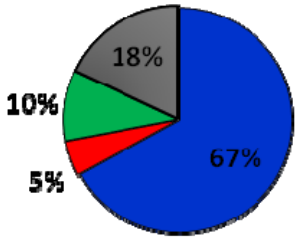
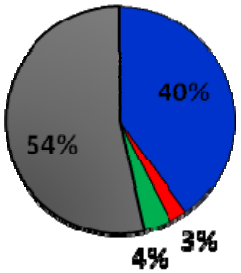
How well do we know the composition of Organic matter ?

Epipel. zone (0-400 m) | **Mesopel. zone (400-1000 m)** | **Bathypel. zone (1000-4000 m)**

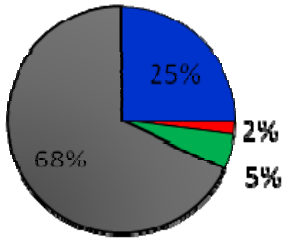
POM

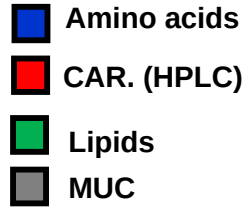


$\delta^{13}C$=-21.6‰
$\Delta^{14}C$= 10 to 120‰
(modern)



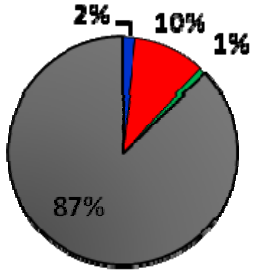
$\delta^{13}C$=-21.6‰
$\Delta^{14}C$=-10 to 120‰
(modern)



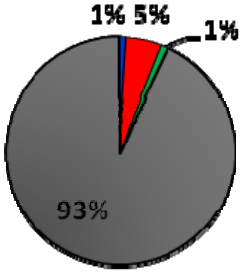


$\delta^{13}C$=-21.3‰
$\Delta^{14}C$=-50 to 110‰
(modern-400 y)

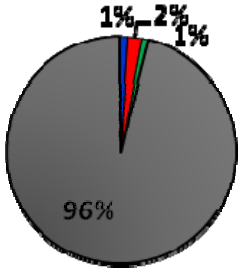
DOM



$\delta^{13}C$=-21.3‰
$\Delta^{14}C$= -191 to -238‰
(1700-2180 y)



$\delta^{13}C$=-21.0‰
$\Delta^{14}C$=-375 to -470‰
(3780-5100 y)



$\delta^{13}C$=-21.2‰
$\Delta^{14}C$=-378 to -533‰
(3810-6120 y)

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

< 50 nmol/L for monomers

Amino acids: similar range with carbohydrates

Lipids: 200-500 nmol/L

→ Concentrations close to the detection limits of the techniques

AND ALWAYS 35 g/L of seasalts

Detection limits

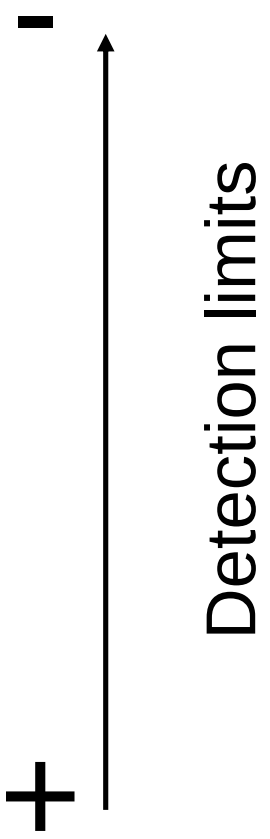
Analytical techniques

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



Conclusion:

a. Very few profiles of these biochemicals published in literature for DOM

b. Impossible to characterize the whole DOM with the current analytical techniques

Do we have any alternatives?

Get info from concentrated OM (i.e High Molecular Weight dissolved Organic matter HMWDOM)

Approaches for the chemical characterization of DOM

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

+

- No contamination and artifacts
- Representative of DOM pool

-

- Low conc. of compounds (nmol)
- Salts (35-38 g/L)

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

+

- Almost no salts
- 5-10 g of material

-

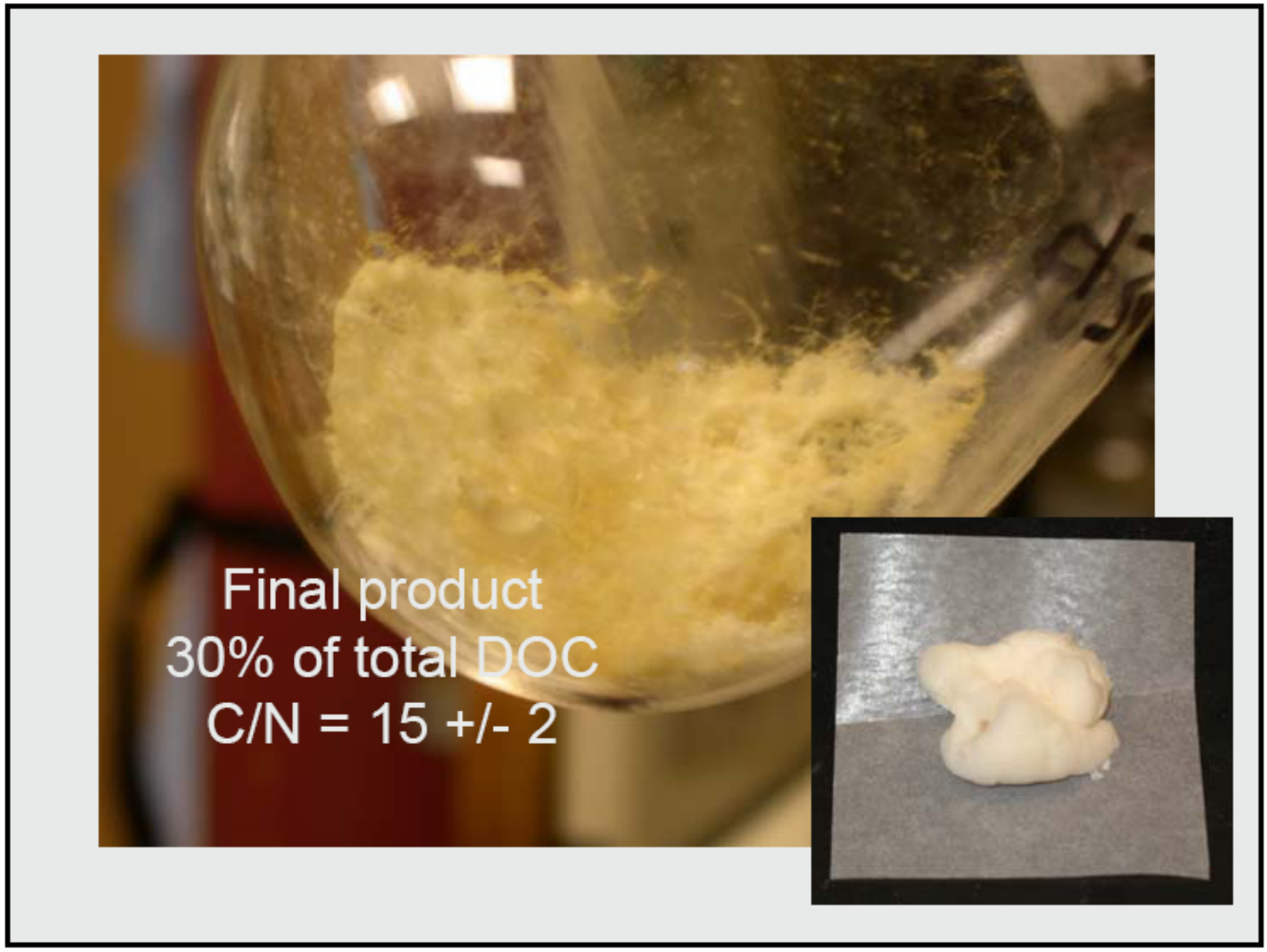
- Large vol. of samples required (>5000L)
- Only 25-30% DOC recovered

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

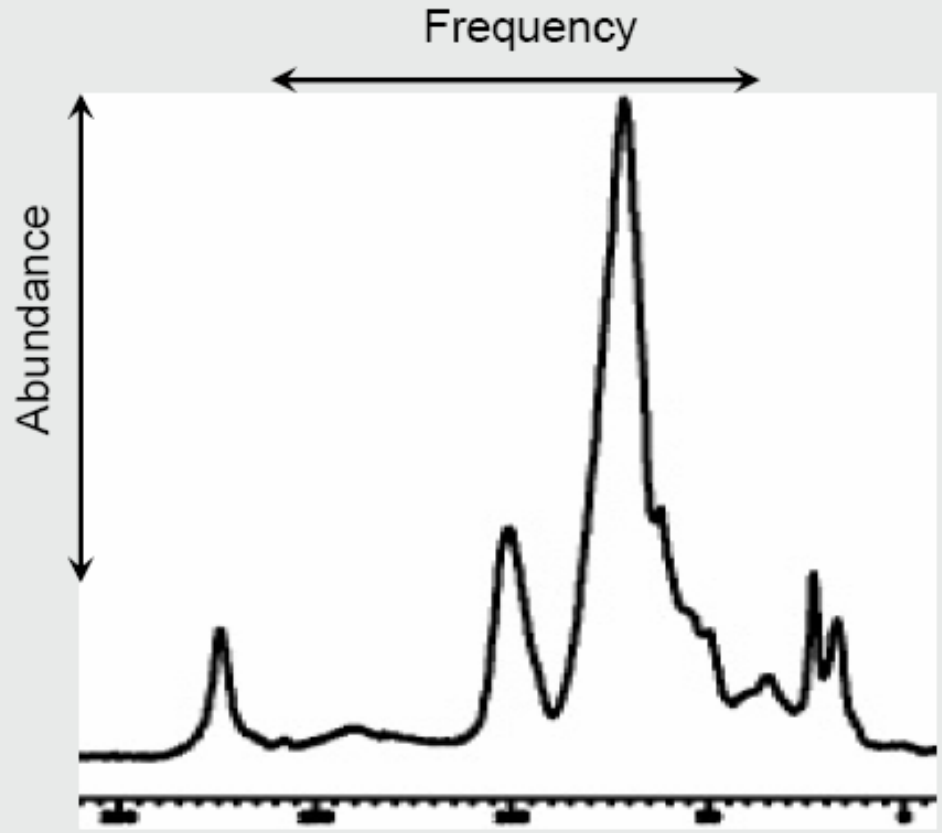
Ultrafiltration of 4000m NPSG water

HMWDOM concentrates from Hawaii

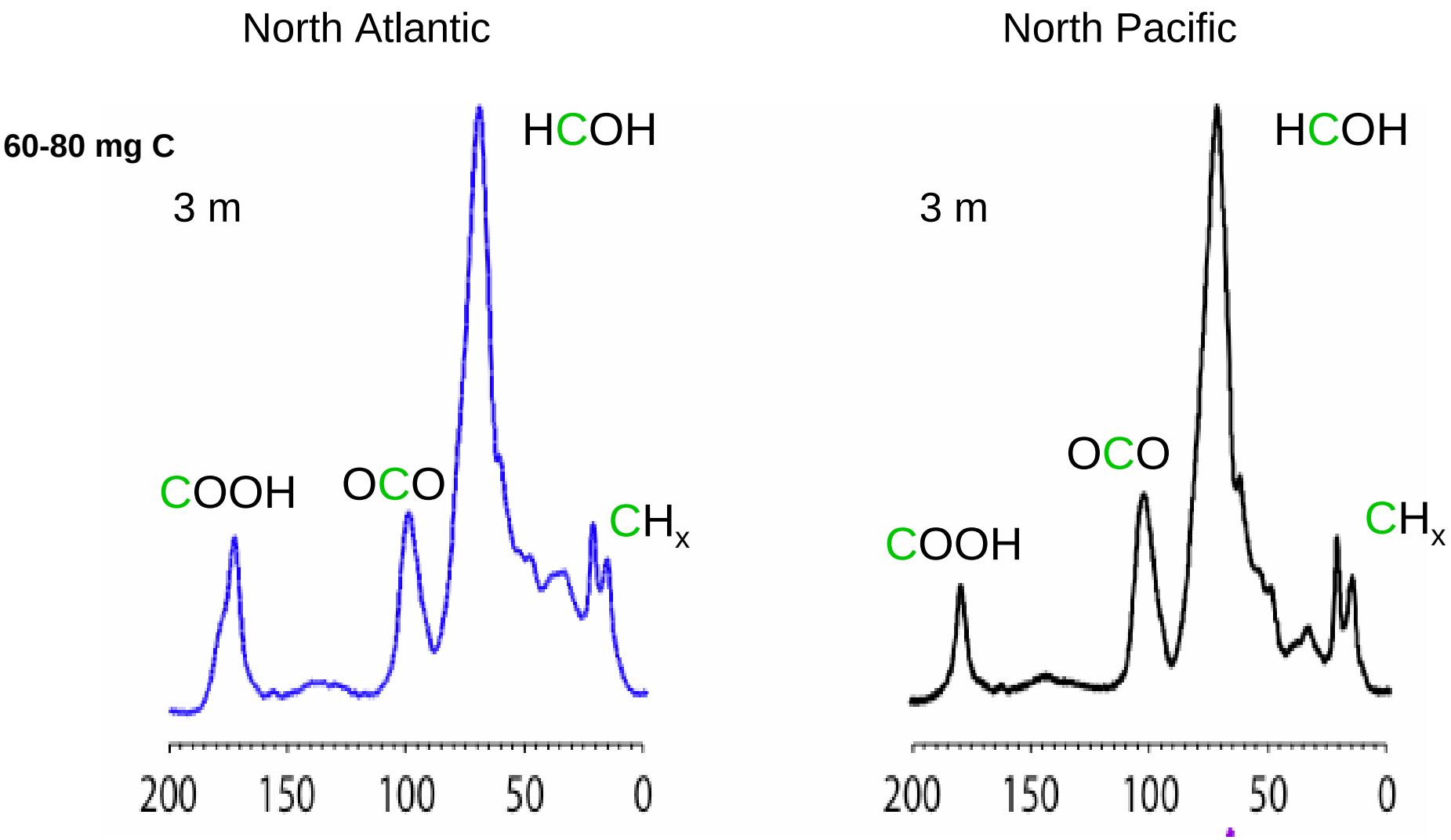
Freeze-dried sample



^{13}C Nuclear Magnetic Resonance Spectrum of
high molecular weight dissolved organic matter (HMWDOM)

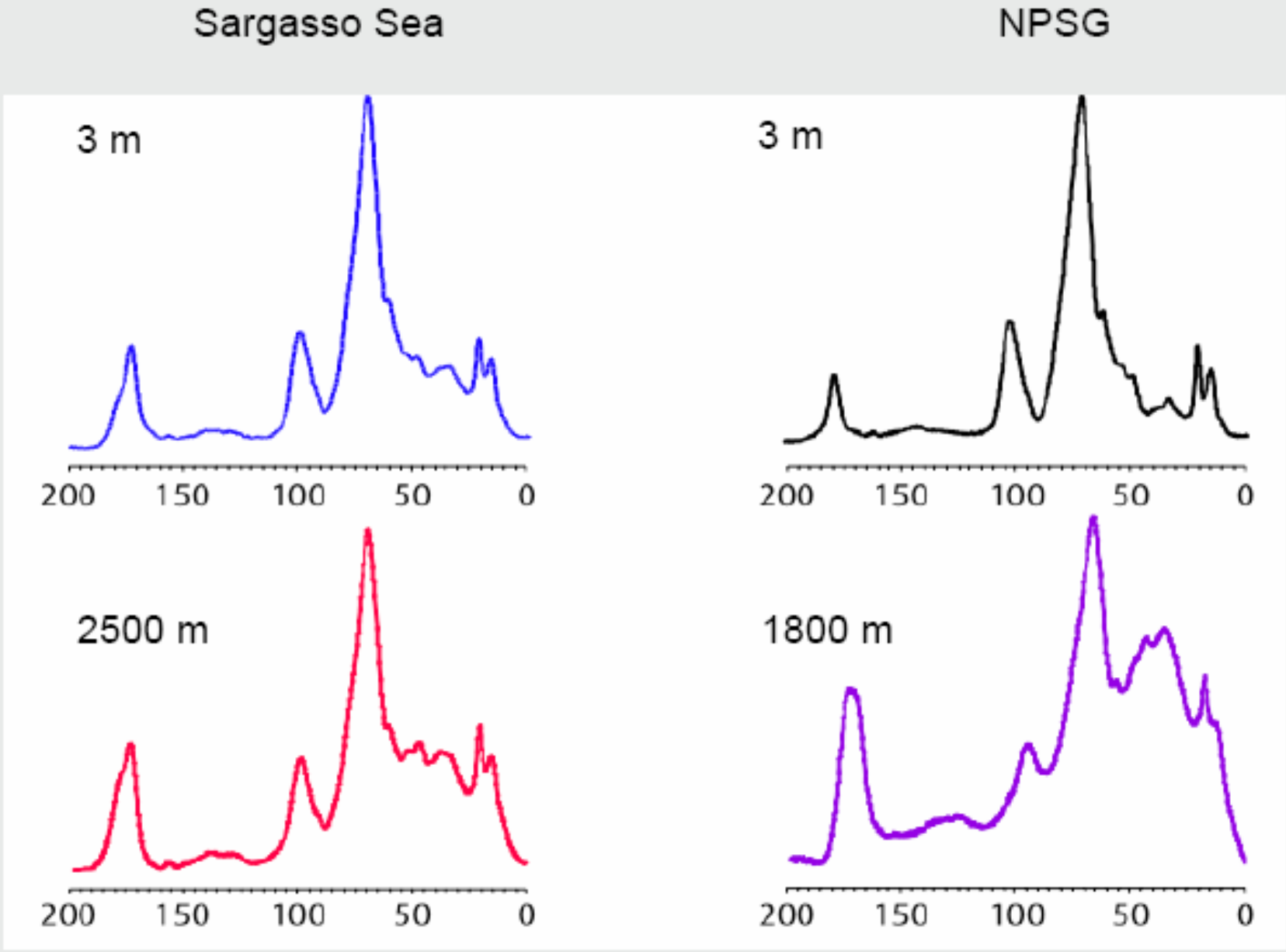


^{13}C **N**uclear **M**agnetic **R**esonance (NMR) spectra of HMWDOM



Benner et al., 1992; McCarthy et al., 1996, Aluwihare et al., 1997……

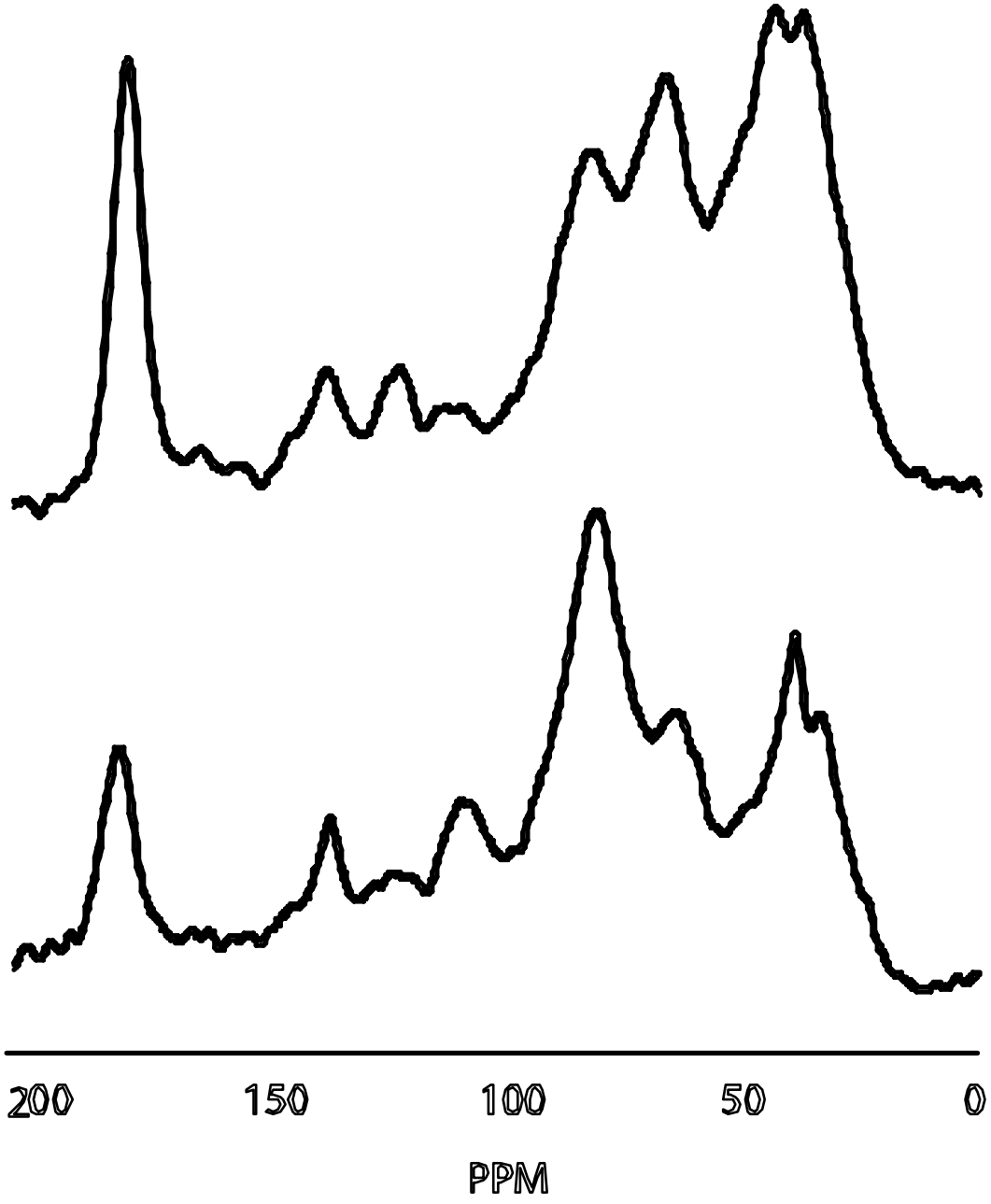
^{13}CNMR of HMWDOM in the deep sea



^{13}CNMR of marine phytoplankton HMWDOM

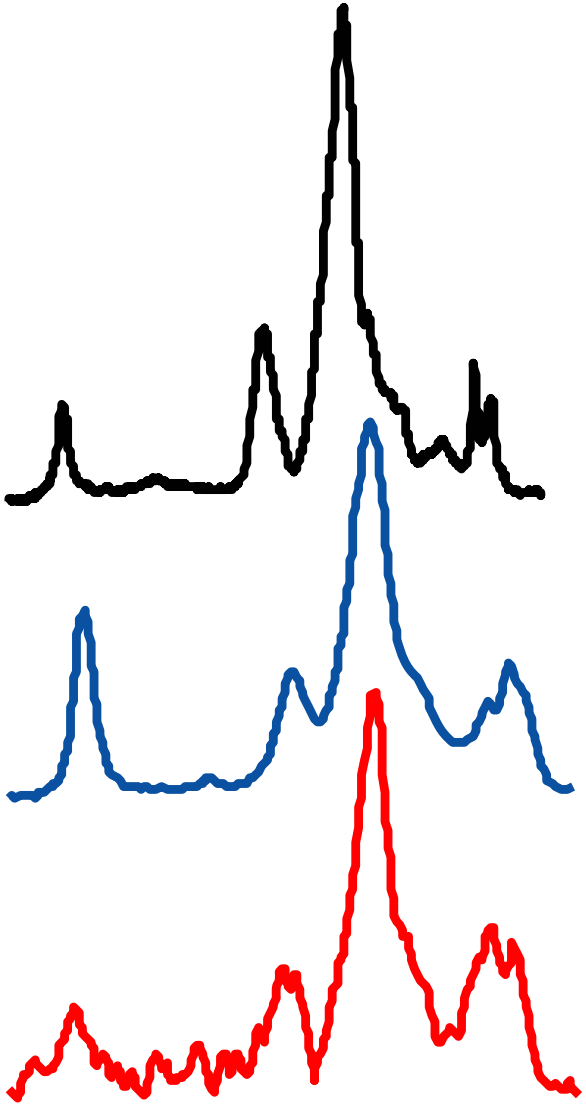
Fragilariopsis cylindrus

Phaeocystis antarctica



**Adina Paytan, 2005*

^{13}CNMR of HMWDOM in different aquatic environments



North Pacific Ocean

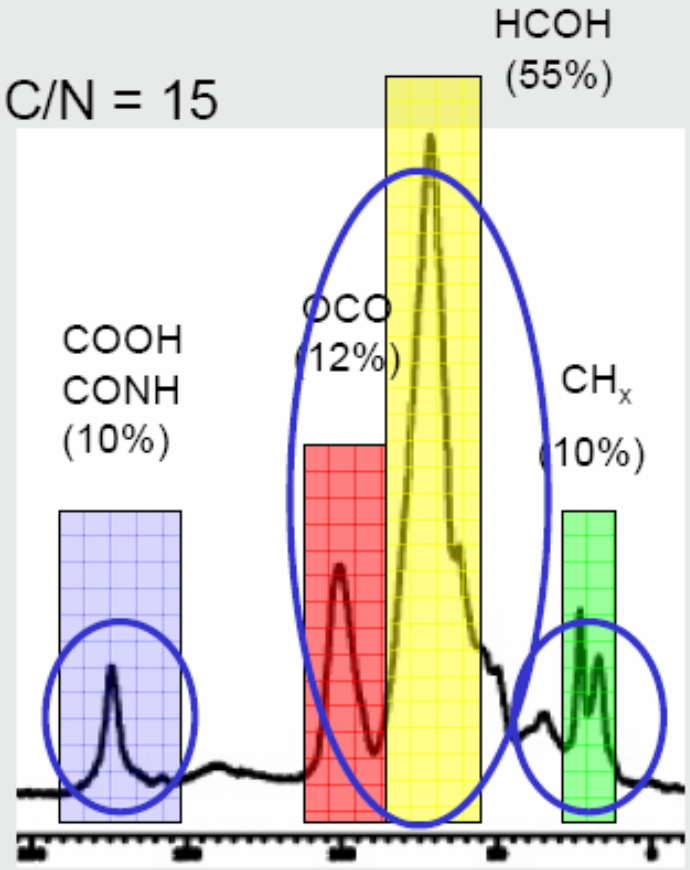
Great Salt Lake

Leenher et al., (2004) Biogeochem. 69, 125-141

Andrews Creek

McKnight et al. (1997) Biogeochem. 62, 99-124

^{13}C Nuclear Magnetic Resonance Spectrum of high molecular weight dissolved organic matter



Proteins
$CH_x(O)$:CON = 3:1
C/N = 4

Carbohydrates
OCO:HCOH=1:5
C and maybe N

Lipids
CH_x:COOH=2-18
CH_x:COH = 2-30
C only

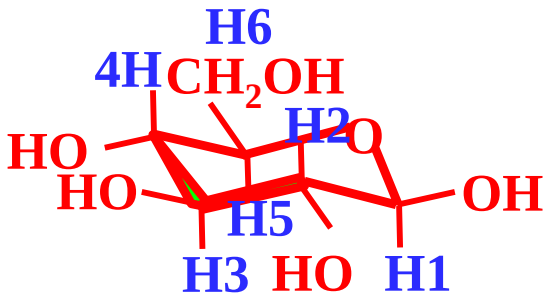
HMWDOM composition by ^{1}HNMR

Carbohydrate (50-70%)

Alkyl (5%)

Acetate (5%)

Proteins + Humics (20-30%)



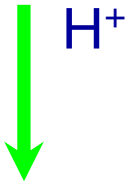
Acylpolysaccharides (APS)

Aluwihare al. (1997) Nature

50-70% of HMWDOM is carbohydrate

But...

HMWDOM



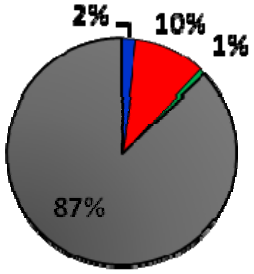
5-20% C (chromatography
HPLC, GC)

Most of the carbohydrate is still unidentified !

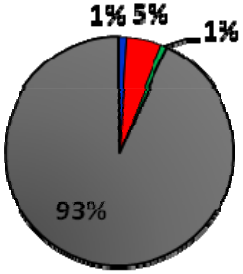
Comparison between DOM and HMWDOM (>1000 Da)

Epipel. zone (0-400 m) | **Mesopel. zone (400-1000 m)** | **Bathypel. zone (1000-4000 m)**

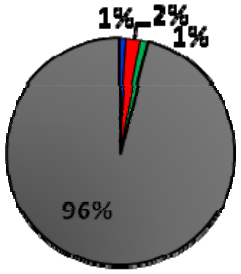
DOM

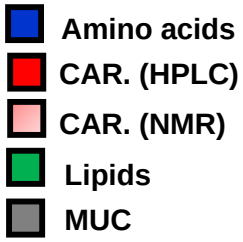


$\delta^{13}C$=-21.3‰
$\Delta^{14}C$= -191 to -238‰
(1700-2180 y)



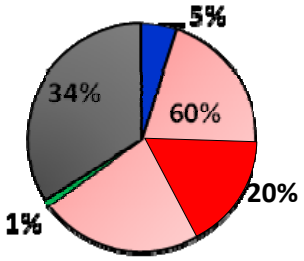
$\delta^{13}C$=-21.0‰
$\Delta^{14}C$=-375 to -470‰
(3780-5100 y)



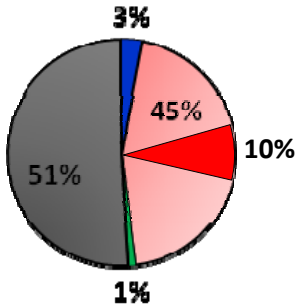


$\delta^{13}C$=-21.2‰
$\Delta^{14}C$=-378 to -533‰
(3810-6120 y)

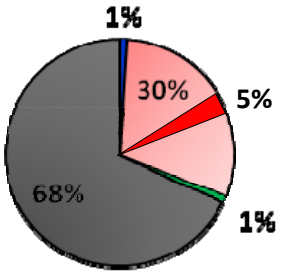
HMWDOM



$\delta^{13}C$=-21.6‰
$\Delta^{14}C$= -5 to 46‰
(modern)



$\delta^{13}C$=-21.6‰
$\Delta^{14}C$=-270 to -381‰
(2530-3850 y)



$\delta^{13}C$=-21.3‰
$\Delta^{14}C$=-262 to -434‰
(2440-4579 y)

Sempéré et al. submitted

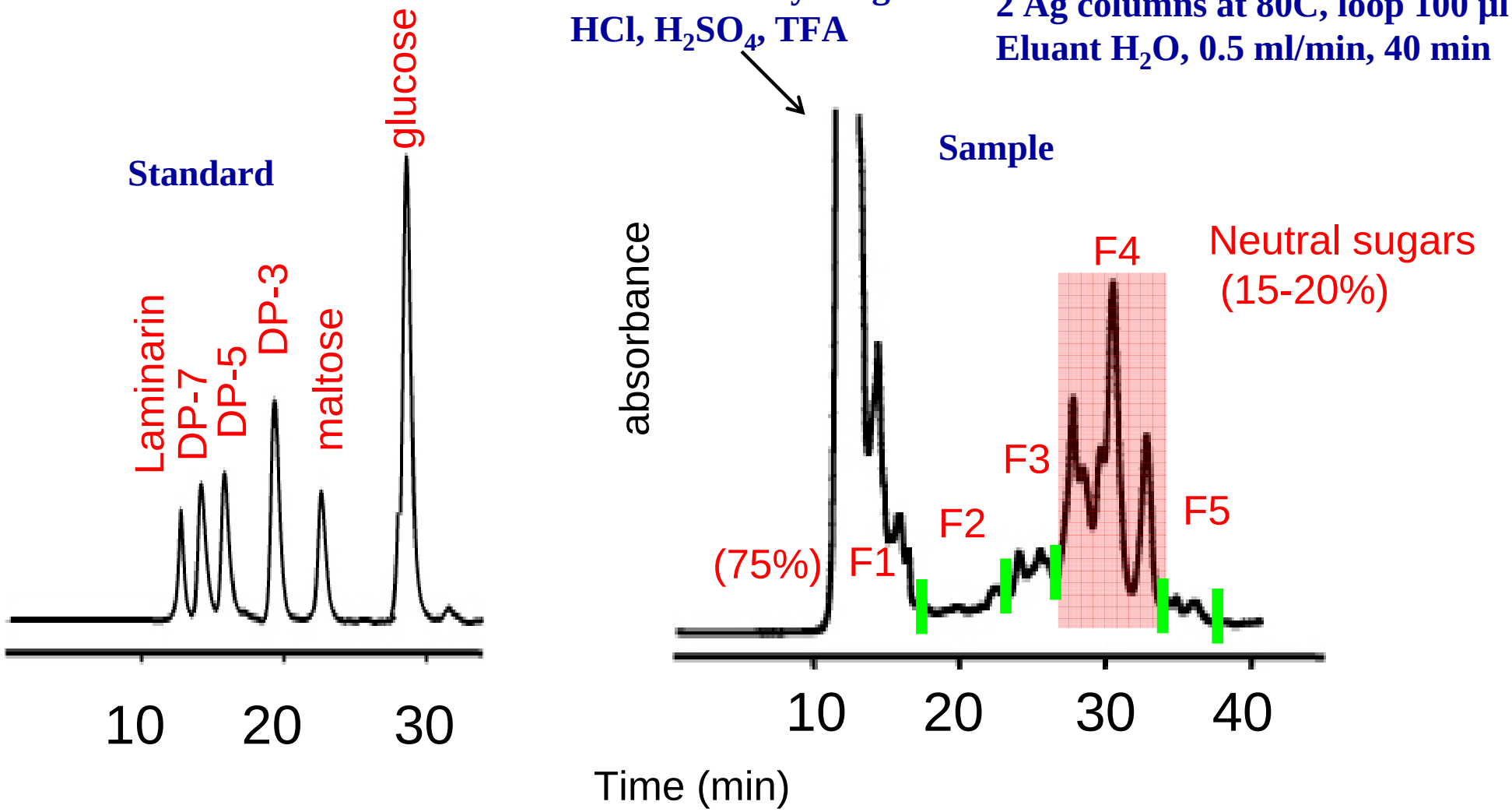
Separation of mono- and oligosaccharides after HMWDOM hydrolysis using ion chromatography

Surface Hawaii sample

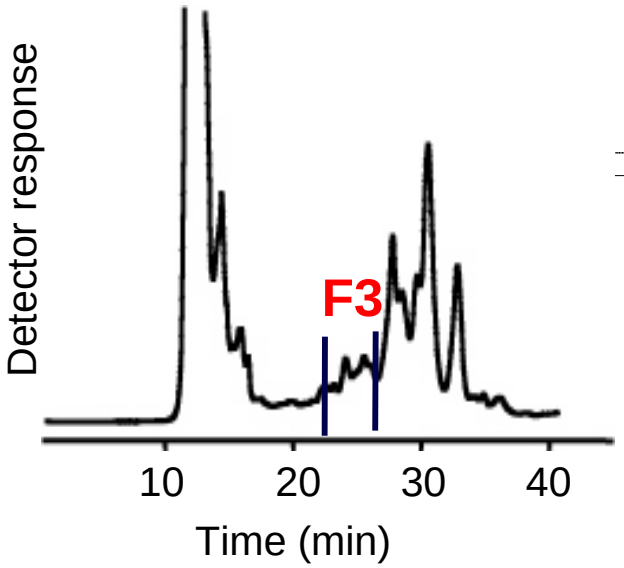
Unhydrolyzed sugar polymer.
WE TRIED everything
HCl, H_2SO_4, TFA

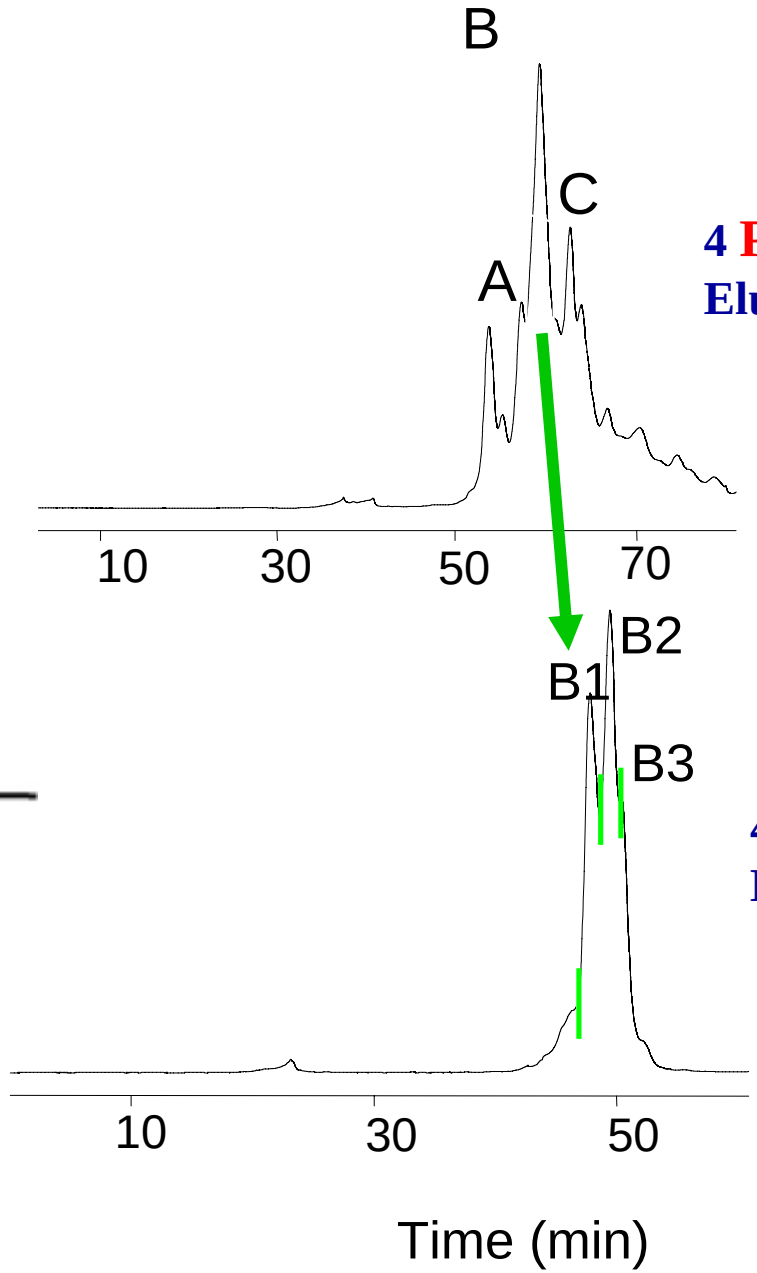
HPLC-RI conditions

2 Ag columns at 80C, loop 100 µl
Eluant H_2O, 0.5 ml/min, 40 min



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





HPLC-RI conditions

4 Pb columns at 80C, loop 100 µl

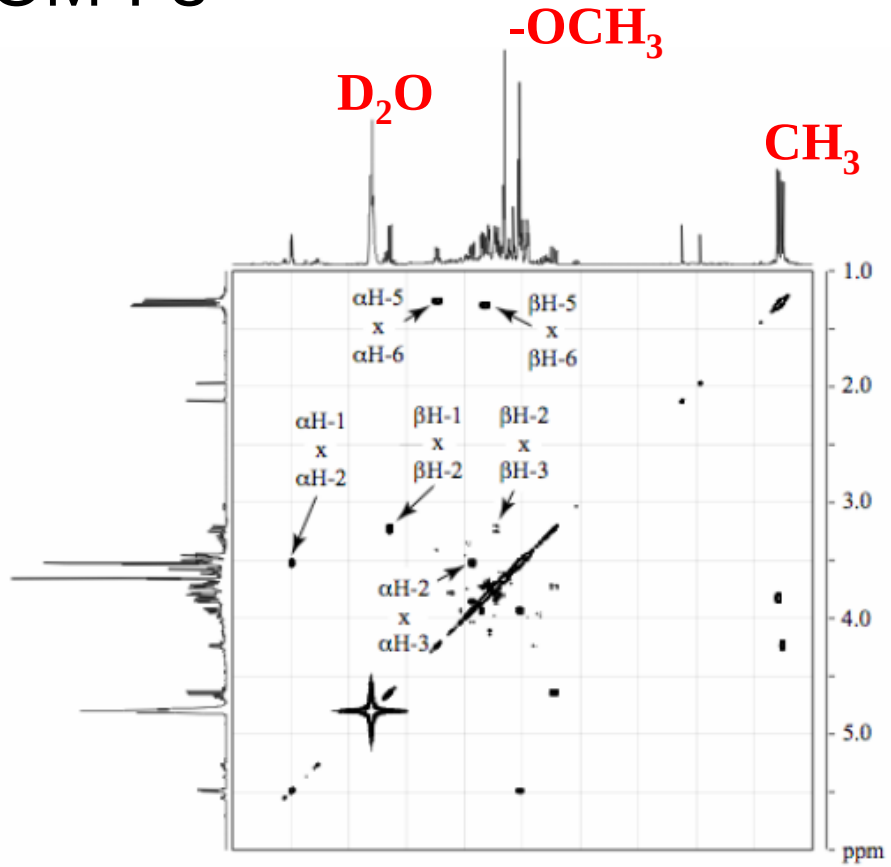
Eluant H_2O, 0.5 ml/min

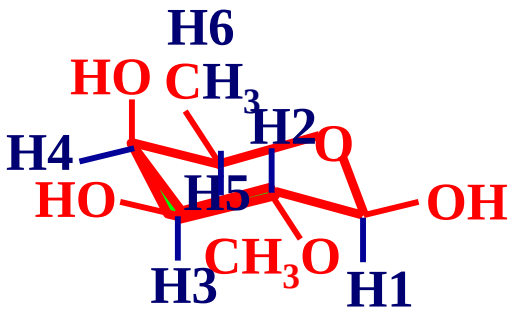
HPLC-RI conditions

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

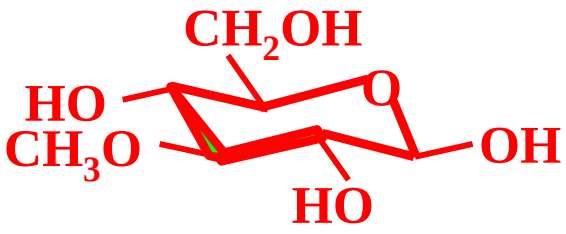




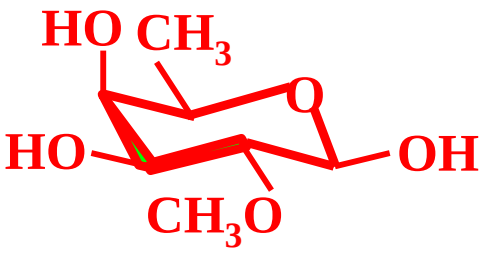
2-OMe fucose = B2 compound

Panagiotopoulos et al (2007)

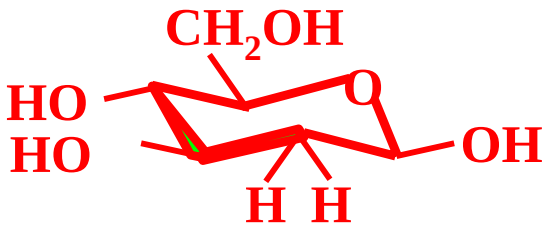
Identification of novel sugar compounds by 2D NMR in HMWDOM-F3



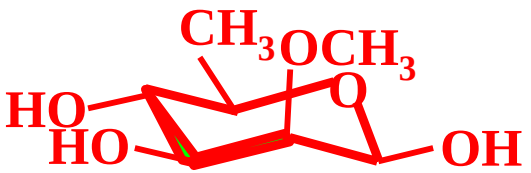
3-OMe glucose



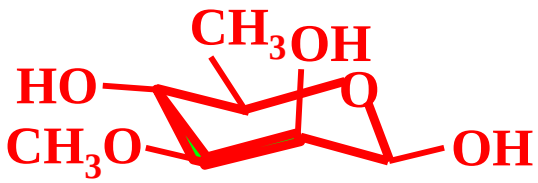
2-OMe fucose



3-deoxy glucose



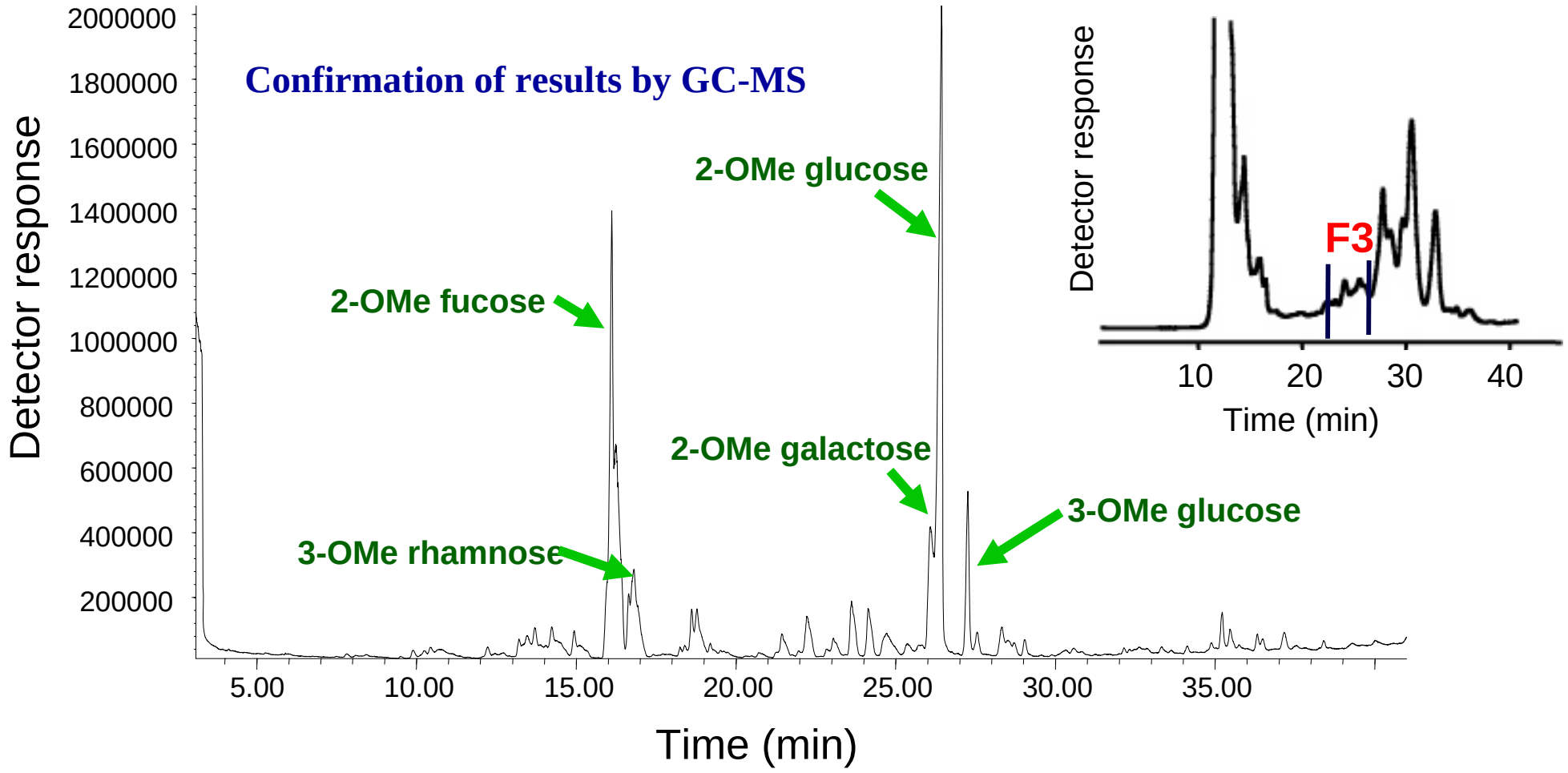
2-OMe rhamnose



3-OMe rhamnose

Panagiotopoulos et al (2007)

GC-MS alditols acetates derivatives in the HMWDOM-F3

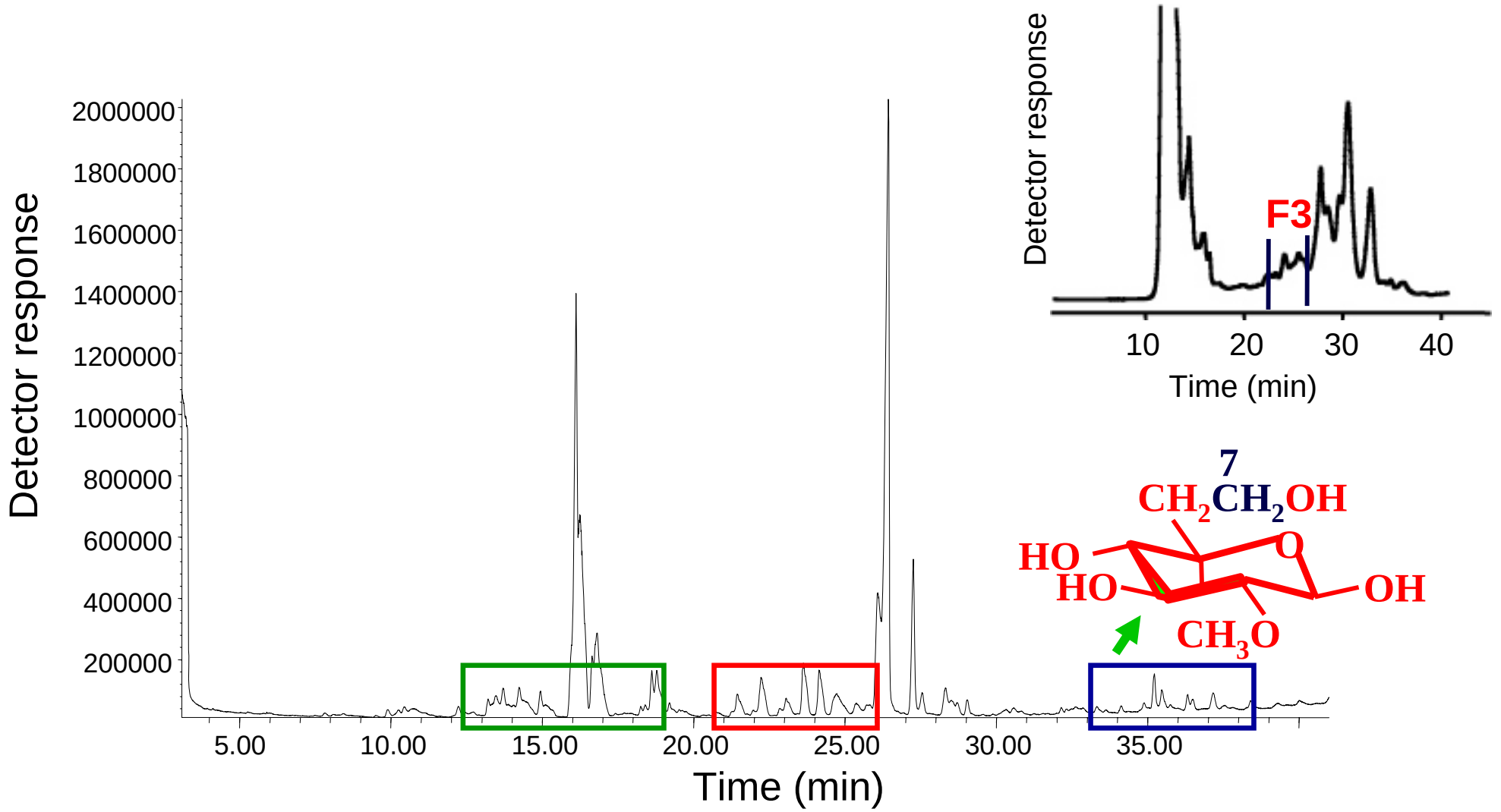


MS can not say the difference between epimers (i.e. glucose, galactose, mannose)

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

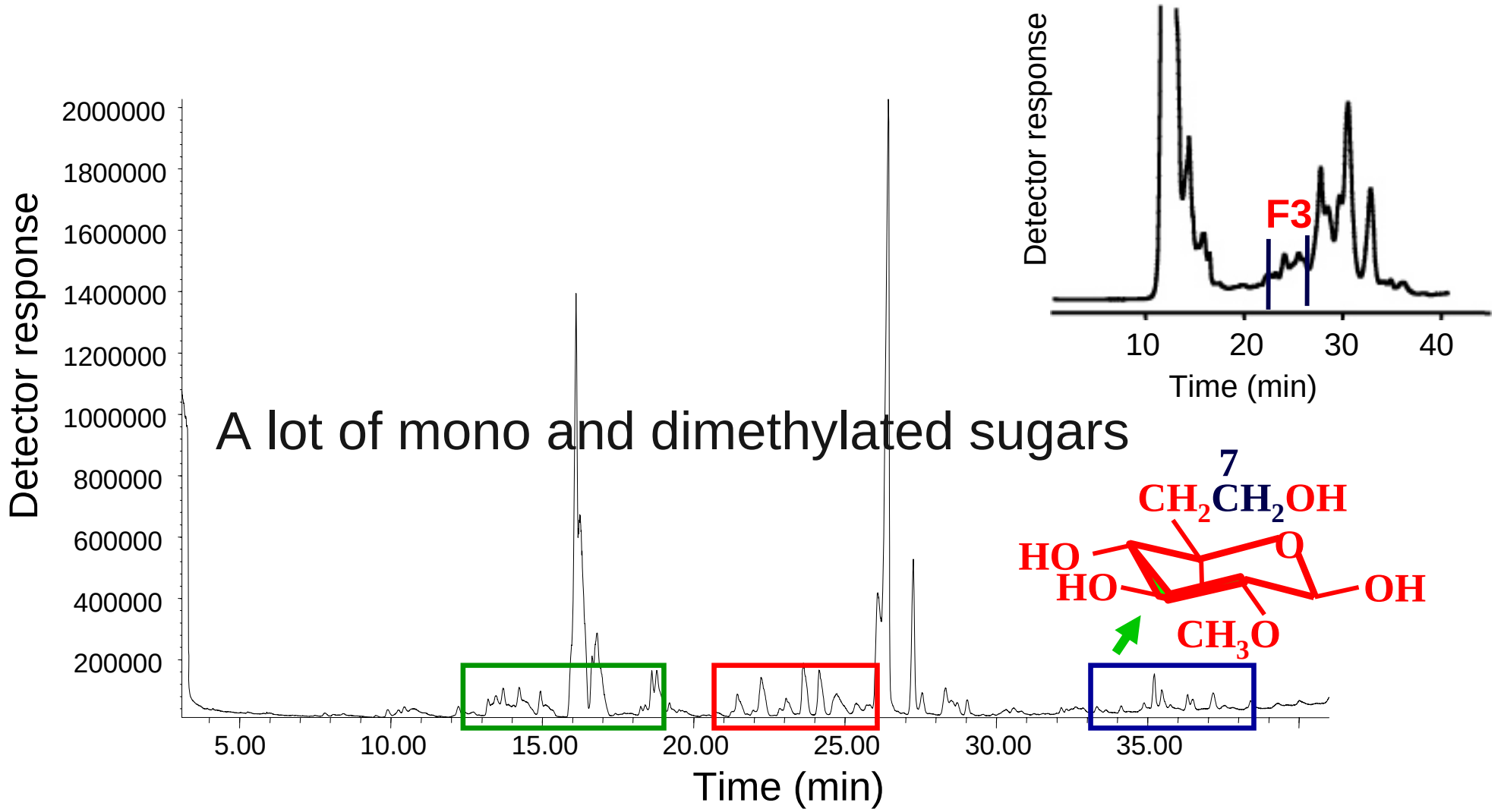


3,6-dideoxyhexose; 2,3-di-O-methylrhmnose; 2,4-di-O-methylrhamnose; 2-O-methyl-pentose

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

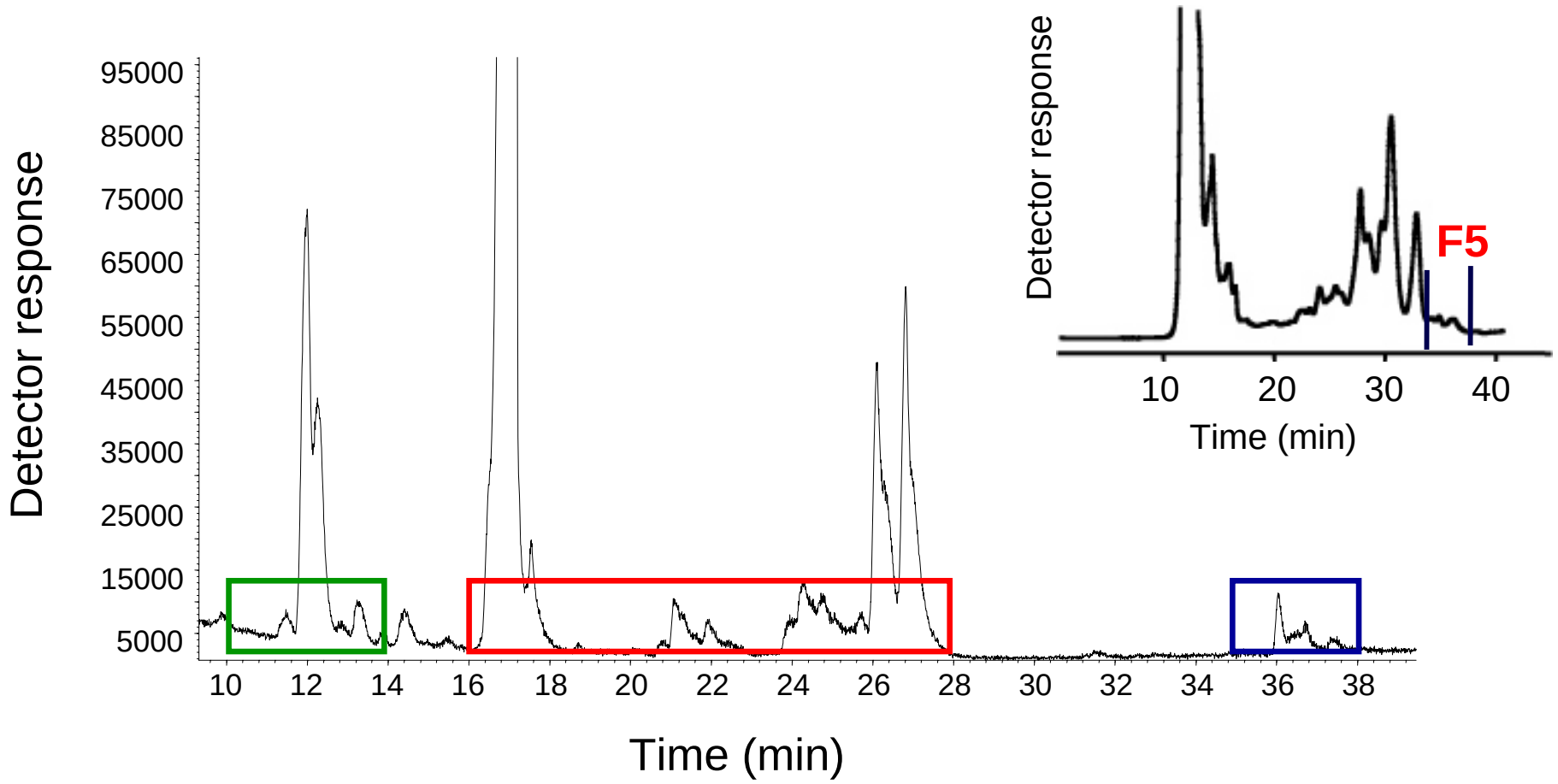


3,6-dideoxyhexose; 2,3-di-O-methylrhmnose; 2,4-di-O-methylrhamnose; 2-O-methyl-pentose

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



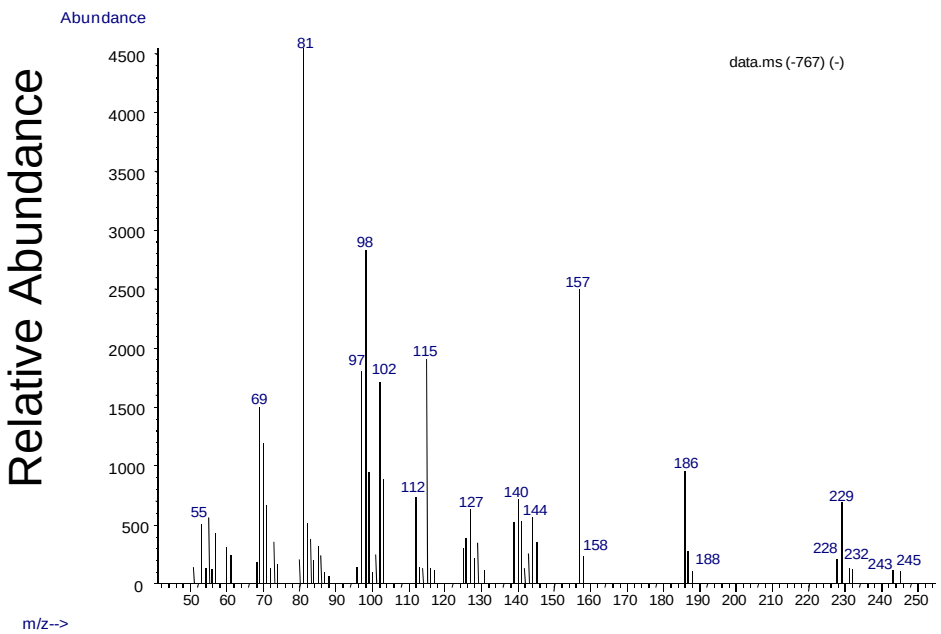
1,6 anhydro hexoses (i.e levoglucosan, galactoglucosan, mannoglucosan etc)

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

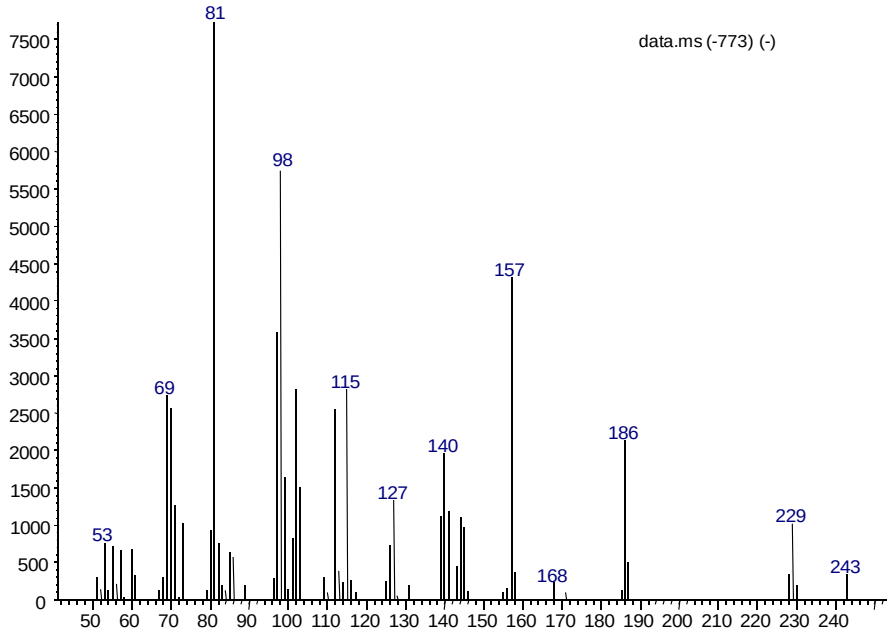
Heptoses

Levoglucosan mass spectra

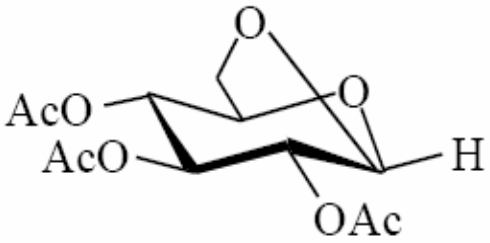
NaBH4 treatment



No NaBH4 treatment

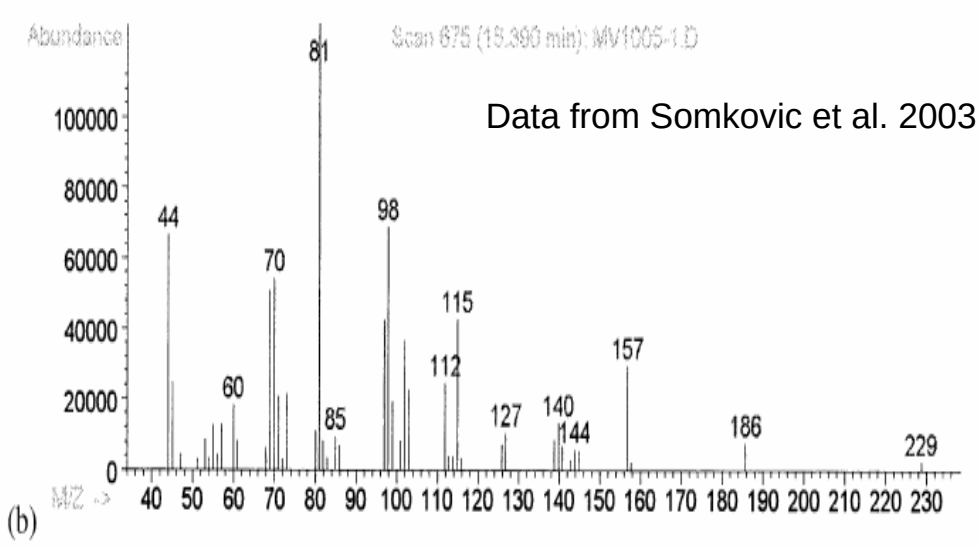


m/z



Levoglucosan

Hexose ring does not open



Data from Somkovic et al. 2003

Biogeochemical importance

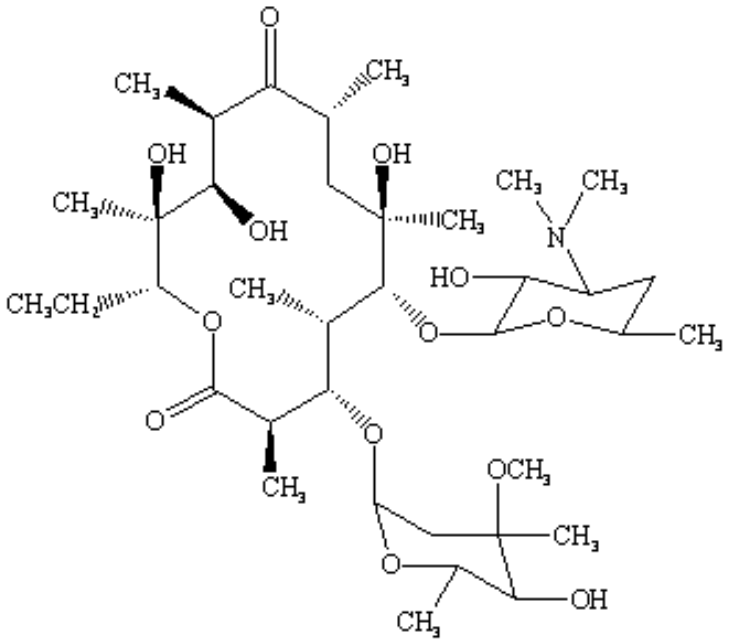
A. Methylated, dimethylated hexoses have been found in bacterial and algal cell walls as part of structural polysaccharides, however their chemical structure and function is poorly understood.
Are these compounds contributors to the refractory DOM ?

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 ?

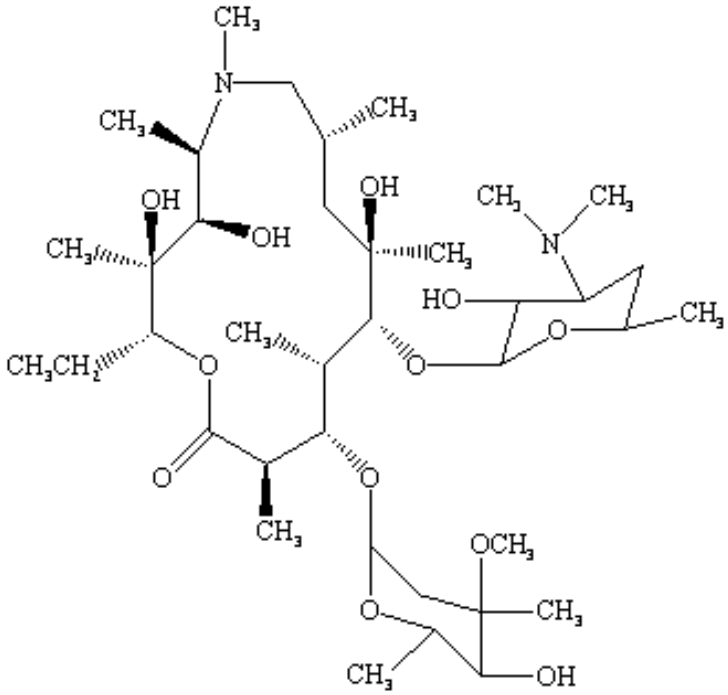
C. Levoglucosan is a component of atmospheric smoke particles derived from wood burning (cellulose degradation product) and this is the FIRST time that has been found in DOM in tiny amounts.
Does black carbon (part of the uncharacterizable DOM carbon) enter the ocean via atmospheric deposition ? (this 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

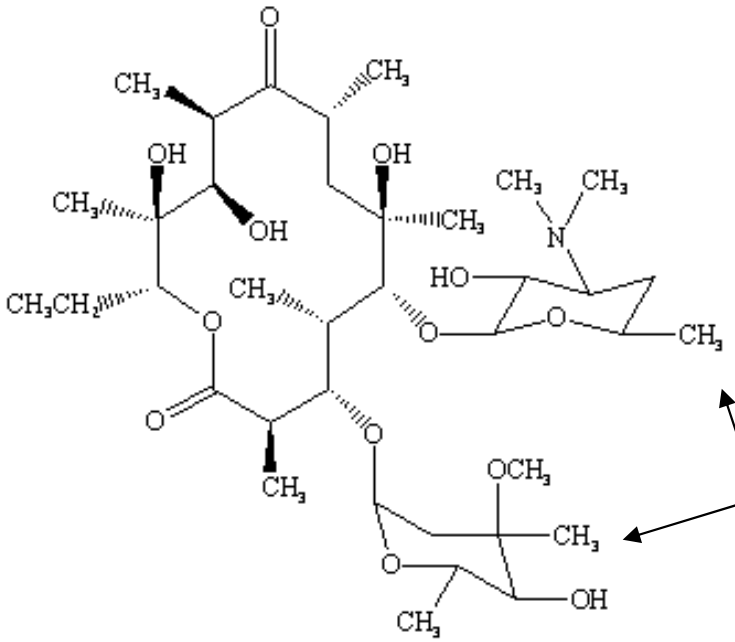


Erythromycin

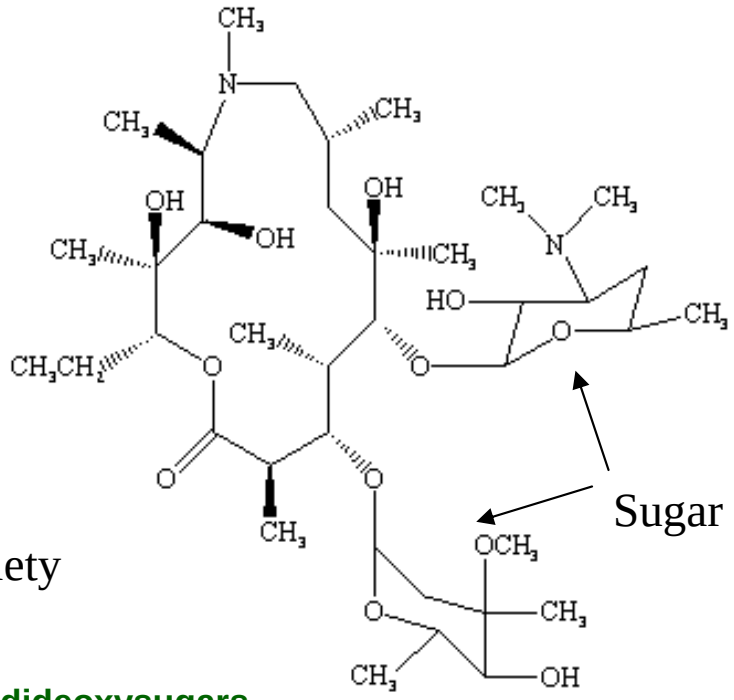


Azythromycin

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)

Is APS important in carbon cycling ?

There are @ 700 GT DOC in the ocean

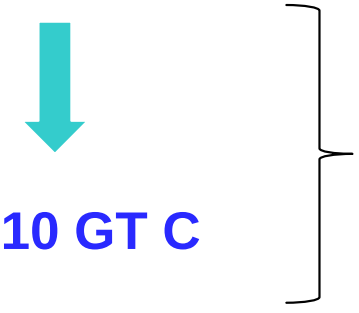
@ 60 GT DOC in the euphotic zone

15-35% recovered by UF

of which @ 20-25 GT is HMWDOC

60-70% are carbohydrates

….and @ 10-15 GT C is APS



POC = 7-10 GT C


Merci de votre attention