Order of Functionality Loss During Photodegradation of Aquatic Humic Substances

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Experimental

Methylation of Nordic HA. Approximately 26 mg of untreated and 45 mg of irradiated (59%IA_{465nm}) Nordic HA, both in $^3$H-saturated form, were dissolved in dimethylformamide and methylated with $^{13}$C-labeled diazomethane, generated from N-methyl-$^{13}$C-N-nitroso-p-toluenesulfonamide (99 atom % $^{13}$C, Cambridge Isotope Laboratories)(Thorn et al., 1987).

Irradiation and Reaction of Laurentian FA with Hydroxylamine. One gram of Laurentian soil FA (ECOLINC, INC., Roxboro, Quebec, Canada) adjusted to pH 6 in 1 L H$_2$O was irradiated without pyrex filter to 58%IA$_{465nm}$. The solution was $^3$H-saturated on the MSC-1 cation exchange resin and freeze dried. The sample was portioned for $^{13}$C NMR analysis and reaction with hydroxylamine. Three hundred mg each of the unreacted and irradiated fulvic acid was dissolved in 150 mL H$_2$O, charged with 80 mg of $^{15}$NH$_3$OH.HCl titrated to pH 5 with 1 N NaOH, and allowed to stir at room temperature for 32 hours. The samples were then $^3$H-
saturated on the MSC-1 cation exchange resin, freeze dried, and dissolved in 2 ml DMSO-d$_6$ for $^{15}$N NMR analysis.

**NMR Spectroscopy.** Liquid state $^{13}$C and $^{15}$N NMR spectra were recorded on a Varian 300 MHz NMR spectrometer at carbon and nitrogen resonant frequencies of 75.4 and 30.4 MHz respectively. DEPTGL (Sørensen et al., 1983) $^{13}$C NMR spectra of the non-irradiated Nordic FA were recorded on a 5-mm broadband probe using a 30,000 Hz spectral window, 0.2 s acquisition time, and 2.0 s delay for proton relaxation, assuming maximum and minimum $^{1}J_{CH}$ values of 200 and 125 Hz, respectively. (DEPTGL: distortionless enhancement by polarization transfer, GL version.) The $^{13}$C NMR spectra of the diazomethylated Nordic HA samples dissolved in dimethylformamide-d$_7$ were recorded on a 5-mm broadband probe with a 45° pulse angle, 0.2 s acquisition time, 5 s pulse delay, and inverse gated decoupling. The 5 s pulse delay is sufficient for complete relaxation of the $^{13}$C-labeled methyl ethers and esters.

Liquid state ACOUSTIC (Patt, 1982) $^{15}$N NMR spectra (direct detection) of hydroxylamine reacted Laurentian FA samples were recorded with a 35,111.7 Hz (1,154.3 ppm) spectral window, 0.2 s acquisition time, 0.5 s pulse delay, and tau delay of 0.1 ms, using a 10-mm broadband probe. (ACOUSTIC: alternating compound one eighties used to suppress transients in the coil.) ACOUSTIC spectra of the Laurentian FA were recorded without paramagnetic relaxation reagent and therefore the NOE is retained. Polarization transfer spectra were also recorded on the hydroxylamine reacted Laurentian FA and Nordic HA samples to detect only nitrogens directly bonded to protons. Refocused INEPT spectra (proton decoupled) of the Laurentian FA were acquired using a 15,649.5 Hz spectral window, 0.957 s acquisition time, and 2.0 s delay for proton relaxation. (INEPT: insensitive nuclei enhanced by polarization transfer.) The polarization transfer time and refocusing delay were set equal to 1/4J, or 2.78 ms.
(\(^1J_{NH} = 90.0\) Hz). DEPT spectra of the Nordic HA were recorded with a 26,000 Hz spectral window, 0.2 s acquisition time, 1.0 s delay for proton relaxation, and \(^1J_{NH}\) of 90.0 Hz.
Figure S1. Liquid state continuous decoupled and DEPTGL $^{13}$C NMR spectra of non-irradiated Nordic FA. Solvent = dimethyl-$^{12}$C$_2$, d$_6$ sulfoxide. LB= line broadening in Hertz.
**Liquid state DEPTGL $^{13}$C NMR spectra of non-irradiated Nordic FA.** The DEPTGL pulse sequence generates subspectra showing all protonated carbons, methine carbons only, methylene carbons only, and methyl carbons only (Sørensen et al., 1983). The spectra indicate that carbons downfield of approximately 140 ppm are not bonded to protons. The O-alkyl carbons are comprised mainly of methine carbons (peak centered at 74.1 ppm in the methine carbon subspectrum), with some contribution of primary alcohols and ethers (peaks at 64.2 ppm and 69.3 ppm in methylene carbon subspectrum). The C-alkyl carbons are resolved into methine (45.1 ppm), methylene (33.7 ppm), and methyl (19.7 ppm) carbons. A quaternary carbon only spectrum (not shown) recorded on the sample using the APT (attached proton test) pulse sequence (Patt and Shoolery, 1982) showed no evidence for quaternary aliphatic carbons.
Figure S2. Liquid state continuous decoupled $^{13}$C NMR spectra of Nordic FA irradiated with pyrex filtered medium pressure Hg lamp. Percentage refers to percent of initial absorbance at 465 nm. LB = line broadening in Hertz.
Figure S3. Quantitative liquid state $^{13}$C NMR spectra of $^{13}$C-diazenylated Nordic HA before and after irradiation to 59%IA$_{465nm}$. LB = line broadening in Hertz.

Liquid State $^{13}$C NMR Spectra of Diazomethylated Nordic HA Before and After Irradiation. The quantitative liquid state $^{13}$C NMR spectra of the $^{13}$C-diazenylated samples in Figure S2 show only the labeled carbons originating from the diazenyl. Spectra consist of three main bands, centered at approximately 52.4 ppm, 56.3 ppm, and 62.0 ppm, corresponding to methyl esters of carboxylic acids, methyl ethers of phenolic hydroxyls, and methyl ethers of phenolic hydroxyls adjacent to two substituents, respectively (Thorn, 1994; Thorn et al., 1987). The $^{13}$C NMR chemical shifts of the methyl ethers and esters of gallic acid illustrate this assignment (Scheme S1). The spectra of the methylated humic acid samples reveal
that after irradiation to 59%IA_{465nm}, the carboxylic acids have undergone significant loss whereas the phenolic hydroxyls have remained mainly intact. The results are consistent with the natural abundance $^{13}$C NMR spectrum of the sample at 59% IA_{465nm} (Figure 4, main text), which shows significant loss of the carboxyl/amide/ester carbons but only minor losses of the substituted aromatic carbons.

![Scheme S1](image_url)

**Scheme S1.** $^{13}$C NMR Chemical Shifts for Methyl Ester and Methyl Ethers of Gallic Acid
Figure S4. Liquid state continuous decoupled $^{13}$C NMR spectra of Nordic humic acids before and after UV irradiation. Irradiated sample processed to remove bicarbonate and LMW carboxylic acids. Solvent = dimethyl-$^{12}$C$_2$-$d_6$ sulfoxide. LB = line broadening in Hertz.
Figure S5. Liquid state continuous decoupled $^{13}$C NMR spectra of Suwannee River XAD-4 acids before and after UV irradiation. Irradiated sample processed to remove bicarbonate and LMW carboxylic acids. Solvent = dimethyl-$^{12}$C$_2$,$^6$ sulfoxide. LB = line broadening in Hertz.
Figure S6. Liquid state continuous decoupled $^{13}$C NMR spectra of Laurentian FA before and after UV irradiation to 58% IA$_{465nm}$. The bicarbonate peak at 161.1 ppm in the spectrum before irradiation was present in the sample as purchased. The irradiated sample was processed to remove bicarbonate and LMW carboxylic acids. Solvent = dimethyl-$^{12}$C$_2$$_{d_6}$ sulfoxide. LB = line broadening in Hertz.
Figure S7. Liquid state ACOUSTIC $^{15}$N NMR spectra of Laurentian soil FA before and after UV irradiation to 58%IA$_{465nm}$, reacted in each case with $^{15}$NH$_2$OH.HCl. Solvent = DMSO-d$_6$. LB = line broadening in Hertz.
LAURENTIAN FA
Before Irradiation
Reacted with $^{15}\text{NH}_2\text{OH}$
INEPT $^{15}\text{N}$ NMR
LB = 50 Hz

After Irradiation
Reacted with $^{15}\text{NH}_2\text{OH}$
INEPT $^{15}\text{N}$ NMR
LB = 50 Hz

Figure S8. Liquid state INEPT $^{15}\text{N}$ NMR spectra of Laurentian soil FA before and after UV irradiation to 58%IA$_{465\text{nm}}$, reacted in each case with $^{15}\text{NH}_2\text{OH}\cdot\text{HCl}$. Solvent = DMSO-$d_6$. LB = line broadening in Hertz.
Figure S9. Liquid state DEPT $^{15}$N NMR spectra of Nordic HA before and after UV irradiation to 75% $\lambda_{465\text{nm}}$ reacted in each case with $^{15}$NH$_2$OH.HCl. Solvent = DMSO-d$_6$. LB = line broadening in Hertz.
NMR Spectra of Laurentian FA and Nordic HA Irradiated and Reacted with Hydroxylamine.

Continuous decoupled liquid state $^{13}$C NMR spectra of the Laurentian FA (Figure S6) indicate that irradiation has resulted in a decrease of the O-alkyl, aromatic/olefinic, and carboxyl/amide/ester carbons, and a shift in the maximum of the ketone peak from 196 to 207 ppm, as observed with the aquatic NOM samples. Oximation analysis of the Laurentian FA by $^{15}$N NMR reveals details observed with the Nordic HA. The ACOUSTIC (NOE-retained) spectrum of the Laurentian FA before irradiation exhibits the main ketoxime and nitrosophenol peaks at 362.7 ppm and 393.8 ppm, respectively (Figure S7). The nitrosophenol peak is in tautomeric equilibrium with its corresponding quinone monoxime. A decrease in the ratio of the nitrosophenol to ketoxime peak is evident in the irradiated sample, demonstrating a relatively greater loss of quinones with respect to ketones, among the quinones and ketones that react with hydroxylamine. The shoulder at 351.4 ppm in the ACOUSTIC spectrum before irradiation is resolved into a distinct peak at 350.9 ppm after irradiation. This may correlate to the loss of specific ketones or possibly signify a photochemical rearrangement of ketones. The trend of increasingly downfield $^{13}$C NMR chemical shifts for diaryl, aryl-alkyl, and dialkyl ketones, respectively, does not apply to the $^{15}$N chemical shifts of the oxime derivatives. Peaks attributable to Beckmann reactions are visible in the ACOUSTIC spectrum before irradiation, from approximately 165 to 215 ppm and 235 to 270 ppm. These are not as clearly resolved as in the ACOUSTIC spectra of the Nordic HA (Figure 5, main text), as the spectra of the Laurentian FA were recorded without paramagnetic relaxation reagent. Possible assignments for peaks from about 180 to 215 ppm include imidates, amidines, imidazoles, isocyanides, and imides (Thorn et al., 1992). The peak from about 235 to 270 ppm and centered at 247.6 ppm correspond to nitriles, from Beckmann fragmentations of oximes. The largest class of oximes that undergo fragmentation to nitriles are oximes that have quaternary carbon centers adjacent to the oxime.
carbon, because of the stability of the carbonium ion which is cleaved. Examples include monoximes of quinones and oximes of -diketones, -ketoacids, -hydroxy ketones, etc. The occurrence of these configurations of ketones in the samples may be inferred from the fragmentation reactions, and considered in future studies on the carbonyl photochemistry of aquatic humic substances. The mechanism for the formation of ammonia, indicated by the peak at 22.6 ppm, is uncertain. Ammonia could be released from a secondary Beckmann reaction product by excess hydroxylamine, or form from direct reduction of the hydroxylamine.

The INEPT spectrum (Figure S8; nitrogens bonded to protons) of the non-irradiated Laurentian FA reacted with hydroxylamine shows three major peaks at 163.8, 132.2, and 107.3 ppm, corresponding to hydroxamic acids (reaction of hydroxylamine with esters), secondary amides (Beckmann rearrangements of oximes), and primary amides (Beckmann fragmentations) or lactams (Beckmann rearrangements), respectively. Decrease of the hydroxamic acid derivative peak after irradiation indicates photodegradation of ester groups. Hydroxylamine may also cleave peptides to form hydroxamic acids, but this requires basic pH (~9.5) and is selective to the asparaginyl-glycyl bond (Bornstein and Balian, 1977).

DEPT $^{15}$N NMR spectra (Figure S9; nitrogens bonded to protons) were recorded on the Nordic HA samples reacted with labeled hydroxylamine, before and after irradiation to 75%IA$_{750nm}$ for which the ACOUSTIC spectra are shown in Figure 5 (main text). Three main peaks assigned as hydroxamic acids (163.1 ppm), secondary amides (131.9 ppm), and primary amides or lactams (110.3 ppm) are visible in the non-irradiated sample. Because the signal to noise ratio of the hydroxamic acid peak in the starting sample is fairly low, it is difficult to conclude whether UV irradiation has resulted in degradation of ester groups. Irradiation does appear to have caused a decrease in the secondary amide peak (131.9 ppm), suggesting degradation of ketones whose oximes are susceptible to the Beckmann rearrangement.
**Irradiation and DOC Fractionation of Filtered Suwannee River Water.**

Suwannee River water was collected on May 4, 1995, near the sampling location for the IHSS materials and filtered through Balston DH (25-μm) and AH (0.3-μm) glass-fiber filters. This is the same water sample from which the XAD-4 acids described in the main text were preparatively isolated. (The Suwannee River NOM and fulvic acid samples examined by $^{13}$C NMR were isolated from separate water samples at different times.) One liter samples of the filtered water were irradiated as described in the main text without the pyrex filter to approximately 75%, 50%, and 25% of initial absorbance at 465 nm. Analytical DOC fractionations were performed on the water samples before and after irradiation by Huffman Laboratories, Golden, CO. The analytical fractionation is based upon DOC sorption to XAD resins and measurement of DOC eluted with base, acid, and organic solvent, without preparative isolation of the fractions. In this procedure, the hydrophobic acid fraction is comprised of both fulvic and humic acids, whereas the hydrophilic acid fraction corresponds to the XAD-4 acids. Before irradiation, the hydrophobic acid, hydrophobic neutral, and hydrophilic acid fractions comprise 27%, 33%, and 35%, respectively, of the total DOC, which is 51mg C/L (Table S1). After irradiation to 25% IA$_{465\text{nm}}$, or, in other words, after 75% loss of absorbance at 465nm, the total DOC has decreased to 26.0 mg C/L, indicating a 51% loss of carbon. The hydrophilic acid fraction is most susceptible to irradiation and shows the greatest loss, from 18 mg C/L to 4 mg C/L, at 25% IA$_{465\text{nm}}$. This may be attributable to the fact that the XAD-4 acids have the highest concentration of carboxylic acids, and readily undergo photochemical decarboxylation. The hydrophobic neutral fraction shows the next greatest loss, from 17 to 9 mg C/L, followed by the hydrophobic acid fraction, from 14 to 10.5 mg C/L at 25% IA$_{465\text{nm}}$. 
The percent of DOC comprised by the hydrophobic neutral fraction in the water collected at this date appears higher than in previous samplings from the Suwannee River. The quantitative liquid state $^{13}$C NMR spectrum reported for the hydrophobic neutral fraction from the May 1995 water sample (Thorn and Cox, 2009) indicates that this fraction contains the ketone, carboxyl, aromatic and O-alkyl carbon functional groups that were observed to undergo degradation in the humic, fulvic, and XAD-4 acids.

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Literature Cited


