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

J. Org. Chem., 2008, 73 (21), 8296-8304 • DOI: 10.1021/jo801397g • Publication Date (Web): 08 October 2008

Downloaded from http://pubs.acs.org on March 25, 2009

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−) 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, 3,4,6,4′-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
A broad range of adverse biological effects, including (neuro-)
developmental toxicity\(^3\) 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\(^2\) 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.\(^7\) 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 (\(O_2^{-}\)), 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 sulfhydrys, 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 \(H_2Q.\) 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 patterns.\(^13,18\) 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.\(^19\) 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 \(H_2O_2/\text{HCl}\) to overcome these problems and to obtain starting materials that can be used to synthesize putative

---


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


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

Chlorination of aromatic compounds with a \( \text{H}_2\text{O}_2/\text{HCl} \) 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). However, controlling the regioselectivity of this chlorination reaction appears to be a challenge, possibly because this chlorination reaction is a radical reaction. Here we report that dimethoxy-benzenes 1, 2, and 5 can be chlorinated regioselectively with \( \text{H}_2\text{O}_2/\text{HCl} \) (Scheme 1A). The best yields were obtained at molar ratios of dimethoxy-benzene/\( \text{H}_2\text{O}_2/\text{HCl} \) of 1:4:8. However, the monochlorinated products 3, 4, and 6 were even isolated when an excess of chlorination reagent (8 equiv of \( \text{HCl} \)) 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 dimethoxy-benzenes 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 \( \text{H}_2\text{O}_2/\text{HCl} \) was employed. Overall, chlorination with \( \text{H}_2\text{O}_2/\text{HCl} \) 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. 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
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 substitution 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 NBS26 and NBS/HBF427) did not yield the desired product. Only Br2 in the presence of iron powder28 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

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(PPh_3)_4 with Na_2CO_3-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 BBr_3 \(^{20,21}\) 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/dicyclohexylphosphino-2',6'-dimethoxy-biphenyl/potassium phosphate, and chlorination of biphenyl-2,5-diol 27 with H_2O_2/HCl/FeCl_3. \(^{32}\) 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, \(^{34,35}\)...
the nonoxygenated phenyl ring tend to exhibit hyperfine splittings in the EPR spectra, but they can contribute to the line width.\textsuperscript{12} Under identical experimental conditions (100 \(\mu\)M, 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\textsuperscript{−} of 13 was 15-fold higher compared to SQ\textsuperscript{−} from 28, suggesting that the electronegative chlorine substituent contributes to this increase in the yield of SQ\textsuperscript{−}. As expected, compound 14, with one chlorine substituent on each phenyl ring gives an approximate 1:2:1 three-line spectrum for its SQ\textsuperscript{−} (Figure 2c). Interestingly, the intensity of this spectrum is about 30% greater than the EPR spectrum for SQ\textsuperscript{−} from 13, again consistent with the electronegative chlorine stabilizing SQ\textsuperscript{−}.

In contrast to SQ\textsuperscript{−} derived from PCB \(p\)-hydroquinones, the EPR spectra of SQ\textsuperscript{−} formed by oxidation of PCB \(o\)-hydroquinones 30 (4′-chloro-biphenyl-3,4-diol) and 9 are quite weak, yielding an irregular spectrum\textsuperscript{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\textsuperscript{−} (1:2:1 three-line, \(a^{H3} = 2.1\) G and \(a^{H6} = 2.1\) G) compared Figure 2f to 2d and 2e. However, the apparent stabilizing effect of these chlorines on the corresponding SQ\textsuperscript{−} is much less for PCB \(o\)-SQ\textsuperscript{−} than PCB \(p\)-SQ\textsuperscript{−}. The greater steady-state concentration for the PCB \(p\)-SQ\textsuperscript{−} compared to PCB \(o\)-SQ\textsuperscript{−} is most likely due to a slower rate of disproportionation for the \(p\)-SQ\textsuperscript{−}.

Because of the dramatic influence of chlorine on the persistence of SQ\textsuperscript{−} derived from PCB \(p\)-hydroquinones (Figure 2b and c), we investigated the influence of additional chlorines on the persistence of the corresponding SQ\textsuperscript{−}. Figure 3 shows the EPR spectra of SQ\textsuperscript{−} 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\textsuperscript{−} increases. As we have reported, the SQ\textsuperscript{−} 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).\textsuperscript{12} Similarly, the SQ\textsuperscript{−} formed by PCB hydroquinones 14, 20, and 25 are identical to the SQ\textsuperscript{−} 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 1:2 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\textsuperscript{−} 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\textsuperscript{−} with increasing degree of chlorination in the oxygenated ring system (Figure 3). The apparent yield of SQ\textsuperscript{−} 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\textsuperscript{−} 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.
The expression for the equilibrium constant for this reaction has the form \( K = \frac{[SQ^-]^2}{[\text{hydroquinone}][\text{quinone}]} \), 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 (5) (2.7 g, 10 mmol) was treated with 2.0 M aq. NaOH (14 mL, 28 mmol) for 14 h, cooled with ice/salt, and then hydrolyzed with an equal volume of ice-cold water. The aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed three times with water (3 × 50 mL). The organic phase was dried over MgSO4 (25 mL), filtered, and the solvent was evaporated under reduced pressure.

#### General Suzuki Coupling Procedure A for the Synthesis of 7, 8, 11, 12, and 19. 4,4′-Dichloro-2,5-dimethoxy-biphenyl (12).

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 CHCl3 (30 mL) and concentrated HCl (24 mL, 280 mmol) was added over 1 h to a rapidly stirred solution of 1-bromo-4-chloro-2,5-dimethoxy-benzene (6) (5.2 g, 20.0 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 × 250 mL) and dried over Na2SO4 (25 mL), 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 7. The organic extracts were combined, washed with 5% NaHSO3 (25 mL), dried over Na2SO4 (25 mL), and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with n-hexanes/CHCl3 = 1:1 (v/v), yielding 5.8 g (98%) of 12.

---

Song et al. JOC Article

**General Demethylation Procedure for the Synthesis of 9, 10, 13, 14, 20, and 25. 4,4′-Dichloro-2,5-dimethoxy-biphenyl (14).**

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.

---

*J. Org. Chem. Vol. 73, No. 21, 2008*
Silica gel with CHCl3 off, and the product was purified by column chromatography on hexanes/ethyl acetate. The mixture was then stirred at 110 °C 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 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 1.03 g (32%) of crude 26 (contained 12.5% of 2,3,5-trichloro-1,4-dimethoxy-benzene) as white solid. Mp 49–51 °C; 1H NMR (400 MHz, CDCl3) δ 7.47–7.38 (m, 4H, 6H), 6.98(s, 1H), 6.91(s, 1H), 5.19(s, 1H), 4.75(s, 1H); 13C NMR (100 MHz, CDCl3) δ 146.4, 145.7, 134.6, 134.5, 130.5, 127.1, 113.1, 112.9, 112.6, 119.0, 117.2, 116.6; MS m/z (relative intensity) 398(47, [M + TMS]+), 348(19), 239(13), 93(27), 73(100, [Si(CH3)3]2). Anal. Calcd for C12H6Cl2O2: C, 56.93; H, 2.39. Found: C, 56.84; H, 1.73.

**General Procedure for the Synthesis of PCB Quinones 15 and 21 from Methylophed PCB Derivatives. 2-Chloro-5-(4-chlorophenyl)-1,4-benzoquinone (15).** 4’-4’-Dichloro-2,5-dimethoxybiphenyl 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 MgSO4, and the product was purified by column chromatography on silica gel with CHCl3/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); 1H NMR (400 MHz, CDCl3) δ 7.44(s, 4H), 7.10(s, 1H), 7.00(s, 1H); 13C NMR (100 MHz, CDCl3) 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]+), 217(100). Anal. Calcd for C12H8Cl2O2: C, 56.93; H, 2.39. Found: C, 56.84; H, 2.35.

**2-Bromo-3,6-dichloro-4-methoxy-phenol (17).** 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-methoxybenzene 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 CHCl3/n-hexanes = 2: 1 (v/v) as eluent to yield 4.9 g (43%) of 17 as white crystals. Mp 126–127 °C; 1H NMR (400 MHz, CDCl3) δ 6.96(s, 1H), 5.60(s, 1H), 3.87(s, 3H); 13C NMR (100 MHz, CDCl3) δ 150.2, 144.2, 123.4, 118.9, 113.1, 112.9, 111.6, 70.4, 151.8, 150.2, 135.6, 134.3, 130.1, 128.5, 124.1, 127.9, 111.6, 61.0, 60.9; MS m/z (relative intensity) 316(100, M+), 301(40, M – Cl), 266(80, M – Cl). Anal. Calcd for C14H11Cl2O2 + 1/8C2H5OH: C, 51.80; H, 3.38.

**Suzuki Coupling Procedure B for the Synthesis of 3,4,6-Trichloro-2,5-dimethoxy-biphenyl (24).** 29–31 K2PO4 (4.25 g, 20 mmol), Pd(OAc)2 (44 mg, 2% molar ratio), and 2-dicyclohexylphosphino-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 MgSO4, and the solvents were removed under reduced pressure. Column chromatography over silica gel with n-hexanes/CHCl3 = 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; 1H NMR (400 MHz, CDCl3) δ 7.50–7.41 (m, AA’X’ system, 3H), 7.33–7.27 (m, AA’X’ system, 2H), 3.93(s, 3H), 3.44(s, 3H); 13C NMR (100 MHz, CDCl3) 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+), 301(40, M – Cl), 266(80, M – Cl). Anal. Calcd for C12H8Cl2O2 + 1/8C2H5OH: C, 51.80; H, 3.38.

**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 × 106 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 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.

Quantitation was accomplished by double integration of the recorded EPR signals using the WinEPR program with 3-carboxypropyl as the standard, ε237 = 2370 ± 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 NIEMS by Duling.45 Correlation coefficients of simulated spectra were typically >0.99.

**Acknowledgment.** This research was supported by grants ES05605, ES012475, and ES013661 from the National Institute of

---


of Environmental Health Sciences, NIH (H.-J.L. and L.W.R.) and R01GM073929 from the National Institute of General Medical Sciences (G.R.B.) and MRI grant 0319176 (S.P.) from the National Science Foundation. Contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS/NIH or NSF.

**Supporting Information Available:** Experimental procedures, and characterization for compounds 3, 4, 7–11, 13, 18–21, 23 and 25; ¹H NMR and ¹³C 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.

JO801397G