Geobiology 2013 Lecture 6
Biogeochemical Tracers
Isotopics #3:
Biosynthetic fractionations and Intramolecular isotopic data, more of multi-element isotopics and the Precambrian C-Cycle

Acknowledgements: John Hayes, David DesMarais

Assigned Reading

• Hayes JM 2001 Fractionation of the isotopes of carbon and hydrogen in biosynthetic processes. Reviews in Mineralogy Stable Isotopic Geochemistry, John W. Valley and David R. Cole (eds.)

Revision of carbon isotopic principles

And examining isotopic fractionation at molecular, organismic and planetary scales
Isotope Effects

EQUILIBRIUM

\[ ^{13}\text{CO}_2 (g) + H^{12}\text{CO}_3^- (aq) \rightleftharpoons ^{12}\text{CO}_2 (g) + H^{13}\text{CO}_3^- (aq) \]

\[ K = \begin{array}{c}
1.0092 \quad (0^\circ C) \\
1.0068 \quad (30^\circ C)
\end{array} \]

KINETIC

\[ \text{H}_3\text{C}-\text{C}-\text{CO}_2\text{H} \xrightarrow{\text{NAD}^+ \text{ NADH} + H^+} \text{CoASH} \xrightarrow{\text{Pyruvate dehydrogenase}} \text{H}_3\text{C}-\text{C}-\text{SCoA} + \text{CO}_2 \]

\( \left( \frac{1^2k}{1^3k} \right)_{C-2} = 1.0232 \)
Mook et al., 1974

\[
\begin{align*}
\text{HCO}_3^- & \quad \text{H}^+ \\
\text{H}_2\text{O} & \quad \text{HCO}_3^- \\
\text{CO}_2(\text{aq}) & \quad \text{H}_2\text{O}
\end{align*}
\]

\[
\text{CO}_2(\text{aq}) \text{ relative to } \text{HCO}_3^-
\]

\[T, ^\circ \text{C}\]
Abundances of $^{13}$C are reported in terms of $\delta$. The zero point of the scale is defined by the VPDB standard. Values of $\delta$ are commonly multiplied by 1000 and thus expressed in parts per thousand, ‰.

$$R = \frac{^{13}C}{^{12}C} \quad \delta^{13}C = \frac{R_{\text{sample}}}{R_{\text{VPDB}}} - 1$$
Isotope effects associated with biochemical reactions cause isotopic variations in nature.

\[
\text{CO}_2 + \begin{array}{l}
\text{CH}_2\text{PO}_3^-\\
\text{C}=\text{O}
\end{array} \text{CHOH} \text{CHOH} \text{CHOH} \text{CH}_2\text{PO}_3^-
\xrightarrow{\text{Rubisco}}
\begin{array}{l}
\text{O}^-\text{O}
\end{array} \text{CHOH} \text{CH}_2\text{PO}_3^- + \begin{array}{l}
\text{O}^-\text{O}
\end{array} \text{CHOH} \text{CH}_2\text{PO}_3^-
\]

\[
\frac{12^k}{13^k} \approx 1.029
\]

Fixed C will be depleted in $^{13}\text{C}$ relative to CO$_2$.

The relative depletion will be 29 parts per thousand.

E.g., if CO$_2$ = 1.000% $^{13}\text{C}$, fixed C = 0.971% $^{13}\text{C}$.
CO₂

C source → Assimilation → Metabolism → Biosynthesis → Biomass

Isotope Effects

External CO₂

Internal CO₂

Fixed C → Metabolites

C₃ Autotroph

Heterotroph

Courtesy of John Hayes. Used with permission.
### Fractionation of C-Isotopes during Autotrophy

<table>
<thead>
<tr>
<th>Pathway, enzyme</th>
<th>React &amp; substr</th>
<th>Product</th>
<th>$\varepsilon$ %</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>$\text{CO}_2 + \text{RUBP}$</td>
<td>3-PGA x 2</td>
<td>10-22</td>
<td>plants &amp; algae</td>
</tr>
<tr>
<td>Rubisco1</td>
<td>$\text{CO}_2 + \text{RUBP}$</td>
<td>3-PGA x 2</td>
<td>30</td>
<td>cyanobacteria</td>
</tr>
<tr>
<td>Rubisco2</td>
<td>$\text{HCO}_3^- + \text{PEP}$</td>
<td>oxaloacetate</td>
<td>22</td>
<td>plants &amp; algae</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>$\text{CO}_2 + \text{PEP}$</td>
<td>oxaloacetate</td>
<td>2</td>
<td>plants &amp; algae</td>
</tr>
<tr>
<td>PEP carboxykinase</td>
<td></td>
<td></td>
<td>10-22</td>
<td></td>
</tr>
<tr>
<td>C4 and CAM</td>
<td>$\text{HCO}_3^- + \text{PEP}$</td>
<td>oxaloacetate</td>
<td>2-15</td>
<td>plants &amp; algae (C4)</td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>$\text{CO}_2 + \text{RUBP}$</td>
<td>3-PGA x 2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Rubisco1</td>
<td></td>
<td></td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>$\text{CO}_2 + 2\text{H}^+ \text{ CoASH}$</td>
<td>AcSCoA pyruvate</td>
<td>15-36</td>
<td>bacteria</td>
</tr>
<tr>
<td>CO dehydrog</td>
<td></td>
<td></td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Pyruvate synthase</td>
<td>$\text{CO}_2 + \text{Ac-CoA}$</td>
<td>oxaloacetate</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PEP carboxylase</td>
<td>$\text{HCO}_3^- + \text{PEP}$</td>
<td>oxaloacetate</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>PEP carboxykinase</td>
<td>$\text{CO}_2 + \text{PEP}$</td>
<td>Oxaloacetate</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Reductive or reverse TCA</td>
<td>$\text{CO}_2 + \text{succinyl-CoA (+ others)}$</td>
<td>$\alpha$-ketoglutamate</td>
<td>4-13</td>
<td>Bacteria esp green sulfur</td>
</tr>
<tr>
<td>3-hydroxypropionate</td>
<td>$\text{HCO}_3^- + \text{acetylCoA}$</td>
<td>Malonyl-CoA</td>
<td></td>
<td>Green non-S</td>
</tr>
</tbody>
</table>
Carbon fixation (C4 & CAM pathways)

Formation of oxaloacetate from PEP (Phosphoenolpyruvate) catalysed by PEP carboxylase

CAM (Crassulacean acid metabolism): Use both C3 and C4 metabolism separated in time
Isotopic consequences of different food sources

Trophic Shifts

GULF OF MEXICO
AUSTRALIA
BERING SEA
MALASIYA
SCOTIAN SHELF

$^{13}$C ENRICHMENT (%) vs. POC
Intramolecular C-isotopic Differences
(DeNiro and Epstein, 1977; Monson and Hayes, 1980, 1982; reviewed Hayes, 2001)

Reactions occur between molecules but isotope selectivity is expressed as chemical bonds that are made or broken at particular carbon positions.

Isotope effects pertain to those specific positions and control fractionations only at that reaction site, not throughout the whole molecule.

To calculate changes in the isotopic compositions of whole molecules we must first calculate the change at the site and then allow for the rest of the molecule because the isotopic shift is diluted by mixing with carbon that is just along for the ride.-------------------Hayes, 2002
Compound Specific Isotope Analysis

Courtesy of John Hayes. Used with permission.
### TABLE 1  Carbon isotopic compositions of individual compounds

<table>
<thead>
<tr>
<th>Peak</th>
<th>( t_R^* ) (s)</th>
<th>Amount (nmol C)</th>
<th>( \delta^{13}C ) (‰)</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.679</td>
<td>1.1</td>
<td>-22.7 ± 1.0</td>
<td>norpristane</td>
</tr>
<tr>
<td>2</td>
<td>1.722</td>
<td>1.0</td>
<td>-30.2 ± 0.3</td>
<td>( C_{19} ) acyclic isoprenoid</td>
</tr>
<tr>
<td>3</td>
<td>1.812</td>
<td>0.7</td>
<td>-25.4 ± 1.0</td>
<td>pristane</td>
</tr>
<tr>
<td>4</td>
<td>2.040</td>
<td>2.0</td>
<td>-31.8 ± 0.8</td>
<td>phytane</td>
</tr>
<tr>
<td>5</td>
<td>2.602</td>
<td>1.0</td>
<td>-29.1 ± 0.6</td>
<td>( C_{22} ) acyclic isoprenoid</td>
</tr>
<tr>
<td>6</td>
<td>3.161</td>
<td>1.3</td>
<td>-23.9 ± 0.6</td>
<td>10( \beta )(H)-des-A-lupane</td>
</tr>
<tr>
<td>7</td>
<td>3.571</td>
<td>1.3</td>
<td>-24.9 ± 1.0</td>
<td>mixture of hydrocarbons</td>
</tr>
<tr>
<td>8</td>
<td>3.688</td>
<td>2.6</td>
<td>-73.4 ± 1.3</td>
<td>( C_{32} ) acyclic isoprenoid</td>
</tr>
<tr>
<td>9</td>
<td>3.883</td>
<td>0.9</td>
<td>-24.2 ± 1.2</td>
<td>isoprenoid alkane</td>
</tr>
<tr>
<td>10</td>
<td>3.957</td>
<td>6.8</td>
<td>-49.9 ± 1.1</td>
<td>17( \beta )(H)-22,29,30-trisnorhopane</td>
</tr>
<tr>
<td>11</td>
<td>3.977</td>
<td>2.0</td>
<td>-60.4 ± 1.8</td>
<td>isoprenoid alkane</td>
</tr>
<tr>
<td>12</td>
<td>4.100</td>
<td>1.6</td>
<td>-43.5 ± 1.0</td>
<td>17( \alpha )(H),21( \beta )(H)-30-norhopane</td>
</tr>
<tr>
<td>13</td>
<td>4.156</td>
<td>2.0</td>
<td>~ -45</td>
<td>17( \beta )(H),21( \alpha )(H)-30-norhopane$\dagger$</td>
</tr>
<tr>
<td>14</td>
<td>4.210</td>
<td>2.9</td>
<td>~ -34</td>
<td>17( \alpha )(H),21( \beta )(H)-hopane$|$</td>
</tr>
<tr>
<td>15</td>
<td>4.256</td>
<td>6.2</td>
<td>-65.3 ± 1.4</td>
<td>17( \beta )(H),21( \beta )(H)-30-norhopane</td>
</tr>
<tr>
<td>16</td>
<td>4.364</td>
<td>1.8</td>
<td>-39.4 ± 0.8</td>
<td>17( \alpha )(H),21( \beta )(H)-homohopane</td>
</tr>
<tr>
<td>17</td>
<td>4.392</td>
<td>1.3</td>
<td>-35.2 ± 1.4</td>
<td>17( \beta )(H),21( \beta )(H)-hopane</td>
</tr>
<tr>
<td>18</td>
<td>4.552</td>
<td>4.2</td>
<td>-36.5 ± 0.5</td>
<td>17( \beta )(H),21( \beta )(H)-homohopane</td>
</tr>
<tr>
<td>19</td>
<td>4.692</td>
<td>15.4</td>
<td>-20.9 ± 0.5</td>
<td>lycopane$|$</td>
</tr>
<tr>
<td>20</td>
<td>5.010</td>
<td>0.5</td>
<td>-27.0 ± 0.4</td>
<td>unknown hydrocarbon</td>
</tr>
<tr>
<td>21</td>
<td>5.408</td>
<td>0.8</td>
<td>-28.8 ± 1.0</td>
<td>unknown hydrocarbon</td>
</tr>
</tbody>
</table>

C-isotopic Composition of Individual Organic Compounds

Three major controls

- Source of carbon and its C-isotopic composition
- Fractionation during assimilation (e.g., heterotrophy, photosynthesis, methanotrophy)
- Fractionation during biosynthesis (lipids)
C-isotopic Composition of Organic Compounds

Source of carbon and its C-isotopic composition

– Inorganic carbon
  • (-7‰ atm. CO₂) assimilated by photosynthesis
    ε → 5-35 per mil depending on pathway extent of consumption
C-isotopic Composition of Individual Organic Compounds

Source of carbon and its C-isotopic composition

– Inorganic carbon
  • (-7‰ atm. CO₂) assimilated by photosynthesis
    \[ \varepsilon \rightarrow 5-35 \text{ per mil depending on pathway extent of consumption} \]

– Organic carbon
  • (-25‰ on average) assimilated during heterotrophy
    \[ \varepsilon \rightarrow -1 \text{ (you are what you eat plus 1 per mil!!)} \]

– Methane carbon
  • (-30 to -100‰) assimilated during methanotrophy
    \[ \varepsilon \rightarrow 0-30 \text{ per mil depending on pathway and extent of consumption} \]
At the very edge of the brine pool, the mussels are especially abundant and happy. This area is often filled with newly settled baby mussels perched on the shells of larger mussels just above the brine.  

_Credit: Penn State University, Dept. of Biology_

Gas hydrates (yellow) are ice with gas trapped inside; exposed beds are accessible to submersibles on the deep sea floor of the Gulf of Mexico. _Ice worms_, a new species only seen in hydrate, were discovered in 1997 by C. Fisher, Penn State University.  

_Credit: I. MacDonald_
Methane-rich water is pumped into the mussel and across its gills. The symbiotic bacteria in the gills use methane as both a carbon and energy source. The mussels, in turn, live off the symbiotic bacteria.

Using the scanning electron microscope, we can see over a dozen mussel gill cells in the panel on the left. On the right is a closer look at the cell with its outer membrane partially removed. Look into the cell to see hundreds of symbiotic bacteria.

Courtesy of Charles Fisher, Penn State University, Dept. of Biology. Used with permission.
Using the scanning electron microscope, we can see over a dozen mussel gill cells in the panel on the left. On the right is a closer look at the cell with its outer membrane partially removed. Look into the cell to see hundreds of symbiotic bacteria.

Courtesy of Charles Fisher, Penn State University, Dept. of Biology. Used with permission.
Aerobic methanotrophy at ancient marine methane seeps: A synthesis

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ABSTRACT

The molecular fingerprints of the chemosynthesis based microbial communities at methane seeps tend to be extremely well preserved in authigenic carbonates. The key process at seeps is the anaerobic oxidation of methane (AOM), which is performed by consortia of methanotrophic archaea and sulphate reducing bacteria. Besides the occurrence of $^{13}$C depleted isoprenoids and $n$-alkyl chains derived from methanotrophic archaea and sul-

Table 1  
Background information on samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Fossil inventory</th>
<th>Carbonate microfabrics</th>
<th>Stable isotopes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tepee Buttes (Campanian)</td>
<td>Lucinid bivalves, gastropods</td>
<td>Clotted micrite, yellow calcite, banded/botryoidal cement, ( \text{in situ} ) brecciation</td>
<td>Yellow calcite ( \delta^{13}C: -45.9 ) to (-31.7% ) (_e), micrite ( \delta^{13}C: -49.7 ) to (-43.1% ) (_e), banded/botryoidal cement ( \delta^{13}C: -45.5 ) to (-13.2% ) (_e)</td>
<td>Kauffman et al., 1996; Shapiro, 2004; Birgel et al., 2006b; Peckmann et al., 2004; Barbieri and Cavalazzi, 2005</td>
</tr>
<tr>
<td>Pietralunga (Miocene)</td>
<td>Lucinid bivalves</td>
<td>Microcrystalline calcite, aragonitic cement, fossilized filaments</td>
<td>Microcrystalline calcite ( \delta^{13}C: -51.5 ) to (-45.8% ) (_e), aragonitic cement ( \delta^{13}C: -43.3 ) to (-40.8% ) (_e)</td>
<td></td>
</tr>
<tr>
<td>Marmorito (Miocene)</td>
<td>None</td>
<td>Microcrystalline dolomite, calcitic veins, ( \text{in situ} ) brecciation</td>
<td>Microcrystalline dolomite ( \delta^{13}C: -40.7 ) to (-38.9% ) (_e), calcitic vein ( \delta^{13}C: -28.5 ) to (-17.3% ) (_e)</td>
<td>Clari et al., 1988; Peckmann et al., 1999</td>
</tr>
</tbody>
</table>

\( \delta^{13}C \) carbonate values in \( \%_e \) relative to V-PDB.

hop-17-(21)-ene XII; \( \delta^{13}C: -32\% \)     

Norlanostane (XVII; \( \delta^{13}C: -80\% \)),  

Lanostane (XVIII; \( \delta^{13}C: -70\% \))

![Image](http://www.sciencedirect.com)

Fig. 1. Partial gas chromatogram (total ion current: TIC) of hydrocarbon fraction from Pietralunga. Istd.: internal standard. Roman numbers: see Appendix.

C-isotopic Composition of Organic Compounds

- Fractionation during biosynthesis (lipids)
Carbohydrate metabolism

DeNiro and Epstein, 1977

Glucose, C$_6$H$_{12}$O$_6$

Pyruvate

Kinetic Isotope Effect

Pyruvate dehydrogenase

Alternate fates

C$_2$ units for biosynthesis and oxidative metabolism

Image by MIT OpenCourseWare.
Precursor = Acetate: \( \text{CH}_3\text{CO}_2^- \)

Product = \( C_{16} \) fatty acid = 8 acetates

\[
\begin{align*}
\text{m} & \quad c \\
m & \quad c & \quad m & \quad c & \quad m & \quad c & \quad m & \quad c & \quad m & \quad c & \quad m & \quad c & \quad m & \quad c \\
\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H} \\
\end{align*}
\]

Methyl (m) and carboxyl (c) positions in acetate are converted to structurally equivalent \( \text{CH}_2 \) positions during biosynthesis.
Isotopic equilibrium:

Equilibration within acetate $\rightarrow$ Biosynthesis without subsequent equilibration $\rightarrow$

Kinetic control throughout, acetate =

Courtesy of John Hayes. Used with permission.
Flows of C at the pyruvate branch point in the metabolism of E. coli grown aerobically on glucose (Roberts 1955). 74% of the pyruvate is decarboxylated to yield Ac-CoA. The observed depletion at odd-numbered positions of FAcids is shown at the right indicating that the isotope effect at C-2 in the pyruvate dehydrogenase reaction is 23‰.
An important consequence of the pyruvate to acetate isotopic fractionation

Alternate carbons derived from acetate carboxyl down acetogenic lipidbackbones are light. In general lipids are also light, but not as light.

In contrast, the carboxyl carbon of amino acids is generally "heavy"
Two Origins for Isoprenoids

Methylenetetrol phosphate pathway, MEP

- **isoprene**
  - head
  - tail

- **isopentenyl pyrophosphate (OPP)**
  - 2 x C5

- **geraniol**
  - head-to-tail
  - C5

- **farnesol**
  - C15
  - tail-to-tail

- **squalene**
  - C30
  - sterols
  - bacteriohopanols

- **phytol**
  - tail-to-tail
  - irregular

- **highly branched isoprenoid**
  - C20

Mevalonic pathway, MVA

- **isopentenyl pyrophosphate (OPP)**
  - 2 x C5

- **phytol**
  - head-to-head
  - C20

- **biphytane**
  - archaea

- **phytoene**
  - and carotenoids

- **pmi**
  - methanogenic archaea

Figure by MIT OpenCourseWare.
Labelling of isopentenylpyrophosphate from MVA pathway
Labelling of isopentenylpyrophosphate from MEP pathway
Labeling of phytol and cholesterol from MVA (a & c) and MEP (b & d)
<table>
<thead>
<tr>
<th>Organism</th>
<th>Pathway</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prokaryotes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>-</td>
<td>Lange et al., 2000</td>
</tr>
<tr>
<td>Aquificales, Thermotogales</td>
<td>MEP</td>
<td>Boucher and Doolittle, 2000</td>
</tr>
<tr>
<td>Photosynthetic bacteria</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Chloroflexus</td>
<td>MVA</td>
<td>Rieder et al., 1998</td>
</tr>
<tr>
<td>Chlorobium</td>
<td>MEP</td>
<td>Boucher and Doolittle, 2000</td>
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<tr>
<td>Gram positive eubacteria</td>
<td>-</td>
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<tr>
<td>Commonly</td>
<td>MEP</td>
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<td>Streptococcus, Staphylococcus</td>
<td>MVA</td>
<td>Boucher and Doolittle, 2000</td>
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<td>Streptomyces</td>
<td>MEP &amp; MVA</td>
<td>Seto et al., 1996</td>
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<td>Spirochaetes</td>
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<td>Borrelia burgdorferi</td>
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<td>Treponema pallidum</td>
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<td>Proteobacteria</td>
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<td>Commonly</td>
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<td>Myxococcus, Nannocystis</td>
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<td>Kohl et al., 1983</td>
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<td>Cyanobacteria</td>
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<td>Disch et al., 1998</td>
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<td>Lange et al., 2000</td>
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<td><strong>Eukaryotes</strong></td>
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<td>Non-plastid-bearing</td>
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<td>Lange et al., 2000</td>
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<td>Plastid-bearing</td>
<td>Plastid</td>
<td>Schwender et al., 2001</td>
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<td>Chlorophyta</td>
<td>MEP</td>
<td>Lichtenhalter et al., 1997</td>
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<td>Streptophyta</td>
<td>MEP</td>
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<tr>
<td>Euglenoids</td>
<td>MVA</td>
<td>Lichtenhalter 1999</td>
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</tbody>
</table>
Twin element stable isotope distributions (δ¹³C and δ¹⁵N, in ‰ versus PDB and air, respectively) of hair samples taken from individual students at the University of Virginia (modified after Macko et al. [1998]). Hair is largely composed of the fibrous protein α-keratin. Its isotope composition is reflective of the recent diet of an individual, generally increasing slightly (1‰ in δ¹³C and 3‰ in δ¹⁵N) with tropic level. The marked variability accords with the great diversity of modern diets. For comparison, George Washington’s hair sample testifies to a rather balanced diet.

Figure by MIT OpenCourseWare.
Multi-element, Compound-specific Isotopic Analyses

Vanillin

Dual isotopic ($\delta^{13}$C and $\delta^{18}$O, in ‰ versus PDB and of standard CO, respectively) for the flavor compound vanillin. The three vanillin extracts from the naturally grown vanilla beans have similar $\delta^{13}$C values, even though they come from geographically widely spaced sites: Mexico and the islands of the Comores and Tahiti. The Mexican sample, however, does differ markedly in $\delta^{18}$O, no doubt owing to major differences in $\delta^{18}$O in the ambient water supply. Not surprisingly, major differences in both $\delta^{13}$C and $\delta^{18}$O are apparent in the synthetic and biotechnological products [Hener et al., 1998]. On the basis of their dual isotopic values, the three samples of unknown origin can be assigned to Mexico.

Figure by MIT OpenCourseWare.
Characterising Cocaine Sources

Figure 2. Identification of geographic regions in South America where coca is commonly grown, based on dual isotope information of cocaine base as well as abundance of minor alkaloid components. Plotted on both axes are mixed expressions, each consisting of an isotope term and a concentration term; the $y$ axis is $[\delta^{15}\text{N} \text{coca} (\%o \text{versus air}) + 0.1 \times \text{relative concentration of truxilline (\%)},$ and the $x$ axis is $[\delta^{13}\text{C} \text{coca} (\%o \text{versus PDB}) - 10 \times \text{concentration of trimethoxycoacaine}].$ Truxilline and trimethoxycoacaine occur as two trace alkaloids in coca leaves. In addition to the obvious benefits for forensics, this illustration demonstrates the potential value of multi-isotope biomarker approaches for the geosciences to distinguish different geographical, climatological, and ecological regimes. Furthermore, it illustrates the importance of innovative data manipulation in biomarker research, especially when multiple isotope dimensions are employed. After Ehleringer et al. [2000].
Multi-element, Compound-specific Isotopic Analyses

Vanillin

Dual isotopic ($\delta^{13}C$ and $\delta^{18}O$, in ‰ versus PDB and of standard CO, respectively) for the flavor compound vanillin. The three vanillin extracts from the naturally grown vanilla beans have similar $\delta^{13}C$ values, even though they come from geographically widely spaced sites: Mexico and the islands of the Comores and Tahiti. The Mexican sample, however, does differ markedly in $\delta^{18}O$, no doubt owing to major differences in $\delta^{18}O$ in the ambient water supply. Not surprisingly, major differences in both $\delta^{13}C$ and $\delta^{18}O$ are apparent in the synthetic and biotechnological products [Hener et al., 1998]. On the basis of their dual isotopic values, the three samples of unknown origin can be assigned to Mexico.

Figure by MIT OpenCourseWare.
### Reservoirs of carbon in the Atmosphere, Hydrosphere and Geosphere.

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Reduced C</th>
<th>Mass, x 10^{18} moles</th>
<th>Total C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxidized C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atmosphere</td>
<td>-</td>
<td>0.06(^{a})</td>
<td>0.06(^{a})</td>
</tr>
<tr>
<td>Biosphere: plants and algae</td>
<td>0.13(^{b})</td>
<td>-</td>
<td>0.13</td>
</tr>
<tr>
<td>Hydrosphere</td>
<td>-</td>
<td>3.3(^{c})</td>
<td>3.3</td>
</tr>
<tr>
<td>Pelagic sediments</td>
<td>60(^{d})</td>
<td>1300(^{d})</td>
<td>1360</td>
</tr>
<tr>
<td>Continental margin sediments</td>
<td>&gt;370(^{e})</td>
<td>&gt;1000(^{e})</td>
<td>&gt;1370</td>
</tr>
<tr>
<td>Sedimentary rocks</td>
<td>750(^{f})</td>
<td>3500(^{f})</td>
<td>4250</td>
</tr>
<tr>
<td>Crustal metamorphic and igneous rocks</td>
<td>100(^{g})</td>
<td>?</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Mantle</td>
<td>-</td>
<td>-</td>
<td>27000(^{h})</td>
</tr>
</tbody>
</table>

Figure 1. Biogeochemical C cycle, showing principal C reservoirs (boxes) in the mantle, crust, oceans and atmosphere, and showing the processes (arrows) that unite these reservoirs. The range of each of these reservoir boxes along the horizontal axis gives a visual estimate of δ13C values most typical of each reservoir. The vertical bars at right indicate the timeframes within which C typically completely traverses each of the four C sub-cycles (the HAB, SED, MET and MAN sub-cycles, see text). For example, C can traverse the hydrosphere-atmosphere-biosphere (HAB) sub-cycle typically in the time scale between 0 to 1000 years.
Figure 2. Biogeochemical C cycle (as in Fig. 1), showing principal C reservoirs (boxes) and their isotopic compositions in the mantle, crust, oceans and atmosphere, and the processes (arrows) that unite these reservoirs. Numbers adjacent to the arrows give estimates of present-day fluxes, expressed in the units $10^{12}$ mol yr$^{-1}$. 

Image courtesy of Mineralogical Society of America. Used with permission.
Figure 3. Relationship between isotopic composition ($\delta^{13}$C$_{\text{carb}}$ and $\delta^{13}$C$_{\text{org}}$) and the fraction of carbon buried as organic matter. The vertical separation between the lines depicts $\varepsilon \Delta$, and thus reflects the combined effects of equilibria between inorganic C species and biological isotope discrimination (see text). A value $\varepsilon_{\text{TOC}} = 30$ is depicted here, and represents the long-term average value during the past 800 Ma (Hayes et al. 1999). The vertical line represents the value of $f_{\text{org}} = 0.2$, which represents the current value for the global C cycle.
Figure 5. Plot of age versus $\delta^{13}$C (crosses) and $\delta^{org}$ for Archean and Proterozoic kerogens. Kerogen data (filled circles) are corrected for the effects of thermal alteration (Des Marais 1997a). Uncorrected data are shown as open circles. Between 2.2 to 2.0 billion years ago, note the high $\delta^{carb}$ values and the virtual disappearance thereafter of $\delta^{org}$ values more negative than -36. Other evidence indicates that atmospheric O2 increased substantially at this time (see text).
Secular variation in $\delta^{13}C$ of purified kerogens (sedimentary organic matter)

Carbon isotope evidence for the stepwise oxidation of the Proterozoic environment

David J. Des Marais, Harald Strauss, Roger E. Summons & J. M. Hayes

The oxidation of the Earth's crust and the increase in atmospheric oxygen early in Earth history have been linked to the accumulation of reduced carbon in sedimentary rocks. Trends in the carbon isotope composition of sedimentary organic carbon and carbonate show that during the Proterozoic epoch (2.5–0.54 Gyr ago) the organic carbon reservoir grew in size, relative to the carbonate reservoir. This increase, and the concomitant release of oxidizing power in the environment, occurred mostly during periods of global rifting and orogeny.

FIG. 4 Quantity of organic carbon in the crust (M) against age. Values of M are calculated according to equation (4) (in the text). Symbols represent calculations of M, assuming sediment half lives (see derivation of equation (4) as follows: ○, 300 Myr; ●, 400 Myr; △, 500 Myr. Rectangles along the bottom margin depict time intervals of enhanced global rifting and orogeny (see text).

Figure 8. Range of δcarb and δCO2 values (open boxes) and δorg values (shaded boxes), together with the processes proposed to explain their distribution prior to 2.2 Ga and subsequent to 2.1 Ga. A temperature of 15°C was assumed for the isotopic equilibrium between δcarb and δCO2. The lines associated with the various groups of autotrophic bacteria and algae illustrate the maximum discrimination expected for each group. The sloped line at right depicts declining discrimination over time, perhaps in response to declining CO2 levels.
Figure 11. Neoproterozoic records of $\delta_{\text{carb}}$ (curve a) and $\delta_{\text{org}}$ (curve b) values (Hayes et al. 1999). Corresponding values for isotopic fractionation, $\varepsilon_{\text{TOC}}$ and $f_{\text{org}}$ during Neoproterozoic time are given by curves c and d, respectively. The periodic negative excursions are typically associated with glacial intervals (see text). Figure modified from Hayes et al. (1999).
Figure 7. The biogeochemical C cycle prior to the advent of oxygenic photosynthesis, showing the much lower global primary productivity and the higher rates of thermal emanation of C (see text). Comparison with Figure 5 illustrates the enhancement of global primary productivity due to the development of oxygenic photosynthesis. Flux estimates are highly approximate, and are shown principally to illustrate the direction and magnitude of change over geologic time.
Biosynthesis of Organic Compounds

An isotope effect causes fractionation.

Carbon Source: $\text{CO}_2$
Intermediate: $\varepsilon_P$
Photosynthate

Lipids: Straight-Chain Polyisoprenoidal

$\frac{13\text{C}}{12\text{C}}$

Autotrophs

Fractionation

$3\%_0$
Biosynthesis of Organic Compounds

Carbon Source  Intermediate  Lipids

Resolve

Heterotrophs

\[ ^{13}C \rightarrow ^{12}C \]

\[ \begin{align*}
\text{Organic Matter} \\
\text{Metabolites} \\
\epsilon_b
\end{align*} \]

\[ \approx 3\% \]

\[ \text{no acyclic isoprenoids} \]
Cell-Sediment

δ, ‰

Nucleic Acids
Proteins
Carbohydrates

Plastidic Isoprenoids
Resistant Biopolymer
Acetogenic Lipids