Factors controlling $^{13}$C contents of sedimentary organic compounds: Principles and evidence

J.M. Hayes

*Biogeochemical Laboratories, Departments of Geological Sciences and of Chemistry, Geology Building, Indiana University, Bloomington, IN 47405, USA*

(Revision accepted May 5, 1993)

ABSTRACT


The carbon isotopic composition of any naturally synthesized organic compound depends on (1) the carbon source utilized, (2) isotope effects associated with assimilation of carbon by the producing organism, (3) isotope effects associated with metabolism and biosynthesis, and (4) cellular carbon budgets. These factors are reviewed and quantitative considerations summarized, particularly with regard to active and passive modes of carbon assimilation by phytoplankton and the existence of discernible regularities in isotope effects associated with lipid biosynthesis. It is concluded that $n$-alkyl lipids are in general depleted in $^{13}$C by about 1.5‰ relative to polyisoprenoid lipids produced by the same organism. The effects of biological reworking on isotopic compositions of organic carbon are examined and it is suggested that enrichment of $^{13}$C in sedimentary organic carbon may result from loss of CH$_4$ from zooplanktonic gut communities. Isotopic methods for estimation of ancient CO$_2$ levels are considered and a hyperbolic form favored for the relationship between concentrations of dissolved CO$_2$ and isotopic fractionation.

Introduction

Each molecule recovered from an ancient sediment carries information about the organism that produced it. Through chemotaxonomic associations, the structure can indicate the identity of the producer. The isotopic composition of the molecule can indicate the isotopic composition of the parent organism and that, in turn, can reveal the carbon source utilized by the producer and thus its position within the ancient ecosystem. Because these factors are in turn dependent on environmental conditions, the distribution of $^{13}$C among natural products is a sensitive paleoenvironmental indicator and can provide a great deal of information about ancient biogeochemical processes.

The principles are straightforward, but details of analysis and interpretation can become intricate. Given the variety of controlling factors, some well organized strategy is required for the interpretation of isotopic data. To disentangle the multiple signals present in the molecular-isotopic record, it has proven useful to consider processes sequentially, focussing first on the products of photosynthetic organisms and determining their isotopic compositions. Isotopic compositions of respiring heterotrophs and of fermentative organisms can then be understood in terms of secondary isotopic fractionations. Quantitative treatment of fractionations associated with equilibrium isotope effects and with branch points in networks of largely irreversible reactions is straightforward, but many persons long interested in sedimentary phenomena are unfamiliar with such considerations. This paper aims to present a useful introduction.
Biogeochemical principles

Isotopic fractionation

Consideration of isotopic compositions must be based on fractionation rather than isotopic composition. It is the difference between two isotopic compositions which is meaningful, not either δ value on its own. We will begin, therefore, by introducing terms for isotopic fractionation and considering the isotopic composition of marine dissolved inorganic carbon (DIC), a prominent reference point for many considerations of fractionation. It is best to express kinetically and thermodynamically controlled fractionations in terms of a ratio of isotope ratios. This is accomplished—indirectly, to be sure—by use of the ε notation:

\[ \varepsilon_{A/B} = (\alpha_{A/B} - 1) \times 10^3 \]  

\[ \alpha_{A/B} = R_A/R_B = \frac{(1000 + \delta_A)}{(1000 + \delta_B)} \]

where \( R \equiv ^{13}C/^{12}C \). As implied by the form of Eq. 1, the units for ε are parts per thousand or ‰, just as for δ. For small values of ε and especially when δ values are near zero, differences between \( \Delta_{A/B} \) and \( \varepsilon_{A/B} \) are small:

\[ \Delta_{A/B} \equiv \delta_A - \delta_B \approx \varepsilon_{A/B} \] (3)

For example, for \( \delta_A = -27 \) and \( \delta_B = -31 \), \( \Delta_{A/B} \) is 4.0 and \( \varepsilon_{A/B} \) is 4.1‰, a difference that is probably small in comparison to related uncertainties. In contrast, for \( \delta_A = 0 \) and \( \delta_B = -28 \), \( \Delta_{A/B} \) is 28.0 and \( \varepsilon_{A/B} \) is 28.8‰. This difference is also not very large, but close comparisons of fractionations associated with carbon fixation, for which these values would be typical, are becoming more common. Confusion will be minimized if ε is uniformly employed in such cases.

Inorganic carbon

The DIC from which marine biomass is synthesized is comprised of multiple chemical species linked by a series of equilibria:

\[ \text{CO}_2(aq) + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \]  

\[ \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \]  

\[ \text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-} \]

Fully deprotonated carbonate can combine with any of a number of divalent cations to form insoluble minerals, calcite and aragonite being the most common:

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \rightleftharpoons \text{CaCO}_3(s) \] (7)

An isotope effect is associated with each of these equilibria. The isotopic compositions—δ values—of dissolved CO2 and carbonate minerals are, therefore, not equal. A characteristic of such equilibrium isotope effects is that the isotopic difference between reactant and product depends only on temperature and not on the distribution of material between product and reactant. Therefore, although the relative abundances of the species indicated in Eqs. 4–7 will be strongly dependent on pH, the isotopic differences between those species will depend only on temperature. Paleobiogeochemists are interested in the substances at the beginning and end of the sequence of carbonate equilibria: \( \text{CO}_2(aq) \) is the substrate for the carbon-fixing enzyme, rubisco, and \( \text{CaCO}_3 \) is the commonly preserved mineral that provides information about overall abundances of \(^{13}\text{C}\) in ancient oceans.

The isotopic differences between these species are summarized most conveniently in terms of equilibrium isotope effects relative to bicarbonate. For dissolved CO2, Mook et al. (1974) have shown that:

\[ \varepsilon_{d/b} = \left[ \frac{\delta_d + 1000}{\delta_b + 1000} - 1 \right] \times 10^3 \]

\[ = 24.12 - 9866/T \]

where the subscripts d and b are shorthand for dissolved CO2 and bicarbonate and T is the absolute temperature. Serious confusion has existed regarding equilibria between bicarbonate and carbonate minerals because isotope effects relating to calcite and aragonite differ and this was not adequately recognized. Recently, however, Morse and MacKenzie (1990) have shown that, for calcite:

\[ \varepsilon_{m/b} = \left[ \frac{\delta_m + 1000}{\delta_b + 1000} - 1 \right] \times 10^3 \]

\[ = -14.07 + 7050/T \]

where the subscript m is shorthand for mineral
calcite. As shown in Fig. 1, the resulting carbon isotopic difference between calcite and dissolved CO$_2$ is large and quite strongly temperature dependent.

Two additional factors complicate the picture. First, it cannot be assumed that equilibrium was attained between calcite and dissolved CO$_2$. Where the preserved carbonate is a product of biomineralization, the $^{13}$C content of CO$_3^{2-}$ at the site of precipitation may have been affected by metabolic processes. For foraminifera, for example, such "vital effects" can be as large as a few permil. Pertinent data have been reviewed recently by Spero et al. (1991). An exemplary case has recently been considered by Jasper et al. (1993), who drew a distinction between $\delta_m$, the isotopic composition of calcite in equilibrium with dissolved CO$_2$, and $\delta_t$, the isotopic composition of a preserved foraminiferal calcite. For Neogloboquadrina dutertrei, a planktonic foram abundant in sediments from the central equatorial Pacific, they showed that available data (Fairbanks et al., 1982) indicated:

$$\Delta_{m/t} \equiv \delta_m - \delta_t = -0.70\,\%$$  \hspace{1cm} (10)

and improved the accuracy of their reconstruction of $\delta_d$ by applying this correction. Second, even if equilibration did occur, the site of that process (i.e., in bottom waters or sedimentary pore waters rather than in the photic zone) may be significant. Thinking again in terms of foraminifera, if the goal is reconstruction of the isotopic composition of dissolved CO$_2$ available to algae in open marine environments, reference to planktonic rather than benthic foraminiferal calcites is clearly preferred. In a study of the Greenhorn Formation, Hayes et al. (1989) found that micritic carbonates were comprised largely of pelleted but otherwise uncorroded coccoliths and that stratigraphic variations in their $\delta$ values were smoother than those of sparry cements or benthic bivalves. To estimate changes in (as distinct from accurate values of) photic-zone $\delta_d$, they therefore used the micritic phase, since it was at least certain that it reflected photic-zone DIC. Where carbonates that formed from sedimentary pore waters are involved, diagenetically induced shifts in $\delta_m$ can be severe and great care must be taken; Irwin et al. (1977) have provided a very useful review of relevant phenomena.

**Organic carbon**

Production of biomass can be viewed as occurring in two distinct steps: assimilation of carbon and biosynthesis of cellular components. An overview is provided in Fig. 2. The $^{13}$C content of each biomolecule depends on:

1. The $^{13}$C content of the carbon source.
2. Isotope effects associated with the assimilation of carbon.
3. Isotope effects associated with metabolism and biosynthesis.
4. Carbon budgets at each branch point.

For an autotroph, the carbon source will be environmental carbon dioxide or bicarbonate. Factors affecting their $\delta$ values have been considered in the preceding section. For a heterotroph, the isotopic composition of the carbon source will be determined by feeding patterns and by isotopic fractionations occurring upstream in the foodweb. Isotope effects associated with assimilation of carbon are of great importance in autotrophic organisms, but assimilation isotope effects per se are significant for heterotrophy only where very small molecules
Fig. 2. An overview of a cellular carbon budget and of pathways leading to biosynthetic products. Kinetic isotope effects are denoted by $\varepsilon$ and are arbitrarily numbered to indicate their independence. Isotopic compositions are denoted by similarly numbered $\delta$ terms. The circled numbers indicate branch points.

(probably three carbons, maximum) are absorbed and processed individually, as in methanotrophs. In heterotrophs that ingest food particles, assimilation "effects," if present at all, derive from selective absorption of specific compounds or compound classes (including, in metazoans, substances that may be produced by gut flora). In all organisms, both autotrophic and heterotrophic, the biosynthesis of cellular components is based on cellular pools of common intermediates; lipids, for example, always deriving from acetate. Isotope effects associated with metabolic reactions that produce or consume such intermediates affect the $^{13}$C contents of the biosynthetic products. Finally, at each point within the cellular reaction network, distribution of carbon among products will affect isotopic compositions. A detailed example follows.

**Fractionation in reaction networks**

Isotopic compositions of organic compounds are shaped within cellular reaction networks. Controlling factors are of two types: isotope effects, specifically kinetic isotope effects except where exchange with bicarbonate is involved, and "branching ratios," the divisions of carbon flow that occur wherever a single reactant leads to multiple products. A schematic view is shown in Fig. 3, which can be taken to represent a point within a sequence of enzymatically catalyzed reactions. Mass balance provides the key to understanding fractionation and is represented by the equation:

$$\phi_R \delta_R = \phi_U \delta_U + \phi_P \delta_P$$

where the $\phi$ terms represent flows of carbon (moles/s) and the $\delta$ terms represent isotopic compositions, the subscripts R, U, and P indicating respectively reactant, unused reactant (which flows to other reaction sites at which it is used to make other products), and product. Because a kinetic isotope effect is associated with the reaction ($C_R \rightarrow C_P$), the product is depleted in $^{13}$C relative to the reactant available at the reaction site. Due to that preferential withdrawal of $^{13}$C-depleted reactant, the pool of $C_R$ at the reaction site is isotopically enriched relative to the external supply. Using $\delta_R^* > \delta_R$ and $\delta_P = \delta_R^* - \varepsilon$

$$\delta_R = \delta_R^* - \varepsilon$$

where $\varepsilon$ indicates the magnitude of the kinetic isotope effect:

$$\varepsilon \equiv (^{12}k/^{13}k - 1) \times 10^3$$

the $k$ terms being rate constants for the indicated isotopic species. Substitutions then transform the mass-balance equation into useful expressions of fractionation. Specifically, the fractional yield of product, $f$, is given by $\phi_P/\phi_R$; by difference, the fraction of incoming $R$ that is unused is $1 - f$; and, since no process acts to change the isotopic composition of the unused reactant, $\delta_U = \delta_R$. Isotopic compositions of materials leaving the reaction site are then given by:

$$\delta_U = \delta_R + f \varepsilon$$

and

$$\delta_P = \delta_R - (1 - f) \varepsilon$$ (14a, 14b)

Fig. 3. A depiction of the flows and isotopic compositions of carbon at a branch point.
Fractionation associated with carbon fixation

Isotopic fractionations accompanying the assimilation of carbon by autotrophic organisms represent a special case. Not only does fractionation depend on mass balance, but the value of $\varepsilon$ itself is variable and dependent on environmental factors. This occurs because assimilation comprises at least two processes, mass transport and carbon fixation, each with its own characteristic isotope effect, and the overall isotope effect depends on interactions between those processes. In some organisms it is necessary to consider isotope effects (plural) associated with fixation of carbon. This is indicated in Fig. 5, in which a distinction is drawn between organisms employing the Calvin Cycle and possessing, therefore, a single pathway through which essentially all fixed carbon flows, and other groups of organisms. Green photosynthetic bacteria (the Chlorobiaceae) fix carbon mainly by reversing the tricarboxylic acid cycle (Sirevag et al., 1977). Together with nonphotosynthetic bacteria that can grow autotrophically while fixing carbon and constructing biomass by use of the "acetyl-CoA pathway" (i.e., acetogenic and methanogenic bacteria, Wood et al., 1986), they form a subset of organisms in which carbon is fixed at three or more different enzymatic sites. The distribution of $^{13}$C between and within biosynthetic products from these organisms can directly reflect isotope effects at and contributions from individual fixation sites.

If mass transport occurs passively, with CO$_2$ reaching the fixation site simply by diffusing in response to concentration gradients, the isotope effect associated with the carbon-fixing enzyme, rubisco, tends to dominate. Relevant flows of carbon and their isotopic compositions are shown schematically in Fig. 6. All are cast in terms of CO$_2$ rather than bicarbonate because, even though the latter substance is much more abundant, molecular CO$_2$ is both the actual substrate utilized by rubisco (Cooper et al., 1969) and the form of inorganic carbon that diffuses passively into and out of cells. The isotope effect associated with

Fig. 4. The relationships between isotopic compositions and fractional yield at a branch point.
Fig. 6. The two-step model for fixation of carbon by autotrophs that obtain CO$_2$ by passive diffusion (Farquhar et al., 1982). The wavy line represents the boundary between a C-fixing cell and the environment. Isotopic compositions of external CO$_2$, CO$_2$ at the fixation site, and fixed carbon are denoted by $\delta$, $\delta_i$, and $\delta_f$, respectively. Flows of carbon (e.g., moles/s) are denoted by $\phi$ and isotope effects by $\epsilon$. Isotopic compositions of carbon travelling the indicated pathways are given by $\delta_e - \epsilon_e$, $\delta_i - \epsilon_i$, and $\delta_f - \epsilon_f$. Isotopic compositions of carbon outside and inside the cell are specified by $c_e$ and $c_i$, respectively.

mass transport is denoted by $\epsilon_i$, so the $\delta$ value of carbon diffusing into the cell is given by $\delta_e - \epsilon_e$. In turn, the isotope effect associated with the fixation reaction is $\epsilon_f$, so the $\delta$ value of fixed carbon is given by $\delta_f - \epsilon_f$. The mass balance for CO$_2$ inside the cell can then be written as:

$$\phi_f(\delta_e - \epsilon_e) = \phi_a(\delta_i - \epsilon_i) + \phi_f(\delta_i - \epsilon_f)$$

If the fluxes of carbon into and out of the cell are proportional to the concentrations, $c_i$ and $c_e$, then $\phi_a/\phi_f = c_i/c_e$ and $\phi_f/\phi_i = 1 - c_i/c_e$. Moreover, $\delta_i$ can be eliminated by the substitution $\delta_i = \delta_f + \epsilon_f$ so that Eq. 15 becomes:

$$\delta_e - \epsilon_i = (c_i/c_e)(\delta_f + \epsilon_f - \epsilon_i) + (1 - c_i/c_e)\delta_f$$

Rearrangement yields an expression for the overall isotope effect—the isotopic difference between fixed carbon and the carbon source—denoted here by $\epsilon_P$ (P for Photosynthesis or Primary Production):

$$\epsilon_P = \delta_e - \delta_i = \epsilon_f + (c_i/c_e)(\epsilon_f - \epsilon_i)$$

This derivation, which follows an approach introduced by Farquhar et al. (1982a), has liberally employed the approximation $\Delta \approx \epsilon$, but it can be shown that the final relationship is exact. According to Eq. 17, values of $\epsilon_P$ ought to vary between $\epsilon_i$ and $\epsilon_f$ depending on the magnitude of the ratio $c_i/c_e$. Since $\epsilon_i$ is very small (0.7%o for diffusion of CO$_2$ in water at 25°C, O'Leary, 1984) and $\epsilon_f$ is about 27%o (Farquhar et al., 1982b), a considerable range is expected.

Observed values of $\epsilon_P$ vary widely, sometimes even beyond the expected range of $0.7 \leq \epsilon_P \leq 27%o$, notably to $\epsilon_P < 0$ (McCabe, 1985). Since all known carbon kinetic isotope effects discriminate against $^{13}$C, the production of organic carbon that is enriched in $^{13}$C relative to the CO$_2$(aq) available to the cell (i.e., $\epsilon_f < 0$, an apparent preference for $^{13}$C) indicates that CO$_2$(aq) was not the carbon source and that some substance relatively enriched in $^{13}$C must have been. As shown by Eq. 8, HCO$_3^-$ is a logical candidate, being enriched by 9%o at 25°C. Thanks in part to such isotopic evidence, it is now recognized that numerous algal species actively transport or “pump” bicarbonate into their cells (Badger, 1987). Carbonic anhydrase subsequently catalyzes production of dissolved CO$_2$ that can serve as a substrate for carbon fixation. As would be expected since such active transport is energetically costly, the process is inducible rather than ubiquitous and is commonly employed when concentrations of CO$_2$(aq) are too low to sustain photosynthesis (Sharkey and Berry, 1985). Ideally, cells that had imported their internal CO$_2$ at significant metabolic cost would lose none of it. In that case (i.e., $\phi_a = 0$), the carbon-flow pathway (Fig. 7) would become a “one-way street.” Every bicarbonate pulled into the cell would be committed to production of organic carbon and the isotopic composition of the biomass would be determined by the isotopic characteristics of the pump. In fact, however, some CO$_2$ leaks from the cell and the isotopic mass balance associated with that leakage forms the principal control on the isotopic composition of the biomass. For the system shown schematically in Fig. 7, a mass balance analogous to Eq. 15 takes the form:

$$\phi_f(\delta_e - \epsilon_d/b - \epsilon_a) = \phi_a(\delta_i - \epsilon_i) + \phi_f(\delta_i - \epsilon_f)$$

where $\epsilon_a$ is the isotope effect associated with the active transport of bicarbonate. Defining $L \equiv \phi_d/\phi_i$ (i.e., $L$ = fraction of imported bicarbonate that leaks from the cell) and making substitutions analogous to those employed above leads to:

$$\epsilon_P = \delta_e - \delta_f = \epsilon_d/b + \epsilon_a + L(\epsilon_f - \epsilon_i)$$

In such systems, $\epsilon_P \rightarrow \epsilon_d/b + \epsilon_a$ as $L \rightarrow 0$ and, because
Fractionation associated with biosynthetic pathways

Products of carbon fixation flow to additional processes that synthesize the compounds needed for construction and maintenance of biomass. These include complex carbohydrates, proteins, and lipids. With the prominent exception of porphyrins, nearly all sedimentary biomarkers derive from lipids. Both for that reason and because diverse information is now available regarding isotopic fractionations associated with the biosynthesis of lipids, we will focus on that class of compounds.

It has long been known (e.g., Abelson and Hoering, 1961) that “lipids are light” (i.e., they contain less of the heavy isotope, $^{13}$C, than do other products of biosynthesis). For prokaryotic organisms, the reaction network within which that isotopic depletion must occur is shown in Fig. 8. Branch points in the flow of carbon are evident (1) downstream from pyruvate and (2) downstream from acetate. In an elegantly simple approach to locating the point of fractionation, DeNiro and Epstein (1977) grew heterotrophic bacteria on glucose (a $C_6$ carbohydrate), on pyruvate, and on acetate, thus providing carbon inputs at two points above and one point below the branch point between pyruvate and acetyl-CoA. The resulting lipids were found to be depleted in $^{13}$C relative to either glucose or pyruvate, the depletions being roughly equal. Lipids from acetate-fed bacteria were equal in isotopic composition to the carbon source. These results indicated clearly that processes responsible for the depletion were at the branch point downstream from pyruvate.

Mechanistically, it appeared that an isotope effect associated with pyruvate→dehydrogenase, the enzyme catalyzing the pyruvate acetyl-CoA reaction, was involved, causing specifically a depletion in $^{13}$C at the carboxyl carbon of acetate and leaving the methyl carbon unfractionated. Although DeNiro and Epstein (1977) miscalculated the fractionations that would derive from the isotope effect they proposed, Monson and Hayes (1980, 1982a) subsequently showed that the qualitatively expected alternating pattern of $^{13}$C depletion was present in acetogenic lipids. From the results of isotopic analyses of specific carbon posi-
Fig. 8. Pathways of lipid biosynthesis in prokaryotic organisms. In heterotrophs, any of the indicated multi-carbon organic compounds might derive from the food source. In Calvin-Cycle autotrophs the effective internal source of organic carbon is a C₆ carbohydrate. For organisms using the reverse-TCA or acetyl-CoA systems of carbon fixation, the methyl and carboxyl positions of acetyl-CoA will derive from two different fixation sites. The letters m and c denote positions in the C₅ and C₂ biomonomers that are derived from the methyl and carboxyl positions in acetyl-CoA and indicate the different m/c ratios in the two lipid families.

Monson and Hayes (1982a) demonstrated, that the methyl position in acetyl-CoA was not isotopically fractionated relative to carbohydrate, thus

$$\delta_m = \delta_{ch}$$

(22)

requiring that the δ value of the carboxyl position would depend on the δ value of the reactant carbon (pyruvate derived without fractionation from carbohydrate, in this case); on the fraction, f, of that reactant which flowed to acetyl-CoA; and on the isotope effect, $e_{pdh}$, associated with reaction catalyzed by pyruvate dehydrogenase.

Further research has revealed significant complexities. Blair et al. (1985) showed that prior results did not exclude the possibility that fractionation occurred downstream from acetate, but then Melzer and Schmidt (1987) showed that kinetic isotope effects associated with pyruvate dehydrogenase (not previously studied in isolation) were precisely in accord with estimates of $e_{pdh}$ derived by Monson and Hayes (1982a). Significant gaps in understanding remain and some of these limit the precision with which results of isotopic analyses of sedimentary materials can be interpreted:

1. All of the preceding investigators assumed
that, in carbohydrates produced by the Calvin Cycle, the abundance of $^{13}$C was equal at all carbon positions. But it has now been shown (Rossmann et al., 1991) that this is not true for maize (a C4 plant) or for sugar beets (C3). In each case, one of the two carbon positions in glucose from which carbon flows without fractionation to the methyl position of acetyl-CoA is depleted in $^{13}$C by 4.4% relative to the molecular average. As a result, one expects on average $\delta_m = \delta_{ch} - 2.2$ and a consequent additional depletion of $^{13}$C in lipids relative to initial carbohydrate. The depletion of $\delta_m$ was not observed by Monson and Hayes (1982a), who, for $\delta_{ch} = -9.96 \pm 0.05$, found $\delta_m = -9.5 \pm 1.0\%$ (uncertainties are standard errors of mean values, $\delta_m$ being based on analyses of two different fatty acids). With allowance for uncertainties in the Rossmann et al. (1991) result, the disagreement between the investigations is probably not statistically significant, but is worth keeping in mind.

(2) As indicated in Fig. 9, pathways of carbon flow are more complex in eukaryotic organisms, mainly because C2 units are produced within the mitochondrion but can be exported from it (as required for lipid biosynthesis) only as part of citrate, the C6 product resulting from the condensation of acetyl-CoA and a C4 species (see Fig. 9). Bonds to the methyl position in acetate are made and broken during the formation of citrate and its subsequent cleavage outside the mitochondrion; these processes provide additional opportunities for isotopic fractionation. In a detailed study of isotopic fractionation accompanying the biosynthesis of fatty acids in yeast, Monson and Hayes (1982b) concluded that the pattern of enrichment and depletion observed in prokaryotes is reversed in eukaryotic products, with the carboxyl position in the C2 biomonomer being enriched in $^{13}$C relative to the methyl position. No subsequent investigation has tested this result directly, but observed isotopic differences between isoprenoidal and n alkyl lipids (discussed below) indicate that it is probably not general.

(3) Not only acetogenic but, as indicated in Fig. 8, polyisoprenoid lipids are produced from acetyl-CoA. In eukaryotic organisms, the latter include abundant sterols as well as acyclic polyisoprenoids such as phytol. Formation of polyisoprenoids is less important in prokaryotes, but acyclic compounds are required by photosynthetic species and pentacyclic hopanoids are also widely distributed. Although both acetogenic and polyisoprenoid lipids derive from acetate, their isotopic compositions are, for several reasons, not expected to be equal. First, as noted in Fig. 8, three of the carbon positions in isoprene, the C5 biomonomer from which polyisoprenoids are built, derive from the methyl position of acetyl-CoA but only two derive from the carboxyl position. As a result, $\delta_{it}$,
the molecular-average \( \delta \) value for polyisoprenoid lipids, will be given by:

\[
\delta_{il} = \frac{3\delta_m + 2\delta_c}{5}
\]

(23)

and will be equal to \( \delta_a \) only if the methyl and carboxyl positions of acetyl-CoA have the same \( ^{13}C \) content, a coincidence that has never been observed. Second, the production of both polyisoprenoid and acetogenic lipids represents a branch point in the flow of \( C_2 \) units. An isotope effect at that branch point could cause isotopic compositions of methyl- and/or carboxyl-derived carbon positions in polyisoprenoids to differ from those of analogous positions in acetogenic lipids.

**Biosynthetic effects in general**

As emphasized in Fig. 2, the \( ^{13}C \) content of any cell is the result of a mass balance between inputs and outputs. For a cell containing \( m_{cell} \) moles of carbon, we can write:

\[
m_{cell} = m_{in} - m_{out}
\]

(24)

and

\[
\delta_{cell} = \frac{m_{in}\delta_{in} - m_{out}\delta_{out}}{m_{in} - m_{out}}
\]

(25)

Values of \( \delta_{in} \) and \( \delta_{out} \) depend on isotope effects associated with the assimilation and excretion of carbon. Associated fractionations will have pancelular effects. Equally,

\[
\delta_{cell} = \Sigma(m_i\delta_i)/\Sigma m_i
\]

(26)

where \( m_i \) and \( \delta_i \) are respectively the molar amount and \( ^{13}C \) content of the \( i \)th biosynthetic product and \( \Sigma \) indicates summation over a complete cellular inventory. Finally, \( \delta_{cell} \) must also be equal to the weighted average \( \delta \) value of the pool of metabolic intermediates which serves as the carbon source for biosynthesis. Conceptually,

\[
\delta_i = \delta_{int} - \Delta_i = \delta_{cell} - \Delta_i
\]

(27)

where \( \delta_{int} \) is the average \( \delta \) value of the pool of metabolic intermediates and \( \Delta_i \) is the overall isotopic fractionation characteristic of the biosynthesis of the \( i \)th product. Isotope effects and branching ratios on pathways linking the pool of metabolic intermediates to biosynthetic product will affect the isotopic compositions of individual molecules but not \( \delta_{cell} \).

**Sedimentary systems**

**Primary and heterotrophic fractionations**

*Ancient systems*

One of the first applications of compound-specific isotopic analyses to the study of a sedimentary record focussed on the Cenomanian–Turonian boundary, specifically in the Greenhorn Formation of the North American Western Interior Seaway (Hayes et al., 1989). The “specific compound” available for analysis was in fact a compound class: the porphyrins. This fraction, thought at the time to derive almost entirely from chlorophylls, was chosen for its significance as an indicator of the isotopic composition of primary materials. Knowledge of \( \delta_{por} \) (where por designates the porphyrin fraction) allowed estimation of \( \delta_p \) values:

\[
\delta_p = \delta_{por} + \Delta_{por}
\]

(28)

where \( \delta_p \) is the isotopic composition of Primary Products (in an open marine setting, the average \( \delta \) value for the biomass of phytoplankton). Comparison of \( \delta_p \) and \( \delta_d \) then allows estimation of Cenomanian and Turonian \( \varepsilon_p \) values. Moreover, comparison of \( \delta_p \) and \( \delta_{TOC} \) (TOC = sedimentary total organic carbon) allows estimation of all secondary isotopic fractionations (\( \equiv \Delta_e \)), i.e., the isotopic shifts associated with reworking of organic carbon prior to burial.

\[
\varepsilon_p \approx \delta_d - \delta_p = \delta_{in} - \varepsilon_{m/d} - (\delta_{por} + \Delta_{por})
\]

(29)

\[
\Delta_e = \delta_p - \delta_{TOC} = \delta_{por} + \Delta_{por} - \delta_{TOC}
\]

(30)

Values of \( \varepsilon_p \) provide information about the nature of primary producer organisms and their environment, those of \( \Delta_e \) about the intensity of reworking and thus trophic structure. Notably, this approach transforms purely chemical data into information about ancient biogeochemical processes, and takes as its starting point the primary production of biomass.

As might be inferred from the preceding discussion of isotope effects associated with lipid biosynthesis, the value of \( \Delta_{por} \) is uncertain. Considering literature reports and analyzing chlorophyllides and biomass from extant organisms, Hayes et al.
(1989) selected $\Delta_{\text{por}} = -0.5\%$o (i.e., porphyrins enriched in $^{13}$C relative to biomass). More recently, Kennicutt et al. (1992) have reported $\Delta_{\text{chl}} = -1.7\%$o (range -5.8 to 0.3%o, $s = 1.7\%o$, $n = 12$), where chl designates chlorophyll and thus indicates that the carbon analyzed included not only the substituted heteroaromatic ring which is the precursor of sedimentary porphyrins but also the phytol ester group. Correction for the presence of that lipid carbon pushes values of $\Delta_{\text{por}}$ beyond $-5\%$. The authors did not discuss the disagreement between their findings and those of previous workers (Hayes et al., 1989, and references cited therein) nor did they discuss mechanisms that would produce the required enrichment of $^{13}$C. The same paper reports that amino acids, which share biosynthetic precursors with the porphyrins, have on average $\Delta_{\text{aa}} = 1.3\%$o. If $\Delta_{\text{por}}$ were $-5\%$, indicated values of $\delta_p$ in many sedimentary systems (Hayes et al., 1987; Freeman et al., 1990; Boreham et al., 1989) would fall below the $\delta$ values of coexisting lipids of probable phytoplanktonic origin (pristane, phytane, steranes) and enormous heterotrophic shifts ($\Delta_T$) would be required to explain observed values of $\delta_{\text{TOC}}$. There are numerous elements of consistency which can be sought in isotopic data sets. If they are absent, caution is in order.

Subsequent investigations of the Greenhorn samples (Hayes et al., 1990), using isotope-ratio-monitoring gcms, have shown that acyclic polyisoprenoids are depleted in $^{13}$C relative to the porphyrin fractions by 3.8 to 5.5%o, averaging 4.5%o ($n = 20$). The same isotopic difference is observed in modern systems (e.g., Madigan et al., 1989) when chlorophyllides and isoprenoids are compared. The hypothesis that sedimentary porphyrins derive mainly from chlorophyll as opposed to alternatives such as cytochromes or hemes is supported by this observation, but the clearest evidence on this point has come from studies of $\delta$ values of individual porphyrins. Simultaneously and independently, research groups at the Bureau of Mineral Resources, Canberra, and the University of Strasbourg showed that C$_{32}$ etioporphyrins were uniquely depleted in $^{13}$C relative to all other porphyrins, notably including C$_{32}$ DPEP structures clearly deriving from chlorophyllides (Boreham et al., 1989; Ocampo et al., 1989). Because the C$_{32}$ etioporphyrins comprise only a few percent of typical porphyrin mixtures, fractions like those examined in the Greenhorn investigation can usually be taken as representative of chlorophyll. The observed depletion of $^{13}$C in the C$_{32}$ etioporphyrins makes derivation from hemes or cytochromes produced by heterotrophs feeding in aerobic portions of the paleoenvironment unlikely and suggests an origin from cytochromes produced abundantly by sulfide-oxidizing bacteria (Prince et al., 1988) which, as a result of their location at the aerobic–anaerobic interface, may utilize a carbon source depleted in $^{13}$C.

**Heterotrophic shifts in modern environments**

In the context of Fig. 2, the outgoing flow of carbon in a heterotrophic organism is comprised of respired CO$_2$ and fecal carbon and CH$_4$ (a product of gut bacteria). Carbon dioxide results mainly from operation of the tricarboxylic acid cycle, in which it is produced by decarboxylation of organic acids. Because the same acids can also flow to biosynthetic uses (chiefly the production of amino acids), each decarboxylation is associated with a branch point. If the decarboxylation reaction has a normal kinetic isotope effect, the respired CO$_2$ will be depleted in $^{13}$C and, by difference, the organism enriched. DeNiro and Epstein (1978) pioneered systematic investigations of this phenomenon and showed that it is quite general. For many animals, biomass is enriched in $^{13}$C relative to the food source by approximately 1%. Examination of isotopic shifts associated with heterotrophy has become a widely used tool for investigation of predator–prey and grazing relationships within ecosystems. A particularly elegant example is provided by Fry’s (1988) investigation of the food web in the water column at Georges Bank in the North Atlantic.

Effects of heterotrophy on the organic carbon being buried in sediments are not well known. As indicated above, Hayes et al. (1989) interpreted the difference between $\delta_p$ and $\delta_{\text{TOC}}$ in terms of heterotrophy, and the isotopic shift is in the direction expected, but the record of molecular structures found in marine sediments has been interpreted very differently, in fact mainly in terms of the preservation of primary products. These
views are not necessarily contradictory—it is possible that little bulk primary material would survive but that a selection of algal biomarkers would (Bradshaw et al., 1992). Investigations focussed on the biomarkers could then suggest that primary primary products were well preserved, but δTOC would in fact reflect secondary overprints. A recent report titled ‘‘Stable carbon isotope ratios of plankton carbon and sinking organic matter from the Atlantic sector of the Southern Ocean’’ (Fischer, 1991) is particularly interesting in this regard. It was found that sinking organic particles (mostly fecal material, though undoubtedly containing phytoplanktonic biomarkers, Bradshaw et al., 1992) were enriched in 13C relative to plankton by 2–5%. The fecal material does not represent heterotrophic biomass. Its enrichment in 13C is very plausible related to the enrichment of 13C noted in sedimentary deposits, many of which contain abundant pelletal material. The enrichment may be associated with heterotrophy, but cannot be due to respiratory metabolism. Instead, we can speculate here that it reflects loss of 13C-depleted CH4 from zooplanktonic gut communities. Shallow subsurface maxima are often found when the distribution of CH4 in marine water columns is examined (Brooks et al., 1981), and at least one study has appeared in which such maxima are specifically associated with high concentrations of zooplankton (Traganza et al., 1979). It appears likely that this mechanism, rather than accumulation of 13C along food chains, provides an explanation for the enrichment of 13C in some sedimentary organic carbon.

Although the Southern Ocean is highly productive and thus of all modern sites one of the most likely to support extensive heterotrophy, it is also true that the observed shifts significantly exceed those ascribed to heterotrophy in the Greenhorn sediments (Hayes et al., 1989) and that a strong correlation between paleontologically assessed trophic complexity and Δr has been observed in recent investigations of the Oxford Clay (Kenig et al., 1993). Additional enrichment of 13C occurred at the sediment–water boundary (Fischer, 1991). Effects of bacterial heterotrophy may also have been underestimated. Results of a recent investigation in which 13C contents of bacterial nucleic acids were used to estimate δ values for bacterial biomass produced under natural conditions indicate isotopic shifts as large as 3‰ (Coffin et al., 1990).

Relationships between carbon skeletons

When considering 13C contents of lipid carbon skeletons isolated from ancient sediments, it is—or would be—often helpful to associate two or more compounds with a single source or closely related sources. When, for example, two steranes or two internally methyl branched alkanes happen to have nearly identical δ values, the structural similarity supports but does not prove the association. In contrast, when fundamentally different structures are possibly to be ascribed to the same source, uncertainties can multiply rapidly. The most basic comparison is that between n-alkyl and isoprenoid carbon skeletons. Should we expect the former always to be depleted in 13C relative to the latter, or vice versa?

Monson and Hayes (1982a) showed that, in Escherichia coli, the carboxyl carbon of acetyl-CoA was depleted in 13C relative to the methyl. Therefore, if no isotope effect intervenes in the synthesis of the C5 biomonomer from acetyl-CoA, we should expect that isoprenoids, with a 3/2 methyl/carboxyl ratio, would be enriched in 13C relative to n-alkyl carbon skeletons, in which the ratio is 1/1. But how much significance can be ascribed to results of analyses of a single bacterial species and, in particular, what differences might occur in eukaryotes? A summary of relevant observations follows:

(1) Wong et al. (1975) found ‘‘organic acids’’ enriched in 13C relative to carotenoids in cultures of the purple photosynthetic bacterium, Chromatium vinosum, thus suggesting that n-alkyl carbon skeletons might be enriched in 13C relative to isoprenoids. However,

(2) Galimov and Shirinskiy (1975) found fatty acids to be depleted in 13C by 1–2‰ relative to polyisoprenoids in every case examined: a cyanobacterium, zooplankton, a vascular plant, and a eukaryotic alga.

(3) Sharkey et al. (1991) examined fractionation of carbon isotopes during the biosynthesis of iso-
prene, C₂H₁₀, by red oak tree leaves. They found that isoprene and carotenoids were depleted in ¹³C relative to total photosynthetically fixed carbon by ≈3% and that fatty acids were depleted by an additional 2%.

(4) Jones et al. (1991) found that 24-ethylcholestanol from lacustrine sediments (and ascribed by them to a terrestrial-plant source) was enriched in ¹³C by 4% relative to n-alkyl plant waxes in the same sediments (Rieley et al., 1991).

Remarkably, there is at present no thorough and systematic investigation of n-alkyl vs. polyisoprenoidal δ values in a suite of biological sources. Presumably this will change. At present, points (2)–(4) above favor the idea that, within a single source, isoprenoid lipids are enriched in ¹³C relative to n-alkyl species.

**CO₂ paleobarometry**

The two-step (mass transport + fixation) model of photosynthetic carbon fixation clearly suggests that εₚ might be pCO₂-dependent. In qualitative terms, if CO₂ is abundant, mass transport is unlikely to be rate limiting and the much larger isotope effect associated with rubisco should be dominant. In semi-quantitative terms (Eq. 17), εₚ should vary with c_i/c_e in systems in which active transport is not a factor. But εₚ tends to maximize at high values of c_e and, because c_e appears in the denominator of the variable term in Eq. 17, the relationship may appear inverted. Questions can also be raised about the proper functional form for c_e vs. εₚ calibrations. McCabe (1985) used a logarithmic form in the first systematic examination of c_e–εₚ relationships. Popp et al. (1989) and Jasper and Hayes (1990) followed that example, but Rau et al. (1989, 1991a,b) preferred a simpler linear form. Freeman and Hayes (1992) considered both logarithmic and linear forms and, finding a marginally better correlation in former, continued the practice begun by McCabe (1985).

When considering short-term variations in εₚ observed during the course of the North Atlantic Bloom Experiment, Rau et al. (1992) took up the idea that c_e – c_i (i.e., the concentration gradient) must reflect CO₂ demand or growth rate and that, under some conditions, this might remain constant. For such cases, modification of Eq. 17 leads to a relationship in which εₚ is expected to increase with c_e. Specifically, if c_i = c_e – γ, where γ represents the hypothetically constant CO₂ demand, then rearrangement of Eq. 17 yields:

\[ \varepsilon_p = \varepsilon_t + \left(\frac{\gamma}{c_e}\right)(c_e - c_t) \]

a form in which maximal values of εₚ and c_e are associated. A more elaborate consideration of this "hyperbolic form" appears in a forthcoming paper by Francois et al. (1993). At least for cases in which construction of an εₚ–c_e relationship does not represent raw empiricism (e.g., Freeman and Hayes, 1992), this approach seems likely to yield the best connection between mechanism and effect.

It is, however, clear that c_e is not the only factor controlling εₚ. Biochemical and biophysical considerations have recently been very thoroughly and helpfully reviewed by Goericke et al. (1993), and other investigators have interpreted variations of εₚ observed in natural systems in terms of varying levels of enzymatic activity and pathways of carbon fixation (Descolas-Gros et al., 1990; Fontugne et al., 1991), growth rate and cell size (Takahashi et al., 1991; Fry and Wainright, 1992), and species-related physiological differences (Falkowski, 1991). Fogel et al. (1992) have called upon many of these factors to explain observed variations in δp in a seasonal study of the complex Chesapeake estuary.

The question to be settled is whether all of these additional factors obscure c_e-related variations in εₚ or whether, in spite of them, useful accurate estimates of ancient c_e levels can be provided. It seems very likely that, if success is achieved, it will be based to a great degree on the use of compound-specific analyses not just to derive εₚ but also to provide as much additional information as possible about the paleoenvironment and the producer community to which it was host.

**Acknowledgements**

I am grateful to Peter Westbroek and Jan de Leeuw for the invitation to participate in this Symposium, and to the many coworkers and collaborators whose work is discussed in the preceding text. Core support for the program in isotopic biogeochemical research at Indiana comes from the National Aeronautics and Space
Administration (NAGW-1940) and from the program in marine chemistry at the National Science Foundation (OCE-9216918, to J.P. Jasper and J.M. Hayes).

References


