

Phylogeny of Bacterial Lipids

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Figure 2 Phylogenetic tree illustrating the major lineages (phyla) of the domain Bacteria. **Wedges shown in black are the 12 original phyla**, as described by Woese (112), **in white are the 14 phyla with cultivated representatives** recognized since 1987, and **in gray are the 26 candidate phyla** that contain no known cultivated representatives. This evolutionary distance dendrogram was constructed by the comparative analysis of over 600 nearly full-length 16S ribosomal RNA gene sequences using the ARB sequence analysis software package (65), selected from a larger database of over 12,000 sequences. A modified version of the “Lane mask” was employed in this analysis (55), along with the Olsen evolutionary distance correction and neighbor-joining tree-building algorithm. Horizontal wedge distances indicate the degree of divergence within a given phylum. The scale bar corresponds to 0.05 changes per nucleotide position. Phylum names are designated by selecting the first applicable option out of the following: (a) their convention in *Bergey’s Manual of Systematic Bacteriology*, if it exists (36); (b) the first described representative genus within the phylum if it has cultivated representatives; (c) the first label given to a candidate phylum if previously published; or (d) the first clones or environment where the first clones were retrieved, for previously unnamed candidate phyla.

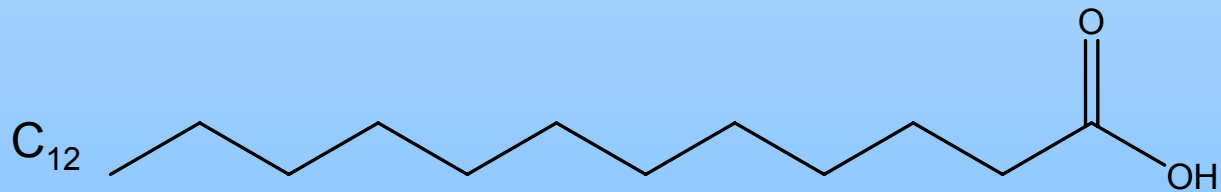
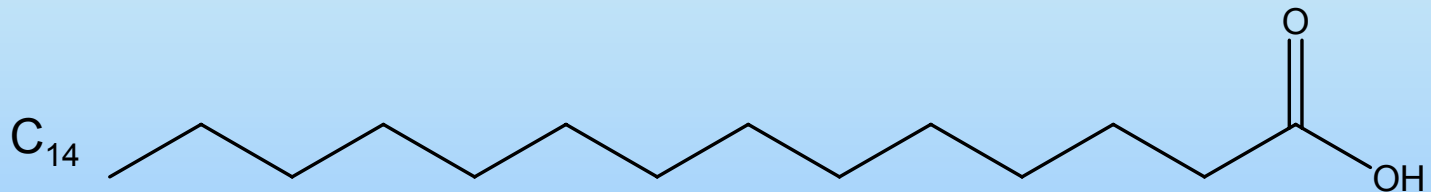
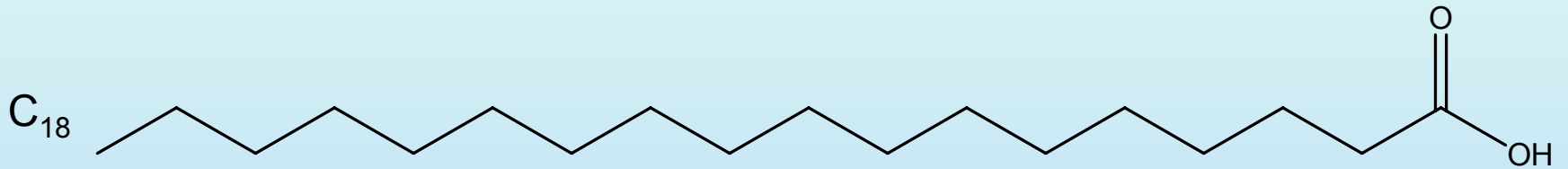
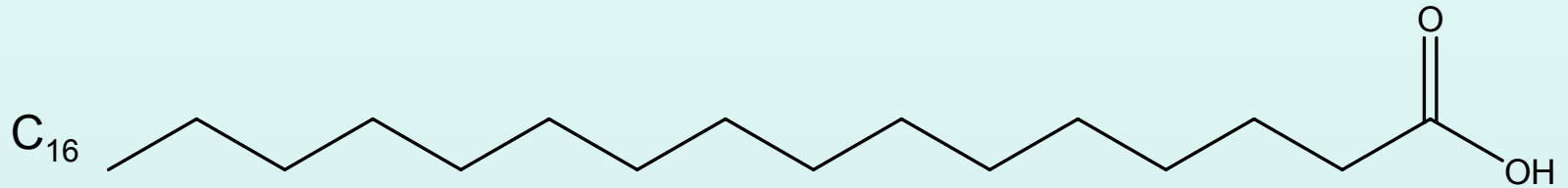
Outline

- Fatty Acids
- Hopanoids
- Pigment Biomarkers
- Porphyrins
- Special Bacterial Lipids
- Steroids?
- Note on Isotopes
- Papers

Fatty Acids

- Higher even-number saturated fatty acids (C_{12-24}) are a significant contribution to total lipid content
- C_{16} is the most common fatty acid in bacteria
 - C_{18} , C_{14} and C_{12} are also common
- C_{20-28} are less common
 - except in extremophiles
- Odd numbered compounds C_{15} and C_{17} have been reported in bacteria

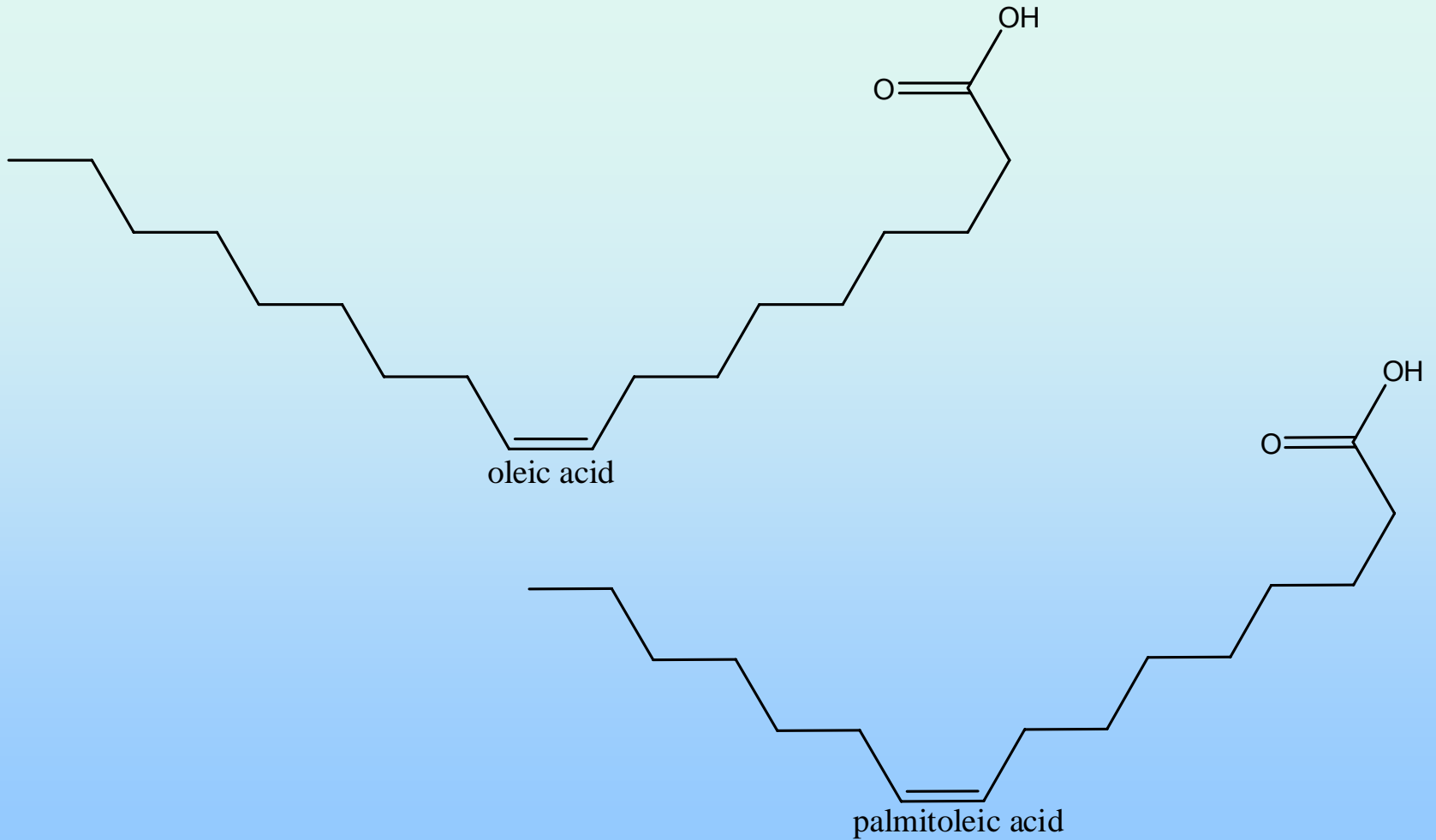
Fatty Acids



Fatty Acids

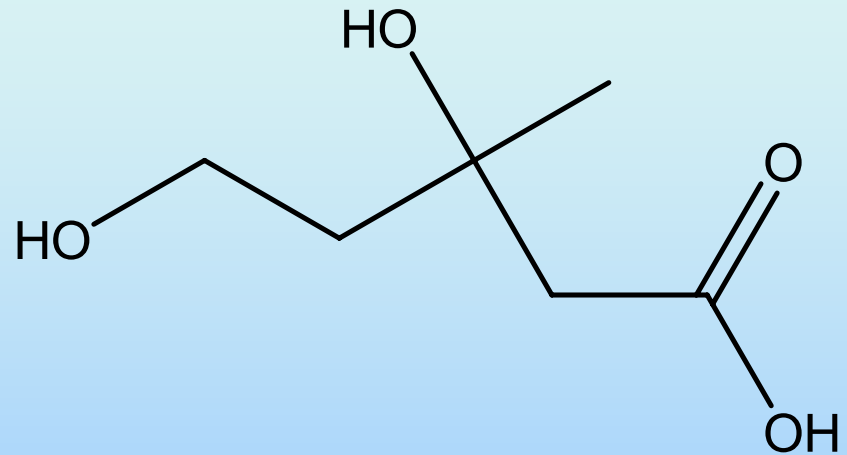
- Unsaturated fatty acids are also important components to the total lipid content of bacteria
- Most common are octadecanoic acid and hexadecanoic acid
 - Occasionally, but not always, oleic acid and palmitoleic acid – specific placement of double bond
- Less common, though still present, are unsaturated carbon compounds with C_{10-14} and C_{20-28}

Fatty Acids



Fatty Acids

- Hydroxy, branched chain and cyclopropane acids have been identified in small amounts in few groups of bacteria
 - Mid-chain monomethyl alkanes are produced by cyanobacteria

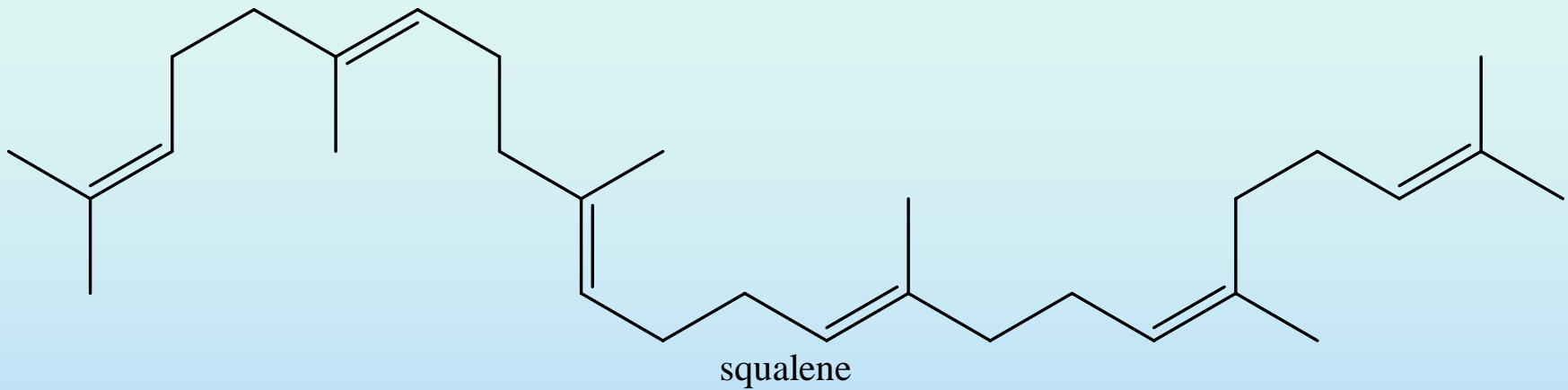


mevalonic acid

Hopanoids

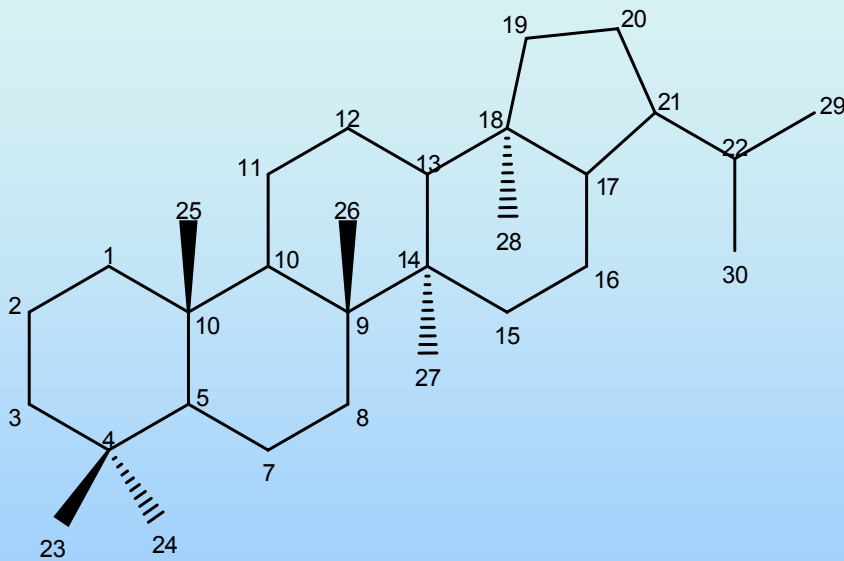
- C_{30} compounds found in diverse bacterial lineages
- Some hopanes are produced by eukaryotes (plants) but C_{31} to C_{35} (homohopanes) are exclusively bacterial and originate from bacteriohopanetetrol and other polyfunctional C_{35} hopanoids

Squalene



- In bacteria, squalene-hopene cyclase is used to turn this compound into generic C₃₀ pentacyclic triterpenoid hydrocarbon skeleton
- Can be highly associated with certain archaea

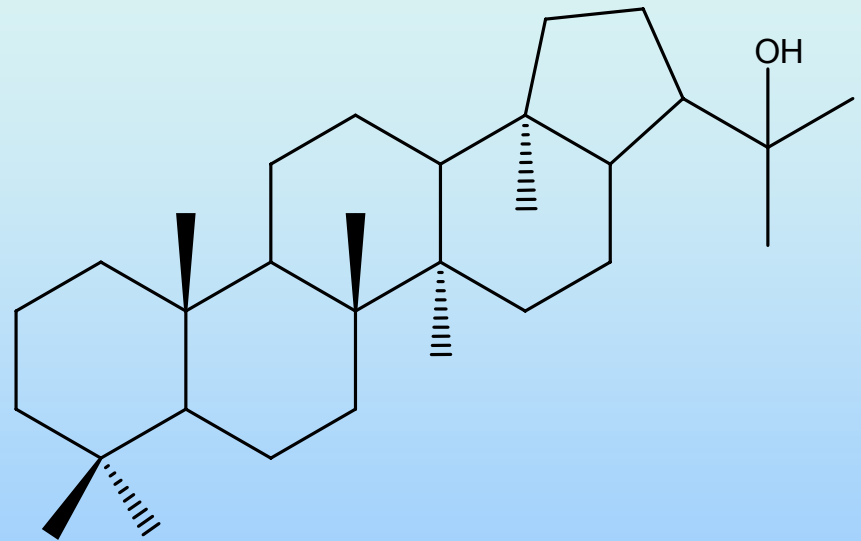
Hopanoids



- General C₃₀ hydrocarbon structure
- Biological stereochemistry ββ(22R)
- Skeleton preserved with some rearrangements in fossil hydrocarbons

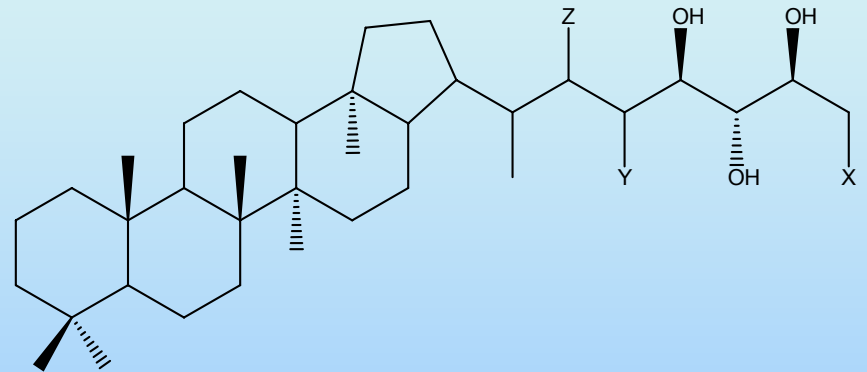
Hopanoids

- Diplopterol
- Synthesized by aerobic bacteria, including methanotrophic species
 - Evidence for anaerobic synthesis
- Can be cyclized directly from squalene (no O₂ required)



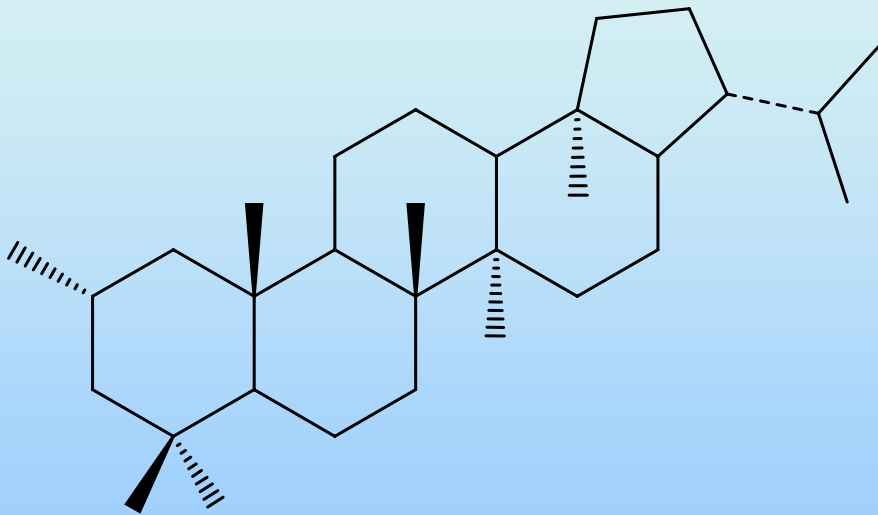
Hopanoids

- Bacteriohopanepolyol - BHP
- Functional hopanoids in Bacteria are often in the form of BHP
- X,Y,Z can be substituted with other -OH groups, sugars, amino acids or other functionalized units
- Side chain carbons are derived from ribose



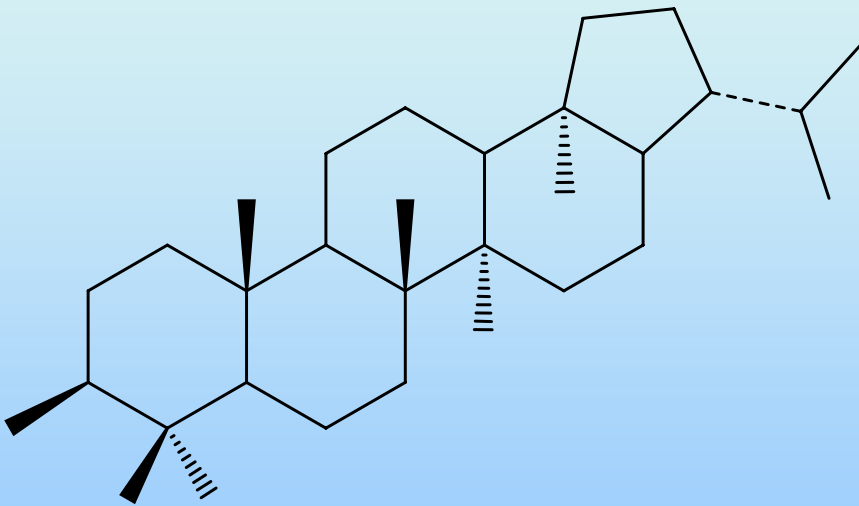
Hopanoids

- 2 α -Me Hopane
- Biomarkers for Cyanobacteria (prochlorophytes)



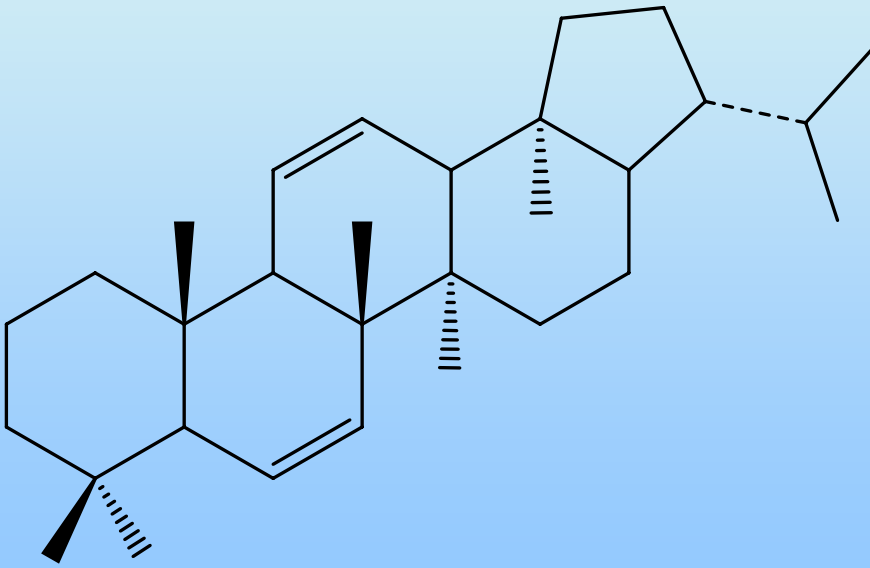
Hopanoids

- 3 β -Me Hopane
- Biomarkers for microaerophilic proteobacteria (methylotrophs, methanotrophs and acetic acid bacteria)

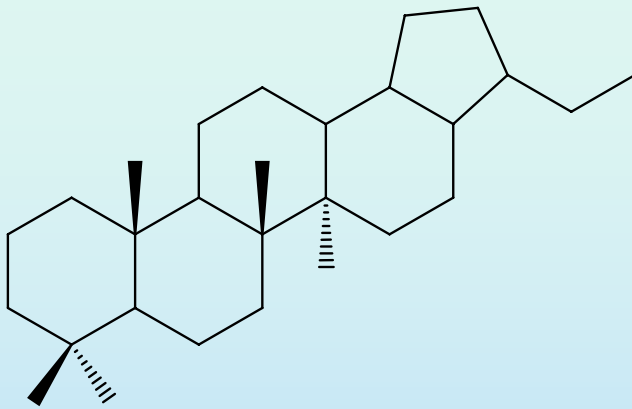


Hopanoids

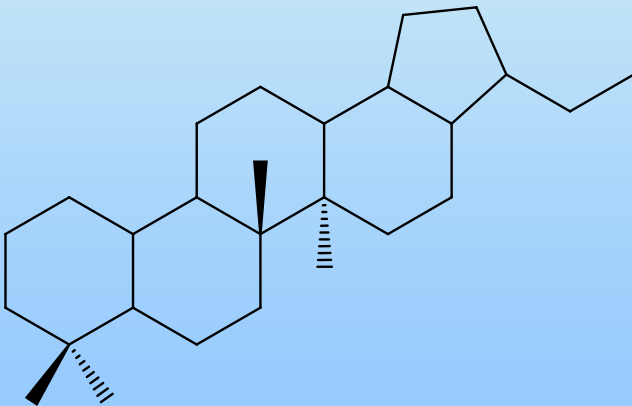
- C₃₀ hydrocarbon structure with Δ^6 and/or Δ^{11}
- Acetic acid bacteria



Hopanoids



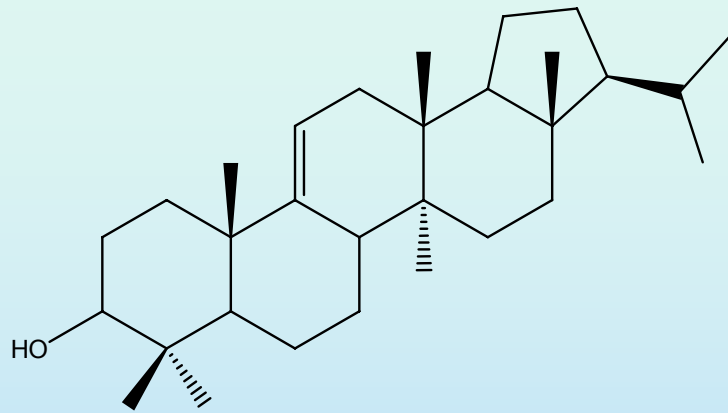
28,30-bisnorhopane



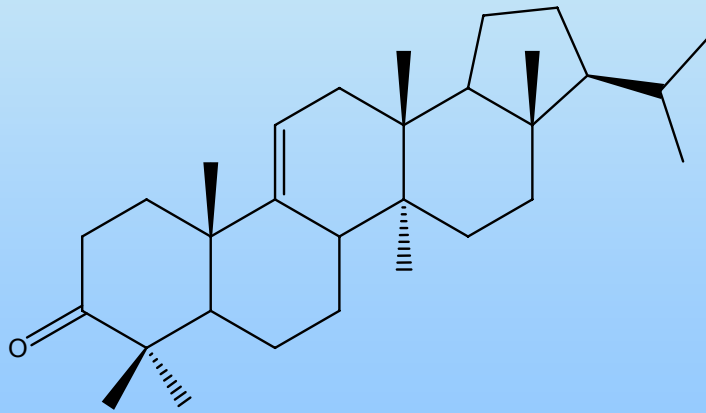
25,28,30-trisnorhopane

- 28,30-bisnorhopane (BNH) and 25,28,30-trisnorhopane (TNH) are believed to originate from chemolithoautotrophic bacteria growing at the oxic-anoxic interface
 - *Beggiatoa*?
 - *Thioploca*?

Hopanoids



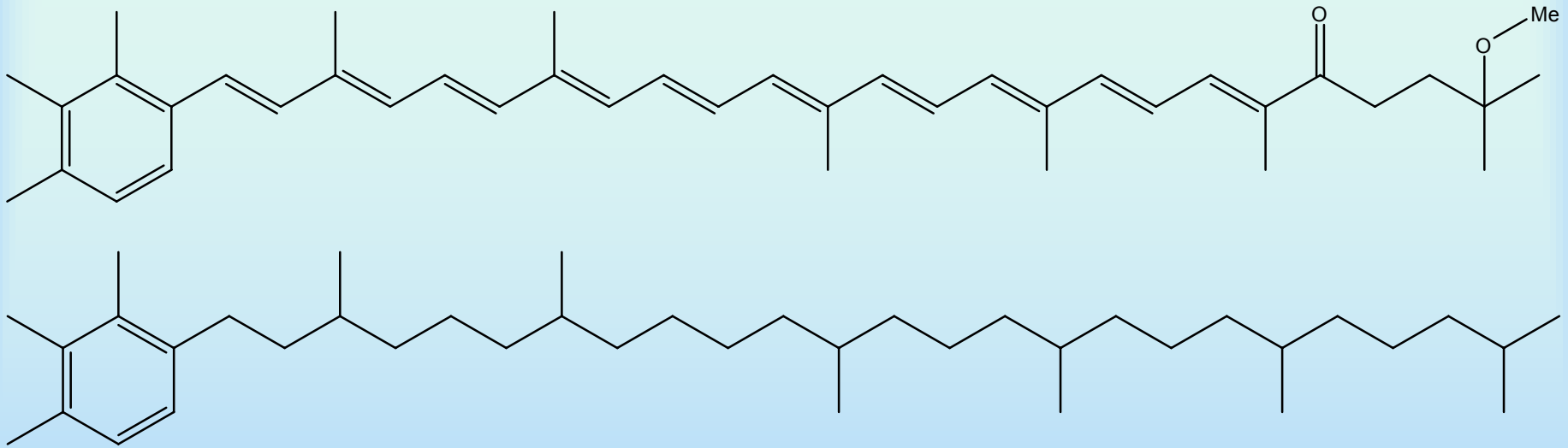
Isoarborinol



Arborinone

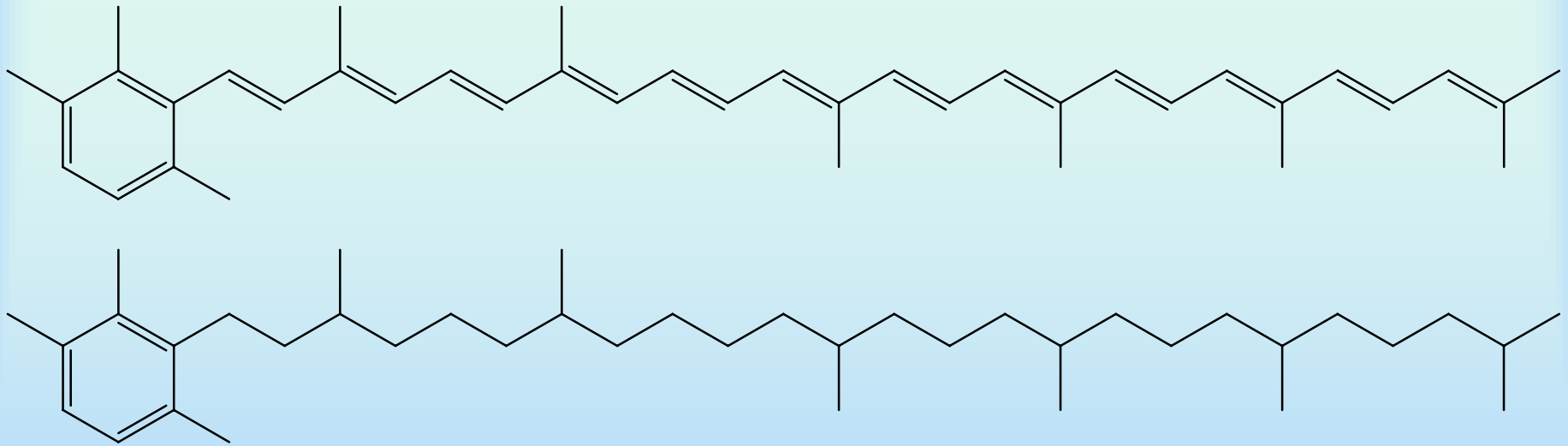
- Isoarborinol and arborinone
- Initially believed to be derived solely from certain families of angiosperms, but identification of derivatives identified in samples predating the evolution of angiosperms indicate a possible bacterial (algal?) source

Pigment Biomarkers



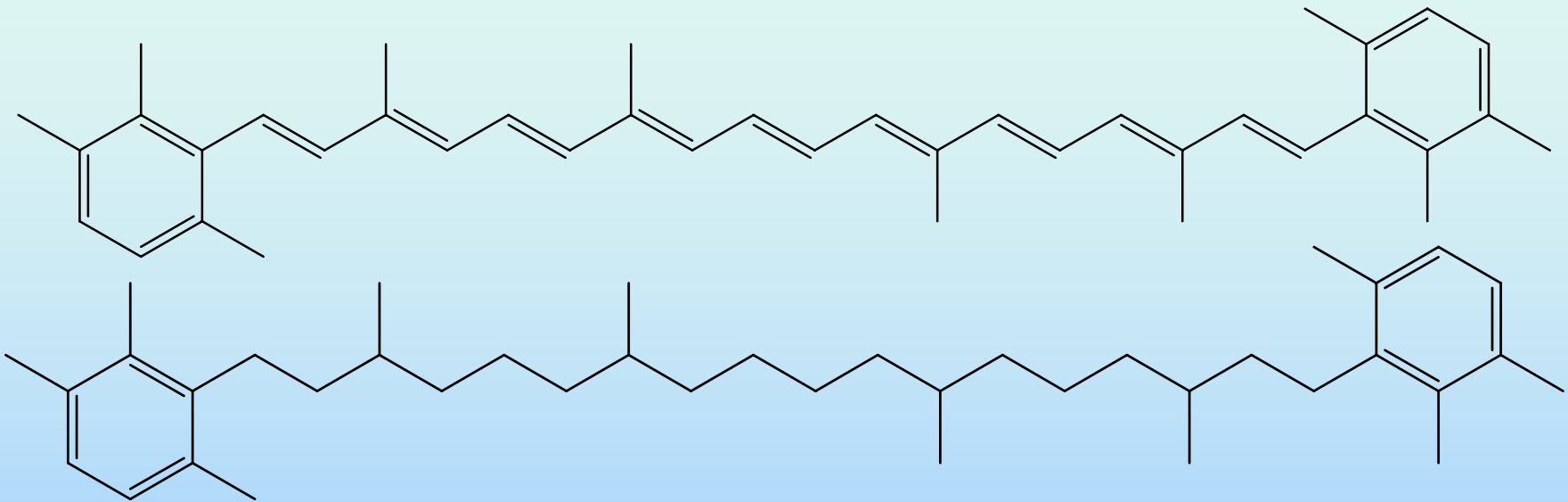
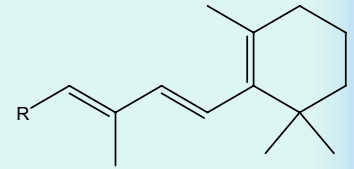
- Okenone/Okenane
- Exclusively known from the planktonic species of *Chromatiaceae* (purple sulfur bacteria)

Pigment Biomarkers



- Chlorobactene/Chlorobactane
- Biomarker for planktonic as well as benthic mat-forming green pigmented species of *Chlorobiaceae* (green sulfur bacteria)

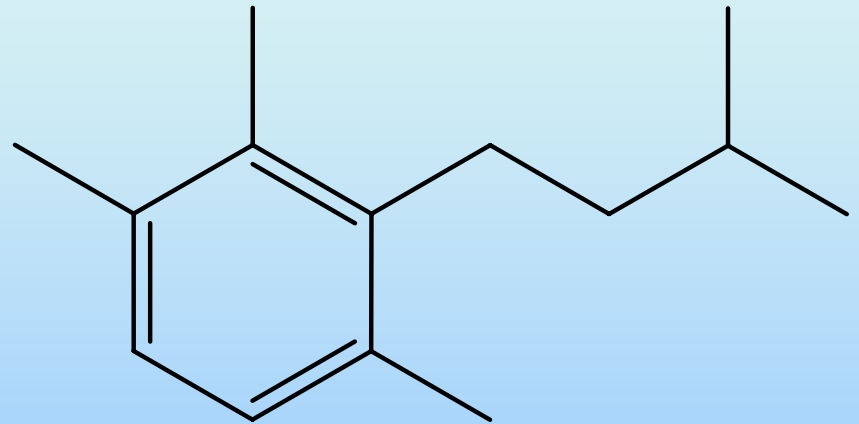
Pigment Biomarkers



- Isorenieratene/Isorenieratane
- Isorenieratane and (β -) isorenieratane are biomarkers for brown pigmented species of *Chlorobiaceae*

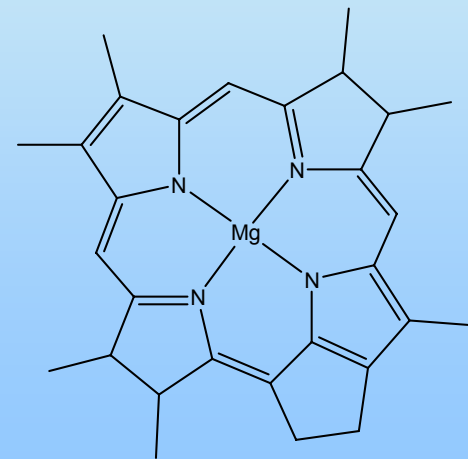
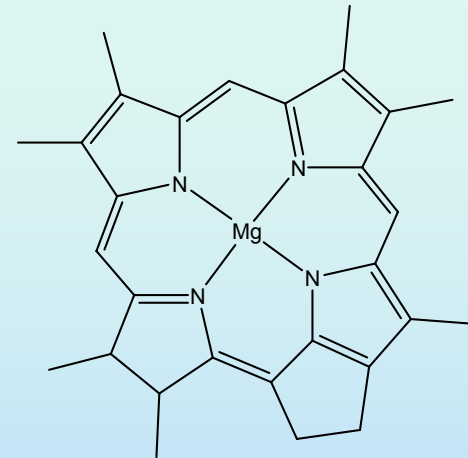
Pigment Biomarkers

- Aryl isoprenoids are products of pigment diagenesis
- 2,3,6- substituted (derived from isorenieratane and/or chlorobactane)

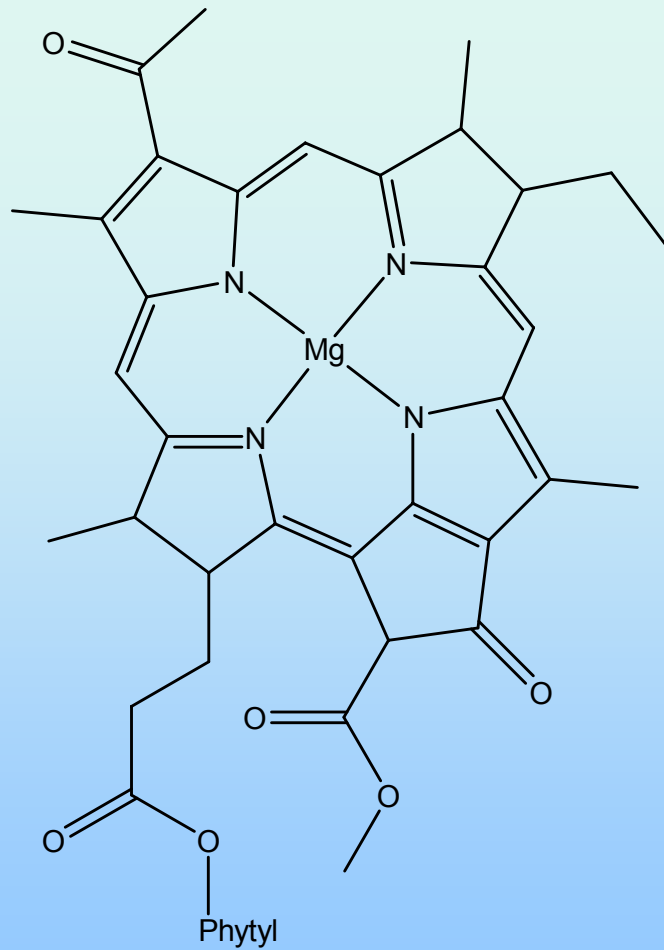


Porphyrins

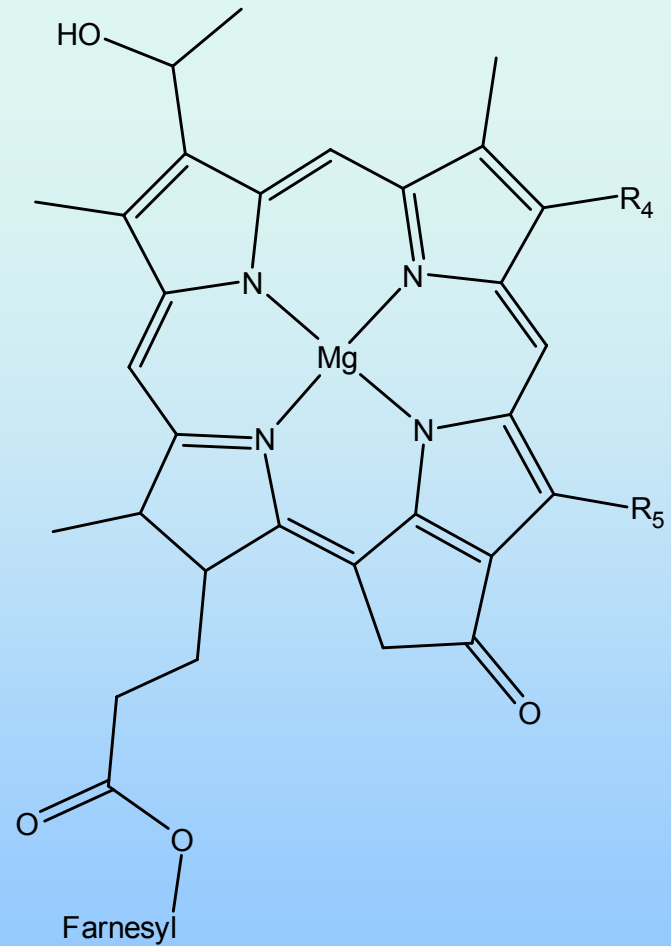
- Bacteriochlorophyll
 - Present in purple sulfur bacteria (a,b), green sulfur bacteria (c,d,e), chloroflexi (c) and heliobacteria (g)
 - c and d as chlorins with one reduced pyrrole ring and the rest as bacteriochlorin with two
- Some bacteria also use chlorophyll



Porphyrins



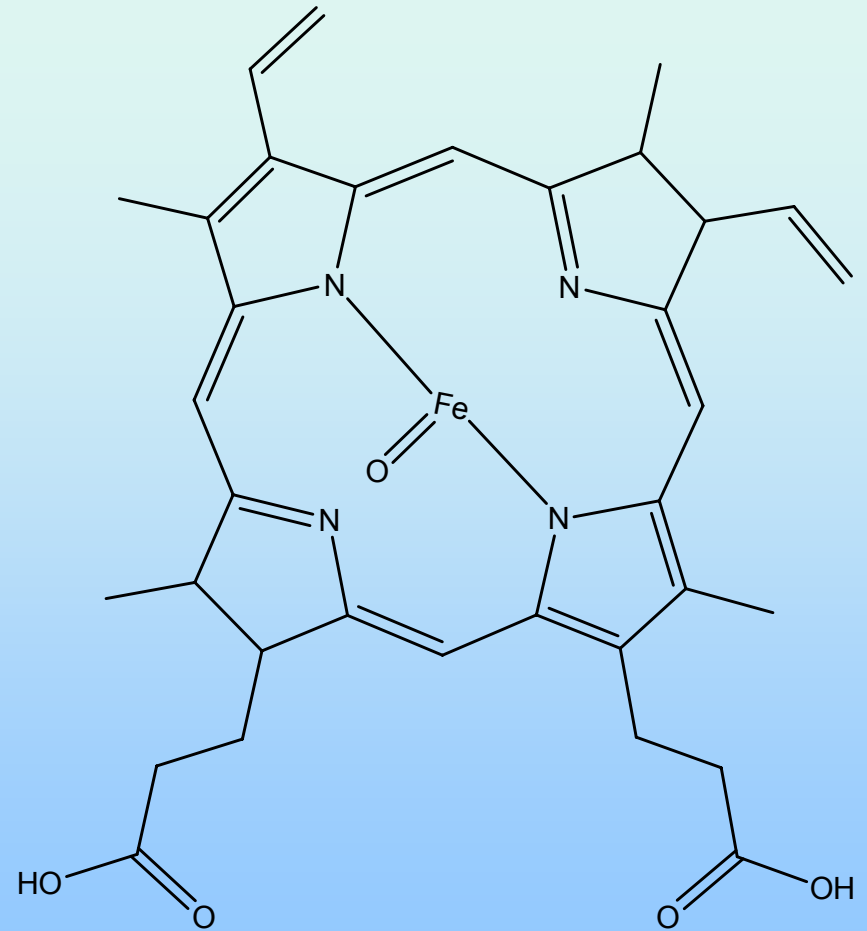
B Chl a – purple sulfur bacteria



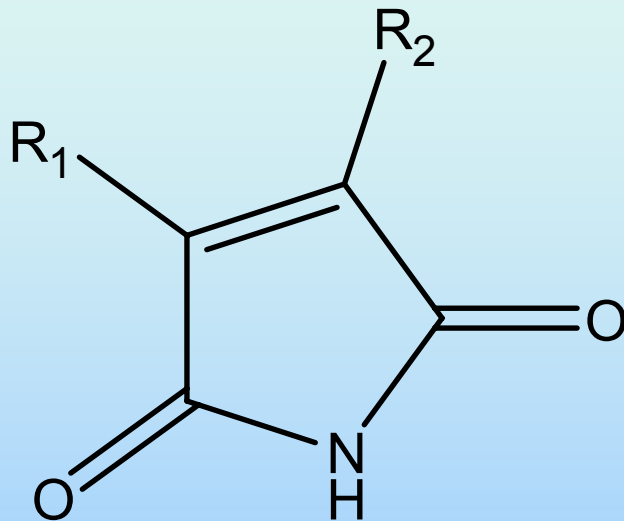
B Chl c – green sulfur bacteria

Porphyrins

- Cytochrome P450
 - Enzyme used in electron transport chain



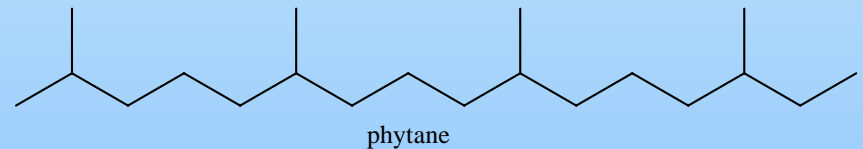
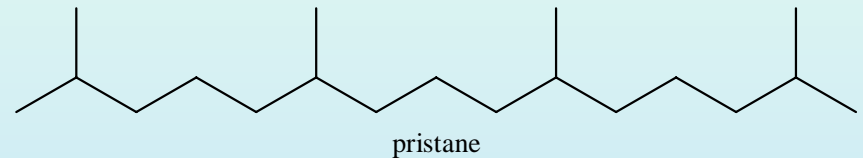
Porphyrins



- Maleimides
- Derive from porphyrins
- R-groups can aid in identification of original compound
 - Often considered biomarkers for bacteriochlorophylls c/d/e in green sulfur bacteria (or chlorophyll a in phytoplankton)

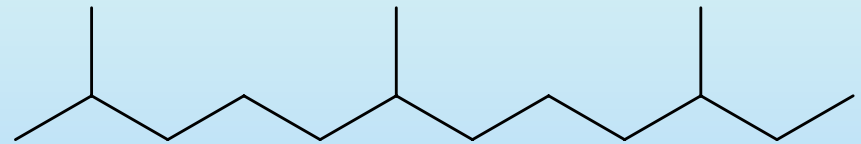
Pristane/Phytane

- Derived from phytyl (phytol) in chlorophyll
 - Oxidic conditions → pristane
 - Suboxidic conditions → phytane
- Careful – can also be derived from other sources



Farnesane

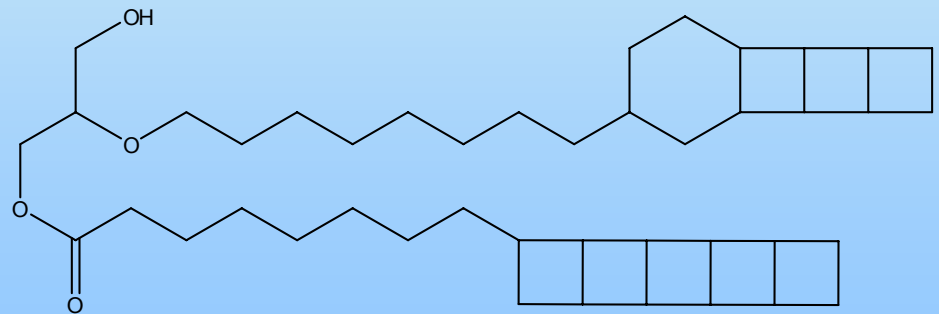
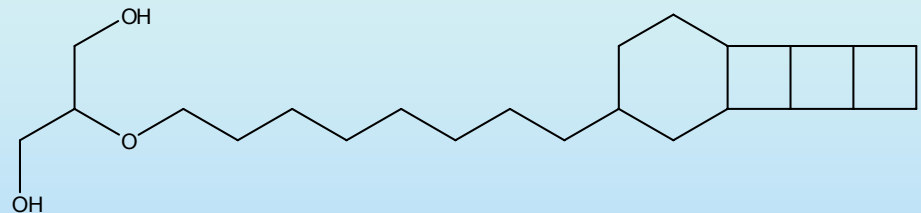
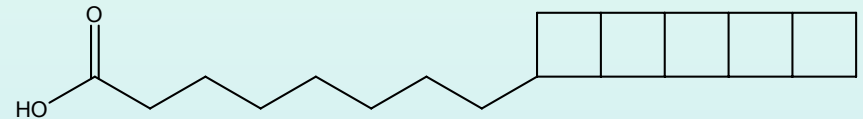
- Similar to pristane/phytane, farnesane is derived from farnesyl as a side chain of bacteriochlorophyll c
 - Green sulfur bacteria



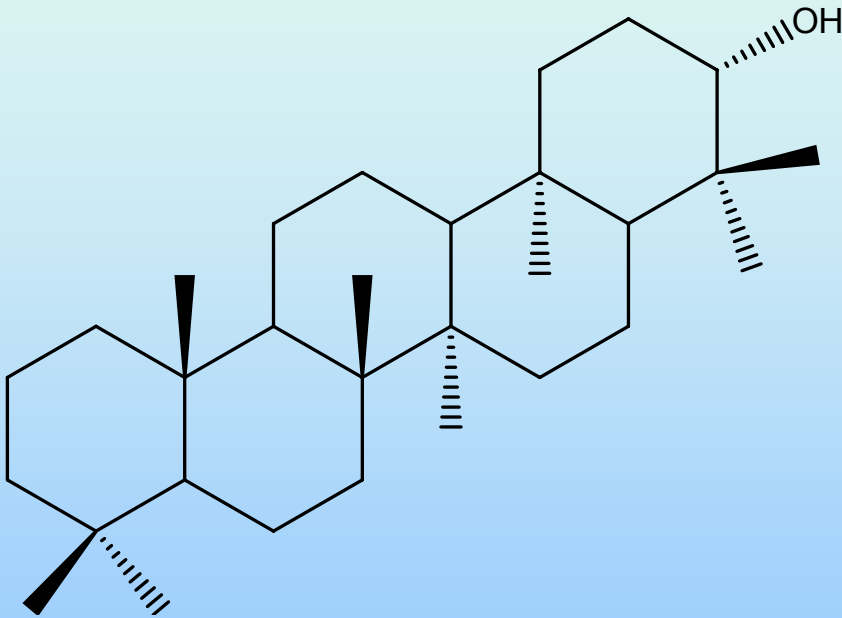
farnesane

Ladderanes

- Lipids specific for bacteria that perform anammox
 - planctomycetes
- Found primarily in anammoxosome as a barrier to diffusion of hydrazine



Tetrahymanol/Gammacerane

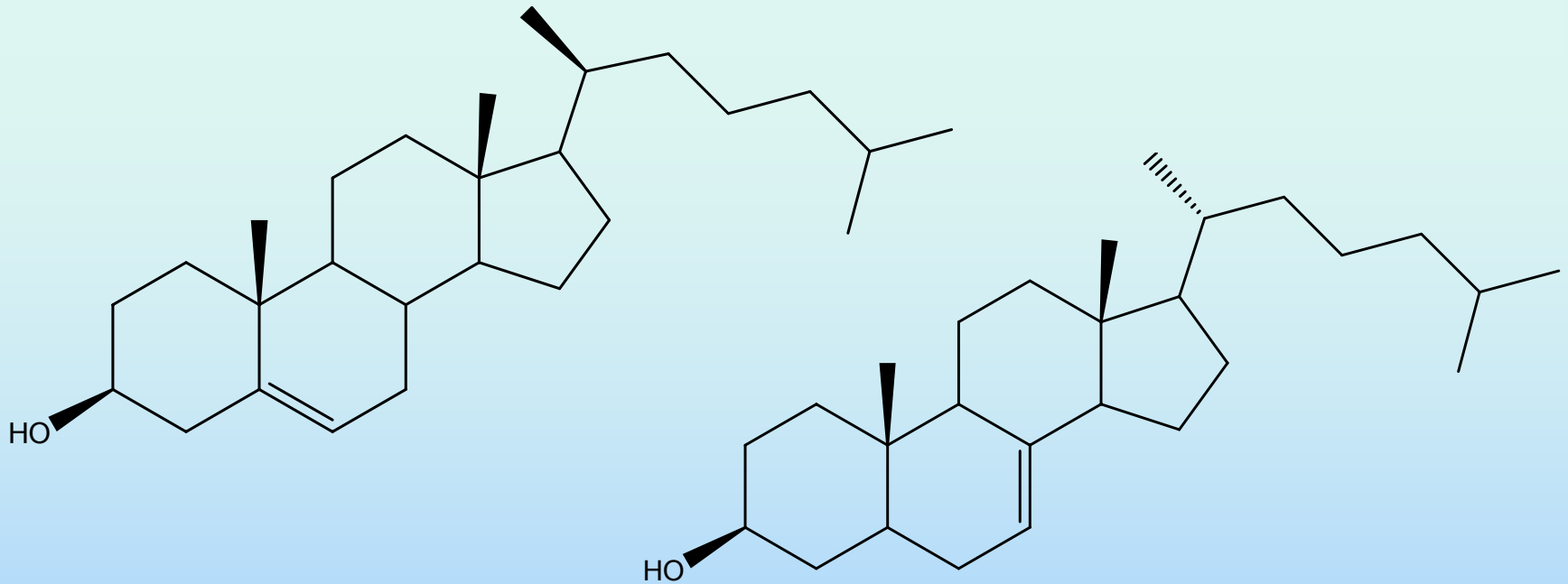


- Tetrahymanol long thought to be a biomarker for bacterial-consuming protozoans
 - Discovered in bacteria, along with a series of related methylated compounds
- Gammacerane is fossil skeleton

Steroids?

- Characteristic of Eukarya; limited proven steroid-producing bacteria
 - Pathways are incomplete
 - May be LGT from Eukarya
- Reports of sterols in cyanobacterial samples
 - Reported in taxonomically diverse cyanobacteria
 - May be contamination

Steroids?



- Cholesterol (left) and lathosterol (right)
 - Common sterols in Eukaryotes and most complete sterol in bacterial species, respectively

Steroids?

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- Comparison of total ion chromatograms and selected ion chromatograms showing differences between possible contaminated cyanobacterial samples and species grown in specified conditions (only sterol is ergosterol from growth media)

A Note on Isotopes

- As with all organisms, the $\delta^{13}\text{C}$ value of organic compounds (such as lipids) in bacteria reflects the $\delta^{13}\text{C}$ of the compounds that they consume
 - Low $\delta^{13}\text{C}$ values (-45 to -80‰) common in methanotrophs
 - Hopanes with $\delta^{13}\text{C} \sim -20$ to -35‰ common in cyanobacteria
- Unlike Archaea, many Bacteria consume a range of organic compounds and tagging of specific carbons can be used to determine biosynthetic pathways for lipids
 - Two pathways for isoprenoid synthesis were identified
 - Fatty acids and isoprenoids can be produced from different pathways and therefore have different bulk $\delta^{13}\text{C}$ values
- However, different growth conditions have also been shown to produce different $\delta^{13}\text{C}$ values for organisms

Papers!

Rohmer et al. 1984

- Hopanoids are phylogenetic sterol ancestors
- BHPs have never been identified in eukaryotes, even in those with hopanoids, must be “prokaryotic metabolites”
- Hopanoid content in bacteria is same order of magnitude as sterol content of eukarya
- Half the strains do not contain detectable hopanoids, but cannot derive taxonomic conclusions from distributions

Pancost et al. 2000

- Fatty acids and methylhopanoids of methanotrophic bacteria not observed, only bishomohopanol (nonspecific bacterial biomarker); $\delta^{13}\text{C}$ values and geochemical evidence are inconsistent with aerobic methanotrophy by bacteria
- $\delta^{13}\text{C}$ values and biomarker evidence suggest a grouping in this area of methotrophy, sulfate-reduction and chemoorganotrophy in a consortium including bacterial acetogens and sulfate-reducers
- Hopanoids are generated at depth under anoxic conditions

Thiel et al. 2003

- ‘Geological’ configuration of $\alpha\beta$ -hopanoids can be produced by living organisms at methane seeps – but not seen in surrounding sediments
- However, the extreme location indicates this will not disrupt the use of this compound as part of a maturity parameter
- $\delta^{13}\text{C}$ values indicate these organisms incorporate methane into biomass
- Location (in anoxic Black Sea gas seeps) indicates that anaerobic bacteria can produce hopanoids in anoxic environments

Harvey et al. 2006

- During incubations (generally):
 - Fatty acid concentrations increased
 - Shifts in fatty acid distribution were substrate-specific
 - A number of site-specific shifts were also observed
- Major bacterial groups show overlap in metabolic capacities – important when using fatty acids to describe community distribution
- The production of certain types of fatty acids shows in response to different substrates is the same across many phylogenetic groups