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Analysis of Ulmic Acid by Mass Spectrometry

ANALYSIS OF ULMIC ACID BY MASS SPECTROMETRY

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ABSTRACT

Fourier Transform Ion Cyclotron Resonance (FT-ICR) and Matrix Assisted Laser Desorption Ionization (MALDI) mass spectrometry are used to systematically study ulmic acid extracts taken from a sediment sample in the Gulf of Mexico. The sample, which is extracted with methanol, is taken from the ecosystem of the bryozoa *Bugula neritina*. Ulmic acid is a loosely defined group of molecules that are the alcohol extracts of naturally occurring organic matter. The FT-ICR analysis focuses on carbon compounds C_{16}-C_{24} and C_{35}. C_{35} is examined by FT-ICR because its structure may represent the bryophan ring, the central structural feature in all bryostatins.

Keywords: Ulmic acid, FT-ICR, MALDI-TOF-MS, bryostatin

INTRODUCTION

Humic substances (HS) are the product of plant and animal decay and are ubiquitous in nature (1, 2, 3). Divided by chemical and physical characteristics, HS are separated into categories called humic acid (HA), fulvic acid (FA), and humin. Both HA and FA belong to the water-soluble class of compounds. This loosely defined group of molecular species can include sugars, lignin, peptides and proteins, antibiotics (i.e., lactams and tetracyclins), carbohydrates

(and cyclitols), lipids, nucleic acids and component units, terpenoids including retinoids and steroids, tetrapyrroles and related compounds. Some of these compounds have a low water solubility and subsequently aggregate in the aqueous phase (4, 5). Ulmic acid is defined as the alcohol-based extract of naturally-occurring organic matter (NOM). There exists little research in the literature on ulmic acid or its chemical composition. Marine natural products (MNP) are typically large organic compounds with low water solubility. Many MNPs are thought to exist in the local ecosystem or produced by bacteria that have a symbiotic relationship with the host organism. Original work in this lab with NOM focused on fundamental physical and chemical characteristics such as studying aggregate sizes and natural chlorination processes (2-5). Recently NOMs have been studied to better understand ecosystems that contain organisms that produce marine natural products. Extracting solvents used to remove MNPs from their host organism have traditionally been methanol, ethanol, or methanol/chloroform.

FT-ICR analysis of molecular samples exhibits mass accuracy and resolution (6). It has been successfully applied to the analysis of Suwannee River humic acid (7) and led to the assignment of thousands of empirical formulas for the HA standard obtained from the International Humic Substance Society located at the Georgia Institute of Technology (Atlanta, GA). HA has a very low solubility in alcohols due to the large number of carboxylate groups. MALDI-TOF-MS is widely used for high-mass nonvolatile molecular species that include sugars, peptides, and proteins. In this lab, FT-ICR and MALDI-MS have recently been incorporated in studies involving the marine natural products bryostatin and ET743 (8-12).

Little is known about the actual production of a marine natural product by a bacterial species. Do the bacteria use enzymes to link together a series of small molecules (i.e., ethanol, acetate) through a complex synthesis to form a large structure such as bryostatin? Does it simply take a single large molecule that is ubiquitous in the environment and perform a few simple steps to reach the final product? Are there any obvious precursors in the host organism's ecosystem? There is no single set of experiments that can completely define the marine geochemistry of an ecosystem and outline the synthesis of a complex molecule. However, systematic data gathering can help scientists define the basic chemical conditions in which the bacterial species resides. In generating the data tables presented here, there is a hope to provide other scientists with a glimpse into the organic constituents present in the *Bugula neritina* ecosystem that are extracted with methanol. Figure 1 presents a possible scheme for either the degradation or synthesis of bryostatin involving smaller organic species. For the degradation one would start at the top of the schematic and follow the route down, and in a synthesis one would start at the bottom and move up. A number of schemes have been developed in which the weakest bonds or reactive centers are considered in order to discover potential precursors identified in the extract.

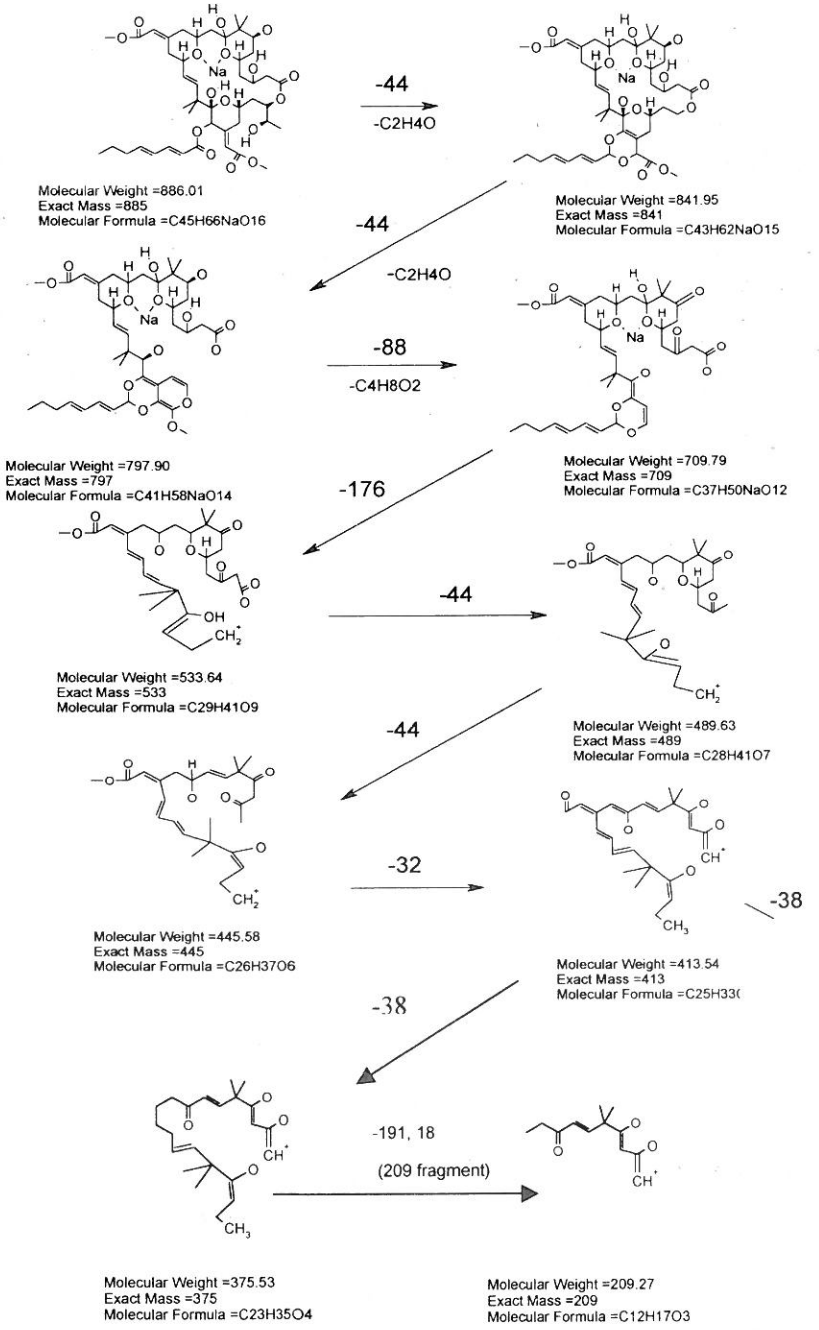

Figure 1. A schematic diagram outlines either the degradation of bryostatin-2 (top to bottom) or the synthesis of bryostatin-2 (bottom to top).

In searching for a potential precursor both here and in past mass spectrometry-based studies, an important structure to consider is the basic bryophan ring (see Fig. 2). While different variations on this structure are possible (i.e., different number of oxygen atoms and hydrogen atoms), the C_{35} ring could serve as a precursor for the bryostatins extracted from *Bugula neritina*. There have been twenty (20) structures of bryostatin identified to date and many speculate that these structures are the product of different genetic strains of *Bugula* or the symbiotic bacterial species that resides within the bryozoa. Work in this lab has demonstrated that common environmental and chemical conditions (i.e., UV light, I_2, pH>10) can account for many of these structural differences. What remains constant in the different bryostatins is the bryophan ring. While FT-ICR may suggest certain empirical formulas and perhaps point to specific functional groups, it does not provide structural information.

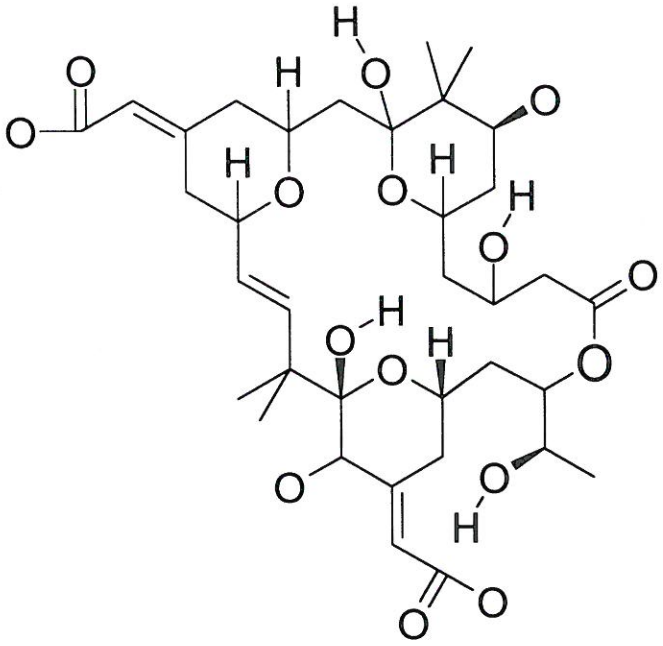

Figure 2. The basic bryophan ring, a C_{35} structure, is a building block for the twenty different bryostatins identified in *Bugula neritina*.

Experimental

A homebuilt 9.4 T FT-ICR MS configured for external ion accumulation located at the National High Magnetic Field Lab (Tallahassee, Florida) was used to determine accurate mass of synthetic and biological bryostatin (13-15). Samples were introduced into the mass spectrometer by direct infusion by microelectrospray ionization (the external electrospray interface has been

described elsewhere (13)). Following passage through a heated metal desolvation capillary (3.8 A heating current), ions were accumulated in a linear octopole ion trap (octopole frequency, 1.5 MHz; amplitude, 240 V_{p-p}) for various periods (depending on the sample) and then transmitted to a three-section open cylindrical 4-inch-diameter Penning trap (trapping voltage, 2 V). Trapped ions were excited (frequency-sweep, 72-320 kHz @150 Hz/μs, 190 V_{p-p}) and detected (320 kHz Nyquist bandwidth) for 0.8 s to yield 512 K time-domain data. Typical chamber base pressure was ~6 × 10^{-10} Torr. Time–domain ICR data were acquired by in-house modular ICR data acquisition system (MIDAS) software (13).

The MALDI-TOF-MS used is located in the University of Georgia (Athens, GA) Chemical and Biological Sciences Mass Spectrometry Facility. The samples were analyzed with a Bruker Autoflex instrument in reflection mode. The nitrogen laser (337 nm wave length) has a maximum output of 125 μJ. The 0.5 μL of sample was spotted on the MALDI target with 0.5 μL of matrix (a saturated solution of alpha-cyano-4-hydroxycinnamic acid in 50:50 acetonitrile:0.1% trifluoroacetic acid.

A sample of naturally occurring organic matter was taken from the Suwannee River delta (Gulf of Mexico). After water was removed by gravity (Buchner funnel), the sample weighed approximately 100 grams. The organic-rich sample was washed with a mildly acidic solution (0.01 N HCl) to remove water-soluble organic and dissolves the relatively high levels of cations, anions, and minerals (i.e. Na^+, Cl^-, $CaCO_3$, $CaSO_4$) present. The remaining sample was again dried by suction, washed, and soaked in 500 mL of methanol for 48 hrs. A 100 mL portion of the methanol was pipetted from the container holding the sample-methanol mixture, and was filtered through a 0.2 μm filter using a syringe pump. A small portion of this was analyzed by FT-ICR MS.

RESULTS AND DISCUSSION

The focus of this paper is to use FT-ICR in the role of elemental analysis for molecular components of ulmic acid taken from an ecosystem in the Gulf of Mexico. The data tables provide a baseline to better understand the organic constituents and potential structures that exist in the sediment of the studied ecosystem. For our research, this ecosystem is important because one of the marine animals that resides there, the bryozoa *Bugula neritina*, contains the FDA-approved natural product bryostatin-1. For example, Figure 3 shows a MALDI-MS analysis of a pentanol extract of sediment from the same ecosystem and illustrates peaks where bryostatin-1 plus Na^+ would be located (927 m/z). The lower mass components correspond to the loss of water molecules. This data suggests that the bacteria tentatively identified in the *Bugula neritina* (16-19) may produce bryostatin and may also reside in the sediment of the same ecosystem. Table I provides a list of common fatty acids (C_{16}-C_{24}) that are produced by a number of plants and would be expected to constitute a portion of ulmic acid.

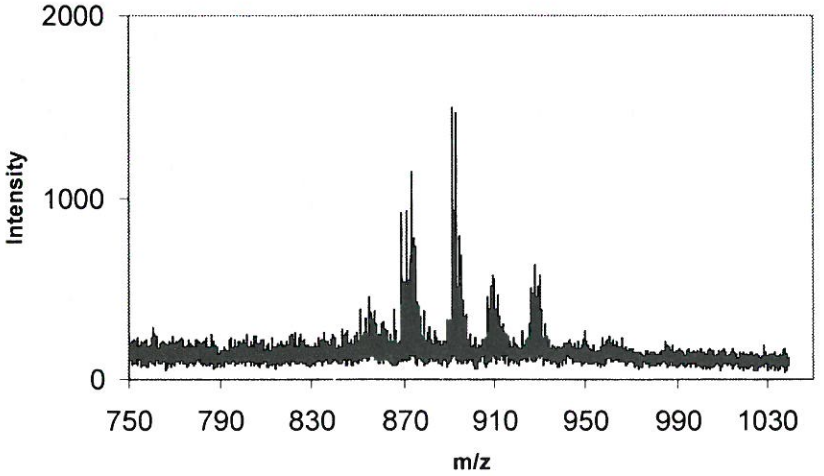

Figure 3. MALDI-MS analysis of Alligator Point sediment located in a *Bugula* ecosystem shows mass spectral features at 927 m/z, which corresponds to the parent ion of bryostatin-1 plus sodium, and other features at 909 m/z (- H_2O), 891 m/z (-$2H_2O$), and 873 m/z (-$3H_2O$). Data of this nature has raised the question if the bacterium that produces bryostatin resides only in bryozoa *Bugula neritina*.

Table I. Common fatty acids with an empirical formula matching species identified in methanol-chloroform extract of the sediment.

Common name	#C	# Double Bonds	Scientific name
Palmitic Acid	16	0	hexadecanoic acid
Palmitoleic Acid	16	1	9-hexadecenoic acid
Stearic Acid	18	0	octadecanoic acid
Oleic Acid	18	1	9-octadecenoic acid
Vaccenic Acid	18	1	11-octadecenoic acid
Linoleic Acid	18	2	9,12-octadecadienoic acid
Alpha-Linolenic Acid (ALA)	18	3	9,12,15-octadecatrienoic acid
Gamma-Linolenic Acid (GLA)	18	3	6,9,12-octadecatrienoic acid
Arachidic Acid	20	0	eicosanoic acid
Gadoleic Acid	20	1	9-eicosenoic acid
Arachidonic Acid (AA)	20	4	5,8,11,14-eicosatetraenoic acid
EPA	20	5	5,8,11,14,17-eicosapentaenoic acid
Behenic acid	22	0	docosanoic acid
Erucic acid	22	1	13-docosenoic acid
DHA	22	6	4,7,10,13,16,19-docosahexaenoic acid
Lignoceric acid	24	0	tetracosanoic acid

Tables II and III provide molecular species that correspond to specific empirical formulas ($C_xH_yO_z$) that have either 16 or 17 carbon atoms. These data show that saturated and unsaturated structures exist as well as structures with multiple oxygen atoms. The results in Tables IVa-e suggest no molecular species containing phosphorus, nitrogen, or chlorine were identified in the methanol extracts. For each carbon backbone (C_{16}-C_{24}), none of these elements, when arranged with different CH or CHO formulas were identified. This indicates that a variety of molecular species, from amino acids and peptides to phospholipids and various thiols, are not present in significant quantities in ulmic acids. This would suggest the structures are quickly digested by microbes in the sediment, completely removed during the initial sample wash, or are simply not disaggregated and extracted by methanol from the sample.

Table II. Organic compounds with 16 carbon atoms are tentatively suggested in an analysis conducted by negative ion-FT-ICR of ulmic acid.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M+H]	Mass from Molar Calculator	Error fr. Molar Calculator (ppm)
$C_{16}H_{31}O_2^-$	255	255.2318	13.0474	256.2396	256.2402	-2.3
$C_{16}H_{31}O_3^-$	271	271.2268	4.6655	272.2346	272.2351	-1.9
$C_{16}H_{31}O_4^-$	287	287.2218	0.5599	288.2296	288.2300	-1.4
$C_{16}H_{31}O_7^-$	335	335.2060	0.1163	336.2138	336.2148	-2.8
$C_{16}H_{29}O_2^-$	253	253.2161	1.6411	254.2239	254.2245	-2.4
$C_{16}H_{29}O_3^-$	269	269.2112	0.2696	270.2190	270.2194	-1.7
$C_{16}H_{29}O_4^-$	285	285.2060	0.1801	286.2138	286.2144	-2.1
$C_{16}H_{29}O_6^-$	317	317.1959	0.2004	318.2037	318.2042	-1.5
$C_{16}H_{27}O_5^-$	299	299.1855	0.1333	300.1933	300.1936	-1.2
$C_{16}H_{27}O_6^-$	315	315.1803	0.1186	316.1881	316.1885	-1.3
$C_{16}H_{25}O_5^-$	297	297.1698	0.2509	298.1776	298.1780	-1.4
$C_{16}H_{23}O_{10}^-$	375	375.1271	0.1291	376.1349	376.1369	-5.4

Table III. Molecular species with 17 carbon atoms identified in the methanol extract.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M+H]	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$C_{17}H_{33}O_2^-$	269	269.2475	4.0190	270.2553	270.2558	-1.9
$C_{17}H_{33}O_3^-$	285	285.2424	4.0844	286.2503	286.2507	-1.7
$C_{17}H_{33}O_4^-$	301	301.2375	0.2429	302.2453	302.2457	-1.1
$C_{17}H_{31}O_2^-$	267	267.2319	0.7214	268.2397	268.2402	-1.9
$C_{17}H_{31}O_3^-$	283	283.2267	0.2666	284.2346	284.2351	-1.9
$C_{17}H_{31}O_4^-$	299	299.2217	0.1663	300.2295	300.2300	-1.8
$C_{17}H_{31}O_5^-$	315	315.2169	0.1446	316.2247	316.2249	-0.8
$C_{17}H_{29}O_6^-$	329	329.1960	0.1555	330.2038	330.2042	-1.2
$C_{17}H_{27}O_5^-$	311	311.1854	0.2647	312.1932	312.1936	-1.5
$C_{17}H_{27}O_6^-$	327	327.1804	0.3213	328.1882	328.1885	-1.0
$C_{17}H_{27}O_7^-$	343	343.1759	0.1356	344.1837	344.1835	0.7
$C_{17}H_{27}O_8^-$	359	359.1712	0.1074	360.1790	360.1784	1.7
$C_{17}H_{25}O_4^-$	293	293.1748	0.1324	294.1826	294.1831	-1.5
$C_{17}H_{25}O_6^-$	325	325.1644	0.1005	326.1722	326.1729	-2.0
$C_{17}H_{25}O_7^-$	341	341.1603	0.1170	342.1681	342.1678	0.8
$C_{17}H_{23}O_7^-$	339	339.1439	0.1105	340.1517	340.1522	-1.2

Table IVa,b,c,d,e. Organic compounds with 18 carbon atoms detected by negative ion-FT-ICR. No evidence was found for C-18 compounds containing sodium, lithium, potassium, chlorine, phosphorous, or nitrogen atoms in the extracts. We also list a number of the structures we searched for in the FT-ICR data but did not identify. In other tables (Tables II, III, V-XIII), we only list empirical formulas that corresponded to spectral features identified.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M+H]	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$C_{18}H_{35}O_2^-$	283	283.2632	11.2384	284.2710	284.2715	-1.7
$C_{18}H_{35}O_3^-$	299	299.2582	2.5327	300.2660	300.2664	-1.2
$C_{18}H_{35}O_4^-$	315	315.2532	1.1591	316.2610	316.2613	-1.1
$C_{18}H_{35}O_5^-$	331	331.2478	0.5405	332.2556	332.2562	-1.8
$C_{18}H_{35}O_6^-$	347	347.2429	0.1170	348.2507	348.2511	-1.3
$C_{18}H_{35}O_7^-$	363	363.2387	0.1685	364.2465	364.2461	1.3
$C_{18}H_{33}O_2^-$	281	281.2476	11.4342	282.2554	282.2558	-1.6
$C_{18}H_{33}O_3^-$	297	297.2424	0.9244	298.2503	298.2507	-1.6
$C_{18}H_{33}O_4^-$	313	313.2375	0.8837	314.2453	314.2457	-1.1
$C_{18}H_{33}O_5^-$	329	329.2324	0.8021	330.2402	330.2406	-1.1
$C_{18}H_{33}O_6^-$	345	345.2279	0.8684	346.2357	346.2355	0.7
$C_{18}H_{33}O_n^-$ (n=7-11)	361, 377, 393, 409, 425	---	-----	-----	-----	-----
$C_{18}H_{31}O_2^-$	279	279.2318	0.7995	280.2397	280.2402	-1.9
$C_{18}H_{31}O_3^-$	295	295.2274	0.2080	296.2352	296.2351	0.3
$C_{18}H_{31}O_4^-$	311	311.2219	0.3925	312.2297	312.2300	-1.1
$C_{18}H_{31}O_5^-$	327	327.2167	0.3382	328.2245	328.2249	-1.2
$C_{18}H_{31}O_n^-$ (n=6-11)	343, 359, 375, 391, 407, 423	---	-----	-----	-----	-----
$C_{18}H_{29}O_n^-$ (n=2-5)	277, 293, 309, 325	---	-----	-----	-----	-----
$C_{18}H_{29}O_6^-$	341	341.1969	0.1698	342.2047	342.2042	1.4
$C_{18}H_{29}O_7^-$	357	357.1915	0.2315	358.1994	358.1991	0.7
$C_{18}H_{29}O_8^-$	373	373.1867	0.2026	374.1946	374.1940	1.4
$C_{18}H_{29}O_9^-$	389	389.1816	0.1355	390.1894	390.1889	1.2

$C_{18}H_{29}O_n^-$ (n=10,11)	405, 421	---	-----	-----	-----	-----
$C_{18}H_{27}O_n^-$ (n=2-5)	275, 291, 307, 323	---	-----	-----	-----	-----
$C_{18}H_{27}O_6^-$	339	339.1806	0.1336	340.1884	340.1885	-0.3
$C_{18}H_{27}O_7^-$	355	355.1760	0.2807	356.1838	356.1835	1.1
$C_{18}H_{27}O_n^-$ (n=8-11)	371, 387, 403, 419	---	-----	-----	-----	-----
$C_{18}H_{25}O_n^-$ (n=2-11)	273, 289, 305, 321, 337, 353, 369, 385, 401, 417	---	-----	-----	-----	-----
$C_{18}H_{23}O_n^-$ (n=2-10)	271, 287, 303, 319, 335, 351, 367, 383, 399	---	-----	-----	-----	-----
$C_{18}H_{23}O_{11}^-$	415	415.1289	0.1767	416.1367	416.1318	11.8
$C_{18}H_{21}O_n$ (n=2-11)	269, 285, 301, 317, 333, 349, 365, 381, 397, 413	---	-----	-----	-----	-----
$C_{18}H_{19}O_n$ (n=2-11)	267, 283, 299, 315, 331, 347, 363, 379, 395, 411	---	-----	-----	-----	-----

Table IVb. No compounds with 18 carbon atoms were identified that also contained nitrogens. Below provides information about some of the structures we searched for in the FT-ICR data.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M + H] or [M + 2H]	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$C_{18}H_{36}NO_n^-$ (n=1-4)	282, 298, 314, 330	---	-----	-----	-----	-----
$C_{18}H_{34}N_2O_n^{2-}$ (n=2,3)	310, 326	---	-----	-----	-----	-----
$C_{18}H_{35}N_2O_n^-$ (n=2,3)	311, 327	---	-----	-----	-----	-----
$C_{18}H_{34}NO_n^-$ (n=1-3)	280, 296, 312	---	-----	-----	-----	-----
$C_{18}H_{32}NO^-$	278	---	-----	-----	-----	-----

Table IVc. No compounds with 18 carbon atoms were identified that also contained chlorine atoms.

Empirical Formula	Nominal Molar Mass	Mass from ICR Data Table	Relative Abundance	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$C_{18}H_{35}ClO_n$ (n=1-3)	302, 318, 334	---	-----	-----	-----
$C_{18}H_{33}ClO_n$ (n=1-3)	300, 316, 332	---	-----	-----	-----
$C_{18}H_{31}ClO$	298	---	-----	-----	-----
$C_{18}H_nCl_2O_2$ (n=32,30,28)	350, 348, 346	---	-----	-----	-----

Table IVd. No compounds with 18 carbon atoms were identified that also contained Na^+, Li^+, or K^+ atoms.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M+H]	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$(C_{18}H_nO_4)^{2-}Li^+$ (n=32,30,28, 26,24,22)	319, 317, 315, 313, 311, 309	---	-----	-----	-----	-----
$(C_{18}H_nO_4)^{2-}$ Na^+ (n=32,30,28, 26,24,22)	335, 333, 331, 329, 327, 325	---	-----	-----	-----	-----
$(C_{18}H_nO_4)^{2-}K^+$ (n=32,30,28, 26,24,22)	351, 349, 347, 345, 343, 341	---	-----	-----	-----	-----

Table IVe. No compounds with 18 carbon atoms were identified that also contained phosphorous atoms.

Empirical Formula (anion)	Nominal Molar Mass	Mass from ICR Data Table (anion)	Relative Abundance	Exper. [M+H], [M+2H], or [M+3H]	Mass from Molar Calculator	Error from Molar Calculator (ppm)
$C_{18}H_{39}P_2O_7^-$	429	---	-----	-----	-----	-----
$C_{18}H_{38}P_2O_7^{2-}$	428	---	-----	-----	-----	-----
$C_{18}H_{37}P_2O_7^{3-}$	427	---	-----	-----	-----	-----

In addition compounds containing sulfur, silicon, sodium, potassium, lithium, bromine, or iodine were not identified in the extract. With one exception (Table X), we did not identify any compounds containing elements other than carbon, oxygen, and hydrogen in any compounds. Tables V through IX identifies C_{19}-C_{24} species. Only a C_{23} species had a mass spectral feature that suggested the presence of compounds containing a non-CHO atom (Table X). This structure provided the correct isotope information (with respect to the Cl-35 and Cl-37 isotopes) and also showed two similar structures in terms of a +2H form. Natural chlorination of NOM has been discussed in a previous publication from this lab (4). Table I provides some common fatty acids routinely found in plants that correspond to structures identified in Tables II-XII.

Figure 4 is a graph that illustrates a typical trend observed involving the number of oxygen atoms for a C_{18} backbone. While FT-ICR does not

allow us to assign exact structures, only providing a molar mass within a high degree of accuracy, the trends observed can suggest certain structures. As observed with the $C_{18}H_{35}O_x^-$ series, an exponential decrease in relative abundance with an increase in the number of oxygen atoms is observed. In separate studies (9, 12, 19) we identified significant quantities of TiO_2 (rutile) in sediment samples from the Gulf of Mexico sediment. TiO_2 is a well-known photocatalyst, allowing oxygenation of the carbon chain to possibly occur when organic compounds are coated on its surface and exposed to UV light. Aliphatic chains of C_{18}, such as oleic and stearic acid, are prominent species in plants and subsequently in naturally occurring organic matter. The oxygenation of stearic acid by a photocatalyst such as TiO_2 (or Al_2O_3, ZnO, etc.) would be one possible explanation for the relative abundance distribution observed. Starting with a singly substituted carboxylate (C_{17}-COO^-), over a period of time, oxygen atoms are added to the structure as its coats metal oxide particles and is struck with UV light from the Sun.

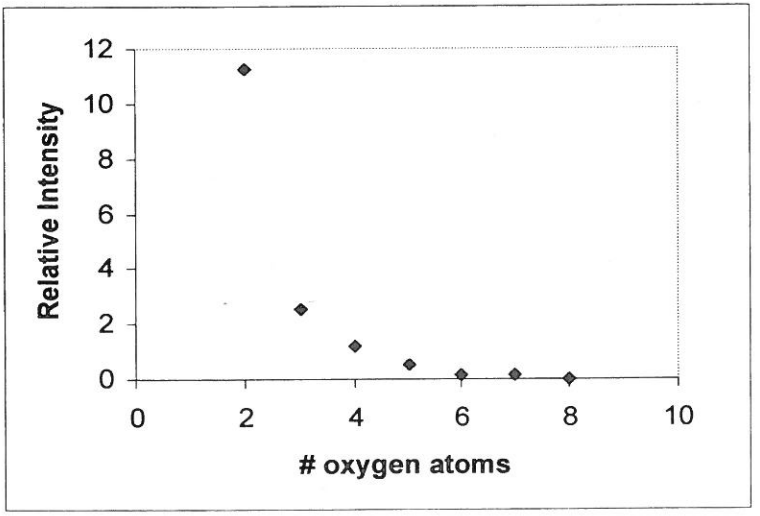

Figure 4. For $C_{18}H_{35}O_x^-$ ($2 < x < 8$) compounds, as the number of oxygen atoms increase the relative ion abundance* (concentration) decreases (*relative abundance is relative to the peak with the highest intensity and is not correlated with any absolute concentrations).

Figure 5 shows the structure of tricarboxylate ($C_{35}H_{39}O_6$, 555.69 Da) that could be functionalized by oxygen atoms in the presence of UV light and a photocatalyst (TiO_2). In terms of analyzing ulmic acid by FT-ICR this structure would be either difficult or impossible to assign by mass spectral feature. First, if all three carboxylates are deprotonated, an m/z of less than 200 occur, which is lower than the limits of our mass spectral window. Second, this species would have some water solubility and its concentration would

be diminished during the water wash phase. In Table XIIa,b,c we show mass spectral features that were identified and the empirical formulas to which they corresponded. In terms of ulmic acid analysis, none of the species identified in the methanol extract came close to having a reasonable number of oxygen atoms (atomic number for Oxygen > 14) on the C_{35} chain to qualify as a close precursor to the bryophan ring. This does not say that the precursor does not exist in the sediment, only that we cannot identify it in a methanol extraction by negative ion mode FT-ICR. In past work, we have shown that bryostatin binds not only sodium, but also Fe^{+3} quite strongly, where either of which would result in a positive complex not recognizable by this mode. The bryophan ring (Figure 2) has a number of esters that can break resulting in a carboxylate and an alcohol (reverse esterfication). Considering this mechanism we selected negative ion mode to search for potential species that might be negatively charged by a carboxylate.

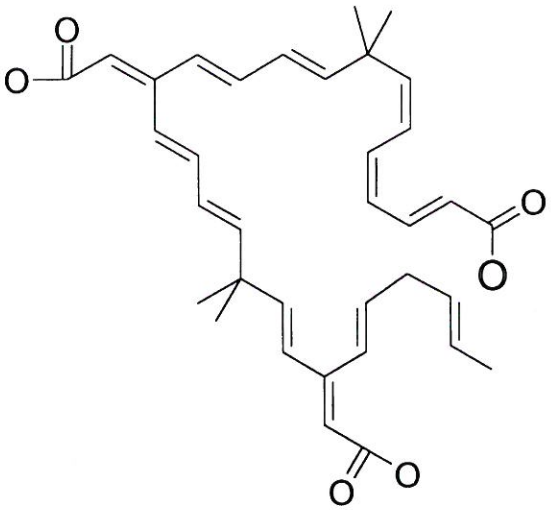

Figure 5. The empirical formula $C_{35}H_{39}O_6$, a tri-carboxylate, would be expected to have some water solubility. In analysis by FT-ICR, it would be a difficult species to identify due to its -3 charge and ICR's lower cut-off of 200 m/z.

CONCLUSIONS

Recently data from our lab proposed a synthesis of bryostatin-1 from a precursor tentatively identified in the sediment of the Bugula ecosystem (20). The data presented here provides us with a better understanding of what structures might be present in ulmic acid. From the empirical formulas, polarity of the solvent, and the negative ion mode employed in the mass spec, it is suggested that many of these structures have alcohols, carboxylates, esters, ethers, and carbonyls as functional groups. The lack of structures containing

phosphorus, sulfur, nitrogen, or halogens indicates the extract contains a relatively select group of molecular compounds in terms of structural characteristics. Bryostatin has FDA approval for orphan drug designation to bryostatin-1 (in combination with Taxol (Paclitaxel) for esophageal cancer). Bryostatin has been labeled the "flagship of marine natural products" (17) with its anti-cancer activity first identified in the late 1960's and its structure identified in the early 1980's (21). In past studies we have identified potential structures that correspond to empirical formulas associated with bryostatin precursors, but in pentanol extracts analyzed in positive ion mode and are typically identified with a Na^+ attached to the parent ion $[M + Na]^+$. This work represents the first mass spectral analysis of ulmic acid. Understanding that naturally occurring cholesterol, with a relatively complex ring structure, is bio-synthesized from the aliphatic hydrocarbon squalene, we believe this data will help unlock the biosynthesis of bryostatin from a specific hydrocarbon.

This full version of this paper contains additional tables and the raw data from the FT-ICR. It can be obtained from the Valdosta State University library under the title "Data used in the Analysis of Ulmic Acid by Mass Spectrometry" by Manning et al. (2005).

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