Organic Geochemistry 75 (2014) 87–98

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

CuO-oxidized dissolved organic matter (DOM) investigated with comprehensive two dimensional gas chromatography-time of flight-mass spectrometry (GC × GC-TOF-MS)

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ARTICLE INFO

Article history:
Received 16 January 2014
Received in revised form 17 June 2014
Accepted 18 June 2014
Available online 25 June 2014

Keywords:
Comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOF-MS)
Cupric oxide (CuO)
Alkaline hydrolysis
Dissolved organic matter (DOM)
Lignin
Lignin dimer
Lignin cyclobutane photodimer
Lignin phenol oxidation products

ABSTRACT

GC × GC-TOF-MS was applied to investigate the molecular diversity of CuO-oxidized, trimethylsilyl (TMS) derivatized DOM isolated from Lake Tahoe's main tributary, the Upper Truckee River (UTR), California. Many (4789) chromatographic peaks were resolved in a single sample at a signal/noise (S/N) ratio of 10 or greater and > 300 (> 6%) were assigned discrete structures. Mapping the two dimensional elution time space revealed 8 homologous series defined by successive CH_2 additions. Series of other compounds related to one another by OCH_3 and OH substitutions were also assigned. Elucidation of the retention time (RT) displacements affected by these molecular transformations guided the discovery of novel compounds as well as those that had previously escaped detection within DOM, including a suite of lignin-derived cyclobutane photodimers. Analysis of RT shifts among sets of benzene polycarboxylic acid (BPCA) isomers revealed the second chromatographic dimension to strongly retain sterically strained isomers, providing a basis for assigning groups of isomers that were otherwise unassignable. The heightened chromatographic resolution also revealed a substantial and hitherto analytically inaccessible diversity of isomers, which appear to be uniquely resolved with the method, but which would fall outside the analytical window of other high resolution MS methods, such as Fourier transform ion cyclotron resonance MS (FT-ICR-MS). The study likely represents the most comprehensive compound-specific elucidation of any natural OM (NOM) sample to date.

1. Introduction

1.1. Importance of dissolved organic carbon (DOC)

DOC is a globally important, actively cycling and chemically complex pool of photosynthetically reduced carbon whose global inventory in the world's oceans, lakes and streams (ca. 670 Gt C; Hansell and Carlson, 1998) rivals that of the carbon content of the Earth's atmosphere (ca. 760 Gt C) and the terrestrial biosphere (ca. 800 Gt C; Falkowski et al., 2000). The ultimate origins of this reduced carbon lie in the biosynthetic pathways of organisms, such as plants and autotrophic algae, where a diverse array of biomolecules, including cellulose, lignin, polysaccharides, lipids and proteins is synthesized prior to release to the dissolved (< 0.7 μm) phase via dissolution (degradative and non-degradative) and exudation (active and passive). Collectively, these dissolved compounds serve as vital growth substrates for heterotrophic microorganisms and enable transmission of carbon to higher trophic levels via the microbial loop (Azam et al., 1983). Despite the chemical energy it contains, however, only a relatively small fraction of DOC is labile or semi-labile and readily accessible to microorganisms (Carlson and Ducklow, 1995). The remaining non-labile DOC is often referred to as semi-refractory or refractory DOC (RDOC; Carlson and Ducklow, 1995) and can act as a carbon sink and persist in the ocean in the dissolved phase on millennial timescales (Williams and Druffel, 1987; Druffel et al., 1992). The compositional and structural reasons underlying its persistence in the environment remain unclear and a comprehensive elucidation of its structure continues to prove elusive. As such, DOM continues to be the subject of study and increasingly powerful and high resolution analytical methods continue to be applied to its characterization.

1.2. Modes of DOM characterization

Bulk DOC parameters are easily measured and include concentration measurements (Hansell et al., 2009) and excitation emission matrix spectroscopy (EEMS; Coble, 2007). Once fractionated

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http://dx.doi.org/10.1016/j.orggeochem.2014.06.010
0146-6380/

via size or chemistry, the fractionated material is available for "bulk" measurements such as elemental ratios, isotopic ratios (which can sometimes differentiate sources) and various means of spectroscopic characterization of functional groups [e.g. nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy].

Some studies have also taken a compound-specific quantitative approach. In these methods, individual compounds such as lipids, lignin phenols, amino acids and neutral sugars are individually resolved and quantified following DOM hydrolysis. Such analyses, however, rarely account for a significant fraction of the total carbon and come only at considerable analytical expense and effort. Emphasizing the limitations of these methods, < 3% of total DOC in the deep ocean can be attributed to individual compounds, while for the surface ocean it is ca. 7% (Benner, 2002). This low level of characterizable carbon is generally thought to result from the biotic and abiotic diagenetic alteration of molecularly recognizable and 'fresh' OM to compounds that become less characterizable with time (Benner, 2002).

In the past decade, the high resolution capability of FT ion cyclotron resonance MS (FT-ICR-MS) has been applied to describe the complexity of natural DOC mixtures in unprecedented detail (Kujawinski et al., 2002; Kim et al., 2003; Koch et al., 2005; Dittmar and Koch, 2006; Sleighter and Hatcher, 2007), especially compounds amenable to electrospray ionization (ESI), which is most commonly employed. A particular advantage of DOM characterization using FT-ICR-MS is that no hydrolysis or derivatization is required before analysis, although pH is adjusted for ESI. The high resolving power, which typically ranges from 300,000–600,000 full width half max (FWHM; Sleighter and Hatcher, 2007), facilitates detection of thousands of discrete molecular masses to a level of accuracy that enables accurate assignment of elemental formulae to thousands of detected mass peaks up to several hundred mass units. As only molecular formulae are resolved, the method provides, however, no information as to the compound structure, or to the number of isomers that give rise to a particular mass signal. It is often implicitly, and sometimes explicitly (Koch et al., 2005), assumed in such studies that compounds with identical mass have identical structure and origin, but the diversity of isomeric structures in DOM appears to have been largely ignored.

1.3. Analysis of CuO-oxidized OM using GC

The number of theoretical plates in GC has made it ideal for resolving complex mixtures. The main limitation with respect to natural OM (NOM) stems from its size and polarity, which is too large and condensed to be amenable to separation in the gas phase. As such, NOM must be first degraded to more volatile components. In oxidative degradation, however, polar and non-volatile alcohols and acids are produced and these are not readily amenable to GC. Therefore, prior to analysis these compounds are converted to e.g. trimethylsilyl (TMS) ethers and esters to make them more volatile.

The most common mode of oxidation relies on alkaline hydrolysis in the presence of CuO at elevated temperature, first described by Pearl (1942) and later adapted by Hedges and Parker (1976) to quantify lignin phenol abundance (as TMS derivatives) in sediments and, subsequently, in oceanic DOM (Meyers-Schulte and Hedges, 1986) for use as an unambiguous tracer of terrestrial OM. The objective of the technique is often the quantification of a small ensemble of 6–11 lignin phenols. However, the method, which cleaves labile bonds, including esters and most ethers (Chang and Allan, 1971), yields many more products that often escape closer examination. Additional compounds previously identified in the mixture include an expansive set of lignin dimers with diverse linkage structures (Goñi and Hedges, 1992), straight chain and branched α,ω-diacids, additional small aromatic acids derived from proteins, phenols, polysaccharides, tannins (Goñi and Hedges, 1995; Goñi et al., 1998), long chain α,ω-diacids and polyhydroxylated fatty acids derived from plant suberin polyesters and cuticular wax (Goñi and Hedges, 1990), and benzene polycarboxylic acids (BPCAs; Dickens et al., 2007). Ca. 120 products from CuO-hydrolyzed OM have been identified. This represents, however, only a small fraction of the thousands of compounds ultimately present in the complex mixtures comprising disparate assemblages of NOM at the Earth's surface. Identification and quantification of additional compounds are limited by low concentration and limited chromatographic resolution. With these analytical challenges in mind and motivated by the desire to access the building blocks of DOM and the information contained therein, a promising new tool has been introduced that offers unique advantages that complement existing high resolution methods of DOM characterization.

1.4. GC × GC-TOF-MS

GC × GC is a powerful technique (Liu and Phillips, 1991) for separation of complex mixtures beyond one dimensional (1-D) GC separation. In GC × GC, two orthogonal columns of dissimilar stationary phase are coupled using a cryo-focusing device that periodically samples the effluent from the primary column by freezing it into a narrow band at the head of a second column, which is significantly (ca. 10×) shorter than the first. The narrow band of primary column effluent is then heated near-instantaneously, frozen, and again heated before chromatography with the second column. Meanwhile, the modulator repeatedly processes a successive interval of primary column eluent every several s (the modulation period) for the duration of the chromatographic run. The maximum chromatographic resolution in such systems is the product of the peak capacity of each respective column, so an enormous increase in theoretical peak capacity is achieved. Additionally, cryofocusing leads to dramatically narrower and sharper peaks, which further increases peak capacity and instrumental sensitivity by increasing S/N. Finally, a high speed TOF detector, which acquires full spectra from m/z 1–1000 at a speed of up to 500 Hz, enables robust spectral deconvolution of chromatographically unresolved peaks. Thousands of peaks can be resolved, each having its own electron ionization (EI) spectrum. Each peak is characterized by a primary column retention time (^{1}RT), of the order of min, and a secondary column retention time (^{2}RT), of the order of s. The EI spectrum of each peak can then be interpreted or searched against external and in-house libraries to enable linking of peaks to chemical structures. Additionally, homologous series are separated along predictable trajectories in 2-D retention time space. In combination with mass spectra, these 2-D trends in separation complement MS information and aid in structure elucidation. Both GC × GC-TOF-MS and FT-ICR-MS commonly resolve as many as 10,000 peaks for environmental samples. As such, the level of chromatographic resolution in comprehensive GC × GC-TOF-MS effectively approaches the level of mass resolution afforded by FT-ICR-MS. Both types of instrumentation provide similarly large quantities of data and reveal different and complementary features of OM complexity and composition. The augmented separation capability of GC × GC systems has made it an attractive platform with which to characterize complex petroleum mixtures and perform chemical class quantitation (Blomberg et al., 1997; Frysinger and Gaines, 1999), investigate unresolved complex mixtures (UCMs) of hydrocarbons in Archean sediments (Ventura et al., 2008) and profile chemical changes associated with dissolution, evaporation and biodegradation associated with hydrocarbon leakage (Wardlaw et al., 2008). The technique has also been used recently to identify dichloromethane (DCM)-extractable compounds in rainwater (Cottrell et al., 2013). It has, however, not