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Factors Affecting the Isotopic Composition of Organic Matter. (1) Carbon Isotopic Composition of Terrestrial Plant Materials

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ABSTRACT

The stable isotope composition of the light elements (i.e., H, C, N, O and S) of organic samples varies significantly and, for C, is also unique and distinct from that of inorganic carbon. This is the result of (1) the isotope composition of reactants, (2) the nature of the reactions leading to formation and post-formational modification of the samples, (3) the environmental conditions under which the reactions took place, and (4) the relative concentration of the reactants compared to that of the products (i.e., [products]/[reactants] ratio). This article will examine the carbon isotope composition of terrestrial plant materials and its relationship with the above factors. $\delta^{13}C_{PDB}$ values of terrestrial plants range approximately from –8 to –38‰, inclusive of C_3-plants (–22 to –38‰), C_4-plants (–8 to –15‰) and CAM-plants (–13 to –30‰). Thus, the $\delta^{13}C_{PDB}$ values largely reflect the photosynthesis pathways of a plant as well as the genetics (i.e., species difference), $\delta^{13}C_{PDB}$ values of source CO_2, relevant humidity, CO_2/O_2 ratios, wind and light intensity etc. Significant variations in these values also exist among different tissues, different portions of a tissue and different compounds. This is mainly a consequence of metabolic reactions. Animals mainly inherit the $\delta^{13}C_{PDB}$ values of the foods they consume; therefore, their $\delta^{13}C_{PDB}$ values are similar. The $\delta^{13}C_{PDB}$ values of plant materials, thus, contain information regarding the inner workings of the plants, the environmental conditions under which they grow, the $\delta^{13}C_{PDB}$ values of CO_2 sources etc., and are unique. Furthermore, this uniqueness is passed on to their derivative matter, such as animals, humus etc. Hence, they are very powerful tools in many areas of research, including the ecological and environmental sciences.

Key Words: stable isotope, terrestrial plant, ecology, environmental science

I. Introduction

Many readers will probably agree without much hesitation that the isotope composition of organic matter is a very useful tool that has provided important insights into the inner workings of plants. At least some readers will probably think of the famous works of Dr. Calvin and his co-workers, who used ^{14}C to decipher the photosynthesis pathway. Actually, this is not the kind of isotope we will examine in this series. We will look at isotopes such as ^{13}C and ^{12}C. There are two kinds of isotopes: radioactive (*e.g.*, ^{14}C) and stable (*e.g.*, ^{13}C and ^{12}C). This series of articles will deal with the composition of stable isotopes of hydrogen (H), carbon (C), nitrogen (N), oxygen (O) and sulfur (S) in organic samples. As shown in Table 1, the isotope compositions of these elements vary greatly in nature. Nowadays, these compositions as well as variations can be routinely determined. One purpose of this series is to summarize the known isotopic data of organic matter along with the ranges of variation and to discuss the factors that determine the isotopic composition of organic matter. The objective is to introduce the art and science of stable isotope chemistry of natural organic substances to relevant researchers, teachers, students and others. Other goals are to equip readers with sufficient knowledge about the art and science of stable isotopes so that they can read relevant literature critically and evaluate the potential value and feasibility of including one or some of these isotopes in their research.

In this first installment, we will only deal with the carbon isotope composition of vegetation and animals of terrestrial environments. There, by now, must be at least thousands of samples of plants materials that have been analyzed to determine their $\delta^{13}C_{PDB}$ values. We will highlight the significance of these $\delta^{13}C_{PDB}$ values and discuss the principles underlying and the factors determining the uniqueness and variation of these values.

II. Terminology and Some Basic Facts

This synthesis and critical review is intended for researchers who are not stable-isotope specialists, such as researchers in the environmental and ecological sciences. In order to facilitate understanding, some facts and terms that

appear in this article will be given here. Readers who are already familiar with them can go on to the next section.

Around 1912, Frederick Soddy named atoms (of an element) with different atomic weight isotopes because these different atoms occupied the same place in the Periodic Table of Elements. Thus, hydrogen has three naturally occurring isotopes with atomic weights of 1(H), 2(D) and 3(T). If we place the atoms or substances containing them in a closed system so that there is no loss and gain in matter, and that if we "count" the number of atoms periodically, then we will find that H and D remains constant, but that T decreases with time. We say that the isotopes H and D are stable, and that T is unstable or radioactive. While the concentration of a radioactive isotope in a sample may largely be a function of its characteristic decay constant, that of a stable isotope is purely a function of its thermodynamic properties. In his landmark article, Urey (1947) concluded that "before the discovery of isotopes, it was generally assumed that all atoms of an element were identical in all respects. With their discovery it was evident that such atoms may differ in atomic weights; but it was believed that their chemical and physical properties were identical except for those properties directly related to mass such as densities of gases and condensed phases, rates of diffusion and evaporation, and others of this kind. As a result of the theoretical and experimental studies reviewed in this paper, we now know that isotopes and isotopic compounds differ in their thermodynamic properties. These differences are small … and (they) generally decrease with increasing atomic weight …." In other words, isotopes and isotopic compounds are, in reality, different chemical entities and can be treated as such. Since the differences are small and generally decrease with increasing atomic weight, only the isotopes of the light elements listed in Table 1 are relevant here. The relative abundance in the stable isotopes of the elements is normally reported as follows:

$$\delta X_{\text{std}} = \left[\frac{(X/x)_{\text{sample}}}{(X/x)_{\text{std}}} - 1 \right] \times 10^{-3} \qquad \textbf{(1a)}$$

where X stands for D, ^{13}C, ^{15}N, ^{18}O, or ^{34}S, x stands for H, ^{12}C, ^{14}N, ^{16}O, or ^{32}S, and "std" stands for standard. In this installment, this formula, of course, becomes

$$\delta^{13}C_{\text{PDB}} = \left[\frac{(^{13}C/^{12}C)_{\text{sample}}}{(^{13}C/^{12}C)_{\text{PDB}}} - 1 \right] \times 10^{-3} \qquad \textbf{(1b)}$$

It is simple mathematical manipulation to convert the δX_{std} value into the relative abundance when the $(X/x)_{\text{std}}$ value is given although there is no need for this conversion. PDB stands for Pee Dee Belemnite. In other words, the standard used to report δ^{13}C-values as given above is the ^{13}C/^{12}C ratio of limestone, composed of Belemnite (*Belemnite Americana*) remains, from the Pee Dee Formation, South Carolina (Craig, 1957: Table 1). It is for the sake of achieving optimal analytical precision and being consistent with the analytical strategy that this expression has become a standard term. The values can be either positive or negative. A positive value means that the sample contains more X (i.e., ^{13}C) than the standard does and *vice versa*.

Similarly, compositional difference of carbon isotopes between sample-A and sample-B is expressed as the difference in their $\delta^{13}C_{\text{std}}$ values (Δ) and can also be either negative or positive:

$$\Delta = (\delta^{13}C_{\text{PDB}})_A - (\delta^{13}C_{\text{PDB}})_B \qquad \textbf{(2)}$$

When Δ is positive, we say that sample-A is enriched in ^{13}C relative to sample-B and *vice versa*. Furthermore, when sample-A and sample-B are genetically related, sample-A is the product, and sample-B is the reactant. This is a very convenient and yet a very good approximate way to obtain a quantified estimate of the enrichment factor. The exact definition of the enrichment factor (or the fractionation factor), however, is the isotope ratio of the product divided by that of the source (Urey, 1947; Craig, 1954), viz,

$$\alpha_{A-B} \equiv \frac{(X/x)_A}{(X/x)_B} = \frac{(^{13}C/^{12}C)_A}{(^{13}C/^{12}C)_B} \qquad \textbf{(3)}$$

It is apparent that Δ is closely related to α, which in turn is the equivalent of an equilibrium constant or the kinetic rate constants of relevant reactions.

III. $\delta^{13}C_{\text{PDB}}$ Values of the Tissues and Compounds of Plants

The $\delta^{13}C_{\text{PDB}}$ values of organic samples are useful as a research tool in the environmental and ecological sciences because they are unique, and because each value carries an imprint of the source and origin of a sample. The ultimate source of organic samples is, of course, almost exclusively plants, the primary producer, and the world's biomass is overwhelmingly dominated by terrestrial plants. The first step toward effective application of the stable carbon isotope method in research is to become familiar with the uniqueness and the ranges of variations of the $\delta^{13}C_{\text{PDB}}$ values of compounds and tissues of plants, and to comprehend the meaning and significance of these values and variations.

The $\delta^{13}C_{\text{PDB}}$ values of leaf samples of plants that have been analyzed so far have a spread of more than 30‰, ranging at least from –8 to –38‰ (Fig. 1). This huge spread is mainly a consequence of differences firstly in the photosynthetic pathways (i.e., C_3, C_4 and CAM) and, secondarily, in the genes and the environmental conditions under which car-

Table 1. Relative Abundance of H, C, N, O and S Stable Isotopes

Element	Stable Isotope	Relative Abundance (%)	*X/x*	Range of δX(‰)
H	H	99.9852 (Lake Michigan water) 99.9842 (SMOW) (calculated from Craig (1961))	D/H 1.4802×10^{-4} (Lake Michigan water)	> 250
	D	0.0148 (Lake Michigan water) 0.0158 (SMOW) (calculated from Craig (1961))	1.5803×10^{-4} (SMOW)	
C	^{12}C	98.892 (Solenhofen limestone CO_2) (Nier, 1950) 98.8888 (PDB-Chicago) (calculated from Craig (1957)) 98.92 (typical C_3 plant) (Farquhar *et al*., 1989) 98.897 (present day free atmosphere CO_2) (Farquhar *et al*., 1989)	$^{13}C/^{12}C$ 1.1204×10^{-2} (Solenhofen limestone)	> 100
	^{13}C	1.108 (Solenhofen limestone CO_2) (Nier, 1950) 1.1112 (PDB-Chicago) (calculated from Craig (1957)) 1.08 (typical C_3 plant) (Farquhar *et al*., 1989) 1.103 (present day free atmosphere CO_2) (Farquhar *et al*., 1989)	1.12372×10^{-2} (PDB-Chicago) 1.093×10^{-2} (typical C_3 plant) 1.115×10^{-2} (present day free atmosphere CO_2)	
N	^{14}N	99.635 (atmospheric nitrogen) (Nier, 1950)	$^{15}N/^{14}N$	> 50
	^{15}N	0.365 (atmospheric nitrogen) (Nier, 1950)	3.6634×10^{-3} (atmospheric N_2)	
O	^{16}O	99.759 (atmospheric O_2) (Nier, 1950) 99.5106 (PDB-Chicago) (calculated from Craig (1957)) 99.80106 (SMOW) (Craig, 1961)	$^{18}O/^{16}O$ 2.0439×10^{-3} (atmospheric O_2)	> 75
	^{17}O	0.0374 (atmospheric O_2) (Nier, 1950) 0.075618 (PDB-Chicago) (calculated from Craig (1957))	4.158×10^{-3} (PDB-Chicago)	
	^{18}O	0.2039 (atmospheric O_2) (Nier, 1950) 0.41376 (PDB-Chicago) (calculated from Craig (1957)) 0.19894 (SMOW) (Craig, 1961)	1.9934×10^{-3} (SMOW)	
S	^{32}S	95.018 (meteoritic sulfur) (Macnamara and Thode, 1950)	$^{34}S/^{32}S$	> 90
	^{33}S	0.750 (meteoritic sulfur) (Macnamara and Thode, 1950)	4.436×10^{-2} (meteoritic sulfur)	
	^{34}S	4.215 (meteoritic sulfur) (Macnamara and Thode, 1950)	4.50045×10^{-2} (Canyon Diablo meteoritic troilite, CDT)	
	^{36}S	0.017 (meteoritic sulfur) (Macnamara and Thode, 1950)		

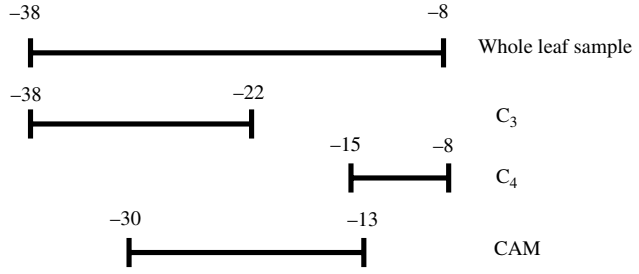


Fig. 1. $\delta_{13}C_{PDB}$ ranges of whole leaf samples of C_3, C_4, and CAM plants.

bon-fixation takes place. The $\delta^{13}C_{PDB}$ values of whole leaf samples of C_3 plants range roughly from –22 to –38‰ and are distinctly different from those of C_4 plants, which range approximately from –8 to –15‰ (Brown and Smith, 1974; Wickman, 1952; Craig, 1953, 1954; Lerman *et al*., 1969; Bender, 1968, 1971; Smith and Epstein, 1971; Bender *et al*., 1973; Smith and Brown, 1973; Lowdon and Dyck, 1974; Osmond *et al*., 1975; Deines, 1980; O'Leary, 1981; Guy and Wample, 1984; Deleens *et al*., 1985; Ehleringer *et al*., 1986; Guy *et al*., 1980, 1986a, 1986b; Leavitt and Long, 1986; Bongi *et al*., 1987; Martin *et al*., 1990; Schleser, 1990; Rao *et al*., 1994; Yeh *et al*., 1995; Livingston and Spittlehouse, 1996; Roux *et al*., 1996; Yeh and Kao, 1996). The $\delta^{13}C_{PDB}$ values of leaf samples of CAM plants range approximately from –13 to –30‰ and bridge over and overlap with those of C_3 and C_4 plants (Fig. 1). Furthermore, the $\delta^{13}C_{PDB}$ values of leaf samples of C_3 plants of a single forest can have large

spread (Yeh *et al*., 1995; Yeh and Kao, 1996; among others). This large difference among different plants grown in a small area and utilizing the same photosynthesis pathway is intriguing to say the least. It is apparent at this point that the $\delta^{13}C_{PDB}$ values of a leaf sample can tell the story of its growth history and origin. This, in fact, is true for plant materials in general, as will become clear later on in this article.

The $\delta^{13}C_{PDB}$ values of various tissues (i.e., leaf, stem, shoot, root, etc.) should, in principle, be within ranges comparable to but perhaps shifted slightly, depending on the case, from those of leaves. This is based on the probable chemical differences among the different tissues of a plant. The results of analyzing different tissue samples (Deines, 1980; Francey *et al*., 1985; Guy *et al*., 1986a; Martin *et al*., 1990; Schleser, 1990; Marino and McElroy, 1991; Bidigare *et al*., 1991) available so far are consistent with this prediction. Within a single plant, in general, shoots and stems all have similar $\delta^{13}C_{PDB}$ values, but roots may have slightly less negative $\delta^{13}C_{PDB}$ values (i.e., may be enriched in ^{13}C) by about one per mil or so compared to the rest (Park and Epstein, 1960; Deines, 1980; Guy *et al*., 1986a). Consequently, the $\delta^{13}C_{PDB}$ values of leaves are close to that of whole plants.

The $\delta^{13}C_{PDB}$ values of different samples of the same tissue, such as different parts of a trunk or leaves of a single plant, have been found to vary significantly. The difference in "tree ring" samples (i.e., cellulose samples of different "rings" of a tree trunk) can be up to about 4‰ but more commonly is 1 to 2‰ (Wilson and Grinsted, 1977; Leavitt and Long, 1986; Livinston and Spittlehouse, 1996; Marshall and Monserud, 1996; Martin and Sutherland, 1990; Sheu *et al*., 1996). This most likely reflects differences in the growth environments under which the cellulose samples were synthesized and in the $\delta^{13}C_{PDB}$ value of the CO_2 used to synthesize the samples. The $\delta^{13}C_{PDB}$ values of leaf samples of the same plant can also differ by more than 1‰ and are believed to be a consequence of differences either in external factors, such as the environmental conditions of carbon fixation, or in the $\delta^{13}C_{PDB}$ values of the source CO_2 internal factors, such as metabolism (Guy *et al*., 1980, 1986a; Deleens *et al*., 1985). Leaf samples of monospecies plants grown in a small area of less than 100×100 m^2 may differ in $\delta^{13}C_{PDB}$ values by more than 2‰ (Yeh and Kao, 1996). In some cases, this seems to be a consequence of the difference in some environmental growth conditions while in others, the difference in the $\delta^{13}C_{PDB}$ values of the source CO_2 appears to be the explanation.

The compositional effect on $\delta^{13}C_{PDB}$ values is most clearly seen in the difference between those of lipids and those of other biochemical compounds (i.e., proteins, carbohydrates, etc.) in the same tissues of a plant. Lipids are generally depleted in ^{13}C (or have larger negative $\delta^{13}C_{PDB}$ values) relative to the other compounds in the same plant. The differences are on the order of several ‰ (Park and Epstein, 1961; Macko, 1994). It had been shown that the metabolic process of synthesizing lipid from carbohydrate can produce a large isotopic difference consistent with the above observation (DeNiro and Epstein, 1977). Isotopic differences among compounds, excluding lipids, appear to be insignificant although significant differences between lignin and cellulose have been reported (Wilson and Grinsted, 1977). The significance of the latter case is not clear, and more studies are needed. Separation and purification of lignin is not an easy process; it involves at least one quite "violent" step and may have some isotope effects (Degens *et al*., 1968). It seems that with the exception of lipids (and perhaps lignin), the $\delta^{13}C_{PDB}$ values of compound groups of carbohydrates (such as cellulose) and proteins from the same plant sample tend to be similar, although the available data are too scanty to give us sufficient confident to draw a definite conclusion.

On the molecular level, the $\delta^{13}C_{PDB}$ values of the components of the compound groups of a plant sample may vary significantly. For example, the $\delta^{13}C_{PDB}$ values of different monosaccharides extracted from a *Sphagnum* sample ranged from –29.0 to –26.1‰, a spread of about 3‰ (Macko *et al*., 1991). The existence of isotopic heterogeneity among different mono-molecular components is to be expected in light of the fact that similar phenomena exist in petroleum related materials. The variation is most likely a consequence of the metabolic processes of the plant and should be viewed as potential powerful tool for deciphering ecophysiological problems in plants, although its significance and meaning is still not clear.

IV. Factors Determining the $\delta^{13}C_{PDB}$ Values of Organic Matter

The isotopic composition of a sample is a function of the isotopic composition of the reactants and their relative abundance, the mechanism of the reactions, the environmental conditions under which the reaction proceeds and the post-production experience of the sample. This is why the isotopic data of a sample carry so much information. In other words, the isotopic composition of a sample contains information about the identity of the reactants, the mechanism of the reaction, the environmental conditions under which the reaction proceeded and what might have happened to the sample since its origination. Under many conceivable circumstances and/or by carefully taking appropriate samples, one or more of these parameters can be inferred from the isotopic composition of the sample.

This is based on knowledge about the isotopic effects (*e.g*., Δ's and α's) of many of these factors. For example, the $\delta^{13}C_{PDB}$ value of a sample of plant tissue is a good indicator of the photosynthetic pathway of the plant (i.e., C_3 or C_4) (Bender, 1971; Troughton, 1972; Bender *et al*., 1973; O'Leary, 1981; Farquhar *et al*., 1989; O'Leary *et al*., 1992). This is possible because we have fairly good knowledge about the isotopic composition of the reactant (i.e., the atmospheric CO_2), the mechanism of the reaction, and the enrichment factors

involved in carbon fixation. One purpose of this article is to familiarize the readers with the known and probable isotope effects of these factors. This will enable the readers to read relevant publications critically and to judge whether the isotopic methods will be useful in their research.

In the case of terrestrial plants, the photosynthetic reactions are mainly interaction between CO_2 and H_2O in the chloroplast. For discussion of carbon isotopes, we need to consider only the CO_2; the reactions include transporting CO_2 to and from the sites of carboxylation, final fixation of the CO_2, respiration of the plants and, depending on the nature of the sample at hand, metabolism.

1. $\delta^{13}C_{PDB}$ Values of Source CO_2

One main factor determining the $\delta^{13}C_{PDB}$ value of a plant sample is the value of the source CO_2, which may vary significantly. This can be seen by examining the expression **(2)**; that is,

$$\Delta_{\text{plant–source } CO_2} = (\delta^{13}C_{PDB})_{\text{plant}} - (\delta^{13}C_{PDB})_{\text{source } CO_2}.$$

By specifying the labels and by performing minor manipulation, the expression becomes:

$$(\delta^{13}C_{PDB})_{\text{plant}} = \Delta_{\text{plant–source } CO_2} + (\delta^{13}C_{PDB})_{\text{source } CO_2}.$$

It is clear that there is a functional relationship between the two $\delta^{13}C_{PDB}$ values.

Terrestrial plants draw CO_2 from ambient air, which can be either normal or modified atmosphere. "Modified atmosphere" is the contemporary atmosphere, which contains either a significant amount of additional CO_2 generated from organic carbon or an abnormally low CO_2 concentration as a consequence of active photosynthetic carbon fixation. A good example of "modified atmosphere" is forest air, which usually contains some metabolic CO_2 and becomes abnormally low in CO_2 content when the rate of carbon-fixation is high. Normal atmosphere is simply the contemporary, well-mixed, bulk air in the open space of the earth's surface. The $\delta^{13}C_{PDB}$ values of CO_2 samples from these two sources differ greatly; thus, the $\delta^{13}C_{PDB}$ values of source CO_2 can vary significantly (Clark and Fritz, 1997). The $\delta^{13}C_{PDB}$ values of plant samples, therefore, vary accordingly even if everything else remains the same in and around the samples.

The $\delta^{13}C_{PDB}$ value of CO_2 in the present day normal atmosphere is around –8‰ (Goodman and Francey, 1988) with slight seasonal fluctuation and spatial difference, while that of organic-carbon-generated CO_2 is much more negative. The $\delta^{13}C_{PDB}$ values of some present day potential CO_2 sources are given in Table 2. The range of the values of present day normal atmospheric CO_2 reflects the effects of the differences in the photosynthetic and human activity in the southern and northern hemisphere and between spring-summer and fall-winter (Mook *et al*., 1983; Keeling and Carter, 1984; Keeling *et al*., 1984, 1989). The range of $\delta^{13}C_{PDB}$ values of potential present day CO_2 sources is about 2‰, and this shall be taken as minimal (Table 2). For example, it is conceivable that the $\delta^{13}C_{PDB}$ values of CO_2 in air in places adjacent to sites of "burning" of organic carbon, such as power plants, may actually be close to the values of organic carbon and could be more than several per mils more negative than that of normal atmospheric CO_2.

The $\delta^{13}C_{PDB}$ values of normal atmospheric CO_2 are subject to temporal variations (Keeling *et al*., 1979; Mook *et al*., 1983; Keeling and Carter, 1984; Friedli *et al*., 1986). The range of these variations is at least 2‰ (Table 2), and input of anthropogenic CO_2 into the atmosphere since the industrial revolution began about two hundred years ago, has shifted the value from circa –6.5 to –7.0‰ in pre-industrial times to circa –8.0 or probably –8.5‰ today (Friedli *et al*., 1986). The longer the geological time considered, the larger the range is likely to be. These temporal variations are relevant when we are dealing with tree-ring and geological material.

2. Δ Values Between Steps from the Source to the Sites of Fixation

The second factor determining the $\delta^{13}C_{PDB}$ values of a plant sample is transportation of CO_2 from ambient air to the

Table 2. Variation in $\delta^{13}C_{PDB}$ Values of Atmospheric CO_2

		Range of $\delta^{13}C_{PDB}$ (‰)	Remark	Reference
	Date			
Diurnal change in $\delta^{13}C_{PDB}$	09-16-1982	–7.0 – –9.5	$\delta^{13}C_{PDB}$ maximum before dawn	Inoue and Sugimura (1984)
of atmosphere CO_2	07-29-1983	–7.0 – –9.0	Minimum during daytime	
	05-18-1955	–7.2 – –10.3		Keeling (1958)
	Age			
Historical and geological	400 – 800 BP	–6.56 – –6.85	Pre-industrial revolution	Friedli *et al*. (1984)
change in $\delta^{13}C_{PDB}$ of	1744 – 1839 AD	–6.48 – –6.62		Friedli *et al*. (1986)
atmosphere CO_2	1843 – 1984 AD	–6.54 – –7.8	Post-industrial revolution	Friedli *et al*. (1986)
	680 – 42890 BP	–6.54 – –6.96		Leuenberger *et al*. (1992)
	Time interval			
Seasonal Change in $\delta^{13}C_{PDB}$	03/1979 – 06/1980	–7.6 – –8.0	Maximum in winter and	Keeling and Carter (1984)
of atmospheric CO_2	03/1977 – 02/1982	–7.3 – –8.1	minimum in summer	Mook *et al*. (1983)

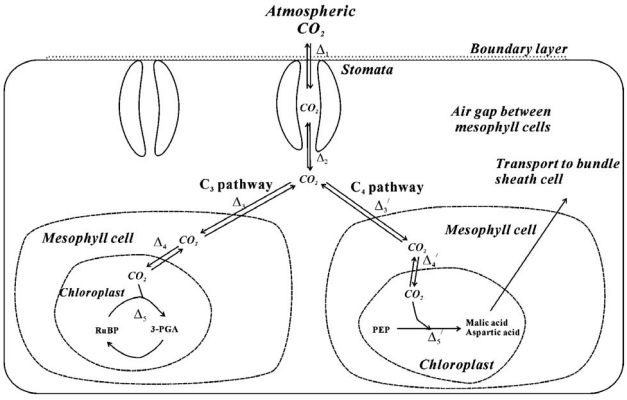


Fig. 2. Stepwise transportation of atmospheric CO_2 into a leaf and the relevant isotope fractionation (Δ) of each step.

sites where carboxylation occurs. This process of transportation is schematically illustrated in Fig. 2. We emphasize that every step a sample experiences, including migration and phase changes, there is a corresponding Δ value although most of these values are only approximately known. Knowledge of the probable processes and the Δ values, even if approximate, is very helpful in understanding the meaning of relevant $\delta^{13}C_{PDB}$ values and in effective application of the isotopic method in research in, for example, the ecology and environmental sciences. The Δ values given in Table 3 are unidirectional only. In reality, reverse flow of CO_2 is not impossible, as shown in Fig. 2, and could complicate things further.

The flow of CO_2 from ambient air to sites of carbon fixation is conceived of as a stepwise process as shown in Fig. 2. Each step is associated with one Δ value for the forward step (Table 3). The Δ value for a backward step, when one occurs, shall, in principle, be the same as that of a forward step but with the opposite sign (i.e., $+ \leftrightarrow -$). In the first step, CO_2 moves from free air through the boundary layer onto the leaf surface. The Δ value of this step shall, in principle, range from –4.4 to –1‰ and was estimated in one case to be about –2.9‰ [i.e., $-2.9 = (x) - (-8.0)_{\text{free-air}}$] (Farquhar, 1983). –4.4‰ is the estimated Δ value for diffusion of CO_2 from free air through stomata into air gaps and –1.0‰ is the estimated value for the diffusion in water (O'Leary, 1981, 1984). In the next step, CO_2 moves from the leaf surface through stomata into the air gap between the mesophyll cells inside the leaf. The Δ value of this step is difficult to get and is not yet available. Theoretically the value will range from –4.4‰ (free air diffusion) to 0‰ (viscous flow) and most likely is close to 0‰. Movement from air gaps through cell walls is the next step which is necessary for CO_2 to reach the sites of carbon fixation. The Δ value of this step is not available but probably is small. The exact nature of the next step is not yet very clear but will depend on whether it is a C_3–, a C_4–, or a CAM-plant. In the case of a C_4-plant, CO_2 is fixed with PEP in the form of bicarbonate (HCO_3^-) ion and, therefore, must undergo hydration prior to carboxylation. The Δ value of hydration depends on how it is "hydrated" (Mook *et al*., 1974; Paneth and O'Leary, 1985; O'Leary *et al*., 1992). The three different methods of hydration, in seawater, "spontaneous" and "carbonic anhydrase-catalyzed," have very different Δ values of +8.0, –6.9 and –1.1‰ (at 25°C), respectively. Theoretically, the Δ value will be close to –1.1‰ (Table 3). In the case of a C_3-plant, CO_2 must migrate through the cytosol and membrane of the chloroplast and be fixed. The Δ values of these "two" steps will also be small but so far are unknown. While many of these Δ values associated with CO_2 flow from air gaps to cells and the organelle's interior are not yet known, experimental results are consistent with theoretical assessment and indicate that they are small. O'Leary's results indicate that the sum of all these Δ values is about –0.7‰ (O'Leary, 1984). In summary, the $\delta^{13}C_{PDB}$ value of CO_2 derived originally from normal atmospheric CO_2 inside cells at sites of carboxylation is now around –12‰ (O'Leary, 1993).

3. Δ Values of Carboxylation Reaction

In a C_3-plant, carbon fixation is accomplished when CO_2 combines with Ribulose 1,5-bisphosphate (RuBP) to generate 2 molecules of 3-carbon sugar (3-PGA). This combination is catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco), and the Δ value of this carboxylation is –29‰ (O'Leary, 1981; Farquhar *et al*., 1989:

Table 3. Reaction Steps and the Associated Isotope Fractionation (Δ) in Plants

Step	Δ‰	Reference
$CO_{2(g)}$ diffusion in free air	–4.4	Craig (1957)
CO_2 diffusion in water	0	O'Leary (1981)
CO_2 diffusion in aqueous solution	–0.7	O'Leary (1984)
$CO_{2(g)} = CO_{2(aq)}$ (at 25 °C)	–1.1	Mook *et al*. (1974)
$CO_{2(aq)} = HCO_3^-{}_{(aq)}$	+9.0	
$CO_{2(g)} = HCO_3^-{}_{(aq)}$	+7.9	
Spontaneous hydration of CO_2	–6.9	Marlier and O'Leary (1984)
Carbonic anhydrase-catalyzed hydration of CO_2	–1.1	Paneth and O'Leary (1984)
Carboxylation of PEP relative to $HCO_3^-{}_{(aq)}$	–2.0	O'Leary (1981) and Farquhar (1983)
relative to $CO_{2(g)}$	~ +5.7	
Carboxylation of RuBP	–27 – –30	Farquhar *et al*. (1989) and Christeller *et al*. (1976)

Table 3).

In the case of a C_4-plant, CO_2 first combines with phosphoenolpyruvate (PEP) to form oxaloacetate in mesophyll cells. This carboxylation reaction is catalyzed by the enzyme phosphoenopyruvate carboxylase (PEP carboxylase) and the Δ value is –2‰. The Δ values of fixation of CO_2 from decarboxylation of C_4 acids in bundle sheath cells through the Calvin cycle is, of course, –29‰ (Table 3).

Since CAM-plants use either both C_3 and C_4 (i.e., facultative CAM) or only C4 (obligatory CAM) carboxylation to fix CO_2, the $\delta^{13}C_{PDB}$ values of CAM-plants depend on the relative importance of C_3 vs. C_4 carboxylation employed by the plant and ranges from that of a C_3-plant to that of a C_4-plant. In other words, it does not matter whether CO_2 is first concentrated at night (CAM) or during daytime (C_4), the Δ value will still be –2‰ as long as PEP is used to do the job.

The Δ values are all instantaneous enrichment factors, and the $\delta^{13}C_{PDB}$ values of the products range from the values of the reactant (i.e., the CO_2), when there is total consumption of the reactant, to that of the reactant plus the Δ value, when the product is infinitesimally small and the reservoir of reactant is so large that its $\delta^{13}C_{PDB}$ value remains constant throughout the time the reaction is in progress. For example, carboxylation of a C_4-plant is so efficient that most of the time, the CO_2 in the cytosol is probably totally combined with PEP, and the $\delta^{13}C_{PDB}$ value of the oxaloacetate is close to that of the CO_2. On the other hand, since CO_2 in free air is very plentiful in comparison with the CO_2 diffused into air gaps, the $\delta^{13}C_{PDB}$ value of the CO_2 in the air gaps will be close to that of the free air CO_2 plus the Δ value.

4. Effects of Photorespiration and Respiration

These processes consume plant material and may affect the $\delta^{13}C_{PDB}$ values of the plant if and only if there is a net loss of carbon. For example, if all the CO_2 generated in these ways is recycled within the cells and re-fixed into sugar, then there will be no change in the $\delta^{13}C_{PDB}$ value of the plant. However, if there is a net loss of CO_2, then the loss may or may not affect the values, depending on the $\delta^{13}C_{PDB}$ value of the CO_2 and the amount that is lost. The magnitude of the effect is proportional to the magnitude of the difference in the $\delta^{13}C_{PDB}$ value between the plant and the respiratory CO_2 as well as to the amount of CO_2 that is lost. It can be argued that the effects cannot be significant because the difference in the values is normally insignificant and the amount of CO_2 lost in comparison with the mass of the plant is very small. However, even if the effect is small, it is still important for understanding the processes.

5. Effects of Metabolism

The first "sugar" in photosynthesis, 3-PGA, undergoes various reactions and generates other sugars, proteins, lipids and other bio-compounds. This metabolic reaction does not affect the $\delta^{13}C_{PDB}$ value of the whole plant sample, but it might affect the specific compounds or molecular samples of a plant. It appears that these reactions have significant isotopic effects only on lipids and on the molecules of a single compound. The mechanism that has effects on lipids is reasonably well understood, as discussed above, but is still poorly understood regarding molecular heterogeneity. Both effects, however, can provide useful information for the ecological and environmental sciences.

6. Other Factors

As long as the $\delta^{13}C_{PDB}$ values of the source CO_2, the Δ values of transportation and carboxylation, the relative abundance of the "reactant(s)" and the "product(s)" are given, the $\delta^{13}C_{PDB}$ value of a plant sample can be determined. Likewise, when these parameters change, the $\delta^{13}C_{PDB}$ values of a plant sample will also change correspondingly as indicated above. We have previously discussed the variation of the $\delta^{13}C_{PDB}$ values of the source CO_2. We will now examine the variability of the remaining parameters and what might be the cause(s) of this variation. It should be clear by now that the $\delta^{13}C_{PDB}$ values of plant samples reflect much more than just about the difference in photosynthesis pathways and variability of the isotopic composition of the source CO_2.

The fact that C_3-plants grown in identical environments can still have large differences in $\delta^{13}C_{PDB}$ values indicates a genetic influence on the values. This effect is at least 2‰. This effect may arise from the difference in efficiency of photosynthesis, leaf structure etc. In any event, it is certain that the genes of an individual plant are an important factor determining the isotopic composition of the plant although we don't know exactly how this occurs yet.

Environmental conditions will, in principle, also be important factors because they may influence the characteristics of CO_2 transport, the carboxylation rate etc. Ambient temperatures, humidity, O_2/CO_2 ratios, irradiation characteristics, the physical-chemical conditions of the soil and wind etc. of the growth environments are likely important and relevant environmental factors. However, only the relationship between the isotopic composition and the ambient humidity is relatively well understood.

It can be shown that the $\delta^{13}C_{PDB}$ values of C_3-plant samples are inversely correlated with the difference between the vapor pressure inside the leave and that of the atmosphere. That is, if everything else remains constant, the $\delta^{13}C_{PDB}$ values of the plant samples will be positively correlated with the humidity of the ambient air (Farquhar and Richards, 1984). It is not yet clear exactly what happens although it seems that the proportion of CO_2 that is fixed at the carboxylation sites is high when the humidity is low and *vice versa*. The effect of ambient temperatures is likely to be complex but is not yet clear due to a lack of relevant data. Available evidence sug-

gests that the effect is small or nonexistent. The salinity of the soil has been shown to have a significant effect. The effect of high salinity is comparable to that of low ambient humidity and *vice versa*. The potential effect of O_2/CO_2 ratios arises from the fact that rubisco is at the same time carboxylase and oxygenase; hence, the presence of O_2 will reduce the efficiency of carboxylation. The effect of irradiation characteristics and wind will be significant, but there still are few relevant data available for assessment (Yeh, 2000).

In summary, when we examine the significance of the $\delta^{13}C_{PDB}$ value of a terrestrial plant sample, it is necessary to take into account the $\delta^{13}C_{PDB}$ values of its source CO_2, the reaction mechanism (i.e., the nature of CO_2 transport and the type of photosynthesis pathway and metabolism), the environmental conditions and the genetics. By designing an experiment creatively or selecting samples cleverly, we can obtain much insight in the domains of ecological and environmental sciences from the $\delta^{13}C_{PDB}$ values of the plant samples. Conversely, with a clever experimental plan, much can be learned about a specific plant physiological phenomenon from the $\delta^{13}C_{PDB}$ values of the experimental products.

V. Concluding Remarks

The $\delta^{13}C_{PDB}$ values of terrestrial plants and their constituents are unique and vary over a large range. A large proportion of this uniqueness and variation can be attributed to known factors, such as the isotopic characteristics of the source CO_2, photosynthetic pathway, environmental conditions, specific metabolism etc. There is, however, much that still has not been explained with any degree of confidence. Nevertheless, the $\delta^{13}C_{PDB}$ values of terrestrial plants are very useful in ecological, environmental and plant-physiological research, among other fields. For instance, the large variation means that the plant materials are practically labeled, and that the labels will follow the materials wherever they go. In other words, the $\delta^{13}C_{PDB}$ values of the plant materials serve as tracers of the sources. One result of this "tracer" property is their applicability in foodchain research. Since the $\delta^{13}C_{PDB}$ values of an animal's tissues closely resemble that of the food the animal take in and since the food (i.e., plant materials) is isotopically labeled, the $\delta^{13}C_{PDB}$ values of the animal's tissue sample provide a clue to the food it has consumed (DeNiro and Epstein, 1978). On the other hand, since the values have the imprints of the CO_2 sources and of the mechanism and the environmental conditions, the $\delta^{13}C_{PDB}$ values of plant materials can provide insights into these aspects. The feasibility of using the $\delta^{13}C_{PDB}$ values of samples to determine the photosynthetic pathway is just one such consequence. The patterns of the $\delta^{13}C_{PDB}$ values of the molecules of specific compounds of a plant sample similarly can provide much insight into the processing going on inside the plants because deciphering the pattern is equivalent to elucidating these processes and *vise versa*.

It is beyond the scope of this installment to discuss applications. They will be dealt with in the future installment, but we surmise that many the readers already have some good ideas in this regard. Applications naturally include the use of relevant organic sediment samples, both bulk organic matter as well as specific compounds, and others (Yeh *et al*., 1995; Huang *et al*., 1999). We feel it appropriate to state that stable isotope compositions of organic matter have great potential for providing important insights into nature, including problems in the areas of ecological and environmental sciences.

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