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Advanced Solid-State NMR Characterization of Marine Dissolved Organic Matter Isolated Using the Coupled Reverse Osmosis/Electrodialysis Method

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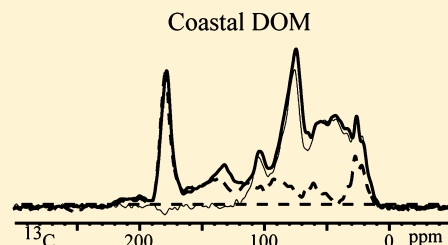
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S Supporting Information

ABSTRACT: Advanced ^{13}C solid-state techniques were employed to investigate the major structural characteristics of two surface-seawater dissolved organic matter (DOM) samples isolated using the novel coupled reverse osmosis/electrodialysis method. The NMR techniques included quantitative ^{13}C direct polarization/magic angle spinning (DP/MAS) and DP/MAS with recoupled dipolar dephasing, ^{13}C cross-polarization/total sideband suppression (CP/TOSS), ^{13}C chemical shift anisotropy filter, CH , CH_2 , and CH_3 selection, two-dimensional ^1H - ^{13}C heteronuclear correlation NMR (2D HETCOR), 2D HETCOR combined with dipolar dephasing, and ^{15}N cross-polarization/magic angle spinning (CP/MAS). The two samples (Coastal and Marine DOM) were collected at the mouth of the Ogeechee River and in the Gulf Stream, respectively. The NMR results indicated that they were structurally distinct. Coastal DOM contained significantly more aromatic and carbonyl carbons whereas Marine DOM was markedly enriched in alkoxy carbon (e.g., carbohydrate-like moieties). Both samples contained significant amide N, but Coastal DOM had nitrogen bonded to aromatic carbons. Our dipolar-dephased spectra indicated that a large fraction of alkoxy carbons were not protonated. For Coastal DOM, our NMR results were consistent with the presence of the major structural units of (1) carbohydrate-like moieties, (2) lignin residues, (3) peptides or amino sugars, and (4) COO-bonded alkyls. For Marine DOM, they were (1) carbohydrate-like moieties, (2) peptides or amino sugars, and (3) COO-bonded alkyls. In addition, both samples contained significant amounts of nonpolar alkyl groups. The potential sources of the major structural units of DOM were discussed in detail. Nonprotonated O-alkyl carbon content was proposed as a possible index of humification.



INTRODUCTION

The marine organic carbon pool is one of the largest dynamic reservoirs of organic carbon on earth¹⁻³ and is comparable in size to that of atmospheric carbon. Dissolved organic matter (DOM) comprises most of marine organic carbon. Although significant progress has been made in understanding the origin, composition, and cycling of marine DOM, a large fraction of it is still poorly characterized.⁴⁻⁶

Progress in the understanding of the chemical structures of DOM has been impeded by (1) the lack of a suitable method for its isolation, and (2) its intrinsic chemical complexity. Marine DOM samples isolated by tangential-flow ultrafiltration and XAD-8 are not representative of native DOM simply because such a small percentage of total dissolved organic carbon (only 5–30%) is recovered by those methods.^{5,7} The recently developed coupled reverse osmosis/electrodialysis (RO/ED) method shows great promise as a solution to the problem of representativeness because this method can collect

70–85% of total dissolved organic carbon from seawater.^{1,8,9} The RO/ED samples contain both the hydrophobic moieties that are preferentially isolated by solid phase extraction (e.g., XAD resins and C_{18} adsorbents) and the more polar, carbohydrate-like moieties preferentially isolated by tangential-flow ultrafiltration.¹ However, a large fraction of inorganic components in RO/ED samples makes structural analyses of the organic matter more challenging.

Isolated DOM samples from different aquatic environments may have distinct chemical and spectroscopic properties consistent with their origins. Detailed characterization of representative DOM samples from different sources will help define the links between source and chemical structure, and

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Table 1. Some Background Information, Mass Percentage of C and N, SUVA₃₀₀, and Molar C/N Ratios of Freeze Dried Samples

sample	latitude	longitude	sampling location	depth (m)	%C ^a	%N ^a	C/N	SUVA ₃₀₀
Marine DOM	31° 30.0'	79° 20.3'	Gulf Stream	20.0	6.84	0.45	17.7	0.399
Coastal DOM	31° 51.6'	81° 09.1'	Ogeechee River Mouth, High Tide	2.0	24.9	1.53	19.0	3.059

^aNote that %C and %N refer to those of organic carbon and nitrogen, respectively.

thus shed light on formation and reformation mechanisms. Solid-state nuclear magnetic resonance (NMR) spectroscopy is a valuable tool for elucidating the structures of natural organic matter (NOM) such as marine DOM. The most frequently used technique, ¹³C cross-polarization/magic angle spinning (CP/MAS), has limitations, however.¹⁰ It can only identify around 10 types of functional groups in NOM, because spectra are broad and heavily overlapped. We have developed and applied a series of advanced solid-state NMR techniques for characterizing complex NOM samples.^{11,12} This new, systematic protocol can identify many more functional groups by selectively retaining certain peaks and eliminating others. We can use two-dimensional ¹H–¹³C heteronuclear correlation NMR (2D HETCOR) or 2D HETCOR combined with spectral editing to detect the proximities and connectivities of different specific functional groups. Moreover, ¹H spin diffusion can be used to probe domains or heterogeneities on a 1–50 nm scale.¹³

In the present study, a DOM near the mouth of a blackwater river ("Coastal DOM") and a DOM from the Gulf Stream open ocean ("Marine DOM") were characterized using advanced solid-state NMR methods. The objectives were (1) to characterize Coastal and Marine DOM in detail using advanced solid-state NMR techniques; (2) to obtain the detailed structural differences between Marine and Coastal DOM; and (3) to explore the potential sources of the major components of the DOM samples. The results underscore the advantages of our approach using advanced techniques over the standard ¹³C CP/MAS technique with regard to accuracy and the level of detail of the structural analysis. This is the first time that RO/ED DOM fractions have been characterized in detail using advanced solid-state NMR techniques.

MATERIALS AND METHODS

DOM Samples. Coastal DOM was collected at a depth of 2 m on the Georgia coast near the mouth of the Ogeechee River. The specific UV absorption coefficient at 300 nm (SUVA₃₀₀) for the original coastal water sample was much greater than that of the other RO/ED samples collected from the open Atlantic Ocean and the Gulf Stream, reflecting a significant input of terrestrially derived organic matter.¹ Marine DOM was collected at a depth of 20 m in the Gulf Stream. Both samples were isolated using the novel RO/ED method, the details of which have been reported previously.^{1,8,9} Basic characteristics and sampling locations are summarized in Table 1.

NMR Spectroscopy. All ¹³C NMR analyses were performed at 100 MHz using a Bruker DSX400 spectrometer equipped with 4-mm sample rotors in a double-resonance probe head. NMR experiments included quantitative ¹³C DP/MAS and DP/MAS with dipolar dephasing, ¹³C cross-polarization and total suppression of sidebands (CP/TOSS) and ¹³C CP/TOSS plus dipolar dephasing, ¹³C chemical-shift-anisotropy (CSA) filter, CH selection, CH₂ selection, spectral editing of immobile CH₂ + CH, ¹H–¹³C 2D heteronuclear correlation NMR (HETCOR), 2D HETCOR with dipolar

dephasing, and ¹⁵N CP/MAS. Experimental details are described in the Supporting Information. Due to the low organic content in particular of the Marine DOM (ca. 1/5 of a full rotor mass) (see Table 1), many of the NMR experiments, in particular ¹⁵N NMR, were more challenging and time-consuming than for humic acids or other nearly purely organic extracts.

RESULTS

Overall Quantitative and Qualitative NMR Analyses. Figure 1a (thin line) and b (thick line) are quantitative ¹³C

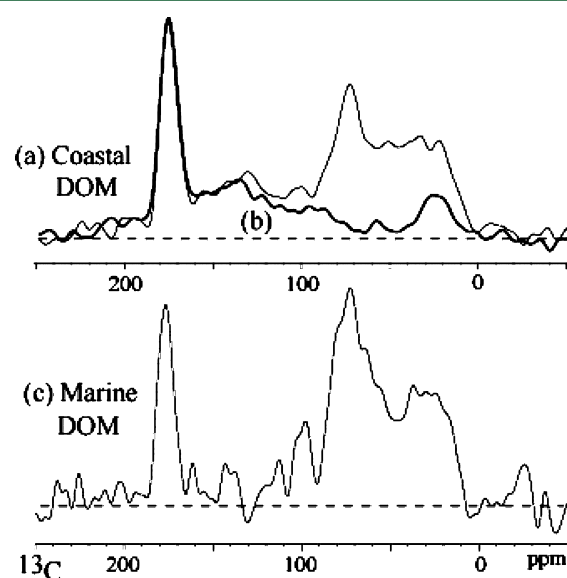


Figure 1. (a) DP/MAS spectrum of Coastal DOM and (b) DP/MAS spectrum after recoupled dipolar dephasing of Coastal DOM. (c) DP/MAS spectrum of Marine DOM. Spinning speed: 14 kHz.

NMR spectra of all C (DP/MAS) and of nonprotonated or mobile C (DP/MAS with recoupled dipolar dephasing), respectively, in Coastal DOM. Figure 1c is the DP/MAS spectrum of Marine DOM. We did not collect the dipolar-dephased DP spectrum of Marine DOM due to its small organic-matter fraction and correspondingly low signal.

The percentages of different functional groups are listed in Table 2. The assignments are as follows: 0–45 ppm, alkyl; 45–60 ppm, NCH and OCH₃; 60–94 ppm, carbohydrate-like C or other O-alkyls; 94–117 ppm, O–C–O anomeric C; 117–150 ppm, aromatic C; 150–164 ppm, aromatic C–O; 164–191 ppm, COO and N–C=O; and 191–220 ppm, ketone, quinone, or aldehyde C. Marine DOM contains much more carbohydrate-like moieties or other O-alkyls (60–94 ppm) but much less aromatic C (117–150 ppm) compared with Coastal DOM. In addition, Marine DOM has less aromatic C–O (150–164 ppm) and ketone, quinone, or aldehyde C (191–220 ppm); this is seen with better signal-to-noise ratio in the cross-polarization spectra of Figure 2. More detailed analysis

Table 2. Integration Results from Quantitative DP/MAS and Semi-Quantitative CP/MAS NMR

sample	technique	ppm								
		220–191	191–164	164–150	150–117	117–94	94–60	60–45	45–30	30–0
		ketone, aldehyde, quinone	COO, NC=O	aroma.C–O	aromatics	OCO	OC	OCH ₃ ,NCH	CCH ₂ C, CCHC,C _q	CCH ₂ C, CCH ₃
Coastal DOM	DP	2%	19%	4%	12%	7%	24%	10%	10%	12%
	CP	2%	14%	2%	10%	8%	29%	13%	12%	10%
Marine DOM	DP	1%	17%	2%	2%	8%	35%	12%	12%	13%
	CP	0%	12%	0%	2%	8%	41%	12%	12%	13%

^aTotal aromaticity (12.2%) = protonated aromatic fraction (1.0%) + nonprotonated aromatic fraction (11.2%). The uncertainties are generally $\pm 1\%$ for small peaks ($<10\%$), $\pm 2\%$ for large peaks, and twice larger for peaks in the DP spectrum of Marine DOM.

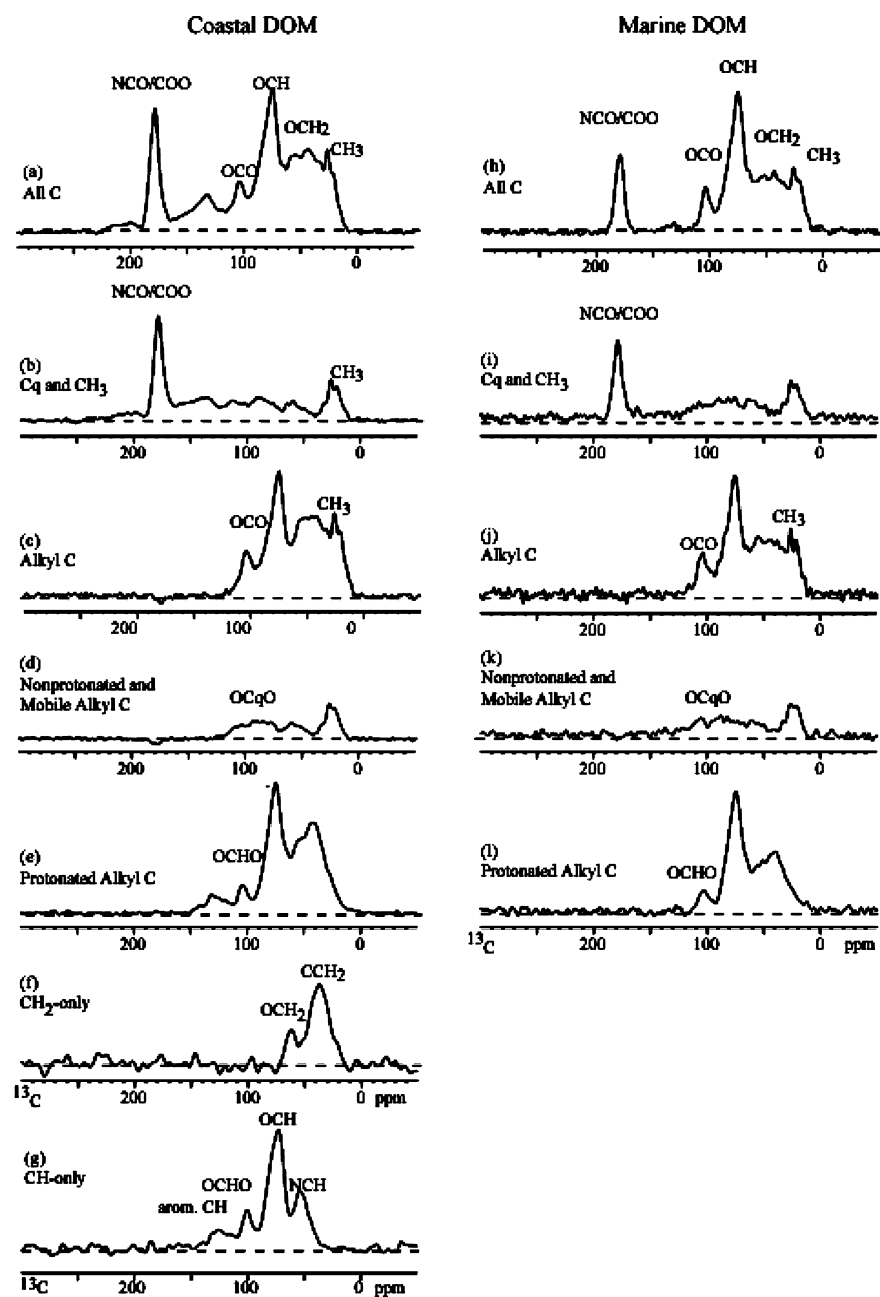


Figure 2. ^{13}C NMR with spectral editing of Coastal DOM and Marine DOM. Left column (a–g): Coastal DOM (a–g); and right column (h–l): Marine DOM. Left column: (a) ^{13}C CP/TOSS, (b) CP/TOSS with 40- μs dipolar dephasing, (c) ^{13}C CSA filter, (d) ^{13}C CSA filter with dipolar dephasing, (e) CH_n -only, (f) CH_2 -only, and (g) CH -only. Right column: (h) ^{13}C CP/TOSS, (i) CP/TOSS with dipolar dephasing, (j) ^{13}C CSA filter, (k) ^{13}C CSA filter with dipolar dephasing, and (l) CH_n -only.

requires the identification of specific functional groups using our spectral-editing techniques and ^1H – ^{13}C 2D HETCOR (below).

We also include the integrals of ^{13}C CP/MAS spectra of Coastal and Marine DOM samples in Table 2. Generally, CP results show trends similar to those of DP with respect to the differences between the two samples. However, ^{13}C CP/MAS is less quantitative than ^{13}C DP/MAS at high spinning speeds for characterizing NOM because it overestimates sp^3 -hybridized carbons and underestimates sp^2 -hybridized carbons.^{14–16}

Detailed NMR Analysis Based on Spectral-Editing Techniques. Figure 2 shows a series of ^{13}C CP/MAS NMR with spectral editing of Coastal DOM (Figure 2a–g) and Marine DOM (Figure 2h–l) which allow for identifications of specific functional groups. Suitably designed radiofrequency pulse sequences select subspectra of specific types of functional groups, such as sp^3 -hybridized only, nonprotonated carbons and mobile groups, immobile CH_n -only (i.e., CH_2 and CH), CH_2 -only, and CH -only carbons. We did not collect CH -only and CH_2 -only spectra for Marine DOM, again due to the limited amount of sample.

Figure 2a and h are the ^{13}C CP/TOSS spectra of Coastal DOM and Marine DOM, respectively, intended to serve as reference spectra for those beneath. Consistent with the DP/MAS spectra (Figure 1 and Table 2), the CP/MAS spectrum of Coastal DOM shows significantly more signals from aromatics and aromatic C–O groups than the Marine DOM. In addition, the CP/MAS spectrum of Coastal DOM contains observable signals of ketones/aldehydes around 200 ppm, whereas that of Marine DOM lacks those signals.

Figure 2b and i are the spectra of ^{13}C CP/TOSS with 40- μs dipolar dephasing, displaying signals of nonprotonated carbons (C_q) and mobile groups such as OCH_3 and CCH_3 (CH_3 bonded to carbon). Significant amounts of nonprotonated carbons are present in both DOM samples. The spectral region around 0–24 ppm is attributed to CCH_3 groups. The signal around 30 ppm with a shoulder to ca. 40 ppm represents mobile CH_2 carbon. The signals between 40 and 50 ppm are attributed to nonprotonated CC_q and those between 50 and 62 ppm are due to OCH_3 groups. The NCH signals, which overlap with those of OCH_3 groups in the routine ^{13}C CP/TOSS spectra (Figure 2a and h), are removed by dipolar dephasing. The signals around 62–92 ppm and 100 ppm are attributed to nonprotonated OC_q and OC_qO , respectively. Note that the signals due to nonprotonated carbons and mobile groups between 0 and 90 ppm are very similar for the two samples. The signals of nonprotonated aromatic and anomeric OC_qO carbons overlap around 100 ppm and this overlap problem can be addressed by our ^{13}C CSA filter technique¹⁷ as shown later. There is no such overlap for the spectrum of Marine DOM due to its low aromatic content (Figure 2h). Significant nonprotonated aromatic carbon resonances between 120 and 162 ppm are present for Coastal DOM (Figure 2b) but almost none for Marine DOM (Figure 2i). Obviously, the majority of the aromatic carbons in Coastal DOM are not protonated, as shown by the comparison of its dipolar-dephased spectrum (Figure 2b) with its reference ^{13}C CP/TOSS spectrum (Figure 2a); nevertheless, the peak at 132 ppm, which disappears after dipolar dephasing, is due to aromatic C–H. In contrast, the small signal between 120 and 150 ppm for Marine DOM (Figure 2h) is primarily due to protonated carbons since its dipolar dephased spectrum (Figure 2i) shows almost no signal in this region. For Coastal DOM, the dipolar-dephased

spectrum also exhibits signals from aromatic C–O around 150 to 162 ppm, N–C=O/COO around 176 ppm, and ketones around 220 ppm. In addition, substantial amounts of aldehydes (protonated C=O groups that resonate between 190 and 220 ppm) can be excluded based on dipolar dephasing, which does not show the corresponding signal decrease (cf. Figure 2a and b). Marine DOM has no significant signal in the aldehyde and ketone region.

Figure 2c and j are the ^{13}C CP/TOSS spectra obtained after a ^{13}C CSA filter. The ^{13}C CSA-filtered spectra allow separation of sp^3 - and sp^2 -hybridized carbons. Using this technique, the overlap of anomeric OCO and aromatic carbon signals between 90 and 120 ppm is resolved, especially for Coastal DOM. The ^{13}C CSA-filtered spectra clearly show signals of sp^3 -hybridized OCO at >100 ppm, indicating that sugar rings are present in both samples. The combination of this CSA filter technique with dipolar dephasing (Figure 2d and k) shows the signals of nonprotonated sp^3 -hybridized carbons, indicating that significant amounts of quaternary OC_qO groups are present in both samples. Interestingly, these two spectra (Figure 2d and k) are quite similar, suggesting similar nonprotonated sp^3 -hybridized carbons in the two samples.

The CH_n -only spectra are shown in Figure 2e and l. The CCH_3 signals are reduced in intensity relative to the corresponding signals in the CP/TOSS spectra due to their low CP efficiency. Obviously, the protonated-carbon spectra of the two samples differ. The signals between 0 and 60 ppm from Coastal DOM are more intense relative to the total signal than those from Marine DOM. In addition, significant protonated aromatic signals of Coastal DOM are present between 117 and 150 ppm, whereas we do not observe any aromatic signals in the CH_n -only spectrum of Marine DOM. The small peak in the region 130–150 ppm in the CP/MAS spectrum of Marine DOM (Figure 2h) corresponds to mobile olefinic $-\text{C}=\text{C}-$ within lipid chains.¹²

We also acquired CH_2 - and CH -only spectra for Coastal DOM (Figure 2f and g). In the CH_2 -only spectrum (Figure 2f), two bands appear, those of CCH_2C around 30 ppm and OCH_2C around 62 ppm; a weak shoulder at 24 ppm is most likely due to residual CH_3 signals. In the CH -only spectrum (Figure 2g) four major bands are present, those of NCH around 55 ppm, OCH around 72 ppm, OCHO around 100 ppm, and aromatic CH around 110–150 ppm. With the detailed information on CH and CH_2 groups, the overlapping CH_n -only spectrum (Figure 2e) can now be reliably assigned: the shoulder below 24 ppm is attributed to residual CCH_3 groups; the band around 33 ppm to CH_2 ; the signals around 40 ppm to CH groups; the dominant peak at 72 ppm to OCH; the peak around 102 ppm to OCHO; and the broad band from 110 to 150 ppm to aromatic CH. Note that the OCH_2C band is buried under the shoulder of the dominant OCH peak at 72 ppm. Clearly, the sum of the signals of CH and CH_2 selection spectra is close to that of the CH_n -only spectrum. In the CH_n -only spectrum of Marine DOM (Figure 2l), we observe a very weak aromatic or olefinic $-\text{C}=\text{CH}-$ band around 120–150 ppm, the OCHO signal around 100 ppm, and the OCH resonance at 72 ppm. However, without assistance of CH and CH_2 selection techniques, we cannot identify OCH_2 , NCH, and CCH_2 specifically, as we can for Coastal DOM, although we know that they are present in the sample indirectly from other spectral-editing techniques.

Structural Information via Short-Range 2D HETCOR. To identify the connectivities or proximities of different

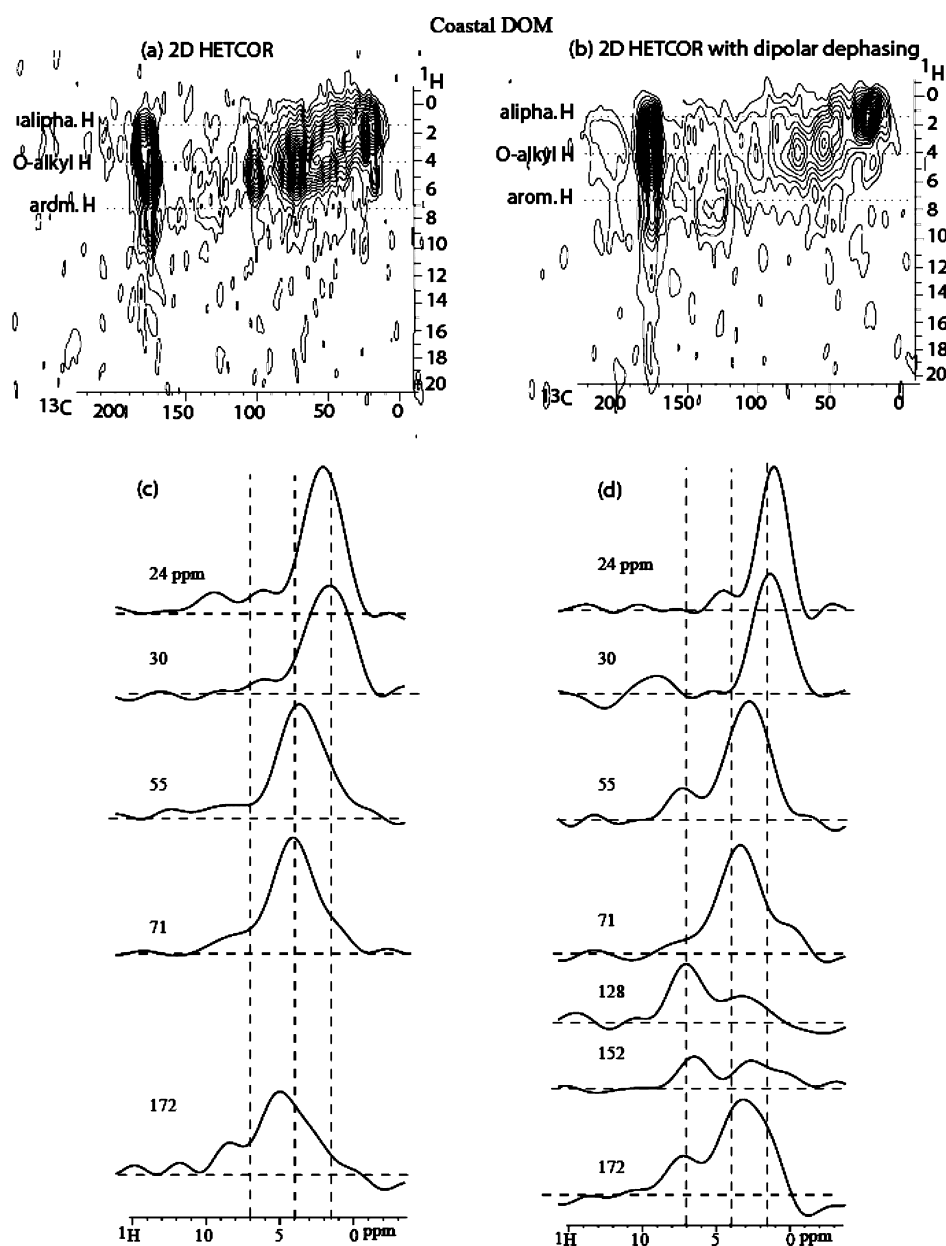


Figure 3. 2D HETCOR spectra of Coastal DOM. (a) 2D HETCOR, (b) 2D HETCOR with 40- μ s dipolar dephasing, (c) ^1H slices of (a), and (d) ^1H slices of (b).

functional groups, 2D ^1H – ^{13}C HETCOR spectra of Coastal DOM were acquired (Figure 3a). 2D HETCOR with 0.5 ms of Lee–Goldburg cross polarization (LGCP) shows correlation peaks primarily for one- and two-bond distances. Proton cross sections at the indicated ^{13}C chemical shifts were extracted to facilitate the identification of connectivities or proximities of different functional groups (Figure 3c). Nonpolar alkyl H resonates between 1 and 3 ppm, alkyl OCH and NCH between 3 and 5.5 ppm, aromatic H between 5.5 and 9 ppm, amide NH near 8 ppm, and COOH between 8 and 12 ppm. ^1H slices extracted at the ^{13}C chemical shifts of 24 and 30 show only alkyl ^1H bands around 2 ppm, indicating that these carbon sites primarily correlate with their directly bonded ^1H . The ^1H slice extracted at 55 ppm of OCH_3 demonstrates that these carbons are mainly associated with their directly bonded protons but their proximity with alkyl protons is also observed, as demonstrated by a shoulder up to 0 ppm. The ^1H slice at

the ^{13}C chemical shift of 71 ppm attributed to OCH groups indicates that the dominant contributions are from the directly bonded O-alkyl protons, although correlations with alkyl and aromatic protons are also observed.

Dipolar dephasing of 40 μ s duration was inserted in the LGCP HETCOR pulse sequence to reveal multibond C–H connectivities of nonprotonated carbons. Except for mobile CCH_3 and CCH_2C groups, nontrivial correlation peaks of nonprotonated carbons with the nearest ^1H that are at a distance of at least two bonds can be obtained (Figure 3b). Again, proton slices were extracted at the indicated ^{13}C chemical shifts (Figure 3d). The proton slices extracted at 24 and 30 ppm attributable to mobile CCH_3 and CCH_2C groups exhibit correlations of these carbons with their own protons. The 55-ppm carbon shows correlations predominantly with aliphatic protons resonating at ~ 3.5 ppm but also with aromatic protons at ~ 7 ppm, consistent with the OCH_3 assignment. This

correlation is due to lignin residues. ^1H slices at the ^{13}C chemical shift of 71 ppm, from OCH groups, show that the dominant contributions are from the directly bonded O-alkyl protons. The ^1H slice extracted at 128 ppm, attributed to nonprotonated carbons, shows major contributions from aromatic protons but also from some O/N-alkyl and alkyl protons. The ^1H slice from 152 ppm, from aromatic C–O, shows contributions from aromatic H, N/O-alkyl H, and alkyl H. The ^1H slice extracted at 172 ppm shows that the COO/N–C=O carbons are primarily correlated with N/O– and other alkyl protons, and somewhat with aromatic protons. We did not extract ^1H slices around 200 ppm attributed to ketones due to the low signal-to-noise ratio. However, based on 2D HETCOR spectrum with dipolar dephasing (Figure 3b), the dominant contributions of ketones are from O/N-alkyl protons, with some from aromatic protons.

Nitrogen Functional Groups Based on ^{15}N CP/MAS.

^{15}N CP/MAS spectra of the two samples are shown in Figure 4.

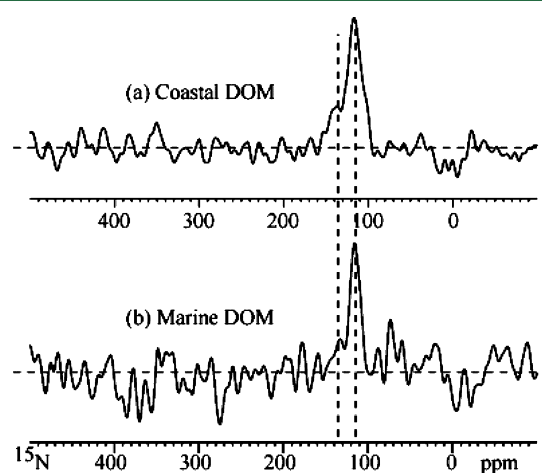


Figure 4. ^{15}N CP/MAS spectra of (a) Marine DOM and (b) Coastal DOM. The right dashed line at 115 ppm marks the typical resonance position of peptides, while the left one around 140 ppm is at a position typical of pyrroles.

We observe signals from 100 to 155 ppm for Coastal DOM, and from 100 to 140 ppm for Marine DOM. Both spectra have a dominant band around 115 ppm corresponding to secondary amides. A shoulder centered at ca. 135 ppm is also observed, which could correspond to protonated pyrrole or indole N, anilide N, or CO–NH–CO–NH as found in hydantoin.¹⁸ The shoulder appears to be larger for Coastal DOM than Marine DOM, which is consistent with the larger fraction of aromatic residues seen in the ^{13}C spectra of Coastal DOM.

Evidence for Major Structural Units of the Two DOMs Based on NMR Results. The detailed NMR structural information provides evidence for the major structural units of the two distinct DOMs. For Coastal DOM, our NMR results are consistent with the presence of the major structural units of (1) carbohydrate-like moieties, (2) lignin residues, (3) peptides or amino sugars, and (4) COO-bonded alkyls. For Marine DOM, they are (1) carbohydrate-like moieties, (2) peptides or amino sugars, and (3) COO-bonded alkyls. The detection of OCH around 72 ppm, OCHO around 100 ppm, and OCH₂C around 62 ppm is consistent with the presence of carbohydrate-like components. The presence of lignin residues in Coastal DOM can be justified by their characteristic OCH₃ peak around 56 ppm and aromatic C–O signals between 150 and 162 ppm.

The NCH peak around 55 ppm in ^{13}C NMR as well as the dominant amide bands in the ^{15}N spectra are consistent with the presence of peptides or amino sugars. The correlation of COO and alkyls revealed in the 2D HETCOR spectra are consistent with the presence of COO-bonded alkyls, which could be terpenoid-derived structures or carboxyl-rich alicyclic molecules (CRAMs) as proposed in the literature.^{19,20} The minor aromatic carbon and absence of aromatic C–O groups in Marine DOM indicates the paucity of lignin. In addition to these major structural units, there is evidence of significant humification in both samples. The presence of significant amounts of nonprotonated (i.e., highly substituted) carbon in both samples is indicative of extensive diagenesis, since the biopolymers from which they presumably are derived contain very few nonprotonated carbons, as demonstrated elsewhere.^{21,22} As we discuss later, the abundance of nonprotonated carbons could be used as an index of humification.

Compared to Marine DOM, Coastal DOM is more complex because it is a mixture of organic matter of more variable terrestrial and aquatic inputs and diagenetic history.¹ Generally, three major differences, some of which were found in other studies,^{1,23,24} exist between the two samples. The quantitative differences can be found in Table 2. First, there are some ketones (2%) in Coastal DOM, but few or none (1%, not significantly different from zero) in Marine DOM. Second, significant amounts of lignin residues are present in Coastal DOM (12% aromatics), but not in Marine DOM (2% aromatics). Third, Coastal DOM contains fewer carbohydrate-like moieties (117–60 ppm: 31% total) than Marine DOM (43%). The COO/N–C=O groups in both DOM are abundant, accounting for about 19% and 17% of total carbons for Coastal DOM and Marine DOM, respectively. Note that the COO/N–C=O peak is the highest in the DP spectrum of Coastal DOM but not in that of Marine DOM.

DISCUSSION

Comparison with UDOM. The two DOM samples in the present study were collected from surface water by the RO/ED technique. In the literature, NMR studies have been carried out on surface water DOM isolated using tangential-flow ultrafiltration, known as UDOM.^{7,25,26} Since the yield of marine DOM is so low (5–30%) using tangential-flow ultrafiltration, UDOM may not be representative of marine DOM. Consistent with the conclusion reached by Koprivnjak et al.,¹ our NMR spectra indicate that RO/ED collected more alkyl carbons than tangential-flow ultrafiltration. These alkyls could be low-molecular-weight COO-bonded alkyl compounds that do not seem to be in UDOM because ultrafiltration can only isolate organic compounds larger than 1 kDa. In contrast, the RO/ED technique can collect 70–80% of DOM and, therefore, is more likely to be representative of native DOM.

Potential Sources of Organic Matter. An understanding of the chemical structure of marine DOM can provide insights into its origins, reactions, and fates. Based on detailed structural information from advanced solid-state NMR, we can try to explore the potential sources or precursors of two distinct DOM samples. Roughly speaking, the removal of aromatics, aromatic C–O groups, and ketones from Coastal DOM would render it structurally more similar to Marine DOM.

Ketones. Our study indicates that in Coastal DOM ketones are primarily adjacent to O/N-alkyl protons (Figure 3b). This contrasts with the conclusions of Leenheer et al.⁴⁴ who found that the majority of ketones in stream humic- and fulvic-acid

standards of the International Humic Substances Society were aromatic ketones. They hypothesized that aromatic ketones could be formed by photolytic rearrangements and oxidation of phenolic ester and hydrocarbon precursors. In addition, aromatic ketones could also originate from oxidation of benzylic hydroxyl groups in lignin side-chains.^{27,28} Mopper and Stahovc²⁹ demonstrated that low-molecular-weight carbonyl compounds such as formaldehyde, acetone, and glyoxal can be formed by the photolysis of seawater DOM. This process was most pronounced in the surface ocean and in coastal regions where terrigenous DOM prevails.³⁰ However, the Marine DOM of our study had no detectable ketone C.

Aromatics. Aromatics in Coastal DOM could be derived from lignin and tannin. It has been well documented that lignin-derived compounds contribute significantly to the aromatics in terrestrial organic matter.²⁸ However, it has also been proposed that most riverine aromatic carbons are derived from tannins, not lignins.³¹ On average, lignin degradation products determined by cupric oxide oxidation account for only 0.5% of the DOC.³¹ Opsahl and Benner demonstrated that lignin derivatives were destroyed by light in a short time.³²

It has been shown that a peak at 105 ppm in the dipolar-dephased spectra is a characteristic marker for tannin; this peak is attributed to C-10 and C-8 in the tannin structure, aromatic carbons two bonds away from oxygen.³³ In addition, there are two well-resolved peaks at 145 and 155 ppm.³³ We do not observe these distinct peaks in the spectra of Coastal DOM, even with the aid of spectral editing (Figure 2), suggesting that tannin cannot be significantly present in this sample, whereas strong evidence supports significant presence of lignin. We observed only a very small amount of aromatic moieties in Marine DOM; these aromatic moieties could be partially accounted for by aromatic amino acids.²²

Carbohydrate-Like Moieties. Significant carbohydrate-like moieties are present in both samples. The compositions and structures of carbohydrate-like moieties in UDOM have been the subject of debate. Initially, it was proposed that carbohydrate, acetate, and lipid are linked together in UDOM.³⁴ In 2002, Mao et al. obtained rather similar ¹³C NMR spectra of the hydrophilic high-molecular weight fraction of fresh biosolids derived from wastewater³⁵ and indicated that the assignments by Aluwihare et al.³⁴ were questionable. The model was the first to feature N-acetylated sugar rings, but it explained the 19-ppm CH₃ resonance in terms of lactic-acid groups with an ether linkage to the sugar rings.³⁵ Aluwihare et al.²⁵ and Quan and Repeta³⁶ later changed their model completely and proposed a revised, simple model containing two types of sugar rings, N-acetylated sugars and deoxy sugars. For Coastal DOM, cellulose and hemicellulose from terrestrial sources could also contribute to carbohydrates. Note that significant nonprotonated O-alkyls are present in both samples, which must be attributed to humification (see below).

Alkyls. Both DOM samples contain significant amounts of alkyl carbons resonating around 0–50 ppm in ¹³C spectra. In addition, ~1/5–1/6 of carbons belong to COO/N–C=O. Using ¹H–¹³C HETCOR NMR we observe that some COO/N–C=O groups are indeed associated with alkyls, indicating the presence of COO-bonded alkyls. This is consistent with (though not proof of) the presence of terpenoid-derived CRAMs;^{20,21} however, it is difficult to prove the presence of ring structures using NMR. Nevertheless, COO/N–C=O groups appear to be associated with a range of protons. Alicyclic rings, such as those found in terpenoid hydrocarbons,

are quite resistant to oxidative degradation, and therefore, these structures could accumulate in DOM.^{19,20,31} It has been demonstrated that terpenoid-derived CRAMs could be present as refractory components in marine DOM.²⁰ The rings of terpenoids are degraded by the Bayer-Villiger oxidative degradation pathway as described by Trudgill.³⁷ The rings are first hydroxylated, oxidized to ketones, converted to lactones, and then opened by hydrolysis to create a degradable short-chain carboxylic acid. As ketones are intermediates in this process, they are not stable and are thus absent in refractory marine DOM.

Potential N Sources. The Marine DOM contains nitrogen primarily from primary and secondary amides. Aluwihare et al.²⁵ indicated that there were two pools of nitrogen in marine organic matter: one from peptide and another from N-acetyl sugar.^{35,38} In addition to amide N, Coastal DOM also contains pyrroles, indole protonated N, anilides,³⁹ or hydantoin-like residues.¹⁸ The significant aromatic moieties in this DOM are consistent with the presence of these N compounds. Marine DOM appears to contain less of these compounds. Aluwihare et al.²⁵ indicated that amides in high-molecular-weight dissolved organic nitrogen in the ocean could be from N-acetyl amino polysaccharides, protein, or nonhydrolyzable amides. Possible environmental sources of pyrrole rings include degraded pigments^{40,41} and Maillard reaction products from protein and carbohydrate starting materials.^{42,43} Anilides could be formed via the reaction of lignin-derived phenolic compounds with ammonia and the COOH or NCO groups of amino acids or peptides.³⁹

Nonprotonated O-Alkyl Carbons—Potential Index of Humification. Our previous study indicated that fresh DOM and algae, which are primarily composed of biopolymers, contain few nonprotonated alkyl carbons,²² while humified DOM contains significant amounts of these. Thus, it seems that the nonprotonated alkyl carbon content, especially in the O-alkyl region (60–120 ppm), can be used as a potential index of humification. This is reasonable because humification leads to greater substitution, branching, and cross-linking. We have chosen nonprotonated O-alkyl carbons as the potential humification index because alkyl carbons in carbohydrates from biopolymers or young DOM are primarily protonated, so nonprotonated O-alkyl carbons must have been generated during the humification process. We have limited the chemical shift range to 60–94 ppm because below 60 ppm, there are signals of mobile OCH₃, CH₂, and CCH₃ groups in the dipolar-dephased spectra, whereas aromatic signals can extend from 94 to 120 ppm. In the present study, by using dipolar dephasing techniques we detected significant nonprotonated OC_q signal around 60–90 ppm, indicating that significant amounts of humified carbons are present in both samples. Interestingly, we note similar nonprotonated-carbon signals between 0 and 90 ppm, suggesting that the two samples could be subject to similar humification. In another study, Helms et al.²¹ hypothesized that nonprotonated aromatics in gilsonite bitumen, either in the form of fused or highly substituted rings, are refractory components in nature. In the future, we will investigate NOM samples from different sources and test the viability of the proposal of nonprotonated O-alkyl carbons as a humification index.

■ ASSOCIATED CONTENT

■ Supporting Information

NMR experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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