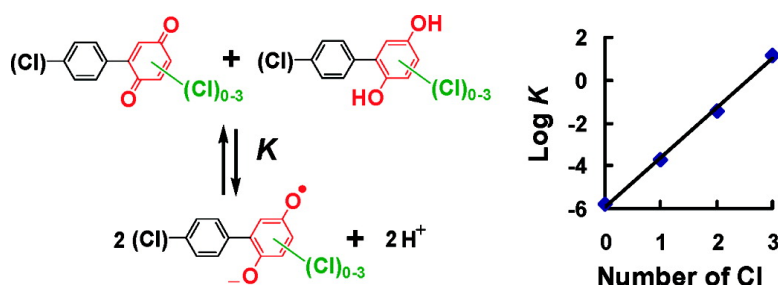Article

Chlorination Increases the Persistence of Semiquinone Free Radicals Derived from Polychlorinated Biphenyl Hydroquinones and Quinones

Yang Song, Garry R. Buettner, Sean Parkin, Brett A. Wagner, Larry W. Robertson, and Hans-Joachim Lehmler

More About This Article

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

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Chlorination Increases the Persistence of Semiquinone Free Radicals Derived from Polychlorinated Biphenyl Hydroquinones and Quinones

Yang Song,[†,‡] Garry R. Buettner,[‡,§] Sean Parkin,[||] Brett A. Wagner,[‡] Larry W. Robertson,[†,§] and Hans-Joachim Lehmler*,[†,§]

Department of Occupational and Environmental Health, The University of Iowa, 100 Oakdale Campus, 124 IREH, Iowa City, Iowa 52242-5000, Free Radical and Radiation Biology Program & ESR Facility, The University of Iowa, Iowa City, Iowa 52242-1101, Interdisciplinary Graduate Program in Human Toxicology, The University of Iowa, Iowa City, Iowa 52242-5000, and Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506-0055

hans-joachim-lehmler@uiowa.edu

Received July 1, 2008
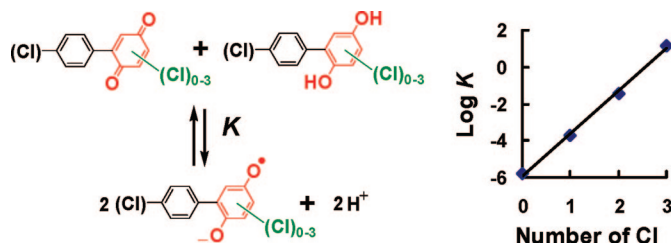
Polychlorinated biphenyls (PCBs) comprise a group of persistent organic pollutants that differ significantly in their physicochemical properties, their persistence, and their biological activities. They can be metabolized via hydroxylated and dihydroxylated metabolites to PCB quinone intermediates. We have recently demonstrated that both dihydroxy PCBs and PCB quinones can form semiquinone radicals ($SQ^{\bullet-}$) in vitro. These semiquinone radicals are reactive intermediates that have been implicated in the toxicity of lower chlorinated PCB congeners. Here we describe the synthesis of selected PCB metabolites with differing degrees of chlorination on the oxygenated phenyl ring, e.g., 4,4′-dichloro-biphenyl-2,5-diol, 3,6,4′-trichloro-biphenyl-2,5-diol, 3,4,6,-trichloro-biphenyl-2,5-diol, and their corresponding quinones. In addition, two chlorinated *o*-hydroquinones were prepared, 6-chloro-biphenyl-3,4-diol and 6,4′-dichloro-biphenyl-3,4-diol. These PCB (hydro-)quinones readily react with oxygen or via comproportionation to yield the corresponding semiquinone free radicals, as detected by electron paramagnetic resonance spectroscopy (EPR alias ESR). The greater the number of chlorines on the (hydro-)quinone (oxygenated) ring, the higher the steady-state level of the resulting semiquinone radical at near neutral pH.

Introduction

Polychlorinated biphenyls (PCBs) are a class of organic compounds containing one to ten chlorine substituents covalently attached to biphenyl.[1,2] PCBs have been produced in industrial scale by batch chlorination of biphenyl in the presence of an iron catalyst. They have been (and still are) used as coolants and insulating fluids for transformers and capacitors. PCBs have also been sold commercially for use as stabilizing additives in flexible PVC coatings of electrical wiring and electronic components, cutting oils, flame retardants, hydraulic fluids, sealants, and adhesives. These industrial applications exploit the physicochemical properties of PCBs, such as the high thermal and chemical stability and high dielectric constant. The commercial production and sale of PCBs were banned in the United States in the late 1970s because of their persistence and

---

† Department of Occupational and Environmental Health, The University of Iowa.

‡ Free Radical and Radiation Biology Program & ESR Facility, The University of Iowa.

§ Interdisciplinary Graduate Program in Human Toxicology, The University of Iowa.

|| University of Kentucky.

(1) Robertson, L. W.; Hansen, L. G. *Recent Advances in the Environmental Toxicology and Health Effects of PCBs*; University Press of Kentucky: Lexington, 2001.

(2) Hansen, L. G. *The Ortho Side of PCBs: Occurrence and Disposition*; Kluwer Academic Publishers: Boston, 1999.

broad range of adverse biological effects, including (neuro-)-developmental toxicity[3,4] and carcinogenesis.[5]

Mechanisms of PCB toxicity are still incompletely understood. One reason is that commercial PCB products contain a complex mixture of many of the possible 209 PCB derivatives or congeners. Depending on the degree of chlorination and the substitution pattern, PCB congeners have different modes of action. In particular, environmentally persistent PCB congeners interact with a number of cellular receptors and cause adverse effects by interfering with inter- and intracellular signaling processes and gene expression. Other PCB congeners, especially lower chlorinated PCB congeners and congeners with vicinal hydrogen atoms, are more rapidly metabolized[2,6] and can form reactive metabolites.[7] These metabolites, which include PCB epoxides, dihydroxy PCBs, and PCB quinones, have been shown to react rapidly with cellular nucleophiles, such as glutathione, amino acids, and DNA, in vitro and in vivo.[7–10] A quinone metabolite of 4-chlorobiphenyl (PCB 3), 2-(4-chlorophenyl)-[1,4]benzoquinone, has also been implicated as the ultimate carcinogen in the Solt-Faber protocol, an in vivo liver carcinogenesis test.[11]

A recent electron paramagnetic resonance (EPR) study from our laboratory demonstrated that lower chlorinated dihydroxy PCBs and PCB quinones derived from PCB 3 and other PCB congeners can form reactive PCB semiquinone free radicals by autoxidation under basic conditions (pH > 7) as well as by (enzymatic) one-electron transfer and comproportionation reactions.[12] This study provides direct evidence that PCB metabolites can form reactive PCB semiquinone radicals, which are likely to play an important but poorly understood role in the toxicity of PCBs. PCB semiquinone radicals can facilitate the generation of superoxide radicals ($O_2^{\bullet-}$), which in the presence of superoxide dismutase forms hydrogen peroxide; H_2O_2 can lead to the production of hydroxyl radicals ($HO^\bullet$). Subsequently, these reactive oxygen species and, possibly, PCB semiquinone radicals themselves can react with cellular nucleophiles, such as protein and nonprotein sulfhydryls, resulting in oxidative stress.[7]

In addition to lower chlorinated PCBs, some higher chlorinated PCBs can be rapidly metabolized to mono- and dihydroxylated PCB metabolites.[13] It is currently unclear how the degree of chlorination in the metabolites of these higher chlorinated PCBs alters the formation, persistence, and properties of the corresponding PCB semiquinone radicals. The apparent persistence of semiquinone radicals in the setting of our experiments is a complicated result of the thermodynamic or kinetic properties, or a combination, of all three redox forms, i.e., Q, SQ$^{\bullet-}$, and H$_2$Q. The work presented here does not address this directly. However, it is clear that the yield of semiquinone radical varies greatly among the (hydro-)quinones studied; both thermodynamic and kinetics will play a role in these apparent yields. Unfortunately the synthesis of oxygenated PCB derivatives with differing degrees of chlorination of the oxygenated ring system still represents a synthetic challenge. PCB catechols, hydroquinones, and quinones have been traditionally synthesized using a variety of approaches, such as the Cadogan[14] and Ullmann coupling reactions.[13] These reactions typically have poor yields, require tedious cleanup procedures and/or result in the formation of undesired[15] and potentially toxic byproducts.[16,17] In addition, chlorinated starting materials with suitable substitution patterns are frequently not available. Novel synthetic strategies to overcome these difficulties are, therefore, needed to make reactive PCB metabolites available for further studies of the corresponding PCB quinones and semiquinone radicals.

Here we report strategies for the synthesis of oxygenated PCB congeners with differing degrees of chlorination (1 to 3 Cl substituents in the oxygenated ring system). In addition, we report an initial investigation of the semiquinone radicals formed by these compounds using electron paramagnetic resonance. We show that the yield of the PCB-derived semiquinone radicals resulting from comproportionation of quinone and hydroquinone depends on the oxygen and chlorine substitution pattern and especially the number of chlorines on the semiquinone ring.

Results and Discussion

Synthesis of PCB Hydroquinones/Quinones with One Chlorine Substituent on the Oxygenated Ring. Metabolism of PCB congeners by cytochrome P-450 enzymes frequently results in the formation of PCB metabolites with 2,5- and 3,4-dihydroxy substitution patters.[13,18] If no chlorine substituents are present in the oxygenated ring system, these catechol and hydroquinone metabolites can be easily synthesized, for example, from 3-bromo-1,2-dimethoxy-benzene and a chlorinated benzene boronic acid using the Suzuki coupling reaction.[19–21] In contrast, the synthesis of PCB metabolites with a single chlorine substituent in the oxygenated ring system is less straightforward because suitable starting materials are not available. Typically, the approaches used for the synthesis of these metabolites result in product mixtures because of poor selectivity and give the desired product in poor yields.[13,14] We investigated the selective chlorination of dimethoxy-benzenes **1** or **2** using H_2O_2/HCl to overcome these problems and to obtain starting materials that can be used to synthesize putative

(3) Kodavanti, P. R. S. In *Molecular Neurotoxicology: Environmental Agents and Transcription-Transduction Coupling*; Zawia, N. H., Ed.; CRC Press: Boca Roton, FL, 2004; pp 151–182.

(4) Schantz, S. L.; Widholm, J. J.; Rice, D. C. *Environ. Health Perspect.* **2003**, *111*, 357–376.

(5) Silberhorn, E.; Glauert, H. P.; Robertson, L. W. *Crit. Rev. Toxicol.* **1990**, *20*, 439–496.

(6) Letcher, R. J.; Klasson-Wehler, E.; Bergman, A. In *The Handbook of Environmental Chemistry, Vol. 3 Part K. New Types of Persistent Halogenated Compounds*; Paasivirta, J., Ed.; Springer Verlag: Berlin, Heidelberg, 2000; pp 315–359.

(7) Robertson, L. W.; Gupta, R. C. In *Molecular Drug Metabolism and Toxicology*; OICA International: London, 2000; pp 16–32.

(8) Lin, P. H.; Sangaiah, R.; Ranasinghe, A.; Upton, P. B.; La, D. K.; Gold, A.; Swenberg, J. A. *Chem. Res. Toxicol.* **2000**, *13*, 710–718.

(9) Arif, J. M.; Lehmler, H.-J.; Robertson, L. W.; Gupta, R. C. *Chem.-Biol. Interact.* **2003**, *142*, 307–316.

(10) Zhao, S.; Narang, A.; Ding, X.; Eadon, G. *Chem. Res. Toxicol.* **2004**, *17*, 502–511.

(11) Espandiari, P.; Glauert, H. P.; Lehmler, H.-J.; Lee, E. Y.; Srinivasan, C.; Robertson, L. W. *Toxicol. Sci.* **2004**, *79*, 41–46.

(12) Song, Y.; Wagner, B. A.; Lehmler, H.-J.; Buettner, G. R. *Chem. Res. Toxicol.* **2008**, *21*, 1359–1367.

(13) Waller, S. C.; He, Y. A.; Harlow, G. R.; He, Y. Q.; Mash, E. A.; Halpert, J. R. *Chem. Res. Toxicol.* **1999**, *12*, 690–699.

(14) Lin, P.-H.; Sangaiah, R.; Ranasinghe, A.; Ball, L. M.; Swenberg, J. A.; Gold, A. *Org. Biomol. Chem.* **2004**, *2*, 2624–2629.

(15) Shaikh, N. S.; Parkin, S.; Lehmler, H.-J. *Organometallics* **2006**, *25*, 4207–4214.

(16) Goldstein, J. A.; Hass, J. R.; Linko, P.; Harvan, D. J. *Drug Metab. Dispos.* **1978**, *6*, 258–264.

(17) Moron, M.; Sundström, G.; Wachtmeister, C. A. *Acta Chem. Scand.* **1973**, *27*, 3121–3122.
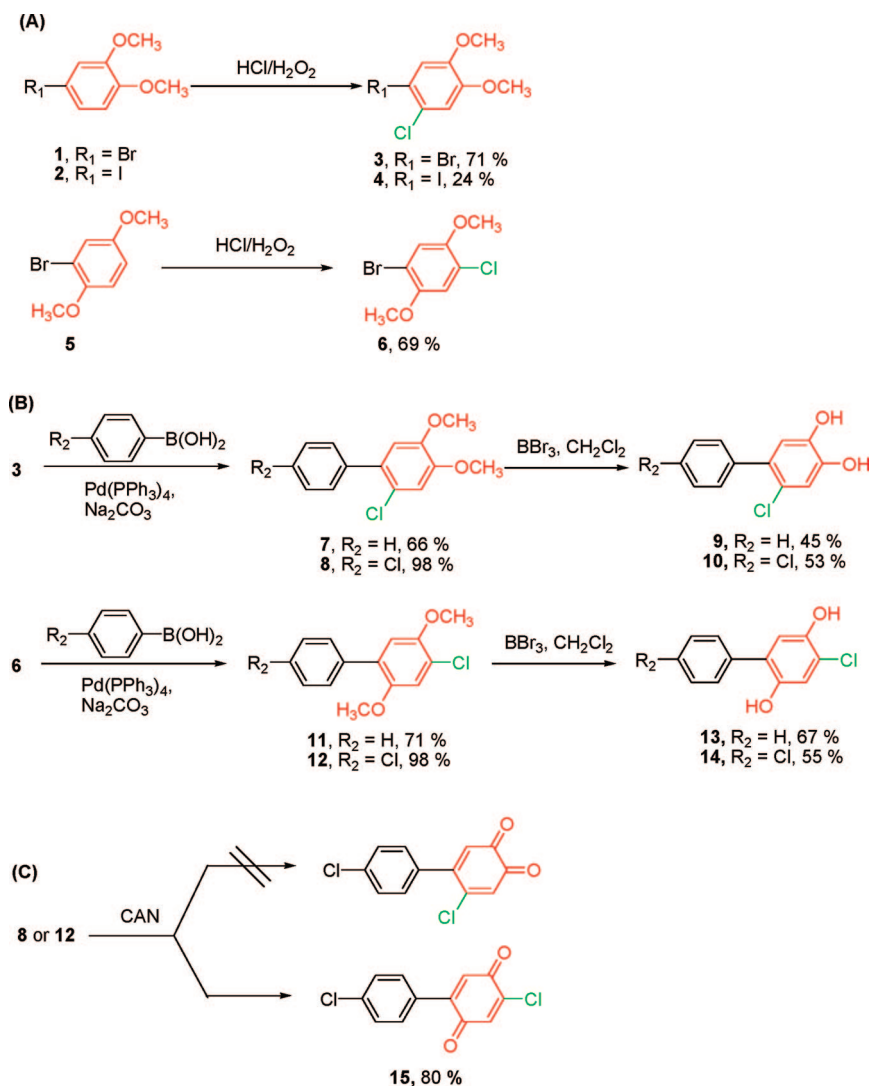
(18) McLean, M. R.; Bauer, U.; Amaro, A. R.; Robertson, L. W. *Chem. Res. Toxicol.* **1996**, *9*, 158–164.

(19) Lehmler, H.-J.; Robertson, L. W. *Chemosphere* **2001**, *45*, 137–143.

(20) Lehmler, H.-J.; Robertson, L. W. *Chemosphere* **2001**, *45*, 1119–1127.

(21) Kania-Korwel, I.; Parkin, S.; Robertson, L. W.; Lehmler, H. J. *Chemosphere* **2004**, *56*, 735–744.

SCHEME 1.   Synthesis of PCB Hydroquinones/Quinones with One Chlorine on the Oxygenated Ring

metabolites of several lower chlorinated biphenyls using the Suzuki coupling reaction.

Chlorination of aromatic compounds with a H$_2$O$_2$/HCl system was developed several decades ago and, for example, used to fully chlorinate dimethoxy-benzene **2** for the synthesis of a dihydroxy metabolite of PCB 136 (2,2′,3,3′,6,6′-hexachlorobiphenyl).[13] However, controlling the regioselectivity of this chlorination reaction appears to be a challenge,[22] possibly because this chlorination reaction is a radical reaction. Here we report that dimethoxy-benzenes **1**, **2**, and **5** can be chlorinated regioselectively with H$_2$O$_2$/HCl (Scheme 1A). The best yields were obtained at molar ratios of dimethoxy-benzene/H$_2$O$_2$/HCl of 1:4:8. However, the monochlorinated products **3**, **4**, and **6** were even isolated when an excess of chlorination reagent (8 equiv of HCl) was employed. The yields for iodo compound **2** (24%) were significantly lower compared to bromo compound **1** (71%).

GC−MS analysis showed that monochlorination of dimethoxybenzenes **1** and **2** resulted in the formation of traces of 4,5-dichloro-1,2-dimethoxy-benzene and 4,5-dibromo- (or 4,5-diiodo-) 1,2-dimethoxy-benzene. Similar side products were observed with dimethoxy-benzene **5**. These side reactions, which are consistent with a radical mechanism, explain the lower yield with the iodo compound **2**. The yield of debrominated side products (i.e., the respective chlorinated dimethoxy benzenes) increased when an excess of H$_2$O$_2$/HCl was employed. Overall, chlorination with H$_2$O$_2$/HCl is not a straightforward approach for the preparation of bromo (or iodo) dimethoxy-benzenes with two or three chlorine substituents because the impurities are difficult to remove.

The brominated dimethoxy-benzenes **3** and **6** were subsequently converted into dihydroxy compounds **9**−**10** and **13**−**14**. These compounds are putative metabolites of 2-chlorobiphenyl (PCB 1), 2,4′-dichlorobiphenyl (PCB 8), 4-chlorobiphenyl (PCB 3), and 4,4′-dichlorobiphenyl (PCB 15), respectively (Scheme 1B). In the first step of the synthesis, the corresponding dimethoxy PCBs were synthesized with the Suzuki coupling of **3** and **6** with benzene or 4-chlorobenzene boronic acid.[19−21] This cross-coupling reaction was highly efficient in the case of 4-chlorophenylboronic acid (98% yield for compounds **8** and **12**), whereas lower yields were obtained for Suzuki coupling reactions involving benzene boronic acid (66% and 71% for compounds **7** and **11**, respectively). These comparatively low yields are probably due to the more difficult purification of lower

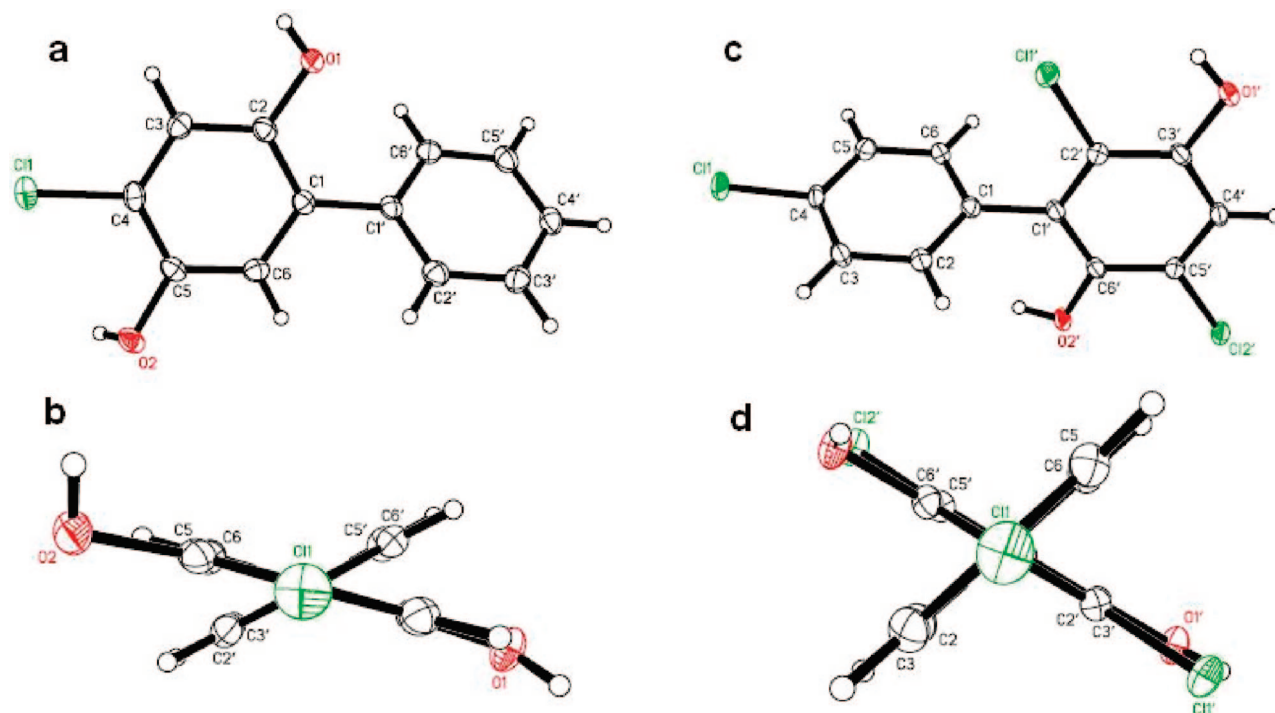(22) Samant, B. S.; Saraf, Y. P.; Bhagwat, S. S. *J. Colloid Interface Sci.* **2006**, *302*, 207–213.

FIGURE 1. Molecular structures derived from the crystal structure of **13** and **20**. (a) Molecular structure of 4-chloro-biphenyl-2,5-diol (**13**) showing the atom-labeling scheme and (b) view of 4-chloro-biphenyl-2,5-diol (**13**) along the C1−C1′ axis illustrating the nonplanar conformation of the molecule. (c) Molecular structure of 3,6,4′-trichloro-biphenyl-2,5-diol (**20**) showing the atom-labeling scheme and (d) view of 3,6,4′-trichloro-biphenyl-2,5-diol (**20**) along the C1−C1′ axis illustrating the nonplanar conformation of the molecule. Displacement ellipsoids are drawn at the 50% probability level.

chlorinated biphenyls with *ortho* substituents, consistent with earlier observations from our laboratory.[19−21] Subsequently, the dimethoxy PCBs were demethylated with BBr₃ in dichloromethane.[20,21] Recrystallization using CHCl₃/*n*-hexanes = 1:3 (v/v) gave the desired PCB catechol and hydroquinone metabolites in moderate-to-good yields. The only exception was compound **9**, which was relatively unstable at ambient temperature.

The structure of PCB hydroquinone **13** was further characterized by single-crystal X-ray diffraction analysis (Figure 1a and b). The solid state twist angle of compound **13**, an important structural determinant of the toxicity of PCBs and their metabolites, is 43.3°.

PCB *o*-quinones are typically synthesized in situ by oxidation of the corresponding catechols with Ag₂O.[11] This approach has the drawback that PCB catechols themselves are unstable and cannot be stored for extended periods of time without considerable degradation. Therefore, we explored the direct oxidation of dimethoxy PCBs **8** or **12** to the corresponding PCB quinones using CAN, a strong one-electron oxidizing reagent that can be used as a reagent for a wide variety of organic transformations, including the oxidation of 1,4-hydroquinones and methoxy compounds to the corresponding quinones.[23] Unfortunately, oxidation of compound **8** with CAN failed to yield the desired PCB *o*-quinone as the major product and in a purity sufficient for biological studies. However, the oxidation of dimethoxy PCB **12** with CAN gave the PCB *p*-quinone **15** in good yield (Scheme 1C).

Synthesis of PCB Hydroquinones/Quinones with Two Chlorine Substituents on the Oxygenated Ring. Reaction of **1**, **2**, and **5** with excess of H₂O₂/HCl resulted in partial sub-

stitution of bromine (or iodine) with chlorine. This approach was abandoned because the resulting byproducts were tedious to separate from the desired products. Chlorination of **1** and **5** with other chlorination reagents, such as NCS, was also not successful. Therefore, we explored a different synthetic strategy and investigated the introduction of a substituent suitable for the Suzuki coupling reaction (e.g., bromine) into a dichlorinated dimethoxy-benzene, such as **16**. Although the bromination of bulky and unreactive aromatic compounds is well documented in the literature, the bromination of compound **16** proved to be a challenge. Bromination of **16** with a series of bromination reagents (such as Br₂/AcOH,[24] pyridinium bromide perbromide,[25] NBS[26] and NBS/HBF₄·Et₂O[27]) did not yield the desired product. Only Br₂ in the presence of iron powder[28] yielded a brominated product in 43% yield (Scheme 2). Surprising, the product was not the desired dimethoxy-benzene **18** but the demethylated product **17**. The dimethoxy-benzene **18** was obtained by methylation with Me₂SO₄. Subsequently, the dimethoxy PCB **19** was obtained in moderate yield by the Suzuki coupling of **18** with 4-chlorobenzene boronic acid.

Demethylation with BBr₃ gave the desired hydroquinone **20**. The structure of compound **20** was confirmed by single-crystal X-ray diffraction analysis (Figure 1c and d). The solid state twist angle of compound **20** is 73.2° and, because of the *ortho*

(23) Nair, V.; Deepthi, A. *Chem. Rev.* **2007**, *107*, 1862–1891.

(24) Clive, D. L. J.; Khodabocus, A.; Vernon, P. G.; Angoh, A. G.; Bordeleau, L.; Middleton, D. S.; Lowe, C.; Kellner, D. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1433–1444.

(25) Shimbashi, A.; Tsuchiya, A.; Imoto, M.; Nishiyama, S. *Bull. Chem. Soc. Jpn.* **2004**, *77*, 1925–1930.
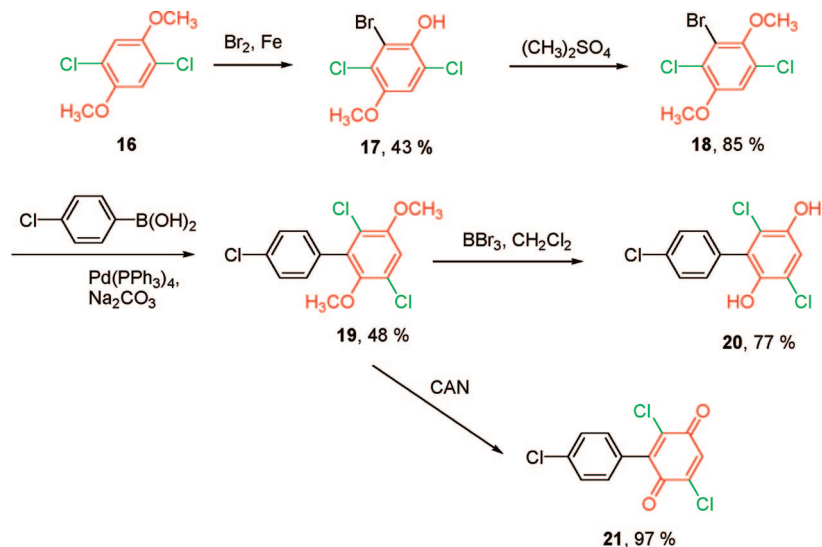
(26) Goldberg, Y.; Bensimon, C.; Alper, H. *J. Org. Chem.* **1992**, *57*, 6374–6376.

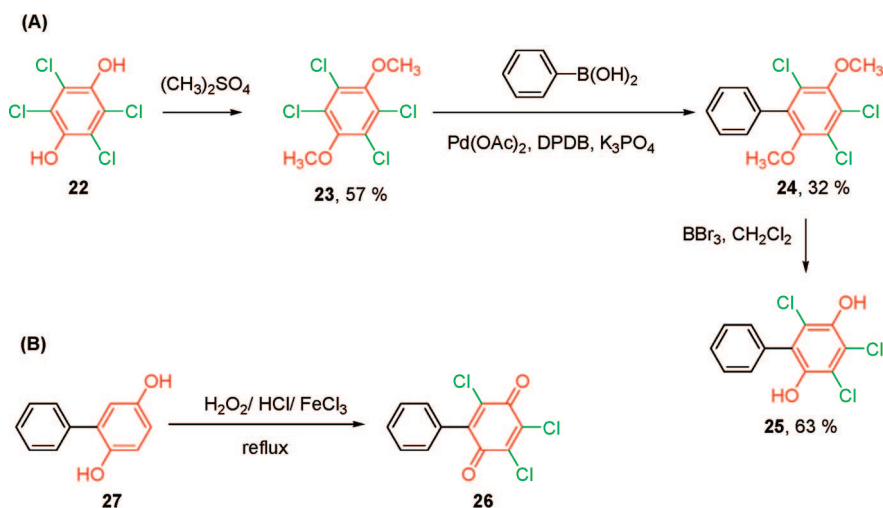(27) Oberhauser, T. *J. Org. Chem.* **1997**, *62*, 4504–4506.

(28) Liu, H.; Bernhardsen, M.; Fiksdahl, A. *Tetrahedron* **2006**, *62*, 3564–3572.

SCHEME 2. Synthesis of PCB Hydroquinones/Quinones with Two Chlorines on the Oxygenated Ring

SCHEME 3. Synthesis of PCB Hydroquinones/Quinones with Three Chlorines on the Oxygenated Ring

chlorine substituent, larger compared to the twist angle of compound **13**. Oxidation of **19** with CAN yielded *p*-quinone PCB **21** (Scheme 2). The oxidation of compounds **19** to **21** was slower than the oxidation of the monochlorinated compounds **12** to **15** (1 vs 3 h), which suggests that multiple chlorine substituents reduce the reaction rate of CAN oxidations.

Synthesis of PCB Hydroquinones/Quinones with Three Chlorine Substituents on the Oxygenated Ring. We explored the synthesis of PCB hydroquinones and quinones with three chlorines on the oxygenated ring via the Suzuki coupling reaction with the goal to exploit the selectivity and versatility of this approach. The Suzuki coupling of a chlorinated (instead of a brominated) benzene derivative with a boronic acid is generally not suitable for the synthesis of PCB derivatives because of the unselective coupling of all chlorine substituents. However, the Suzuki coupling of symmetrical chlorinated dimethoxy-benzenes, such as 2,3,5,6-tetrachloro-1,4-dimethoxy-benzene **23**, with benzene boronic acids can be used for the synthesis of the desired dimethoxy biphenyls, such as **24** (Scheme 3A). The Suzuki coupling reaction of **23** with benzene boronic acids using Pd(PPh₃)₄ with Na₂CO₃−toluene[19−21] or Pd(OAc)₂ with di-*tert*-butylphosphino-biphenyl as ligand and potassium phosphate as base did not yield the desired product

24. However, crude compound **24** (containing 12.5% of 2,3,5-trichloro-1,4-dimethoxy-benzene) was obtained with Pd(OAc)₂ in 32% yield using 2-dicyclohexylphosphino-2',6'-dimethoxy-biphenyl (DPDB) as the ligand and potassium phosphate as the base.[29−31] Subsequently, demethylation of crude **24** with BBr₃[20,21] yielded 3,4,6-trichloro-biphenyl-2,5-diol **25** in good yield (Scheme 3A).

Several approaches were investigated for the synthesis of 2,3,5-trichloro-6-phenyl-[1,4]benzoquinone **26**, including CAN oxidation of **24** (as described above), coupling of chloranil and benzene boronic acid with Pd(OAc)₂/2-dicyclohexylphosphino-2',6'-dimethoxy-biphenyl/potassium phosphate, and chlorination of biphenyl-2,5-diol **27** with H₂O₂/HCl/FeCl₃.[32] However, the purification of **26** proved to be difficult because the compound rapidly degraded during handling, even at subambient temperatures.

EPR Studies of PCB Semiquinones. Dihydroxylated aromatic compounds, for example dopamine,[33] catechol estrogens,[34,35]

(29) Zapf, A.; Ehrentraut, A.; Beller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4153–4155.

(30) Wolfe, J. P.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 2413–2416.

(31) Wolfe, J. P.; Singer, R. A.; Yang, B. H.; Buchwald, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 9550–9561.

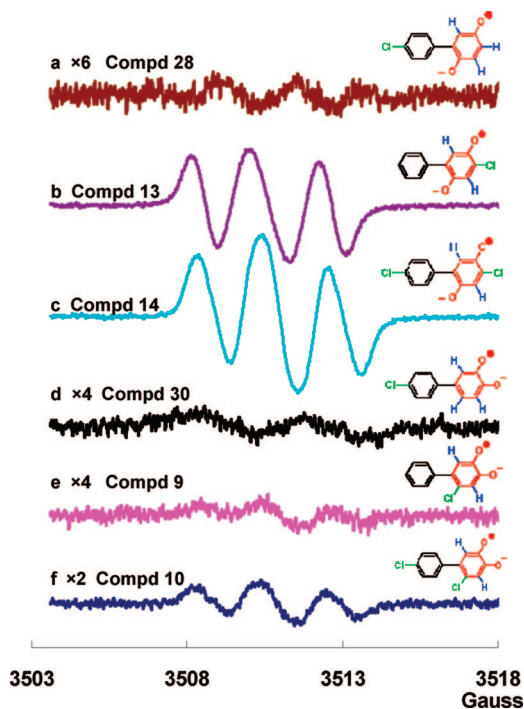(32) Wilson, J. G. *Aust. J. Chem.* **1972**, *25*, 2383–2391.

FIGURE 2. EPR spectra of semiquinones generated from PCB hydroquinones with differing numbers and positions of chlorine on the phenyl rings. Final concentrations of the original hydroquinones were 100 μM in phosphate buffer, pH = 7.4. (a) SQ$^{\bullet-}$ generated from 4′-chloro-biphenyl-2,5-diol (**28**). (b) SQ$^{\bullet-}$ generated from 4-chloro-biphenyl-2,5-diol (**13**). (c) SQ$^{\bullet-}$ generated from 4,4′-dichloro-biphenyl-2,5-diol (**14**). (d) SQ$^{\bullet-}$ generated from 4′-chloro-biphenyl-3,4-diol (**30**). (e) SQ$^{\bullet-}$ generated from 6-chloro-biphenyl-3,4-diol (**9**). (f) SQ$^{\bullet-}$ generated from 6,4′-dichloro-biphenyl-3,4-diol (**10**).

or stilbene catechols,[36] form reactive semiquinone radicals that play an important role in their toxicity. Similarly, PCB hydroquinones, catechols, and quinones can form semiquinone radicals[12] that have been implicated as reactive intermediates in PCB toxicity.[9,37,38] Unfortunately, little is known about the effects of chemical structure (e.g., substitution pattern and degree of chlorination) on the properties of PCB semiquinone radicals (SQ$^{\bullet-}$) and, ultimately, their toxicity. Here we investigate the SQ$^{\bullet-}$ formed from PCB hydroquinones and quinones with varying numbers of chlorines on the oxygenated ring.

Autoxidation of the 2,5-dihydroxylated PCB metabolite **28** (4′-chloro-biphenyl-2,5-diol) resulted in a weak but distinct approximate 1:3:3:1 four-line spectrum ($a^{H3} = a^{H4} = 2.1$ G and $a^{H6} = 2.5$ G at pH 7.4[12,39]), Figure 2a. This spectrum is typical for oxygenated PCB metabolites with no chlorine substituent on the oxygenated phenyl ring.[12,40] We have shown previously that neither chlorine nor hydrogen substituents on

(33) Kalyanaraman, B.; Premovic, P. I.; Sealy, R. C. *J. Biol. Chem.* **1987**, *262*, 11080–11087.

(34) Seacat, A. M.; Kuppusamy, P.; Zweier, J. L.; Yager, J. D. *Arch. Biochem. Biophys.* **1997**, *347*, 45–52.

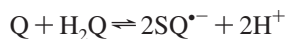(35) Kalyanaraman, B.; Sealy, R. C.; Sivarajah, K. *J. Biol. Chem.* **1984**, *259*, 14018–14022.

(36) Kalyanaraman, B.; Sealy, R. C.; Liehr, J. G. *J. Biol. Chem.* **1989**, *264*, 11014–11019.

(37) Sadeghi-Aliabadi, H.; Chan, K.; Lehmler, H.-J.; Robertson, L. W.; O'Brien, P. J. *Chem.-Biol. Interact.* **2007**, *167*, 184–192.

(38) McLean, M. R.; Twaroski, T. P.; Robertson, L. W. *Arch. Biochem. Biophys.* **2000**, *376*, 449–455.

(39) Ashworth, P.; Dixon, W. T. *J. Chem. Soc., Perkin Trans. 2* **1972**, 2264–2267.

(40) Pedersen, J. A. *Handbook of EPR Spectra from Quinones and Quinols*; CRC Press, Inc.: Boca Raton, FL, 1985.

the nonoxygenated phenyl ring tend to exhibit hyperfine splittings in the EPR spectra, but they can contribute to the line width.[12] Under identical experimental conditions (100 μM, pH 7.4), the autoxidation of 4-chloro-biphenyl-2,5-diol (**13**) gave an approximate 1:2:1 three-line spectrum with $a^{H3} = 1.7$ G and $a^{H6} = 2.3$ G (Figure 2b). Interestingly, the yield of SQ$^{\bullet-}$ of **13** was 15-fold higher compared to SQ$^{\bullet-}$ from **28**, suggesting that the electronegative chlorine substituent contributes to this increase in the yield of SQ$^{\bullet-}$. As expected, compound **14**, with one chlorine substituent on each phenyl ring gives an approximate 1:2:1 three-line spectrum for its SQ$^{\bullet-}$ (Figure 2c). Interestingly, the intensity of this spectrum is about 30% greater than the EPR spectrum for SQ$^{\bullet-}$ from **13**, again consistent with the electronegative chlorine stabilizing SQ$^{\bullet-}$.

In contrast to SQ$^{\bullet-}$ derived from PCB *p*-hydroquinones, the EPR spectra of SQ$^{\bullet-}$ formed by oxidation PCB *o*-hydroquinones **30** (4′-chloro-biphenyl-3,4-diol) and **9** are quite weak, yielding an irregular spectrum[12] and a 1:2:1 three-line spectrum ($a^{H3} = 2.1$ G and $a^{H6} = 2.1$ G), respectively (Figure 2d and e). Introducing a second chlorine such that both the oxygenated and nonoxygenated rings have a chlorine (**10**) results in a somewhat stronger EPR spectrum for the corresponding SQ$^{\bullet-}$ (1:2:1 three-line, $a^{H3} = 2.1$ G and $a^{H6} = 2.1$ G) compare Figure 2f to 2d and 2e. However, the apparent stabilizing effect of these chlorines on the corresponding SQ$^{\bullet-}$ is much less for PCB *o*-SQ$^{\bullet-}$ than PCB *p*-SQ$^{\bullet-}$. The greater steady-state concentration for the PCB *p*-SQ$^{\bullet-}$ compared to PCB *o*-SQ$^{\bullet-}$ is most likely due a slower rate of disproportionation for the *p*-SQ$^{\bullet-}$.

Because of the dramatic influence of chlorine on the persistence of SQ$^{\bullet-}$ derived from PCB *p*-hydroquinone (Figure 2b and c), we investigated the influence of additional chlorines on the persistence of the corresponding SQ$^{\bullet-}$. Figure 3 shows the EPR spectra of SQ$^{\bullet-}$ formed by autoxidation of a series of PCB *p*-hydroquinones and quinones with 0 to 3 chlorines on the oxygenated phenyl ring; as the number of chlorines increases, the persistence of the corresponding SQ$^{\bullet-}$ increases. As we have reported, the SQ$^{\bullet-}$ formed from 4′-chloro-biphenyl-2,5-diol (**28**) and 2-(4-chloro-phenyl)-[1,4]benzoquinone (**29**) are essentially identical (Figure 3a and b).[12] Similarly, the SQ$^{\bullet-}$ formed by PCB hydroquinones **14**, **20**, and **25** are identical to the SQ$^{\bullet-}$ formed by the corresponding PCB quinones **15**, **21**, and **26**, respectively. As anticipated, the spectra change with increasing chlorine (and decreasing hydrogen) substitution from 1:3:3:1 four-line (**28** and **29**) to 1:2:1 three-line (**14** and **15**, $a^{H3} = 1.7$ G and $a^{H6} = 2.3$ G) to 1:1 two-line (**20** and **21**, $a^{H4} = 1.9$ G) to one-line spectra (**25** and **26**, no hyperfine splitting). Another interesting phenomenon is that the g-factor of each of the SQ$^{\bullet-}$ changed, with the increasing of numbers of chlorine: 2.0047 (**28** and **29**); 2.0050 (**14** and **15**); 2.0051 (**20** and **21**); and 2.0054 (**25** and **26**). This systematic increase in g-value is consistent with the increased spin−orbit coupling that is expected with increasing number of chlorines.

The most intriguing observation is the apparent exponential increase in the initial yield of SQ$^{\bullet-}$ with increasing degree of chlorination in the oxygenated ring system (Figure 3). The apparent yield of SQ$^{\bullet-}$ increases from 0.000 092/0.000 12 for **28/29** to 0.23/0.23 for **25/26**. Thus, as the degree of chlorination increases, the persistence of the radical also increases. The three-dimensional structure did not influence SQ$^{\bullet-}$ persistence (e.g., the dihedral angle; Figure 1b and d). To examine this systematically, we generated each of the radicals shown in Figure 3 via the comproportionation reaction of quinone and hydroquinone.

$$Q + H_2Q \rightleftharpoons 2SQ^{\bullet-} + 2H^+$$

The expression for the equilibrium constant for this reaction has the form $K = [SQ^{\bullet-}]^2/[\text{hydroquinone}][\text{quinone}]$, assuming constant pH. EPR spectra were collected and quantitated from solutions with equal concentrations of PCB hydroquinone and PCB quinone and the resulting concentrations used to estimate the equilibrium constant for the comproportionation reaction of each set of PCB hydroquinones and quinones of Figure 3. As seen in Figure 4, the estimated equilibrium constant increases by approximately $10^{2.3} = 200$ with each addition of chlorine to the oxygenated ring. It is quite amazing that nearly 64% of the trichloro-hydroquinone/quinone equilibrium system (**25/26**) exists as the semiquinone radical. These observations demonstrate the additive nature of the ring chlorines and the size of the MO coefficients to the persistence of the corresponding radical.

In summary, in this work we have presented efficient and high-yielding synthetic routes for the preparation of a series of oxygenated PCB metabolites with differing degrees of chlorination. The synthesis of these compounds is straightforward. These oxygenated PCBs readily react with oxygen or via comproportionation to yield corresponding semiquinone free radicals. The greater the number of chlorines on the oxygenated ring, the more persistent the semiquinone radical.

Experimental Section

General Procedure for the Preparation of Chlorinated Bromo-(or Iodo-)dimethoxy-benzenes 3, 4, and 6. 1-Bromo-4-chloro-2,5-dimethoxy-benzene (6).[13] Hydrogen peroxide (30%, 15 mL, 145 mmol) was added over 1 h to a rapidly stirred solution of 1-bromo-2,5-dimethoxy-benzene **5** (7.6 g, 35 mmol) in CHCl3 (30 mL) and concentrated HCl (24 mL, 280 mmol). After 16 h at room temperature, the mixture was extracted with CHCl3 (3 × 30 mL). The organic extracts were combined, washed with 5% NaHSO3 (25 mL), dried over Na2SO4, and filtered, and the solvent was evaporated under reduced pressure. The product was purified by column chromatography on silica gel with *n*-hexanes/CHCl3 = 1:1 (v/v), yielding 5.7 g (69%) of **6** as white crystals. Mp 134−135 °C; ^{1}H NMR (400 MHz, CDCl3) δ 7.12(s, 1H), 6.94(s, 1H), 3.85(s, 3H), 3.84(s, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 150.3, 149.6, 121.8, 117.4, 114.3, 109.6, 57.1, 57.0; MS *m/z* (relative intensity) 250(61, M$^{\bullet+}$), 235(7). Anal. Calcd for C8H8BrClO2: C, 38.18; H, 3.21. Found: C, 38.33; H, 3.03.

General Suzuki Coupling Procedure A for the Synthesis of 7, 8, 11, 12, and 19. 4,4′-Dichloro-2,5-dimethoxy-biphenyl (12).[19−21] Sodium carbonate (20 mL, 2 M aq) was added to a solution of 1-bromo-4-chloro-2,5-dimethoxy-benzene (**6**) (5.2 g, 20.0 mmol) and Pd(PPh3)4 (0.72 g, 3% molar ratio) in toluene (80 mL). A solution of a 4-chloro-phenylboronic acid (4.7 g, 30.0 mmol) in ethanol/toluene/H2O = 8:1:1 (40 mL total) was added slowly to the mixture under a nitrogen atmosphere. The reaction mixture was maintained at 80 °C for 12 h. Hydrogen peroxide (30%, 2.0 mL) was added slowly to the warm reaction mixture to destroy unreacted boronic acid. The mixture was stirred at room temperature for an additional 4 h and diluted with diethyl ether (120 mL). The reaction mixture was extracted once with NaOH (40 mL, 2 M aq) and three times with water (40 mL). The organic phase was dried over MgSO4, and the solvents were removed under reduced pressure. Column chromatography over silica gel with *n*-hexanes/CHCl3 = 3: 1 (v/v) as eluent followed by recrystallization with *n*-hexanes/CHCl3 = 3: 1 (v/v) yielded 5.8 g (98%) of **12** as white solid. Mp 123−124 °C; ^{1}H NMR (400 MHz, CDCl3) δ 7.46−7.37(m, 4H), 7.02(s, 1H), 6.89(s, 1H), 3.89(s, 3H), 3.77(s, 3H); ^{13}C NMR (100

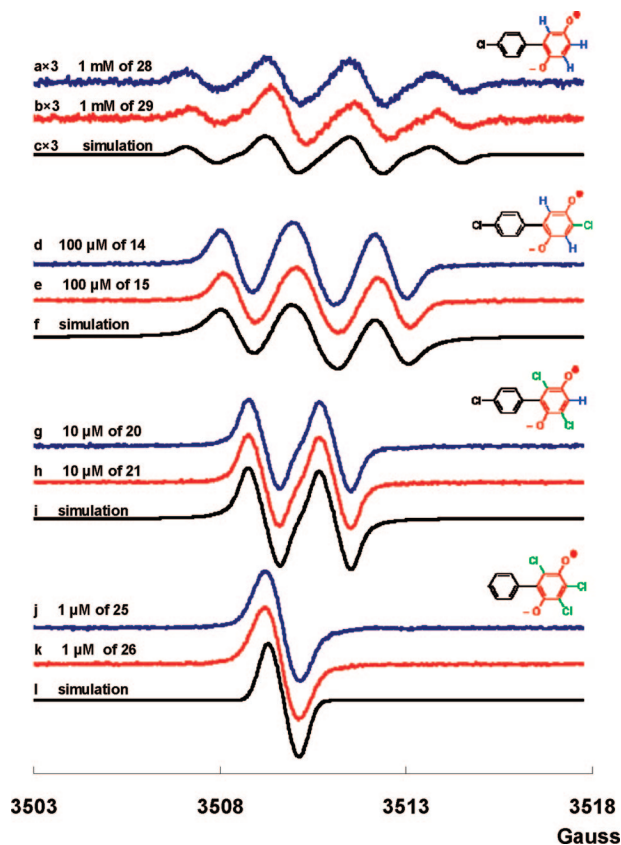(41) Yamazaki, I.; Piette, L. H. *J. Am. Chem. Soc.* **1965**, *87*, 986−989.

FIGURE 3. EPR spectra of semiquinones generated from PCB hydroquinones and quinones with differing numbers of chlorine on the oxygenated phenyl rings. (a) SQ$^{\bullet-}$ generated from 1 mM of 4′-chloro-biphenyl-2,5-diol (**28**) (yield: 0.000 092). (b) SQ$^{\bullet-}$ generated from 1 mM of 2-(4-chloro-phenyl)-[1,4]benzoquinone (**29**) (yield: 0.000 12). (**c**) Computer simulation of SQ$^{\bullet-}$ with three hydrogens. (d) SQ$^{\bullet-}$ generated from 100 μM of 4,4′-dichloro-biphenyl-2,5-diol (**14**) (yield: 0.004 5). (e) SQ$^{\bullet-}$ generated from 100 μM of 2-chloro-5-(4-chloro-phenyl)-[1,4]benzoquinone (**15**) (yield: 0.003 5). (f) Computer simulation of SQ$^{\bullet-}$ with two hydrogens. (g) SQ$^{\bullet-}$ generated from 10 μM of 3,6,4′-trichloro-biphenyl-2,5-diol (**20**) (yield: 0.026). (h) SQ$^{\bullet-}$ generated from 10 μM of 2,5-dichloro-3-(4-chloro-phenyl)-[1,4]benzoquinone (**21**) (yield: 0.025). (i) Computer simulation of SQ$^{\bullet-}$ with one hydrogen. (j) SQ$^{\bullet-}$ generated from 1 μM of 3,4,6-trichloro-biphenyl-2,5-diol (**25**) (yield: 0.23). (k) SQ$^{\bullet-}$ generated from 1 μM of 2,3,5-trichloro-6-phenyl-[1,4]benzoquinone (**26**) (yield: 0.23). (l) Computer simulation of SQ$^{\bullet-}$ with no hydrogen. These spectra were collected using a modulation amplitude of 1.0 Gauss. Lowering the modulation amplitude to 0.1 Gauss (for spectra d and e) or 0.01 Gauss (for spectra g, h, j, and k) did not reveal any possible hyperfine splittings from the chlorines on the semiquinone moiety.

MHz, CDCl3) δ 150.7, 149.6, 136.3, 133.6, 131.0, 128.9, 128.5, 122.3, 115.2, 114.4, 57.2, 56.6; MS *m/z* (relative intensity) 282(88, M$^{\bullet+}$), 267(43), 232(100), 217(30), 189(17), 161(11) 126(16). Anal. Calcd for C14H12Cl2O2: C, 59.36; H, 4.27. Found: C, 59.24; H, 4.19.

General Demethylation Procedure for the Synthesis of 9, 10, 13, 14, 20, and 25. 4,4′-Dichloro-biphenyl-2,5-diol (14).[20,21] Boron tribromide (18.0 mL, 1 M in *n*-hexanes, 2.2 equiv) was added to a solution of the 4,4′-dichloro-2,5-dimethoxy-biphenyl (**12**) (2.36 g, 8.3 mmol) in anhydrous dichloromethane (50 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 14 h, cooled with ice/salt, and hydrolyzed with an equal volume of ice-cold water. The aqueous phase was extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with water (3 × 25 mL) and dried over MgSO4, and the solvent was removed under reduced pressure. The product was purified by column chromatography on silica gel with *n*-hexanes/ethyl acetate
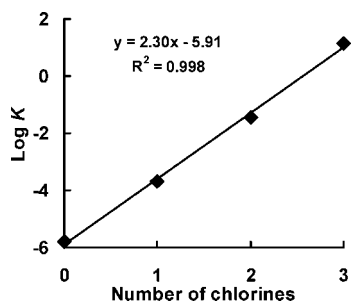
FIGURE 4. The equilibrium constant for the comproportionation reaction to form SQ$^{\bullet-}$ increases with the number of chlorines on the oxygenated ring. Assay conditions: quinone and hydroquinone mixture in 100 mM PBS buffer, pH 7.4. (a) Zero chlorine, 50 μM of **28** and 50 μM of **29** yields [SQ$^{\bullet-}$]$_{ss}$ = 63 nM. (b) One chlorine, 5 μM of **14** and 5 μM of **15** yields [SQ$^{\bullet-}$]$_{ss}$ = 70 nM. (c) Two chlorines, 500 nM of **20** and 500 nM of **21** yields [SQ$^{\bullet-}$]$_{ss}$ = 87 nM. (d) Three chlorines, 50 nM of **25** and 50 nM of **26** yields [SQ$^{\bullet-}$]$_{ss}$ = 65 nM. $K = $[SQ$^{\bullet-}$]^2/[hydroquinone][quinone]. This equilibrium constant will actually be dependent on pH because the pK_a's for the hydroquinones will be above pH or near 7, whereas the pK_a of the semiquinone radicals will be somewhat acidic; for example, the pK_a of the semiquinone radical of unsubstituted benzoquinone/hydroquinone is 4.25.[41]

= 1:1 (v/v) as eluent followed by recrystallization using *n*-hexanes/CHCl$_3$ = 3: 1 (v/v) yielded 1.18 g (55%) of **14** as white solid. Mp 118−120 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.47−7.38(m, 4H), 6.98(s, 1H), 6.91(s, 1H), 5.19(s, 1H), 4.75(s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 146.4, 145.7, 134.7, 134.5, 130.5, 129.6, 127.6, 119.8, 117.2, 116.6; MS *m/z* (relative intensity) 398(47, [M + TMS$_2$]$^{\bullet+}$), 348(19), 239(13), 93(27), 73(100, [Si(CH$_3$)$_3$]$^+$). Anal. Calcd for C$_{12}$H$_8$Cl$_2$O$_2$: C, 56.48; H, 3.16. Found: C, 56.31; H, 3.12.

General Procedure for the Synthesis of PCB Quinones 15 and 21 from Methoxylated PCB Derivatives. 2-Chloro-5-(4-chloro-phenyl)-[1,4]benzoquinone (15).[42] 4,4′-Dichloro-2,5-dimethoxy-biphenyl **12** (0.54 g, 1.9 mmol) was dissolved in acetonitrile (10 mL) at 50 °C, a solution of CAN (5.8 g, 10.0 mmol) in water (15 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. The solution was extracted with chloroform (2 × 20 mL) and washed with water (2 × 50 mL). The combined organic phase dried over MgSO$_4$, and the product was purified by column chromatography on silica gel with CHCl$_3$/*n*-hexanes = 2:1 (v/v) as eluent, yielding 0.38 g (80%) of **15** as a yellow solid. Mp 137−139 °C (lit.[43] 147−148 °C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.44(s, 4H), 7.10(s, 1H), 7.00(s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 184.3, 179.7, 145.4, 144.2, 137.3, 134.2, 132.3, 130.9, 130.5, 129.2; MS *m/z* (relative intensity) 254(30, [M + 2H]$^{\bullet+}$), 217(100). Anal. Calcd for C$_{12}$H$_6$Cl$_2$O$_2$: C, 56.93; H, 2.39. Found: C, 56.84; H, 2.35.

2-Bromo-3,6-dichloro-4-methoxy-phenol (17).[28] A solution of bromine (7.0 g, 44 mmol) in dichloromethane (10 mL) was added slowly over 15 min to a solution of 1,4-dichloro-2,5-methoxy-benzene **16** (8.5 g, 41 mmol) and iron powder (1.24 g, 22 mmol) in dichloromethane (50 mL) heated under reflux. After 4 h, another portion of iron powder (1.24 g, 22 mmol) was added and the reaction heated for an additional 4 h. The reaction mixture was allowed to cool to ambient temperature, the iron powder was filtered off, and the product was purified by column chromatography on silica gel with CHCl$_3$/*n*-hexanes = 2: 1 (v/v) as eluent to yield 4.8 g (43%) of **17** as white crystals. Mp 126−127 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.96(s, 1H), 5.60(s, 1H), 3.87(s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 150.2, 144.2, 123.4, 118.9, 113.1, 112.9, 57.4; MS *m/z* (relative intensity) 270(80, M$^+$), 255(100), 227(20). Anal. Calcd for C$_7$H$_5$BrCl$_2$O$_2$: C, 30.90; H, 1.85. Found: C, 30.84; H, 1.73.

(42) Lopez-Alvarado, P.; Avendano, C.; Menendez, J. C. *Synth. Commun.* **2002**, *32*, 3233–3239.

(43) Bagli, J. F.; L'Ecuyer, P. *Can. J. Chem.* **1961**, *39*, 1037–1048.

Suzuki Coupling Procedure B for the Synthesis of 3,4,6-Trichloro-2,5-dimethoxy-biphenyl (24).[29−31] K$_3$PO$_4$ (4.25 g, 20 mmol), Pd(OAc)$_2$ (44 mg, 2% molar ratio), and 2-dicyclohexy-lphosphino-2′,6′-dimethoxybiphenyl (164 mg, 4% molar ratio) were added to a solution of 1,2,4,5-tetrachloro-3,6-dimethoxy-benzene (**23**) (2.62 g, 10.0 mmol) and phenylboronic acid (1.83 g, 15.0 mmol) in toluene (50 mL) under nitrogen atmosphere. The reaction mixture was then stirred at 110 °C for 48 h. Hydrogen peroxide (2.0 mL, 30%) was added slowly to the warm reaction mixture to destroy unreacted boronic acid. The mixture was stirred at room temperature for an additional 4 h and diluted with diethyl ether (60 mL). After filtration, the solvent was extracted once with NaOH (20 mL, 2 M aq) and three times with water (20 mL). The organic phase was dried over MgSO$_4$, and the solvents were removed under reduced pressure. Column chromatography over silica gel with *n*-hexanes/CHCl$_3$ = 3:1 (v/v) as eluent followed by recrystallization with *n*-hexanes/CHCl$_3$ = 3:1 (v/v) yielded 1.03 g (32%) of crude **24** (contained 12.5% of 2,3,5-trichloro-1,4-dimethoxy-benzene) as white solid. Mp 49−51 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ7.50−7.41(m, AA′XX′ system, 3H), 7.33−7.27(m, AA′XX′ system, 2H), 3.93(s, 3H), 3.44(s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 151.8, 150.2, 135.6, 134.3, 130.1, 128.45, 128.41, 127.9, 127.2, 111.6, 61.0, 60.9; MS *m/z* (relative intensity) 316(100, M$^{\bullet+}$), 301(40, M − CH$_3$), 266(80, M − CH$_3$Cl). Anal. Calcd for (C$_{14}$H$_{11}$Cl$_3$O$_2$ + 1/8C$_{14}$H$_{11}$Cl$_3$O$_2$): C, 51.80; H, 3.44. Found: C, 51.79; H, 3.38.

2,3,5-Trichloro-6-phenyl-[1,4]benzoquinone (26).[32] Biphenyl-2,5-diol (**27**) (2.0 g, 10.7 mmol) was suspended in glacial acetic acid (30 mL), and concentrated HCl (20 mL), followed by anhydrous FeCl$_3$ (3.4 g), was added to the solution. Hydrogen peroxide (20 mL, 30%) was dropped slowly into the reaction mixture (20 min), and the mixture was heated under reflux for an additional 1 h. The mixture was filtered and extracted with CHCl$_3$ (3 × 20 mL). The combined organic phases were washed with saturated NaHCO$_3$ (20 mL) and water (3 × 20 mL) and dried over MgSO$_4$, and the solvents were removed under reduced pressure. Recrystallization with *n*-hexanes/ethyl acetate = 4: 1 (v/v) yielded 2.8 g (91%) of **26** as a yellow solid. Mp 94−95 °C (lit.[32] 112−114 °C); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.58−7.46(m, 3H), 7.40−7.29(m, 2H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 175.6, 171.9, 143.9, 141.6, 140.6, 140.5, 130.5, 130.3, 129.8, 128.5; MS *m/z* (relative intensity) 288(100, [M + 2H]$^{\bullet+}$), 251(90, M − Cl), 233(52); HRMS(EI) calcd for C$_{12}$H$_5$Cl$_3$O$_2$ *m/z* ([M]$^{\bullet+}$) 285.9355, found 285.9350.

EPR Spectroscopy. Electron paramagnetic spectroscopy was performed using a Bruker EMX spectrometer equipped with a High Sensitivity cavity and an Aqua-X sample holder. Spectra were obtained at room temperature. Typical EPR-parameters were 3510.3 G center field; 15 G scan width; 9.854 GHz microwave frequency; 20 mW power; 2 × 10^5 receiver gain; modulation frequency of 100 kHz; modulation amplitude of 1.0 G; with the conversion time and time constant both being 40.96 ms with 5 scans for each 1024-point spectrum. Stock solutions (200 mM each) of PCB hydro-quinones and quinones were prepared in DMSO. All experiments were carried out in 100 mM phosphate buffer containing 250 μM DETAPAC (diethylenetriaminepentaacetic acid) as described previously.[12]

Quantitation was accomplished by double integration of the recorded EPR signals using the WinEPR program with 3-carbox-yproxyl as the standard, $\varepsilon_{234} = 2370 \pm 50$ M^{-1} cm^{-1}.[44] To obtain the best quantitative information on all samples and standards, they were dissolved in identical aqueous buffer; after setup, the 4-bore Aqua-X holder was not removed from the cavity for all samples.

Spectral simulations of EPR spectra were performed using the WinSim program developed at the NIEHS by Duling.[45] Correlation coefficients of simulated spectra were typically >0.99.

(44) Venkataraman, S.; Martin, S. M.; Schafer, F. Q.; Buettner, G. R. *Free Radical Biol. Med.* **2000**, *29*, 580–585.

Supporting Information Available: Experimental procedures, and characterization for compounds **3**, **4**, **7**−**11**, **13**, **18**−**21**, **23** and **25**; ^{1}H NMR and ^{13}C NMR spectra for compounds **3**, **4**, **6**−**15**, **17**−**21**, and **23**−**26**; GC−MS chromatograms and MS spectra for compounds **9**, **24**, and **26**; crystallographic data for **13**, **17**, and **20** in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

(45) Duling, D. R. *J. Magn. Res. Series B* **1994**, *104*, 105–110.