

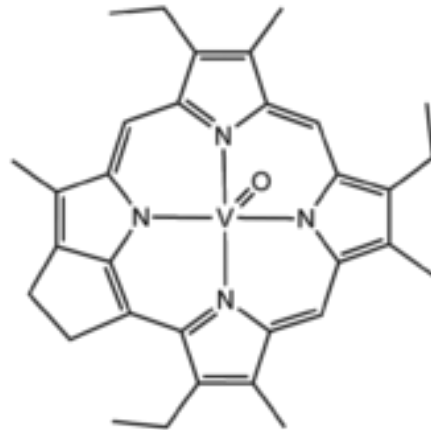
Introduction to biomarkers- Life, molecules and the geological record



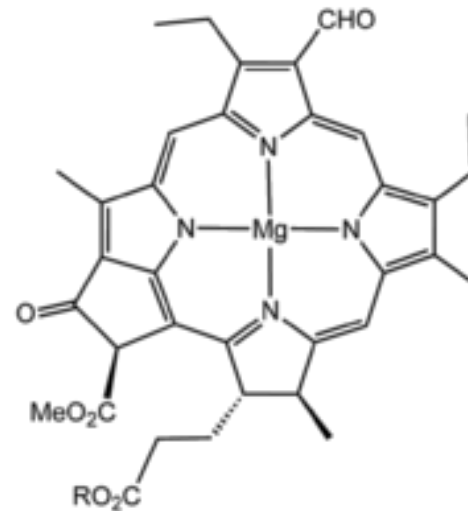
Alfred Treibs
1899-1983

Explored the composition of porphyrins in oil and proposed the “Treibs Hypothesis” that porphyrins are diagenetic alteration products of chlorophylls, and therefore porphyrin containing organic matter in ancient rocks is derived from plant material

Vanadyl porphyrin

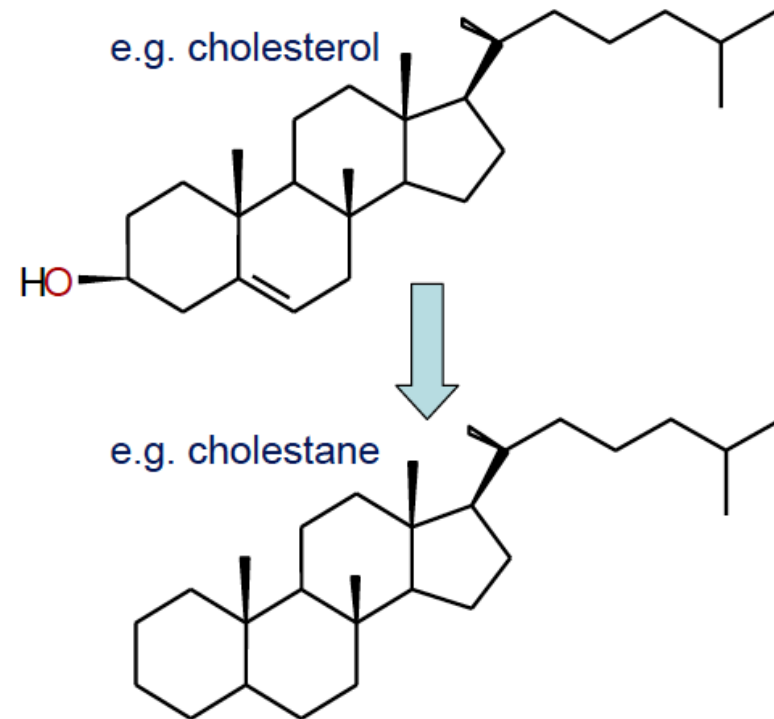


Chlorophyll-b



Life, Molecules and the Geological Record

- Life leaves molecular residues (*Chemical Fossils*) as well as visible shapes/objects (*Fossils*) in the sedimentary record.
- These molecular residues, when characterised as specific molecules (*Biomarkers*) by their structures and isotopic content, may give precise indications of their biosynthetic origins in particular organisms, as well as the environmental conditions that the organisms experienced.



**Definition of a biomarker
(or “molecular marker” or “geochemical fossil”):**

“A molecule whose carbon skeleton can unambiguously be linked to that of a known biological precursor compound”

More generally:

“Organic compounds found in sediments which have properties that can be directly related to a known biological precursor”

Biological marker molecules

- Living organisms biosynthesize a very small subset of the billions of molecules that can be assembled in theory from C, H, O, N, S, P etc.
- These molecules can be regarded as biomarkers. Their presence in an environment reflects their synthesis by the parent organisms.
- Some biomolecules are produced only by a certain species or classes of organism, and hence indicate the presence or prior existence of those organisms.
- Other biomolecules are produced by many species of organism and are indicative of the general level of biological activity.
- Molecular signatures can comprise the only means to decipher past ecosystems and biological inputs for organisms composed only of soft parts (i.e., leave no morphological or isotopic imprint).

Lipids

- Lipids present in the water column and in sediments can originate from all three domains of life (i.e., eukaryotes, bacteria, archaea).
- Certain lipids are synthesized by only one domain.
 - Steroids are almost exclusively synthesized by eukaryotes
 - Hopanoids are exclusively synthesized by bacteria
 - Acyclic and cyclic isoprenoid ether lipids are restricted to the archaea.

Occurrence:

- Ubiquitous
- 10-20% of TOC in most organisms
- Extensively studied classes of compounds
 - - analytically accessible
 - - diagenetically and chemically [relatively] stable
 - - structurally extremely diverse (high potential as "biomarkers")

Function:

- - Long-term energy storage
- - membrane fluidity regulators
- - membrane rigidity/barrier to proton exchange
- - pigments
- - hormones
- - vitamins

For further information see- <http://lipidlibrary.aocs.org/>

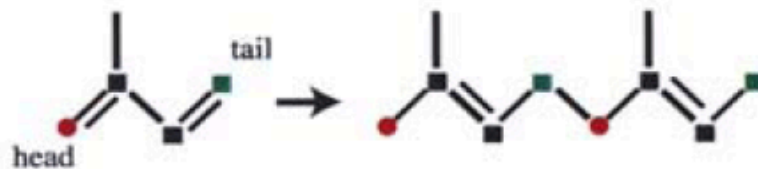
Lipid biosynthesis

Occurs via two main pathways:

1. Polyketide Biosynthesis: The polymerization of acetate; products typically have even carbon numbers.



2. Isoprenoid synthesis: The polymerization of isoprene; products typically have 10, 15, 20 carbon atoms.



Group I. Polyketide lipids

Compounds whose structure is based on repeat units of acetate.

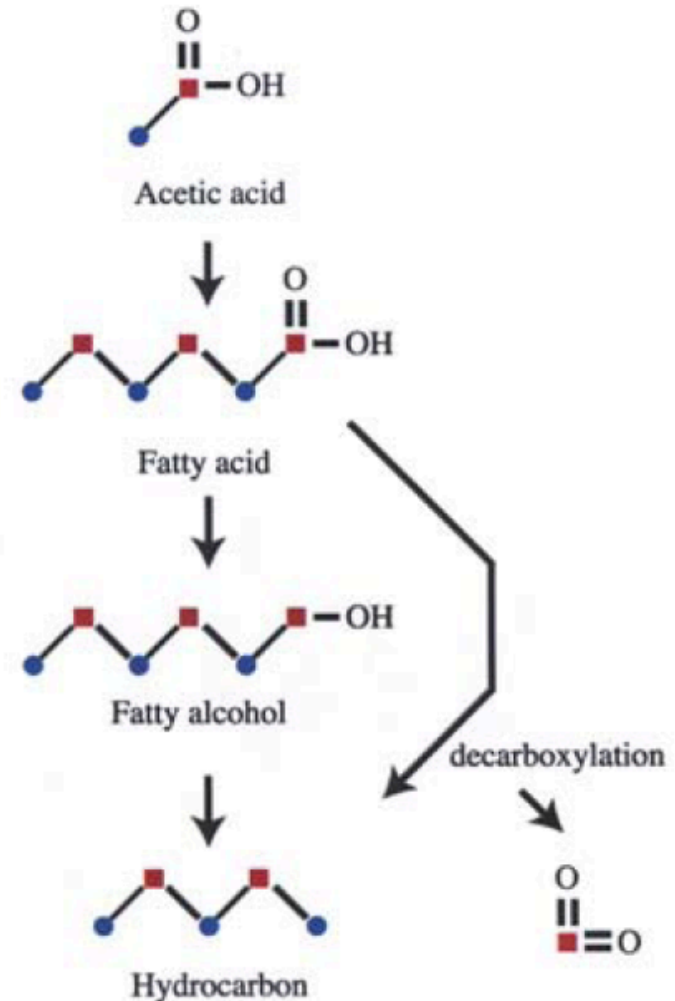
Products usually have an even number of carbons.

Common compound classes:

- Fatty acids
- Fatty alcohols
- Hydrocarbons (*n*-alkanes)

Nomenclature

- *Normal* alkanes and alcohols are denoted: $n\text{-C}_x$ where x = number of carbon atoms
- e.g. $n\text{-C}_{15}$ = normal pentadecane
- Fatty acids are often unsaturated
- $\text{C}_{x,y}$ where y = number of double bonds
- double bonds are usually *cis*
- Δ denotes the position of unsaturation from the COOH end of the molecule
 - e.g. Oleic acid is ${}^9\Delta\text{C}_{18:1}$
- ω denotes unsaturation from the methyl end
- Carbon Preference Index (CPI), Odd/Even Predominance (OEP) and Average Chain Length (ACL) used to describe distributions.

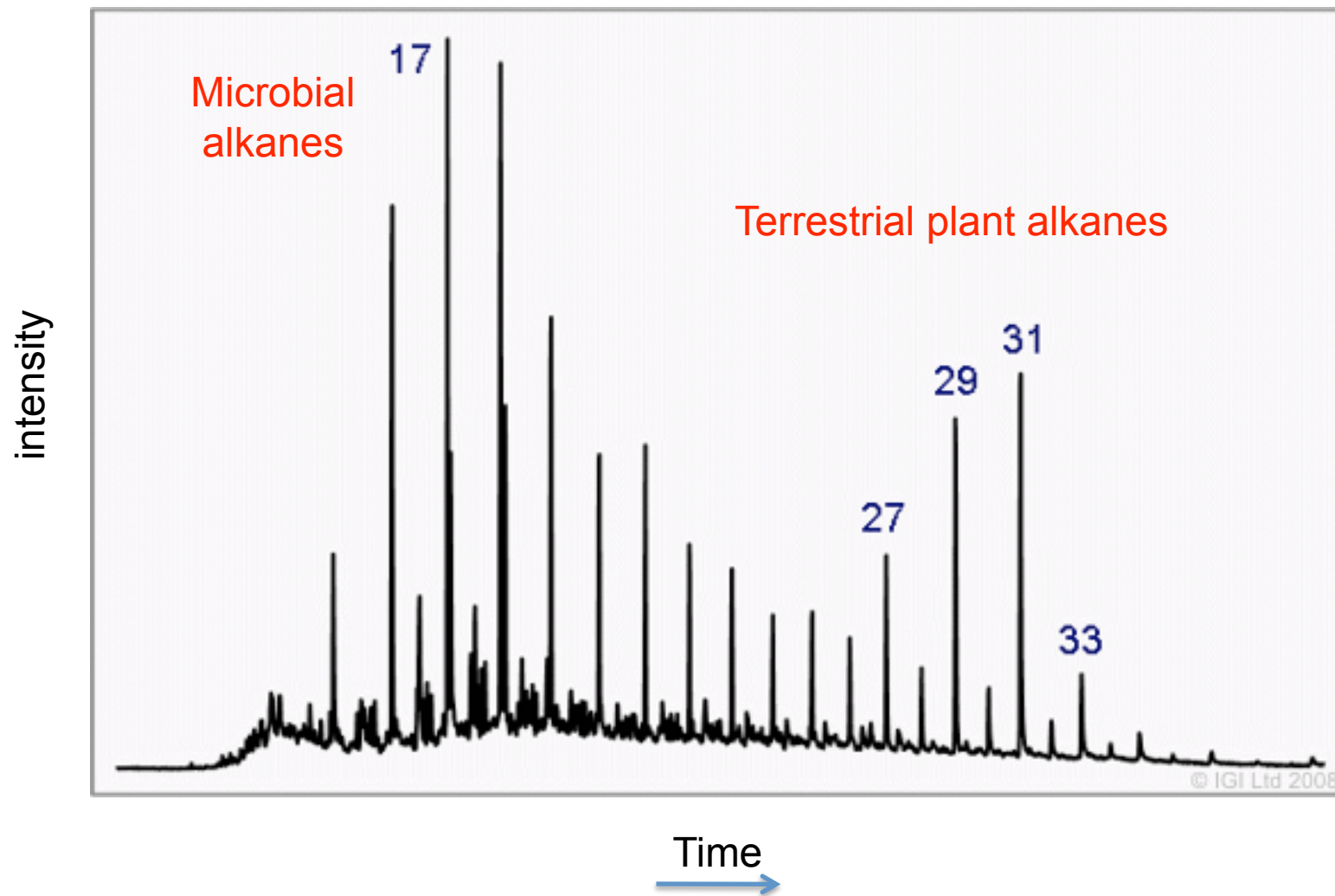


Generalized Lipid Distributions

	Acids	Alcohols	Hydrocarbons
Phytoplankton	even/odd CPI 16:0, 16:1 18:0, 18:1	even/odd CPI	odd/even nC17, nC18
Bacteria	iso + anteiso	?	CPI = 1 nC13-nC30 nC17-nC20
Zooplankton	same as phyto		same as phyto
Higher plants	even/odd CPI max C28-C30	C28, C30, C32	odd/even max C29-C31

- Lipids in higher plants mainly associated with leaf cuticles ("waxes")
- Serve as physical protection

Distribution of n-alkanes in immature sedimentary rocks



Esterified lipids

Diglycerides and Triglycerides ("fats")

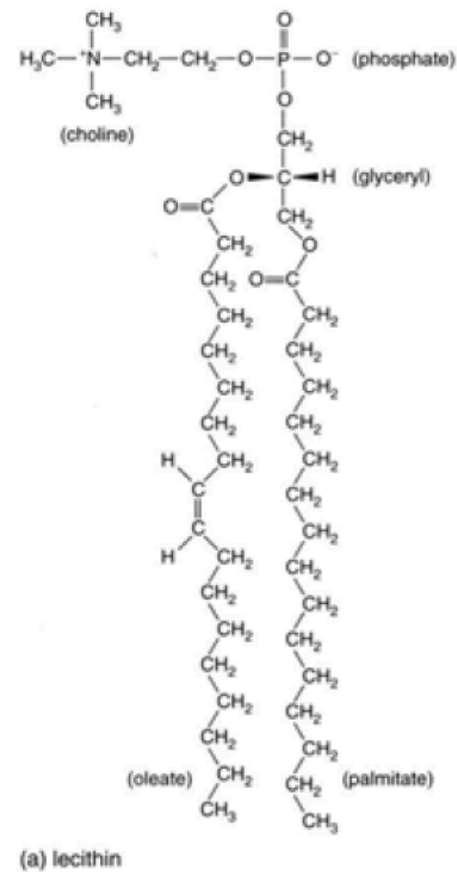
- Esters of glycerol + fatty acids
- formed by condensation ($-H_2O$) reactions
- fatty acids usually straight-chain (C_{14} - C_{18}) with various levels of unsaturation

Wax esters

- Esters comprising of a fatty acid + fatty alcohol

Phospholipids

- Fatty acid(s) + phosphoric acid + glycerol (+ basic nitrogen)



Phospholipid

Phospholipids in POM from the North Pacific Subtropical Gyre

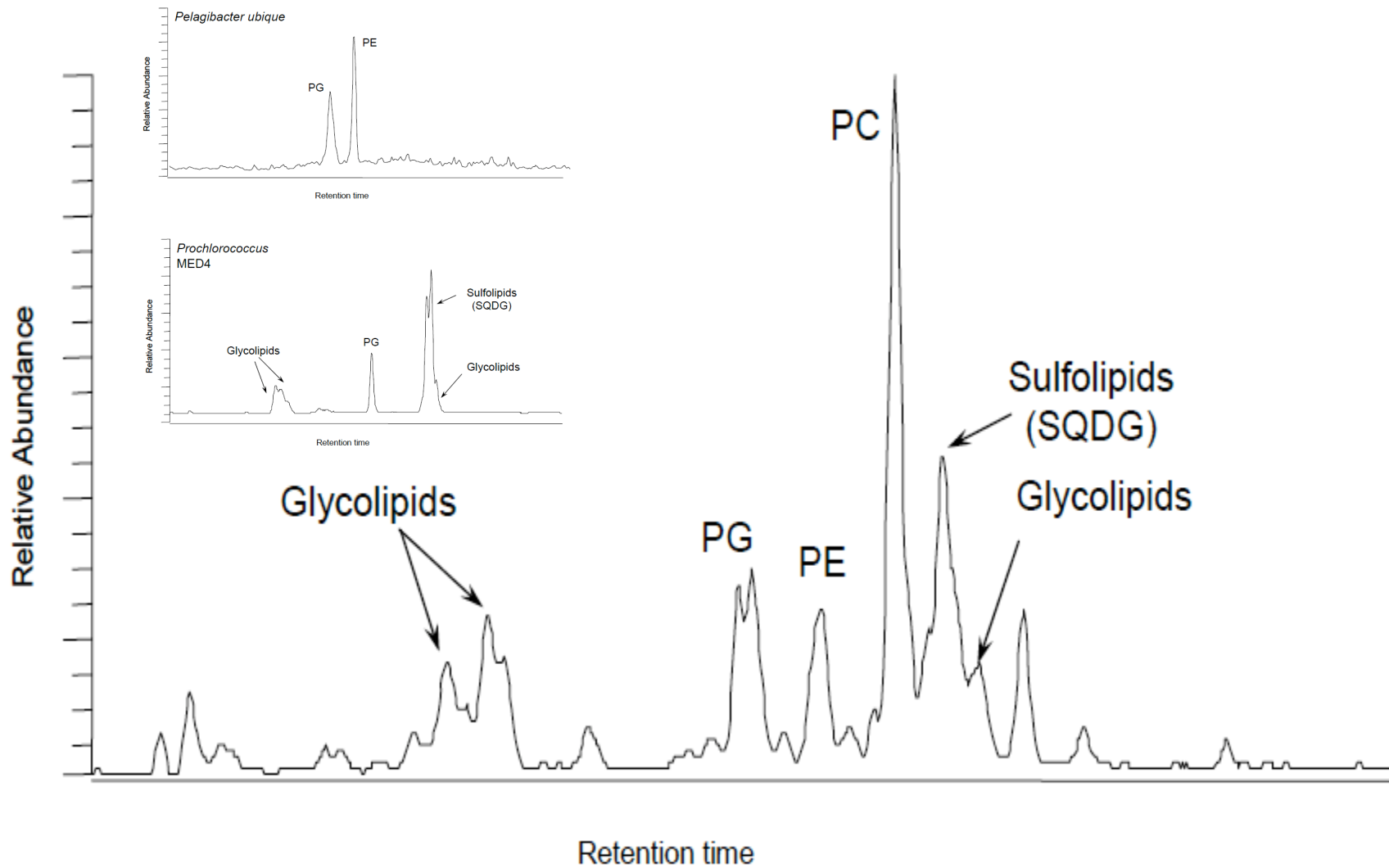
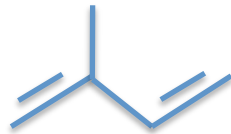


Figure from Ben van Mooy

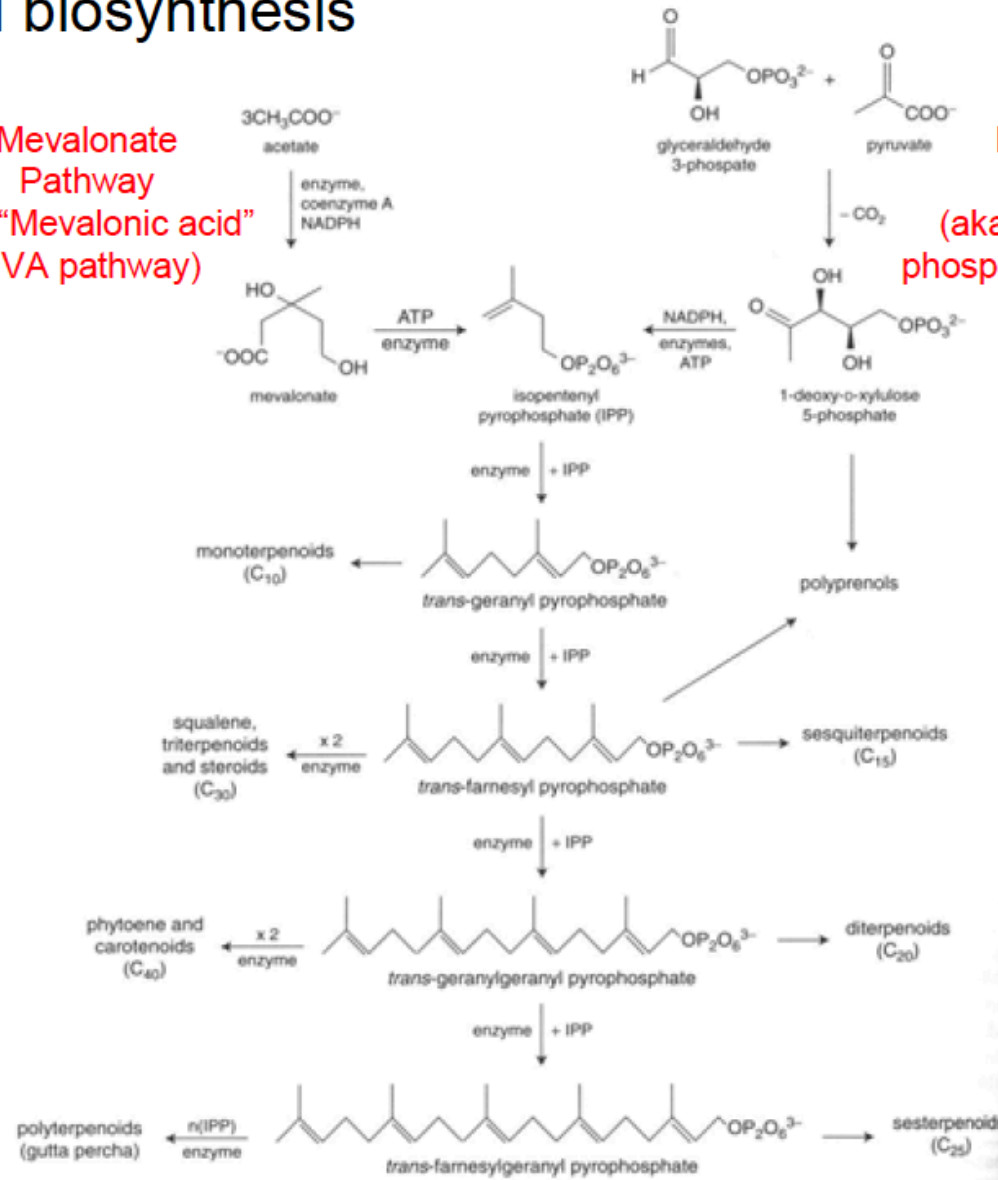
Isoprenoid lipid biosynthesis



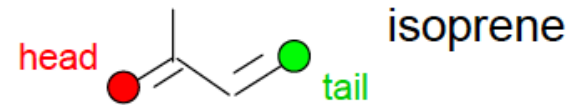
isoprene

Mevalonate Pathway
(aka "Mevalonic acid" MVA pathway)

Deoxyxylulose Pathway
(aka "methylerythritol-phosphate" MEP pathway)

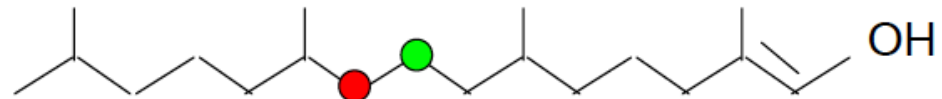


Isoprenoid lipids - Configurations



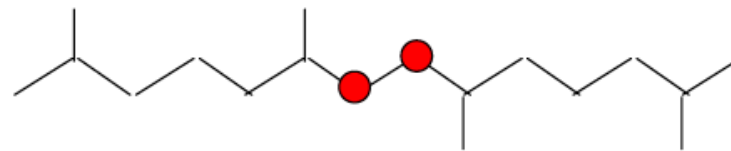
Regular isoprenoids

- head-to-tail
 - e.g. phytol

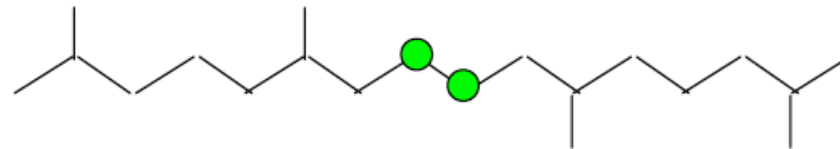


Irregular isoprenoids

- head-to-head



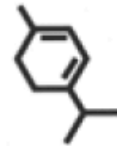
- tail-to-tail



Group II. Polyisoprenoid lipids

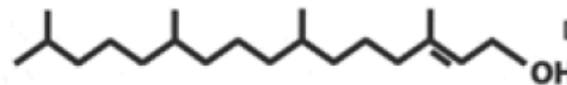
Biosynthetically related to the polymerization of isoprene (C₅)

- dimer - monoterpene (C₁₀)
 - e.g. essential oils
- trimer - sesquiterpenes (C₁₅)
 - e.g. farnesol, abeitic acid
- tetramer - diterpenes (C₂₀)
 - phytol
- hexamer - triterpenes (C₃₀)
 - steroids, hopanoids
- octamer - tetraterpenes (C₄₀)
 - carotenoids, ether lipids
- polymer - polyterpenes
 - e.g. natural resins (polycadinene)



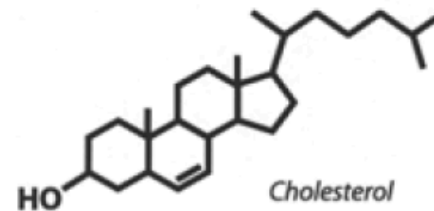
α-terpinene

Monoterpenes C₁₀



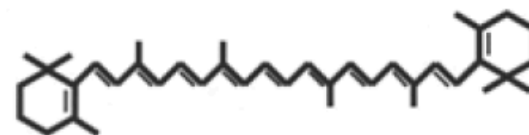
phytol

Diterpenes C₂₀



Cholesterol

Triterpenes C₃₀



β-carotene

Tetraterpenes C₄₀

Isoprenoid lipids

Cyclic Triterpenoids (C30)

- Squalene - main biosynthetic precursor to cyclic triterpenes.
- An irregular isoprenoid (tail-to-tail)
- *Pentacyclic triterpenoids*
- oleanane type
- ursane type
- lupane type
- hopane type
- gammacerane type
- arborane type
- primary sources: bacteria, higher plants

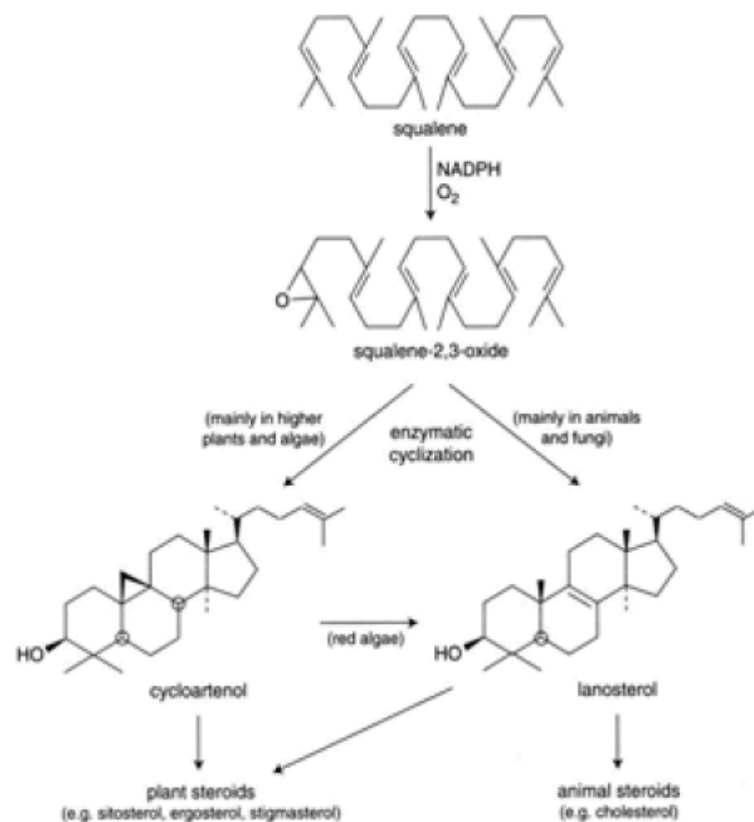


Fig. 2.21 Steroid biosynthesis from squalene (after Harwood & Russell 1984).

Pentacyclic triterpenoids

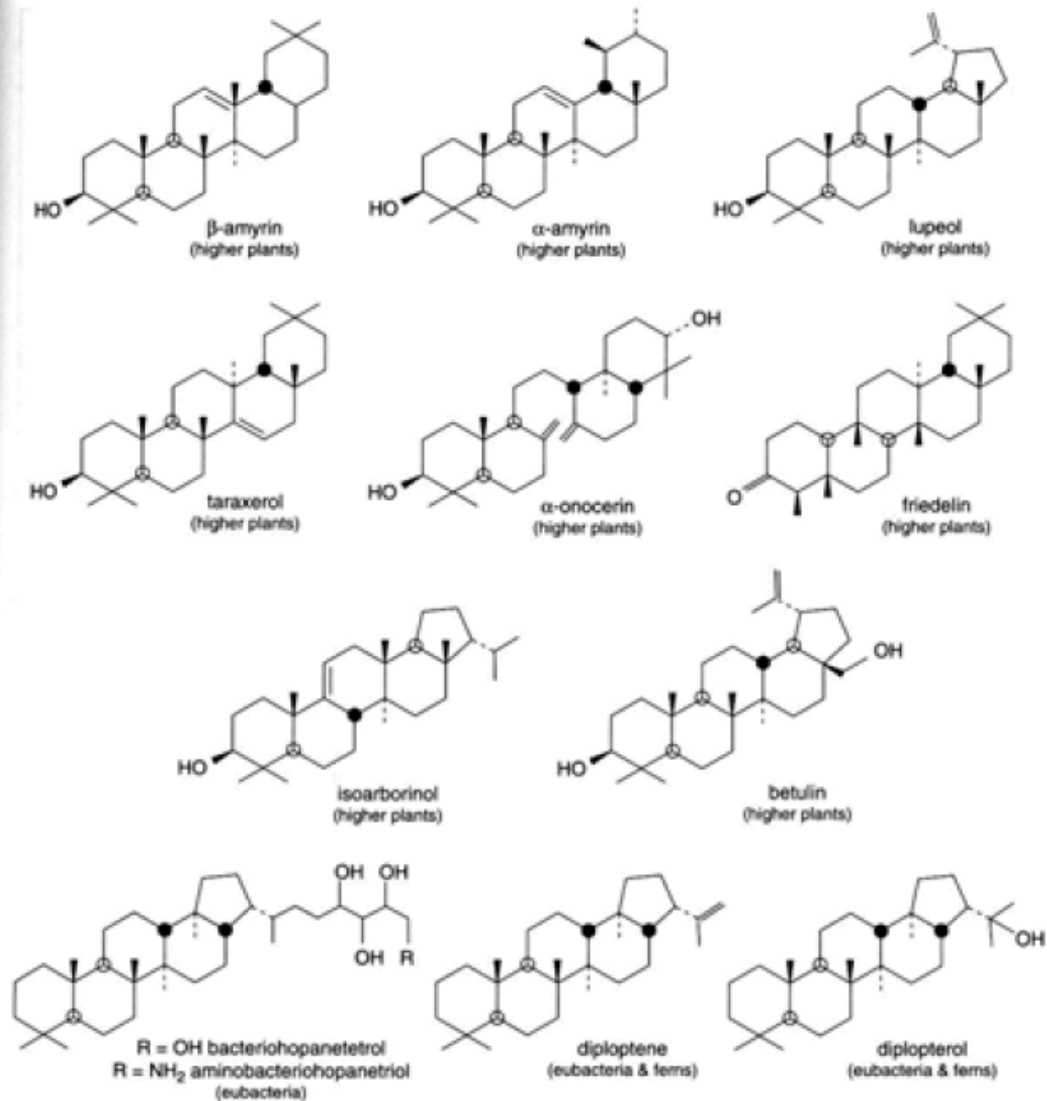
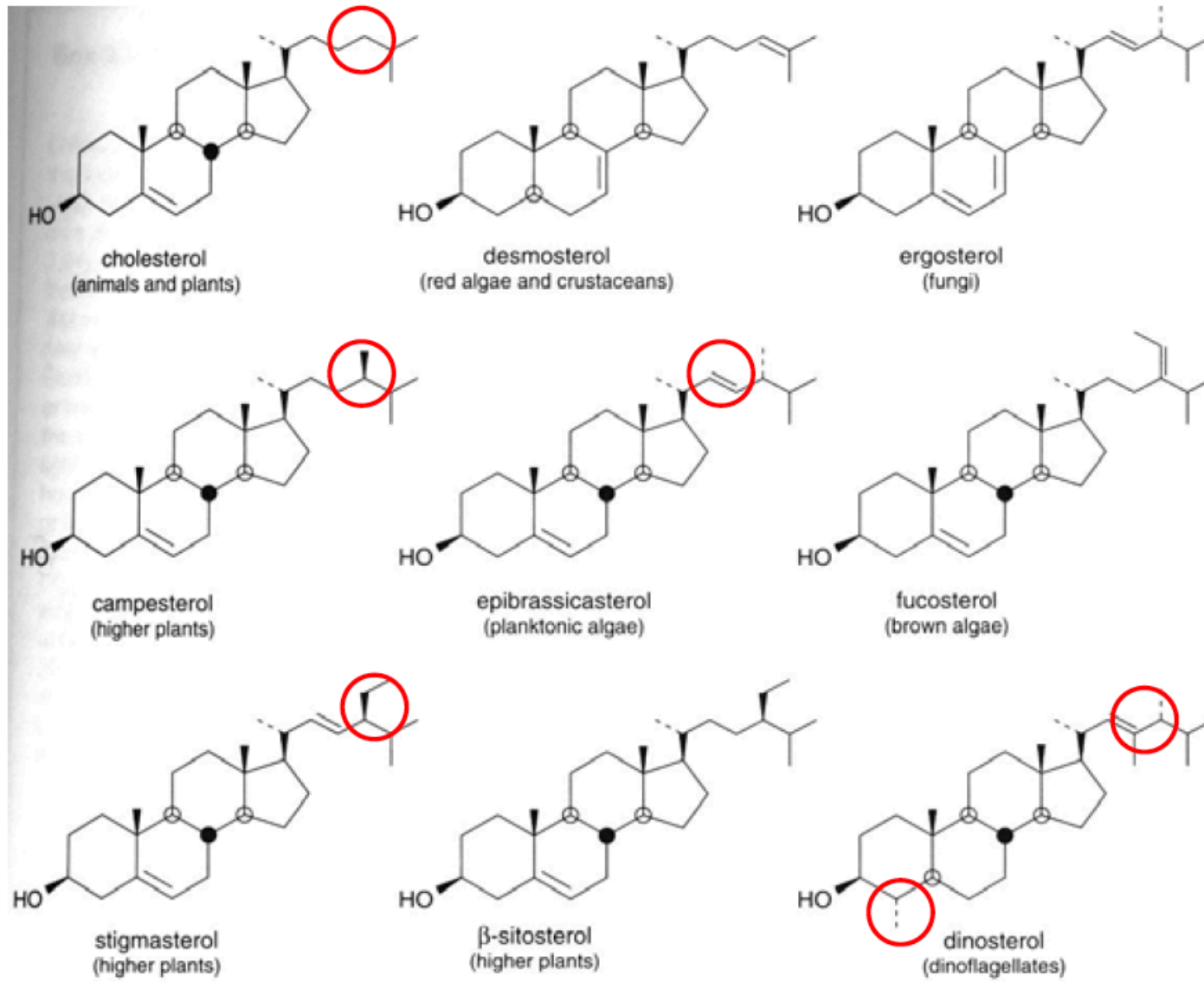
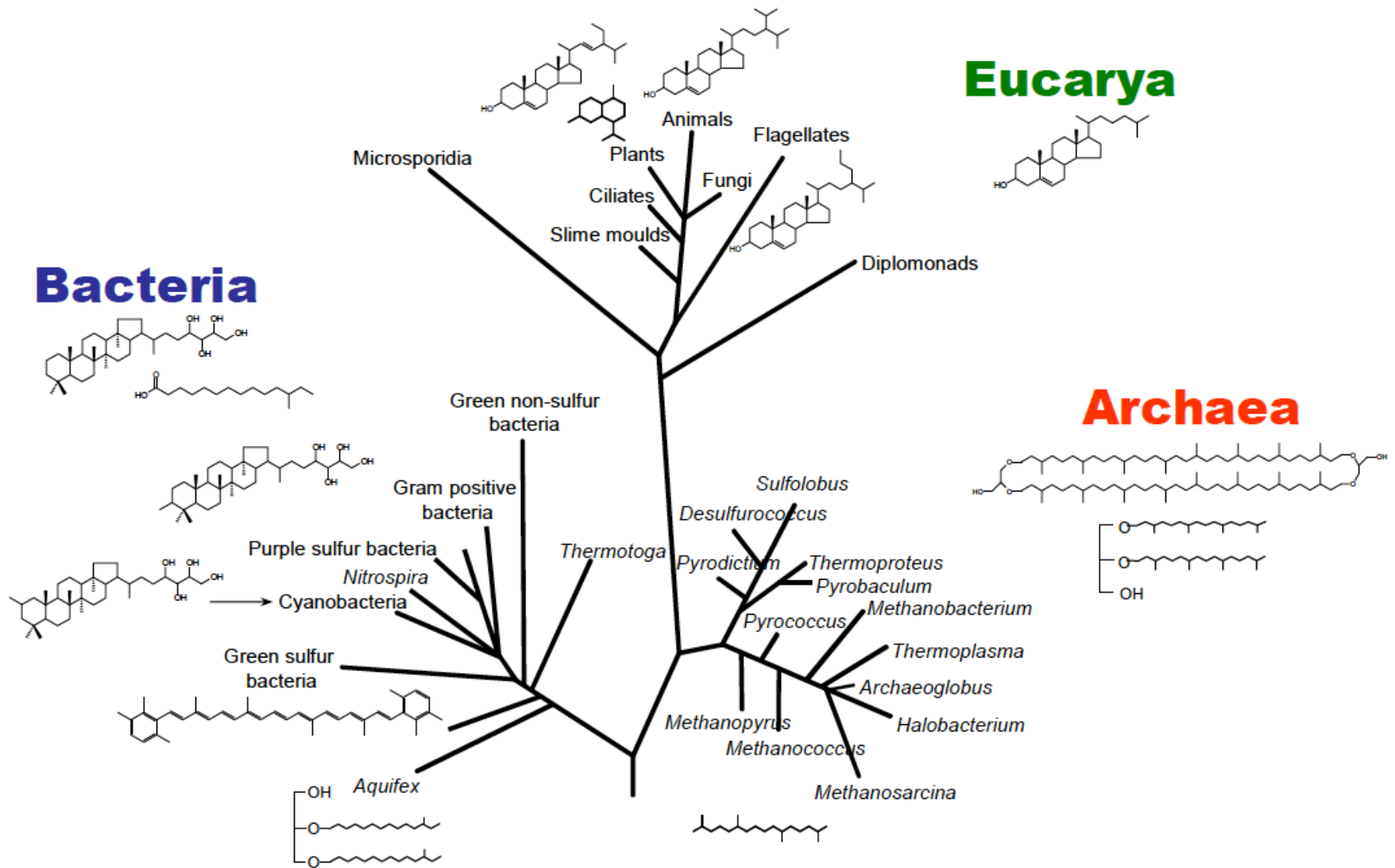


Fig. 2.19 Some geochemically important polycyclic triterpenoids and their major sources.

Steroids



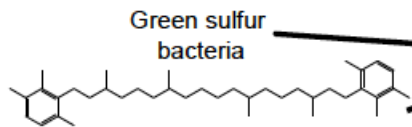
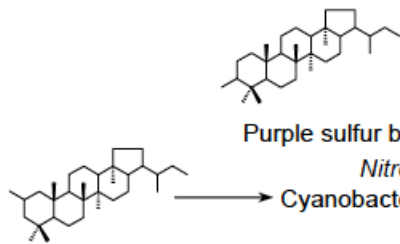
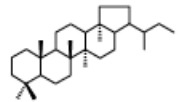
The Biological Precursors...



rRNA tree modified after Woese

...and their Molecular Fossils

Bacteria



Aquifex

Green non-sulfur bacteria

Gram positive bacteria

Purple sulfur bacteria

Nitrospira

Cyanobacteria

Green sulfur bacteria

Green non-sulfur bacteria

Thermotoga

Sulfolobus

Desulfurococcus

Pyrodictum

Thermoproteus

Pyrobaculum

Pyrococcus

Methanobacterium

Thermoplasma

Archaeoglobus

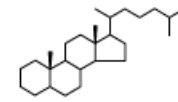
Halobacterium

Methanopyrus

Methanococcus

Methanosarcina

Eucarya



Microsporidia

Slime moulds

Ciliates

Plants

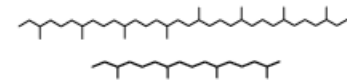
Animals

Fungi

Flagellates

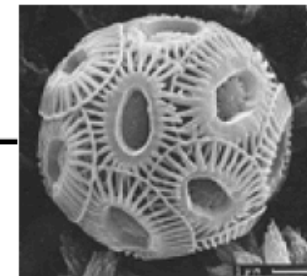
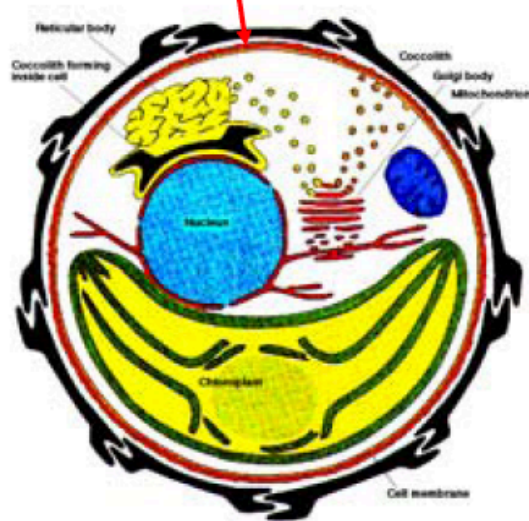
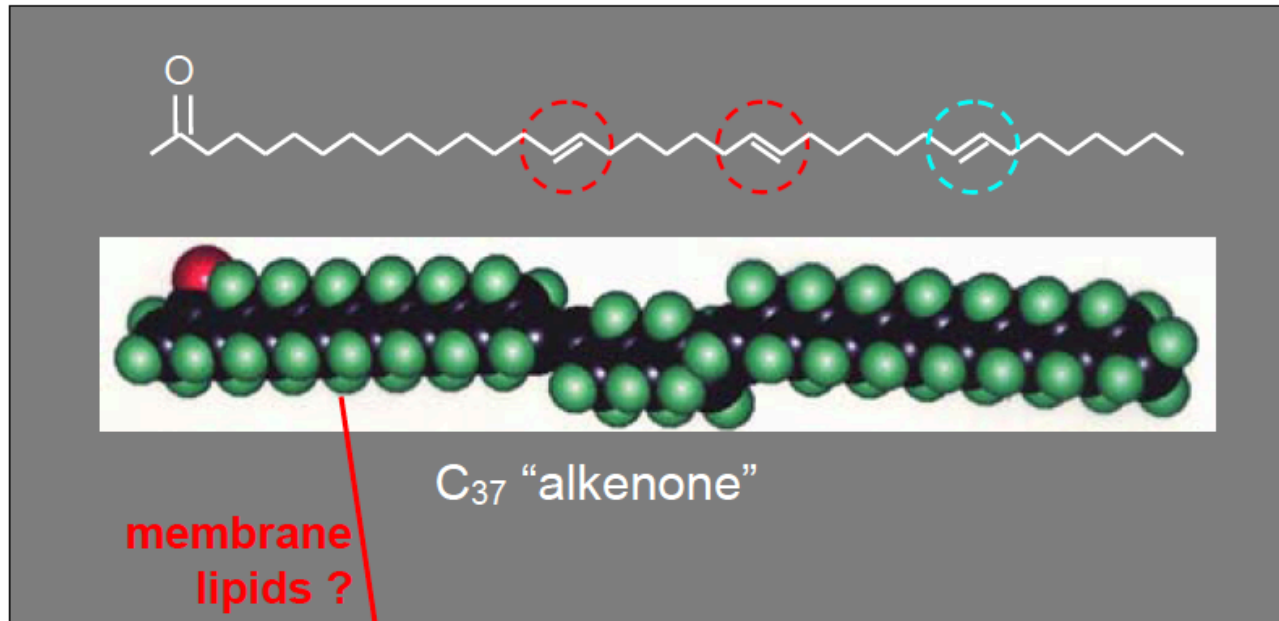
Diplomonads

Archaea



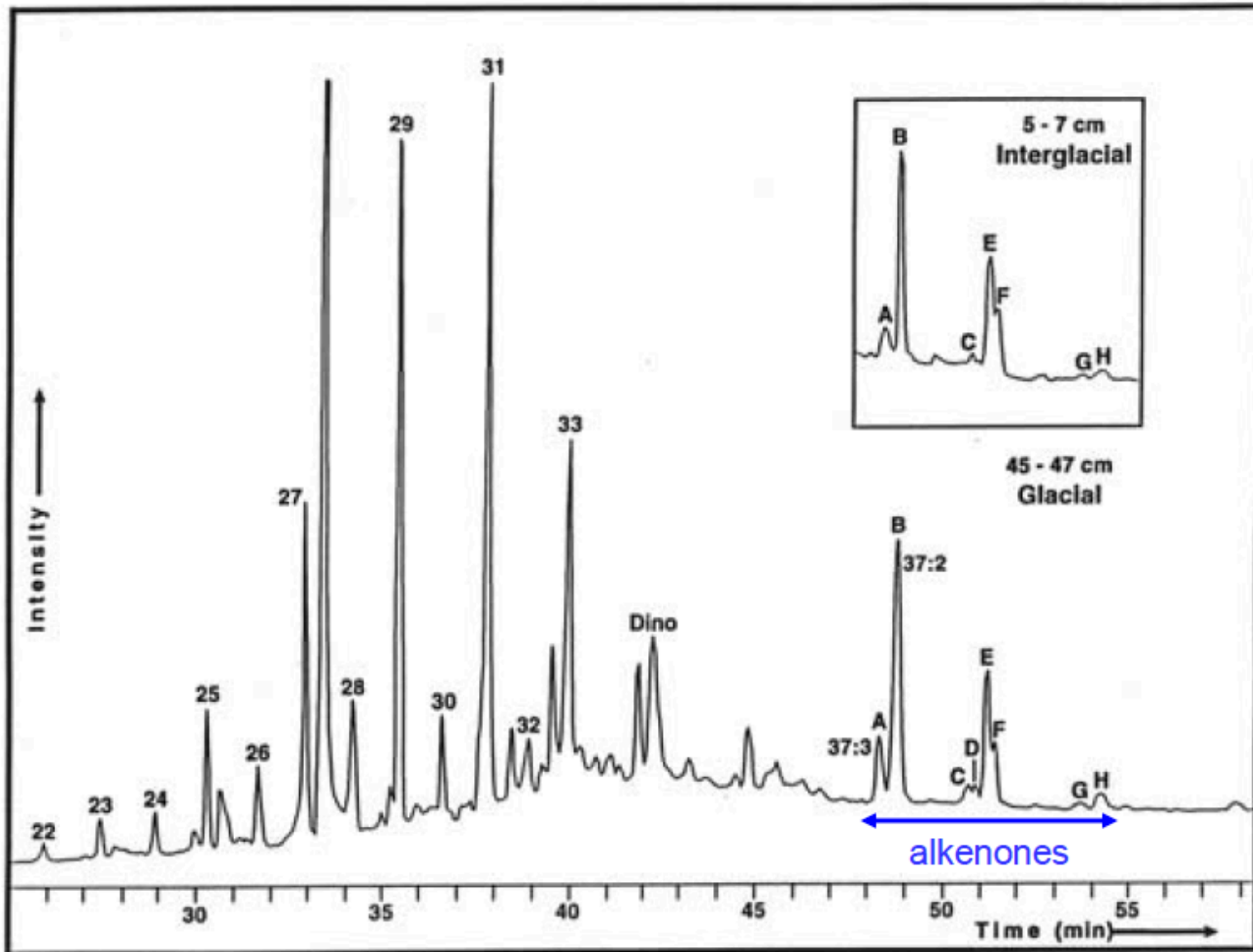
rRNA tree modified after Woese

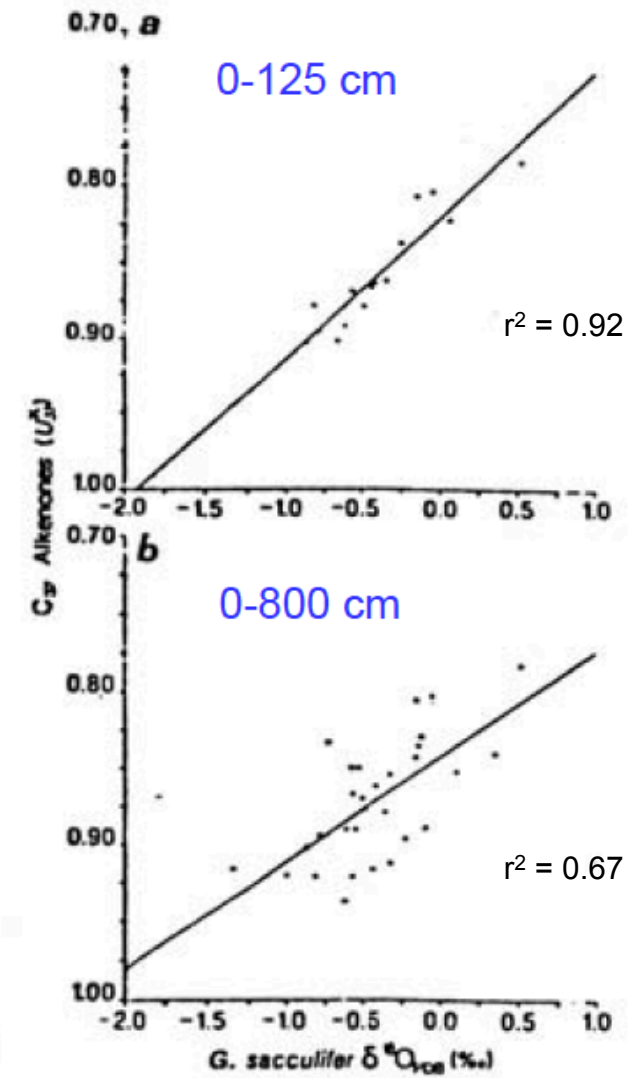
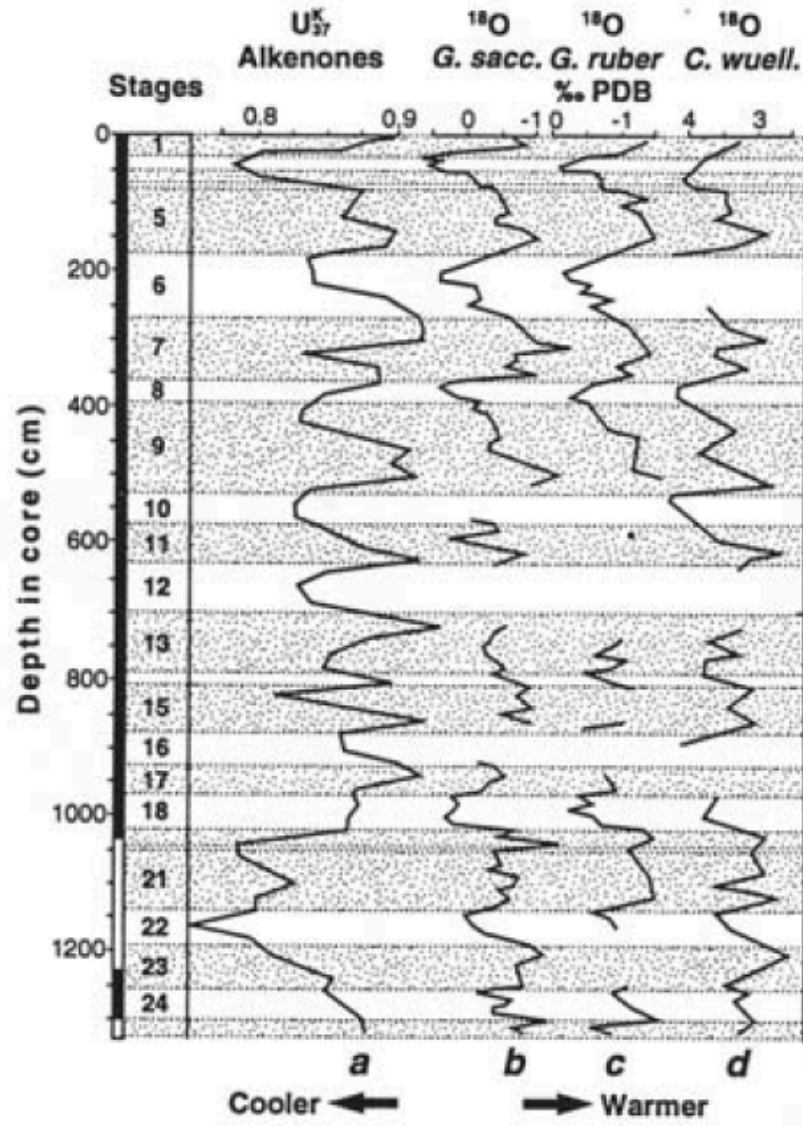
Alkenones – Magical Molecules!



Photomicrograph of *E. Huxleyi*

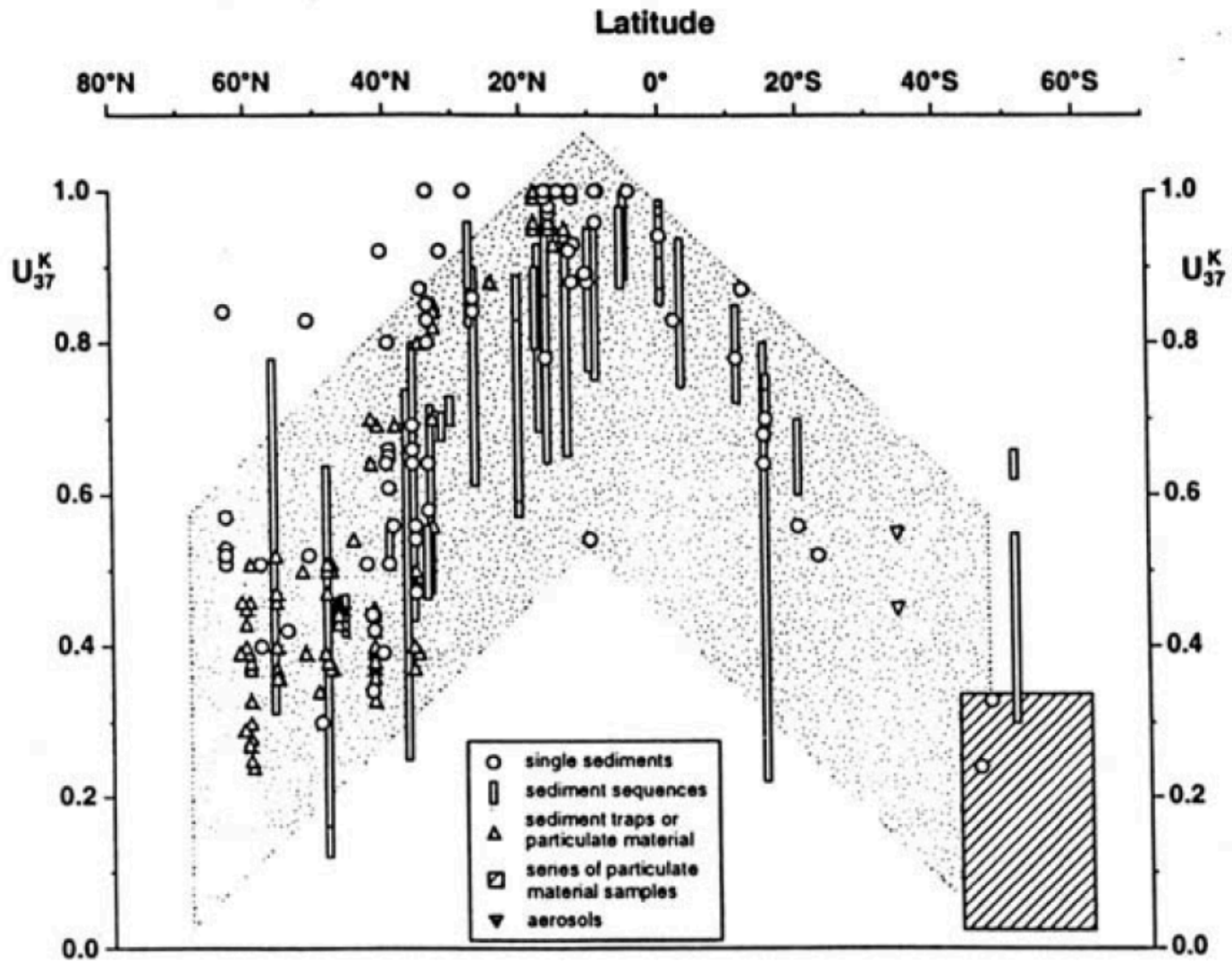
Gas chromatograph of TLE of Kane Gap sediments





Brassell et al., 1986

Latitudinal variations in U_{37}^K values of sediments and particulate samples



Alkenone Unsaturation as an Indicator of SST

Fundamental relationship

- A decrease in temperature leads to an increase in the degree of unsaturation
- Initial ratio:

$$U_{37}^K = [C37:2]-[C37:4]/[C37:2+C37:3+C37:4]$$

(Brassell et al., 1986)

- Modified to:

$$U_{37}' = [C37:2]/[C37:2 + C37:3]$$

(Prahl and Wakeham, 1987)

- *Ratio can be measured very precisely (GC-FID)*

Calibration

- Most commonly used:

$$U_{37}' = 0.033T + 0.043 \text{ (Prahl and Wakeham, 1987)}$$

$$U_{37}' = 0.033T + 0.044 \text{ (core-top calibration of Muller et al. 1998).}$$

- Accuracy of SST estimation: $\pm 1^\circ\text{C}$ (in open ocean, temperate and sub-polar waters)

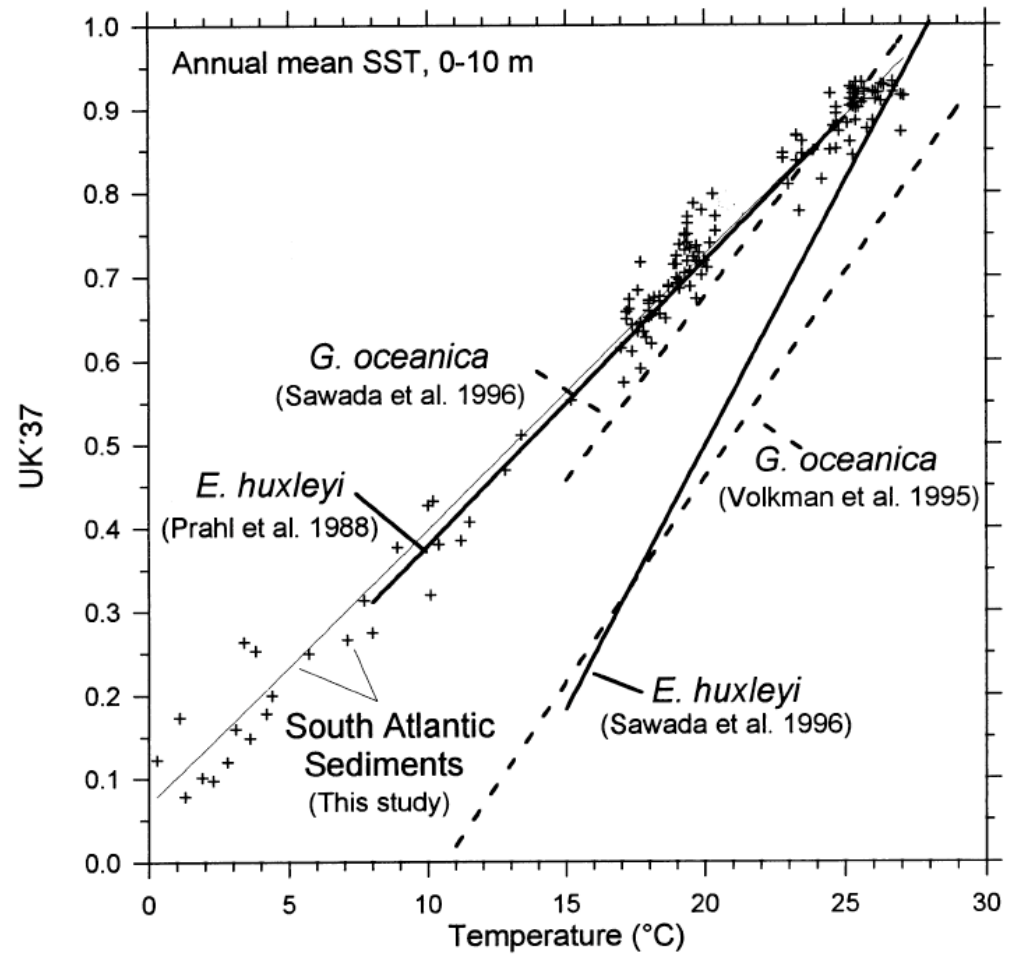
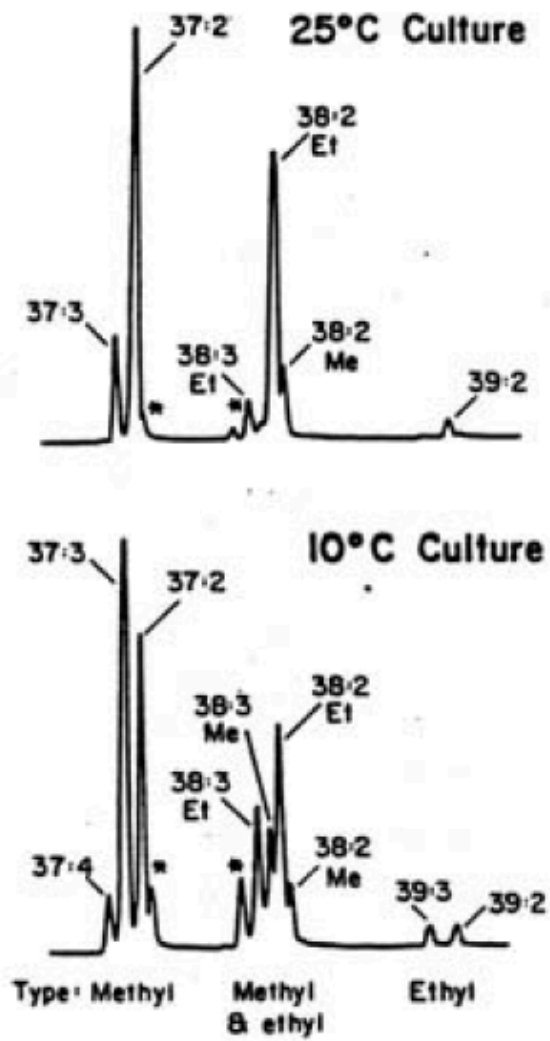
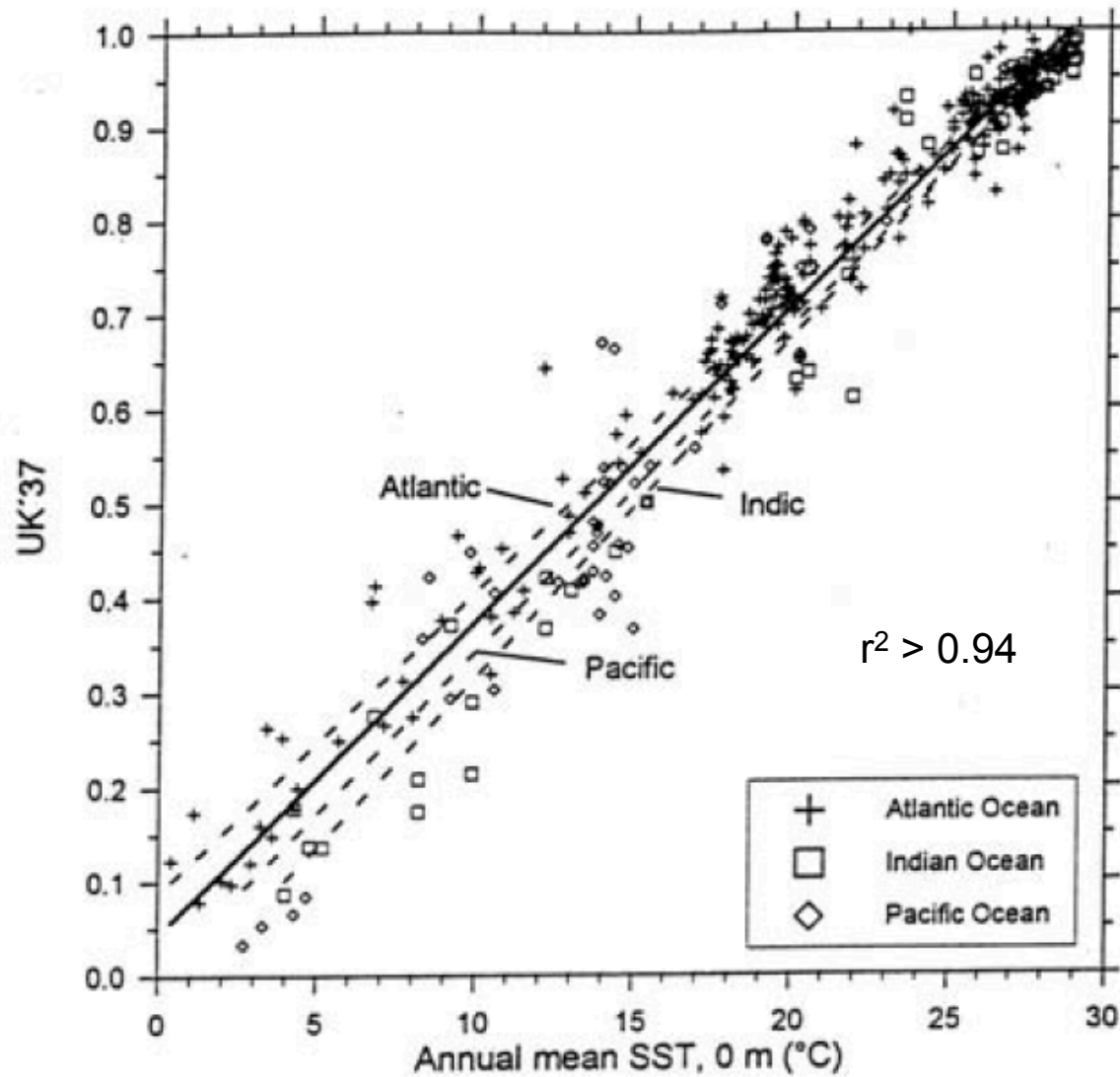


Fig. 6. Comparison between the U_{37}^K -SST calibration for the eastern South Atlantic using annual mean SST at 0–10 m water depth (crosses and thin solid line) and published culture calibrations for *E. huxleyi* (thick solid lines) and *G. oceanica* (dashed lines) (see Table 1 for the equations). The excellent agreement between the sediment-based calibration and the culture equation of Prahl et al. (1988) indicates that the North Pacific strain cultured by these authors is also representative for the alkenone-synthesizing algae in the South Atlantic.

Prahl & Wakeham, 1988

Global core-top calibration of UK_{37} vs SST

(Muller et al., 1998)



Introduction to biomarkers- Summary

“Biomarkers” or “chemical fossils” are organic compounds with a particular carbon skeleton that can be related to a known biological precursor.

Some biomarkers are synthesized by a restricted number of organisms while others are widely distributed.

Biomarkers are particularly useful in identifying sources of organic matter in samples where the morphological remains of organisms has long since disappeared.

In contemporary systems, biomarkers are used to explore the distribution of organisms, their metabolic state, or determine rates for specific processes.

In sediments, application of biomarkers assumes that their distribution in contemporary organisms is similar to their distribution in organisms living in the distant past. e.g. biosynthetic pathways are conserved over millions of years.

Biomarkers are also used as proxies for environmental parameters such as temperature, salinity, presence of oxygen & oxycline depth, etc.

One of the most useful biomarkers has been long chain, unsaturated alkenones which are useful proxies for sea surface temperature.