IMBER IMBIZO — Integrating biogeochemistry and ecosystems in a changing ocean — 9-13 Nov 08 — Miami (FL,USA)

Novel sugar compounds isolated from high molecular weight dissolved organic matter (HMWDOM)

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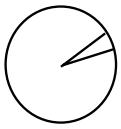
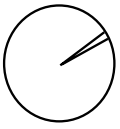
(1) LMGEM, Center of Oceanology Marseille
(2) Woods Hole Oceanographic Institution

How well do we know the composition of Organic matter ?

DOC

Surface DOC  15-30% C characterized (AA-C + Lip-C +Sugar-C)

Deep DOC  < 10% C characterized (AA-C + Lip-C +Sugar-C)

→ Most of the DOC remains uncharacterizable at the molecular level

Benner (2002)

Natural Concentrations of compounds in DOM

Carbohydrates: 100-800 nmol/L for polysaccharides
< 10 nmol/L for monomers

Amino acids: similar range with carbohydrates

Lipids: 200-500 nmol/L

Concentrations close to the detection limits of the techniques

Pulsed amperometric detection (PAD) ~ 2-10 nmol/L (sugars)

Flame ionization detection (FID/MS) ~ 100-300 nmol/L (lipids)

Fluorescence/UV-visible ~ 200-1000 nmol/L (Amino acids)

NMR ~ mg-g/L

- Detection limits +

Impossible to characterize the whole DOM with the current techniques

Get info from concentrated OM [i.e. High Molecular Weight dissolved Organic matter (HMWDOM)] > 1000Da)

Approaches for the chemical characterization of DOM

(1) Direct analyses of DOM (0.5-1 mg/L DOC)

+	-
• No contamination and artifacts	• Low conc. of compounds (nmol)
• Representative of DOM pool	• Salts (35-38 g/L)

(2) Analyses of concentrated DOM (HMWDOM > 1kDa)

+	-
• Almost no salts	• Large vol. of samples required (>5000L)
• 5-10 g of material	• Only 25-30% DOC recovered

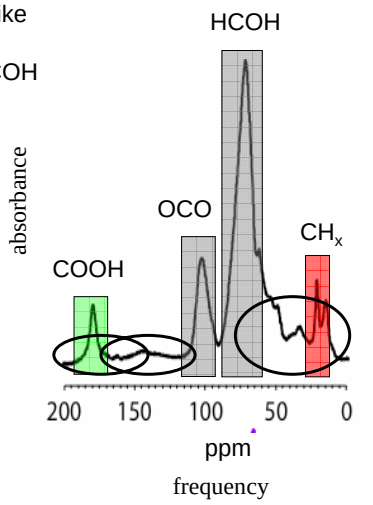
NMR; MS-MS; $\Delta^{14}C$ on individual comp. etc

HMWDOM composition by ^{13}CNMR

HMWDOM is mostly a mixture of carbohydrate like molecules [acyl polysaccharides (APS)]. HMWDOM comprize sugars (50-70%; OCO, HCOH groups), carboxylic + acetate (5%; COOH, CH_3CO), alkyl (5%; CHx), proteins and humics (20%)

However, after acid hydrolysis (HCl, TFA, H_2SO_4 etc) only 15-20% of HMWDOM is recovered as neutral sugars.

APS are present in both fresh and marine waters and their inventory is large (10-15 GTC at least)



Aluwihare et al., (1997); Repeta et al.(2002)

Chemical composition and age of HMWDOM

HMWDOM

Δ^{14}C (‰) = 46 to -381 (<10-3850 yr)

(3-3600 m)

Hydrolysis desalting (resins)

50-70% HMWDOC (APS)

Elution NH_4OH | **Elution H_2O**

Amino acids and humics

Δ^{14}C (‰) = -416 to -428 (4320-4480 yr)
(3-3600 m)

Rich in –COOH Aliphatic component

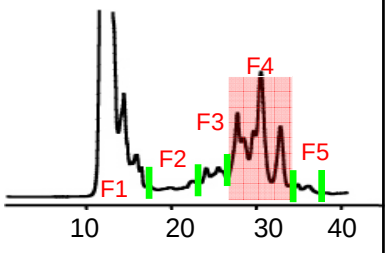
Total Carbohydrates

Rich in –CHOH Hydrophilic component

HPLC-RI

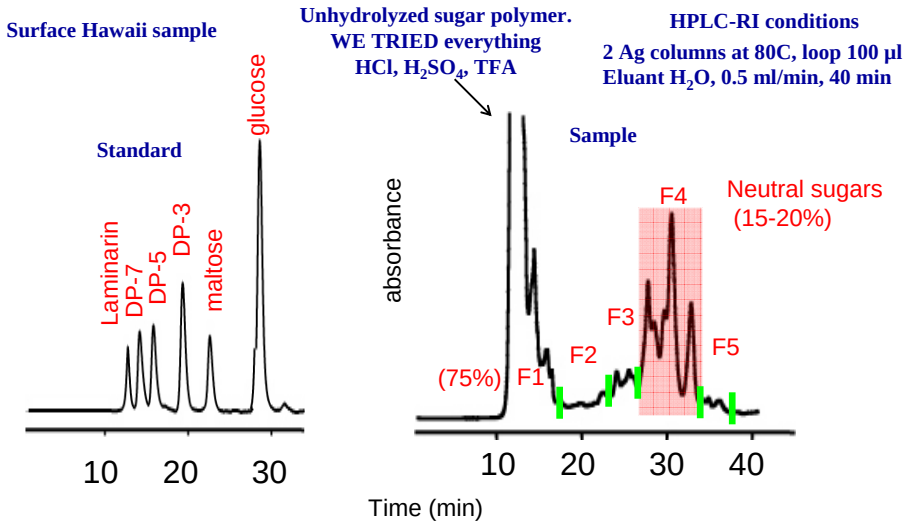
Arabinose
Fucose
Galactose
Glucose
Mannose
Rhamnose
Xylose

Δ^{14}C (‰) = 57 to -145 (<5-1280 yr)
(3-3600 m)

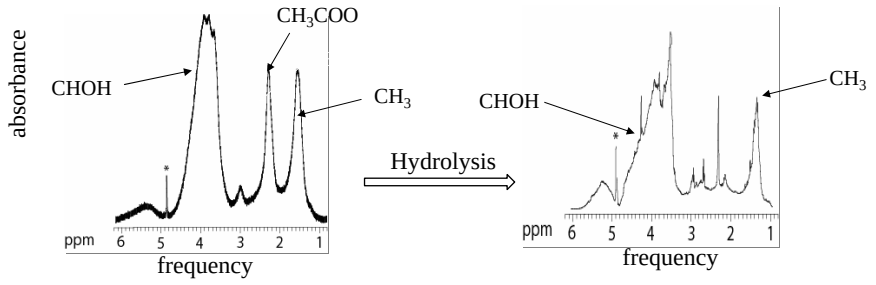


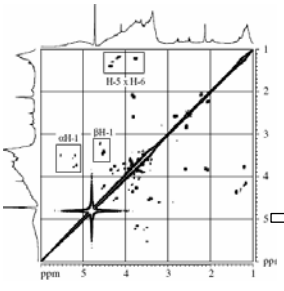
Repeta and Aluwihare (2006)

Separation of mono- and oligosaccharides after HMWDOM hydrolysis using ion chromatography



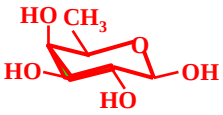
Chemical composition of the HMWDOM- F1 by NMR





Unhydrolyzed sugar polymer
most AcOH is gone

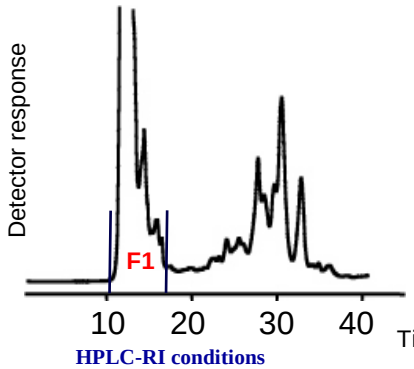
absorbance

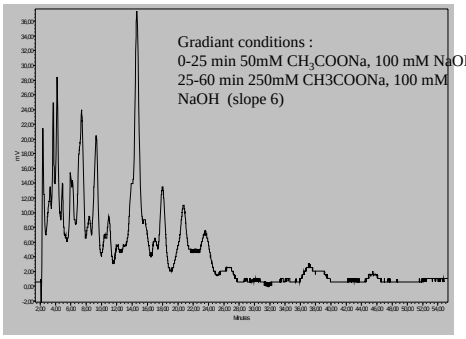


6-deoxy sugars (fucose or rhamnose like) represent 25-30% of the unhydrolyzed polymer (HMWDOM-F1)

Panagiotopoulos et al (2007)

Chemical composition of the HMWDOM- F1 by anion exchange chromatography





HPLC-RI conditions

2 Ag columns at 80°C, eluant H_2O (isocratic elution), 0.5 ml/min, 40 min

NON-DESTRUCTIVE TECHNIQUE

HPAEC-PAD conditions

1 Anion exchange column at 17°C, eluant NaOH and CH3COONa (gradient mode), 0.7 ml/min, 60 min

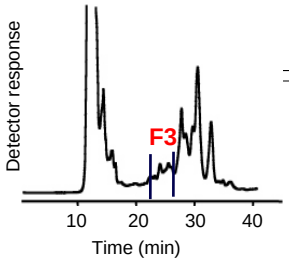
DESTRUCTIVE TECHNIQUE

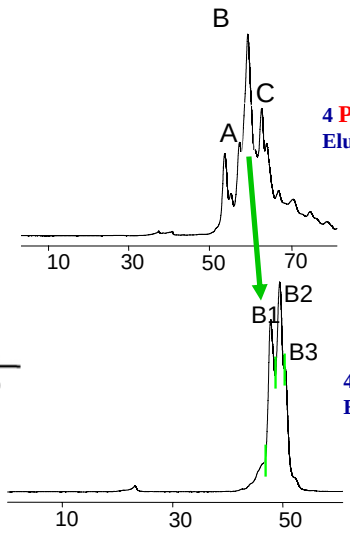
Combination HPAEC with MS (HPAEC-MS/MS)

Technological challenge:

Use of strong non-volatile eluents (i.e NaOH, CH3COOH)
Desalting prior MS

Chemical composition of the HMWDOM- F3 using ion chromatography and NMR





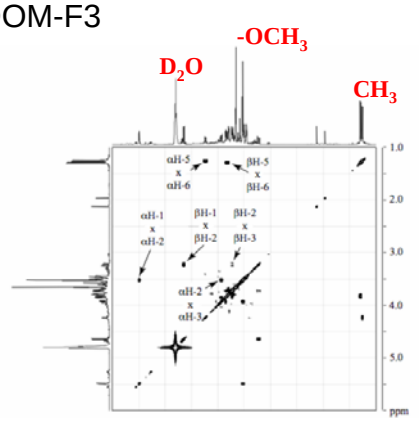
HPLC-RI conditions

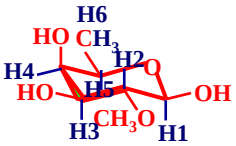
4 Pb columns at 80C, loop 100 µl
Eluant H_2O, 0.5 ml/min

HPLC-RI conditions

4 Ag columns at 80C, loop 100 µl
Eluant H_2O, 0.5 ml/min

Identification of novel sugar compounds by 2D NMR in HMWDOM-F3

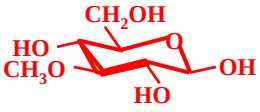




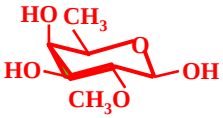
2-OMe fucose = B2 compound

Panagiotopoulos et al (2007)

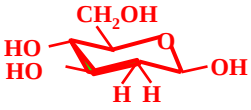
Identification of novel sugar compounds by 2D NMR in HMWDOM-F3



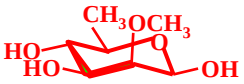
3-OMe glucose



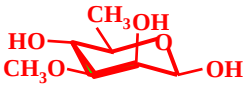
2-OMe fucose



3-deoxy glucose



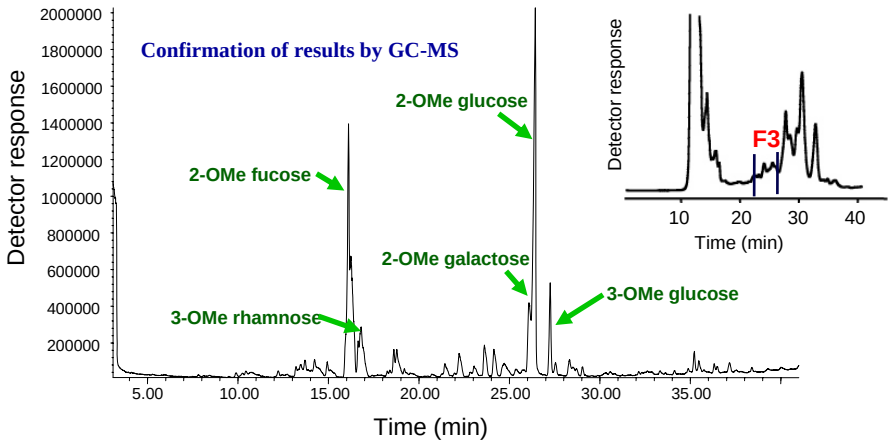
2-OMe rhamnose



3-OMe rhamnose

Panagiotopoulos et al (2007)

GC-MS alditols acetates derivatives in the HMWDOM-F3

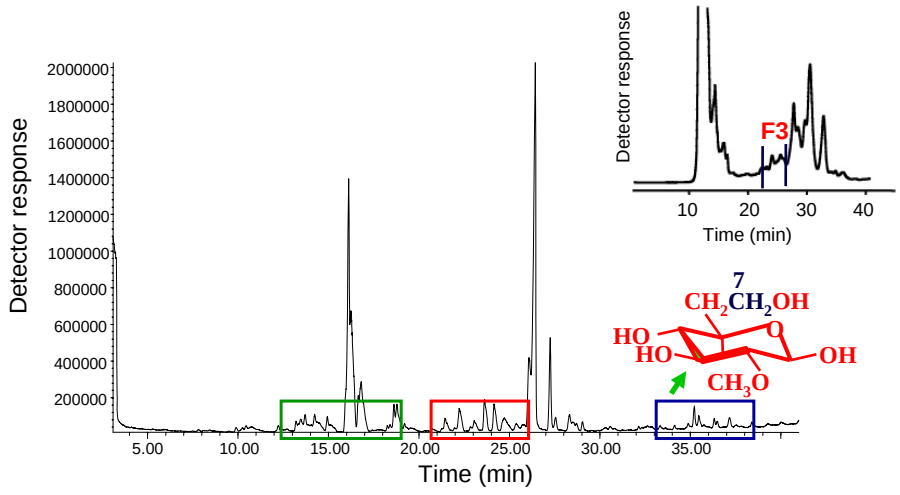


MS can not say the difference between epimers (i.e. glucose, galactose, mannose)

Lack of authetic sugar standard (differences only in retention times)

Determine the absolute configuration of sugars (D or L). Other derivatization procedures (e.g. trimethylsilylated dithioacetals)

GC-MS alditols acetates derivarives in the HMWDOM-F3

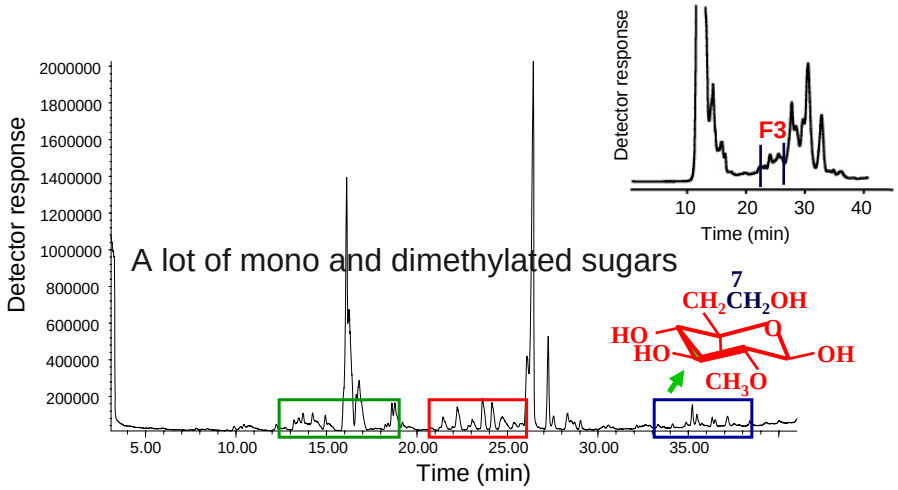


3,6-dideoxyhexose; 2,3-di-O-methylrhmnose; 2,4-di-O-methylrhamnose; 2-O-methyl-pentose

2,6-di-O-methylhexose; 3,6-di-O-methylhexose; 2,3-di-O-methylhexose; 6-O-methylhexose

Glucosamine, 2-O-methylheptoses

GC-MS alditols acetates derivarives in the HMWDOM-F3

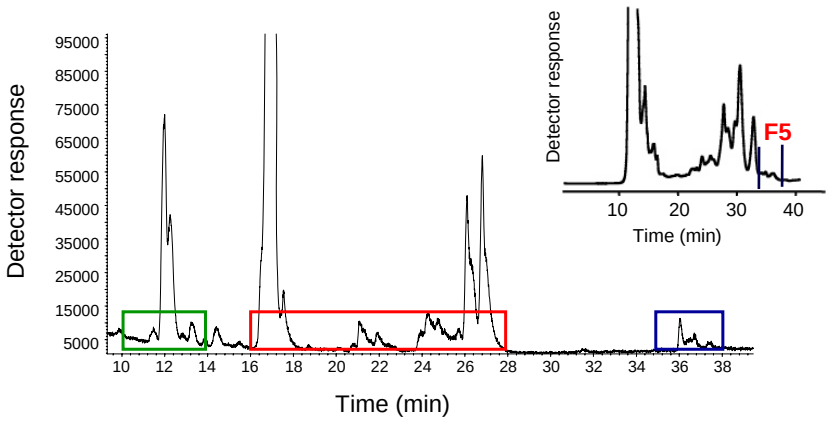


3,6-dideoxyhexose; 2,3-di-O-methylrhmnose; 2,4-di-O-methylrhamnose; 2-O-methyl-pentose

2,6-di-O-methylhexose; 3,6-di-O-methylhexose; 2,3-di-O-methylhexose; 6-O-methylhexose

Glucosamine, 2-O-methylheptoses

GC-MS alditols acetates derivarives in the HMWDOM-F5

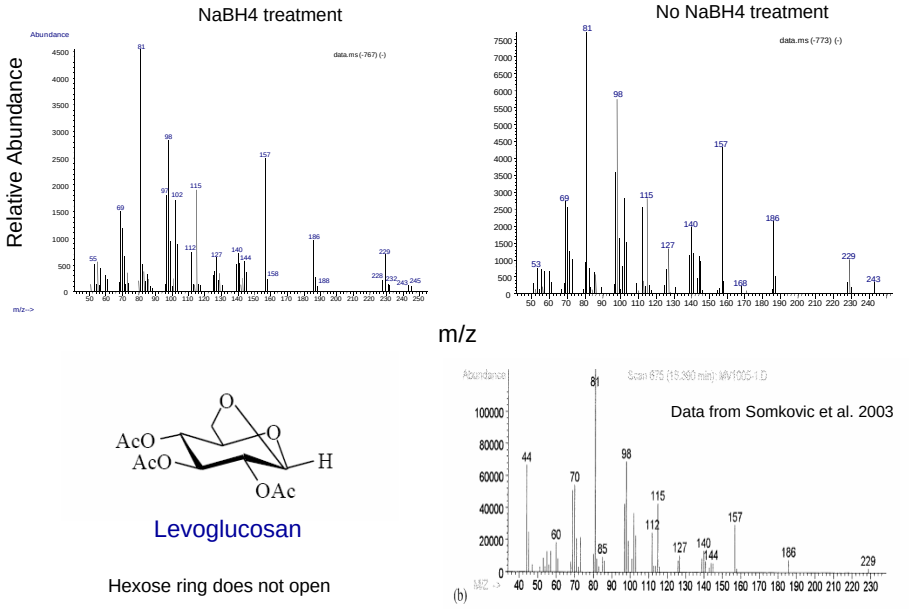


1,6 anhydro hexoses (i.e levoglucosan, galactoglucosan, mannoglucosan etc)

Hexoses, Pentoses (leftovers from the F4 neutral sugar fraction)

Heptoses

Levoglucosan mass spectra



Levoglucosan

Hexose ring does not open

Biogeochemical importance

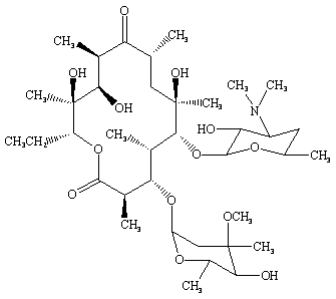
A. Methylated, dimethylated hexoses have been found in bacterial and algal cell walls as part of structural polysaccharides, however their chemical structure and function is poorly understood.
Are these compounds contributors to the refractory DOM ?

B. 3-6-dideoxysugars and heptoses have been as antigenic polysaccharides in Gram-negative bacteria as well as in antibiotics.
It is fundamental to get more info about bacteria structures. Do bacteria assimilate metylated or deoxysugars ?

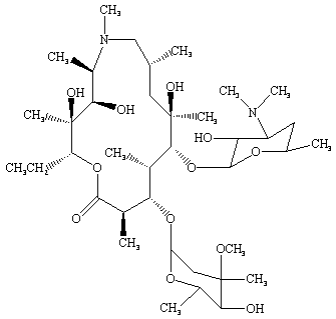
C. Levoglucosan is a component of atmospheric smoke particles derived from wood burning (cellulose degradation product) and this is the FIRST time that has been found in DOM in tiny amounts.
Does black carbon (part of the uncharacterizable DOM carbon) enter the ocean via atmospheric deposition ? (this was assumed but not proven by molecular level analysis by Masiello & Druffel, 1998).

Measurements of $d^{13}C$ of pure levoglucosan in surface and deeper DOM samples

Beyond Biochemistry

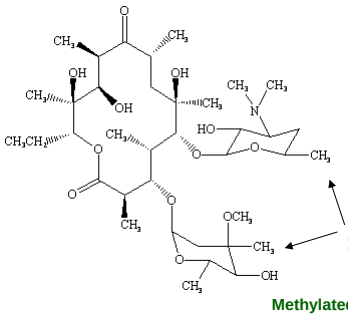


Erythromycin

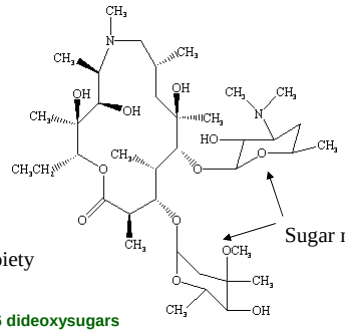


Azythromycin

Beyond Biochemistry



Sugar moiety



Sugar moiety

Methylated 2,6 or 3,6 dideoxysugars

Erythromycin

Azythromycin

ANTIOBIOTICS: Antibacterial agents

The sugars moieties in antiobiotics actively contribute as recognition elements to the mechanism of action of the respective drug and their removal often results in the loss of all biological activity.

IDENTIFY new sugar compounds in the HMWDOM may help discovering new antiobiotic analogs (pharmaceutical chemistry etc..)

Much more to do….

A. Technological development of the HPAEC-MS/MS for structural characterization of the unhydrolyzed HMWDOM-F1 fraction.

B. Chemical characterization of the F2 fraction

C. Dig out more sugars from the F3 fraction and get someway authentic sugar standards (buy them or synthetize them). Perform $\Delta^{14}C$ measurements on individual methyl, deoxy sugars.

D. Start to identify all sugar components in marine bacteria and algae

E. Isotopic analysis of levoglucosan in surface and deep samples (info about black carbon)