12.007 Geobiology
Spring 2009

Geobiology 2009 Lecture 12

Biogeochemical Tracers

Isotopics #2: C, H, O and N

Acknowledgements: John Hayes, Karen Casciotti, Steve Macko, John Hedges

Assigned Reading

- Stanley 2nd Ed Chapter 10, pp 221-240

- 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.)

- Hayes Concepts and Calculations from Stellar

Geobiology 2009 Lecture 12

Biogeochemical Tracers

Isotopics #2: C, H, O and N

When comprehensively understood, isotopic fractionation processes that take place in modern organisms can be a very powerful means to reconstruct ancient ecosystems

…….the example of aerobic methane oxidation

Geobiology 2009 Lecture 12

Biogeochemical Tracers

Isotopics #2: C, H, O and N

Homework #4 Due 4/16/09

An hypothesis paper by Catling et al (Astrobiology 5, 415, 2005) discuss the essential role of oxygen in the development of complex life and the concept of planetary 'oxygenation time'. In no more than 5 pages synthesize the arguments presented, offer criticisms and speculate about the possibility for forms of 'weird life' that might be able to develop complexity via alternative terminal electron acceptors.

Geobiology 2009 Lecture 12

Biogeochemical Tracers

Isotopics #2: C, H, O and N

Need to Know:

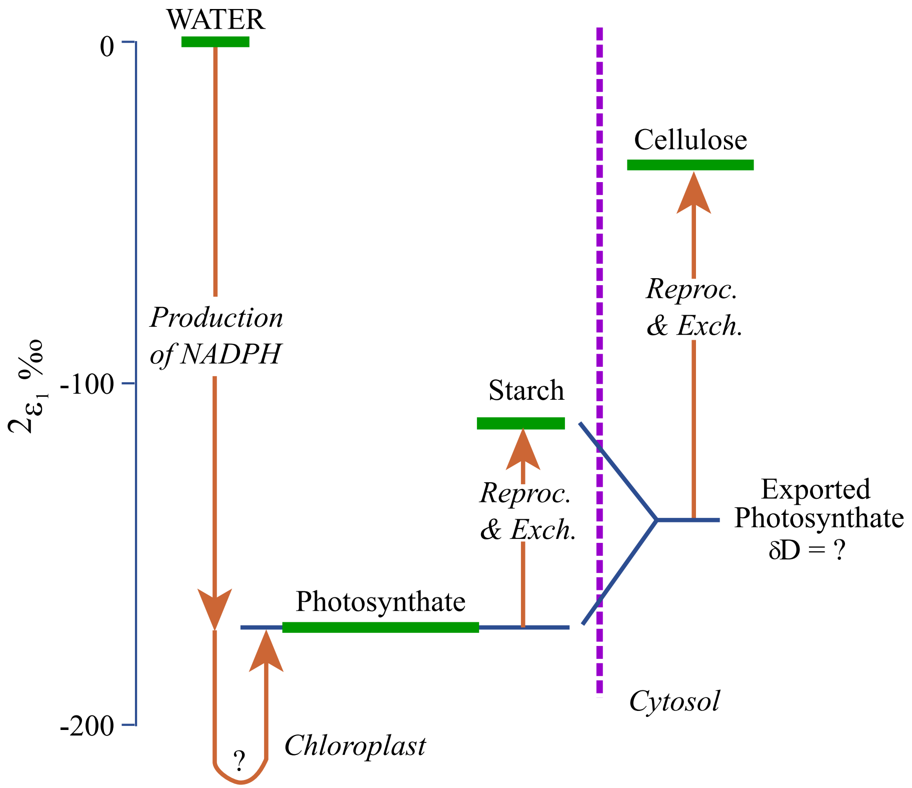
Ballpark delta values of C, O, N, H in marine and terrestrial biomass

How H&O are fractionated in the hydrological cycle

How (roughly) C&H fractionation occurs in organic matter

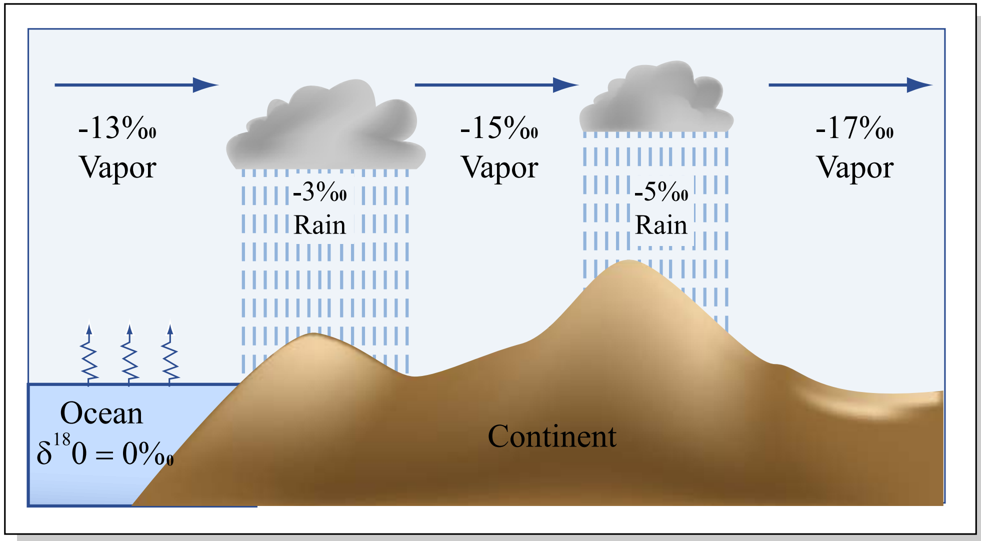
How (roughly) N is fractionated on land and in the ocean

Hydrogen isotopic signatures inherited from water through reactions of NADPH



Relationships between the hydrogen isotopic compositions of initial photosynthate and related products and of water available within plant cells. The uncertainty indicated on the pathway between water and photosynthate reflects the possibility of offsetting fractionations in the production of NADPH and the subsequent transfer of H^- to photosynthate.

Figure by MIT OpenCourseWare.



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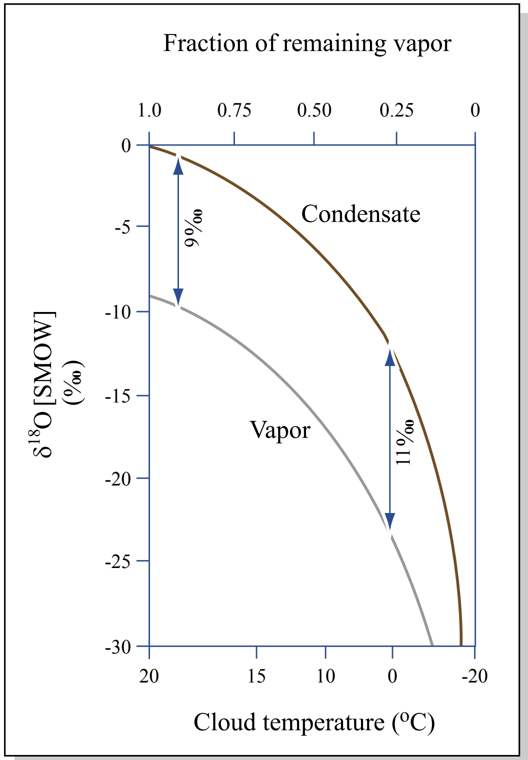

Figure by MIT OpenCourseWare.

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Graph of deuterium per mil over oxygen-18 per mil.

The major nonbiological fractionation process affecting stable hydrogen (and oxygen) isotopes is the hydrologic cycle in which water molecules containing lighter isotopes (^{1}H & ^{16}O) are preferentially evaporated and retained in a cloud (vs. ^{2}H & ^{18}O).

The net result of this fractionation process is that precipitation at increasingly inland, higher altitude (cooler) sites is depleted in both ^{18}O and ^{2}H (D).

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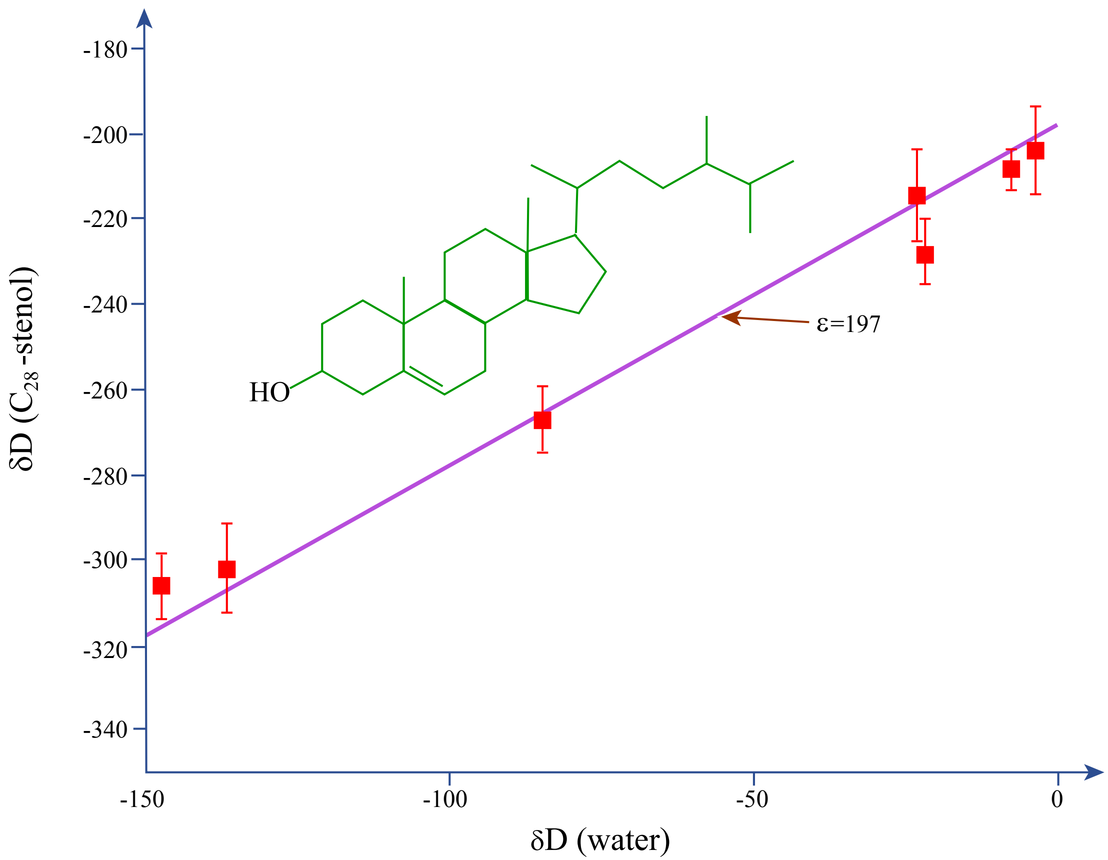
Graphs of isotopic enrichment of water at various stages of incorporation into plant matter.

In general, tree sap isotopically resembles local meteoric water, whereas leaf water is isotopically enriched. Organic matter is depleted in D versus the leaf water from which it is biosynthesized (values given above are typical for N. American plants).

In general, lipids are depleted in D versus lignin and cellulose from the same plant, whereas cellulose is slightly more depleted than coexisting lignin (Rundle et al., 1989).

Cellulose is usually analyzed in nitrated form (NO_3 replacing OH on each C), so that only the nonexchangeable H directly bound to C is analyzed.

Because the D in cellulose nitrate reflects local water, woods can be used as a proxy for the hydrogen isotope composition of past environments in which the wood was made



Relationship between D/H ratios (δD, in ‰ Vienna standard mean ocean water (SMOW)) for the C_{28} stenol biosynthesized by phytoplankton grown in different lakes and marine enviroments and that of the ambient waters (modified after *Sauer et al*. [2001]). The line represents constant fractionations ε. It is apparent that the sterol is recording the D/H ratio of the ambient water. Hence the δD value of sedimentary sterols should permit proxy estimation of the δD of past water bodies, a key feature of paleohydrology.

Simplified Terrestrial Nitrogen Cycle

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Please see http://www.windows.ucar.edu/tour/link=/earth/climate/images/nitrogencycle_jpg_image.html

Some Typical ^{15}N values

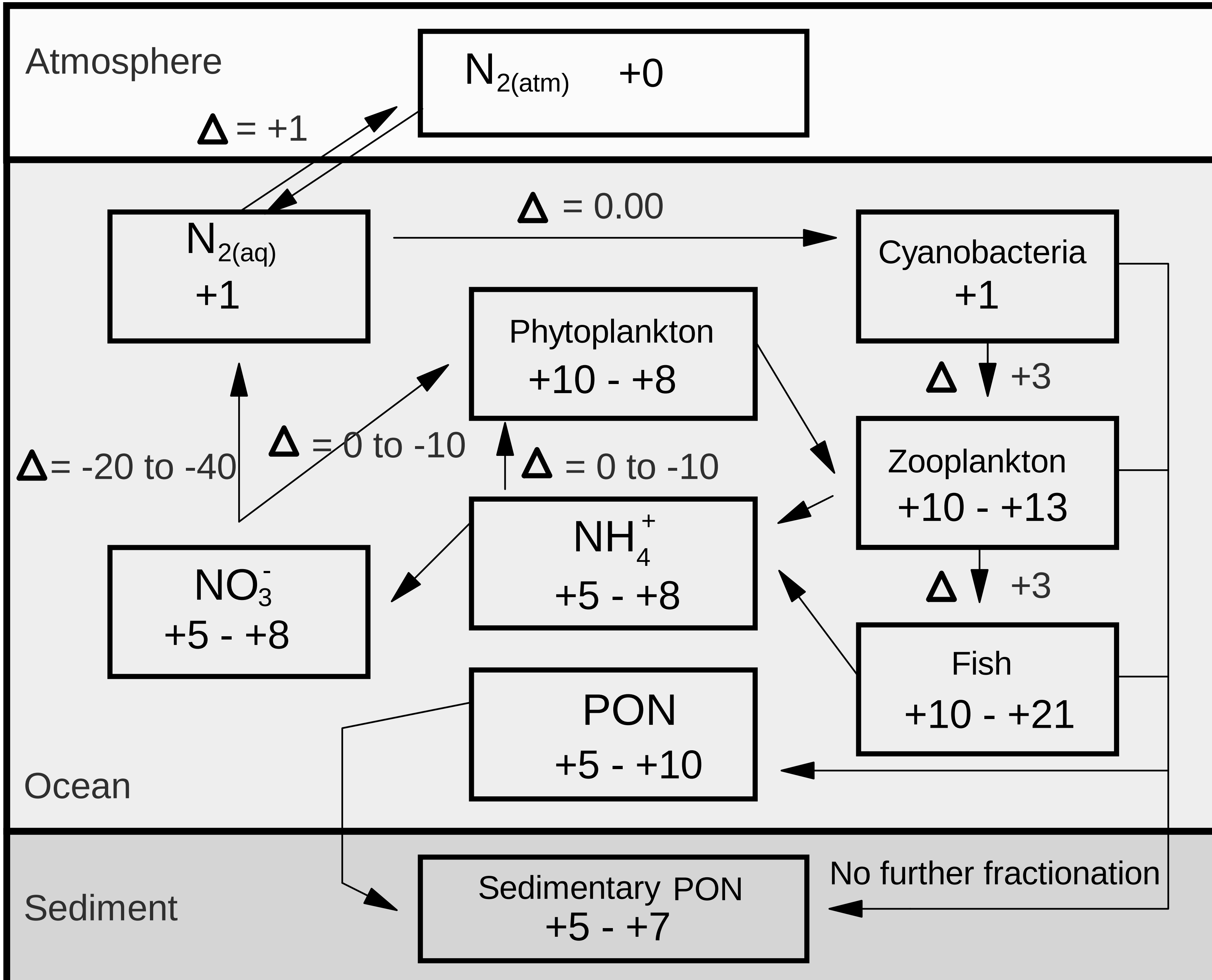
Atm. N_2 ‘fixed’’ by terrestrial flora (Nif in legumes) ~ 0 per mil

Atm. N_2 ‘fixed’ by marine bacteria and Cb ~ 0 per mil

Nitrate or ammonium utilized by terrestrial flora and converted to organic nitrogen ~0 per mil

Dissolved nitrate in the ocean ~ +7 to +10 per mil

Marine organisms high in the trophic structure (eg fish) +10 to + 20 per mil



Atmosphere
$N_{2(atm)}$ +0
Δ = +1
Δ = 0.00
$N_{2(aq)}$
+1
Cyanobacteria
+1
Phytoplankton
+10 - +8
Δ +3
Zooplankton
+10 - +13
Δ = 0 to -10
Δ= -20 to -40
Δ = 0 to -10
NH_4^+
+5 - +8
NO_3^-
+5 - +8
Δ +3
Fish
+10 - +21
PON
+5 - +10
Ocean
Sediment
Sedimentary PON
+5 - +7
No further fractionation

U. Weismann, "Biological nitrogen removal from wastewater", Adv. Biochem.Engr. 51:113-154 (1994)

Nitrification:

$$NH_4^+ + 1.5\ O_2 \text{ ----> } NO_2^- + H_2O + 2\ H^+$$

lowers pH

$$NO_2^- + 0.5\ O_2 \text{ -----> } NO_3^-$$

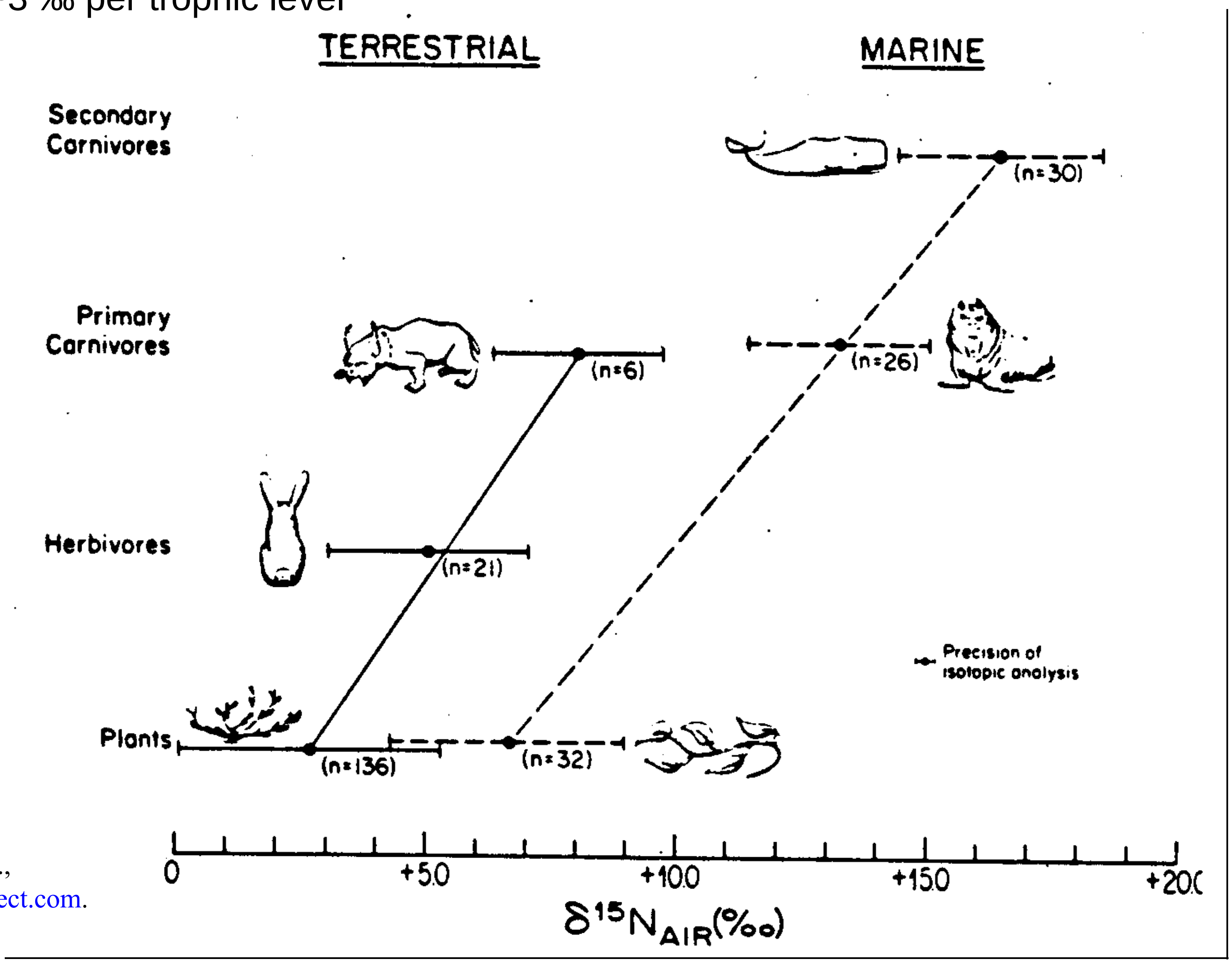
Denitrification:

$$5\ HOAc + 8\ NO_3^- \text{ ----> } \boxed{\overset{\text{escapes}}{4\ N_2}} + 10\ CO_2 + 6\ H_2O + 8\ OH^-$$

MUST have carbon source, e.g., acetate   raises pH

Biogeochemistry of the Stable Nitrogen Isotopes

An important use of stable nitrogen isotopes is as an indicator of trophic level in natural systems with known nitrogen sources and relatively simple food webs. The following figure is from Schoeninger and DeNiro (1984) GCA 48, 625-639. Average δ15N trophic offset is ~3 ‰ per trophic level



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Multi-element, Compound-specific Isotopic Analyses

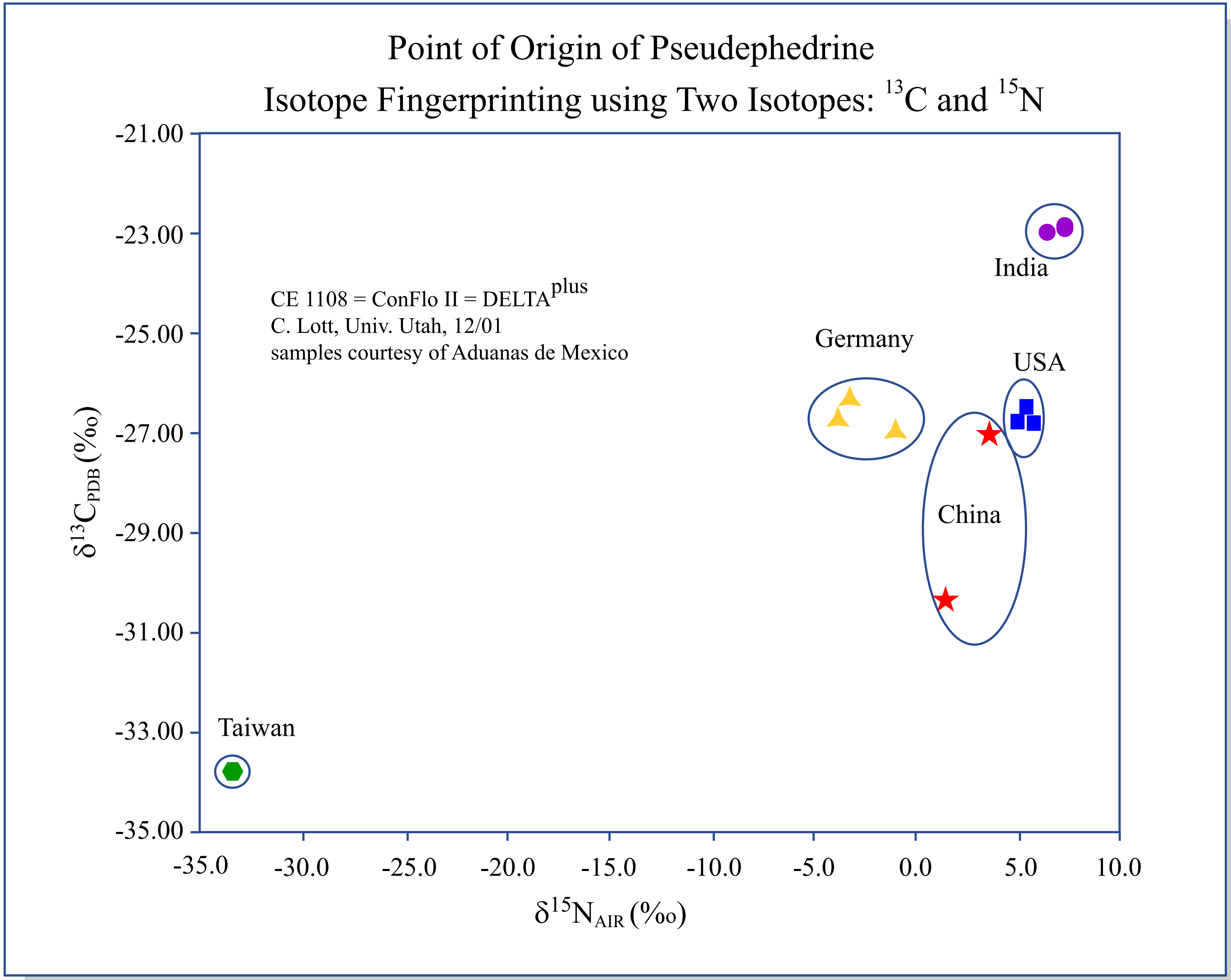

Figure by MIT OpenCourseWare.

Characterising Cocaine Sources

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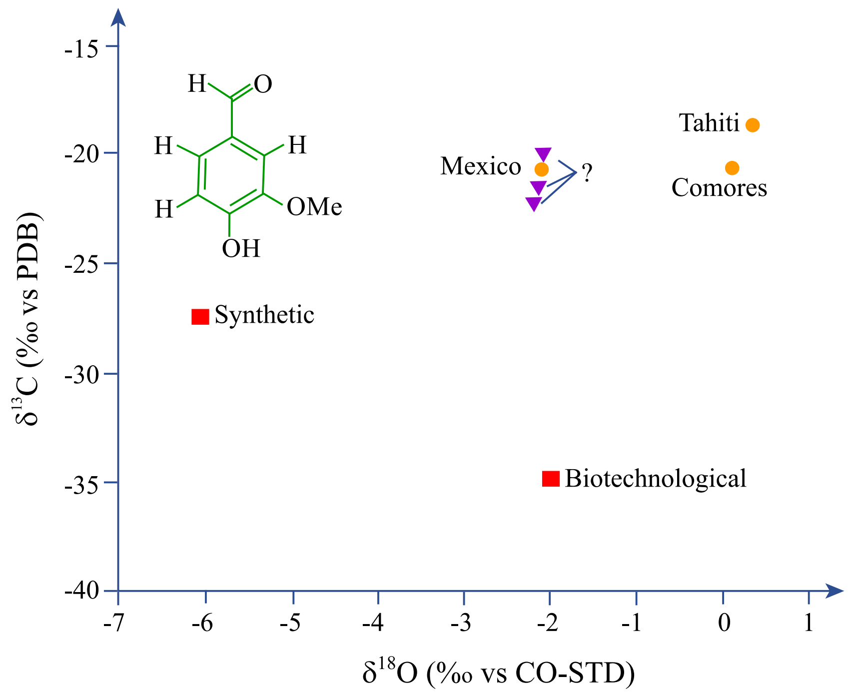
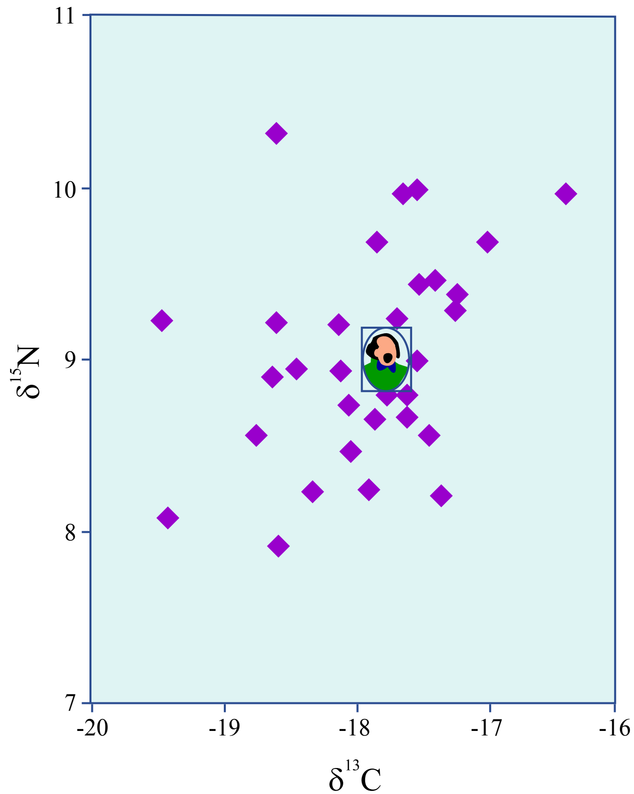
Map and graph to illustrate the identification of geographic regions in South American where coca is commonly grown, based on dual isotope information of cocaine base as well as abundance of minor alkaloid components.

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.

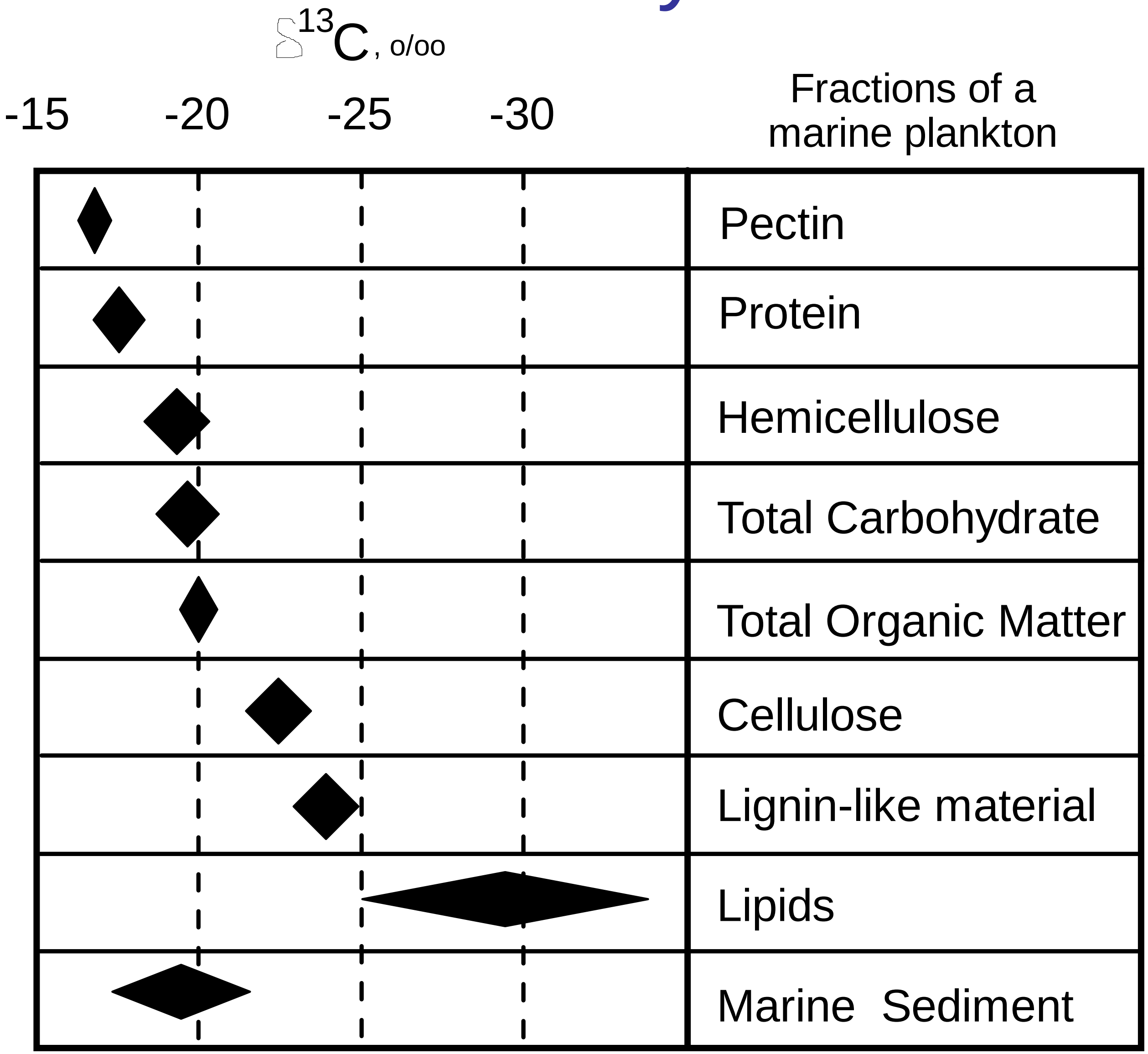
Figure by MIT OpenCourseWare.



Twin element stable isotope distributions ($\delta^{13}C$ and $\delta^{15}N$, 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 $\delta^{13}C$ and 3‰ in $\delta^{15}N$) 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.

Organismal Variability in Bulk C-Isotopes



Degens, 1969

Polyisoprenoid lipids

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Incorporation of CO2 into the TCA cycle.

Polymethylenic
= acetogenic lipids

Compound Specific Isotope Analysis

Illustration and graph removed due to copyright restrictions.

C-isotopic Composition of Organic Compounds

Three major controls

- Source of carbon and its C-isotopic composition
- Fractionation during assimilation (eg 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. CO_2) assimilated by photosynthesis

    $\varepsilon \rightarrow$ 5-35 per mil depending on pathway extent of consumption

C-isotopic Composition of Organic Compounds

- Fractionation during photosynthesis

Pathway, enzyme	**React & substr**	**Product**	**ε ‰**	**Organisms**
C3 Rubisco1 Rubisco2 PEP carboxylase PEP carboxykinase	 CO_2 +RUBP CO_2 +RUBP HCO_3^- +PEP CO_2 +PEP	 3-PGA x 2 3-PGA x 2 oxaloacetate oxaloacetate	10-22 30 22 2	 plants & algae cyanobacteria plants & algae plants & algae
C4 and CAM PEP carboxylase Rubisco1	 HCO_3^- +PEP CO_2 +RUBP	 oxaloacetate 3-PGA x 2	2-15 2 30	 plants & algae (C4)
Acetyl-CoA CO dehydrog Pyruvate synthase PEP carboxylase PEP carboxykinase	 CO_2 + 2H+ CoASH CO2 + Ac-CoA HCO_3^- +PEP CO_2 +PEP	 AcSCoA pyruvate oxaloacetate Oxaloacetate	15-36 52 2	bacteria
Reductive or reverse TCA	CO2 + succinyl-CoA (+ others)	α-ketoglutarate	4-13	Bacteria esp green sulfur
3-hydroxypropionate	HCO_3^- + acetylCoA	Malonyl-CoA		Green non-S

C-isotopic Composition of Organic Compounds

Source of carbon and its C-isotopic composition

- Inorganic carbon
  - (-7‰ atm. CO_2) assimilated by photosynthesis

    $\varepsilon \rightarrow$ 5-35 per mil depending on pathway extent of consumption

- Organic carbon
  - (-25‰ on average) assimilated during heterotrophy

    $\varepsilon \rightarrow$ -1 (you are what you eat plus 1 per mil!!)

- Methane carbon
  - (-30 to -100‰) assimilated during methanotrophy

    $\varepsilon \rightarrow$ 0-30 per mil depending on pathway and extent of consumption http://www.astrobio.net/news/modules.php?op=modload&name=News&file=article&sid=34

Image removed due to copyright restrictions.

Café Methane

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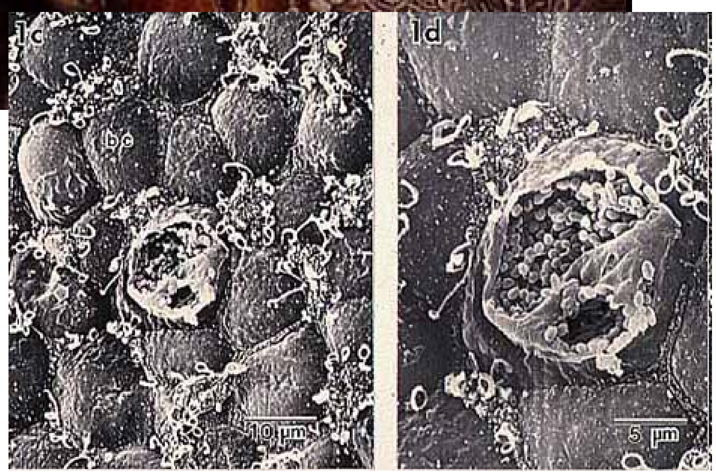
At the very edge of the brine pool, the mussels are especially abundant and happy. This area is often filled with newly settled baby mussels perched on the shells of larger mussels just above the brine.
Credit: Penn State University, Dept. of Biology

Image removed due to copyright restrictions.

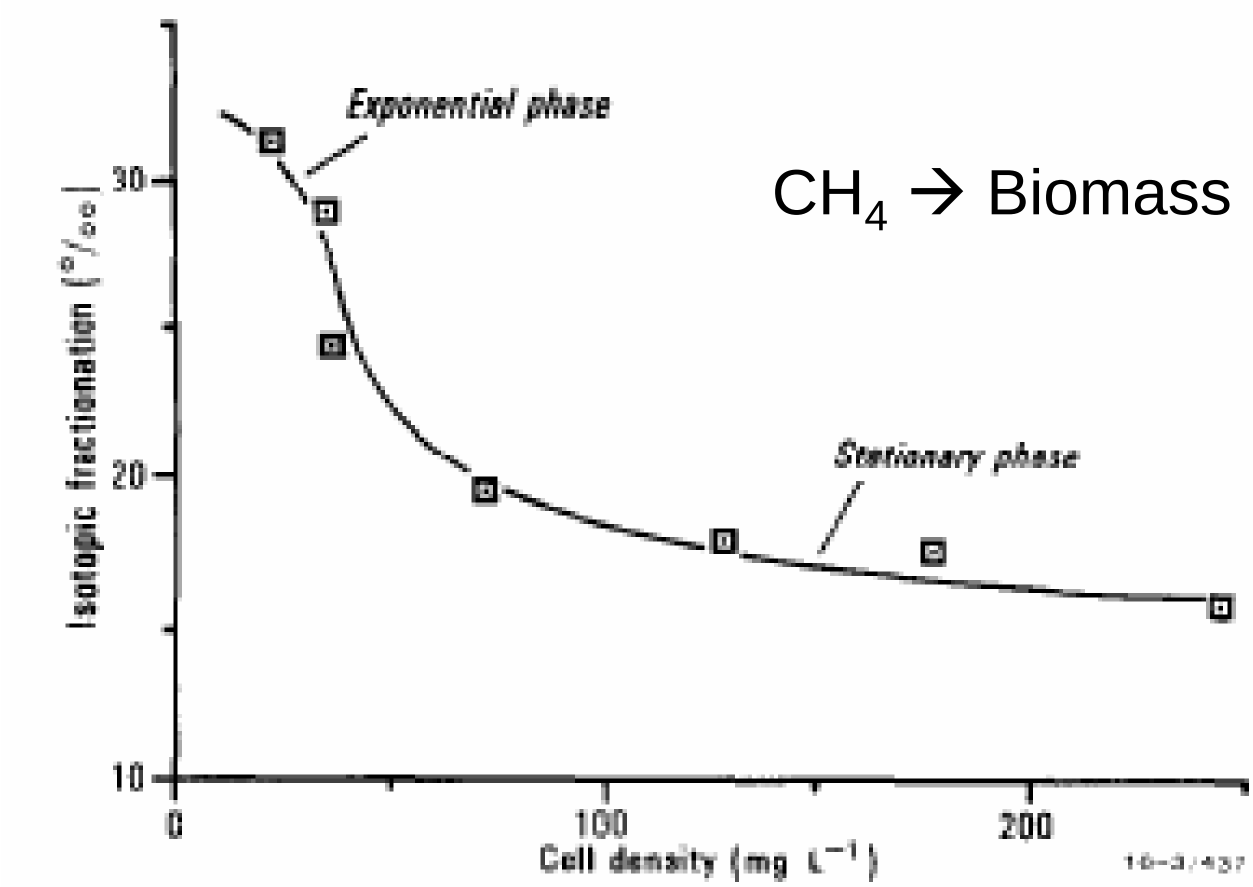
Gas hydrates (yellow) are ice with gas trapped inside; exposed beds are accessible to submersibles on the deep sea floor of the Gulf of Mexico. Ice worms, a new species only seen in hydrate, were discovered in 1997 by C. Fisher, Penn State University.
Credit: I. MacDonald

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.

Fractionation Maximal Early in Growth



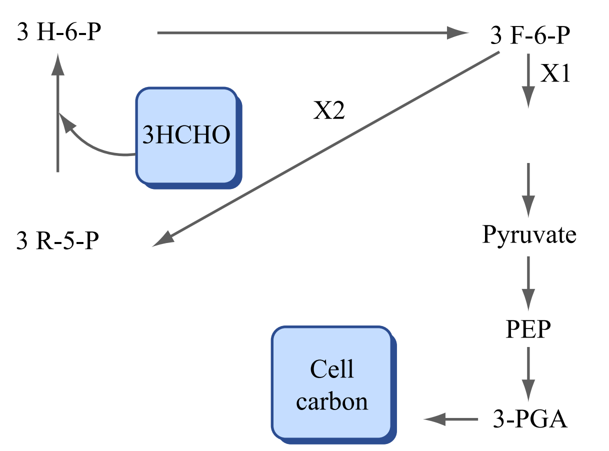
$CH_4 \rightarrow$ Biomass $\varepsilon \sim 32‰$

C-isotopic fractionation in biomass of M. capsulatus as function of growth stage

C-isotopic Composition of Organic Compounds

- Fractionation during methane consumption

$CH_4 > CH_3OH > HCHO > HCOOH > CO_2$



Assimilation pathway of carbon from methane oxidation for RuMP Type I and Type X methanotrophs such as *Methylococcus capsulatus*.

Figure by MIT OpenCourseWare.

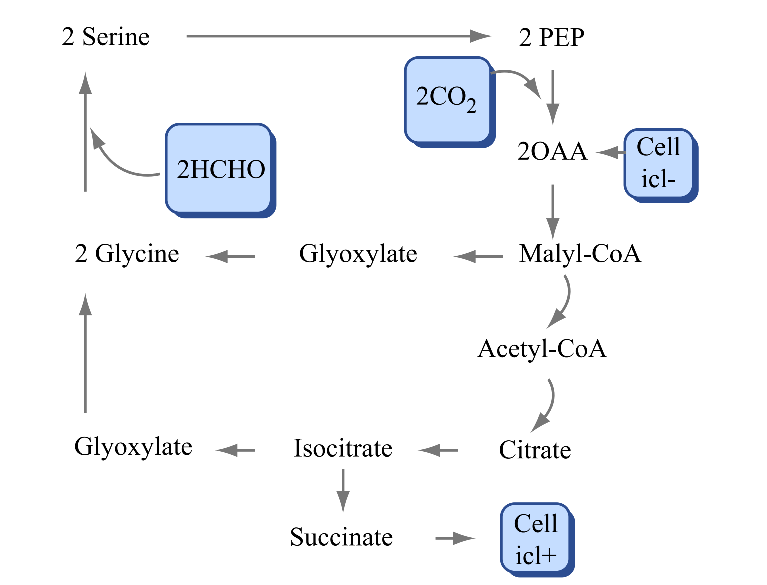
Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers

ROGER E. SUMMONS,[1] LINDA L. JAHNKE,[2] and ZARKO ROKSANDIC[1]

C-isotopic Composition of Organic Compounds

- Fractionation during methane consumption

$CH_4 > CH_3OH > HCHO > HCOOH > CO_2$



Assimilation pathway of carbon from methane oxidation for Type II methanotrophs such as *Methylobacterium organophilum* and *Methylosinus trichosporium.*

Figure by MIT OpenCourseWare.

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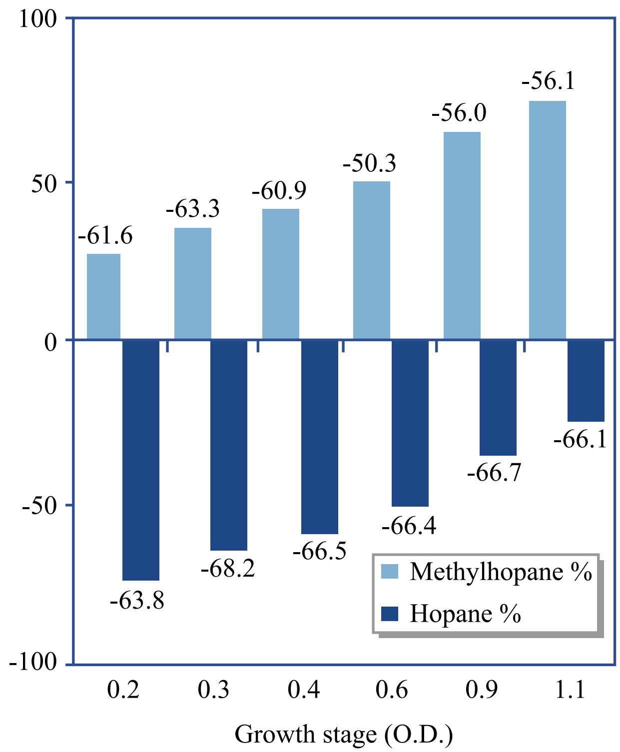
Biosynthetic pathways to hopanols and methyl sterols in Methylococcus capsulatus

Biomass-triterpenoids ε ~ 36‰



Changes in the proportions and isotopic compositions of the hopane skeletons of bacteriohopanepolyols during growth of *M. capsulatus*. The data were obtained by CSIA analysis of the hopane-29-ols prepared by cleavage of the polyhydroxy side-chain using periodate oxidation-$NaRH_1$, reduction.

Figure by MIT OpenCourseWare.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1995, p. 576–582 Vol. 61, No. 2
0099-2240/95/$04.00+0

Identification of Methanotrophic Lipid Biomarkers in Cold-Seep Mussel Gills: Chemical and Isotopic Analysis

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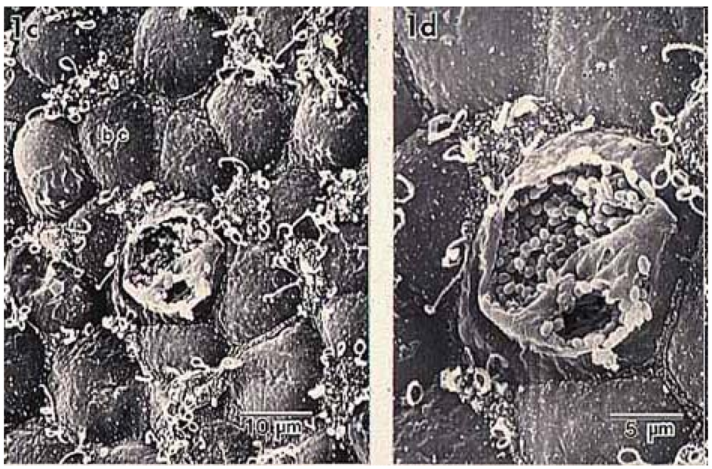

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Using the scanning electron microscope, we can see over a dozen mussel gill cells in the panel on the left. On the right is a closer look at the cell with its outer membrane partially removed. Look into the cell to see hundreds of symbiotic bacteria.

Courtesy of Charles Fisher, Penn State University, Dept. of Biology. Used with permission.

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Organic Geochemistry 39 (2008) 1659–1667

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Organic Geochemistry

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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-

C-isotopic Composition of Organic Compounds

- Fractionation during biosynthesis (lipids)

Intramolecular C-isotopic Differences

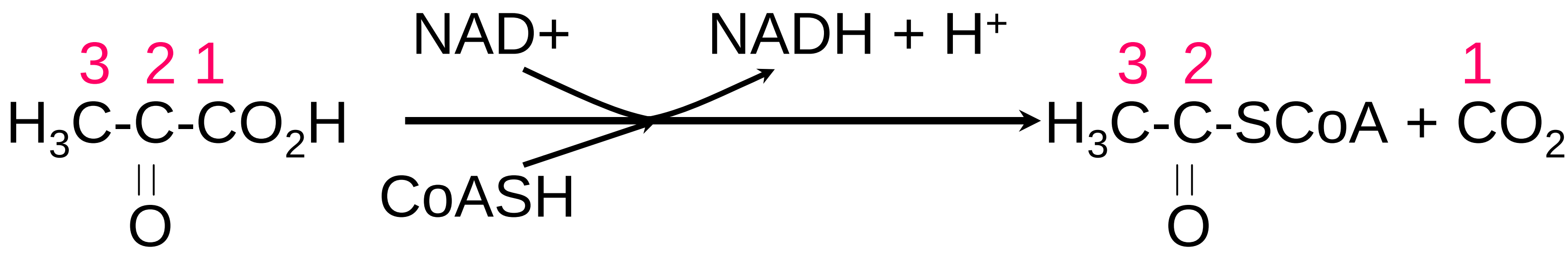
(DeNiro and Epstein, 1977; Monson and Hayes, 1980, 1982; reviewed Hayes, 2001)

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

Isotopic Fractionations in Biosynthetic Reactions



Pyruvate Dehydrogenase

$$({}^{12}K/{}^{13}K)_{C\text{-}2} = 1.0232$$

The species containing carbon-12 at position 2 reacts **1.0232 times** more rapidly that the species containing carbon-13 at that position

It is termed "a 23‰ isotope effect"

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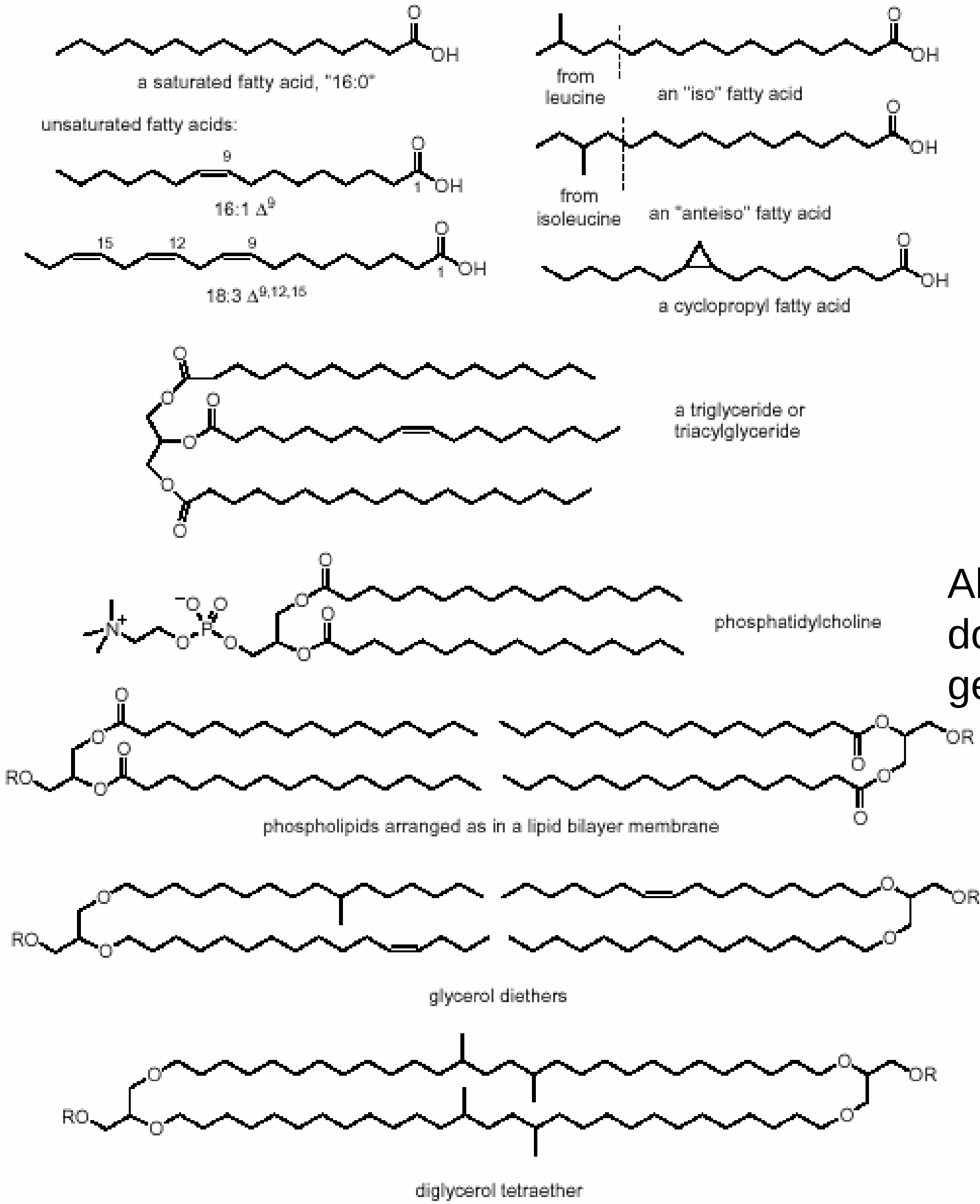
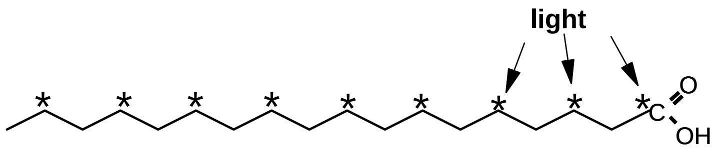
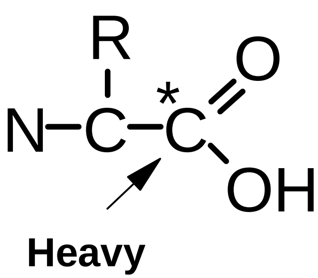
Flows of C at the pyruvate branch point in the metabolism of E. coli grown aerobically on glucose (Roberts 1955). 74% of the pyruvate is decarboxylated to yield Ac-CoA. The observed depletion at odd-numbered positions of FAcids is shown at the right indicating that the isotope effect at C-2 in the pyruvate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

An important consequence of the pyruvate to acetate isotopic fractionation



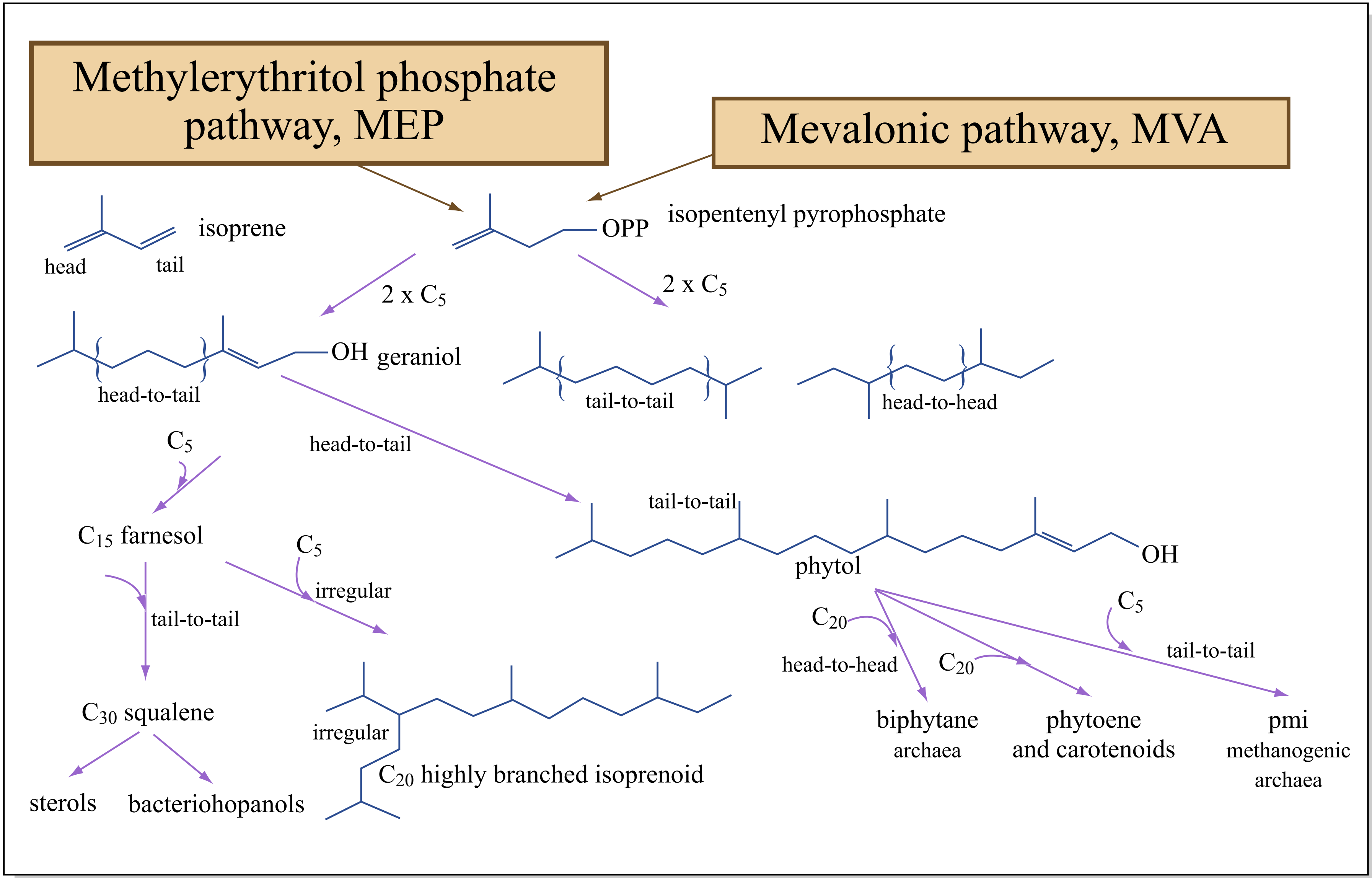


Alternate carbons derived from acetate carboxyl down acetogenic lipidbackbones are light. In general lipids are also light, but not as light.



In contrast, the carboxyl carbon of amino acids is generally "heavy"

Two Origins for Isoprenoids



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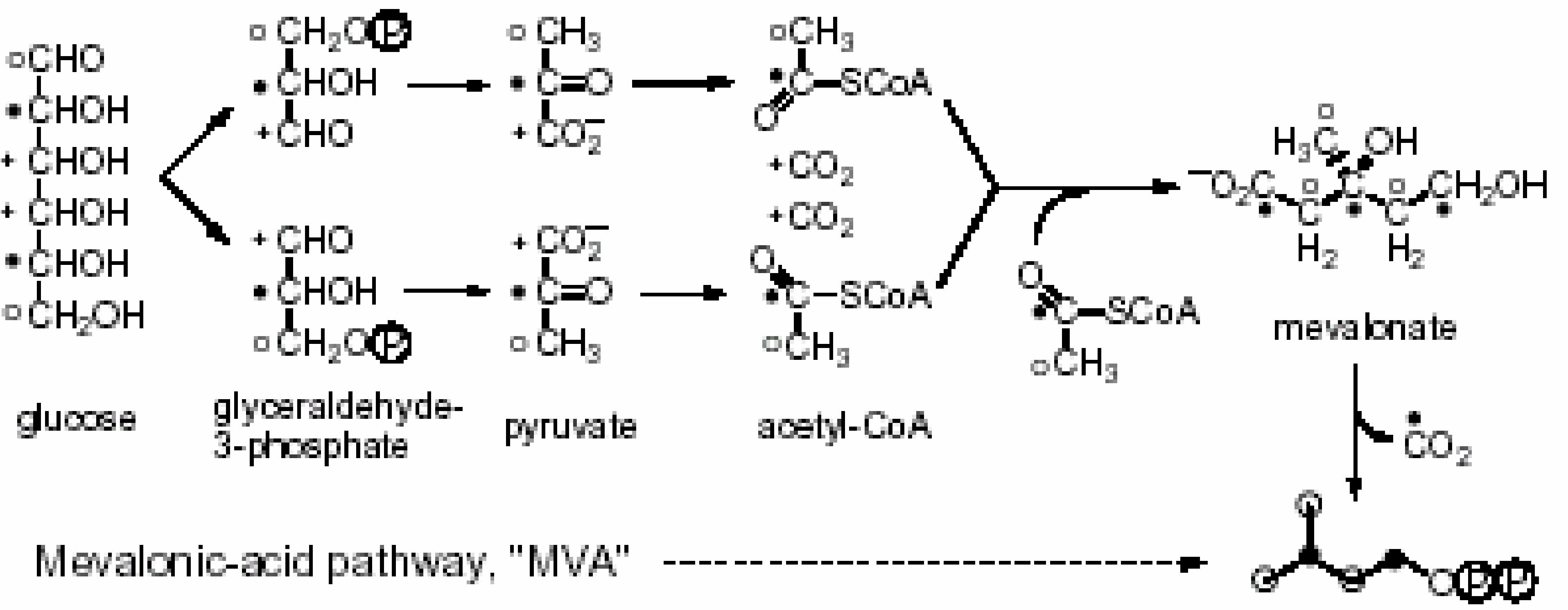
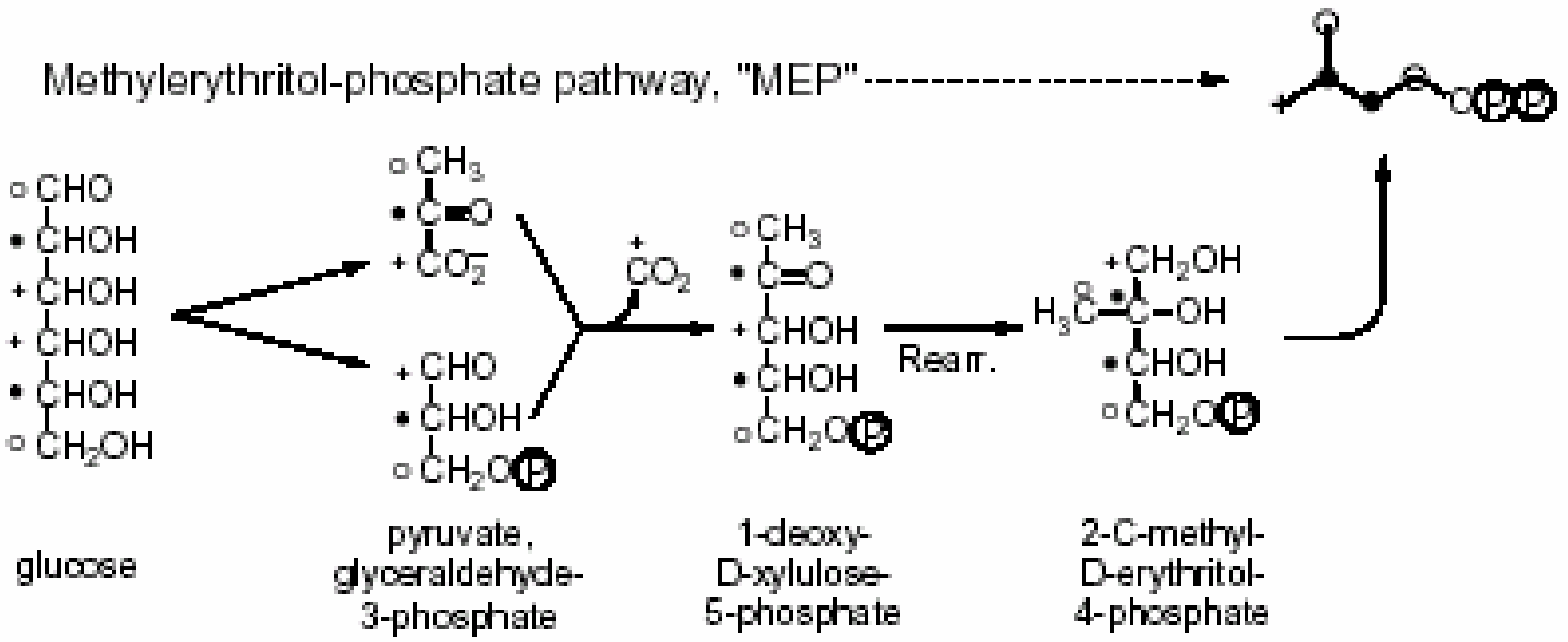
Labelling of isopentenylpyrophosphate from MVA pathway



Labelling of isopentenylpyrophosphate from MEP pathway



Labeling of phytol and cholesterol from MVA (a & c) and MEP (b & d)

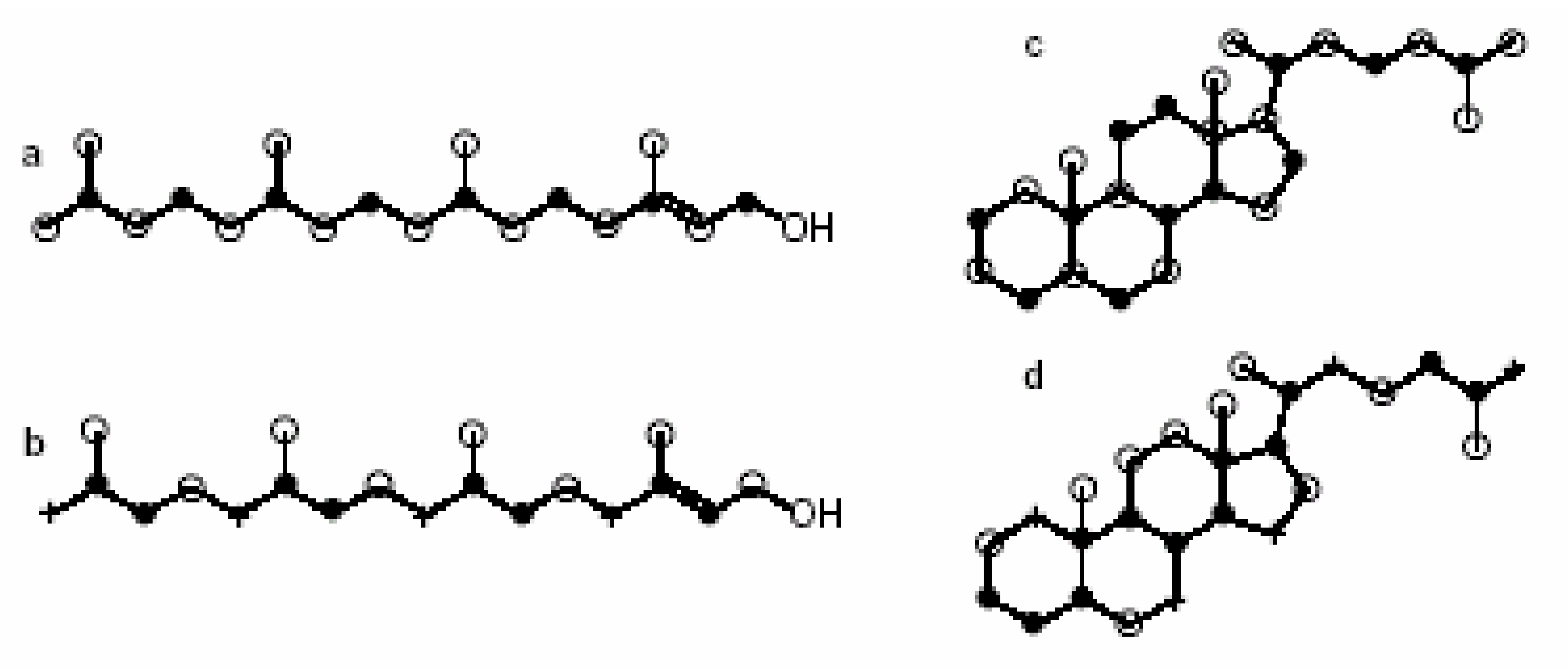


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Table of pathways used for the biosynthesis of isoprenoid lipids in a variety of prokaryotic and eukaryotic organisms.