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Two Categories of $^{13}C/^{12}C$ Ratios for Higher Plants[1]

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ABSTRACT

$^{13}C/^{12}C$ ratios have been determined for plant tissue from 104 species representing 60 families. Higher plants fall into two categories, those with low δ_{PDB_1} ^{13}C values (−24 to −34‰) and those with high δ ^{13}C values (−6 to −19‰). Algae have δ ^{13}C values of −12 to −23‰. Photosynthetic fractionation leading to such values is discussed.

Carbon isotope fractionation is associated with photosynthesis. This fractionation results in lowering the $^{13}C/^{12}C$ ratio by about 20 per mille for land plants and 10 per mille for marine plants relative to atmospheric CO_2. A model has been proposed by Park and Epstein (12) to delineate the processes associated with this fractionation. To understand more fully the carbon isotope record and its implications for plant physiology, a more extensive investigation of the $^{13}C/^{12}C$ ratio in plants was undertaken. One hundred and four species representing 60 families have been investigated and the $^{13}C/^{12}C$ ratio for these samples shows a much wider variation than previously reported. These results bear on more recent ideas regarding the biochemical mechanisms or pathways of carbon fixation as well as showing the relevance of $^{13}C/^{12}C$ studies to biological processes.

MATERIALS AND METHODS

Plant material (in most instances a green leaf) was air-dried at room temperature, combusted at 800 C in an excess of oxygen, and isotope ratios of the CO_2 evolved were measured on a Nier-type mass spectrometer modified according to McKinney *et al.* (10). Only the organic tissues of calcareous algae were used for the $^{13}C/^{12}C$ measurements. The $CaCO_3$ was removed by reaction with dilute HCl. Results are reported in terms of $\delta^{13}C$ relative to a carbonate standard.

$$\delta^{13}\ C‰ = \left[\frac{R\ \text{sample}}{R\ \text{standard}} - 1\right] \times 1000$$

where R = mass 45/mass 44 of sample or standard CO_2. The standard is carbonate from the fossil skeleton of *Belemnitella americana* from the Peedee formation of South Carolina (PDB_1).

Thus a value of −10‰ means that $^{13}C/C^{12}$ ratio of the sample is less than that of the standard by 10 per mille or 1%. The precision of measurement is ±0.1‰ of the ratio. Sample replication, including all errors of sample preparation, was ± 0.5‰. Details of the procedures are described elsewhere (12).

RESULTS

Table I lists our data in order of decreasing $^{13}C/^{12}C$ ratios. Data from Table I are recorded in Figure 1 to demonstrate that on the basis of carbon isotope abundance our samples fall into three broad classes. The first class is highest in ^{13}C content and is composed of aquatics, desert and salt marsh plants, and tropical grasses. Another class is low in ^{13}C content and comprises the bulk of the plant kingdom. There is no overlap in $\delta^{13}C$ values between these two groups of plants. The algae are put into a separate group and are generally intermediate between the two higher plant groups. They belong to a separate and primitive plant subkingdom and will be discussed apart from the others. In spite of the close phylogenetic relationship between our groups I and II, some fundamental process is different in the two groups.

The four plants with highest $\delta^{13}C$ values are aquatic monocots, whereas most plants exhibiting $\delta^{13}C$ values of −5.6 to −18.6‰ are terrestrial plants including monocots and dicots. *Welwitchia* was the only gymnosperm with a high $\delta^{13}C$ value. Bender (2) has recently reported high $\delta^{13}C$ values for a number of the panicoid grasses and our results are in agreement with hers. On the average the $\delta^{13}C$ values of dicots are slightly more negative than those of the monocots. Wickman (17) also reported aquatic monocots and dicots with relatively high $^{13}C/^{12}C$ ratios. Most higher plants, including all lower vascular plants and all gymnosperms except *Welwitchia,* have $\delta^{13}C$ values of less than −23‰. Festucoid grasses (17), including bamboo, are in this group as are the palms. Dicots with $\delta^{13}C$ values close to −23‰ are plants from xeric and salt marsh habitats. Cultivated plants and mesophytes are somewhat more reduced in ^{13}C. There appears to be no relationship between the $\delta^{13}C$ and phylogeny. Our results for marine algae are in good agreement with published values (3, 13, 14). Freshwater algae (*Spirogyra* and *Chlorococcum*) did not differ in $\delta^{13}C$ from marine algae.

Atmospheric carbon dioxide does not change isotopically with geography or topography (7). Urban air is the exception, due to fossil fuel combustion increasing the ^{12}C content. Plant tissues reflect differences in isotopic composition of the carbon fixed in photosynthesis. In only five cases was the same species collected from more than one geographic area. Table II indicates that a systematic difference did exist with plants from the Los Angeles area consistently 0.4 to 1.2‰ lighter than corresponding species from Utah or Texas. This small difference may be accounted for by the smaller $\delta^{13}C$ values of atmospheric CO_2 in southern California than for less polluted rural areas. The values listed in Table I are from California.

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Table I. *Plant* $\delta^{13}C$ *Values*

Family	Species	$\delta^{13}C‰$
	I. High $^{13}C/^{12}C$ Plants	
	Gymnospermae	
Welwitschiaceae	*Welwitchia mirabilis* Hook.	−14.4
	Monocotyledoneae	
Potamogetonaceae	*Cymodocea manatorum* Aschers.	−5.6
Hydrocharitaceae	*Thallasia testudinum* Konig and Sims	−9.3
Potamogetonaceae	*Zostera marina* L.	−10.0
	Diplanthera wrightii (Aschers.) Aschers.	−10.9
Cyperaceae	*Carex* sp.	−11.5
Gramineae	*Spartina alterniflora* Loisel.	−13.1
	Cynodon dactylon (L.) Pers.	−13.4
	Zea mays L.	−14.0
	Saccharum sp.	−13.9
Potamogetonaceae	*Phyllospadix torreyi* Wats.	−14.0
Gramineae	*Sorghum* sp.	−14.4
	Distichlis spicata (L.) Greene	−14.7
	Monanthochloë littoralis Engelm.	−15.3
	Cymbopogon citratus Stapf	−14.8
	Stenotaphrum secundatum (Walt.) Kuntze	−15.7
Cyperaceae	*Cyperus* sp.	−15.9
Bromeliaceae	*Tillandsia usneoides* L.	−18.6
	Dicotyledoneae	
Amaranthaceae	*Amaranthus edulis* Speg.	−15.4
Chenopodiaceae	*Kochia scoparia* (L.) Schrad.	−14.0
	K. childsii Hort.	−14.8
	Atriplex vesicaria (Benth.) Heward	−15.1
	A. lentiformis ssp. *brewerii* Hall and Clements	−16.4
	A. nummularia Lindl.	−16.7
	A. halimus L.	−17.1
	A. polycarpa S. Wats.	−17.6
	A. semibaccata R. Br.	−18.3
	A. canescens ssp. *typica* (Pursh) Nutt.	−18.0
	A. canescens ssp. *linearis* (Pursh) Nutt.	−12.6
Saxifragaceae	*Philadelphus microphyllus* Hitch.	−17.1
	II. Low $^{13}C/^{12}C$ Plants	
	Bryophyta	
Sphagnaceae	*Sphagnum magellanicum* Brid.	−26.0
	Psilotinae	
Psilotaceae	*Tsmesipteris fowerakeri* Barb.	−29.0
	Sphenotinae	
Equisetaceae	*Equisetum arvense* L.	−28.6
	Gymnospermae	
Taxodiaceae	*Metasequoia glyptostroboides* Hu and Cheng	−25.4
Ginkgoaceae	*Ginkgo biloba* L.	−25.6
Araucariaceae	*Araucaria bidwillii* Hook.	−25.9
Podocarpaceae	*Podocarpus elata* R. Br.	−26.6
Cycadaceae	*Cycas revoluta* Thunb.	−27.0
Cupressaceae	*Cupressus sempervirens* L.	−29.7
Gnetaceae	*Gnetum africanum* Rodin.	−30.2
Pinaceae	*Pinus halepensis* Mill.	−30.8
	Monocotyledoneae	
Gramineae	*Triticum aestivum* L.	−23.7
	Stipa columbiana Macoun	−24.2
Palmae	*Trachycarpus khasianus* H. Wendl.	−25.3
	Caryota mitis Lour.	−26.7
Gramineae	*Agropyron spicatum* (Pursh) Scribn. and Sm.	−27.1
	A. intermedium (Host) Beauv.	−28.8
Iridaceae	*Iris spuria* L.	−27.4
Typhaceae	*Typha* sp.	−27.6
Gramineae	*Uniola paniculata* L.	−27.7
	Bromus tectorum L.	−28.0
	Poa secunda Presl.	−28.2
	Bambusa vulgaris Schrad.	−29.5
Pontederiaceae	*Eichhornia* sp.	−31.8

Table I—*Continued*

Family	Species	$\delta^{13}C‰$
	Dicotyledoneae	
Plumbaginaceae	*Limonium commune* S. F. Gray	−23.2
Aizoaceae	*Mesembryanthemum chilense* Mol.	−23.6
Verbenaceae	*Avicennia nitida* Jacq.	−23.8
Compositae	*Artemisia pycnocephala* D.C.	−24.2
Chenopodiaceae	*Salicornia bigelovii* Torr.	−25.2
Rutaceae	*Citrus* sp.	−25.6
Magnoliaceae	*Magnolia grandifolia* L.	−26.1
Leguminosae	*Pisum sativum* L.	−26.1
Cucurbitaceae	*Cucurbita* sp. (squash)	−26.2
Frankeniaceae	*Frankenia grandifolia* Cham. and Schl.	−26.4
Chenopodiaceae	*Suaeda fruticosa* (L.) Forsk.	−26.5
Fagaceae	*Quercus palustris* Cockerell	−26.5
Batidaceae	*Batis maritima* L.	−26.7
Aceraceae	*Acer rubrum* L.	−26.7
Oleaceae	*Olea europaea* L.	−26.8
Compositae	*Achillea tomentosa* L.	−27.6
	Helianthus annuus L.	−27.8
	Baccharis pilularis D.C.	−28.1
Bombacaceae	*Chorisia speciosa* St. Hil.	−28.0
Proteaceae	*Grevillea lanigera* (Meissn.) A. Cunn.	−28.3
Salicaceae	*Populus alba* L.	−28.4
Leguminosae	*Arachis hypogaea* L.	−28.5
	Genista monosperma Lam.	−28.6
Euphorbiaceae	*Ricinis communis* L.	−28.7
Ericaceae	*Arctostaphylos pumila* Nutt.	−28.7
Cruciferae	*Raphanus* sp.	−28.8
Fagaceae	*Quercus engelmannii* Greene	−28.9
Rhamnaceae	*Ceanothus* sp.	−29.1
Casuarinaceae	*Casuarina stricta* Dry.	−29.1
Chenopodiaceae	*Beta vulgaris* L.	−30.1
Convolvulaceae	*Dichodra* sp.	−30.3
Proteaceae	*Hakea leucoptera* R. Br.	−30.4
Platanaceae	*Platanus occidentalis* L.	−30.5
Compositae	*Chrysothamnus nauseousus* (Pall.) Britton	−30.5
Solanaceae	*Nicotiana tobaccum* L.	−30.7
Compositae	*Achillea lanulosa* Nutt.	−31.6
Scrophulariaceae	*Mimulus lewisii* Pursh.	−32.3
Myrtaceae	*Eucalyptus globulus* Labill.	−33.3
Scrophulariaceae	*Mimulus cardinalis* Dougl.	−34.1
Compositae	*Achillea borealis* Bong.	−34.3
	III. Algae	
(Division)		
Chlorophycophyta	*Acetabularia* sp.	−12.3
Phaeophycophyta	*Sargassum* sp.	−16.3
Chlorophycophyta	*Entermorpha marginata* J. Agardh	−16.6
Phaeophycophyta	*Macrocystis pyrifera* (L.) C. A. Agardh	−17.5
Rhodophycophyta	*Corallina chilense* Descaisne	−18.6
	Gigartina cristata (Setchell) Setchell and Gardner	−20.2
Cyanophycophyta	Blue-green sp. (mud)	−21.3
Rhodophycophyta	*Grateloupia setchellii* Kylin	−22.7
Chlorophycophyta	*Chlorococcum* sp. (fresh-water)	−21.6
	Spirogyra sp. (fresh-water)	−21.7

DISCUSSION

Since 1965, when Kortshak (8) first described labeling of malate and aspartate as the first products of photosynthesis in sugarcane, much work has been done on differences between the Calvin cycle and the C_4-dicarboxylic acid pathway of carbon fixation (6). Thus some species may fix a great deal of carbon via P-enol pyruvate carboxylase rather than by ribulose-1,5-diP carboxylase. They also have bundle sheath chloroplasts which may lack grana (9) and, if so, are deficient in photosystem II activity (18). Species exhibiting the C_4-dicarboxylic acid pathway can fix CO_2 at very low ambient concentrations (11). This syndrome (16) also includes that group of plants with relatively large $\delta^{13}C$ values.

Many of our samples were selected to ascertain whether it is possible to predict, *a priori*, the $\delta^{13}C$ value of the species.

Table II. *Geographical Influence on $\delta^{13}C$ Values*

	$\delta^{13}C$‰
Zea mays, L. Kaysville, Utah	−13.6
Chino, California	−14.0
Sorghum sp., Kaysville, Utah	−13.8
Chino, California	−14.4
Distichilis spicata, (L.) Green, Port Aransas, Texas	−14.0
Pt. Mugu, California	−14.7
Monanthochloë littoralis, Engelm. Port Aransas, Texas	−14.1
Pt. Mugu, California	−15.3
Salicornia bigelovii, Torr. Port Aransas, Texas	−24.7
Pt. Mugu, California	−25.2

translocation step determines how rapidly CO_2 in the cytoplasm is removed from the plant system to avoid a build-up of ^{13}C in the cells. All three steps affect the final fractionation that is associated with the fixation of CO_2 by plants. The relative rates and efficiency of these various steps determine the isotopic composition of the final plant. In principle, this allows for plants to have the entire range of $\delta^{13}C$ values from −1 to −38‰ (12).

Adaptations leading to high $^{13}C/^{12}C$ ratios seem to be a response to life under difficult conditions (aquatic or xeric habit for instance). Hall and Clements (5) indicate that *Atriplex canescens* subspecies *typica* is wide-ranging throughout the Great Basin, whereas subspecies *linearis* is restricted to more arid regions of the Pacific Southwest. Subspecies *linearis* is over 5‰ richer in ^{13}C than subspecies *typica*, implying

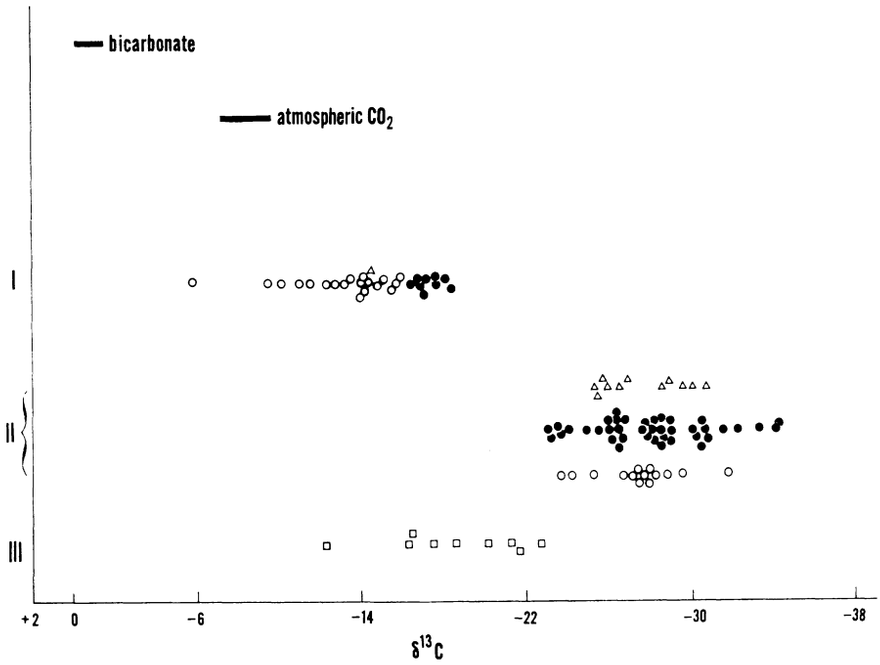

FIG. 1. $\delta^{13}C$ values of plant groups. Monocotyledoneae (○); Dicotyledoneae (●); algae (□); Bryophyta, Gymnospermae. (△).

For example, those plants which are known to have agranal bundle sheath chloroplast morphology were found to have $\delta^{13}C$ values in group I. Conversely, it was possible to predict which plants had the above morphology from the $\delta^{13}C$ value. Similarly, isotope ratios could be used to predict which plants would exhibit high CO_2 compensation and which low CO_2 compensation.[3]

In the Park and Epstein model (12), uptake of CO_2 into the cytoplasm involves isotopic fractionation due to the greater frequency of $^{12}CO_2$ colliding with the cell membrane as compared with $^{13}CO_2$. After passing through the membrane, the dissolved CO_2 is partitioned into enzyme-catalyzed conversion to starch and into removal of some of the dissolved CO_2 through the vascular system resulting in excretion through the roots. Galimov (4) found CO_2 in the soil to be lighter than atmospheric CO_2 but heavier than organic carbon, thus confirming prediction from the model. The ribulose-1,5-diP carboxylase reaction had an experimentally determined isotope fractionation associated with it of about 17 per mille. The rather different physiological adaptations for the two subspecies. This example also indicates that a great deal of $\delta^{13}C$ variation can occur within a species. Within the genus *Atriplex* there are species exhibiting $\delta^{13}C$ values as light as −29‰ (16). Growth under difficult environmental conditions might indicate an adaptation for more efficient photosynthetic carbon fixation which could be reflected in high $^{13}C/^{12}C$ ratios. Sculthorpe (15) reported that *Thallasia* and *Typha* produce the highest dry weight per unit area of all plants, with *Eichhornia* a close second. *Thallasia* has a high $\delta^{13}C$ value, but the latter two do not. Thus, the difference between the two plant groups is not necessarily reflected in efficient dry matter production, although efficiency may be indicated under certain restricted environmental conditions. *Casuarina* through convergent evolution has some morphological similarity to *Equisetum* even though these genera are only distantly related. Close similarity in $^{13}C/^{12}C$ ratios for the two genera might argue for considerable similarity in physiology as well. *Artemisia, Chrysothammus, Philadelphus,* and *Atriplex* are all found in the Great Basin. Striking differences in $\delta^{13}C$ values between these species might indicate a rather different evolutionary history resulting in different physiological adaptations to the xeric environment. Our modern desert flora have evolved dur-

[3] We thank W. M. Laetsch (dimorphic chloroplasts) and E. B. Tregunna (CO_2 compensation measurements) for their cooperation in making these estimations.

ing late Pliocene and Pleistocene times (*1*); thus, it seems possible that distinguishing ancient land and marine flora on the basis of $^{13}C/^{12}C$ ratios may still be a valid approach, unless similar adaptations evolved during other hot, dry periods of the past (*e.g.*, Permian).

The $\delta^{13}C$ of bicarbonate is 7 to 8‰ greater than that of CO_2 (Fig. 1). If algae or aquatic plants utilize bicarbonate, they would be expected to have a relatively larger $^{13}C/^{12}C$ ratio than plants incorporating atmospheric CO_2. Since *Cymodocea* has a $\delta^{13}C$ value greater than atmospheric CO_2, it is possible that this plant utilized bicarbonate as the carbon source for photosynthesis. Some emergent plants, *e.g.*, *Eichhornia* and *Sphagnum*, have relatively low $^{13}C/^{12}C$ ratios and probably fix atmospheric CO_2. The similarity in $\delta^{13}C$ values observed between fresh-water and marine algae indicates utilization of a similar carbon source in ocean and fresh water. The narrow range of values measured for the algae thus follows predictions made from the model. Most higher plants exhibiting relatively high $\delta^{13}C$ values are terrestrial and utilize atmospheric CO_2. To demonstrate that this is indeed the case we grew corn seedlings for several weeks in acid-washed quartz sand watered only with distilled water and could observe no significant change in $\delta^{13}C$ from the −14‰ reported for field-grown corn.

Plants high in ^{13}C differ from plants low in ^{13}C in anatomy, physiology, biochemistry, and ecology as well as in isotopic ratios. Adaptations leading to high $^{13}C/^{12}C$ ratios seem to be a response to life under stress, such as aquatic or xeric habit. That such adaptations are a relatively recent development in the evolution of angiosperms can be shown by the large variation in $\delta^{13}C$ within families (*Chenopodiaceae*), within genera (*Atriplex*), and even within species (*Atriplex canescens*). Carbon isotopic ratios allow one to predict aspects of plant physiology. For instance, one can easily determine if a particular brand of sucrose was obtained from sugarcane (high ^{13}C) or from sugarbeet (low ^{13}C)—a distinction difficult, if not impossible, to make using classical chemical techniques.

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