Lecture 2

- The biomarker concept
- Acetogenic lipids- most common form of sedimentary lipid
  - Fatty acids and derivative lipids
  - Fatty alcohols and wax esters (bacteria, algae, zooplankton, insects)
  - Non-isoprenoid alkanes, n-alkanes, branched alkanes (C_{15}-C_{20} Cb, algae, leaf wax, bacterial wax?)
    - Cyanobacterial hydrocarbons
  - Polymethylenic biopolymers (algeanans marine and non-marine microalgae; cutans)

References

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Please see the book cover of
"An Introduction to Organic Geochemistry".

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Please see the book cover of Gaines, S. M., G. Eglinton, J., Rullkötter. "Echoes of Life" In *Echoes of life: What Fossil M oleculesReveal About Earth History*. Oxford University Press, New York.2009. New York.

Introduction To Organic Geochemistry 2nd Edition Killops S and Killops V

An Introduction to Organic Geochemistry explores the fate of organic matter of all types, biogenic and man-made, in the Earth System.

The global carbon cycle and related elemental cycles.
The influence of the evolution of life on the carbon cycle.
Production and chemical composition of biogenic matter.
Degradation vs. preservation of sedimentary organic matter in various environments.
Biological and thermal alteration in sediment, soil and water column.
Molecular and isotopic stratigraphy.
Greenhouse gases and palaeoclimatic variation.
Man's influence on biogeochemical cycles and global climate change.
Factors affecting the behaviour of pollutants in the environment.

References

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Please see the book cover of "The Biomarker Guide: Volume 1".

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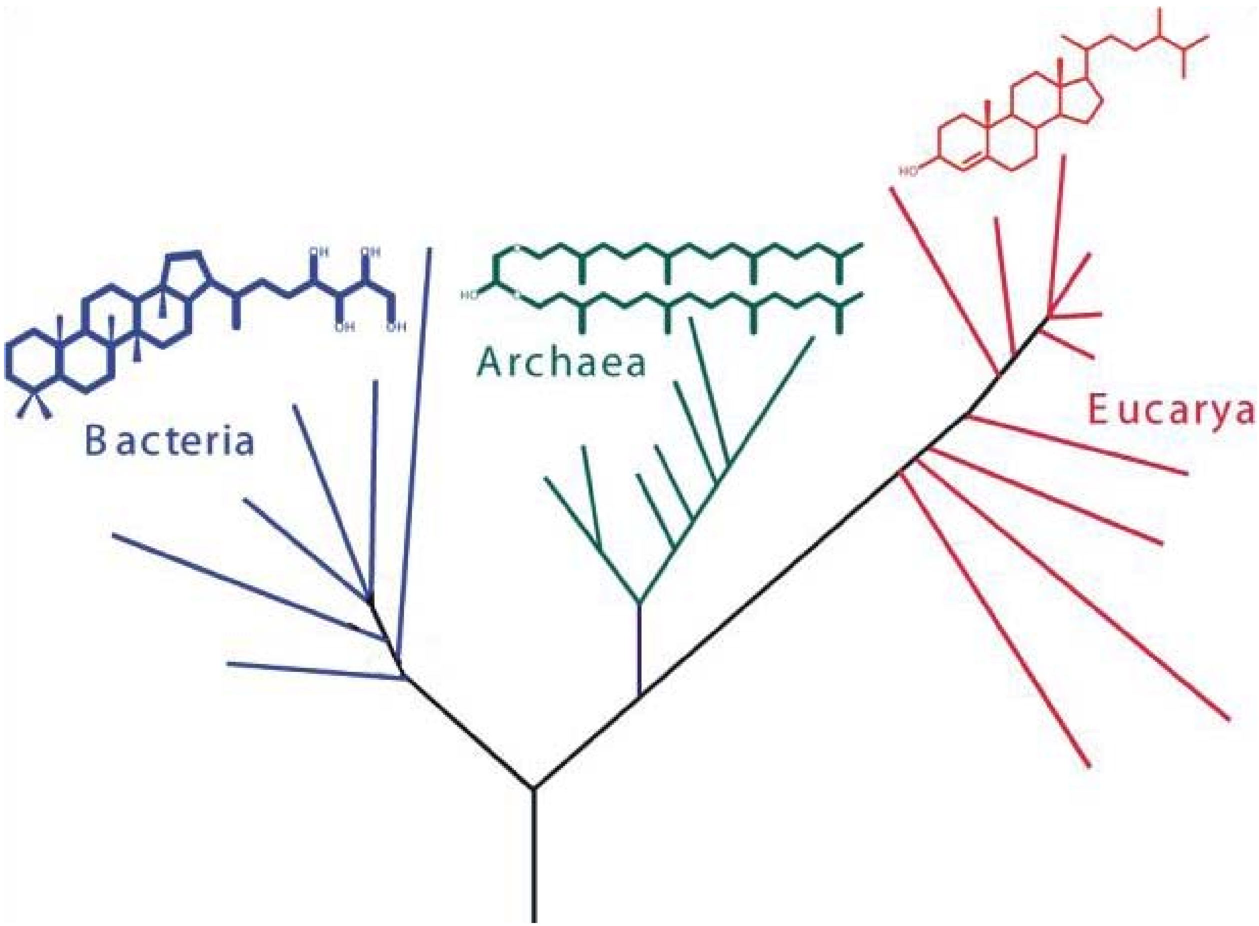
Please see the book cover of "The Biomarker Guide: Volume 2".

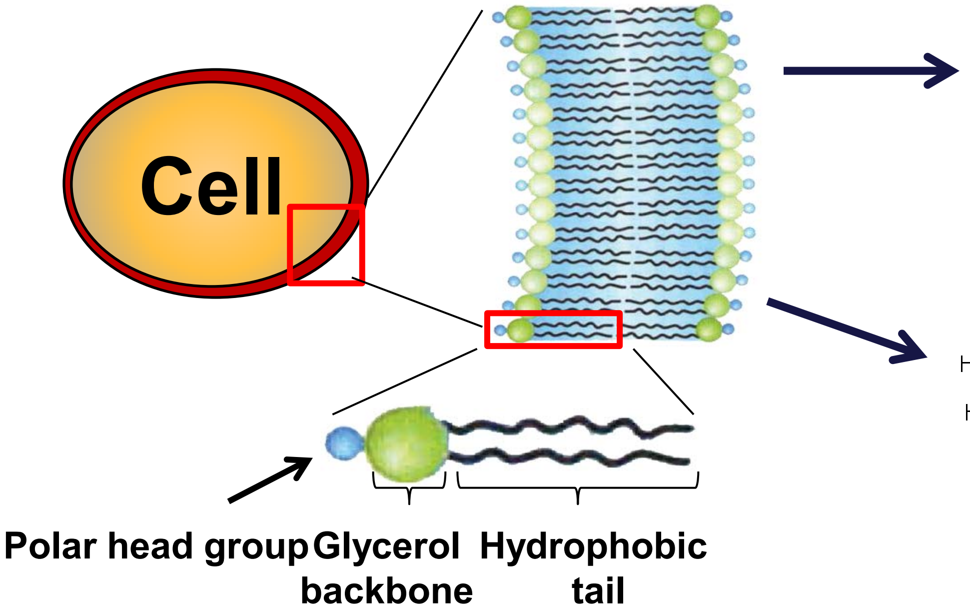
Biomarkers (chemical/molecular fossils)



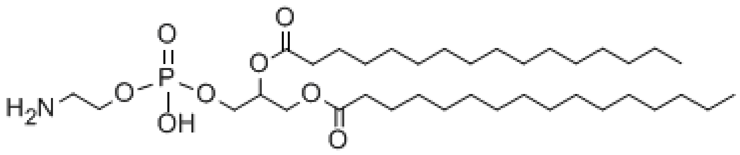
Biomarkers can be related to specific biological sources and can provide environmental or age information

Lipid biomarkers

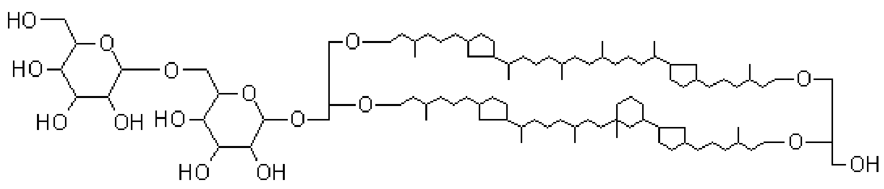
Bi- or monolayer structure



Bacteria/Eukarya



Archaea



- Membrane constituents (e.g. steroids, hopanoids)
- Energy sources (e.g. triacylglycerols of fatty acids)
- Protective coatings (e.g. waxes on leaves, fruit)
- and others……

Cycling of biomarkers in the environment

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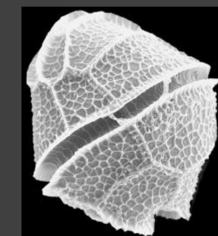
Diagenetic reactions of biomarkers in sediments

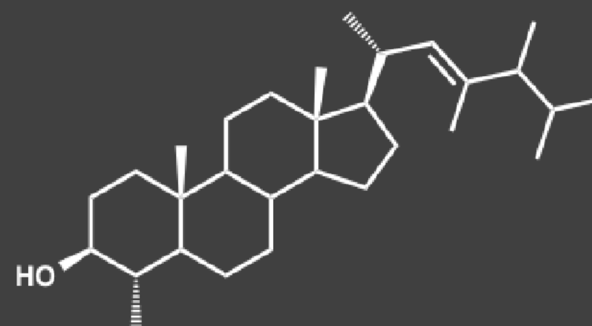
1. Oxidation/mineralization
2. Sulfurisation
3. Aromatization
4. Defunctionalisation
5. Isomerization
6. Catagenesis (e.g., C-C bond cleavage)

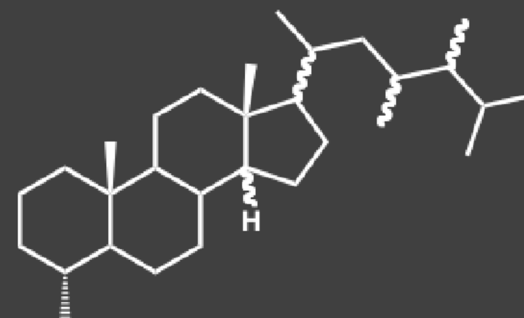
The biomarker concept: source

T-Rex

biolipid: Dinosterol







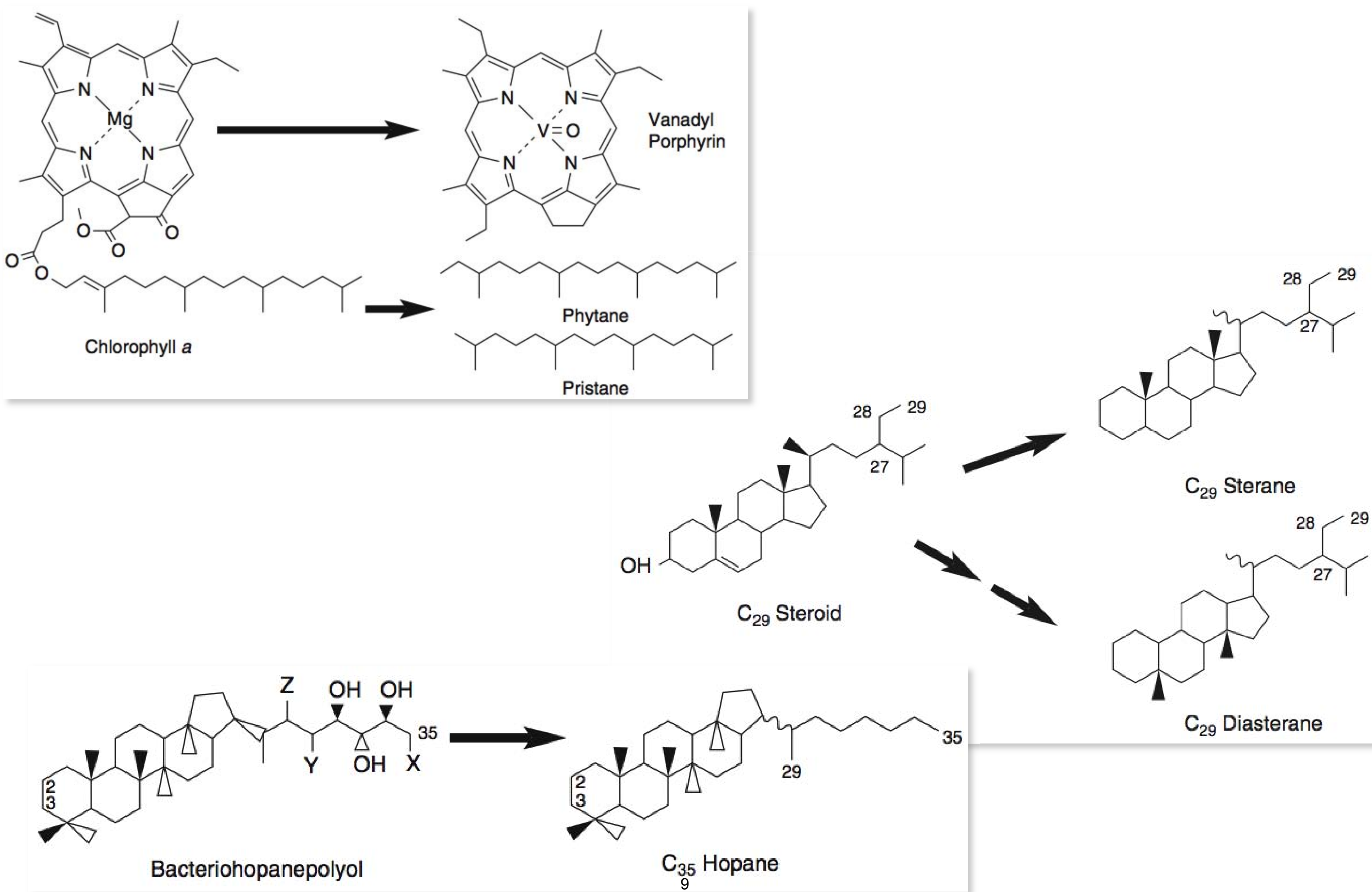
molecular fossil: Dinosterane

Increasing burial (age)

Dia- and Catagenesis

Increasing loss of information

The biomarker concept



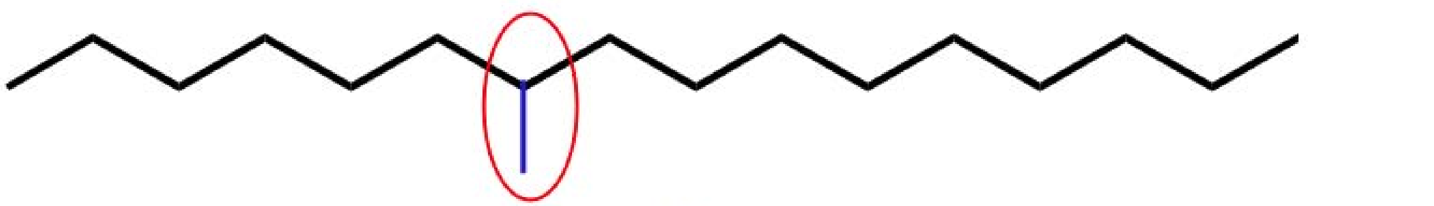
Information content of biomarkers

1. Carbon skeleton
2. Type and position of functional groups
3. ^{13}C content
4. ^{14}C content
5. Other isotope content (e.g., D, ^{15}N, ^{34}S)

Carbon skeleton information of biomarkers: Species specific biosynthesis

$C_{17}H_{36}$

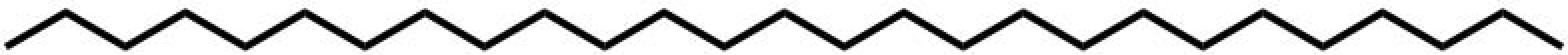
Specific algae



$C_{17}H_{36}$

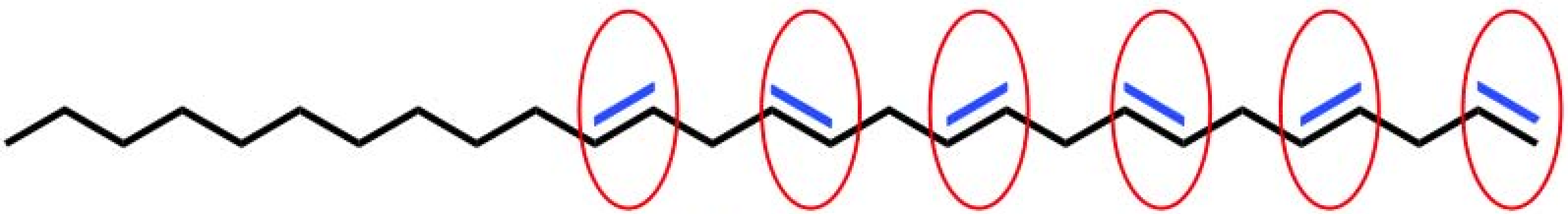
Cyanobacteria

Functional group information of biomarkers: Species specific biosynthesis



$C_{27}H_{56}$

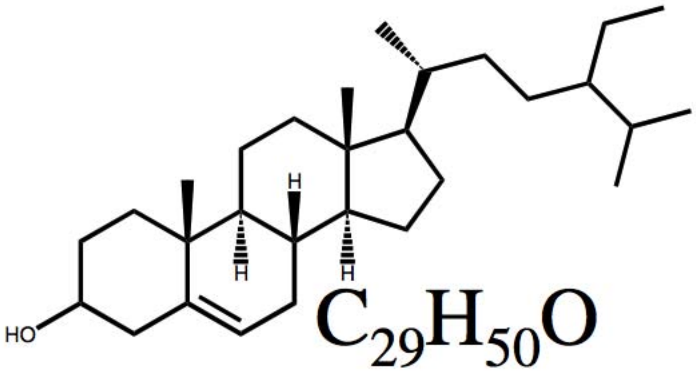
Terrestrial higher plants



$C_{27}H_{44}$

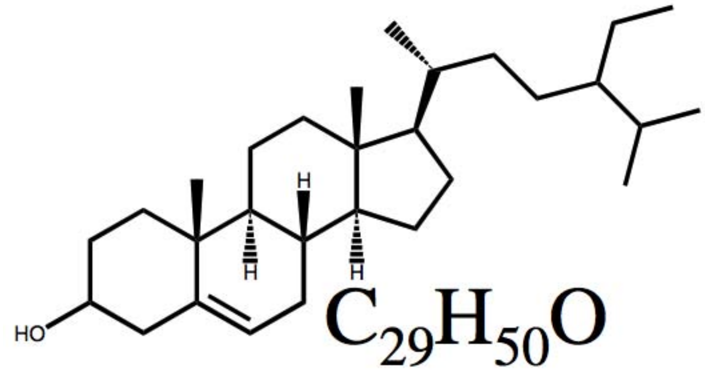
Rhizosolenia setigera (a marine diatom)

Stable carbon isotopic information of biomarkers



Terrestrial higher plants

$\delta^{13}C$ = -31‰



Marine algae

$\delta^{13}C$ = -22‰

Largely controlled by:
Species specific biosynthesis
Many environmental factors
Carbon source

Radiocarbon content of biomarkers: Age

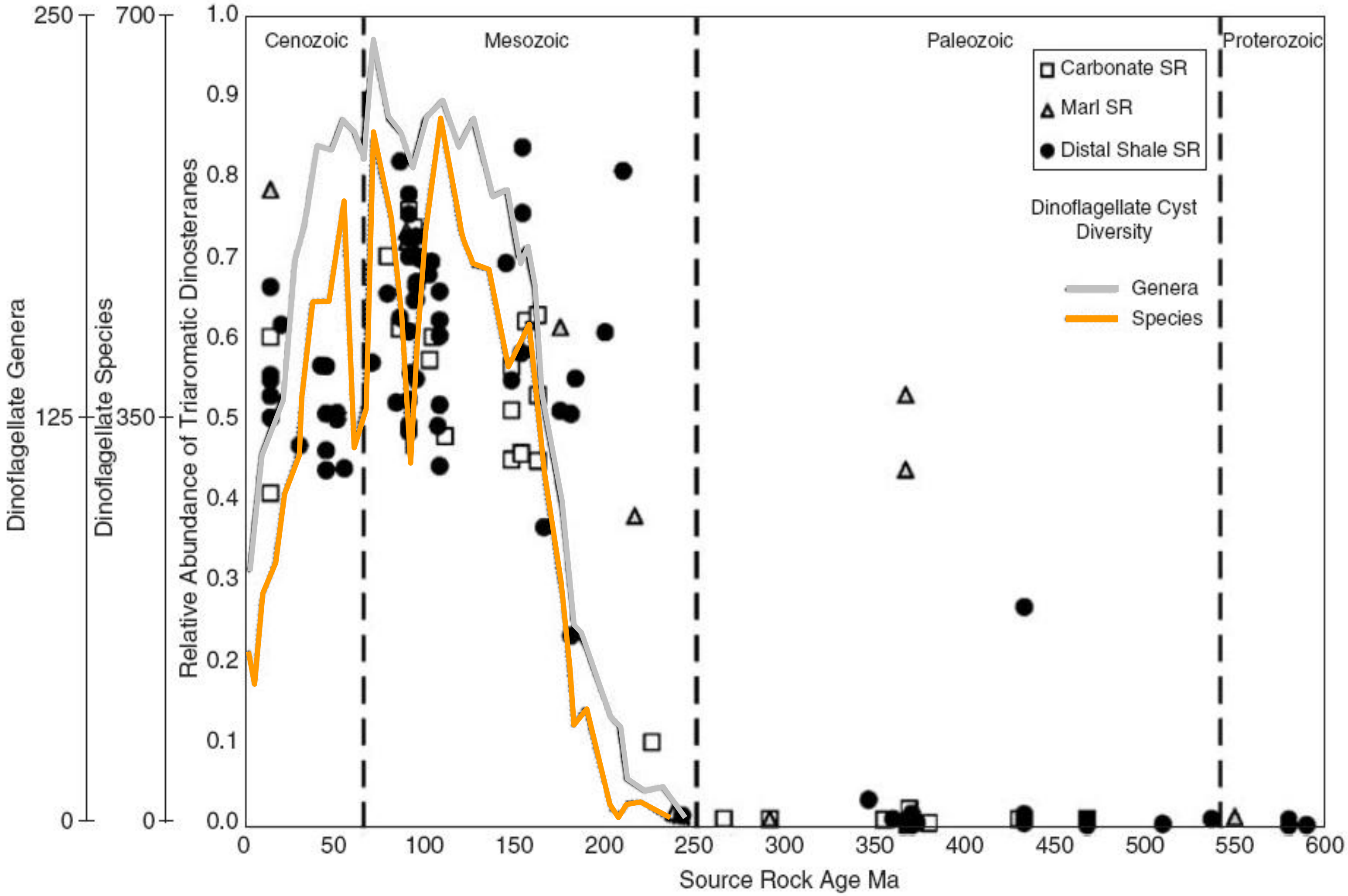
$C_{27}H_{56}$

Terrestrial higher plants
$\delta^{14}C$ = -50‰ (ca.

$C_{27}H_{56}$

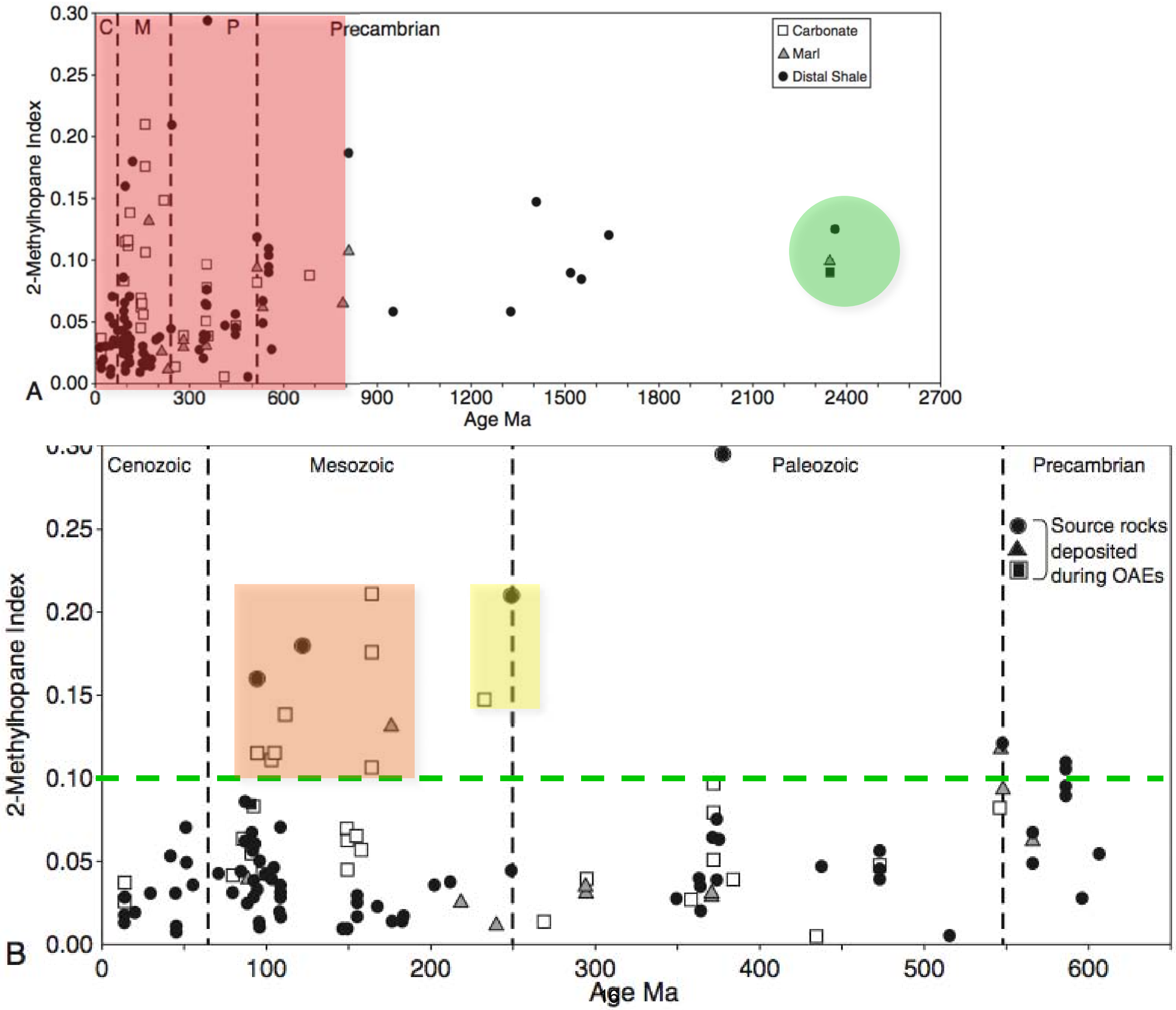
Petroleum
$\delta^{13}C$ = -1000‰ (radiocarbon dead)

Biomarkers & phytoplankton evolution



After Knoll et al. (2007)

Biomarkers for cyanobacteria



After Knoll *et al.* (2007)

Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E and K), monoglycerides, diglycerides, phospholipids, and others. The main biological functions of lipids include energy storage, as structural components of cell membranes, and as important signaling molecules.

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Please see: http://en.wikipedia.org/wiki/Lipid

- Lipids have high H/C ratio
- Are rich in energy (petroleum & natural gas)
- Often stable to microbial decay
- Often stable at high temp and pressures
- Carry informative isotopic signals for C, H, sometimes N, O and S

Acetogenic Lipids

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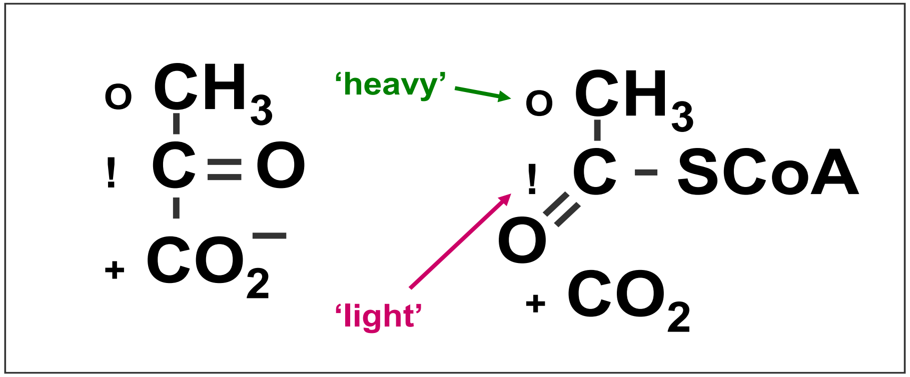
Please see:

http://www.cyberlipid.org/cyberlip/desc0004.htm#top

Acetogenic Lipids

n-Alkyl lipids are essentially polymers of acetate – acetogenic lipids



The acetate building block is Acetyl Coenzyme-A which is formed from pyruvate

Acetate Methyl-C and Carboxyl-C are isotopically distinct and determined by its metabolic source and the profound isotope effect of pyruvate dehydrogenase

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Acetogenic Lipids

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Please see Figure 26 on
http://rimg.geoscienceworld.org/cgi/content/full/43/1/225.

Additional carbons from amino acid „starters" and S-adenosylmethionine (SAM)

Diagnostic unsaturation present in biological and environmental samples but is not preserved in ancient sediments

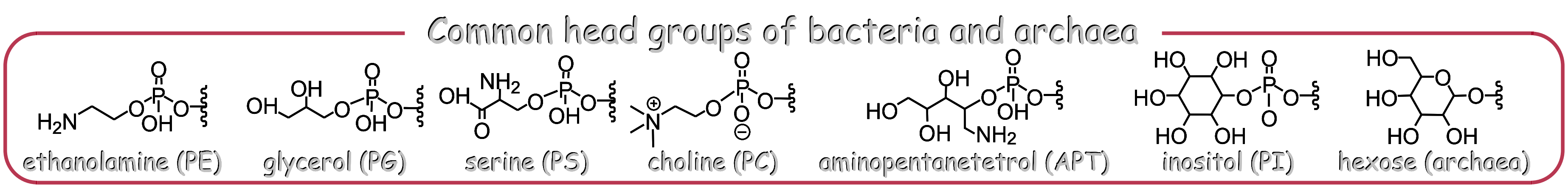
JM Hayes

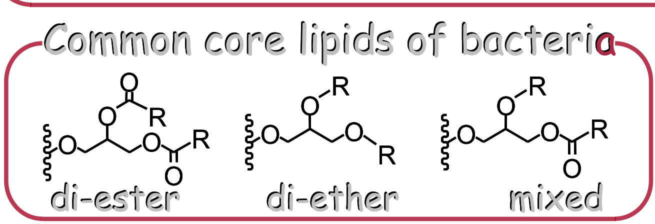
Acetogenic Lipids

Most commonly found as complex polar lipids in membranes; main component of the lipid bilayer

Glycerol esters (bacteria & eukaryotes)

Glycerol ether lipids (thermophilic bacteria and some SRB)





Acetogenic Lipids

Bacteria & eukaryotes

sn-1, 2-diacylglycerols

Thermophilic bacteria and some SRB

sn-1-akylglycerol monoethers

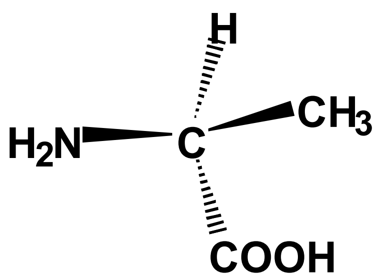
sn-1,2-dialkylglycerol diethers

sn-1,2-alkylacyl glycerols

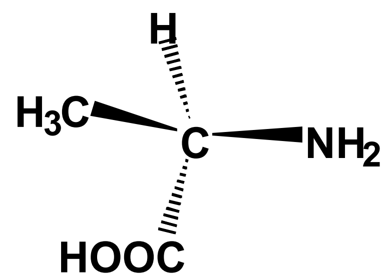
Archaea

sn-2,3-diakylglycerol diethers

Enantiomers of Alanine



L-alanine



D-alanine

L-amino acids predominate in biology

L-amino acid XS in Murchison meteorite

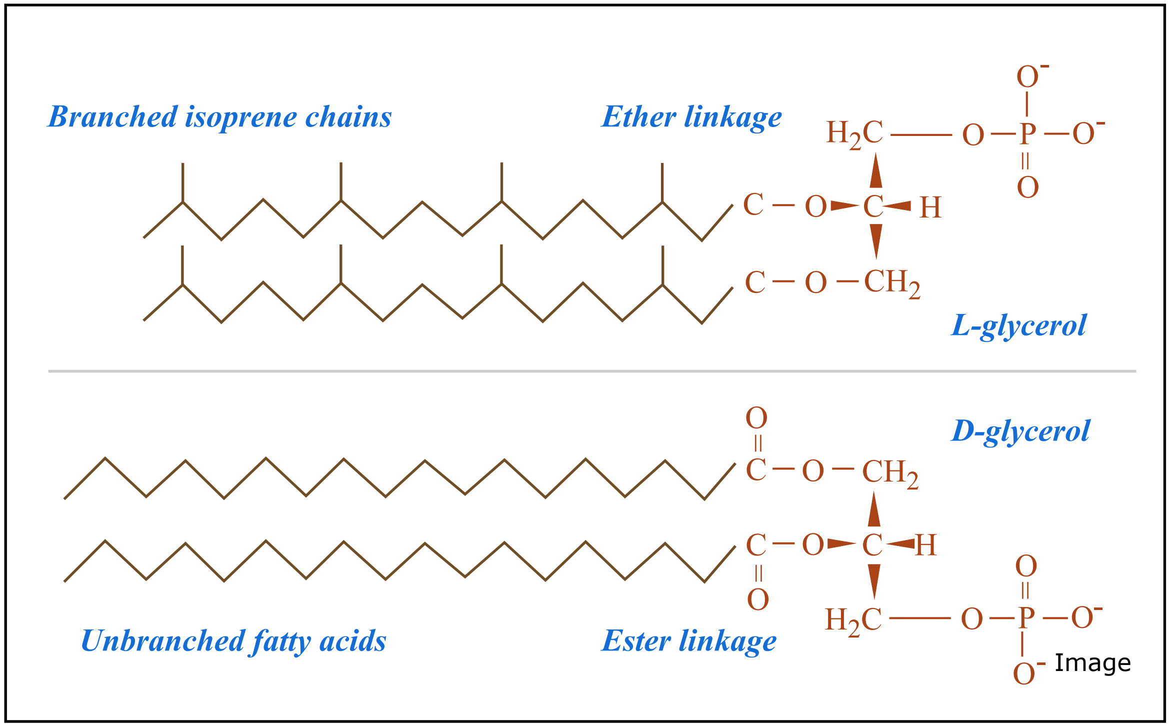
(Engel & Macko a-aa's; Cronin & Pizzarello non-protein aa's)

Non-biological processes can yield enantiomeric excess

Asymmetric catalysis and autocatalysis

Soai & Sato: slight chiral excess propagated during autocatalytic syntheses

Pizzarello and Weber: AA enantiomeric excess promotes asymmetry in aldol condensations of glycoaldehyde



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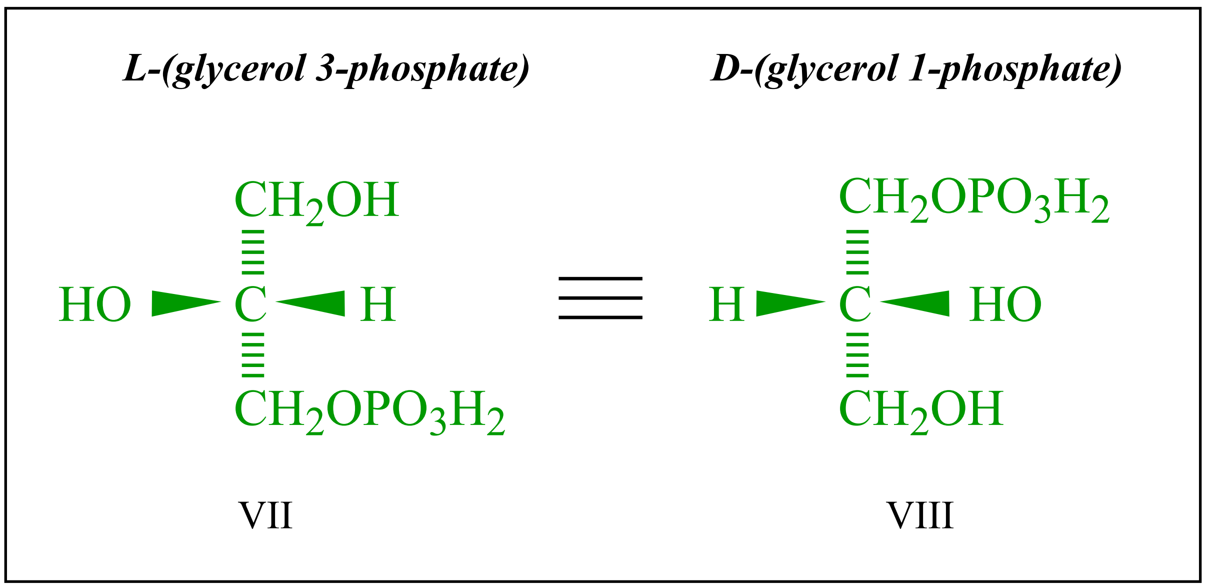
Archaea

Bacteria & Eukarya

Lip-1.13. *Stereospecific Numbering.* In order to designate the configuration of glycerol derivatives, the carbon atoms of glycerol are numbered stereospecifically. The carbon atom that appears on top in that Fischer projection that shows a vertical carbon chain with the hydroxyl group at carbon-2 to the left is designated as C-1. To differentiate such numbering from conventional numbering conveying no steric information, the prefix *'sn'* (for *stereospecifically numbered*) is used. This term is printed in lower-case italics, even at the beginning of a sentence, immediately preceding the glycerol term, from which it is separated by hyphen. The prefix *'rac-'* (for *racemo*) precedes the full name if the product is an equal mixture of both antipodes; the prefix *'X-'* may be used when the configuration of the compound is either unknown or unspecified (cf. Lip-1.10).
Examples:

(a) *sn*-glycerol 3-phosphate for the stereoisomer (VII = VIII), previously known as either L-α-glycerophosphate or as D-glycerol 1-phosphate;
(b) *rac*-1-hexadecylglycerol;
(c) 1,2-dipalmitoyl-3-stearoyl-*X*-glycerol.



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L-(glycerol 3-phosphate) D-(glycerol 1-phosphate)

Cis- and trans- isomerism

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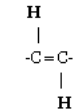
Trans fatty acids do occur in nature but, our diet, largely result from processing



Trans fats in food

Though some *trans* fats are found naturally (in the milk and body fat of ruminants such as cows and sheep), the majority are formed during the manufacture of processed foods (see below for details). In unprocessed foods, most unsaturated bonds in fatty acids are in the *cis* configuration.

Trans fat from partially hydrogenated vegetable oils has displaced natural solid fats and liquid oils in many areas. Partial hydrogenation increases the shelf life and flavor stability of foods containing these fats. Partial hydrogenation also raises the melting point, producing a semi-solid material, which is much more desirable for use in baking than liquid oils. Partially hydrogenated vegetable oils are much less expensive than the fats originally favored by bakers, such as butter or lard. Because they are not derived from animals, there are fewer objections to their use.

In the US, snack foods, fried foods, baked goods, salad dressings, and other processed foods are likely to contain *trans* fats, as are vegetable shortenings and margarines. Laboratory analysis alone can determine the amount. Outside the US, trans fats have been largely phased out of retail margarines and shortenings. US food manufacturers are now also phasing out trans fats, but at present, most US margarines still have more *trans* fat than butter. In the 1950s advocates said that the *trans* fats of margarine were healthier than the saturated fats of butter, but this has been questioned. See the saturated fats page for details.

A *trans* configuration of hydrogen atoms

Chemistry of *trans* fats

Trans fatty acids are made when manufacturers add hydrogen to vegetable oil, in the presence of small amounts of catalyst metals such as nickel, palladium, platinum or cobalt -- in a process described as partial hydrogenation. If the hydrogenation process were allowed to go to completion, there would be no *trans* fatty acids left, but the resulting material would be too solid for practical use. A claimed exception to this is Kraft Foods' new *trans* fat free Crisco which contains the wax-like fully hydrogenated cottonseed oil blended with liquid vegetable oils to yield a shortening much like the previous Crisco which was made from partially hydrogenated vegetable oil. However *any* hydrogenated or partially hydrogenated oil will contain trace amounts of the metals used in the process of hydrogenation. In a natural fatty acid, the hydrogen atoms usually form a double bond on the same side of the carbon chain. However, partial hydrogenation reconfigures most of the double bonds that do not become chemically saturated, so that the hydrogen atoms end up on different sides of the chain. This type of configuration is called *trans* (which means "across" in Latin). The structure of a *trans* unsaturated chemical bond is shown in the diagram.

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Please see the images on
http://library.med.utah.edu/NetBiochem/FattyAcids/4_1.html
and http://library.med.utah.edu/NetBiochem/FattyAcids/4_1d.html.

WAX → http://www.cyberlipid.org/index.htm

Wax Components

Compound	***General Structure***
n-Alkanes	$H_3C[CH_2]_nCH_3$
Ketones	R^1COR^2
Secondary alcohols	$R^1CH(OH)R^2$
β-Diketones	$R^1COCH_2COR^2$
Monoesters	R^1COOR^2
Primary alcohols	RCH_2OH
Aldehvdes	RCHO
Alkanoic acids	RCOOH
Dicarboxylic acids	$HOOC[CH_2]_nCOOH$
ω-Hydroxy acids	$HOCH_2[CH_2]_nCOOH$

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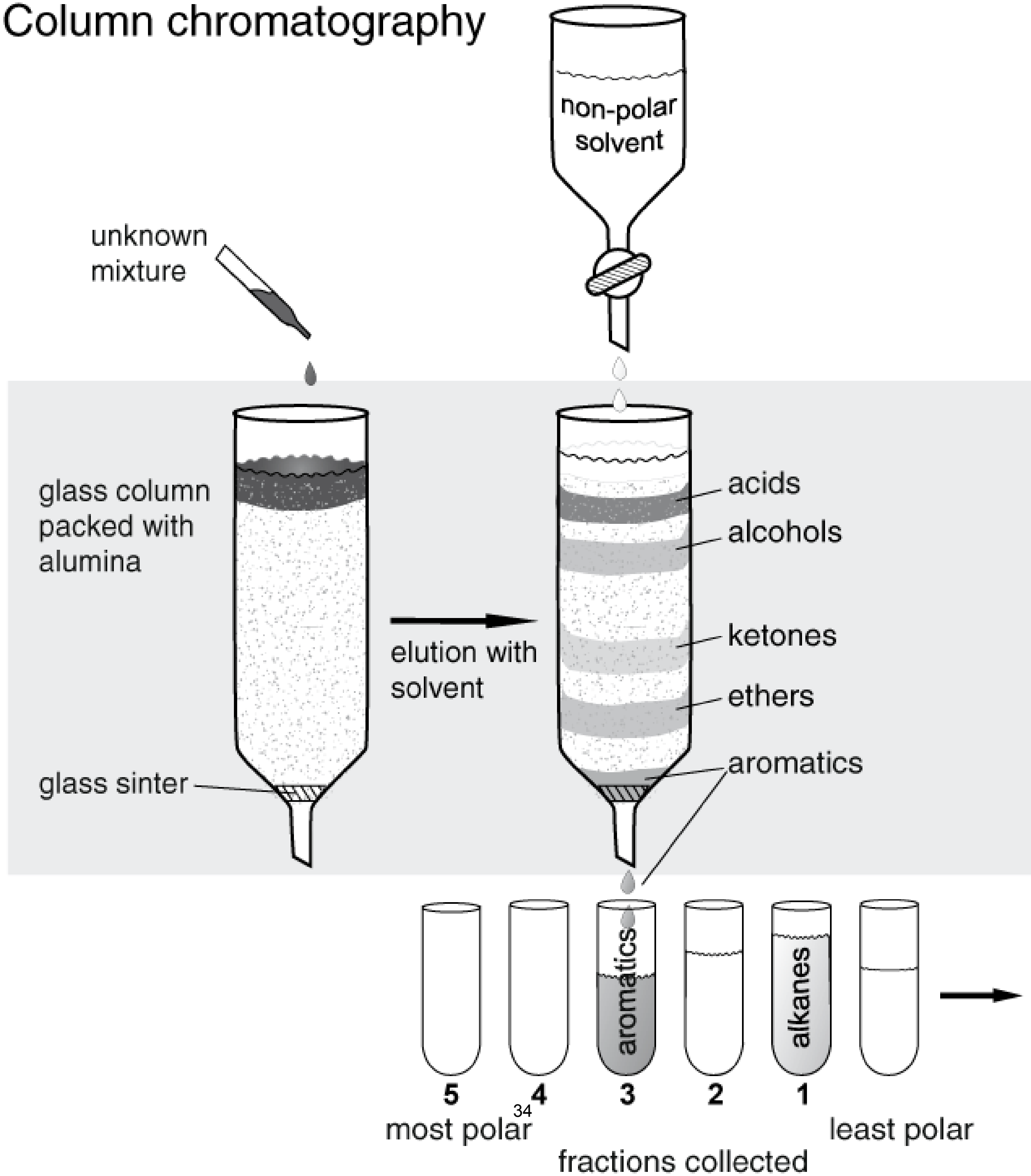
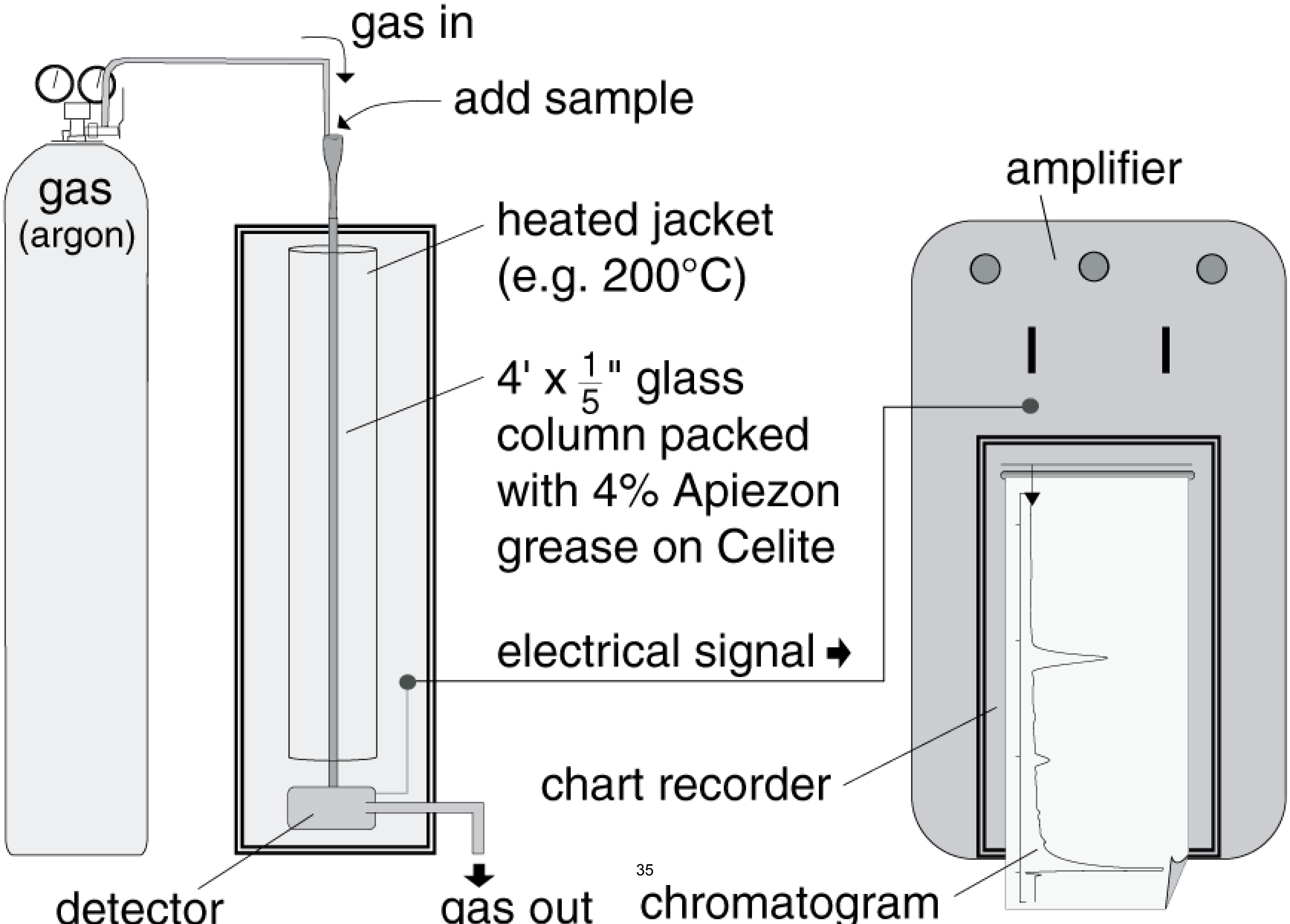
Please see:
http://www.cyberlipid.org/wax/wax0001.htm

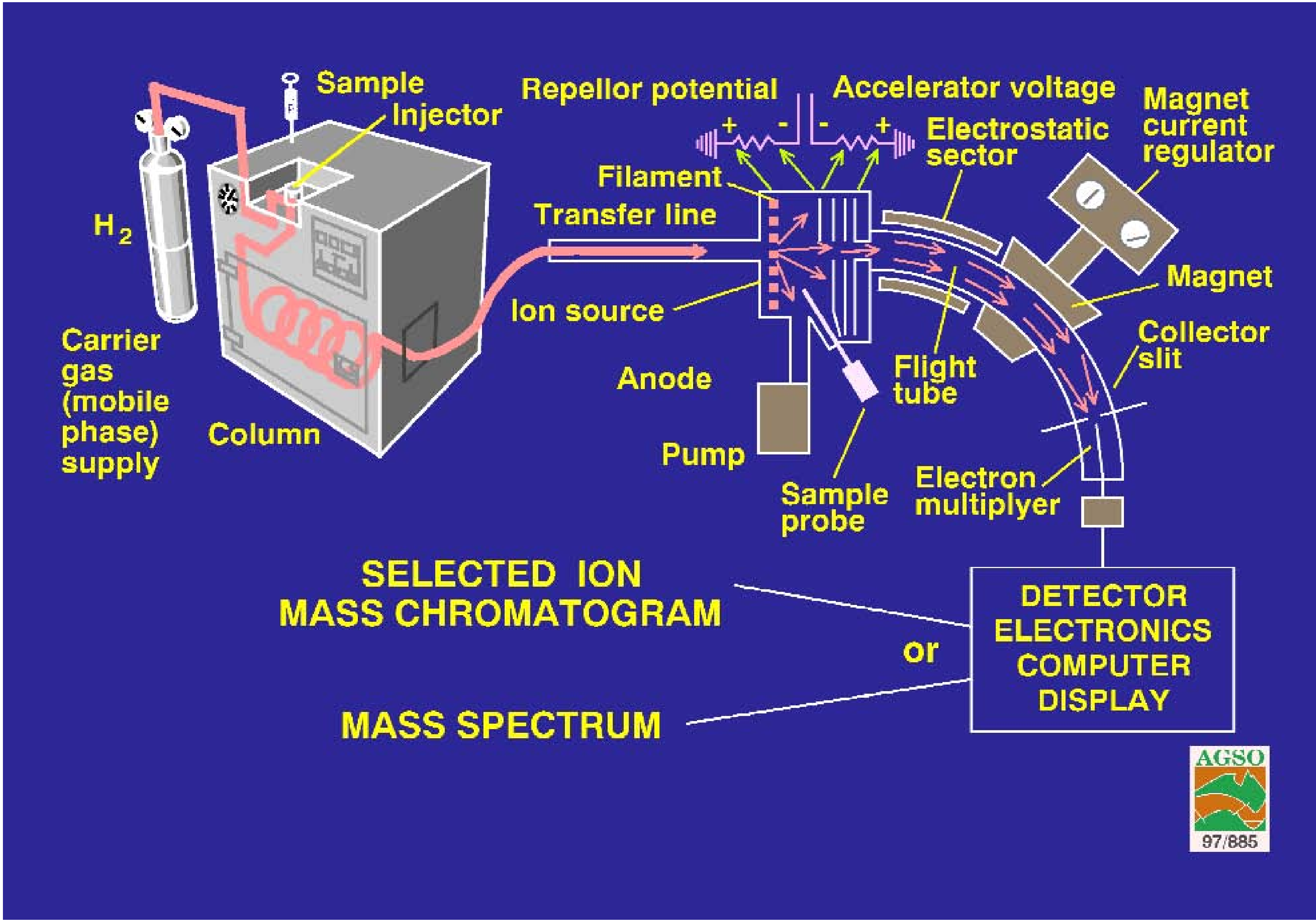
Overview of analytical approaches

Column chromatography



Gas chromatograph, 1950s




Sample
Injector
Repellor potential
Accelerator voltage
+
-
-
+
Electrostatic
sector
Magnet
current
regulator
Filament
Transfer line
H_2
Ion source
Magnet
Collector
slit
Carrier
gas
(mobile
phase)
supply
Anode
Flight
tube
Column
Pump
Electron
multiplyer
Sample
probe
SELECTED ION
MASS CHROMATOGRAM
or
MASS SPECTRUM
DETECTOR
ELECTRONICS
COMPUTER
DISPLAY
AGSO
97/885

Acetogenic lipids in microbial mats

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Please see:
http://www.rcn.montana.edu/resources/features/features.aspx?nav=11&area=32.

http://www.rcn.montana.edu/resources/features/features.aspx?nav=11&area=32

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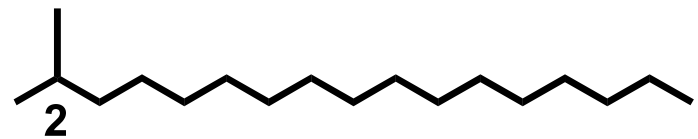
Please see:
http://www.americansouthwest.net/maps/lower-geyser-basin-map.gif.

Hydrocarbons

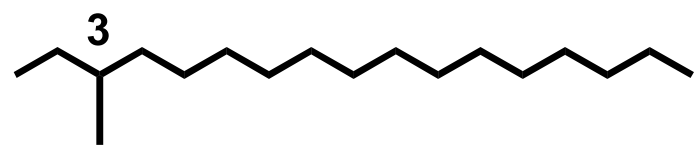
ACYCLIC & MONOCYCLIC ALKANES

n-alkane C_{17}

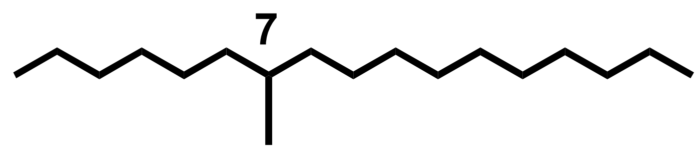
2-methyl- or isoalkane C_{18}



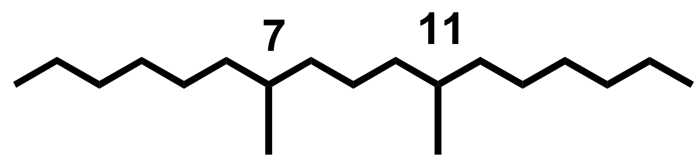
3-methyl- or anteisoalkane C_{18}



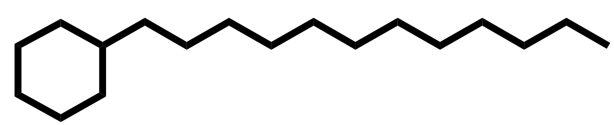
7-methyl alkane C_{18}



7,11-dimethyl alkane C_{19}



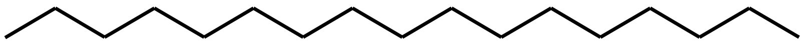
cyclohexyl alkane C_{18}



Hydrocarbon biomarkers of living organisms (Hedges, Wakeham & Keil)

Organism	Major Hydrocarbons	Comments
Diatoms Dinoflagellates	n-$C_{21:6}$ (HEH)	Also smaller amounts of pristane and n-alkanes of C_{21}-$C_{30}{}^{+}$
Red, green and yellow algae	n-C_{15} and n-C_{17}	Some of these algae types also contain unsaturated n-C_{17} hydrocarbons
Cyanobacteria	n-C_{15} and n-C_{17}	Also sometimes contain ***n***-$C_{19:1}$ and n-$C_{19:2}$, and 7-and 8-methylheptane
Bacteria	n-alkanes	Typically produce smooth distributions of n-alkanes over the range of C_{15}-$C_{30}{}^{+}$
Zooplankton	pristane in copepods, HEH in zooplankton eating diatoms	Pristane is derived from the phytol side-chain of chlorophyll pigments
Vascular land plants (especially leaf cuticles)	n-alkanes in the range of n-C_{25} to n-$C_{35}{}^{+}$	Characterized by a high odd carbon preference index (CPI) of 5-10

1. $CPI_{20\text{-}36}$ is defined as (Σodd n-alkanes/Σeven n-alkanes), in this case over the range of alkanes having 20 to 36 carbons per molecule.



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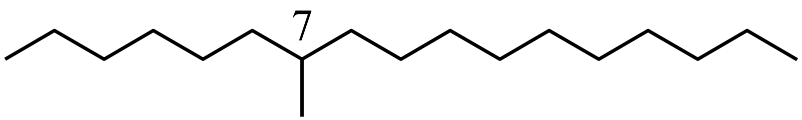

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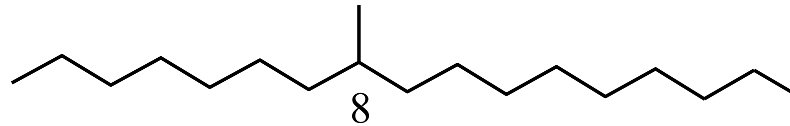

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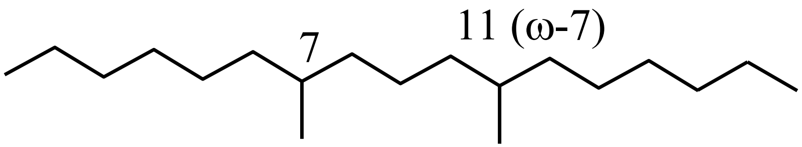

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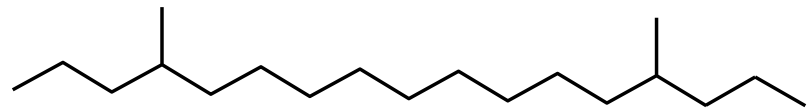

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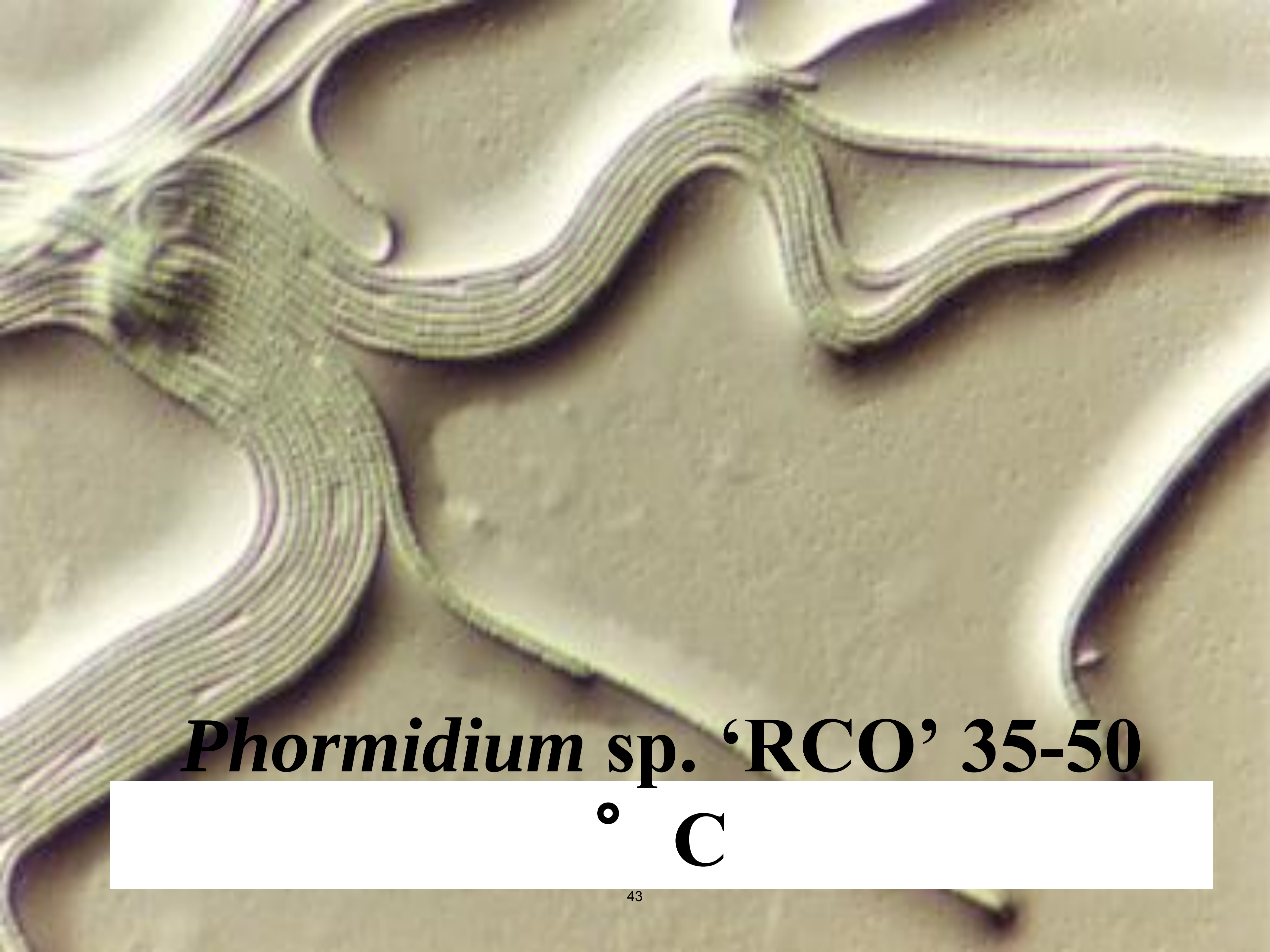
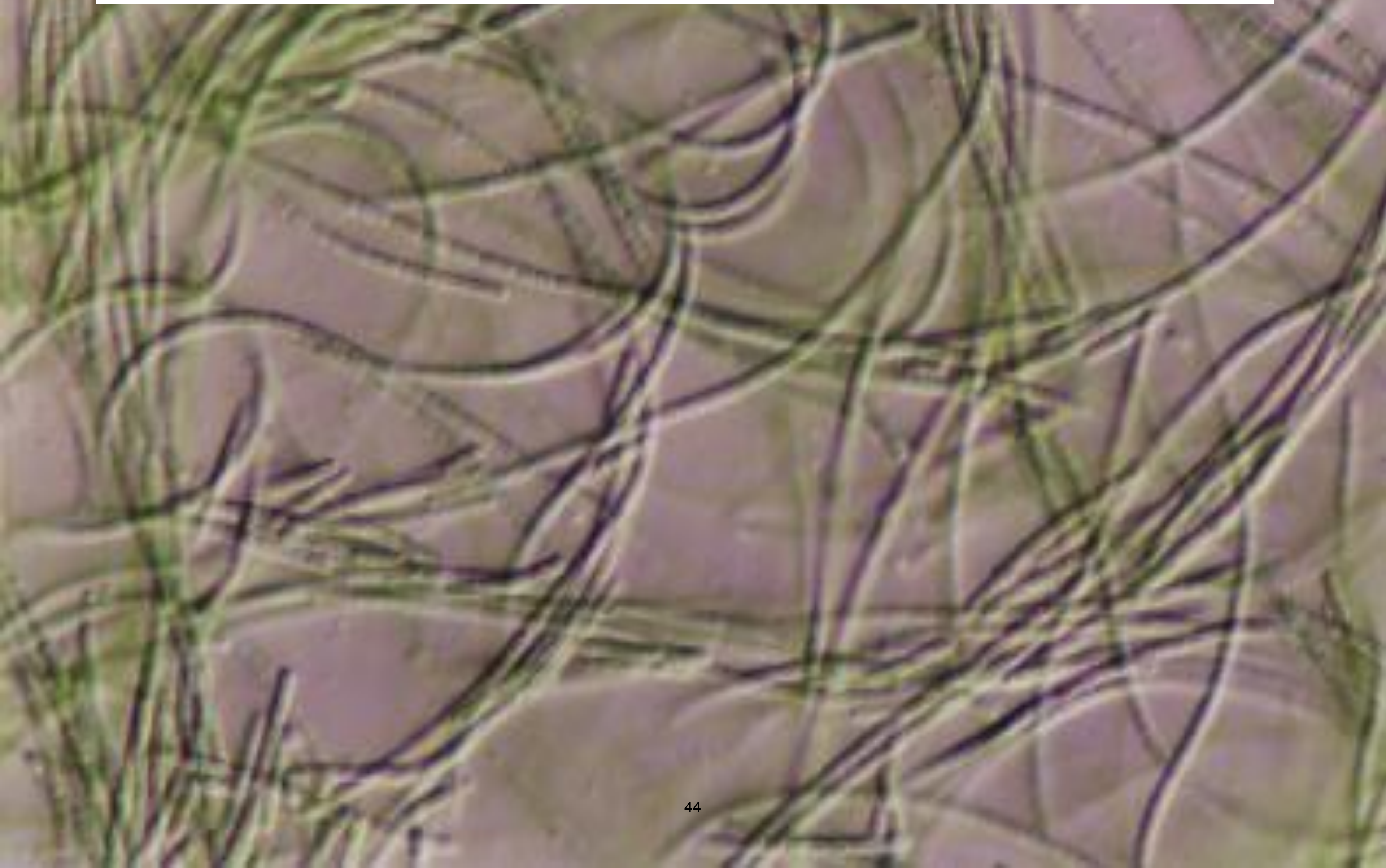
Common hydrocarbons of Oscillatoriaceae (eg *Phormidium* sp.) cyanobacteria and Yellowstone cyanobacterial mats

– Analyses of cultures and environmental samples by Linda Jahnke et al.



Phormidium sp. ‘RCO’ 35-50 ° C

Phormidium sp. ‘RCG’ 35-45 °C



CYANOBACTERIAL HYDROCARBONS

GENUS	*n*-A	MA	DMA
Oscillatoria Guerrero Negro Isol.	$C_{15\text{-}19,\ 18:1}$		
Oscillatoria Yellowstone Isol.	C_{17}	C_{18} (7-9)	
Synechococcus YS ATCC27180	$C_{15\text{-}20}$	$C_{18\text{-}20}$	
Cyanothece Guerrero Negro 1	$C_{15\text{-}17+\text{ enes}}$		
Cyanothece Guerrero Negro 2	$C_{15\text{-}17+\text{ enes}}$		
Microcoleus Shark Bay Isol.	$C_{17,\ 17:1}$		
Microcoleus Guerrero Negro Isol.	$C_{15\text{-}17+17:1}$		

CYANOBACTERIAL HYDROCARBONS

GENUS	*n*-A	MA	DMA
Phormidium luridum	C_{17}	C_{18} (7+8)	C_{19} (7,11+)
Phormidium Yellowstone Isol.	$C_{16\text{-}19}$		
Chlorogloeopsis fritschii	C_{17}	C_{18} (4+3)	C_{19} (4,x)
Chlorogloeopsis Yellowstone Isol.	C_{17}	C_{18} (4+5)	$C_{19}+C_{20}$
Anabaena (Anc-2)		$C_{17:1}+C_{18:1}$	
Pseudanabaena Guerrero Negro Isol.	$C_{15\text{-}19}$	$C_{17\text{-}19}$	
Pseudanabaena [illegible]	C_{17}	C_{18} (6-8)	C_{19} (7,11+)

Serve a, yet to be determined, physiological function!!!!!!

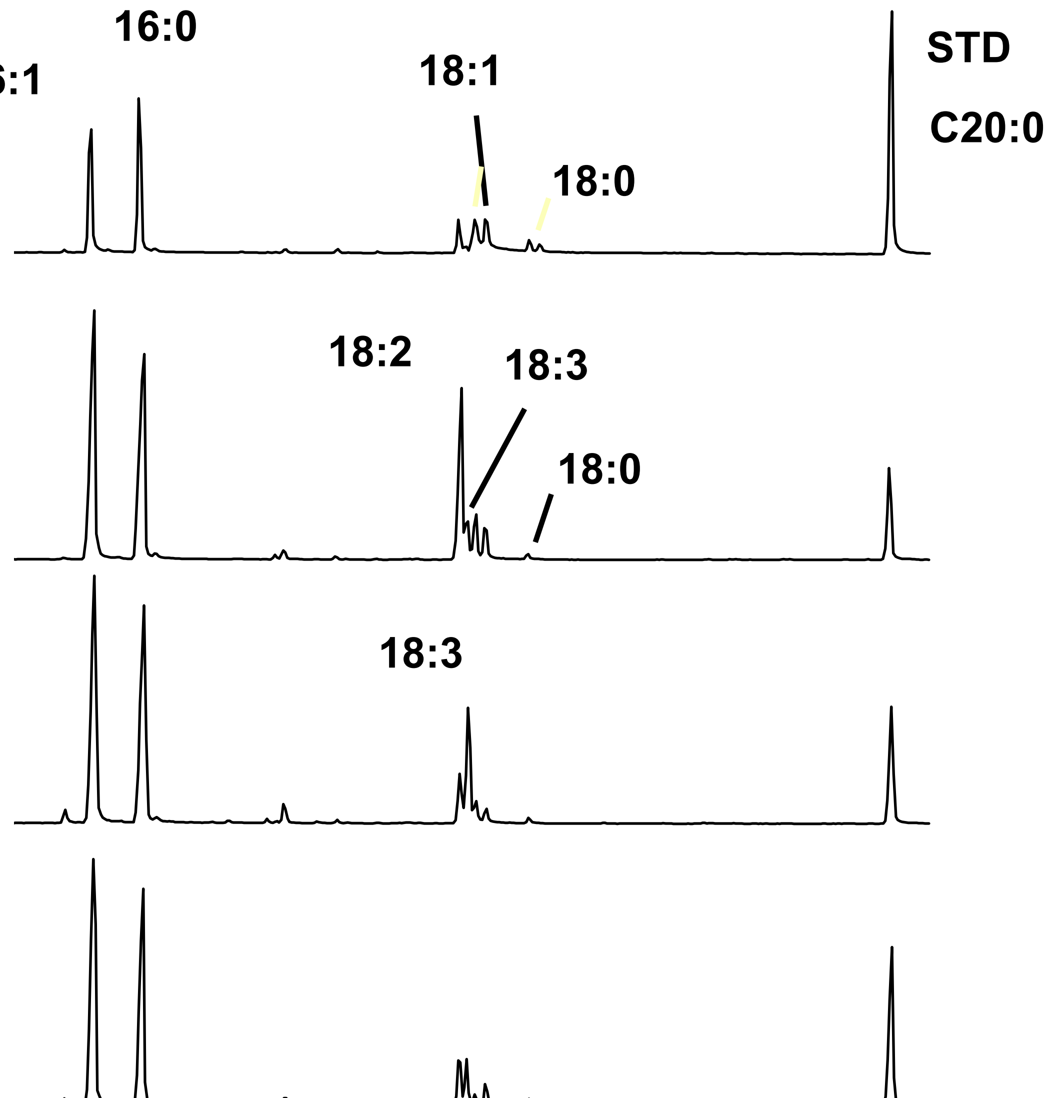
Phormidium luridum pCO_2 culture experiments produce different FAMES

Standing
Hi $NaHC0_3$ P4

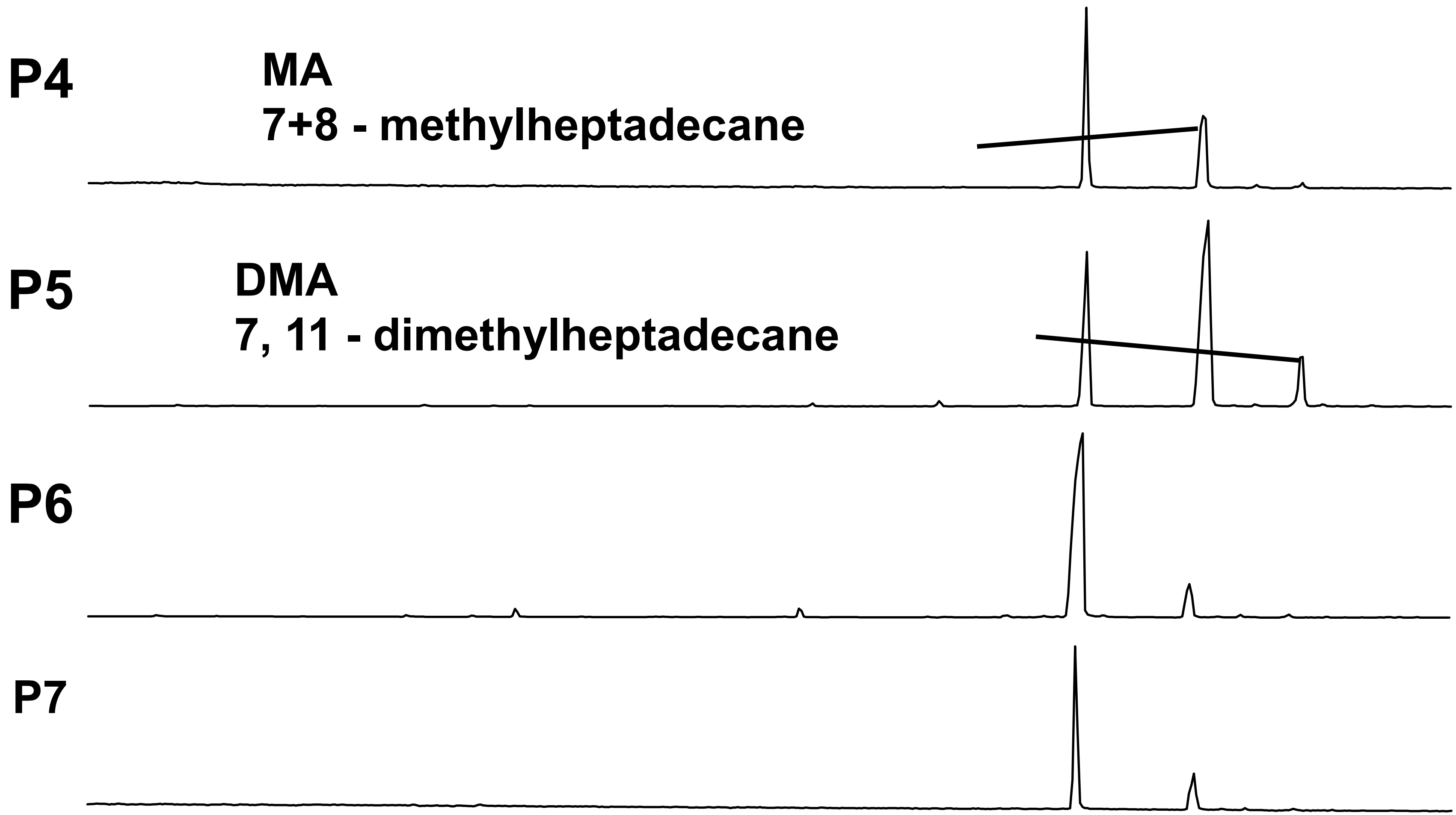
Standing
CO_2 in air P5

Bubbled
CO_2 in N_2 P6

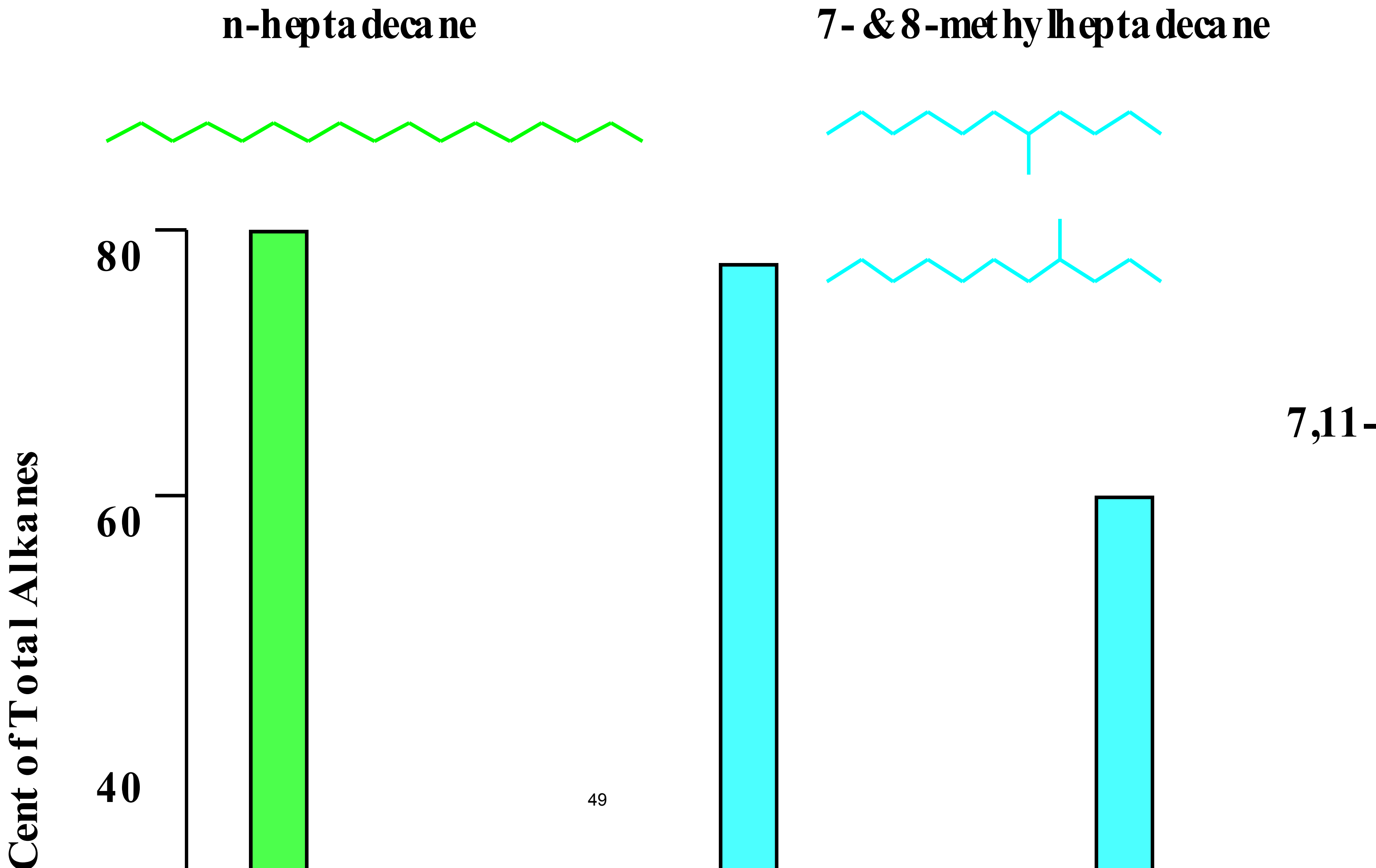
Bubbled
CO_2 in air P7



Phormidium luridum culture experiments → different hydrocarbons



Effect of CO_2 Level on Branched Alkane Synthesis i



Environmental conditions for *Phormidium* mat sites.

	OCTOPUS SPRING	FOUNTAIN PAINT POTS
Temperature, ° C	46°	45°
pH	8.5	8.7
DIC, mM	5.2	5.7
$d^{13}C_{DIC}$, ‰	-0.23	+0.48
CO_2 (aqueous), μM*	30	21
$d^{13}C_{CO2}$, ‰*	-7.1	-6.4

* CO_2 and $\delta^{13}C_{CO2}$ calculated according to Mook et al. (1974)

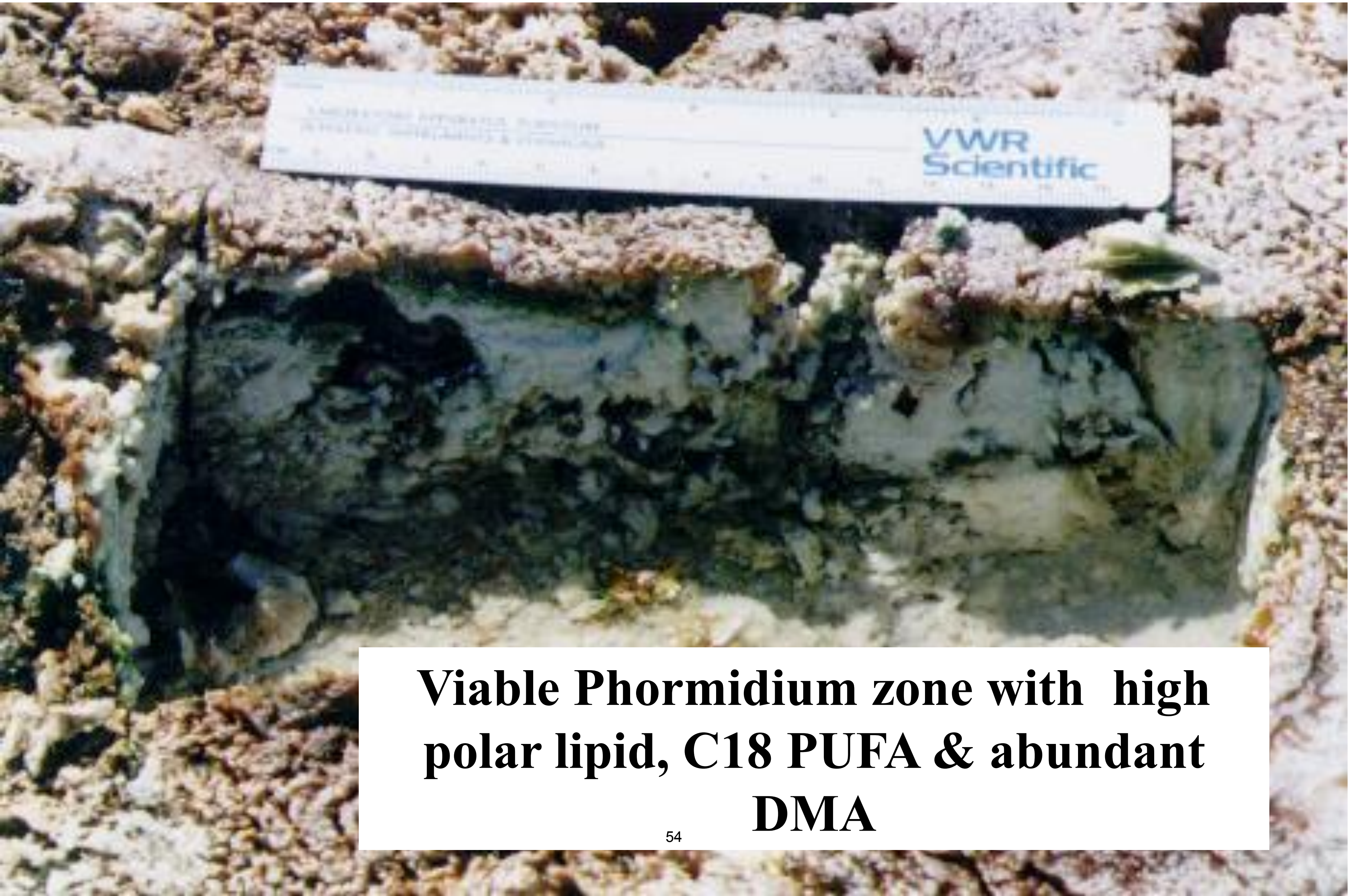
Lipid compositions of Octopus Spring and Fountain Paint Pots mat layers.

Mat Layer		TOC μg mg^{-1} dry wt	Lipid Component μg mg^{-1} TOC					
			Polar Fatty Acid			WXE	Alkane[a]	BHP
			Total	PUFA	BFA			
OCTOPUS SPRING								
OS-1	Coniform tips	334	19.7	1.69	1.16	0.80	0.86	0.70
OS-2	Green base	151	12.8	0.46	0.99	1.53	0.28	0.57
OS-3	Red layer	367	20.2	0.28	2.02	5.10	0.12	0.30
FOUNTAIN PAINT POTS								
FPP-1	Orange surface	216	11.4	1.80	0.83	1.07	0.76	1.40
FPP-2	Green-white zone	82	7.0	0.99	0.31	2.85	0.34	1.90
FPP-3	Flesh-colored	94	9.6	0.29	0.98	3.46	0.48	0.95

FPP4 Submerged Phormidium mat 45° C

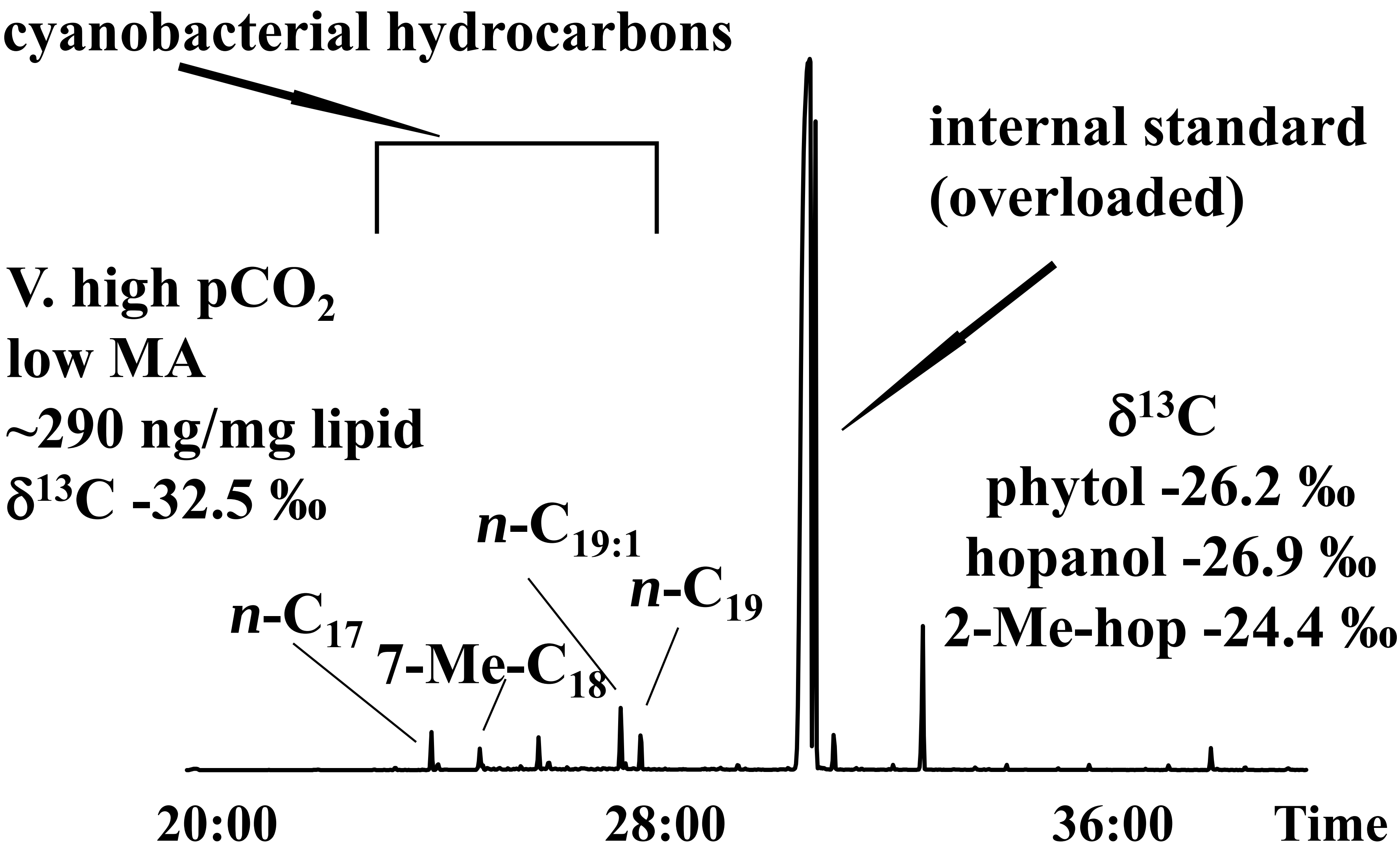
Silicified Phormidium Mat FPP5

FPP 5 Silicified Phormidium mat

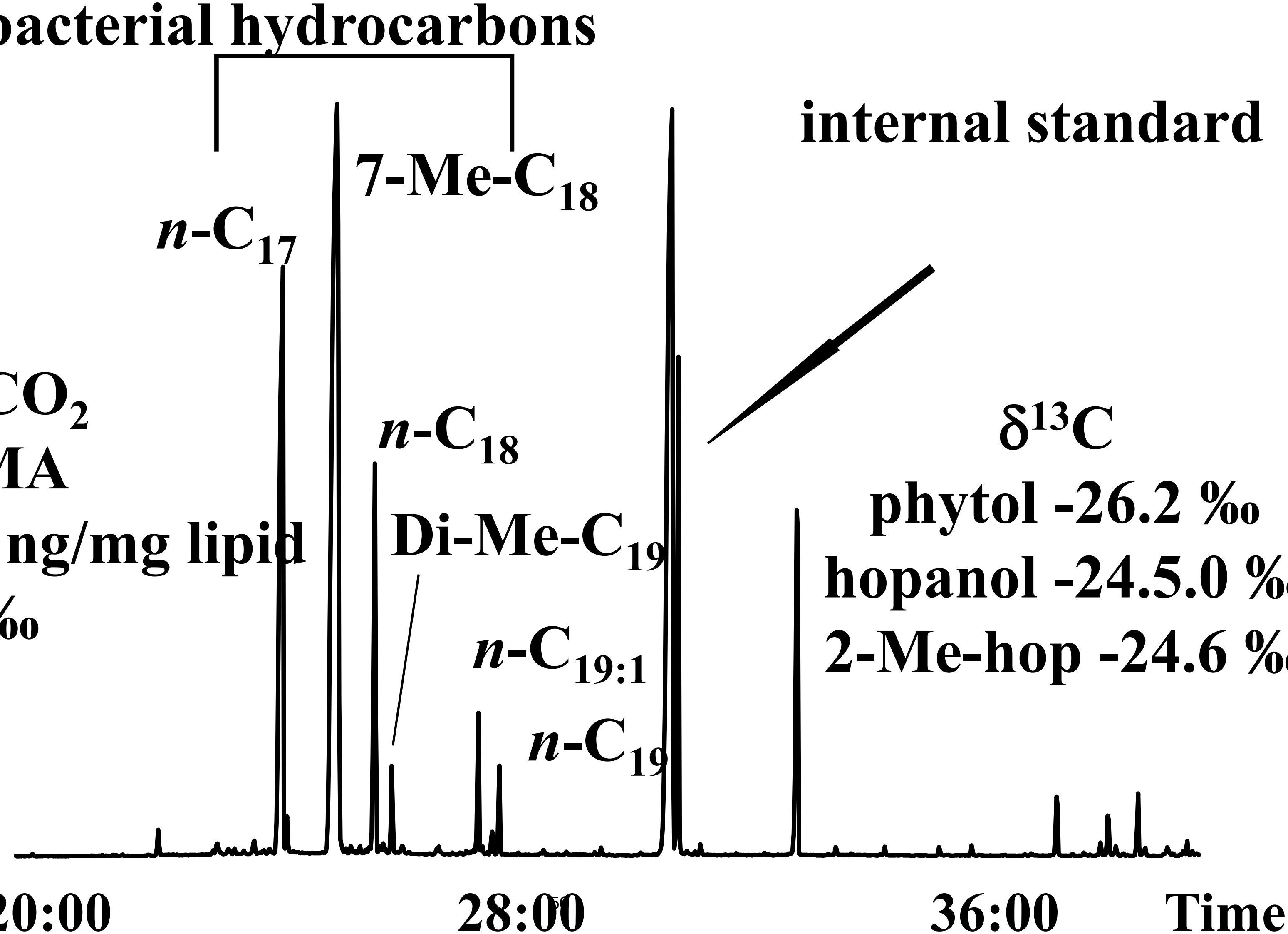


Viable Phormidium zone with high polar lipid, C18 PUFA & abundant DMA

FPP Submerged Mat F4-2



FPP Exposed & Silicified Mat F5



low pCO_2
high MA
~1907 ng/mg lipid
-26.5 ‰

$\delta^{13}C$
phytol -26.2 ‰
hopanol -24.5.0 ‰
2-Me-hop -24.6 ‰

Lipid Biomarker Diversity Associated With Cyanobacteria.

	Alkanes			Bacteriohopanepolyols (BHP)			
Cyanobacterium	Normal	Methyl	Dimethyl	$2MeC_{31}$	C_{31}	$2MeC_{32}$	C_{32}
Phormidium luridum	+	+	+	−	−	+	+
Chlorogloeopsis fritschii	+	+	+	−	−	+	+
Synechococcus lividus	+	−	−	−	−	+	+
Cyanothece RCB4*	+	−	−	−	−	+	+
Phormidium RCG3*	+	+	+	−	−	−	+
Phormidium FPGF4*	+	+	+	−	−	−	+
Phormidium FPOS4*	+	+	−	+	+	+	+
Phormidium OSS4*	+	+	±	+	+	±	±
Oscillatoria amphigranulata	+	+	+	+	±	+	±
Fischerella sp.	+	+	+	−	−	−	−
Phormidium RCO4*	+	−	−	−	−	−	−

**YNP cyanobacteria isolated for this study. Suffix codes refers to isolation source mat (RC, Rabbit Creek Spouter; FP, Fountain Paint Pots; OS, Octopus Spring).*

Mono-, di- and trimethyl-branched alkanes in cultures of the filamentous cyanobacterium Calothrix scopulorum

Jürgen Köster, John K. Volkman, Jürgen Rullkötter, Barbara M. Scholz-Böttcher, Jörg Rethmeier

Organic Geochemistry 30 (1999) 1367-1379

Table 1
Literature reports of dimethylalkanes in cyanobacteria, microbial mat samples and seawater particles[a]

Sample	C_{17}[b]	C_{18}[b]	C_{19}[b]	C_{20}[b]	References
Phormidium luridum			7,11		Summons et al. (1996; 1998)
Calothrix sp.			2,15 2,16		Summons (pers. comm.)
Calothrix scopulorum strain Hi 41			**4,13 5,13** 4, 5 3,14 4,14 5,6 3,15 3,4		This work
Gavish Sabkha (Sinai) mats			**6,10**[c]	7,10 6,10	de Leeuw et al. (1985)
Icelandic hot spring mats			**5,13 4,13** 5,12 6,12	7,12	Robinson and Eglinton (1990)
Orakai Karako (NZ) hot spring microbial mat			**5,12 4,13** 5,6 3,12 4,5	Present?	Shiea et al. (1990)
Abu Dhabi mat (modern)			**6,11 6,12** 7,11 6,7 4,12 5,6	**6,12 6,13** 6,7 7,12 5,13	Kenig et al. (1995)
Hao (French Polynesia) mat			**6,10**[c]		Kenig et al. (1995)
Alboran Sea sinking particles	**3,13**	**3,14**	**3,15**		Dachs et al. (1998)
Lake Vanda, Antarctica		**2,6**			Matsumoto et al. (1984)

[a] Major isomers are shown in bold; isomers are listed in order of decreasing abundance, additional isomers may be present in low concentrations.
[b] Value refers to total carbon numbers (i.e. C_{17} is a dimethylpentadecane).
[c] This isomer may be 6,12-dimethylheptadecane (Kenig et al., 1995).

Peak no. in Fig. 1	Compound	Position of methyl groups
1	*n*-heptadecane	
2	7-methylheptadecane	7
3	6-methylheptadecane	6
4	5-methylheptadecane	5
5	4-methylheptadecane	4
6	2-methylheptadecane	2
7	3-methylheptadecane	3
8	5,13-dimethylheptadecane	5,ω5
9	4,13-dimethylheptadecane	4,ω5
10	4,14-dimethylheptadecane	4,ω4
11	5,6-dimethylheptadecane	5,6
12	3,14-dimethylheptadecane	3,ω4
13	4,5-dimethylheptadecane	4,5
14	3,15-dimethylheptadecane	3,ω3
15	3,4-dimethylheptadecane	3,4
16	4,12,13-trimethylheptadecane	4,ω5,ω6
17	4,5,13-trimethylheptadecane	4,5,ω5
18	4,5,14-trimethylheptadecane	4,5,ω4
19	2,13,14-trimethylheptadecane	2,ω4,ω5
20	3,13,14-trimethylheptadecane	3,ω4,ω5
21	3,4,14-trimethylheptadecane	3,4,ω4
22	3,4,15-trimethylheptadecane	3,4,ω3

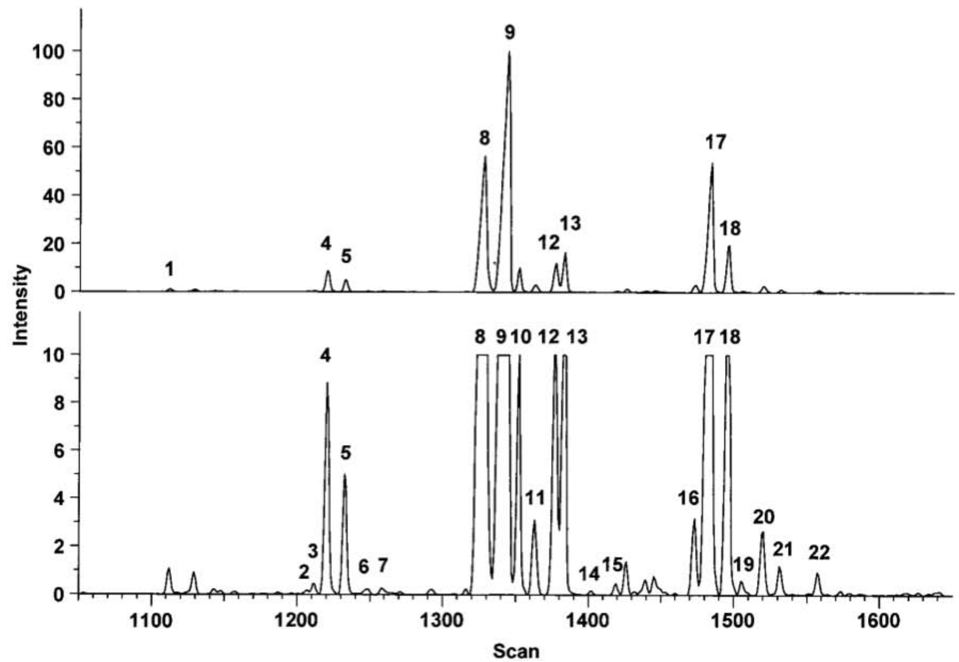


Fig. 1. Partial capillary gas chromatogram showing the distribution of aliphatic hydrocarbons in *C. scopulorum* strain Hi 41. The vertical scale of the lower trace is enhanced by a factor of 10 to show the minor constituents. Peaks are identified in Table 2.

Hydrocarbons of *Calothrix scopulorum*

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Please see: Figure 2, Köster, Jürgen, et al. "Mono-, Di- and Trimethyl-Branched Alkanes in Cultures of the Filamentous Cyanobacterium Calothrix Scopulorum." *Organic Geochemistry* 30, no. 11 (November 1999): 1367-1379.

12.158 Molecular Biogeochemistry
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