Organic Geochemistry Wk. 2

• Polyisoprenoid lipids
  – Structural diversity and biosynthesis
  – Hydrocarbons
  – Complex lipids in archaea
  – Isoprenoids of plants and algae
  – Polyisoprenoids as environment and process indicators
    • Lacustrine environments – botryococcenes etc
    • Methanogenesis
    • Anaerobic oxidation of methane
  – Fossil record of Archaea
2- carbon molecule be the major building block for the complex 27- carbon, 4- ringed structure of the cholesterol molecule?

**BLOCH, LYNEN, AND THE CORNFORTH / POPJAK TEAM**

In the late 1930s, another young Jewish émigré from Germany, Konrad Bloch, joined Clarke’s department as a graduate student. Bloch had already completed most of his thesis research at the University of Basel and had published two papers on that research. Still, the Basel faculty rejected it as “insufficient” (10). Bloch many years later learned that only one examiner on his committee had objected and that was on the grounds that the thesis failed to cite some important references – papers authored by that examiner! Looking back, Bloch realized that this may have been providential. Had he passed he decided to stay on in Germany. At any rate, when Bloch came to New York in 1936, Clarke, a guardian angel to refugee scientists, admitted him to his program and the Ph.D. was awarded about 2 years later. At that point, Schoenheimer offered a Bloch position in his
Biosynthesis of isoprenoids (carotenoids, sterols, prenyl side-chains of chlorophylls and plastoquinone) via a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green alga *Scenedesmus obliquus*

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Courtesy of the National Library of Medicine.
Figure 4  Hypothetical scheme for the biosynthesis of IPP from pyruvate and GAP in *Scenedesmus*

○, label from [1-\(^{13}\)C]glucose. TPP, thiamin diphosphate.
Common Acyclic Isoprenoids

- isoprene
- phytol
- pristane
- crocetane
- 2,6,10,15,19-pentamethylicosane (PMI)
- 2,6,10,14,18-pentamethylicosane
- squalane
- farnesane
Less Common Acyclic Isoprenoids

C30 HBI

C25 HBI

C20 HBI

Diatom sources

Botryococcus braunii

botryococcane

lycopene

tomato carotenoid

Probable algal hydrocarbon
? from lycapodiene

lycopane
Polar Lipid Precursors of Acyclic Isoprenoids

archaeol \rightarrow \text{phytane}

caldarchaeol \rightarrow \text{biphytane}

chrenarchaeol
Stereochemistry of archaeal and bacterial lipids
Polar Lipid Precursors of Acyclic Isoprenoids

Common head groups of bacteria and archaea
- Ethanolamine (PE)
- Glycerol (PG)
- Serine (PS)
- Choline (PC)
- Aminopentanetetrol (APT)
- Inositol (PI)
- Hexose (archaea)

Common core lipids of archaea
- Archaeol
- Caldarchaeol
Favored Mass Spectrometric Fragmentations
Crocetane – Phytane Distinction

GC-FID

Full Scan (RIC)

169 Da (RIC from FS)

169 Da (SIR)

183 Da (SIR)

GC and GC-MS (SIR)

GC-MS-MS

(a) 282-169; 0.6%, 1.9

(b) 196-127; 100%, 2.1

(c) 196-126; 63%, 2.3

(d) 168-182; 13%, 11.7

(e) 182-127; 40%, 0.1

Crocetane – Phytane Distinction
Crocetane – Phytane Distinction

(a) Barney Ck 169 Da
(b) Barney Ck 168-126 Da
(c) Barney Ck 196-127 Da
(d) Monterey 196-127 Da

Crocetane
Phytane

nC$_{18}$
**Regular C$_{25}$ vs PMI Distinction**

### Ace Lake Modern Sed$_{PMI}$

- **(a) 266-197 Da**
  - 100%

- **(b) 252-197 Da**
  - 2.4%

- **(c) 352-267 Da**
  - 4%

### Wilkinson 1 C$_{25}$ reg

- **(a)**
  - 100%

- **(b)**
  - 68%

- **(c)**
  - 26%

### W. Terrace 1 C$_{25}$ reg

- **(a)**
  - 100%

- **(b)**
  - 76%

- **(c)**
  - 26%

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One thin peak + one compound

All fat peaks = more than one compound
Regular $C_{25}$ vs PMI & HBI Distinction

2,6,10,14,14-pentamethylicosane
Carbon chains of *Halobacterium* core lipid

2,6,10,15,19-pentamethylicosane (PMI)
Found as a free hydrocarbon in some methanogens

A ‘highly branched isoprenoid’ (HBI)
from a diatom
Distinguishing C25 Isoprenoids

Note peak shapes

Partial 183 Da (SIR) chromatograms of (a) Monterey Formation showing elution position of PMI; (b) Byilkaoora-3 showing elution position of I25 reg; (c) Monterey + Byilkaoora-3 mixture showing relative elution order of PMI and I25 reg isomers (NB. only partially resolved); (d) West Terrace-1 which has a peak at the same position as the I25 reg isomer and no peak at the earlier retention time of PMI. Unknown peaks 1 (Monterey) and 2 (West Terrace-1) elute after I25 reg. Chromatogram time range = 36 sec.
E-3, 7R, 11R, 15-tetramethylhexadec-2-enol = phytol

reduction/dehydration/reduction

oxidation/decarboxylation/reduction

phytane

6(R), 10(S) - pristane

6(S), 10(R) - pristane

6(R), 10(R) - pristane

6(S), 10(S) - pristane
Pristane to Phytane Ratio Pr/Ph

• An empirical parameter that was originally used to classify Australian oils; high in oils from land plant OM (Powell & McKirdy, 1973)

• Empirical correlation with depositional environment (Didyk et al., 1978)
  – <1 → strongly reducing or evaporitic environments (correlates with Gammacerane)
  – 1-4 reducing marine and lacustrine environments
  – >4 terrestrial aquatic environments
  \[ \delta^{13}C \] of Pr and Ph generally similar

• Pr/Ph probably reflects redox control on diagenesis of phytol
Botryococcus braunii isoprenoids
$C_{30}$ Botryococcene $C_{31}$-$C_{33}$ Botryocananes
lake sediments (Maoming) and Oils (Duri of Sumatra)

some cultured *B. braunii* strains

lake sediments (Maniguin) and Oils (Duri of Sumatra)

lake sediments (Maniguin) and Oils (Minas and Duri of Sumatra)
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Fig. 1. Structures of the parent C$_{20}$, C$_{25}$ and C$_{30}$ carbon skeletons. The C$_{20}$ alkane is a major constituent of the hydrocarbons isolated from Rozel Point crude oil. Its structure was elucidated by Yon et al. (1982). In marine sediments and seawater, the C$_{25}$ and C$_{30}$ hydrocarbons mainly occur as highly unsaturated alkenes.
The 18S ribosomal DNA molecular phylogeny and lipid composition of over 120 marine diatoms showed that the capability to biosynthesize highly branched isoprenoid (HBI) alkenes is restricted to two specific phylogenetic clusters, which independently evolved in centric and pennate diatoms.
Fig. 1. Neighbor-joining phylogenetic tree based on nearly complete 18S rRNA sequences of diatoms. Some of the sequences were published before (5); 86 others (see table S1 for details) were determined in this study. The sequences of Coccoid haptophyte and Emiliania huxleyi were used as outgroups but were pruned from the tree. Bolidomonas mediterranea is a sister group of the diatoms. The tree was created with the use of the Jukes Cantor model. HBI-biosynthesizing strains are indicated in red. Diatoms in green were tested but did not contain HBI alkenes; diatoms in black were not tested for the presence of HBI alkenes. The scale bar indicates 10% sequence variation. The inset shows the structure of C25 HBI alkane (27) and parent skeleton of C25 HBI unsaturated alkenes (7–11) produced by diatoms. Note that the odd non HBI-biosynthesizing Rhizosolenia strain, R. robusta, falls completely out of the Rhizosolenia phylogenetic cluster, indicating that its morphological classification as a Rhizosolenia diatom is probably wrong.
Methane seeps: 
Anaerobic oxidation of methane (AOM)

Image courtesy of Victoria Orphan. Used with permission.
Sediment Core from a methane-rich Monterey cold seep

This is a chemistry “profile” from the core

Bacteria feed on methane and sulfate

Image courtesy of Victoria Orphan. Used with permission.
Anaerobic oxidation of methane
The “consortium hypothesis”

Reconstructed-ion-current chromatograms of trimethylsilylated total lipid extracts from (A) a sample 13±15 cm below the sediment surface at a site of active methane seepage (ERB-PC26) and (B) a control sample 33±36 cm below the sediment surface in the same basin but remote from any site of methane release (ERB-HPC5). Analytical conditions for both sediment extracts were identical (similar amounts of extracted sediment, identical dilutions prior injection into the GC). Compound 1=archaeol, compound 2=sn-2-hydroxyarchaeol.
Archaeal / Bacterial 16S rRNA methane seep phylotypes affiliated with AOM

Image courtesy of Victoria Orphan. Used with permission.

modified from Orphan et al. (2001)
Fluorescent In Situ Hybridization (FISH)

Target CELL

PROBE

Labeled CELL

RIBOSOMES

Visualize labeled cells by EPIFLUORESCENCE

Image courtesy of Victoria Orphan. Used with permission.
Archaeal / Bacterial 16S rRNA methane seep phylotypes affiliated with AOM

Image courtesy of Victoria Orphan. Used with permission.
Distribution of anaerobic methane-oxidizing consortia

**ANME-2** / **Desulfosarcina**

Hydrate Ridge, Oregon

Image courtesy of Victoria Orphan. Used with permission.

Up to 80% total biomass in sample
FISH-SIMS

1) identify target cells using FISH and epi-fluorescence microscopy

2) map and photo document aggregate location using light microscopy

Image courtesy of Victoria Orphan. Used with permission.
Secondary Ion Mass Spectrometry (SIMS)
CAMECA ims-1270

3) relocate target (reflected light) in CAMECA ims-1270.
Sputter sample with Cs⁺ beam.

4) measure $\delta^{12}$C and $\delta^{13}$C for target cells vs. time.

Image courtesy of Victoria Orphan. Used with permission.
Heterogeneous composition of ANME-2 archaea and Desulfosarcina in AOM aggregates

Depth profile ANME-2/DSS aggregate 1.2 µm optical sections (confocal)

Distance of ion beam penetration (µm)

Image courtesy of Victoria Orphan. Used with permission.
$^{13}$C compositions of archaeal lipids from different marine sedimentary environments

<table>
<thead>
<tr>
<th>AOM Environment</th>
<th>Archaeol</th>
<th>OH-archaeol</th>
<th>Crocetane</th>
<th>PMI</th>
<th>Phytanol</th>
</tr>
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<tbody>
<tr>
<td>Eel River Basin</td>
<td>-100</td>
<td>-106</td>
<td>-92</td>
<td>-92</td>
<td>-88</td>
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<tr>
<td>Santa Barbara</td>
<td>-119</td>
<td>-128</td>
<td>-119</td>
<td>-129</td>
<td>-120</td>
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<tr>
<td>Hydrate Ridge</td>
<td>-114</td>
<td>-133</td>
<td>-118</td>
<td>-114</td>
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<tr>
<td>Guaymas Basin</td>
<td>-81</td>
<td>-85</td>
<td>---</td>
<td>---</td>
<td>---</td>
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<tr>
<td>Kattegat</td>
<td>---</td>
<td>---</td>
<td>-100</td>
<td>-47</td>
<td>---</td>
</tr>
<tr>
<td>Mediterranean mud volcanoes</td>
<td>-96</td>
<td>-77</td>
<td>-64</td>
<td>-91</td>
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</tr>
</tbody>
</table>

Comparative Analysis of Methane-Oxidizing Archaea and Sulfate-Reducing Bacteria in Anoxic Marine Sediments

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The oxidation of methane in anoxic marine sediments is thought to be mediated by a consortium of methane-consuming archaea and sulfate-reducing bacteria. In this study, we compared results of rRNA gene (rDNA) surveys and lipid analyses of archaea and bacteria associated with methane seep sediments from several different sites on the Californian continental margin. Two distinct archaeal lineages (ANME-1 and ANME-2), peripherally related to the order Methanosarcinales, were consistently associated with methane seep marine sediments. The same sediments contained abundant 13C-depleted archaeal lipids, indicating that one or both of these archaeal groups are members of anaerobic methane-oxidizing consortia. 13C-depleted lipids and the signature 16S rDNAs for these archaeal groups were absent in nearby control sediments. Concurrent surveys of bacterial rDNAs revealed a predominance of δ-proteobacteria, in particular, close relatives of Desulfosarcina variabilis. Biomarker analyses of the same sediments showed bacterial fatty acids with strong 13C depletion that are likely products of these sulfate-reducing bacteria. Consistent with these observations, whole-cell fluorescent in situ hybridization revealed aggregations of ANME-2 archaea and sulfate-reducing Desulfosarcina and Desulfococcus species. Additionally, the presence of abundant 13C-depleted ether lipids, presumed to be of bacterial origin but unrelated to ether lipids of members of the order Desulfosarcinales, suggests the participation of additional bacterial groups in the methane-oxidizing process. Although the Desulfosarcinales and ANME-2 consortia appear to participate in the anaerobic oxidation of methane in marine sediments, our data suggest that other bacteria and archaea are also involved in methane oxidation in these environments.
ANME-2/Desulfosarcina/Bacteria probes

Diverse archaeal/bacterial associations

Eel River Basin

Image courtesy of Victoria Orphan. Used with permission.

Fig. 1. Gas chromatograms of the hydrocarbon fractions extracted from the total sediment ("free") and from the residual matter after decalcification ("total"). $\bullet = n$-alkanes (selected carbon numbers denoted); S, 2,6,10,15,19,23-hexamethyl-tetracosane (squalane). Peak heights of crocetane and PME in the "free" fraction are cut at 75%; the peak of PME in the GC trace of the "total" fraction is cut at 50% peak height.
Table 1. Isotopic composition of selected biomarkers ($\delta^{13}$C [%o] vs. PDB).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Free</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hydrocarbons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n$-C$_{18}$</td>
<td>n.a.</td>
<td>−44.2</td>
</tr>
<tr>
<td>Crocetane</td>
<td>−108.3</td>
<td>−115.6</td>
</tr>
<tr>
<td>PME</td>
<td>−105.5</td>
<td>−112.2</td>
</tr>
<tr>
<td>$n$-C$_{26}$</td>
<td>−30.3</td>
<td>−32.0</td>
</tr>
<tr>
<td>$n$-C$_{29}$</td>
<td>−30.4</td>
<td>−38.4</td>
</tr>
<tr>
<td>Hop-17(21)-ene</td>
<td>n.a.</td>
<td>−83.2</td>
</tr>
<tr>
<td><strong>Alcohols</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anteiso-$C_{15}$</td>
<td>n.a.</td>
<td>−88.3</td>
</tr>
<tr>
<td>$n$-C$_{16}$</td>
<td>n.a.</td>
<td>−87.6</td>
</tr>
<tr>
<td>10-Methyl-$C_{16}$</td>
<td>n.a.</td>
<td>−87.8</td>
</tr>
<tr>
<td>Phytanol</td>
<td>n.a.</td>
<td>−108.5</td>
</tr>
<tr>
<td>$n$-C$_{18}$</td>
<td>n.a.</td>
<td>−66.8</td>
</tr>
<tr>
<td>$n$-C$_{26}$</td>
<td>n.a.</td>
<td>−51.3</td>
</tr>
<tr>
<td>Ether lipid*</td>
<td>n.a.</td>
<td>−108.2</td>
</tr>
</tbody>
</table>

Standard deviations ($\sigma$) are below ±1%o for all compounds except $n$-octadecanol (±6.5%o), and $n$-hexacosanol (±2.8%o); ‘free’ = compounds extractable from the untreated rock; ‘total’ = compounds obtained from the total rock after carbonate dissolution; n.a. = not analysed. *: ‘Ether lipid’ refers to the compound tentatively assigned as 1-O-hexadecyl-2-O-phytanylglycerol.
Accumulating evidence suggests that methane has been released episodically from hydrates trapped in sea floor sediments during many intervals of rapid climate warming. Here we show that sediments from the Santa Barbara Basin deposited during warm intervals in the last glacial period contain molecular fossils that are diagnostic of aerobic and anaerobic methanotrophs. Sediment intervals with high abundances of these compounds indicate episodes of vigorous methanotrophic activity in methane-laden water masses. Signals for anaerobic methanotrophy in 44,100-year-old sediment are evidence for particularly intense methane emissions and suggest that the basin's methane cycle can profoundly affect oxygen budgets in the water column.