Nature, origin and average age of estuarine ultrafiltered dissolved organic matter as determined by molecular and carbon isotope characterization

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Abstract

The Ems-Dollart estuary (on the border of the Netherlands and Germany) was chosen for a pilot study to characterize ultrafiltered dissolved organic matter (UDOM) in estuarine systems. UDOM samples were taken from four locations with salinities varying from 0.43 to 20%. The UDOM in these samples was concentrated using cross-flow ultrafiltration and represents the size fraction of DOM between 1000 Dalton and 0.2 µm. The samples were analyzed by analytical pyrolysis, stable carbon isotope and radiocarbon analysis, and solid state 13C NMR. All UDOM samples throughout the estuary showed relatively similar pyrolyzates and 14C activities (∼87%). Solid-state 13C NMR spectra were also similar, except for the sample with the highest salinity. UDOM δ13C values ranged from −27.78 to −25.40% with increasing salinity. This suggests mixing of river DOM with seawater DOM of comparable ages. The absence of pyrolysis products of unaltered polysaccharides, lignins, proteins and lipids indicates that the Ems-Dollart estuary UDOM consists of a large fraction of refractory organic matter and a very small fraction of fresh organic matter.

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1. Introduction

One of the major reactive reservoirs of organic carbon on Earth is dissolved organic carbon (DOC) in the oceans (Hedges, 1992). Studies on DOC have been important research topics in oceanography for several decades and these topics have gained increasing attention because of efforts to better understand the global carbon cycle. The study of DOC is particularly difficult because DOC occurs in very low concentrations (ca. 1 mg C/l) in the oceans and is comprised of a complex mixture of organic compounds. Given the importance of marine dissolved organic matter (DOM) to the global carbon cycle, we investigated the chemical composition of DOM to better understand its source, reactivity and fate.
The mean average age of oceanic dissolved organic carbon (DOC) is over 1000 years (Kirchman et al., 1991). DOC consists of a labile fraction with a high turnover rate and a refractory fraction. The labile fraction is a possible source of nutrition for the microbial loop (Kirchman et al., 1991; Amon and Benner, 1994, 1996). The turnover rate of marine DOC is highly variable. It varies from less than a day up to more than 1000 years. Williams and Druffel (1987) calculated that in the central North Pacific Ocean upper mixed layer water, 56% of the DOC was less than 30 years old, and the remaining 44% was older than 6000 years. Thus the refractory fraction probably consists mainly of selectively preserved constituents in the water column. Studies by Meyers-Schulte and Hedges (1986) and Williams and Druffel (1988) have shown that marine DOM is comprised mainly of polymeric materials derived from marine sources. Only very low concentrations of terrestrial DOM can be detected in the humic fraction of DOM from the pelagic Pacific Ocean (Meyers-Schulte and Hedges, 1986; Opsahl and Benner, 1997). Furthermore, carbon isotope analyses indicated that marine DOC is predominantly autochthonous ($\delta^{13}C = -21$ to $-24\%$; Williams and Gordon, 1970; Benner et al., 1997). Such results further suggest a marine origin of DOM. Direct molecular-level analyses have provided important information on the concentrations and dynamics of non-polymeric compounds, but these comprise a relatively small fraction (<15%) of the DOM (Williams and Druffel, 1988).

In estuaries, DOM can originate from a number of autochthonous as well as allochthonous sources (Menzel, 1974; Duce and Duursma, 1977; Handa, 1977; Whittle, 1977). Autochthonous sources are associated with processes such as excretion by organisms living in the estuary, autolysis of dead organisms and microbial decomposition. Allochthonous sources include organic matter from the sea, rivers and marshes, and precipitation of low-molecular weight DOM, thus forming colloidal DOM. Removal of DOM in an estuary is caused by decomposition, heterotrophic intake by living organisms, transformation of DOM to POM and transport towards the sea (Williams, 1975). Studies concerning the sources of organic matter and processes within the estuaries will provide more insight in the mechanisms that control the global carbon cycle and the oxygen cycle, since 80% of the global organic matter burial and 90% of the global sedimentary mineralization are believed to be concentrated in the coastal zone (Pernetta and Milliman, 1995).

DOC concentrations in estuarine and deltaic waters are generally relatively high. In many cases an estuarine system is characterized by high DOC concentrations in the river water and decreasing DOC concentrations towards the marine end of the estuary. In the Ems-Dollart estuary (Laane, 1980) as well as in other estuaries, like Galveston Bay (Guo and Santschi, 1997), an inverse linear relationship exists between DOC concentration and salinity. Therefore, DOC in the Ems-Dollart estuary seems to be biologically inactive and behaves conservatively during mixing and transport processes. This would suggest that marine DOM contains an important terrestrial component. However, based on stable carbon isotope and lignin contributions, most of the land-derived organic matter discharged by rivers to the marine environment is deposited close to shore (Gearing et al., 1977; Hedges and Mann, 1979). It is not clear what happens to terrestrial DOM in estuaries. On the one hand, indications exist that a fraction of riverine DOM is removed by flocculation, precipitation and adsorption to particles, thus reducing the impact of riverine DOM to the ocean carbon cycle. On the other hand, experiments have shown that DOM behaves more conservatively (e.g. Mantoura and Woodward, 1983), suggesting a more significant contribution of riverine DOM to oceanic DOC (Lee and Wakeham, 1992).

Although data exist on the quantification and composition of suspended organic matter in estuaries (e.g. Laane, 1982; Eisma et al., 1991; Qian et al., 1996), molecular composition of colloidal material in estuaries (Sigleo et al., 1982) and bulk chemical characterization of DOM in estuaries (Laane, 1980), no studies concerning the molecular composition of DOM in estuaries have been conducted. Therefore a pilot study was initiated to investigate the molecular composition of DOM in estuaries aiming to determine the contribution of terrestrial DOM to marine DOM in estuaries. The Ems-Dollart estuary has been chosen for this pilot study. It is very well characterized and, compared with other estuaries like those of the Scheldt, Meuse and/or Rhine, it represents a relatively unpolluted area.

The aim of the present study is to determine the origin and fate of organic matter in the Ems-Dollart estuary by means of stable carbon isotope measurements, $^{14}$C dating, solid-state $^{13}$C-NMR and molecular characterization using analytical pyrolysis.

2. Experimental

2.1. Site description

The Ems-Dollart estuary is part of the Wadden Sea, a coastal sea separated from the North Sea by a large number of barrier islands. The Wadden Sea is located along the northern coasts of the Netherlands and Germany and the western coast of Denmark.

The salinity at the freshwater end of the estuary is less than or equal to 0.5%, whereas the salinity at the mouth of the estuary is greater than 35%. For most of its length, the Ems-Dollart is a fully mixed estuary, both horizontally and vertically (Dorrestein, 1960; Dorrestein...
and Otto, 1960). Stratification only occurs in parts of the river Ems and in the southern part of the Dollart. Here the estuary is partly mixed. In a one-dimensional model of the Ems estuary Dorrestein and Otto (1960) calculated the average freshwater flushing time to be 84 tidal periods, assuming no freshwater inflow from the Dollart and a mean freshwater inflow from the Ems of 100 m$^3$ s$^{-1}$.

2.2. Sampling and sample processing

During a cruise with the RV *Navicula* at the end of September 1995, surface water samples were collected using an air-pressure driven membrane pump and a polyethylene-coated tube at four different locations in the Ems-Dollart estuary (Fig. 1), representing different water characteristics. Sample 1 was taken from the Ems river upstream from a lock. It represents a pure riverine water sample without any tidal and thus seawater influence. Sample 2 represents a riverine water sample with tidal influence. Sample 3 was taken from a point in the river Ems that was reported by Eisma et al. (1991) to be a suspended matter maximum. This point is the boundary between water masses with low salinity and water masses with higher salinity. Finally, sample 4 was taken from the sea end of the estuary, representing river water that has been mixed thoroughly with seawater.

Immediately following sampling, water samples were prefiltered to separate DOM from POM using a Sartorius II Concentrator tangential-flow system equipped with a polypropylene 0.2 μm pore sized Sartorius membrane filter cartridge.

Ultrafiltered dissolved organic matter (UDOM) was obtained by concentrating these prefiltered samples using a custom-made Amicon SP-60 tangential-flow ultrafiltration system equipped with two Amicon S10N1 spiral-wound polysulfone 1000 Dalton cut-off filters. Tangential-flow ultrafiltration is a relatively new method that has been applied successfully to concentrate a fraction of DOM from seawater (Benner, 1991; Benner et al., 1992). The samples were concentrated to about 2 l and were stored frozen at −20°C for transport to the laboratory. In the laboratory, most salts were removed from the concentrated samples by diafiltration with 10 volumes (i.e. 20 l) of distilled water. Although generally about 10% of the DOC is lost during diafiltration (Benner, 1991), it is essential to remove most of the salts before the analyses by analytical pyrolysis. Subsequently, water was removed by evaporation in vacuo at 30°C using a rotary evaporator followed by freeze-drying of the samples.

2.3. Solid-state $^{13}$C NMR

Dried samples of UDOM were subjected to solid-state $^{13}$C NMR in a 9-mm diameter ceramic rotor packed with Teflon tape as filler. The small amount of material, generally 50 mg dry weight per sample (TOC of only 7 to 19%; Table 1), necessitated such an approach to optimize the weak signals. The rotor was spun at 3.5 kHz at the magic angle (54.7°) in the probe of a Chemagnetics, Inc., M-100 spectrometer operating at 25.2 MHz for carbon. The standard cross-polarization pulse sequence with 1 ms contact time and 0.7 s cycle time was used and approximately $10^5$ acquisitions were accumulated to obtain the spectra. Exactly 512

<table>
<thead>
<tr>
<th>Sample</th>
<th>Salinity</th>
<th>Sample volume</th>
<th>Dry weight UDOM sample</th>
<th>$C_{org}$ UDOM sample</th>
<th>Ultrafiltered [DOC]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.43</td>
<td>50</td>
<td>316</td>
<td>15.3±1</td>
<td>80.6</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>20</td>
<td>288</td>
<td>10.6±1</td>
<td>127</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>25</td>
<td>270</td>
<td>19.3±1</td>
<td>174</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>50</td>
<td>356</td>
<td>7.1±1</td>
<td>42.1</td>
</tr>
</tbody>
</table>

Fig. 1. Map of the sampling area.

Table 1
Hydrographic data
data points were acquired and zero-filled to 4096 data points prior to filtering with 75 Hz linebroadening and Fourier transformation. The chemical shifts were referenced externally to hexamethylbenzene.

2.4. Determination of \( C_{\text{org}} \), \( \delta^{13}C \) values and \( ^{14}a \) values (AMS)

Dried samples of UDOM were acidified with dilute hydrochloric acid to remove carbonates, dried over KOH and placed in quartz tubes together with oxidized copper wire. The tubes were evacuated and sealed and placed in a furnace at 800°C for 6 h. The combustion gases were led over a copper oven at 600°C and a silver oven at 400°C to remove nitrogen oxide, sulfur oxides and halogens. Carbon dioxide was trapped in liquid air. The amount of CO2 was determined by expanding the gas into a known volume and measuring its pressure. The resulting graphite/catalyst mixture was pressed into a target for graphite/catalyst mixture was pressed into a target for the Tandem-Accelerator Mass Spectrometer (AMS; HVEE BV, Amersfoort; Aerts-Bijma et al., 1997; Wijma and van der Plicht, 1997).

From the measured \( ^{14}C:^{12}C \) ratio \( (^{14}R) \) of the sample and a known standard the \( ^{14}a \) value, expressed as percent of modern carbon (pMC), is calculated as:

\[
^{14}a = (^{14}R_{\text{sample}}/^{14}R_{\text{standard}})(1-25\%)/(1+\delta^{13}C))^{2} \times 100 \text{ pMC}
\]

The standard to which \( ^{14}C \) data are referred to is oxalic acid from 1950. According to the official definition by Stuiver and Pollach (1977) a correction has to be made for the time of measurement after 1950, but the effect of this is negligible. The \( ^{14}a \) value is related to the age of the sample, since due to radioactive decay \( (T_{1/2} = 5730) \), \( ^{14}C \) is disappearing from the sample during time. Because the \( ^{14}C \) concentration is also affected by isotopic fractionation, a correction for this is made with the factor \( (1-25\%)/(1+\delta^{13}C) \).

When comparing marine and terrestrial samples, samples with the same age do not have the same \( ^{14}a \) value because the carbon source for organisms in the ocean usually has a lower \( ^{14}C:^{12}C \) ratio than CO2 in the atmosphere, due to the so called reservoir effect. Therefore, and because the measured samples are probably mixtures of components with different ages, \( ^{14}a \) values are reported in addition to the average ages, which are merely reported in order to compare the \( ^{14}C \) isotope data presented in this study with the existing oceanographic literature.

2.5. Curie-point pyrolysis-gas chromatography (CuPy-GC)

Curie-point pyrolysis-gas chromatography (CuPy-GC) analyses were performed using a Hewlett-Packard 5890 gas chromatograph, equipped with a FOM-4LX pyrolysis unit (Boon et al., 1987) and a cryogenic unit. A 30 m fused silica capillary column coated with chemically bound DB 1701 (0.25 mm i.d., film thickness 0.25 μm) was used for all samples. Some selected samples were also studied using a 25 m fused silica capillary column coated with chemically bound CP Sil-5 (0.32 mm i.d., film thickness 0.45 μm). Helium was used as a carrier gas. A flame ionization detector (FID) at 320°C was used for detection. The GC was temperature programmed as follows: initial temperature 0°C (5 min); heating rate 3°C/min; final temperature 300°C (10 min). Samples were pressed onto flattened ferromagnetic wires (Curie temperatures of 610°C) and placed into the pyrolysis unit. The pyrolysis unit was connected to a FOM high frequency generator that heated the wires inductively in 0.15 s to the Curie temperature. This temperature was maintained for 9 s.

2.6. Curie-point pyrolysis-gas chromatography/mass spectrometry (CuPy-GC/MS)

Curie-point pyrolysis-gas chromatography/mass spectrometry (CuPy-GC/MS) analyses were carried out in the same way as the CuPy-GC analyses. A Hewlett-Packard 5890 gas chromatograph was connected to a VG Autospec Ultima mass spectrometer operated at 70 eV with a mass range of \( m/z \) 700–35 and a cycle time of 1 s.

3. Results and discussion

DOM from the Ems-Dollart estuary was concentrated and isolated using tangential-flow ultrafiltration. Benner et al. (1992) have shown that tangential-flow ultrafiltration is a successful method to recover a substantial fraction of DOM from seawater. It concentrates compounds on the basis of molecular size and shape. Ultrafiltration is therefore not as chemically selective as the XAD isolation method which concentrates mainly hydrophobic components of DOM. Ultrafiltration is also a much gentler isolation method that does not require large manipulations of pH like the XAD isolation method. Ultrafiltration (1000 Dalton cut-off filter) has been shown to isolate a much larger fraction (up to ~30%) of seawater DOM (Benner et al., 1992) than conventional DOM isolation methods. The 70% that could not be isolated contains particles smaller than 1000 Dalton. Therefore, the isolated UDOM is not actually dissolved, but more of colloidal nature. In Table 1, dry weights of the recovered Ems-Dollart UDOM samples are listed together with the ultrafiltered DOC concentrations calculated from the percentages of organic carbon present in the UDOM samples. No DOC concentrations were measured in this study, so no
estimates were made for the recovery of the UDOM. It should be realized, however, that the data obtained do not represent the total size spectrum of the DOM, but the size fraction of DOM ranging from 1000 Dalton to 0.2 μm. From the study by Santschi et al. (1995) it has become clear that this fraction may be relatively less refractory than the smaller DOM size fraction.

From Table 1, it may be observed that the concentration of ultrafiltered DOC (UDOC) increases with increasing salinity up to the suspended matter maximum (sample point 3). After the suspended matter maximum, the concentration of UDOC decreases rapidly. This may be explained by coagulation of low-molecular weight (LMW) DOM to high-molecular weight (HMW) DOM and particulate organic matter (POM) across the salinity gradient and dilution of UDOM with seawater and seawater UDOM after this point.

3.1. Solid-state $^{13}$C NMR data

The solid-state $^{13}$C NMR spectra shown in Fig. 2 for three samples (the spectrum of sample 3 was nearly identical to that of sample 2) show a remarkable degree of similarity. Although the poor signal-to-noise ratio precludes more precise comparisons and reporting of integrated intensities, the three spectra appear to be composed mainly of aliphatic carbons (0–100 ppm; Table 2). The next most intense signals are those assigned to carboxyl/amide carbons at 175 ppm. The generally high intensity for these signals is indicative of the fact that the UDOM is composed of either highly carboxylated materials or that amide functionalities such as found in proteinaceous materials are important. Aromatic carbons are represented between 100 and 160 ppm, and their intensity is generally greater than would be observed in oceanic UDOM (Benner et al., 1992) and similar to what is expected for riverine DOM (Malcolm, 1990; Hedges et al., 1992). In the aliphatic region, a significant signal is observed at about 72 ppm, attributable to hydroxylated aliphatic carbons as would be found in carbohydrates or alcohols.

3.2. Carbon isotope data

The $\delta^{13}$C and $^{14}$C data of the UDOM samples are shown in Table 3. Sample 1 has been taken from a location in the river Ems without any tidal influence. Therefore, the UDOM in this sample is assumed to consist exclusively of terrestrial UDOM. It may therefore be regarded as a terrestrial end member, its $\delta^{13}$C value (~27.8‰) thus represents the value of terrestrial UDOM present in the Ems-Dollart estuary. The $\delta^{13}$C values show that from the most upstream station to the turbidity maximum (station 3), there is no significant increase in $\delta^{13}$C values. This implies that there is almost no mixing of riverine UDOM with marine UDOM. A clear marine contribution is seen in the UDOM from station 4 ($\delta^{13}$C = 25.4‰). The $\delta^{13}$C value of UDOM from this station indicates mixing of fluvial UDOM with c. 45% marine UDOM, assuming that North Sea UDOM has a $\delta^{13}$C value of ~22 to ~23‰ (le Clercq et al., 1997; van Heemst et al., 1999b). These results are in agreement with those of Eisma et al. (1991), who also found an increase in $\delta^{13}$C values of suspended organic matter with increasing salinity, indicating mixing of river organic matter with North Sea organic matter. This kind of conservative behavior of UDOM does not mean that is the case in all estuaries. Guo et al. (1996) measured $\delta^{13}$C values of UDOM isolated from the surface waters from the Middle Atlantic Bight of Cape Hatteras. They determined $\delta^{13}$C values of ~24.9, ~26.6, ~30.8, ~30.1, ~26.8, ~27.8 and ~22.9‰ for the UDOM samples collected from water with salinities of 5.0, 9.1, 10.4, 15.0, 18.2, 25.0 and 35.28‰, respectively. In contrast with the present study, the $\delta^{13}$C values that were determined by Guo et al. (1996) seem to indicate a non-conservative behavior of UDOM in the Middle Atlantic Bight.

Samples 1 to 3 also show no change in their $^{14}$C values (87 and 86% pMC; Table 3). Furthermore, the $^{14}$C activity in the fluvial UDOM (sample 1) is equal to that of dissolved inorganic carbon (DIC) at this station, at first sight suggesting that it is recently formed material.
by photosynthetic assimilation of DIC. However, these equal $^{14}a$ values may be better explained by assuming that the UDOM consists of old organic material giving accidentally the same $^{14}a$ value as the DIC. The downstream UDOM sample has a $^{14}a$ value of 87% pMC, equal to the upstream samples. Thus, the marine UDOM component must have a very similar age as the riverine component (around 87% pMC), since no change in $^{14}a$ is observed. Although a significant change in $^{14}$inorg is observed throughout the estuary, the $^{14}$org shows relatively similar values. This means that no relationship exists between the $^{14}$C of the organic and the inorganic matter. This implies the refractory nature of the UDOM present in the Ems-Dollart estuary.

### 3.3. CuPy-GC/MS results

Chromatograms of the pyrolyzates of the Ems-Dollart UDOM samples are shown in Fig. 3. These pyrolyzates are dominated by alkylphenols, alkylbenzenes, alkylpyroles and pyrolysis products of carbohydrates (alkylcyclopentenones, alkylcyclopentanones, alkylfurans and alkylcyclopentadienes). Unaltered polysaccharides yield vast amounts of acetic acid, alkylfurans, alkylfuraldehydes and deanhydromonosaccharides like levoglucosenone, 1,4:3,6-dianhydro-2,6-glucopyranose and levoglucosan (Pouwels et al., 1989; Pastorova et al., 1994) upon pyrolysis. None of these compounds except for small amounts of furans were encountered in any of the pyrolyzates of the Ems-Dollart UDOM, thus no unaltered polysaccharides were present in detectable amounts. The presence of relatively large amounts of salts in the UDOM samples might have influenced the formation of specific pyrolysis products, like the deanhydromonosaccharides. However, laboratory experiments involving addition of salt to a mixture of albumin bovine serum and starch still yielded these deanhydromonosaccharides upon pyrolysis of the mixture (van Heemst et al., 1999a).

Aliphatic amino acids in peptide and protein material show very characteristic pyrolysis products which can be monitored by mass chromatography of masses m/z 195+209 (Boon and De Leeuw, 1987). These pyrolysis products are indicative for the presence of intact proteins. None of these compounds was encountered in the pyrolyzates of the Ems-Dollart samples, therefore the contribution of fresh proteins to the samples was negligible.

No large amounts of lipids like fatty acids, phytadienes and sterols were encountered in any of the UDOM pyrolyzates. Overall, no major differences between the different chromatograms of the pyrolyzates of the samples are observed. These data correspond to earlier findings by Duursma (1961) and Laane (1980) that DOM behaves conservatively during transport and mixing in the Ems-Dollart estuary and that it is biochemically inert. Furthermore, Sigleo et al. (1982) concluded that the molecular composition of estuarine colloidal matter was very similar throughout the estuary. As was already concluded from the carbon isotope data, the pyrolysis data seem to imply as well that the Ems-Dollart UDOM appears to be refractory in nature, because of the absence of unaltered polysaccharides, proteins and lipids.

### Table 2
Relative intensities of the various NMR regions for UDOM samples

<table>
<thead>
<tr>
<th>UDOM sample</th>
<th>Aldehyde/ketone (190–220 ppm) %</th>
<th>Carboxyl/amide (160–190 ppm) %</th>
<th>Aromatic (100–160 ppm) %</th>
<th>Alkoxyl (60–110 ppm) %</th>
<th>Paraffinic (0–60 ppm) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>19</td>
<td>18</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>19</td>
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<tr>
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<td>4</td>
<td>21</td>
<td>19</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

### Table 3
UDOM sample isotope data

<table>
<thead>
<tr>
<th>UDOM sample</th>
<th>$^{13}$Corg (%)</th>
<th>$^{13}$Cinorg (%)</th>
<th>$^{14}a_{org}$ (‰pMC)</th>
<th>$^{14}C_{org}$ age (year BP)</th>
<th>$^{14}a_{inorg}$ (‰pMC)</th>
<th>$^{14}C_{inorg}$ age (year BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$-27.78 \pm 0.01$</td>
<td>$-11.40 \pm 0.02$</td>
<td>$87 \pm 1$</td>
<td>$1080 \pm 100$</td>
<td>$87 \pm 1$</td>
<td>$1080 \pm 100$</td>
</tr>
<tr>
<td>2</td>
<td>$-27.15 \pm 0.02$</td>
<td>n.d.</td>
<td>$87 \pm 1$</td>
<td>$1080 \pm 100$</td>
<td>n.d.</td>
<td>$1080 \pm 100$</td>
</tr>
<tr>
<td>3</td>
<td>$-27.12 \pm 0.02$</td>
<td>$-10.23 \pm 0.02$</td>
<td>$87 \pm 1$</td>
<td>$1200 \pm 100$</td>
<td>$90 \pm 1$</td>
<td>$850 \pm 100$</td>
</tr>
<tr>
<td>4</td>
<td>$-25.40 \pm 0.02$</td>
<td>$-7.43 \pm 0.02$</td>
<td>$87 \pm 1$</td>
<td>$1080 \pm 100$</td>
<td>$80 \pm 10$</td>
<td>$10 &gt;Modern$</td>
</tr>
</tbody>
</table>

a n.d., not determined.
Mass chromatography was used to monitor specific chemical classes of compounds, like alkylphenols (Fig. 4), pyrolysis products of (partly) altered polysaccharides (Fig. 5) and alkylpyrroles (Fig. 5). The origin and geochemical importance of the different compound classes is discussed in the following paragraphs.

3.3.1. Alkylphenols

Mass chromatograms reflecting C0–C2 alkylphenols in the pyrolyzates of all four Ems-Dollart UDOM samples are shown in Fig. 4. The samples show very similar alkylphenol distribution patterns. These patterns are very similar to those of alkylphenols in the pyrolyzates of oceanic UDOM like the Pacific Ocean UDOM (van Heemst et al., 1993, 1996), suggesting similar precursors of algal, and thus non-lignin, origin. Alkylphenols in pyrolyzates are generally formed upon pyrolysis of lignins (Meuzelaar et al., 1982; Saiz-Jimenez and de Leeuw, 1986b) or degraded lignins (e.g. Saiz-Jimenez and de Leeuw, 1984b). In pyrolyzates of lignins and degraded lignins, methoxyalkylphenols and dihydroxyalkylbenzenes are encountered in addition to alkylphenols. However, in the pyrolyzates of the UDOM samples, neither methoxyalkylphenols nor dihydroxyalkylbenzenes were observed. No specific lignin pyrolysis products were encountered in the pyrolyzates of the Ems-Dollart estuarine samples, despite the relatively large or exclusive contribution of terrestrial organic matter brought in by the river Ems. Thermochemolysis results using tetramethylammonium hydroxide on these samples by van Heemst et al. (1999c) reveal the presence of only traces of contributions from lignin, mostly the oxidized lignin forms (weight percentages of approximately 2–4%). Fresh lignin can thus be excluded as major precursor of the alkylphenols present in the UDOM pyrolyzates studied.

It has been shown that in addition to lignins, polyphenolic type macromolecules rich in tyrosine moieties resulting from degradation of proteins also produce alkylphenols upon pyrolysis (van Heemst et al., 1999a). An origin from cross-linked proteins or proteins/polysaccharides seems to be the best option, since the findings by van Heemst et al. (1999a). On the basis of the presence of these non-lignin phenols in the pyrolyzates of the samples, it may be concluded that the UDOM from the Ems-Dollart is old and transformed. However, it is difficult to determine a terrestrial or marine origin, only on the basis of the pyrolysis results, because the
precursors (polysaccharides, proteins, etc.) are present in organic matter both on land and in the sea. The precursors of the alkylphenols may thus represent heavily degraded proteins and/or lignins originating from peat and soil organic matter in the hinterland.

3.3.2. Alkylpyrroles

The mass chromatograms shown in Fig. 5 reveal distribution patterns of C0-C2 alkylpyrroles in the Ems-Dollart UDOM pyrolyzates. The precursors of the alkylpyrroles are yet unknown nitrogen containing compounds. These compounds are not likely to be derived from the degradation products of pigment molecules produced by bacteria and plants (e.g. chlorophyll-a) based on comparison of the alkylpyrrole distributions in pyrolyzates of the UDOM samples with those in the pyrolyzates of appropriate standards (Sinninghe Damsté et al., 1992). Nitrogen-containing compounds are encountered in pyrolyzates of a number of macromolecules, like chitin, proteins and macromolecules present in bacterial cell walls. A specific pyrolysis product of chitin is 2-(N-acetylamino)-levoglucosan (van der Kaaden et al., 1984; Baas et al., 1995). This compound was not encountered in the pyrolyzates of the UDOM samples. Therefore, chitin was excluded as precursor for the nitrogen-containing compounds encountered in the Ems-Dollart UDOM samples. No fresh proteins are present in the Ems-Dollart UDOM samples because of the absence of the specific protein pyrolysis products mentioned before. However, in pyrolyzates of albumin bovine serum, pyrrole and methylpyrroles have been encountered (van Heemst et al., 1999a). It is unlikely that proteins have survived serious biological degradation, but some moieties present in proteins could have been preserved.

3.3.3. Alkylcyclopentanones

In the pyrolyzates of all Ems-Dollart UDOM samples alkylcyclopentanones are encountered as important pyrolysis products (Figs. 3 and 5). The nature of the precursor of these compounds is not completely understood. Alkylcyclopentenones have been encountered in pyrolyzates of soil organic matter (Saiz-Jimenez and de Leeuw, 1984a, 1986a). They have also been encountered in pyrolyzates of amyllose (van der Kaa den et al., 1983) and as burned sugar aroma components (Mills and Hodge, 1976). It is believed that the precursors of the alkylcyclopentanones in the UDOM samples are related to refractory organic matter, possibly of polysaccharide origin.

![Sample 1](image1)
![Sample 2](image2)
![Sample 3](image3)
![Sample 4](image4)

Fig. 5. Mass chromatograms (m/z 67+80+94) of alkylcyclopentenones, alkylpyrroles and alkylcyclopentenones.

![Sample 1](image5)
![Sample 2](image6)
![Sample 3](image7)
![Sample 4](image8)

Fig. 6. Mass chromatograms (m/z 78+92+106+120) of C0-C3 alkylbenzenes.
3.3.4. Alkylbenzenes

All pyrolyzates contain relatively large amounts of alkylbenzenes. The mass chromatograms shown in Fig. 6 reveal distribution patterns of C0–C3 alkylbenzenes in the pyrolyzates of the Ems-Dollart UDOM samples. All chromatograms of the pyrolyzates show very similar distribution patterns of the C1–C3 alkylbenzenes, suggesting similar precursors. The exact precursors of the alkylbenzenes are unknown, but they might be related to aromatic moieties present in the macromolecular structure of organic matter (Hartgers et al., 1994). These compounds have been reported to occur in other pyrolyzates of UDOM and POM samples, e.g. in pyrolyzates of UDOM from the Pacific Ocean (van Heemst et al., 1993), of sediment trap material from the Mediterranean (Peulvé et al., 1996) and of organic matter present in algae (van Heemst et al., 1996). In ocean water UDOM, and thus probably also in Ems-Dollart UDOM, the precursor of the alkylbenzenes is part of the refractory UDOM. This is based on increasing relative amounts of the alkylbenzenes in pyrolyzates of UDOM samples of increasing depth (van Heemst et al., 1993), suggesting selective preservation of the precursors of these alkylbenzenes.

4. Conclusions

The chemical composition of UDOM (fraction of 1000 Dalton to 0.2 µm) from the Ems-Dollart estuary does not change significantly throughout the estuary. However, an increase in δ13C values of UDOM is observed with increasing salinity, suggesting mixing of river UDOM with seawater UDOM of similar molecular composition in the estuary. This change in δ13C is not paralleled by a change in the 14C activities of the UDOM samples. The 14C activities are the same for all four stations, probably because the UDOM from the North Sea has the same 14C activity.

UDOM from the Ems-Dollart estuary contains aromatic moieties of unknown origin and moieties derived from altered polysaccharides and proteins.

The negligible contribution of unaltered polysaccharides, proteins and lipids emphasizes the refractory nature of UDOM from the Ems-Dollart estuary.

This refractory nature may be due to cross-linking reactions between proteins and polysaccharides, resulting in the formation of refractory organic matter in marine and terrestrial (soil) organic matter.

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