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$^{13}C/^{12}C$ Ratio Changes in Crassulacean Acid Metabolism Plants[1]

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ABSTRACT

$^{13}C/^{12}C$ ratios have been found in totally combusted leaves of Crassulacean acid metabolism plants to range from −14 to −33 δ ^{13}C‰ compared with a limestone standard. Crassulacean acid metabolism plants apparently utilize both ribulose-1,5-diphosphate carboxylase and phosphoenolpyruvate carboxylase to assimilate atmospheric CO_2 and, depending on environmental conditions, have $^{13}C/^{12}C$ ratios indicative of either carboxylase or to any intermediate value. The degree of discrimination against ^{13}C and the resultant $^{13}C/^{12}C$ ratio from the photosynthetically fixed CO_2 is influenced by environmental conditions and is not a specific and fixed characteristic of a Crassulacean acid metabolism plant. Certain Crassulacean acid metabolism plants may shift their ratios as much as 17 δ ^{13}C‰ in specific environments.

In the process of photosynthesis, plants which incorporate CO_2 by the C_4-dicarboxylic acid cycle[3] have a $^{13}C/^{12}C$ ratio closer to the atmospheric ratio than plants which incorporate CO_2 by the pentose cycle (2). Both groups of plants, however, discriminate in favor of ^{12}C over ^{13}C to a predictable and reproducible degree (2, 3, 12, 15–17). Compared to a limestone standard, total combustion of leaves from C_4 plants give values ranging from −10 to −18 δ ^{13}C ‰ and pentose plant values ranging from −23 to −34 δ ^{13}C ‰. Other work has shown that the δ ^{13}C value of various plant parts such as leaves, kernels, stems, and roots or of the major constituents such as proteins or sugars vary slightly but fall within the predictable range for each plant (1, 6, 8, 17), further confirming the distinctive values for both C_4 and pentose plants and for each plant. Again, while the same species grown in different geographical areas or under different environmental conditions may show some differences in isotopic content (3, 12, 16), the shifts in δ ^{13}C values are within those characteristic of C_4 or pentose plants, and the changes are minor. Furthermore, in plants grown under controlled changes in temperature and oxygen, very little effect on the isotopic content of the plants has been observed (4, 13).

More recently this isotopic consistency within a group of higher plants does not appear to hold true for Crassulacean acid metabolism plants (5, 10). In a study of the pathways of carbon assimilation with isolated CAM plant leaf cells (10, 11), it was noted that certain CAM plants had δ ^{13}C values ranging from −14 to −30. The literature on plants which may be CAM was surveyed and values of δ ^{13}C ranging all the way from those typical of C_4 plants to those of pentose plants have been reported (3, 10, 12, Table I). The thesis of this study with CAM plants is to document this wide range of C isotope discrimination, to examine some influences of environment upon the variation in δ ^{13}C, and to consider possible explanations for these variations.

MATERIALS AND METHODS

A number of plants either suspected of being CAM or determined to be CAM in this study were grown under a variety of experimental conditions including controlled temperature and light conditions in both Athens, Georgia and Madison, Wisconsin. Leaf samples were taken from new growth developed while under the controlled conditions for 1 to 2 months. Leaf samples were dried at 60 C, totally combusted, and analyzed with a precision of ±0.1 ‰ for carbon isotope composition, according to the method described by Bender (2, 3). The isotope measurements were made with a Nuclide Corporation RMS 6-60 isotope ratio mass spectrometer located at the University of Wisconsin. The isotopic composition is conventionally expressed as δ ^{13}C, the difference in per mille of the $^{13}C/^{12}C$ ratio of a standard, Pee Dee Belemnite limestone, which is set at zero.

$$\delta^{13}C\ (‰) = \left[\frac{^{13}C/^{12}C\ \text{sample}}{^{13}C/^{12}C\ \text{standard}} - 1\right] \times 10^3$$

On this scale, as the values become more negative, a greater preference for ^{12}C is indicated resulting in an exclusion of ^{13}C.

Light conditions in Georgia in the greenhouse varied from day to day and reached 7000 ft-c in midday with a day temperature of 28 C and night temperature of 16.5 C. The growth chamber light was constant at 2500 ft-c (Sylvania Gro-Lux supplemented with tungsten), and the temperature was as specified in the tables.

In the Wisconsin greenhouse sunlight was the light source and day temperatures ranged from 21 to 37 C, while the night temperature was maintained at 20 to 21 C. Light conditions in Wisconsin under constant conditions were 200 to 300 ft-c (Sylvania Gro-Lux), and the temperature was approximately 21 C. Heat was supplied by fuel oil which is known to evolve small amounts of ethylene. Concentration of CO_2 under the conditions of this growth is known to increase up to 500 μl/l of air.

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[3] Abbreviations: C_4: dicarboxylic acid cycle; pentose cycle: reductive pentose phosphate cycle; RuDP: ribulose diphosphate; PEP: phosphoenolpyruvate; CAM: Crassulacean acid metabolism.

RESULTS AND DISCUSSION

The major plant families which contain CAM species (9, 10, 14) were used to compile the data shown in Table I. These plants have δ ^{13}C values ranging from −13.8 to −30.5 ‰. The environmental conditions under which most of these plants were grown is not known, but with the limited growth data their δ ^{13}C values were noted and were assumed to be characteristic of the particular species. CAM is known to be strongly influenced by environment (9), thus we do not know if the literature values quoted here were obtained with plants exhibiting CAM. In Table I, however, we have determined that some plants were in CAM at the time of sampling and their δ ^{13}C values ranged from −13.8 to −29.2 ‰.

Table I. *Summary of Mass Spectrometric Analyses of $^{13}C/^{12}C$ Ratios for CAM Plants*

Family	Plant	$\delta^{13}C$	Suspected (s) or Determined (d) to be CAM
Aizoaceae	*Mesembryanthemum chilense* Mol.	−23.6 (12)[1]	s
Bromeliaceae	*Tillandsia usneoides* L.	−18.6 (12)	s
	Ananas comosus	−14.6	d
Cactaceae	*Opuntia strobiliformis* A. Berg.	−15.7 (3)	s
	O. humifusa Raf.	−13.8 (3)	s
	Cereus peruvianus Hort. ex Foerst	−15.2 (3)	s
	Schlumbergera bridgesii (*Zygocactus truncatus*)	−18.1, −19.5 (10)	s
Compositae	*Senecio gregori* (S. Moore) Jacobsen	−19.5 (3)	s
Euphorbiaceae	*Euphorbia tirucalli* L.	−15.3 (3)	s
	Pedilanthus tithymaloides var. *cucullatus* L.	−15.9 (3)	s
Crassulaceae	*Sempervivum calcareum* Ford.	−16.8 (3)	s
	Sedum 1871 *spurium coccineum*	−29.9 (12)	s
	S. spurium Bieb.	−27.6, −28.9, −29.9 (3, 10)	s
	S. spectabile Bor.	−26.6 (3)	s
	S. rubrotinctum Clausen	−13.8 (3, 10)	s, d
	S. telephium	−29.2 (10)	d
	S. pulchellum	−29.6 (10)	s
	S. telephoides	−22.6, −22.7 (10)	d
	Kalanchoë tubiflora Hamet	−14.2 (3)	s
	K. daigremontiana	−25.8, −25.9 (10)	d
	Crassula argentea	−17.2 (10)	d
	C. tomentosa L.	−18.9 (3)	s
	Nananthus malherbi	−30.5	s
	Echeveria cilva White	−18.1 (3)	s
	Titanoposis calcareum	−18.7	s
Liliaceae	*Yucca filamentosa* L.	−27.1 (3)	s
	Aloe arborescens Mill. Gard. Dict.	−24.5 (3)	s
	A. broomii	−23.2	s
	Sanseveria fasciata Poupion	−21.8	s
	Haworthia attenuata	−17.1 (3)	s
	Asclepiadaceae Stapelia semota N. E. Brown	−17.6	s

[1] Numbers in parentheses indicate reference.

Table II. *Mass Spectrometric Analyses of $^{13}C/^{12}C$ Ratios of CAM Plants Grown in Known Environments*

Family	Plant	Wisconsin	Georgia		
		24 hr-200 ft-c, 20 C	12 hr dark, 20 C / 12 hr-2500 ft-c, 30 c	24 hr-2500 ft-c, 20 C	24 hr-2500 ft-c, 30 C
		$\delta^{13}C$			
Crassulaceae	*Kalanchoë tubiflora* Hamet		−24.0[1]	−27.7	−25.6, −27.1
	K. daigremontiana Hamet & Perrier		−25.0[1]	−24.5	−25.8
	K. fedtschenkoi	−33.3			
	K. pinnata Pers.	−29.9			
	K. verticillata Elliott	−24.5			
	Sedum rubrotinctum Clausen		−15.2[1]	−22.2	−15.7
	S. telephium		−26.9[1]	−26.3	−28.5
	S. telephoides		−26.4[1]	−23.6	−25.2
Cactaceae	*Zygocactus truncatus*		−16.5[1]		−20.8

[1] These plants were exhibiting a diurnal acid cycle typical of CAM metabolism when samples were taken for $^{13}C/^{12}C$ ratio analysis.

Since CAM plants, like C_4 plants, fix CO_2 into C_4-dicarboxylic acids (9, 11, 14), one might expect them to be similar in carbon isotope discrimination to C_4 plants rather than similar to pentose plants. Instead, even within the same genus one can find $^{13}C/^{12}C$ ratios across the entire range of values, *e.g. Sedum rubrotinctum* is −13.8, *S. telephoides* is −22.6, and *S. telephium* is −29.2 ‰. Each of these *Sedum* species was exhibiting CAM when analyzed (Table I).

The δ ^{13}C values for C_4, pentose, and CAM plants from Table I and our data on other CAM plants are summarized in Figure 1. The C_4 and pentose plants fall into two distinct δ ^{13}C value groups, as shown previously (2, 3, 12, 15). Previous workers have used the $^{13}C/^{12}C$ ratio, obtained with a particular plant, to classify the plant as either a C_4 or a pentose plant. Clearly from a $^{13}C/^{12}C$ ratio one obtains with a particular plant, you would be required to know it is not a CAM plant in order to classify it as a C_4 or a pentose plant on the basis of its $^{13}C/^{12}C$ ratio alone.

In the course of these investigations we noted that some CAM plants did not exhibit the same $^{13}C/^{12}C$ ratio when grown under different environments (Tables II, III, and IV); *e.g. Kalanchoë pinnata* −15.2 to −29.9, *K. verticillata* −15.8 to −25.4, *K. fedtschenkoi* −16.3 to −33.3, *S. rubrotinctum* −13.8 to −23.6, or *Zygocactus truncatus* −16.5 to −22.2 δ ^{13}C. One possible explanation for such variations was the fact that most of the data in Tables I, II, and III were obtained with different plants within a given species, which may not have been isogenetic. Thus we selected a single mother plant for each genus in Table IV and vegetatively propagated each. These plants are considered isogenetic, and the data in Table IV show that variations are induced environmentally and are not due to genetic differences. The *K. verticillata* and *K. fedt-*

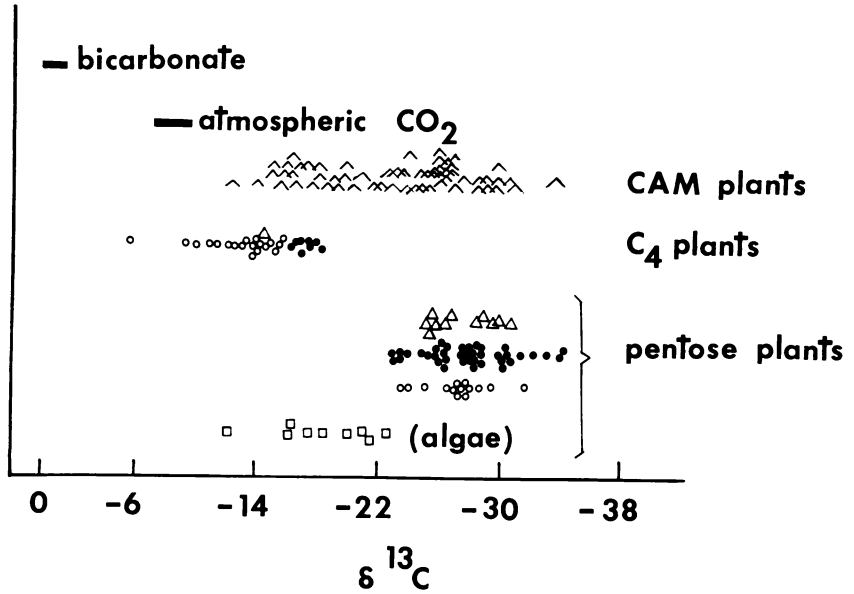

FIG. 1. Adapted from Smith and Epstein (12). □: Algae; ○: Monocotyledoneae; ●: Dicotyledoneae; △: Bryophyta, Gymnospermae; Λ: Crassulacean acid metabolism. δ ^{13}C relative to Pee Dee Belemnite standard.

Table III. *Mass spectrometric analyses of $^{13}C/^{12}C$ Ratios of Various CAM Plants Grown in Greenhouses*

Plant	Wisconsin	Georgia		
		March 1972	August 1972	March 1973
		$\delta^{13}C$		
Sedum telephoides		−22.6	−26.0	−23.1
S. telephium		−29.2	−26.6	−24.9
S. rubrotinctum	−13.8		−18.8	−19.7
Kalanchoë tubiflora	−14.2	−17.5	−25.1	−18.0
K. daigremontiana	−17.2	−25.9	−22.8	−16.4
K. fedtschenkoi	−16.3			−26.9
K. verticillata	−15.8			−18.4
K. pinnata	−15.2			
Crassula argentea		−17.2		−16.3
Zygocactus truncatus		−18.1		−19.4
Bryophyllum crenatum		−19.2		

schenkoi also were clones from the Wisconsin plants in Tables II and III.

The CAM plants grown in the Wisconsin greenhouse (Table III) had δ ^{13}C values near those of C_4 plants, while clonal offspring of these plants in low light and at a constant temperature (Table II) had δ ^{13}C values near those of pentose plants. Thus both light and temperature seem to affect the final δ ^{13}C of a given CAM plant. When pentose plants are compared to C_4 plants, both a lower light saturation and a lower temperature optimum for growth are exhibited by pentose plants (5). So CAM plants may exhibit δ ^{13}C values similar to pentose plants or to C_4 plants under proper environmental conditions (Tables II and III). However, at this point in our research we are not certain which factor(s) in the environment is the principal factor in deciding the final δ ^{13}C of a specific CAM plant.

If the sites of isotopic discrimination in plants are considered in the model proposed by Park and Epstein (7), we think fractionation at the site of enzymatic CO_2 fixation is the dominant factor in ultimately determining the final δ ^{13}C of a plant. It has been shown recently that PEP carboxylase from a C_4 plant has only a 3 ‰ discrimination for ^{12}C at 25 C and pH 8.5, while RuDP carboxylase from a C_4 plant at pH 8.2 has a 33.7 ‰ and 18.3 ‰ enrichment discrimination at 24 C and 37 C, respectively, in reference to dissolved CO_2 in the reaction mixtures (18). Earlier work (7) also showed that RuDP carboxylase from a pentose plant at 25 C and pH 7.4 enriched the ^{12}C by 8.2 ‰ with respect to dissolved CO_2 in the reaction mixture. Thus the molecular site for most of the ^{13}C *versus* ^{12}C discrimination apparently is at the level of the two primary leaf carboxylases. CAM plants may utilize both carboxylases for net CO_2 uptake (5, 9, 11, 14). Note that RuDP carboxylase enriches less in ^{12}C as the temperature increases (18) so that at higher temperatures, which are optimal for C_4 plants and for some CAM plants (5), less ^{12}C enrichment would occur. A shift in growing temperature could thereby shift the final δ ^{13}C of a CAM plant. With C_4 plants most of the CO_2 enters the plants through PEP carboxylase which has a low ^{12}C enrichment, and C_4 plants also exhibit a high temperature optimum for photosynthesis (5) which would favor less ^{12}C enrichment by RuDP carboxylase. So both the primary sites of CO_2 fixation and the growth temperature are the dominant factors in determining the ^{12}C enrichment in C_4 plants, and similar reasoning could be applied to pentose plants with their lower temperature optimum and their low activity of PEP carboxylase (5).

Hence, an explanation of variable δ ^{13}C values is that a CAM

Table IV. *$^{13}C/^{12}C$ Ratios of Isogenetic CAM Plants (Georgia)*

Plant	Greenhouse	Continuous light—24 hr, 24–25 C		
	March 1973	90 ft-c	120 ft-c	370 ft-c
	$\delta^{13}C$			
Sedum telephium	−24.9	−29.0	−25.8	−26.4
S. telephoides	−23.1	−24.1	−22.0	−23.4
S. rubrotinctum	−19.7	−20.8	−21.6	−23.6
Kalanchoë tubiflora	−18.0	−19.1	−18.9	−23.7
K. daigremontiana	−16.4	−19.4	−20.1	−27.5
K. fedtschenkoi	−26.9		−22.2	−30.0
K. verticillata	−18.4		−21.0	−25.4
Crassula argentea	−15.9	−18.2	−16.5	−18.0
Zygocactus truncatus	−19.4		−19.1	−22.2

plant grown under environmental conditions favoring CO_2 fixation through PEP carboxylase (high light intensity and high temperature) will have a $^{13}C/^{12}C$ ratio similar to C_4 plants; whereas a CAM plant grown under environmental conditions favoring CO_2 fixation through RuDP carboxylase (low light intensity and low temperature) will have a $^{13}C/^{12}C$ ratio similar to a pentose plant. However, different CAM plants grown in the same environment have different δ ^{13}C values (Table I, *Sedum* sp.) which lead to the postulation that different CO_2 fixation pathways predominate in specific CAM plants, and the relative amounts of carbon flowing through each pathway can be shifted by the environment.

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