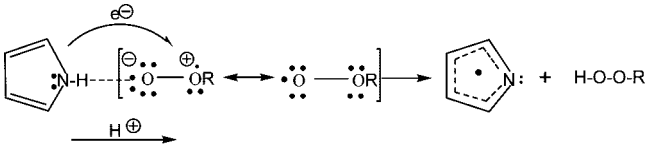


FIGURE 9. PCET mechanism for reaction of a pyrrolic molecule with a peroxyl radical.

The antioxidant mechanism of **BRDE** with peroxyl radicals in chlorobenzene is best described by the PCET mechanism. A scheme for the mechanism is proposed in Figure 9, where a N−H group of a pyrrolic compound like **BRDE** is H bonded to a peroxyl radical, known to be highly polarized with the terminal oxygen bearing higher negative charge.[40,41] In this associated complex, proton vibrational motion is coupled to electron transfer so a kinetic isotope effect would be expected.[27] Thus it appears that the observation of a DKIE is important to distinguish experimentally between PCET and sequential electron transfer followed by proton transfer. There is an interesting analogy between this mechanism and PCET observed in a N−H to oxygen H bonded amidium carboxylate interface[27] in which a N−H/N−D DKIE of 1.2 at 27 °C was observed.[27a,b]This complex has received considerable theoretical interest,[27c–e] and this model system was reviewed as a model for radical processes in biological systems.[27f] Recent NMR evidence for such a complex between a hydrogen bond donor and peroxyl radicals indicates a significance bond strength for a complex[44] and provides support for the role of such H bonded complexes in PCET reactions.

Our results show that some previous antioxidant mechanisms for reactions of bile pigments with peroxyl radicals, such as HAT from the connecting $-CH_2-$ group of bilirubin[8b] or addition of peroxyl radicals to the pyrrolic rings[8g] are untenable.

The popular **DPPH•** radical for monitoring HAT activities of phenols gives anomalous kinetic solvent effects which makes it unreliable for determination of HAT activities in protic solvents. In contrast, an oxygen-centered radical, 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl, provides reliable HAT kinetic data that agree with theoretical predictions and is recommended for such studies using stopped-flow kinetics.

Experimental Section

Materials: Bilirubin dimethyl ester and biliverdin dimethyl ester were obtained from a commercial supplier and used as received. The commercial inhibitors, 2,2′,5,7,8-pentamethyl-6-hydroxychroman (PMHC), 2,6-di-*tert*-butyl-4-methoxyphenol (DBHA), 2,6-di-*tert*-butylphenol (DTBP), 2,4,6-trimethylphenol (TMP), and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were recrystallized from methanol before use. AIBN was recrystallized from methanol from a solution prepared at room temperature and cooled. Commercial styrene was separated from added stabilizer by bulb-to-bulb vacuum distillation at room temperature. The distilled styrene was passed "neat" through chromatographic silica gel immediately before use. Cumene, purest grade, was passed through silica gel before use. Solvents used were of HPLC purity. Diphenylpicrylhydrazyl radical (98%) was used as received. The concentration of **DPPH•** was determined from its molar absoptivity value, ϵ, of 11 500 M^{-1} cm^{-1} (λ_{max} = 516 nm).[17l] 2,6-Di-*tert*-butyl-4-(4′-methoxyphenyl)phenol, the phenolic precursor of **DBMP•**, was synthesized according to the procedure outlined by Kawamura et al.[39] by the aryl coupling reaction from 2,6-di-*tert*-butylphenol and 4-bromoanisole in the presence of tri-(4-methoxy)phenylphosphine catalyzed by cesium carbonate and palladium acetate. The product recrystallized from hexane was identical to that reported earlier.[17n] The reported procedure[17n] was followed to prepare the **DBMP•** radical. The concentration of **DBMP•** was determined from its molar absorptivity value, ϵ, of 24 000 M^{-1} cm^{-1} (λ_{max} = 370 nm).[17n] Syntheses and characterization of pyrrolic compounds **2** and **3** are given in the Supporting Information. The synthesis of **4** was reported earlier;[8f] however, an alternative method was used in this work, which is also reported in the Supporting Information. The synthesis of **5** was reported earlier.[23] The syntheses of the monopyrroles **6**, **7**, and **8** were reported previously; however, the details are given herein along with complete characterization.

(40) Barclay, L. R. C. The Structure of Organic Peroxyl Radicals In *Peroxyl Radicals*; Alfassi, Z., Ed; Wiley: Chichester, England, 1997; Chapter 3, pp 27−48.

(41) The polarity of peroxyl radicals has been proposed to account for some significant observations such as their diffusion from nonpolar regions of lipid membranes[40] or micelles,[42] and hydrogen bonding by water could account for their reduced reactivity resulting in lower termination rate constants in aqueous lipid systems.[43] Recently, direct evidence was provided for hydrogen bonding of alkyl peroxyl radicals with an alcohol of high H-atom donating ability by showing a large increase in the esr lifetime of *sec*-alkyl peroxyls and a enthalpy change of−3.4 kcal/mol in the H-bonded complex.[44] These results add support for our proposed scheme since it is most likely that similar H-bonding occurs between pyrrolic N−H groups and peroxyl radicals as shown in Figure 9.

(42) Yazu, K.; Yamamoto, Y.; Niki, N.; Miki, K.; Ukegawa, K. *Nihon Yukagakkaishi* **2000**, *49*, 611–615.

(43) Barclay, L. R. C.; Baskin, K. A.; Locke, S L.; Vinqvist, M. R. *Can. Chem.* **1987**, *67*, 1366–1369.

(44) Mugnaini, V.; Lucarini, M. *Org. Lett.* **2007**, *9*, 2725–2728.

(45) Wayner, D. D. M.; Burton, G. W. *Handbook of Free Radicals and Antioxidants in Biomedicine*; CRC Press: Boca Raton, FL, 1989; Vol. 3, pp 223−232.

Deuteration of Inhibitors: Inhibitors were dissolved in hexadeuterated acetone and D_2O until all O−H or N−H hydrogen atoms were deuterated. The acetone was then distilled, and the sample was dissolved in chlorobenzene with traces of D_2O. NMR spectra confirmed deuterated O−H/N−H groups in both hexadeuterated acetone/D_2O and chlorobenzene solutions. The N−H's for **BVDE** are not distinguishable using NMR spectroscopy, and therefore, the N−H groups were assumed to be deuterated by using the above procedure.

Autoxidation/Inhibition Procedures: Autoxidations were carried out at 30 °C under 760 Torr of air in a dual-channel oxygen uptake apparatus equipped with a sensitive pressure transducer described previously.[45] The procedures for conditioning the apparatus and conducting an inhibition experiment have been described previously.[2c]

Stopped-Flow UV−Vis Experiments: Experiments were conducted at 30 °C under an argon atmosphere. Samples were rapidly mixed using a stopped-flow assembly and monitored using a UV−vis spectrometer.

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Supporting Information Available: Syntheses and characterization of pyrrolic compounds. Calculations of ionization potentials and the twist angles for **8** and the derived radical. The structures of **8** (parent) and the derived radical with the related twist angles. Detailed tables for oxygen uptake kinetics and stopped flow kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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