Carbon Isotopes as Biogeochemical Recorders of Ancient Biological Fractionation:

Are they set in stone?

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1. Introduction

This essay aims to elucidate the use of carbon isotopes as biogeochemical recorders of life over Earth's history, and detail some of the major problems associated with using $^{13}C/^{12}C$ analysis - with particular reference to the early evolution of life.

The rise of life on Earth remains one of the major enigmas of modern science. The answer, if we ever get one, will have to come from the Earth's rock-record. The geological time period which hosts the oldest rocks on Earth, between 3.9 and 2.5 *Ga*[1], is termed the 'Archaean'. Geologists employ a host of different techniques to scrunitize Archaean rocks for signs of early life. Unfortunately, several severe limitations are placed upon any study of ancient rocks.

First and foremost amongst these is the limited spatial and temporal extent of the available data. That is, the Archaean geologist has a limited number of outcrops to work with, all of which may represent different depositional environments, times and locations and none of which need necessarily be representative. Tied to this problem is the lack of old rocks: while the age of the Earth is fairly well constrained at 4.56 *Ga*, the oldest crustal rocks found to-date have an age of 'merely' 3.85 *Ga*. This leaves a gap of over half a billion years of Earth history for which even the rock record cannot provide any clues.

Secondly, there are problems with the rocks themselves. As rocks get older, their exposure to elements of weathering and erosion – such as chemical leeching and frost - generally increases. Of even more concern to Archaean geologists is the degree of *metamorphic* alteration and heating suffered by ancient rocks subsequent to their formation; a suite of rocks may undergo several episodes of severe temperature and pressure changes before becoming exposed for study at the Earth's surface. Examples include the nearby intrusion of a body of hot magma, subduction to greater depths within the Earth's crust, and so on. The probability of these tectonic events occurring increases with the age of a rock.

The last major problem concerns the specific study of ancient signs of life. Having finally managed to isolate a relatively undeformed, ancient, well-preserved rock deposited in an environment deemed suitable for life, *what do you do?* An obvious approach might seem to examine the rock for microfossils. However, early biological structures – if preserved - may well have been far simpler and perhaps very different from microorganisms known today. A second, more subtle approach is to search for *biosignatures*. And what better signature to use than isotopes of that element thought to be quintessential to life: carbon.

[1] 1 Ga = 10^9 years

2. Carbon isotopes as a proxy for Early Life

The validity of using carbon isotopes in the search for early life hinges on two assumptions, both of which are unconditionally true for all known forms of life:

(i) Early life was carbon-based; and

(ii) Early life employed the process of metabolism, and this early metabolism exerted a fractionation effect on isotopes of carbon.

The unique properties of carbon which make it a suitable building-block for life are not shared by any other element. In any event, it is safe to assume that carbon-based life today must also have evolved from a carbon-based ancestor. The second assumption is perhaps harder to justify. It is first important to distinguish between *anabolic* and *catabolic* processes, which together form the keystones of metabolism. *Anabolism* is the biological process whereby the functional and structural materials of life, such as cell components, are biosynthesized. *Catabolism*, on the other hand, involves the transformation of energy from outside sources - such as sunlight, heat or chemical bonds in molecules absorbed from the environment – into a compact and transportable form that life-sustaining reactions can use. Organisms can be categorized on the basis of catabolic and anabolic processes, as shown schematically in Figure 1.

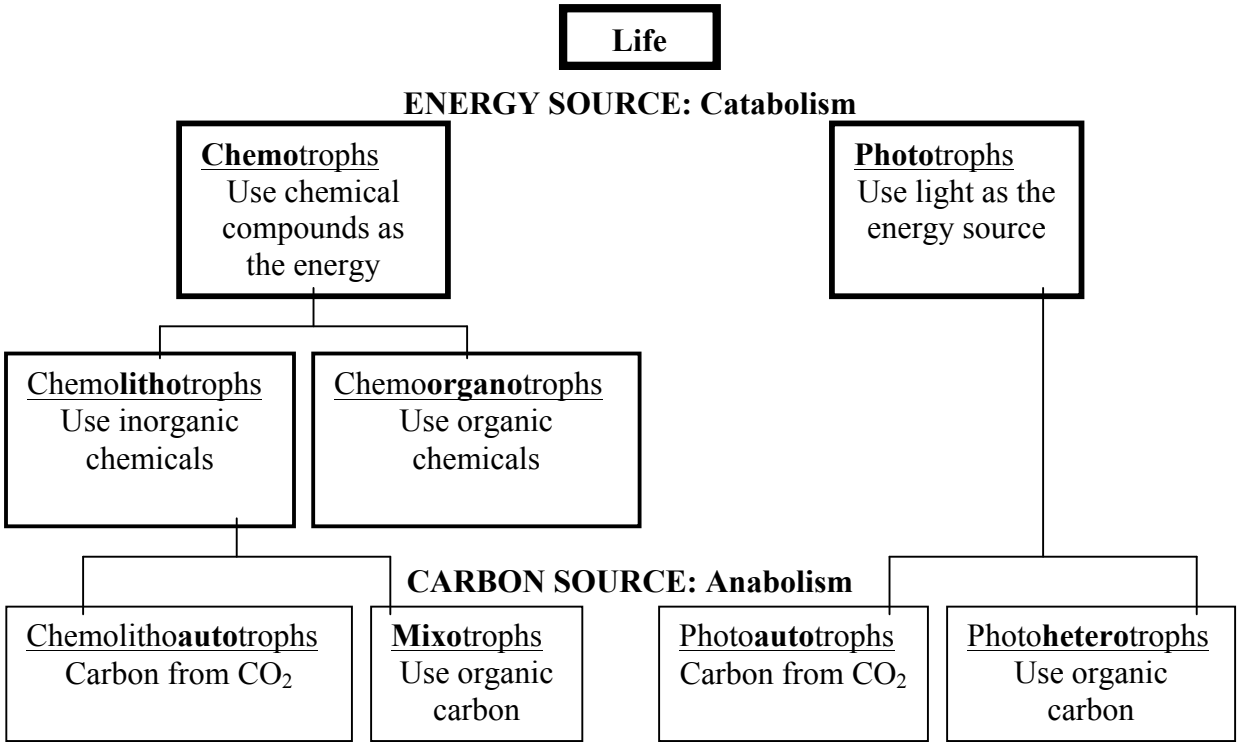

Figure 1: *Classification of life in terms of metabolic energy- and carbon- sources*

Autotrophic organisms are those that anabolise (or 'fix') CO_2 from their environment into biosynthetic end-products. It is likely that early forms of life also derived their carbon from CO_2, as opposed to obtaining it heterotrophically from organic molecules. CO_2 is abundant in most terrestrial environments and was probably never a limiting nutrient at any time over the course of Earth's history, as suggested by the high CO_2 concentrations found on the lifeless inner planets of our Solar System today (*Table 1*).

Table 1: *Atmospheres of the Solar System's inner planets.* (Adapted from: Hubbard, 1994)

Planet	Mercury	Venus	Earth	Mars
Major (>1 %) constituents	-	CO_2, N_2	N_2, O_2	CO_2, N_2, Ar
Atmospheric pressure	~0	90 atm	1 atm	70-10 mbar[2]
Weight% CO_2	-	97	0.03	95

The first carbon-fixing enzymatic carboxylation reaction in the primary metabolism of autotrophic organisms imposes a kinetic isotope effect favoring biological uptake of the lighter isotope, ^{12}C. Although autotrophic organisms employ a diverse array of carboxylation reactions, the direction of fractionation appears to be universally in favor of the lighter isotope species – it is only the amount of fractionation that is process- or species- specific (*Table 2*; see also Figure 4 in Schidlowski, 2001).

Table 2: *Pathways of biological carbon fixation responsible for the anabolism of carbon into living biomass.* (Adapted from: Schidlowski, 2001 and Hayes, 1994)

(1) CO_2 + ribulose-1,5-biphosphate -> phosphoglycerate

- Green plants [Green plants relying on reaction (1) exclusively are termed C3 plants]
- Eukaryotic algae
- Cyanobacteria
- Purple photosynthetic bacteria (*Chromatiaceae*)
- Purple nonsulfur bacteria (*Rhodospirillaceae*)
- Chemoautotrophic bacteria

(2) CO_2/HCO_3^- + phosphoenolpyruvate/pyruvate -> oxaloacetate

- Green plants [C4 and CAM plants combine reactions (2) and (1)]
- Anaerobic bacteria
- Facultatively aerobic bacteria

(3) $CO_2 + CO_2$ -> acetyl coenzyme A/acetate

- Green photosynthetic bacteria (*Chlorobiacceae*) [via succinyl coenzyme A and α-ketoglutarate]
- Anaerobic bacteria (*Acetobacterium woodii*, *Clostridium acidiurici*)
- Methanogenic bacteria [via C1 acceptors]

[2] 1 atm = 1013 mbar. Martian atmospheric pressure varies seasonally.

Table 2 *(continued)*

(4) CO_2 + acetyl coenzyme A -> pyruvate/phosphoenolpyruvate

- Green photosynthetic bacteria (*Chlorobiacceae*) [combine reactions (2), (3) and (4)]
- *Clostridium kluyveri*
- Autotrophic sulfate reducing bacteria
- Methanogenic bacteria

(5) CH_4 -> formaldehyde (HCHO)
HCHO + ribulose monophosphate -> hexulose monophosphate

- Type I methanotrophic bacteria

(6) CH_4 -> formaldehyde (HCHO)
HCHO + glycine -> serine

- Type II methanotrophic bacteria

Given that early life almost certainly employed metabolic processes by way of autotrophic carbon fixation, and that all such processes exhibit strong kinetic fractionations, we can conclude that the biological fractionation of carbon is a valid proxy for the existence of life. An assessment of whether or not biological fractionation occurred in the past requires knowledge of two key quantities, namely the carbon isotope ratios in (i) the biosphere; and (ii) the atmosphere during the time-period of interest. It is here that we are forced to turn to the geological record.

3. Evidence for biological fractionation in the Geological Record

An examination of sedimentary rocks can reveal a host of information about the environment in which they were deposited. Two distinct families of carbon-bearing molecules found in sedimentary rocks, namely organic carbon (C_{org} in kerogen) and carbonate (C_{carb} in $CaCO_3$), are arguably representative for the biological ('sink') and source carbon reservoirs respectively. If so, a quantitative comparison of their corresponding isotope ratios ($\delta^{13}C_{org}$ versus $\delta^{13}C_{carb}$) could be used to test for the presence of life. In order to evaluate this claim, an examination of the various processes involved in the accumulation of C_{carb} and C_{org} in the sedimentary rock record is required.

3.1. The formation of carbonates

Today, marine carbonate sediments are composed principally of the skeletal remains of calcite or aragonite secreting organisms. However, calcium carbonate can also precipitate inorganically on pre-existing grains or as inter-grain cement. In either event, the amount of fractionation ε is on the order of 1‰ and can therefore be considered negligible compared to biological fractionation effects (Morse and MacKenzie, 1990). The vast majority of exogenous carbon in surficial reservoirs is found as dissolved inorganic carbon ('DIC'; consisting of $CO_{2(aq)}$, $HCO_3^-{}_{(aq)}$ and $CO_3^{2-}{}_{(aq)}$) in the oceans. Since C_{carb} is derived almost exclusively from DIC, we can use Archaean $\delta^{13}C_{carb}$ values as reliable indicators of the isotope ratios in the external CO_2 pool on early Earth.

3.2. The formation of kerogen

Today, only a minor fraction of dead organic matter is bound for preservation in the rock record. The vast majority (99.0 – 99.9%; Ronov *et al.*, 1990) is recycled into the biosphere and atmosphere through decomposition. Schidlowski (2001) argues that the lack of variation exhibited by the sedimentary organic carbon content over time, which oscillates around 0.5 wt.%, attests to the perseverance of autotrophic processes since 3.8 *Ga*. However, he ignores the fact that such a pattern of organic carbon preservation - should it exist - also necessitates the operation of more complex heterotrophic metabolic processes to account for the low time-invariant C_{org} influx into the Earth's crust.

Besides microbial degradation, several other controls on the C_{org} distribution in marine sediments exist. These include: (i) overlying productivity; (ii) water depth; and (iii) sedimentation rate. Although one or more of these factors could conceivably exert a fractionation effect upon the accumulated C_{org}, we may assume that such an effect would shift the entire $\delta^{13}C_{org}$ record, therefore not invalidating comparisons between modern-day and ancient isotope ratios.

Kerogen is a heterogenous geopolymer representing the residuum of living substances, and is characterized as a chemically inert, acid-insoluble polycondensed aggregate of aliphatic and aromatic hydrocarbons. Primary biogenic matter undergoes multiple stages of transformation during diagenesis from freshly deposited sediment to sedimentary rock, with kerogen being the end-product. However, several geological processes may affect

this conversion (*Figure 2*; compare with Figure 3 in Schidlowski, 2001). Principle processes that complicate the interpretation of isotope biomarkers include:

(i) **Contamination** by addition of inorganic externally-derived hydrocarbons; and

(ii) **Metamorphism** due to changes in pressure and/or temperature.

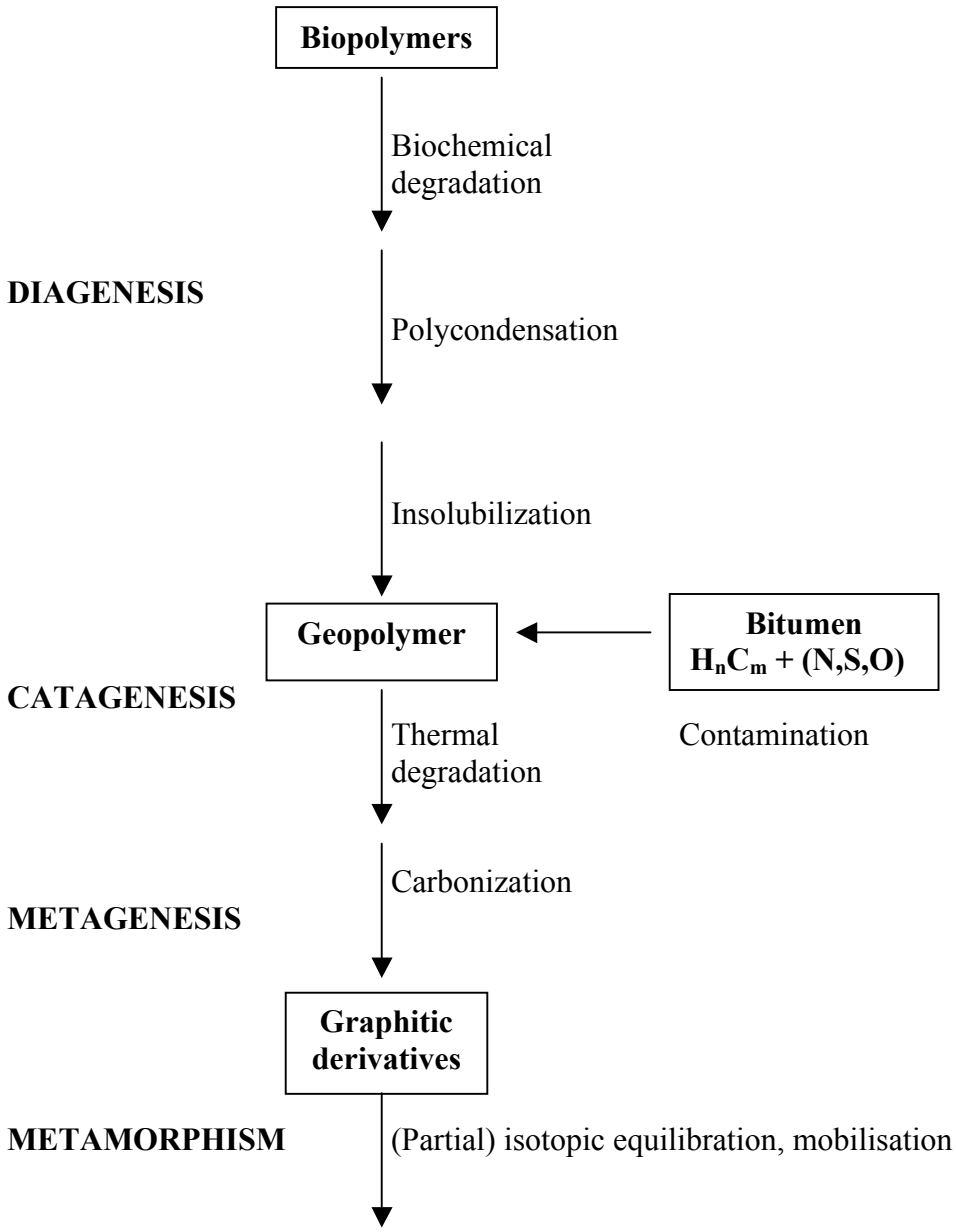

Figure 2: *Generalized scheme of the evolution of organic matter*. (Adapted from: Schopf, 1983).

Pioneering work by des Marais *et al.* (1992) and more recent work (eg. Brochs *et al.*, 1999) have been successful in utilizing *H/C* molar ratios and other constraints to identify the least altered kerogen fraction, in order to better define the $\delta^{13}C$ of genuinely pristine kerogen components. Even with the ability to minimize kerogen contamination however, the value for its' original diagenetic $\delta^{13}C$ is not set in stone. Indeed, the very processes that make the identification and interpretation of Archaean rocks so challenging often also exert a redistributive effect on any pre-existing isotope patterns. One such process of particular importance to Archaean isotope studies is metamorphism. Unlike more localized effects, metamorphism can affect entire terranes spanning hundreds of km^2.

It is common for sedimentary rocks to contain other sources of carbon in minerals or fluid inclusions, in addition to biological carbon derivatives such as kerogen and graphite. In such cases, thermodynamic equilibria generally predict that $^{13}C/^{12}C$ ratios of C_{org} increase during metamorphism. Because of the slow reaction kinetics involved, the isotopic redistribution rarely comes to a completion.

One metamorphic terrane where isotope re-equilibration is thought to have occurred is the Isua Supracrustal Suite in southwest Greenland. This region has received considerable interest because it hosts the oldest known (~3.8 *Ga*) sedimentary rocks on the planet. The average isotopic composition of sedimentary organic carbon over the last 3.5 *Ga* is representative of a biological pedigree, with $\delta^{13}C_{org}$ = -26 ± 7 ‰[3]. However, both the $\delta^{13}C_{org}$ and $\delta^{13}C_{carb}$ records exhibit a pronounced discontinuity at 3.5 *Ga*, with the older Isua rocks displaying values of $\delta^{13}C_{org}$ = -13.0 ± 4.9 ‰ (see Figure 4 in Schidlowski, 2001). The pronounced upward shift in $\delta^{13}C_{org}$ at Isua is commonly attributed to amphibolite-facies[4] metamorphism. Schidlowski (various publications, 1975-2001), amongst others (eg. Veizer and Hoefs, 1976; Hayes *et al.*, 1983; Mojzis *et al.*, 1996), argues that the lowest values for $\delta^{13}C_{org}$ at Isua reflect the most pristine – or least exchanged – isotopic state. Since the lowest values of reduced (graphitic) carbon fall into the range –22 to –28 ‰ while $\delta^{13}C_{carb}$ falls in the range –10 to 0 ‰, these workers argue that the Earth's sedimentary rock record exhibits proof of continuous habitation – and associated biological fractionation - since 3.8 *Ga*.

However, a case can be made that the Isua graphite is of abiotic origin. We now set out to show that isotopically light carbon can also be produced from degassing of CO_2 and/or CH_4 from a carbon-bearing rock during metamorphism. We assume a continuously reactive carbon residue and immediate loss of gas from the system. In our simple model, there does not exist a continuous isotopic equilibrium between the evolved gas and the carbon residue.

[3] All $\delta^{13}C$ values quoted relative to PDB.

[4] 'Amphibolite-facies metamorphism' refers to changes to rocks that occur at temperatures over ~500 ºC and pressures over ~3 kbar.

4. Mimicking biological isotope signatures: Rayleigh degassing of CO_2 from an inorganic carbon source

The Rayleigh distillation equation for the distillation of CO_2 from residual graphite is:

$$R_f = R_i F^{(\alpha - 1)} \qquad (1)$$

Where: R_i is the isotope ratio ($^{13}C/^{12}C$) in the graphite prior to oxidation and subsequent distillation of CO_2;
R_f is the isotope ratio in the graphite following distillation;
F is the mole fraction of graphite remaining;
α is the equilibrium isotope fractionation factor ($R_{CO2} / R_{graphite}$) between the evolved CO_2 and the residual graphite;

In our model we use an equilibrium fractionation factor for CO_2 – graphite partitioning at the estimated temperature of metamorphism (~ 400 ˚C). Experimental work by Chacko *et al.* (1991) suggests an appropriate value for α = 1.0111 ± 0.0027 ‰ under these conditions. This corresponds to a fractionation effect ε = 11.1 ‰ between CO_2 and graphite. We derive our initial value for R_i from the lower limit of abiotic carbon $\delta^{13}C_{carb}$ = -10 ‰ using the standard isotope equation:

$$\delta = (R / R_{std} - 1)\ 1000 \qquad (2)$$

Substitution into equation (1) yields:

$$\delta_f = [1000\ (F^{(\alpha - 1)} - 1)] + \delta_i F^{(\alpha - 1)} \qquad (3)$$

If we now assume that 10% of the original carbon remains in the rock as graphite (F = 0.1) then equation (3) yields a final $\delta^{13}C$ of δ_f = -35 ‰. We can therefore not discount the possibility that the observed isotope patterns at Isua are abiological, and result through the action of metamorphism-induced oxidation reactions that proceed by Rayleigh distillation during hydrocarbon maturation.

Light isotopic carbon trends at Isua could also be an artifact of open-system behavior. Metasomatism, a process that often accompanies metamorphism, involves the introduction of fluids into a geological system. It is easy to see how equilibration with an externally derived isotopically light carbonaceous fluid could give rise to the isotope ratios resembling those in a biological system.

Conclusion

Carbon isotopes have proved to be an extremely powerful tool in the search for life in the rock record. However, it is vital to appreciate the limitations imposed upon their interpretation. Any study wishing to use isotopes as biogeochemical indicators for life in rocks must take into account the post-depositional history of the rocks, and address the restrictions resulting therefrom. As such, there exists much scope for quantitative studies of both open-system (eg. metasomatic) and closed-system (eg. metamorphic) effects on isotope ratios in geological systems. As for finding evidence of life in that elusive period of Earth history separating the oldest rocks (3.8 *Ga*) and the oldest fossils (3.5 *Ga*): it remains, as yet, like squeezing blood from a stone.

References

Brocks J.J., Logan G.A., Buick R., Summons R.E., 'Archean Molecular Fossils and the Early Rise of Eukaryotes', *Science*, 285, pp. 1033-1036.

Buick R., 'Life in the Arcaean'. *In:* Briggs E.G., Crowther P.R., 'Palaeobiology II', Blackwell Science, Malden (2001).

Chacko T., Mayeda T.K., Clayton R.N., Goldmisth J.R., *Geochim. Cosmochim. Acta*, 5 (1994), pp. 2867-2882.

Des Marais D.J., Straus H., Summons R., Hayes J.M., 'Carbon isotope evidence for the stepwise oxidation of the Proterozoic environment', *Nature*, 359, pp. 605-609.

Hayes J.M., 'Global methanotropy at the Archaean-Proterozoic transition. *In:* Bengtson S. (Ed.), "Early Life on Earth" (Nobel Symposium 84), Columbia University Press, New York (1983), pp. 220-236.

Hubbard W.B., 'Planetary Interiors'. Van Nostrand Reinhold, New York, 1992.

Mojzsis S.J., Arrhenius G., McKeegan K.D., Harrison T.M., Nutman A.P., Friend R.L., 'Evidence for life on Earth before 3800 million years ago', *Nature*, 384, pp. 55-59.

Morse J., MacKenzie F., "Geochemistry of Sedimentary Carbonates", Elsevier, New York (2002), pp. 707.

Ronov A.B., Yaroshevsky A.A., Migdisov A.A., "Chemical Structure of the Earth's Crust and Chemical Balance of Major Elements", Izdatel'stvo, Moscow (1990), pp. 181.

Satish-Kumar M., Wada H., Santosh M., 'Constraints on the application of carbon isotope thermometry in high- to ultrahigh-temperature metamorphic terranes', *J. metamorphic Geol.*, 20 (2002), pp. 335-350.

Schidlowski M., 'Carbon isotopes as biogeochemical recorders of life over 3.8 Ga of Earth history: evolution of a concept', *Precambrian Research*, 106 (2001), pp. 117-134.

Schopf J.W. (Ed.), "Earth's Earliest Biosphere: It's Origin and Evolution", Princeton University Press, Princeton, New Jersey, pp. 98-100.

Veizer J., Hoefs J., 'The nature of 18O/16O and 13C/12C secular trends in sedimentary carbonate rocks', *Geochim Cosmochim. Acta*, 40 (1976), pp. 1387-1395.