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Characterization of methyl sugars, 3-deoxysugars and methyl deoxysugars in marine high molecular weight dissolved organic matter

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy shows that > 50% of the carbon in marine high molecular weight dissolved organic matter (HMWDOM) from surface waters is a compositionally well-defined family of acylated polysaccharides that is conserved across ocean basins. However, acid hydrolysis of HMWDOM, followed by chromatographic analysis, recovers only 10–20% of the carbon as neutral, amino and acidic sugars. Most carbohydrate in HMWDOM therefore remains uncharacterized. Here, we use acid hydrolysis followed by Ag⁺ and Pb²⁺ cation exchange chromatography to separate HMWDOM hydrolysis products for characterization using 1-D and 2-D NMR spectroscopy. In addition to neutral sugars identified in past studies, we find 3-*O*-methylglucose, 3-*O*-methylrhamnose, 2-*O*-methylrhamnose and 2-*O*-methylfucose. We also find 3-deoxysugars to be present, although their complete structures could not be determined. Methyl sugars are widely distributed in plant and bacterial structural carbohydrates, such as cell wall polysaccharides, and their presence in HMWDOM suggests that structural carbohydrates may contribute to DOM in surface seawater. We find most HMWDOM carbohydrate is not depolymerized by acid hydrolysis and that the non-hydrolyzable component includes 6-deoxysugars.

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1. Introduction

Dissolved organic matter (DOM) is a large reservoir of carbon that affects light penetration (Nelson

et al., 1998; Nelson and Siegel, 2002), microbial activity (Williams, 1981; Azam et al., 1983; Ducklow and Carlson, 1992), carbon–nitrogen–phosphorus storage (Benner, 2002; Bronk, 2002; Karl and Björkman, 2002; Mopper and Kieber, 2002), trace metal bioavailability (Rue and Bruland, 1997; Wells, 2002) and the retention of hydrophobic organic contaminants (Guo and Santschi, 1997 and references therein) in seawater. Although the influence of the dissolved organic matter reservoir on ocean biogeochemical cycles has been widely recognized, we have only a broad understanding of its

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chemical composition, sources or fate. Molecular level analysis of DOM hydrolysis products (simple sugars, amino acids and lipids) in the surface and deep ocean recovers only 4–11% and 1–2% of the total carbon respectively, and most DOM remains unidentified at this level of characterization (Lee and Bada, 1977; Wicks et al., 1991; Benner, 2002; Eglinton and Repeta, 2004).

The introduction of large volume ultrafiltration techniques was a major advance in DOM studies and has made gram quantities of the high molecular weight fraction of DOM (HMWDOM) available for wet chemical and spectral analysis. Ultrafiltration through a 1 nm pore size membrane nominally retains organic matter of > 1 kDa MW, and routinely isolates 20–35% of total marine DOM. Marine HMWDOM is chemically distinct from humic substances and is enriched in carbohydrates (Benner et al., 1992). ^{13}C NMR spectral analysis of HMWDOM in surface waters shows that > 50% of the carbon consists of compositionally well-defined acylpolysaccharides (APS) that are conserved across ocean basins (Aluwihare et al., 1997; Eglinton and Repeta, 2004). However, after acid hydrolysis, only 10–20% of HMWDOM carbon (HMWDOC) can be recovered as simple neutral sugars (arabinose (I), fucose (II), galactose (III), glucose (IV), mannose (V), rhamnose (VI) and xylose (VII); see Appendix 1) using gas chromatography (GC) or high pressure liquid chromatography (HPLC; Panagiotopoulos and Sempéré, 2005 and references therein). Another 5–8% of HMWDOC is recovered as acetic acid (Aluwihare et al., 1997), and a further 3–5% is recovered as amino acids (McCarthy et al., 1996). Lipids represent < 2% of the HMWDOC (Mannino and Harvey, 1999; Wang et al., 2004). It is notable that the distribution of major sugars released by acid hydrolysis of HMWDOM is nearly the same irrespective of where the sample is collected or the specific hydrolysis conditions used for depolymerization (Sakugawa and Handa, 1985; McCarthy et al., 1996; Aluwihare et al., 1997; Borch and Kirchman, 1997; Panagiotopoulos and Sempéré, 2005). Most of the carbohydrate in HMWDOM is therefore not characterized using conventional HPLC or GC techniques. Reconciling the discrepancy between the amount of carbohydrate measured using ^{13}C NMR spectroscopy and the amount of carbohydrate hydrolysis products measured with chromatographic techniques represents a major challenge for HMWDOM characterization.

Other approaches such as direct temperature-resolved mass spectrometry (DT-MS) and ^{15}N NMR indicate the presence of a much broader range of sugars than has been observed with GC and HPLC, including methyl sugars, methyl-deoxy-sugars, amino sugars and furfurals (Boon et al., 1998; Minor et al., 2001; Minor et al., 2002; Aluwihare et al., 2005). DT-MS studies characterize classes of sugars via diagnostic fragment ions but do not allow full identification of specific sugars or determination of their abundance. The ^{15}N NMR spectroscopy technique can be used to quantify nitrogen-containing sugars indirectly, but likewise does not give information on the presence or amounts of specific amino sugars. For example, Aluwihare et al. (2005) inferred from ^{15}N and ^1H NMR analysis that 25–30% of HMWDOM occurs as unspecified *N*-acetyl-aminosugars. Chromatographic analysis has identified glucosamine and galactosamine in HMWDOM, but at concentrations of $\leq 2\%$ HMWDOC (Aluwihare et al., 1997; Aluwihare et al., 2002). Similar inconsistencies have also been reported for plankton and sinking particulate organic matter (Hedges et al., 2001; Hedges et al., 2002). Although a clear explanation for these discrepancies has not yet been found, two likely scenarios exist.

First, sugars other than neutral sugars, such as amino sugars, sugar alcohols, methylated sugars, uronic acids, sulfonated sugars, furfurals, etc., may constitute a considerable fraction of HMWDOM carbohydrate but lie outside the analytical window of the HPLC or GC techniques used for HMWDOM characterization. A second likely explanation is that HMWDOM carbohydrate is simply not depolymerized with acid hydrolysis. In this study, we use Ag^+ and Pb^{2+} ion chromatography to show that acid hydrolysis does not depolymerize most carbohydrate in HMWDOM. We also report the spectroscopic analysis of new methyl, deoxy and methyl deoxy sugars isolated from HMWDOM hydrolysis products. These sugars represent 2–5% of HMWDOM carbohydrate, but may also be present in the unhydrolyzed carbohydrate fraction.

2. Methods

2.1. Sampling

Seawater was drawn from the 15 m seawater intake at the Natural Energy Laboratory of Hawaii Authority in Kona, Hawaii in February 2002. The

samples were filtered to remove bacteria and small particles using a cleaned (10% HCl) Suporflow dual stage (0.8 μm and 0.2 μm) Gelman polyether sulfone cartridge filter (Chisolm Corp., Lincoln, RI) fitted to an Advanta stainless steel housing. HMWDOM samples were collected using a large volume cross flow ultrafiltration system consisting of a stainless steel centripetal pump and membrane housings and a fluorinated high density polyethylene reservoir. The system was plumbed with teflon tubing and fitted with PVDF valves. We used four ultrafiltration membranes [90 ft² (8.4 m²) each; Separation Engineering, Escondido, CA] with a 1 nm pore size that nominally retain organic matter of molecular weight greater than 1000 Da (> 98% rejection of vitamin B₁₂). Membranes were cleaned using isopropanol, detergent (0.01% micro), HCl (0.01 N) and NaOH (0.01 N), stored in sodium azide (0.55 mM) and rinsed with water and seawater immediately before use. The four filters were plumbed in parallel. Surface seawater (28,900 L) was concentrated over several days to approximately 40 L, frozen and returned to Woods Hole for further processing. The HMWDOM samples were concentrated to 2 L using a smaller system fitted with a 1 nm pore size, 10 ft² (0.93 m²) membrane, desalted using repeated ($\times 10$) diafiltration with high purity, low carbon content, deionized water (Milli-Q water, 2 L) and reduced to 2 L. The final concentrate gave no visible precipitate (AgCl) when an aliquot of permeate was mixed with a 5 mg/50 ml solution of AgNO₃. After diafiltration was complete, the sample (2 L) was lyophilized to a fluffy off-white powder (9.4 g) that was 35% by weight carbon with a C/N ratio of 13.

2.2. Sample preparation

HMWDOM samples (200–300 mg) were hydrolyzed in 2 M trifluoroacetic acid (TFA). Approximately 14.5 g (TFA) was added to 43 g of water in a 250 mL round bottomed flask. The solution was heated and HMWDOM added as a solution in approximately 2–3 mL water. The flask was flushed with He, sealed and placed in a sand bath at 120 °C. After 4 h, the dark brown solution was cooled, frozen at –20 °C and freeze dried. The dried sample was dissolved in 2–3 mL water and applied to a 6–8 mm i.d. column packed with 4 g anion exchange resin (OH[–] form, Bio-Rex[®] 5 resin, Bio-Rad Laboratories, Hercules, CA). The column was preconditioned by rinsing with

15 mL NaOH (0.5 M) followed by Milli-Q water until the pH returned to ca. 7. The column was drained of water and the sample was applied. Carbohydrates were eluted with 20–40 mL water, collected, concentrated to a small volume (few ml) using rotary evaporation and freeze dried. Sample recovery (by weight) after hydrolysis and ion exchange was 87%.

2.3. Chromatography

Chromatography was performed with a Waters pump (Model 510) and a Shodex RI-71 refractive index detector. Sample injection was performed with a manual Rheodyne injector fitted with a 100 μL sample loop. Carbohydrates were separated by degree of polymerization on sulfonated styrene-divinylbenzene gel analytical columns (30 cm \times 7.8 mm i.d.; particle size 8 μm ; cross linking 8%) in the silver or lead form (Supelcogel[™] Ag or Pd, Supelco, Inc., Bellefonte PA). Column temperature was controlled using an oven (Tiberline TL-50) set at 80 °C. Sugars were eluted with water at a flow rate of 0.5 ml min^{–1}. Prior to use, the mobile phase was degassed with He for 30 min. The total weight of the sample injected in to the column did not exceed 10 mg/injection. Columns were regenerated at the end of each day by washing overnight with water at 0.2 ml min^{–1}. Retention times were measured using arabinose (I), fucose (II), galactose (III), glucose (IV), mannose (V), rhamnose (VI) and xylose (VII), 3-O-methylglucose (VIII) and DP (degree of polymerization, or number of monosaccharides) 2–7 maltooligosaccharides (IX through XII; Sigma, St. Louis, MO, USA). Data acquisition and processing were performed with Agilent ChemStation software (Agilent Technologies).

2.4. Mass spectrometry

Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) analysis was performed at the Center for Complex Carbohydrate Research in Athens, GA (USA), using a Voyager DE mass spectrometer (Applied Biosystems) operated in the positive ion mode. Aqueous solutions of samples were diluted 1:1 with 2,5-dihydroxybenzoic acid, applied to a MALDI plate and desorbed with a nitrogen laser having a guide wire reading of 0.005% and laser intensity of approximately 2630. Ions formed by the pulsed laser beam were accelerated through 20 kV with an

acquisition range of 149–2000 Da. The mass spectrometer was calibrated with angiotensin I.

2.5. NMR spectroscopy

Spectra were recorded with a Bruker Avance DPX 400 MHz spectrometer using XWIN-NMR 3.0 software and an inverse broadband (BBI) 5 mm probe tuned for ^{13}C with a Z-gradient coil. Approximately 1–2 mg of the isolated compounds were dissolved in 1–2 ml D_2O (99.9 atom% D, Aldrich) and dried under a N_2 stream. Dissolution/drying was repeated to ensure complete replacement of all exchangeable hydrogen with deuterium. Samples were finally dissolved in 0.75 ml D_2O (freshly opened ampoules, > 99.96 atom% D) and transferred to 5 mm glass NMR tubes (Model 535-PP, Wilmad) using combusted (450 °C) NMR pipettes; ^1H chemical shift was set against the residual HOD signal (δ 4.80 ppm) and ^{13}C chemical shifts were derived from the transmitter frequency.

The proton spectral width and file size were set to achieve ca. 0.3 Hz resolution, with subsequent equivalent zero filling bringing the final resolution to ca. 0.14 Hz. Experiments employed some suppression of the residual water signal, either a simple 30° pulse and presaturation during 1 s relaxation or WATERGATE (WATER suppression by GrADient Tailored Excitation; Bruker sequence “p3919gp”) sequence with gradient strengths reduced for minimal collateral suppression (particularly of the anomeric proton signals). Acquisition times ranged from 10 min to several days depending on the amount of material available. ^1H – ^1H gs-COSY (gradient selected correlation spectroscopy; Bruker sequence “cosygp”) spectra were acquired with a 45° mixing pulse to produce 512 or 1024 FID (free induction decay) of 32 or 64 summed scans, resulting in 2×8 Hz raw resolution and 2×2 Hz final resolution after zero filling and symmetrization. Interscan relaxation time was 3 s. Acquisition times were 15–30 h. The ^1H – ^{13}C gs-HSQC (phase sensitive echo/antiecho-TPPI gradient-selected heteronuclear single quantum coherence; Bruker sequence “invietgpsi”) spectra were acquired with delays optimized for $^1J_{^{13}\text{C}^1\text{H}} = 145$ Hz; 1024 FIDs of 144 summed scans and no zero filling resulted in 2×24 Hz final resolution. Interscan relaxation time was 2 s and acquisition times were 4 days. All gradient experiments used 1500 μS 100 point sine shaped gradient pulses.

3. Results and discussion

3.1. Isolation of mono- and oligosaccharides from HMWDOM

To further investigate the composition of HMWDOM carbohydrates, we used Ag and Pb cation exchange chromatography to separate oligosaccharides from monosaccharides released by acid hydrolysis. The mechanism of separation is based on the strength of the complex formed between the hydroxyl groups of the sugars and the metal (Ag^+ or Pb^{2+}) and the technique easily separates glycans via their degree of polymerization (DP) as shown in Fig. 1a [R_t monosaccharides: (glucose, IV) > disaccharides (maltose, IX) > trisaccharides (maltotriose, X) > oligosaccharides (maltopentaose, XI; maltoheptaose, XII) > polysaccharides (laminarin, XIII)]. Monosaccharides are resolved via the sequence of axial and equatorial (ax, eq) vicinal hydroxyl groups that form the complexes with the metal cation (Angyal, 1989). The column selectivity

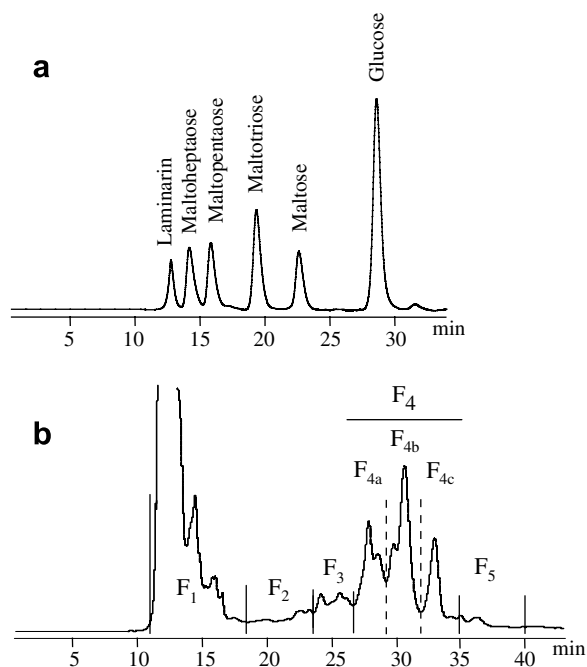


Fig. 1. (a) Separation of glucose (IV; 28.2 min), maltose (IX; 22.6 min), maltotriose (X; 19.3 min), maltopentaose (XI; 15.8 min), maltoheptaose (XII; 14.2 min) and laminarin (XIII; 12.8 min) with Ag^+ cation exchange chromatography (80 °C, two columns in series). Sugars were eluted with high purity water at 0.5 ml min^{-1} and detected with a refractive index (RI) detector. (b) Typical separation of hydrolyzed HMWDOM using same columns and conditions.

can be manipulated by changing the complexing cation (K^+ , Ca^{2+} , Ag^+ , Pb^{2+}) and resolution is generally improved when large cations are used (e.g. Pb^{2+} or Ca^{2+}), because stronger complexes are formed.

Fig. 1b depicts a typical chromatogram of hydrolyzed HMWDOM using two Ag columns in series. Unhydrolyzed or partially hydrolyzed HMWDOM fractions F_1 (59%, 11–18.5 min) and F_2 (15%, 18.5–23.6 min) were eluted before disaccharides and methyl sugars (F_3 ; 5%, 23.6–26.8 min), neutral sugars (F_4 ; 20%, 26.8–35 min) and other hydrolysis products (F_5 ; 2%, 35–40 min). The seven major neutral sugars characteristic of HMWDOM are eluted in fraction F_4 and collected in three sub-fractions (F_{4a} – F_{4c}). Fraction F_{4a} includes glucose (27.8 min) and rhamnose (28.5 min), fraction F_{4b} xylose (29.5 min), galactose (30.5 min) and mannose (30.5), while fraction F_{4c} contains fucose and arabinose which co-elute at 33.1 min.

The 1H NMR spectrum of fraction F_3 shows it to be a complex mixture of methylated, 6-deoxy- and 3-deoxysugars and the fraction was further separated into 10 additional fractions using four Pb cation exchange columns in series. The three largest peaks were collected as fractions F_{3A} – C (Fig. 2a) and were purified one more time using four Ag columns in series. Fig. 2b depicts a typical separation of the F_{3B} fraction purification. Only the largest peaks were collected for detailed compositional analysis (see below).

3.2. Characterization of methyl, methyldeoxy, and deoxysugars in HMWDOM

3.2.1. Fraction F_{3A}

Separation of fraction F_{3A} on four Ag columns yielded three peaks. The major peak was collected as fraction F_{3A2} and had an 1H NMR spectrum with signals for the anomeric protons at δ 5.27 ppm (H-1, α anomer: $J_{1,2} = 3.6$ Hz) and 4.69 ppm (H-1, β anomer: $J_{1,2} = 7.9$ Hz), H-C-OH protons at 3.34–3.93 ppm and one O-methyl ($-OCH_3$) group at 3.65 ppm (Table 1). Protons H-2 through H-6 were assigned from COSY experiments, while HSQC experiments indicated the presence of seven carbon atoms consistent with an O-methylated sugar. On the basis of its NMR spectrum, fraction F_{3A2} was identified as a mixture of α - and β -3-O-methylglucose (VIII). Additional confirmation was given by examination of the first order coupling constants and co-elution with

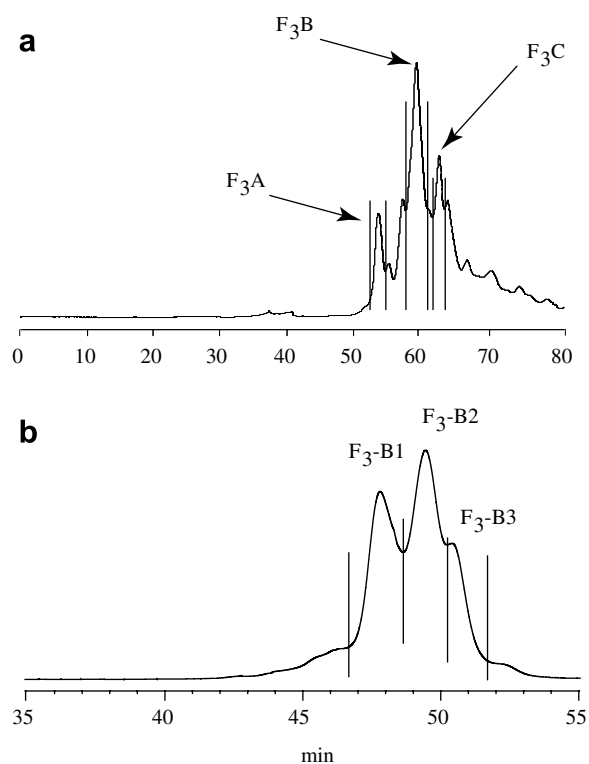


Fig. 2. (a) Separation of fraction 3 (F_3 ; Fig. 1b) with cation exchange HPLC-RI. Conditions: four Pb columns in series; column temperature, elution mode, flow rate and detection as for Fig. 1. (b) Separation of F_{3B} fraction on four Ag columns in series; NB expansion of 35–55 min section. No peaks detected before 35 min. Conditions same as for Fig. 1.

authentic 3-O-methylglucose on HPLC using four Ag columns connected in series (retention time of 46.2 min).

Fraction F_{3A3} was an α/β mixture of at least three different sugars. The major anomers appeared as a broad singlet at 5.34 ppm (α anomer) and 4.82 ppm (β anomer). We were unable to measure the coupling constant for the β anomer due to interference with the solvent peak (water, 4.80 ppm). We observed a sharp O-methyl singlet at 3.68 ppm and the 1H COSY NMR spectrum allowed us to partially assign two coupled spin systems: $\alpha H-1$ (5.34 ppm) \rightarrow $\alpha H-2$ (4.29 ppm) \rightarrow $\alpha H-3$ (2.54, 1.88 ppm) \rightarrow $\alpha H-4$ (4.17 ppm) and $\beta H-1$ (4.82 ppm) \rightarrow $\beta H-2$ (3.60 ppm) \rightarrow $\beta H-3$ (2.32, 1.67 ppm) \rightarrow $\beta H-4$ (3.58 ppm; Table 1). Unlike other sugar fractions we isolated from HMWDOM, we observed for this sugar strong cross peaks in the COSY spectrum between the equatorial proton (H_{eq}) $\alpha H-3_{eq}$ at 2.59 ppm (14.9, 5.5 Hz) and the axial proton (H_{ax}) at $\alpha H-3_{ax}$ at 1.90 ppm (13.0, 5.5 Hz) with $\alpha H-2$ at 4.29 ppm,

Table 1
¹H and ¹³C NMR chemical shifts for HMWDOM sugars^{a,b}

Fraction/compound	Nucleus	1	2	3	4	5	6; 6'	O-Methyl
α -F3-A2	H	5.27	3.63	3.53	3.53	3.88	3.88; 3.81	3.65
	C	92.5	71.4	83.1	69.4	71.9	60.9	60.3
3-O-Methyl- α -glucose ^c	H	5.25	3.64	3.53	3.53	3.89	3.88; 3.81	3.68
	C	92.4	71.4	83.1	69.4	71.9	60.9	60.3
β -F3-A2	H	4.69	3.34	3.34	3.53	3.53	3.93; 3.78	3.65
	C	96.3	73.9	85.8	69.4	76.3	61.1	60.3
3-O-Methyl- β -glucose ^c	H	4.70	3.35	3.34	3.53	3.53	3.94; 3.77	3.68
	C	96.3	73.9	85.7	69.4	76.2	61.1	60.3
α -F3-A3	H	5.34	4.29	2.54; 1.88^d	4.17	NDⁱ	NDⁱ	3.68
	C	92.9						60.5
β -F3-A3	H	4.82	3.60	2.32; 1.67^d	3.58	NDⁱ	NDⁱ	3.68
α -F3-B1	H	5.21	4.23	3.53	3.53	3.94	1.32	3.50
	C	94.3	66.8	79.9	71.6	68.8	17.2	56.5
α -3-O-Methylrhamnose ^c	H	5.16	4.18	3.48	3.46	3.89	1.27	3.45
	C	94.3	66.9	79.9	71.6	68.7	17.2	56.5
β -F3-B1	H	4.91	4.25	3.37	3.45	3.47	1.34	3.50
	C	94.0	67.3	82.4	72.5	ND	17.2	56.5
β -3-O-Methylrhamnose ^c	H	4.86	4.18	3.31	3.39	3.41	1.29	3.45
	C	93.9	67.4	82.3	71.3	72.5	17.2	56.5
α -F3-B2	H	5.32	3.60	3.87	3.39	3.87	1.30	3.51
	C	91.1	81.2	70.1	73.0	68.6	17.2	59.1
α -2-O-Methylrhamnose ^f	H	5.17	3.70	3.89	3.37	3.81	1.26	3.45
	C	99.8	81.0	70.6	73.1	70.0	17.4	59.4
α -F3-C1	H	5.49	3.52	3.93	3.86	4.24	1.26	3.54
	C	89.8	77.7	68.9	72.4	66.6	15.8	57.8
α -2-O-Methylfucose ^g	H	5.65	3.45	4.00	3.81	4.37	1.17	3.47
	C	97.6	79.9	70.7	74.4	68.8	17.9	59.8
α -Fucose ^h	H	5.25	3.83	3.92	3.85	4.25	1.27	
	C	92.7	68.5	69.7	71.3	66.8	16.0	
β -F3-C1	H	4.64	3.23	3.73	3.79	3.83	1.29	3.66
	C	96.4	81.9	73.0	71.9	71.2	15.8	60.6
β -Fucose ^h	H	4.60	3.50	3.70	3.79	3.86	1.29	
	C	96.7	72.2	73.4	71.9	72.3	16.0	

^a Numbering of C 1-6 and H1-6 given in Appendix 1.

^b Chemical shifts for fractions isolated from HMWDOM in bold face type.

^c From authentic 3-O-methylglucose.

^d H(equatorial); H(axial).

^e Popper et al. (2004) [Data for pure α and β -3-O-methylrhamnose; trivial name acofriose].

^f Schäffer et al. (2002) [Data for α -2-O-methylrhamnose as terminal sugar in a glycan polymer].

^g Glushka et al. (2003) [Data for α -2-O-methylfucoheptasaccharide].

^h From authentic fucose.

ⁱ Not determined.

and between β H-3_{eq} at 2.34 ppm (1.7, 14.4 Hz) and β H-3_{ax} at 1.70 ppm (1.2, 12.5 Hz) with β H-2 at 3.35 ppm that are characteristic of a 3-deoxysugar. Fig. 3 compares the ¹H COSY NMR spectrum for 3-deoxyglucose (top; XIV) and fraction F₃A3 (bottom) and highlights the presence of the CH₂ protons as cross peaks in the 3.5–4.5 ppm \times 1.5–2.6 ppm region (compare to Fig. 4). The large coupling con-

stants of 12.5–14.4 Hz are characteristic of H_{eq}/H_{ax} coupling, while the smaller coupling constants are typical of H_{eq}/H_{ax} coupling to neighboring (H-2, H-4) protons. We were unable to identify the major compound in F₃A3 beyond the level of an O-methyl-3-deoxysugar. Comparison with the NMR spectra for authentic 3-deoxyglucose shows that F₃A3 is not O-methyl-3-deoxyglucose, based on differences

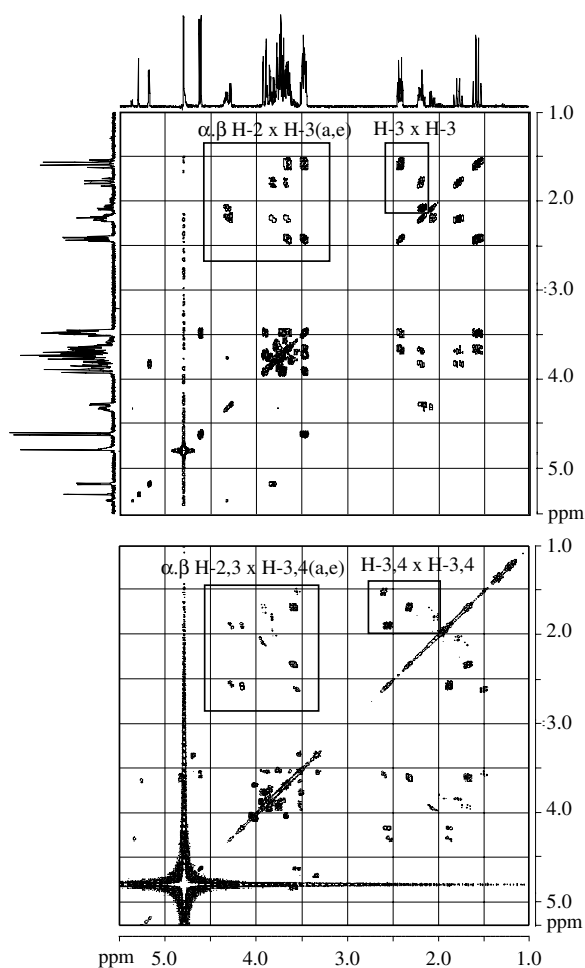


Fig. 3. ^1H NMR COSY spectra of 3-deoxyglucose (top panel) and fraction $\text{F}_3\text{A2}$ (bottom panel). The 1-D ^1H NMR spectrum of 3-deoxyglucose (**XIV**) is projected on the X- and Y-axes of the top panel for reference. Cross peaks due to coupling of axial and equatorial protons H-3 between 1.5 and 2.6 ppm are characteristic of 2-, 3-, and 4-deoxysugars. Cross peaks in this region are not observed in the spectra of hexoses (glucose, galactose) and 6-deoxysugars (see Fig. 4 for comparison).

in cross peaks at 3.5–4.5 ppm \times 1.5–2.6 ppm (Fig. 3). We also identified a third deoxysugar which, from the ^1H COSY NMR spectrum, appears to have the system: $\beta\text{H-1}$ (4.69 ppm) \rightarrow $\beta\text{H-2}$ (3.34 ppm) \rightarrow $\beta\text{H-3}$ (3.54 ppm) \rightarrow $\beta\text{H-4}$ (2.62, 1.52 ppm). From the COSY spectrum, we assigned this as a 4-deoxysugar, but the assignment is not definitive.

3.2.2. Fraction F_3B

Separation of F_3B on four Ag columns resulted in two major peaks collected as fractions $\text{F}_3\text{B1}$ and $\text{F}_3\text{B2}$ (Fig. 2b). The ^1H NMR spectra showed the two fractions to be closely related. Fraction

$\text{F}_3\text{B1}$ was obtained as a mixture of α and β anomers, for which we were able to assign protons H 2–6 from the COSY spectrum and all carbons through HSQC experiments. The ^1H NMR spectrum of fraction $\text{F}_3\text{B1}$ displayed signals for two anomeric protons at δ 5.21 ppm (H-1, α -anomer; d, $J_{1,2} = 1.6$ Hz) and 4.91 ppm (H-1, β -anomer; d, $J_{1,2} = 1.0$ Hz), H–C–OH protons at 3.37–4.25 ppm, one *O*-methyl at 3.50 ppm and two methyls at 1.32 ppm ($\alpha\text{H-6}$, $J_{5,6} = 5.4$ Hz) and 1.34 ppm ($\beta\text{H-6}$, $J_{5,6} = 6.4$ Hz) (Table 1). We noted the very small coupling constant for the β anomer, the α/β anomeric ratio > 1 and the smaller coupling constant of $J_{5,6}$ for the β relative to the α anomer, which are all characteristics of rhamnose (**VI**). First order coupling constants for α $\text{F}_3\text{B1}$ (H-1, 1.6 Hz; H-2, 3.6 Hz; H-3, 9.7 Hz; H-4, 9.7 Hz; H-5, 6.4 Hz) were within 1–2 Hz of authentic rhamnose (α -6-deoxymannose) reported by Bock and Thøgersen (1982); (H-1, 1.6 Hz; H-2, 3.5 Hz; H-3, 9.5 Hz; H-4, 9.5 Hz; H-5, 6.2 Hz). We also noted the ca. 10 ppm downfield shift in the HSQC spectrum for C-3 ($\alpha\text{C-3}$, 79.9; $\beta\text{C-3}$, 82.3) relative to rhamnose ($\alpha\text{C-3}$, 71.1; $\beta\text{C-3}$, 73.8 ppm), indicating methylation at this site (Deepak et al., 2003). In the HSQC spectrum we observed a correlation between $\beta\text{H-3}$ (3.37 ppm) and a carbon at 82.4 ppm, which we assigned as $\beta\text{C-3}$, further establishing the site of methylation. There is a cross peak between H-4 (3.45 ppm) and a carbon at 71.4 ppm, which we assigned as $\beta\text{C-4}$, in good agreement with the values assigned by Popper and Fry (2003) and Popper et al. (2004) for 3-*O*-methylrhamnose released by hydrolysis of lower land plant primary cell walls. We therefore identified $\text{F}_3\text{B1}$ as a mixture of α - and β -3-*O*-methylrhamnose (**XV**; Table 1). Mass spectrometry of $\text{F}_3\text{B1}$ showed a molecular ion at m/z 176, in agreement with our identification. However, for $\alpha\text{F}_3\text{B1}$, protons $\alpha\text{H-3}$ and $\alpha\text{H-4}$ have the same chemical shift and for this anomer we are unable to establish the site of *O*-methylation (3-*O*-methyl vs 4-*O*-methyl). Our assignment of 3-*O*-methylation therefore rests on our assignments for the β anomer.

We used the same reasoning to identify $\text{F}_3\text{B2}$ as a α/β mixture of 2-*O*-methylrhamnose (**XVI**; Zdorovenko et al., 2001; Schäffer et al., 2002). Fractions $\text{F}_3\text{B1}$ and $\text{F}_3\text{B2}$ have very similar NMR spectra, but fraction $\text{F}_3\text{B2}$ was obtained as a mixture of at least four different sugars, leading to some ambiguity in our interpretation of the COSY and HSQC spectra. We were only able to make full assignments for the most abundant α anomer (α $\text{F}_3\text{B2}$). However, due

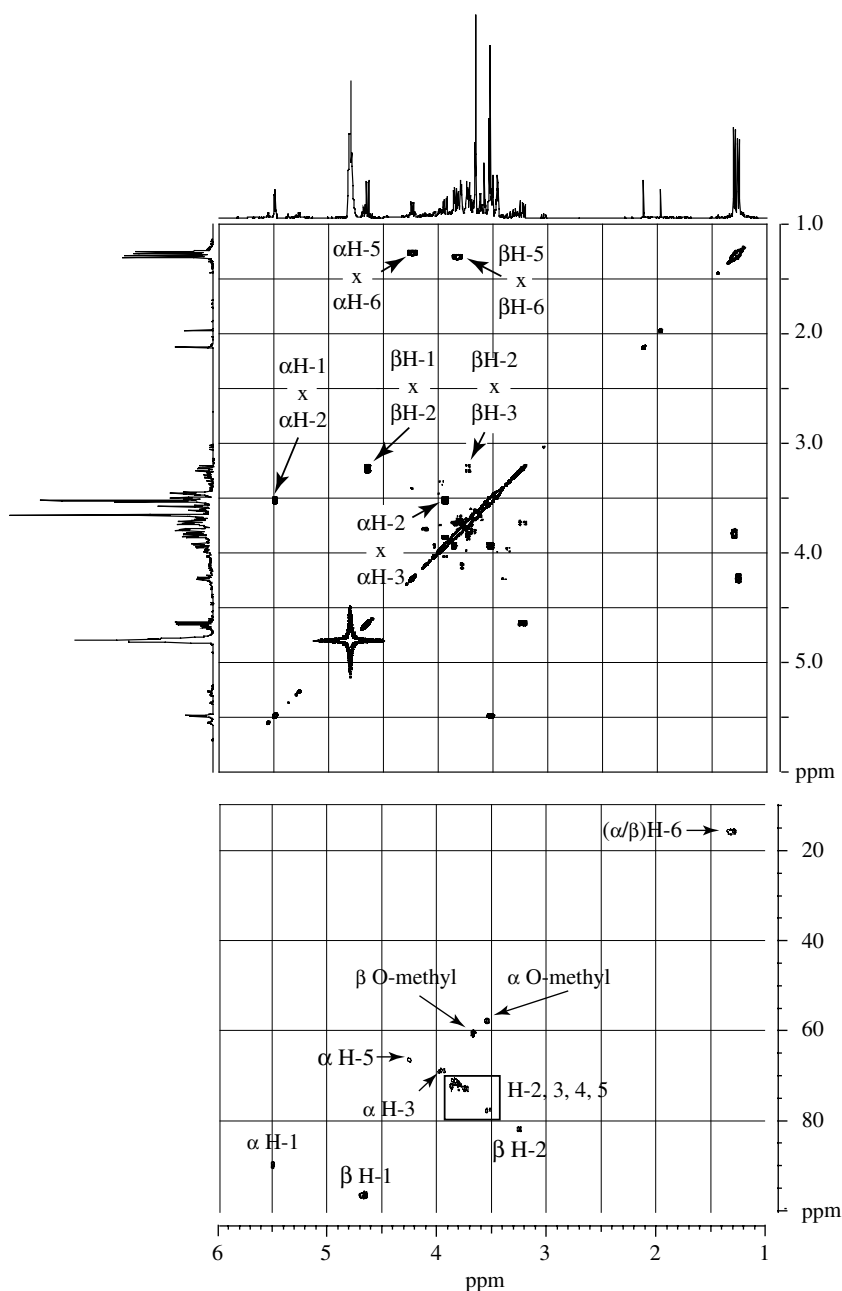


Fig. 4. ^1H NMR COSY (top) and HSQC spectra (bottom) of fraction $\text{F}_3\text{C1}$ (2-*O*-methylfucose; XVII). The 1-D ^1H NMR spectrum of $\text{F}_3\text{C1}$ is projected on the *X*- and *Y*-axes of the top panel for reference. Cross peaks in COSY spectrum are due to coupling between adjacent protons and are used to assign signals in the 1-D spectrum. ^1H NMR spectra assignments are then used to determine carbon chemical shifts in the HSQC spectrum (lower panel).

to the complexity of the spectrum, our assignments of H-3 to H-5 should be viewed as tentative. We found two anomeric protons at 5.32 ppm (H-1, α -anomer; d, $J_{1,2} = 1.6$ Hz) and 4.92 ppm (H-1, β -anomer; broad singlet), *H*-C-OH protons at 3.39–3.87 ppm,

one *O*-methyl group at 3.51 ppm and two methyls at 1.31 ppm ($J_{5,6} = 5.3$ Hz) and 1.33 ppm ($J_{5,6} = 6.7$ Hz) for the α and β anomers, respectively. We found good agreement between our spectral data and 2-*O*-methylrhamnose as the terminal sugar in a

glycan polymer (Schäffer et al., 2002). Because the 2-*O*-methylrhamnose reported by Schäffer et al. (2002) is glycosylated at C-1, we expected a difference in the chemical shift for H-1 and the C-1 chemical shift to appear downfield by 6–7 ppm of our value for F₃B1 (Deepak et al., 2003). Other ¹H and ¹³C values should remain near those for α -2-*O*-methylrhamnose (Table 1); 2-*O*-methylrhamnose was previously identified in HMWDOM from the NE Atlantic Ocean using gas chromatography mass spectrometry of the alditol peracetate (Aluwihare et al., 2002).

The mass spectrum of fraction F₃B2 has a molecular ion at *m/z* 176, as expected for a methylated deoxysugar.

3.2.3. Fraction F₃C

Separation of F₃C on four Ag columns yielded one large peak collected as fraction F₃C1. The ¹H NMR spectrum has major signals for the anomeric protons at δ 5.49 ppm (H-1, α anomer, $J_{1,2} = 1.4$ Hz) and 4.64 ppm (H-1, β anomer, $J_{1,2} = 8.0$ Hz), H-C-OH protons at 3.23–4.24 ppm, two *O*-methyl groups at 3.54 and 3.66 ppm and two methyls at 1.26 and 1.29 ppm ($J_{5,6} = 6.5$ Hz; Table 1). The strong coupling between the methyl at 1.26 ppm and the upfield quartet ($J_{5,6} = 6.5$ Hz) at 4.24 ppm is typical of an α fucose-like configuration. Protons H-2 through H-6 were assigned from COSY experiments, while HSQC experiments indicated the presence of fourteen carbon atoms, consistent with a mixture of α and β anomers of methylfucose (Fig. 4). In Table 1 we compare chemical shift data for F₃C1 with ¹H NMR data we acquired for authentic fucose. We find excellent agreement between fucose and F₃C1, with the greatest difference at H-2, suggesting 2-*O*-methylation in F₃C1. We measured first order coupling constants for β F₃C1 of H-1 (8.0 Hz), H-2 (9.6 Hz), H-3 (3.4 Hz), H-4 (1 Hz), H-5 (6.6 Hz) compared to H-1 (8.2 Hz), H-2 (10.0 Hz), H-3 (3.6 Hz), H-4 (0.8 Hz), H-5 (6.6 Hz) for authentic β -fucose (Bock and Thøgersen, 1982). Integration of the *O*-methyl and 6-methyl groups gave a 1:1 quantitative relationship and we note that C-2 signals (α C-2 = 77.7; β C-2 = 81.9) are shifted approximately 10 ppm downfield from C-2 in fucose (α C-2 = 68.6 ppm, β C-2 = 72.2 ppm), confirming *O*-methylation at C-2. On the basis of its NMR spectrum, we assigned F₃C1 as a mixture of α and β 2-*O*-methylfucose (XVII). To the best of our knowledge, there are no reports of 2-*O*-methylfucose ¹H and ¹³C NMR spectra with which to compare our data. However, NMR

data for 2-*O*-methylfucosyl-containing heptasaccharide isolated from red wine rhamnogalacturonan II are in good agreement with our values (Glushka et al., 2003; Table 1).

3.3. *O*-Methyl and deoxysugars in structural polysaccharides

The components 2-*O*-methylrhamnose and 3-*O*-methylrhamnose have been reported in structural polysaccharides from a large number of cyanobacteria (Painter, 1983; Shekharam et al., 1987; Ogawa et al., 1997) and vascular plants (Bacon and Cheshire, 1971; Casagrande et al., 1985; Popper and Fry, 2003; Popper et al., 2004; Sasaki et al., 2005). These two sugars, together with 3-*O*-methylglucose and 2-*O*-methylfucose are common in lipopolysaccharides, part of the outer cell membrane of gram negative bacteria (Kennedy and White, 1983; Brade et al., 1988). 2-*O*-methylfucose is also a constituent of rhamnogalacturonan II pectic polysaccharides, part of the primary walls of dicotyledenous and monocotyledenous plants and gymnosperms (Glushka et al., 2003) and 2- and 3-*O*-methylrhamnose, along with 2- and 3-*O*-methylfucose, have been putatively reported in cyanobacterial mats and in recent marine sediments, where they comprise up to 5–15 wt% of the total neutral monosaccharide yield (Klok et al., 1983; Klok et al., 1984), as well as in humic and fulvic acids extracted from soil (Ogner, 1980). Given the wide distribution of these methyl sugars in biological and geological materials, they provide little information about the specific source of HMWDOM carbohydrate. However, to the best of our knowledge they have only been reported to occur in structural polysaccharides, and not in storage polysaccharides. Their occurrence in HMWDOM suggests that specific degradation-resistant structural polysaccharides contribute to DOM.

Only one methyl sugar, 2-*O*-methylrhamnose, has been previously reported as a trace constituent of HMWDOM (Aluwihare et al., 2002) and we are not aware of reports of 3-deoxysugars in HMWDOM. Methyl and 6-deoxysugars may nevertheless be important in the uncharacterized fraction of HMWDOM and might serve to give the ¹H NMR spectrum of HMWDOM its characteristic appearance (Aluwihare et al., 1997). Fractions F₁ and F₂ from our Ag column separation of acid-hydrolyzed HMWDOM were eluted with \geq DP 3 glucans, which have a nominal molecular weight > 0.5 kD

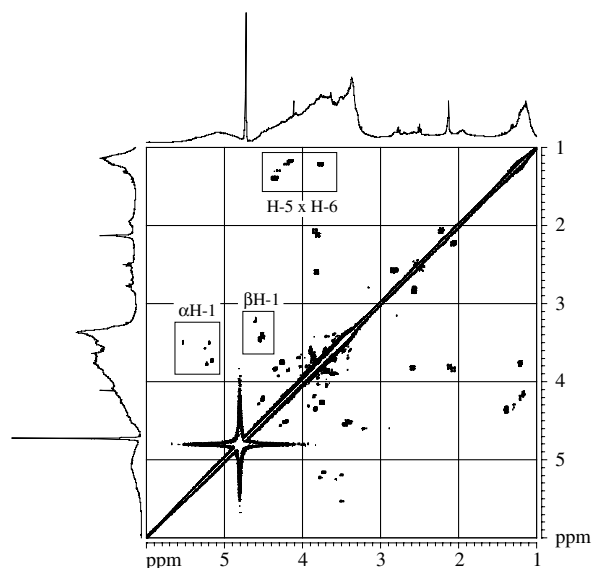


Fig. 5. ^1H NMR COSY spectrum of fraction F₁ (11.8–18.5 min). The spectrum displays numerous cross peaks for α and β anomeric protons ($\alpha\text{H-1}$, 5.23–5.52; $\beta\text{H-1}$, 4.54–4.64) and $\alpha/\beta\text{H-2}$ protons (3.22–3.80 ppm) indicate heterogeneity in either the monosaccharide composition or glycosidic linkages of HMWDOM carbohydrate. Strong cross peaks are also apparent between 1.23–1.42 ppm and 3.79–4.38 ppm from 6-deoxysugars that are part of the non-hydrolyzable fraction of HMWDOM carbohydrate.

(Fig. 1b). ^1H NMR spectra of F₁ (Fig. 5) and F₂ display none of the spectral fine structure characteristic of DP 1–4 mono- and oligosaccharides that would be expected if these fractions contained significant amounts of degraded simple sugars. The two fractions represent 74% of the total HMWDOM, demonstrating that commonly used hydrolytic protocols [2N TFA; 1–6 N HCl (Repeta, unpublished results)] do not depolymerize most HMWDOM. The recalcitrance of HMWDOM carbohydrate towards acid hydrolysis explains most of the discrepancy between NMR measurements of HMWDOM carbohydrate and GC or HPLC measurements of simple sugars. Previous GC and HPLC analyses of HMWDOM hydrolysis products have also missed methyl sugars identified in our current study, further lowering the amount of carbohydrate reported using these methods.

3.4. Hydrolysis resistant carbohydrate in HMWDOM

Qualitative information on the composition of HMWDOM carbohydrate that is resistant to acid hydrolysis can be obtained from the NMR spectra

of fraction F₁. The COSY spectrum of hydrolyzed HMWDOM fraction F₁ (Fig. 1b) shows strong cross peaks for 6-deoxysugars (1.2–1.4 ppm \times 3.3–4.4 ppm; Fig. 5). The most intense cross peaks appear at 1.34 \times 4.32 ppm and 1.23 \times 3.79 ppm, similar to α and β fucose (1.26 \times 4.24 ppm and 1.29 \times 3.83 ppm, respectively). The COSY spectrum further shows at least eight distinct α anomeric and four distinct β anomeric protons. These protons could represent a mixture of different α - and β -linked sugars or positional isomers, but nevertheless suggest a structurally complex polysaccharide.

We did not observe cross peaks in the COSY spectrum that are characteristic of lipids. No cross peaks were observed between 1.3 ppm ($-\text{CH}_2-$) and signals < 1 ppm (CH_3) or 1.5–3 ppm ($-\text{CH}_2-\text{OH}$; $-\text{CH}_2-\text{COOH}$) that we expect to see if ether or ester bound lipids were present. The COSY spectrum of HMWDOM does not suggest a major contribution from lipids to the peak at 1.3 ppm in the ^1H NMR spectrum. In support of this, Quan (2005) noted the production of acetic acid as a major oxidation product of HMWDOM previously treated with sodium periodate and ascribed this to the oxidation of 6-deoxysugars at C-5. Quan (2005) did not recover lipids from HMWDOM periodate oxidation products, even though lipids should have survived the reaction conditions. Quan's (2005) data support the COSY NMR assessment that lipids are only a minor component of HMWDOM. If we assume that all the carbon represented by the 1.3 ppm peak is from 6-deoxysugars, then 6-deoxysugars represent 25–30% of the total uncharacterized HMWDOM carbohydrate.

4. Conclusions

Previous characterization of HMWDOM using DT-MS has shown that HMWDOM carbohydrate includes methyl- and methyldeoxysugars. We confirm these results with the identification of 2- and 3-*O*-methylrhamnose, 2-*O*-methylfucose and 3-*O*-methylglucose in HMWDOM. We also isolated fractions that have NMR spectral characteristics of 3- and 4-deoxysugars, although the full structure of these components could not be determined. We are not aware of reports of *O*-methylrhamnose and *O*-methylfucose in algal or bacterial storage polysaccharides, but these sugars are commonly found in structural polysaccharides from higher plant cell walls and our analyses point to degradation-resistant cell wall polysaccharides as an impor-

tant component of HMWDOM. Separation of HMWDOM after acid hydrolysis using Ag^+ and Pb^{2+} cation exchange chromatography shows that most carbohydrate is not depolymerized using commonly used hydrolysis protocols. New strategies need to be developed if HMWDOM is to be characterized at the level of constituent simple sugars. The 2-D NMR techniques provide some insight into the composition of the non-hydrolyzed component and show it to be a heterogeneous mixture containing up to 25–30% 6-deoxysugars, most likely rhamnose and fucose.

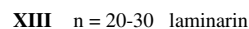
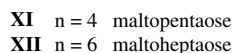
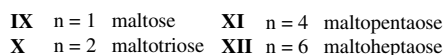
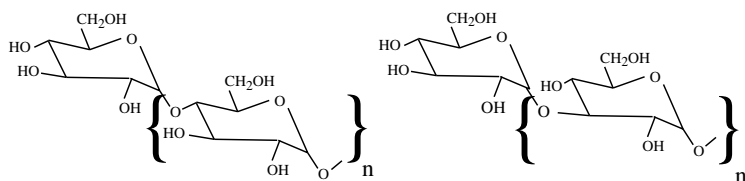
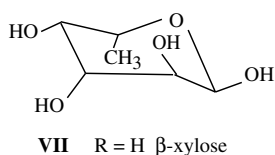
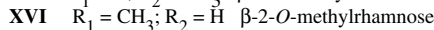
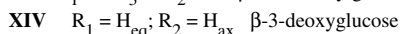
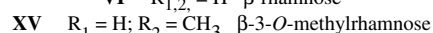
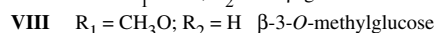
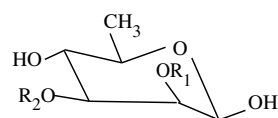
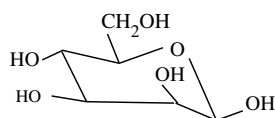
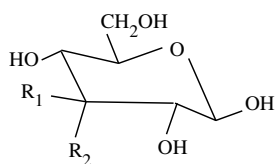
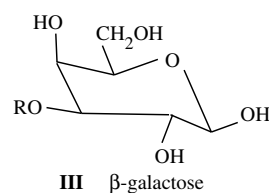
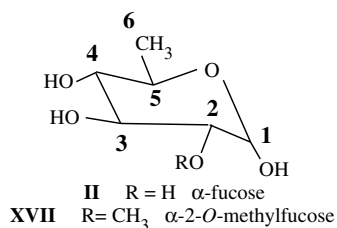
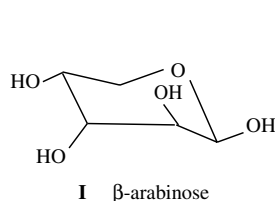
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Appendix I

Structures of methyl- deoxy- and methyldeoxysugars. Positions 1–6 are noted in bold numerals on structures **II** and **XVII**; H_{ax} and H_{eq} in β -3-deoxyglucose (**XIV**) denote configuration (axial and equatorial) of H-3 protons.



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References

- Aluwihare, L.I., Repeta, D.J., Chen, R.F., 1997. A major biopolymeric component to dissolved organic carbon in surface water. *Nature* 387, 166–169.
- Aluwihare, L.I., Repeta, D.J., Chen, R.F., 2002. Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight. *Deep-Sea Research II* 49, 4432–4437.
- Aluwihare, L.I., Repeta, D.J., Pantoja, S., Johnson, C.G., 2005. Two chemically distinct pools of organic nitrogen accumulate in the ocean. *Science* 308, 1007–1010.
- Angyal, S.J., 1989. Complexes of metal cations with carbohydrates in solution. *Advances in Carbohydrate Biochemistry* 47, 1–43.
- Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L., Thingstad, F., 1983. The ecological role of water column microbes in the sea. *Marine Ecology Progress Series* 10, 257–263.
- Bacon, J.S.D., Cheshire, M.V., 1971. Apiose and mono-*O*-methyl sugars as minor constituents of the leaves of deciduous trees and various other species. *Biochemical Journal* 124, 555–562.
- Benner, R., 2002. Chemical composition and reactivity. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 59–90.
- Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., Hatcher, P.G., 1992. Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* 255, 1562–1564.
- Bock, K., Thøgersen, H., 1982. Nuclear magnetic resonance spectroscopy in the study of mono- and oligosaccharides. *Annual Reports on NMR Spectroscopy* 13, 1–57.
- Boon, J.J., Klap, V.A., Eglinton, T.I., 1998. Molecular characterization of microgram amounts of oceanic colloid organic matter by direct temperature-resolved ammonia chemical ionization mass spectrometry. *Organic Geochemistry* 29, 1051–1061.
- Borch, N.H., Kirchman, D.L., 1997. Concentration and composition of dissolved combined neutral sugars (polysaccharides) in seawater determined by HPLC-PAD. *Marine Chemistry* 57, 85–95.
- Brade, H., Brade, L., Rietschel, E.Th., 1988. Structure–activity relationships of bacterial lipopolysaccharides (endotoxins). *Zentralblatt für Bakteriologie Mikrobiologie und Hygiene A* 268, 151–179.
- Bronk, D., 2002. Dynamics of DON. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 153–247.
- Casagrande, D.J., Ferguson, A., Boudreau, J., Predny, R., Folden, C., 1985. Organic geochemical investigations in the Okefenokee swamp, Georgia: the fate of fatty acids, glucosamine, cellulose, and lignin. In: Cross, T. (Ed.), *Proceedings of the 9eme Congres International de Stratigraphie et de Géologie du Carbonifère*, 1979, pp. 193–204.
- Deepak, D., Arun, S., Sapna, S.S., Anakshi, K., Sanjay, S., 2003. Structure elucidation of oligosaccharides containing 2-deoxy sugars by NMR techniques. *Trends in Carbohydrate Chemistry* 4, 25–32.
- Ducklow, H.W., Carlson, C.A., 1992. Oceanic bacterial production. In: Marshall, K.C. (Ed.), *Advances in Microbial Ecology*. Plenum Press, pp. 113–181.
- Eglinton, T.I., Repeta, D.J., 2004. Marine Organic Geochemistry. In: Elderfield, H. (Ed.), *Treatise on Geochemistry*, vol. 7. Elsevier, pp. 145–180.
- Glushka, J.N., Terrell, M., York, W.S., Malcolm, A. O'Neill, Gucwa, A., Darvill, A.G., Albersheim, P., Prestegard, J.H., 2003. Primary structure of 2-*O*-methyl- α -*L*-fucose-containing side chain of the pectic polysaccharide, rhamnogalacturonan II. *Carbohydrate Research* 338, 341–352.
- Guo, L., Santschi, P.H., 1997. Composition and cycling of colloids in marine environments. *Reviews of Geophysics* 35, 17–40.
- Hedges, J.I., Baldock, J.A., Gélinas, Y., Lee, C., Peterson, M., Wakeham, S.G., 2001. Evidence for non-selective preservation of organic matter in sinking marine particles. *Nature* 409, 801–804.
- Hedges, J.I., Baldock, J.A., Gélinas, Y., Lee, C., Peterson, M., Wakeham, S.G., 2002. The biochemical and elemental compositions of marine plankton: a NMR perspective. *Marine Chemistry* 78, 47–63.
- Karl, D.M., Björkman, K.M., 2002. Dynamics of DOP. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 249–366.
- Kennedy, J.F., White, C.A., 1983. Bioreactive carbohydrates. In: Horwood, E. (Ed.), *Chemistry, Biochemistry and Biology of Carbohydrates*. Halsted Press, p. 331.
- Klok, J., van der Knaap, J.M.M., de Leeuw, J.W., Cox, H.C., Schenck, P.A., 1983. Qualitative and quantitative characterization of the total organic matter in a recent marine sediment. In: Bjørøy, M. (Ed.), *Advances in Organic Geochemistry*. J. Wiley & Sons Ltd, pp. 813–818.
- Klok, J., Cox, H.C., Baas, M., de Leeuw, J.W., Schenck, P.A., 1984. Carbohydrates in recent marine sediments-II. Occurrence and fate of carbohydrates in a recent stromatolitic deposit: Solar lake, Sinai. *Organic Geochemistry* 7, 101–109.
- Lee, C., Bada, J.L., 1977. Dissolved amino acids in the equatorial Pacific, the Sargasso Sea, and Biscayne Bay. *Limnology and Oceanography* 22, 502–510.
- Mannino, A., Harvey, H.R., 1999. Lipid composition in particulate and dissolved organic matter in the Delaware Estuary: sources and diagenetic patterns. *Geochimica et Cosmochimica Acta* 63, 2219–2235.
- McCarthy, M., Hedges, J.I., Benner, R., 1996. Major biochemical composition of dissolved high molecular weight organic matter in seawater. *Marine Chemistry* 55, 281–297.
- Minor, E.C., Boon, J.J., Harvey, H.R., Mannino, A., 2001. Estuarine organic matter composition as probed by direct temperature-resolved mass spectrometry and traditional geochemical techniques. *Geochimica et Cosmochimica Acta* 65, 2819–2834.
- Minor, E.C., Simpouw, J.-P., Boon, J.J., Kerkhoff, A.E., van der Horst, J., 2002. Estuarine/marine UDOM as characterized by size-exclusion chromatography and organic mass spectrometry. *Marine Chemistry* 78, 75–102.
- Mopper, K., Kieber, D.J., 2002. Photochemistry and the cycling of carbon, sulfur, nitrogen and phosphorus. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 455–489.

- Nelson, N.B., Siegel, D.A., 2002. Chromophoric DOM in the Open Ocean. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 547–578.
- Nelson, N.B., Siegel, D.A., Michaels, A.F., 1998. Seasonal dynamics of colored dissolved material in the Sargasso Sea. *Deep-Sea Research I* 45, 931–957.
- Ogawa, K., Yamaura, M., Maruyama, I., 1997. Isolation and identification of 2-*O*-methyl-L-rhamnose and 3-*O*-methyl-L-rhamnose as constituents of an acidic polysaccharide of *Chlorella vulgaris*. *Bioscience, Biotechnology and Biochemistry* 61, 539–540.
- Ogner, G., 1980. Analysis of the carbohydrates of fulvic and humic acids as their partially methylated alditol acetates. *Geoderma* 23, 1–10.
- Painter, T.J., 1983. Algal Polysaccharides. In: Aspinall, G.O. (Ed.), *The Polysaccharides*, vol. 2. Academic Press, pp. 195–285.
- Panagiotopoulos, C., Sempéré, R., 2005. Analytical methods for the determination of sugars in marine samples: a historical perspective and future directions. *Limnology Oceanography Methods* 3, 419–454.
- Popper, Z.A., Fry, S.C., 2003. Primary cell wall composition of bryophytes and charophytes. *Annals of Botany* 91, 1–12.
- Popper, Z.A., Sadler, I.H., Fry, S.C., 2004. 3-*O*-Methylrhamnose in lower land plant primary cell walls. *Biochemical Systematics and Ecology* 32, 279–289.
- Quan, T.M., 2005. Chemical characterization of dissolved organic matter (DOM) in seawater: structure, cycling, and the role of biology. Ph.D. Thesis, Massachusetts Institute of Technology/Woods hole Oceanographic Institution.
- Rue, E.L., Bruland, K.W., 1997. The role of organic complexation on ambient iron chemistry in the Equatorial Pacific Ocean and the response of a mesoscale iron addition experiment. *Limnology Oceanography* 42, 901–910.
- Sakugawa, H., Handa, N., 1985. Chemical studies on dissolved carbohydrates in water samples collected from North Pacific and Bering Sea. *Oceanologica Acta* 8, 185–196.
- Sasaki, G.L., Gorin, P.A.J., Reis, R.A., Serrato, R.V., Elffio, S.L., Iacomini, M., 2005. Carbohydrate, glycolipid, and lipid components from the photobiont (*Scytonema* sp.) of the lichen, *Dictyonema glabratum*. *Carbohydrate Research* 340, 1808–1817.
- Schäffer, C., Wugeditsch, T., Kählig, H., Scheberl, A., Zayni, S., Messner, P., 2002. The surface layer (S-layer) glycoprotein of *Geobacillus stearothermophilus* NRS 2004/3a. *Journal of Biological Chemistry* 277, 6230–6239.
- Shekharam, K.M., Venkataraman, L.V., Salimath, P.V., 1987. Carbohydrate composition and characterization of two unusual sugars from the blue green alga, *Spirulina platensis*. *Phytochemistry* 26, 2267–2269.
- Wang, X.-C., Altabet, M.A., Callahan, J., Chen, R.F., 2004. Stable carbon isotopic compositions of high molecular weight dissolved organic matter from four US estuaries. *Geochimica et Cosmochimica Acta* 68, 2681–2691.
- Wells, M.L., 2002. Marine colloids and trace metals. In: Hansell, D., Carlson, C. (Eds.), *Biogeochemistry of Marine Dissolved Organic Matter*. Academic Press, pp. 367–404.
- Wicks, R.J., Moran, M.A., Pittman, L.J., Hodson, R.E., 1991. Carbohydrate signatures of aquatic macrophytes and their dissolved degradation products as determined by a sensitive high-performance ion chromatography method. *Applied Environmental Microbiology* 57, 3135–3143.
- Williams, P.J.le B., 1981. Microbial contribution to overall marine plankton metabolism: direct measurements of respiration. *Oceanologica Acta* 4, 359–364.
- Zdorovenko, E.L., Ovod, V.V., Zatonksy, G.V., Shashkov, A.S., Kocharova, N.A., Knirel, Y.A., 2001. Location of the *O*-methyl groups in the *O* polysaccharide of *Pseudomonas syringae* pv. phaseolicola. *Carbohydrate Research* 330, 505–510.