Photosensitizing properties of 2,4-dichlorobenzoic acid and chlorinated biphenyl carboxylic acids, potentially key components of chromophoric dissolved organic matter†

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2,4-Dichlorobenzoic acid and a suite of models of chlorinated biphenyl carboxylic acids were found to be efficient sensitizers of the reactive oxygen species singlet oxygen ($^1O_2$).

A recent report by Repeta *et al.* identified 2,4-dichlorobenzoic acid (2,4-DCBA) and isomers of tetrachlorobiphenylcarboxylic acid (CBPAs) as components of marine chromophoric dissolved organic matter (CDOM).[1] While not the first report of these chlorinated compounds in natural waters,[2,3] the high concentration in ocean water ($<$1–10 μg L^{-1})[1] coupled with their high extinction coefficients, suggests that such compounds may account for a large percentage of the total absorbance of solar radiation by marine CDOM ($>$2%).[1] Along with being the dominant light absorber in most surface waters, CDOM is also the principle photosensitizer, having been implicated in the formation of numerous reactive species including singlet oxygen ($^1O_2$), hydroxyl radical and superoxide.[4] Because CDOM is a complex mixture, it has been difficult to ascertain which functional groups are responsible for the sensitization of reactive species. The identification of individual components of CDOM by Repeta *et al.*, however, allows us to study the photosensitizing ability of these specific compounds. In particular, we have investigated the ability of these compounds to sensitize the formation of $^1O_2$, a reactive oxygen species found in sunlit surface waters known to cause the degradation of anthropogenic and naturally occurring organic substrates.[5–12]

The structural assignment of the chlorinated biphenyl carboxylic acid components of CDOM is ambiguous[1] and the structures proposed are not commercially available. This prompted the use of model compounds that incorporate the essential structural characteristics of the compounds proposed (Table 1).

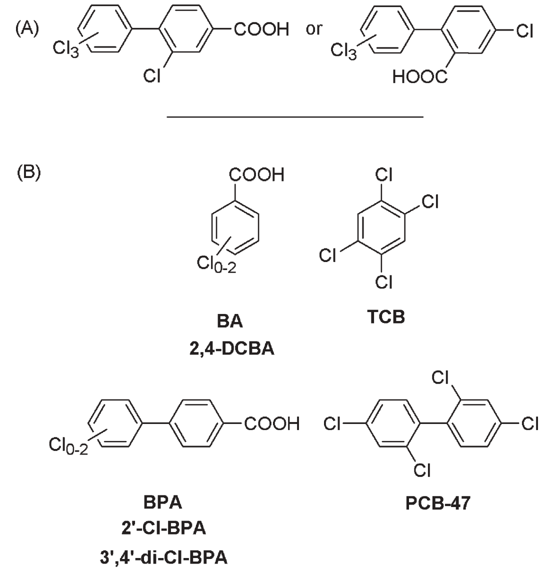
Generation of $^1O_2$ was accomplished with a laser flash photolysis apparatus,[7,13,14] using the 266 nm‡ output of a Nd:YAG laser as the excitation source. The phosphorescent signal of $^1O_2$ at 1270 nm was detected 90° to the incident laser beam with an ultra-sensitive Ge photodiode detector. The signal was collected using a digital oscilloscope, and four transients were averaged to enhance the signal to noise ratio. Representative transients can be seen in Fig. 1, with all solutions optically matched to an absorbance of 1 AU.

The data was fit to an exponential growth and decay curve (eqn. (1)), where A_g and A_∞ are the amplitude of the exponential growth of $^1O_2$ and the amplitude at $t = \infty$, respectively; τ_g and τ_d are the lifetime of the growth and decay of $^1O_2$, and t is the time.[15] The amount of $^1O_2$ formed was calculated from the difference between A_g and A_∞ as the use of the integrated area of the total signal would incorporate any quenching of $^1O_2$ by the substrate, artificially lowering the measured amount of $^1O_2$.

$$\text{Signal} = \left(A_g - A_\infty\right)\left(\frac{\tau_d}{\tau_g - \tau_d}\right)\left(e^{-t/\tau_g} - e^{-t/\tau_d}\right) + A_\infty \qquad (1)$$

The quantum yield (Φ) of $^1O_2$ formation was calculated by comparing the amount of $^1O_2$ produced by each substrate (S) to that generated by perinaphthenone, a well-defined reference (R), in

Table 1 (A) Structures of the biphenyl carboxylic acids proposed to be components of marine CDOM by Repeta *et al.*[a] (B) Model compounds of proposed marine CDOM components used in this study[b]



BA
2,4-DCBA
TCB
BPA
2'-Cl-BPA
3',4'-di-Cl-BPA
PCB-47

[a] Proposed chlorine substitution pattern is either 2′,3′,5′; 2′,4′,5′ or 2′,4′,6′. [b] BA = benzoic acid; 2,4-DCBA = 2,4-dichlorobenzoic acid; TCB = 1,2,4,5-tetrachlorobenzene; BPA = 4-biphenylcarboxylic acid; 2′-Cl-BPA = 2′-chloro-4-biphenylcarboxylic acid; 3′,4′-di-Cl-BPA = 3′,4′-dichloro-4-biphenylcarboxylic acid; PCB-47 = 2,2′,4,4′-tetrachlorobiphenyl.

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† Electronic supplementary information (ESI) available: molar absorptivities of each substrate in the various solvents at 300, 266 and 254 nm and details of the environmental half-life calculations. See http://dx.doi.org/10.1039/b506971h

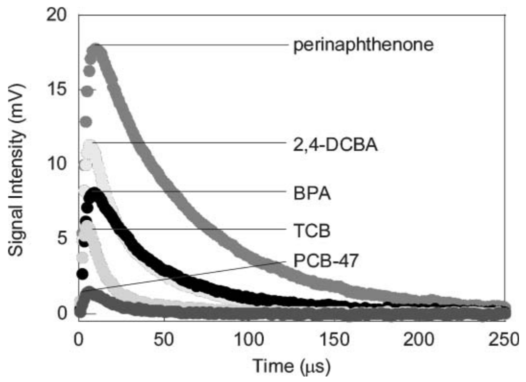


Fig. 1 Representative $^1O_2$ phosphorescence transients for models of the proposed components of CDOM in acetonitrile, with perinaphthenone used as a reference sensitizer. Transients were obtained using an excitation wavelength of 355 nm, with all solutions optically matched to an absorbance of 1 AU.

the corresponding solvent (eqn. (2)).

$$\Phi_S = \frac{(A_g - A_\infty)_S}{(A_g - A_\infty)_R}\Phi_R \qquad (2)$$

The calculated Φ of $^1O_2$ formation for each of the model CDOM components in acetonitrile (CH_3CN), ethanol (EtOH), or deuterium oxide (D_2O) are listed in Table 2.

The Φ of $^1O_2$ production for these substrates are quite high, indicating that these components of CDOM are efficient generators of $^1O_2$, and will contribute significantly to the steady-state $^1O_2$ concentration in sunlit surface waters. The values measured here are of similar magnitude to literature values for biphenyl, 4-chlorobiphenyl and 4,4′-dichlorobiphenyl (0.48, 0.56 and 0.58 in CH_3CN, respectively),[19] with the exception of PCB-47, which has a much lower yield of $^1O_2$. This is believed to be due to the presence of two *ortho*-chlorine substituents. The excited state of biphenyl compounds is thought to be planar; a substitution pattern involving *ortho*-substituents induces strain in this excited state, decreasing its lifetime, and thus decreasing the amount of $^1O_2$ generated.[20–23]

Table 2 Quantum yield of $^1O_2$ formation and direct photolysis for the model CDOM compounds. Errors represent the standard deviation of multiple experiments

Compound	Solvent	Φ Formation of $^1O_2$[a]	Φ Direct photolysis[b]
2,4-DCBA	CH_3CN	0.63 ± 0.03	nd[c]
	EtOH	0.64 ± 0.02	nd
	D_2O–H_2O[d]	0.45 ± 0.02	0.0055 ± 0.0005
BA	CH_3CN	0.42 ± 0.03	nd
	EtOH	0.39 ± 0.02	nd
	D_2O–H_2O	0.37 ± 0.03	0.0027 ± 0.0006
TCB	CH_3CN	0.47 ± 0.02	0.26 ± 0.02
BPA	CH_3CN	0.49 ± 0.04	nd
	EtOH	0.68 ± 0.06	nd
	H_2O	nd	0.00020 ± 0.00005
2′-Cl-BPA	CH_3CN	0.58 ± 0.02	0.008 ± 0.001
	EtOH	0.76 ± 0.07	0.028 ± 0.002
	H_2O	nd	0.08 ± 0.01
3′,4′-di-Cl-BPA	CH_3CN	0.49 ± 0.03	nd
	EtOH	0.66 ± 0.08	nd
	H_2O	nd	0.0011 ± 0.0001
PCB-47	CH_3CN	0.084 ± 0.007	0.089 ± 0.005

[a] Φ of $^1O_2$ production by perinapthenone in CH_3CN = 0.99,[16,17] EtOH = 0.92,[16] and H_2O = 0.98.[17] [b] Values are corrected for screening by substrate.[18] The actinometer was photolyzed in H_2O, and the direct photolysis quantum yields of TCB, 2′-Cl-BPA and PCB-47 were not corrected for differences in the index of refraction between CH_3CN or EtOH and H_2O. [c] nd = not determined. [d] Singlet oxygen quantum yields were measured in D_2O due to the extended lifetime of $^1O_2$ relative to H_2O.

The efficiency of $^1O_2$ formation does not differ substantially between the phenyl and the biphenyl derivatives. Also, when comparing BA to 2,4-DCBA, addition of chlorine substituents increases the quantum yield of $^1O_2$ production. The biphenyl compounds, however, do not follow this trend, indicating that chlorination of the carboxylic acid-substituted ring may be more influential on the efficiency of $^1O_2$ formation than chlorination of the non-acid substituted ring.

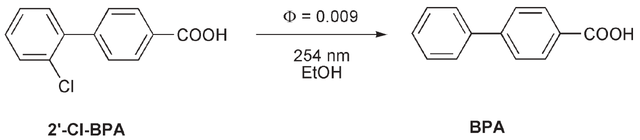
We were interested in the photostability of 2,4-DCBA and the chlorinated biphenyl carboxylic acids, as this will directly influence their persistence in surface waters. Thus, direct photolysis quantum yields were determined. Solutions of each substrate (5–50 μM depending on the molar absorptivity at 254 nm) were photolyzed in unstoppered quartz test tubes using the 254 nm output of low pressure mercury vapor bulbs in a photochemical reactor (Rayonet). Samples were held vertically, and the sample holder was spun on a turntable to ensure even irradiation. Aliquots were removed at various timepoints and analyzed for substrate degradation *via* HPLC with UV-detection. The pseudo-first-order degradation of substrate was compared to that of a reference compound with a known Φ of direct photolysis (uridine, $\Phi_{\text{direct}} = 0.0185$)[24] according to eqn. (3), yielding the Φ of direct photolysis.

$$\Phi_{\text{direct, S}} = \frac{k_S \varepsilon_R^{254\text{nm}}[\text{R}]}{k_R \varepsilon_S^{254\text{nm}}[\text{S}]}\Phi_{\text{direct, R}} \qquad (3)$$

Direct photodegradation was found to be orders of magnitude less efficient than formation of $^1O_2$ for all compounds except TCB and PCB-47, for which the two processes are competitive. The direct photolysis yields for 2′-Cl-BPA and PCB-47 are of similar magnitude to the direct photolysis Φ reported for 2-chlorobiphenyl in H_2O (0.021).[20] The elevated yields for these two compounds are again believed to be attributable to the presence of *ortho*-chlorine substituents and the strain these bulky substituents place on the planar excited state causing rapid degradation.[20–22] The calculated environmental half-lives for direct photolysis at 30° N range from 4 h for 2′-Cl-BPA to 3 years for BPA, again emphasizing the enhanced photolability of compounds containing *ortho*-chlorine substituents (see ESI† for calculation details.)

Loss of *ortho*-chlorine substituents upon photolysis of chlorinated biphenyl compounds is well-known,[20–22,25] and during photolysis of 2′-Cl-BPA in EtOH, the production of BPA was observed. The identity of this photoproduct was confirmed by HPLC chromatographic overlap with an authentic standard of BPA and ESI-TOF MS analysis of the photolysate. The yield of BPA from 2′-Cl-BPA degradation was 31% (Scheme 1).

Thus, the direct photolysis of 2′-Cl-BPA in EtOH yields BPA, and although this product was not observed in photolyses conducted in H_2O, dechlorination products such as hydroxyl-substituted derivatives are still anticipated,[20,25] and these products may still function as $^1O_2$ sensitizers.



Scheme 1 Conversion of 2′-Cl-BPA to BPA in photolyses conducted in EtOH at 254 nm. The quantum yield of dechlorination was calculated as the percent yield of BPA multiplied by the total direct photolysis Φ.

The photoreactivity of both 2,4-DCBA and the CBPAs in the aquatic environment depends not only on their quantum yields of $^1O_2$ formation and direct photolysis, but also on their ability to absorb solar radiation. The chlorine substituents on 2,4-DCBA increase the efficiency of $^1O_2$ formation, but also cause the absorption to undergo a bathochromic shift relative to BA, allowing for greater solar radiation absorption. Similarly, the biphenyl compounds generally absorb longer wavelengths more efficiently than the phenyl compounds. The absorbance of both 2′-Cl-BPA and PCB-47 above 300 nm, however, is substantially less than the other biphenyl compounds due to the non-planarity of the molecules, and thus the amount of solar radiation absorbed by biphenyl compounds containing *ortho*-substituents is expected to be diminished relative to biphenyl compounds with other substitution patterns.

This work suggests that chlorinated benzoic and biphenyl carboxylic acids, if present in the marine environment at the levels found by Repeta *et al.*, will be significant sources for photochemically generated $^1O_2$. Based on the Φ of $^1O_2$ formation by samples of marine CDOM ($\sim$0.06[11]), the Φ of $^1O_2$ production by 2,4-DCBA, and assuming 2,4-DCBA is responsible for 2% of the total absorbance by CDOM, this single chromophore would account for approximately 15% of the $^1O_2$ generated by marine CDOM. This work also indicates that these compounds are weakly photolabile, which should lead to vertical concentration gradients as they are depleted from marine surface waters. Additionally, the magnitude of $^1O_2$ formation will depend on the substitution pattern, as any CBPA containing *ortho*-chlorine substituents absorb less solar radiation, and are expected to undergo chlorine loss in conjunction with sensitization of $^1O_2$. Their degradation products as well as the other isomers of CBPAs and BPA itself, however, are also efficient $^1O_2$ sensitizers.

Notes and references

‡ The use of wavelengths shorter than 300 nm (266 and 254 nm), although not environmentally relevant, were used to calculate the quantum yields of $^1O_2$ formation and direct photolysis due to increased absorption by the substrates (See Table S1. ESI†). Quantum yields of single chromophores are generally wavelength independent, and are thus applicable to environmental systems.[26]

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