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Structure Elucidation and Characterization of Polychlorinated Biphenyl Carboxylic Acids as Major Constituents of Chromophoric Dissolved Organic Matter in Seawater

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Chromophoric or colored dissolved organic matter (CDOM) is one of the principal light adsorbing components of seawater, particularly in the ultraviolet, where it attenuates over 90% of downwelling ultraviolet radiation. In highly productive coastal regions and throughout most of the global ocean, in situ biological production is the major source of CDOM. However, little is known about CDOM composition on the molecular level, and there are only a few reports that link CDOM composition to autochthonous biological sources. Here we report the isolation and characterization of CDOM components from one coastal and two open-ocean sites. Each sample contains a complex mixture of light absorbing (300−400 nm) components, including 2,4-dichlorobenzoic acid and a suite of novel, polychlorinated biphenyl carboxylic acids that closely resemble polychlorinated biphenyls (PCBs) of anthropogenic origin. However, the global inventory and isomer distribution of dissolved chlorinated aromatic acids suggest they are derived from in situ biological production rather than anthropogenic contaminants. These novel chlorinated aromatic acids account for a significant amount of CDOM adsorption in the ultraviolet.

Introduction

Chromophoric or colored dissolved organic matter (CDOM) influences the structure of aquatic ecosystems, complicates satellite measurements of ocean productivity, and affects the bioavailability and fate of important trace organic and metal species (*1*−*4*) in seawater. CDOM absorbs much of the ultraviolet and near-ultraviolet radiation penetrating the ocean, and CDOM absorption in the visible may substantially reduce the penetration of photosynthetically available radiation in some areas as well, limiting primary production (*5*). Photochemical transformation of CDOM leads to the production of low molecular weight organic compounds that act as substrates for microbial production thereby enhancing biological cycling of recalcitrant organic carbon (*6*, *7*). Photochemical transformation of CDOM also leads to the production of reactive oxygen species that oxidize iron, copper, and other trace metals thereby affecting their cycling and biological availability (*8*).

The sources of marine CDOM are not well-known or understood. Degradation of higher plant carbon in soil yields humic substances, structurally complex macromolecular organic matter rich in aromatic and carboxylic acid functional groups, both of which absorb strongly in the ultraviolet (*9*). Rivers supply a significant amount of humic substances leached from soils and decomposing terrestrial organic matter to the coastal ocean (*10*). Comparisons between the chemical composition, stable and radioisotopes, and optical properties of freshwater and coastal CDOM show strong similarities between these two environments, and terrestrial organic matter is thought to be the major source of CDOM in the coastal zone. However, terrestrial humic substances are rapidly removed from seawater by photobleaching and do not supply a significant amount of CDOM to the open ocean (*11*).

In highly productive coastal regions and throughout most of the global ocean, in situ biological production is the major source of CDOM. At some open ocean sites, CDOM fluctuates on seasonal cycles that can be coupled to cycles of marine organic matter production and remineralization (*12*, *13*). CDOM concentrations are high in the chlorophyll maxima of the Arabian Sea and were correlated with the upwelling of colder, nutrient rich subsurface water, consistent with a source related to biological activity (*14*). Likewise, in situ measurements of CDOM absorption at 412 nm were positively correlated with oxygen super saturation and chlorophyll-*a* absorption at a highly productive coastal site, suggesting rapid in situ production of CDOM associated with primary production (*15*). However, few discrete components of CDOM have been reported. Excitation-emission matrix spectroscopy has identified the presence distinct excitation maxima at 275 nm, 312, and 398 nm that are attributed to proteins, marine humic substances, and chlorophyll (*16*, *17*). Proteins, amino acids, marine humic substances, and chlorophyll have all been identified in the dissolved or colloidal phase but together account for only a small fraction of CDOM absorption at open ocean sites.

Several studies have used absorption and fluorescence spectroscopy in combination with capillary gel electrophoresis and high-pressure liquid chromatography (HPLC) to further characterize CDOM. Marine CDOM extracted from seawater by adsorption onto hydrophobic resin has been separated by reverse phase HPLC (*18*). Different fractions display different fluorescence spectra with multiple maxima between 300 and 600 nm (λ_{ex} 300−450 nm) (*19*). Separation by gradient elution HPLC showed improved resolution of a very complex mixture of CDOM components, but most components remained unresolved. Using HPLC, marine CDOM appears as a complex mixture of > 30 discrete components superimposed on a broad, featureless baseline. Fluorescence

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bands observed in the total CDOM extracts could be chromatographically separated into different fractions, and distinct CDOM components were isolated from marine and freshwaters, suggesting compositional differences for at least some CDOM components in different environments (*20*).

Here we report the chemical characterization of CDOM in samples from the Pacific and Atlantic Oceans. We used hydrophobic resins to remove a fraction of CDOM from filtered, acidified seawater and purified the adsorbed components by reverse phase HPLC. Purified CDOM components were analyzed by UV/vis spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry to determine their partial structures. We find that coastal seawater samples have an unresolved mixture of CDOM that we attribute to humic substances as well as a mixture of discrete components resulting from in situ biological production. In open ocean samples the humic substances component is substantially reduced, and a suite of discrete compounds some of which we identified as 2,4-dichlorobenzoic acid and tetrachlorobiphenyl carboxylic acids dominates absorption in the ultraviolet.

Experimental Section

Samples were taken from a number of sites to compare CDOM characteristics in different oceanic regimes. Samples were collected in September 2002 from Vineyard Sound near Woods Hole, MA, in June 2002 from the North Pacific Ocean, and in March 2003 from the Bermuda Biological Station in St. Georges, Bermuda. Surface seawater samples were collected using laboratory or shipboard seawater supply systems and filtered through a cleaned (10% HCl) 0.2 μm filter cartridge (Criticap, Gelman Corp.) into 200 L fluorinated HDPE containers. The 1800 m North Pacific Ocean sample was collected using 30 L rosette mounted Niskin bottles and treated as described above. After filtration, all samples were immediately acidified to pH 3 using either glacial acetic acid or concentrated HCl.

Chromophoric dissolved organic matter was extracted by solid-phase extraction onto C_{18} silica gel after the method of Armador et al. (*21*). The C_{18} silica gel (Aldrich) was cleaned by sequential washing with (per 10 g sorbent) the following: 50 mL of hexane, 50 mL of acetone, 50 mL of methanol, 50 mL of low carbon deionized (Milli-Q) water, 25 mL of 0.1 M HCl, Milli-Q water (until pH is neutral), and 25 mL of methanol. Extraction columns were prepared by dry packing approximately 20−25 g of air-dried resin into 50 mm i.d. glass columns fitted with glass wool plugs at both ends. To extract CDOM, filtered, acidified seawater was pumped through the columns at 20−60 mL/min. In most cases CDOM was eluted immediately after sample collection. However, for the Pacific samples, columns with extracted CDOM were stored in the dark at −20 °C until further processing. Samples were handled in the dark or in opaque containers during all steps of collection and manipulation. Combusted glassware was used throughout. The columns were eluted with 20−100 mL of Milli-Q water (pH 3) to remove salts and then 100 mL of methanol (Fisher Scientific, HPLC grade) and 100 mL of 11.8 N ammonium hydroxide (Fisher Scientific, certified ACS) to elute CDOM. This paper reports only the results from the methanol extract. Cooling or concentrating the methanol extract resulted in the formation of a fluffy white precipitate, presumed to be residual salts. The sample was dissolved in a small amount of methanol and chilled to −30 °C, and the precipitate was separated by centrifugation. Methanol soluble CDOM and washes (2×) were withdrawn by pipet and retained for analyses.

We analyzed several different types of sample blanks to monitor the presence of contaminants in our samples and to determine if specific CDOM components could be introduced by contamination. Solid-phase extraction columns packed with C_{18} silica gel were taken into the field, returned to the laboratory, and processed as samples. We also filled sample containers with artificial seawater (30 L) made from combusted (450 °C, overnight) salts and low carbon, deionized (Milli-Q) water. These blanks were processed in an identical manner to samples, using the same equipment, resins, acids, and solvents as our samples. Blanks contained very low levels of CDOM (<1% of sample amounts) which, when analyzed by HPLC yielded compounds with different retention times and UV spectra than CDOM components reported in this study.

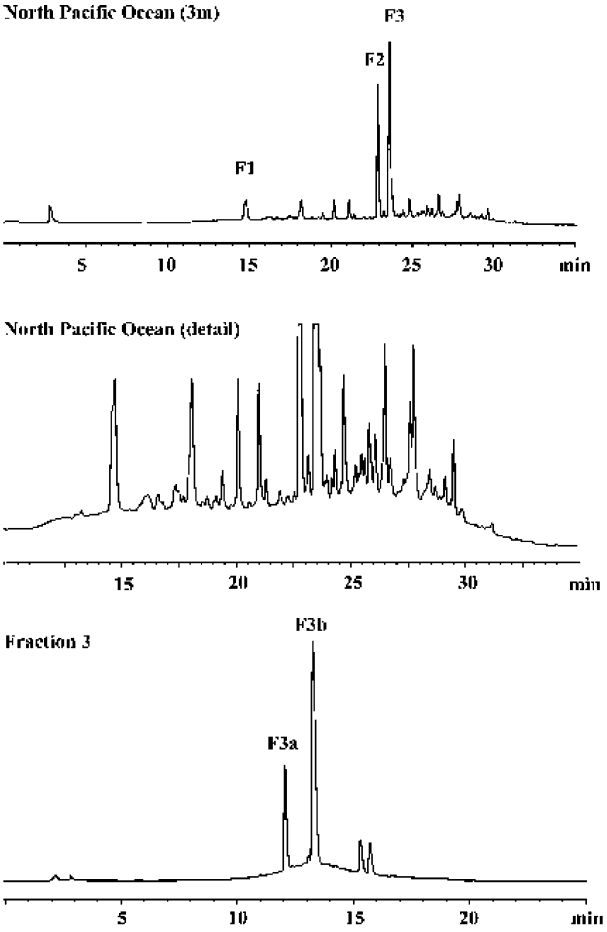

FIGURE 1. Separation of CDOM by reverse phase HPLC. Details of the separation conditions are given in the text. Samples collected from the North Pacific Ocean show the presence of a few major (top panel) and >90 minor (middle panel) CDOM components at 240 nm. The major components 1, 2, 3a, and 3b were purified (bottom panel) and analyzed by NMR and UV/vis spectroscopy and mass spectrometry and shown to be 2,4-dichlorobenzoic acid and a suite of tetrachlorobiphenyl carboxylic acids.

UV/vis absorbance spectra of total methanol extracts and HPLC purified compounds were acquired in methanol on a HP8452A diode array spectrophotometer. CDOM was separated into pure compounds by reverse-phase high performance liquid chromatography (RP-HPLC). Samples were purified on a 15 cm column (4.5 mm i.d. Supelco Discovery HS; Figure 1). Initial separations were performed on a gradient from 100% aqueous ammonium acetate (50 mM, pH = 8) (Fisher Scientific, HPLC grade) to 100% methanol with a flow rate of 1 mL/min (ammonium acetate (aq):methanol; time)/(100:0; 2.5 min)/(40:60; 17.5 min)/(0:100; 27 min). Fractions collected from the this separation were further purified using a 50 mM ammonium acetate:acetonitrile (Fisher Scientific, HPLC grade):methanol gradient at a flow rate of 1 mL/min (70:25:5; 2.5 min)/(0:70:30; 12.5 min)/(0:15:85; 25 min) (Figure

TABLE 1. Sample Location, Depth, Volume, and CDOM Extraction Details

sample	depth (m)	vol (L)	lat/long	resin (g)	flow (mL/min)
Woods Hole	3	760	41°31′ N, 70°40′ W	50	60
Bermuda	3	400	38°20′ N, 64°48′ W	50	60
North Pacific	3	40	24°25′ N, 156°18′ W	20	20
North Pacific	1800	70	24°25′ N, 156°18′ W	20	20

1). Purified CDOM components were dried under nitrogen to remove methanol and lyophilized to remove the aqueous ammonium acetate.

NMR spectra were acquired on a Bruker Avance 400 DPX spectrometer at 400 MHz. Samples were dissolved in perdeuterated methanol (Aldrich, 99.95 at. % D), and chemical shifts were referenced to methanol at 3.5 ppm. Liquid chromatography-MS-MS spectra were obtained on a Micromass (Manchester, England) Quattro II mass spectrometer (triple quadrupole) with electrospray ionization (ESI). The LC was conducted with a Thermo Hypersil-Keystone (Bellefonte, PA) Beta Basic C_{18} using a solvent program of 50 mM ammonium acetate in water to methanol (100:0; 2.5 min/40:60; 15 min/0:100; 25 min/0:100; 35 min).

In preparation for GC-MS analysis, CDOM was methylated to convert carboxylic acids into methyl esters. Methanol solutions of CDOM were treated with 5% acetyl chloride in methanol at 50 °C overnight. The resulting acidic solution was neutralized with 200 mM ammonium carbonate and extracted twice with dichloromethane. The dichloromethane solution was evaporated to approximately 10 μL prior to splitless injection into a Hewlett-Packard 5972 GC-MS, which was equipped with a Supelco (Bellefonte, PA) Equity-5: 30 m l, 0.25 mm i.d., 0.25 μm coating. Helium was used as a carrier gas at a velocity of 40 cm/s, and 70 eV electron ionization mass spectra were collected. A temperature gradient program of 50 °C (hold 2 min)/ramp at 8 °C to 300 °C/hold 300 °C for 10 min. The compounds of interest were identified through the use of extracted ion chromatograms for the predicted molecular weights of the derivatized products.

Results and Discussion

Up to 70% of chromophoric dissolved organic matter (CDOM) can be removed from seawater by adsorption onto hydrophobic resins at low pH (*21*). Using this approach, we isolated CDOM from oligotrophic marine sites in the North Pacific Ocean near Hawaii and the North Atlantic Ocean at the Bermuda Biological Station. Coastal seawater was sampled from Woods Hole, MA (Table 1). Ultraviolet-visible spectra of the methanol extracts for all samples show a logarithmic-like decrease in absorption with increasing wavelength, similar to whole seawater absorption spectra (*4*, *11*).

Using HPLC, we characterized the CDOM mixture in each of our samples. Samples from Bermuda and the North Pacific Ocean have only small amounts of unresolved CDOM, while the sample of coastal seawater from Woods Hole clearly shows the presence of a complex mixture of unresolved CDOM components presumably derived from terrestrial humic substances (Figure 2). Over 90 components with adsorption maxima between 240 and 400 nm were separated in samples from the North Pacific Ocean and Woods Hole. All components display strong absorption bands in the ultraviolet and near-ultraviolet. Further separation of some fractions show the presence of multiple coeluting compounds, at least some of which were found to be structurally related. For example, Fraction 3 from the North Pacific surface water sample was separated into two major and at least five minor components (Figure 1, bottom). The mixture of CDOM components

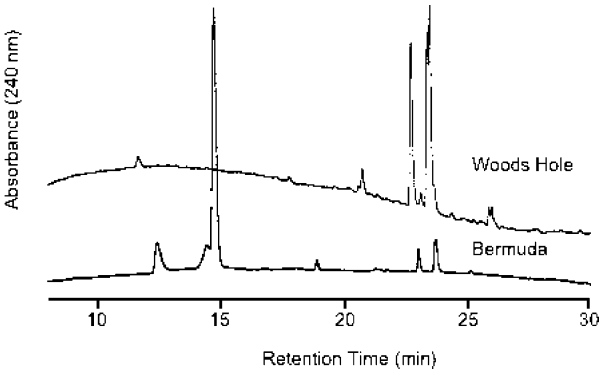

FIGURE 2. Separation of CDOM from Woods Hole (top) and Bermuda seawater (bottom) by HPLC. The Woods Hole sample shows both high relative concentrations of tetrachlorobiphenyl carboxylic acids and the baseline rise characteristic of humic substances. The sample is influenced by CDOM sources from the adjacent, heavily vegetated coast and high in situ biological production. The Bermuda sample is more characteristic of open ocean CDOM samples, with low relative amounts of humic substances and high concentrations of dichlorobenzoic acid and tetrachlorobiphenyl carboxylic acids from local marine production.

present in our samples is therefore greater than the 90 compounds distinguished in Figure 1.

Proton nuclear magnetic resonance (^{1}H NMR) spectra of the total methanol extracts are characterized by complex and unresolved resonance between 7 and 8 ppm and show that at a major portion of CDOM adsorption arises from substituted aromatic compounds. The complexity of NMR spectra is related to the complexity of the CDOM mixture separated by HPLC. Samples with complex mixtures of CDOM components show much greater complexity in the 7−8 ppm region of the ^{1}HNMR spectrum. Samples from the Pacific and Atlantic Oceans have the same major components by HPLC, which we purified for structural characterization.

Fraction 1 was identified as 2,4-dichlorobenzoic acid (Figure 3), with λ_{max}(MeOH) 206 and 228 nm; ^{1}H NMR (CD_3OD) δ 7.45 (1H, d, J = 8.37 Hz, H-6), 7.42 (1H, d, J = 1.94 Hz, H-3), 7.30 (1H, dd, J = 8.37, 1.94 Hz, H-5) (Figure 4): GC/MS of the methyl ester yields (*m*/*z*, % rel int.) 204(20), 173(100), 145(25), 109(25), 74(23). Coinjection of Fraction 1 with authentic 2,4-dichlorobenzoic acid yielded one peak on two HPLC systems which separate 2,4-, 2,5-, and 3,4-dichlorobenzoic acid isomers. We also observed no change in the ^{1}HNMR spectrum upon mixing of Fraction 1 with authentic 2,4-dichlorobenzoic acid.

Fraction 3b was identified as an isomer of tetrachlorobiphenyl carboxylic acid (Figure 3) with λ_{max}(MeOH) 211 nm;^{1}H NMR (CD_3OD) δ 7.60 (1H, d, J = 1.94 Hz), 7.57 (1H, s), 7.44 (1H, dd, J = 8.37, 1.94 Hz), 7.38 (1H, s), 7.32 (1H, d, J = 8.37 Hz) (Figure 4): GC/MS of the methyl ester yields (*m*/*z*, % rel int.) 350(35), 319(100), 254(60), 219(10), 184(35). Negative ion mode liquid chromatography/mass spectrometry of Fraction 3b shows prominent molecular ions at *m*/*z* 333 ($^{35}Cl_4$ M+; (observed abundance/theoretical abundance for C_{12}, H_6, O_2, Cl_4) 79/78), 335 ($^{35}Cl_3{}^{37}Cl$ M+, 100/100), 337 ($^{35}Cl_2{}^{37}Cl_2$ M+; 47/48), and 339 ($^{35}Cl^{37}Cl_3$ M+; 10/10), with a major ion cluster at *m*/*z* 291 from loss of carbon dioxide, confirming the presence of a carboxylic acid. Further evidence comes from the mass spectrum of the methylated derivative, which displays prominent molecular ions at *m*/*z* 348 ($^{35}Cl_4$ M+), 350 ($^{35}Cl_3{}^{37}Cl$ M+), 352 ($^{35}Cl_2{}^{37}Cl_2$ M+), and 354 ($^{35}Cl^{37}Cl_3$ M+) and major ion clusters at *m*/*z* 317 ($-OCH_3$), 254 ($-C_2O_2ClH_3$), 219 ($-C_2O_2Cl_2H_3$), and 184 ($-C_2O_2Cl_3H_3$) as well as ions with *m*/*z* <184 resulting from fragmentation of the biphenyl ring. From the lack of coupling between the

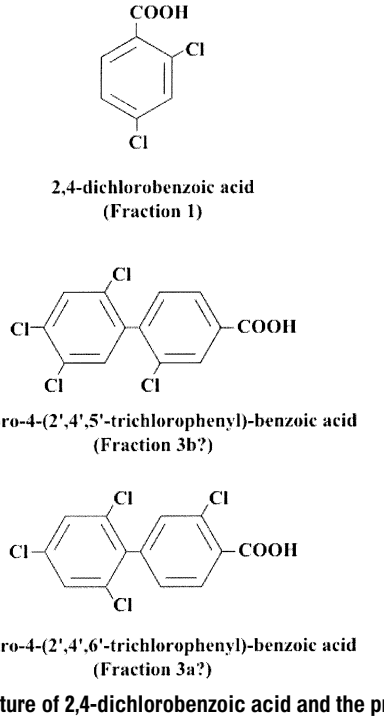

FIGURE 3. Structure of 2,4-dichlorobenzoic acid and the proposed structures of chlorinated biphenyl carboxylic acids isolated from seawater.

two protons at 7.57 and 7.38 ppm on the trichlorophenyl ring we infer these protons are in a *para* orientation, suggesting a 2′,4′,5′ substitution. Further, the upfield shift in the *ortho* coupled doublet to 7.32 ppm is most similar to NMR spectra of 3,4-disubstituted benzoic acids. Our data support a structural assignment for Fraction 3b of either 3-chloro-4-(2′,4′,5′-trichlorophenyl)benzoic acid or 4-chloro-3-(2′,4′,5′-trichlorophenyl)benzoic acid. We know of no previous reports of tetrachlorobiphenyl carboxylic acids in the environment, and no authentic standards of these compounds are presently available. Therefore, the specific isomer of tetrachlorobiphenyl carboxylic acid in Fraction 3b cannot be assigned at this time.

Fraction 3a was separated from Fraction 3b by reverse phase HPLC using a mixture of water, acetonitrile, and methanol (Figure 1). The similar chromatographic properties of Fraction 3a to Fraction 3b suggests a close structural similarity, which was confirmed by spectral analyses. Purified Fraction 3b had λ_{max}(MeOH) 210 nm; ^{1}H NMR (CD_3OD) δ 7.53 (1H, d, J = 1.93 Hz), 7.48 (2H, d, J = 1.93 Hz), 7.46 (1H, d, J = 8.37), 7.34 (1H, dd, J = 8.37, 1.94 Hz), 7.20 (1H, d, J = 1.94 Hz) (Figure 4). GC/MS of the methyl ester yields (*m/z*, % rel int.) 350(50), 319(100), 254(55), 219(15), 184(30). Fraction 3a is almost certainly a second isomer of tetrachlorobiphenyl carboxylic acid. We observe *meta* coupling of 1.9 Hz between protons on the trichlorophenyl substituent, suggesting 3′,5′,6′- or 2′,4′,6′- chlorination. The NMR data, by analogy with NMR data for 2,4-dichlorobenzoic acid suggests this compound is either 2-chloro-4-(3′,5′,6′- or 2′,4′,6′)-trichlorophenyl)benzoic acid or 4-chloro-(3′,5′,6′- or 2′,4′,6′)-trichlorophenyl)benzoic acid (Figure 3). Our assessment is preliminary, and full assignment must await comparison to authentic standards.

We did not obtain mass spectral data on Fraction 2, and we are only able to identify this compound as a tetrasubstituted biphenyl carboxylic acid, with λ_{max}(MeOH) <210 nm; ^{1}H NMR (CD_3OD) δ 7.62 (1H, d, J = 1.94 Hz), 7.47 (2H, s), 7.46 (1H, dd, J = 8.37, 1.94 Hz), 7.26 (1H, d, J = 8.37) (Figure 4). From the upfield shift in the *ortho* coupled doublet to 7.26 ppm, the similarity of the NMR spectra to fraction 3b, and the lack of coupling observed for the protons at 7.47 ppm, we infer this compound is the second isomer of chloro-(2′,4′,5′-trichlorophenyl)benzoic acid as discussed above (Figure 3).

We quantified the amount of 2,4-dichlorobenzoic acid and tetrachlorobiphenyl carboxylic acids in our sample by calibrating our HPLC system with authentic 2,4-dichlorobenzoic acid. Values of 2,4-dichlorobenzoic acid (2,4-DCBA) range from almost not detectable in our Woods Hole seawater sample (<0.1 μg/L), to 0.4 μg/L in Pacific surface water, to 8.3 μg/L in Bermuda seawater. Likewise, we observe variable concentrations of total tetrachlorobiphenyl carboxylic acids (ΣTCBCAs) ranging from 1 μg/L in Woods Hole Seawater to 4.2 μg/L in the Pacific Ocean. We collected very different sized samples from each of our sites, using slightly different column aspect ratios and amounts of C_{18} silica gel. Therefore, some of the observed differences in concentration may result from differences in extraction efficiencies.

Chlorinated aromatic acids contribute only a small fraction of the total dissolved organic carbon in seawater, but their contribution to CDOM absorption is significant, particularly in open ocean waters. High concentrations of 2,4-DCBA and TCBCAs were measured in both surface and deep water samples, with total concentrations (2,4-DCBA +ΣTCBCAs) ranging from 1 to 10 μg/L (Table 2). Using the concentration and extinction coefficient of 2,4-DCBA we calculate that this compound alone contributes 2% of the total CDOM absorption at 280 nm (UV-A/UV-B) in our Bermuda sample extracts. The NMR spectra of whole sample extracts show that 2,4-DCBA +ΣTCBCAs are only a portion of the substituted aromatic compounds present in seawater. The contribution of substituted aromatic compounds to CDOM absorption in the ultraviolet is therefore several times higher than the contribution from 2,4-DCBA +ΣTCBCAs alone.

Of the compounds identified in this study, only 2,4-DCBA is a known anthropogenic product. This compound is used commercially in the production of pharmaceuticals and is an impurity in some herbicides. Low concentrations of 2,4-DCBA measured in groundwater, rivers, and lakes have been attributed to anthropogenic contamination as well as biological and photochemical degradation of anthropogenically produced polychlorinated biphenyls (PCBs) (*22*−*26*). However, 2,4-DCBA in some pristine waters has been attributed to direct biological production (*27*). Tetrachlorinated biphenyl carboxylic acids have not been previously reported as products of either anthropogenic production or the degradation of PCBs.

One approach to distinguishing anthropogenic and natural sources of TCBAs in the ocean is to determine the global inventory of these compounds. If the inventory of TCBAs is much larger than the historical production of PCBs, then natural sources must be important. Our analyses of TCBAs only include four samples from two ocean basins and is not sufficient for a high quality global assessment. However, our data does provide a preliminary basis for making such an assessment. Assuming the concentration of ΣTCBCAs averages 1 μg/L (Table 2) for the upper 1800 m of the global ocean, we calculate a global ocean inventory of approximately 300 MT for these compounds, over 2 orders of magnitude higher than the estimated 0.6−1.5 MT of PCBs that have been produced by commercial synthesis (*28*). Most of the global PCB inventory resides in terrestrial reservoirs, and estimates suggest only a few percent of anthropogenic PCB production has been transported the ocean. The global inventory of TCBCAs is therefore >10 000 times the total marine inventory of PCBs.

Our estimate of the marine inventory for chlorinated aromatic acids is probably conservative, as either a higher average concentration (Table 2) or the occurrence of

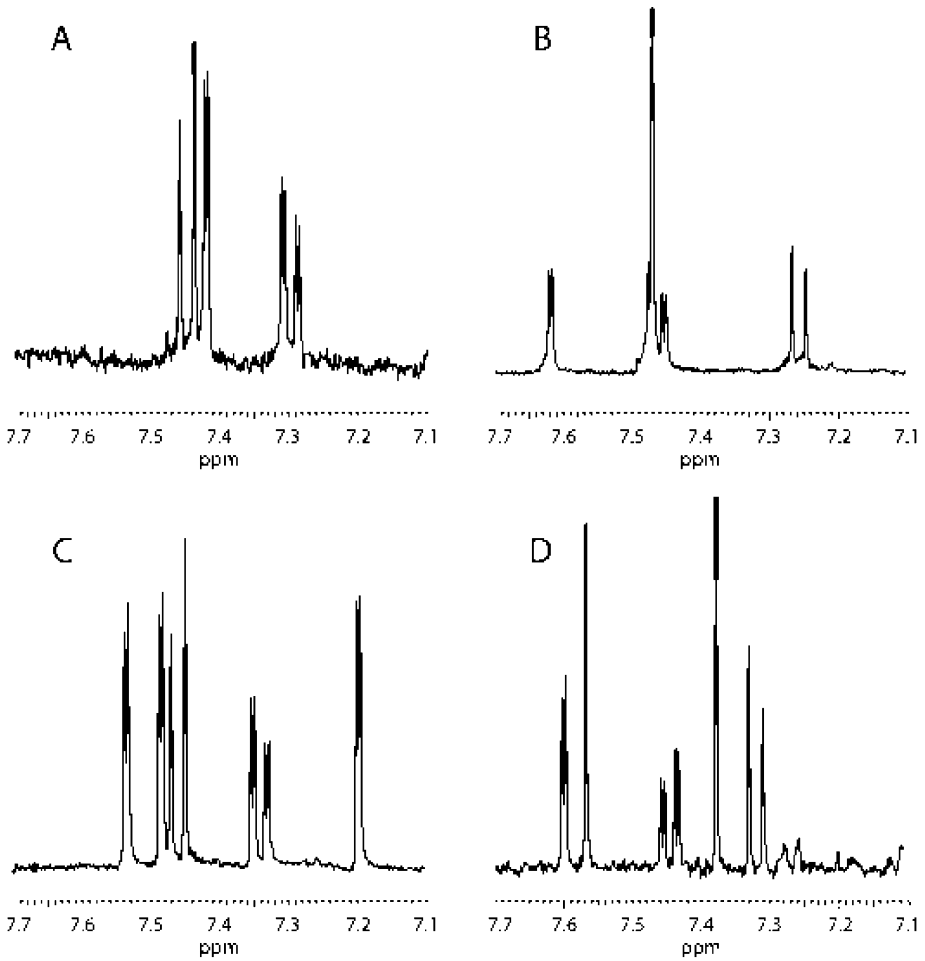

FIGURE 4. Proton nuclear magnetic resonance spectra of (A) CDOM Fraction 1, identified as 2,4-dichlorobenzoic acid, (B) CDOM Fraction 2, an isomer of tetrachlorobiphenyl carboxylic acid, (C) CDOM Fraction 3a, an isomer of of tetrachlorobiphenyl carboxylic acid, and (D) CDOM Fraction 3b, an isomer of of tetrachlorobiphenyl carboxylic acid. Samples were dissolved in deuterated methanol, and chemical shifts are relative to methanol at 3.5 ppm. Chemical shifts and coupling constants are provided in the text.

TABLE 2. Concentrations (μg/L) of Dissolved Chlorinated Aromatic Acids in Seawater

sample	2,4-DCBA	Fraction 2	Fraction 3a	Fraction 3b
Pacific Ocean, 3 m	0.4	1.2[a]	1.0	1.0
Pacific Ocean, 1800 m	1.0	1.6	1.3	1.3
Bermuda, 3 m	8.3	0.4	0.9[a]	
Woods Hole, 3 m		0.3	0.3	0.4

[a] Values were calculated with the assumption that TCBCAs have twice the molar extinction coefficient as 2,4-dichlorobenzoic acid. Compounds TCBCA-2 and TCBCA-3 coelute on the HPLC system shown in Figure 1 and were quantified by further separation on a second chromatographic system. However, these two compounds were not quantified separately for the Bermuda sample.

chlorinated aromatic acids at depths > 1800 m will substantially increase the amount of these compounds sequestered in the ocean. Concentrations of PCBs in heavily impacted coastal waters are < 1 ng/L and fall rapidly with depth. PCBs have been reported to depths of 1500 m in the coastal ocean, but concentrations below the mixed layer are only 1−10 pg/L (*29*). We further note that only a few, specific tetrachlorinated biphenyl carboxylic acid isomers are found in our samples, while many of the products expected from carboxylation of the most common PCB congeners in the environment are absent. Anthropogenic inputs of 2,4-DCBA and partial degradation of anthropogenically produced PCBs may be minor sources for chlorinated aromatic acids in seawater, but they cannot account for the observed distribution and concentration of 2,4-DCBA and TCBCAs in our samples. The distribution of chlorinated aromatic acids in our samples, their global marine inventory, and presence of only specific chlorinated aromatic acids suggest these compounds are produced in situ by marine microbes.

A wide variety of organochlorine natural products have been identified in marine organisms, including simple chlorinated aromatic compounds (*30*, *31*). Halogenated organic compounds are thought to serve as chemical defense agents in marcofauna and flora, but halogenated organic compounds are also common in microorganisms, where their role is unclear (*30*). We know of no reports that describe naturally occurring polychlorinated biphenyl-like compounds in marine organisms. However, a series of recent papers have described a suite of halogenated dimethyl bipyrroles that are widely found in marine biological samples, including tissue samples of marine mammals and birds (*32*). Halogenated dimethyl bipyrroles have not been measured in seawater, but they do occur even in low trophic levels in marine food webs and are most likely produced by marine phytoplankton or bacteria (*33*). The tetrachlorobiphenyl carboxylic acids reported here are also widely distributed in the marine environment, and the large global inventory suggests they are most likely produced by marine microorganisms.

Chlorofluorocarbon (CFC) induced depletion of stratospheric ozone, particularly at high latitudes, has raised concern over potentially deleterious impacts to marine ecosystems from enhanced exposure to harmful ultraviolet

radiation (*34*). Colored dissolved organic matter is the principal light-absorbing component in the UV-A and UV-B and limits the penetration of UV radiation in the water column. In response to increased levels of UV radiation, marine microbes may regulate their biosynthesis of UV adsorbing compounds such as mycosporine-like amino acids (*35*). Low concentrations of dissolved mycosporine-like amino acids have been measured in productive coastal waters, and the release of UV adsorbing compounds may be one strategy by which marine microbes limit damage by harmful UV radiation (*36*). The chlorinated aromatic acids reported here also limit the depth of UV penetration in the marine water column. Further work is needed to establish the specific biological sources, cycling,and fate of these compounds as well as their role in attenuating ultraviolet radiation with depth in the water column.

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