12.158 Lecture 7

- More on cyclic terpenoids
  - Analytical methods for hopanoids
  - LCMS Insights
  - Tricyclic and tetracyclic terpanes
  - Plant sesqui-, di-, triterpenoids
BHP Ring Configurations

\[ \Delta^6 \text{ and/or } \Delta^{11} \]

ACETIC ACID BACTERIA
(Methanotroph)

C-2 Me
CYANOBACTERIA
Summons et al., 2000

C-3 Me
METHANOTROPHS
(Acetic Acid bacteria)

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**BHP Side-Chain Structures**

**TETRA:** $X=\text{OH}, \text{NH}_2$, composite; $Y=Z=\text{H}$

**PENTA:** $X=\text{OH}, \text{NH}_2$, composite; $Y=\text{OH}$, $Z=\text{H}$

$X=\text{OH}$, $Y=\text{H}$, $Z=\text{OH}$

**HEXA:** $X=\text{NH}_2$; $Y=Z=\text{OH}$
Analysis of BHP

• Highly functionalised, amphiphillic
• Not amenable to conventional GC-MS
• Side chain cleavage (Rohmer et al., 1984)
  • Periodic acid/sodium borohydride
  • Product structure directly related to number and position of functional groups in side chain
• Specific nature of functional groups lost
Rohmer periodic acid oxid & red

TETRA

PENTA

HEXA

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Talbot LCMS Methodology

- Extraction:
  - ultrasonication & Soxtherm in 2:1 chloroform/MeOH
- Acetylation (acetic anhydride/pyridine)
- RP-HPLC (Adapted from Schullenberg-Schell et al., 1989)
  - 15 cm C\text{18} column with 1 cm guard column
  - Ternary solvent system

<table>
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<tr>
<th>Time (min)</th>
<th>0-5</th>
<th>10</th>
<th>40</th>
<th>70</th>
<th>75</th>
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<tbody>
<tr>
<td>Water (%)</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>10</td>
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<td>Methanol (%)</td>
<td>90</td>
<td>80</td>
<td>59</td>
<td>59</td>
<td>90</td>
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<td>Propan-2-ol (%)</td>
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<td>15</td>
<td>40</td>
<td>40</td>
<td>0</td>
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</tbody>
</table>

- Positive ion APCI (Talbot et al., 2001 J. Chromatogr. A)
A/B Fragment

\[ R = H \]

\[ R = CH_3 \]

D/E 277

Microcystis sp.

IIIa 655 0.1
IIIb or IIIc

IIIj 714 98

Synechocystis sp.

IIIe 1002 74

IVb or IVc

O. amphigranulata

IIIa 655

IVb or IVc

IIIe 1288

Base Peak
Mehay Modifications to Talbot et al APCI LC-MS
Elucidating Hopanoid Biosynthesis with Mutant Bacteria
Proposed hopanoid biosynthetic pathway

- **Biosynthesis**
- **Not biosynthetic**
- **Transport**
- **Not verified**
Triterpenes in \textit{shc} and \textit{ΔhpnP} mutants

\textbf{TIE-1}

- 2-methyldiploptene
- diploptene

\textbf{Δ hpnP}

- diploptene

\textbf{Δshc}

- squalene

\textbf{Time (minutes)}
Deletion of HpnH
Only C30 (core triterpenoids) present in this mutant

Deletion of HpnG
Only adenosylhopane present in this mutant

Normal complement of BHP present in this mutant
accumulation of ribonyl hopane in the mutant.
Formation of aminotriol appears blocked.

Novel unknown BHP in addition to the normal complement of BHP present in this mutant
Other sedimentary hopanoids

Hopanoic acids in Mesozoic sedimentary rocks: their origin and relationship with hopanes Paul Farrimond, Tony Griffiths, Efthymios Evdokiadis

Fig. 4. An m/z 191 mass chromatogram (top) showing the distribution of hopanoic acids (as methyl esters) in a Triassic sample (Serpiano shale) from Switzerland. The m/z 470, 484, 498, 512 and 526 mass chromatograms represent the molecular ions of C 3135 hopanoic acid methyl esters, including A-ring methylated hopanoic acids (shaded peaks; m/z 205) which are labelled ‘‘Me’’ and with the numerical code of their nonmethylated equivalent hopanoic acid

Bisnorhopananes $\rightarrow$ Dinorhopananes

28,30-dinorhopane: free hydrocarbon in highly anoxic sediments; Monterey Fm., Kimmeridge F.m, U-Shale

29,30-dinorhopane; member of homologous series of 3-norhopananes; direct biological precursors? High in carbonates

$384.374 > 191.179$

30-nor-$\alpha\beta$-hopane
Birba 3 (sapropelic laminite)
Stalvies, Love

Bisnorhopanes → Dinorhopanes

a) Kerogen-bound hopanes

m/z 191

b) Free hopanes

m/z 191

NB C_{29}H>C_{30}H
Other Dinorhopanes and Trinorhopane

25, 28, 30-trinor-αβ-hopane
often found with 28,30-dinorhopane
sometimes referred to as C27T
major ions 370 M⁺, 177 A+B, 163 D+E

25, 30-dinor-αβ-hopane
biodegradation product of 30-nor-αβ-hopane
major ions 384 M⁺, 177 A+B, 163 D+E
Other Triterpenoids- Rearranged Hopanoids

AGSOstd_vial2B 1 mg + 50 ng D4, 1/85 ul

17α(H) diahopane

18α(H) -22,29,30-trisnor neohopane (Ts)

18α(H)-30-nor neohopane (C$_{29}$Ts)
Rearranged Hopanes

\[ \alpha\beta(22S) \text{ hopane} \]

\[ 17\alpha(H) \text{ diahopane} \]

\[ 18\alpha(H)-22,29,30\text{-trisnor neohopane (Ts)} \]

\[ 18\alpha(H)-30\text{-nor neohopane (C}_{29}\text{ Ts)} \]
Tricyclics or cheilanthanes extend to $C_{40}$ or $C_{45}$ mostly marine and mature regular isoprenoid branching (not like squalene) unknown source organism
NWE019
Marine Shale
Source

Oil derived from
a clastic source

C23
C24
C29
C30

VA031
Carbonate
Source

Oil derived from
a carbonate-rich
source

C23
C24
C29
C30
Tricyclic Terpanes

C24 Tricyclic Terpane
All tricyclic terpanes having:
13\(\alpha\), 14\(\beta\)
13\(\alpha\), 14\(\alpha\)
13\(\beta\), 14\(\beta\)
13\(\beta\), 14\(\alpha\)
exist in oils.

Cheilanthanes = Tricyclic Terpanes
Tricyclic Terpanes
Extended Tricyclic Terpanes in Petroleum
AN160
Early Cretaceous Lacustrine

AL007
Silurian Marine

* = # asymmetric carbons in side chain

20min
95.45min
Tricyclic Terpanes Are Powerful Lithology Indicators in Sediments & Oils

Pristane/Phytane vs. C₁₉/C₂₃ Tricyclic Terpanes

- terrestrial
- marine
Tricyclic Terpanes Are Powerful Lithology Indicators in Sediments & Oils
Diagnostic Tricyclic Terpane Ratios

- Marine Distal Shales
- Paralic/Deltaic Shales
- Carbonates
- Marls
- Coals/Resins
- Lacustrine
Application to Oman Oils – Nafun (Pre-salt) versus Ara (Intra-salt) biomarker parameters
191 Da mass chromatogram (above) and partial m/z 191 mass chromatogram (below). Peaks in the partial mass chromatogram are normalized to the C24 tetracyclic terpane -- the most abundant terpane in the data. The oil shown is a lacustrine oil from the Cuyo Basin, Argentina.
Principal Component analysis of 800 oils scores & loadings for OilMod variables