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VARIATIONS IN THE $^{13}C/^{12}C$ RATIOS OF PLANTS IN RELATION TO THE PATHWAY OF PHOTOSYNTHETIC CARBON DIOXIDE FIXATION

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Abstract—Additional data is presented to show that plants of genera which are known to follow the C_4-dicarboxylic acid pathway in photosynthetic carbon dioxide fixation have $\delta^{13}C$ values in the −10 to −20‰ range. Plants which follow the Calvin cycle in photosynthesis have $\delta^{13}C$ values of −22 to −33‰ Plants of families which show Crassulacean acid metabolism also have many examples enriched in ^{13}C.

INTRODUCTION

IN THE process of photosynthesis plants discriminate in favor of the light isotope of carbon, ^{12}C, with the result that all plants contain relatively less ^{13}C than the atmosphere. The isotopic composition is conventionally expressed as $\delta^{13}C$, the difference in per mil of the $^{13}C/^{12}C$ ratio of the sample relative to a standard which in this work is carbon dioxide prepared from the Pee Dee belemnite (PDB) limestone.[1]

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

In a preliminary study of carbon isotope ratios in the Gramineae,[2] it was found that there were two distinct groupings of $\delta^{13}C$ values. Grasses with $\delta^{13}C$ values in the −22 to −30‰ range (compared to the PDB standard), the range in which the majority of the terrestrial plants previously analysed had been found [3–5] are grasses of subfamilies reported to follow the Calvin cycle in photosynthetic carbon dioxide fixation.[6] Plants studied included species of *Dactylis*, *Avena*, *Triticum*, *Hordeum* and *Phleum*. It was pointed out that[2] grasses found to have $\delta^{13}C$ values in the −10 to −19‰ range, relatively enriched in ^{13}C, the range previously considered to be characteristic of marine plants, are members of subfamilies in which the C_4-dicarboxylic acid pathway is operative in photosynthetic carbon dioxide fixation.[6] The plants studied included the genera *Saccharum*, *Sorghum*, *Zea*, *Paspalum*, *Digitaria*, *Setaria* and *Panicum*, members of the Panicoid and Chloridoid–Eragrostoid sub-groups Several of the species were species which had been included in the photosynthetic studies.

These results prompted the extension of such studies to additional plant families; species have again been found for which there is a parallel between carbon isotope ratio and

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[6] M D HATCH, C R SLACK and H S JOHNSON, *Biochem J* **102**, 417 (1967).

the reported pathway of photosynthetic carbon dioxide fixation. In addition many plants of the families which show Crassulacean acid metabolism also show enrichment in the heavy isotope of carbon.

RESULTS

The $\delta^{13}C$ values obtained are summarized in Tables 1 and 2. The grasses of the Panicoid and Chloridoid–Eragrostoid subfamilies which are enriched in ^{13}C are species of the groups which follow the C_4-dicarboxylic acid pathway in photosynthesis.[6,7] One particular species, *Cynodon dactylon*,[7] has been cited as a plant of high photosynthetic capacity and is assumed to follow the C_4 pathway.

Three grasses were found to be divergent. *Panicum scribnerianum* and *P. pacificum* are depleted in ^{13}C and they have already been shown to be exceptions and to follow the Calvin

TABLE 1 MASS SPECTROMETRIC ANALYSES OF PLANTS OF THE GRAMINEAE

Tribe	Genus and species	$\delta^{13}C$‰ relative to PDB
Arundinareae	*Arundinaria* sp.	−29·2
Zizanieae	*Zizaniopsis miliacea* Doell & Aschers	−26·3
	Zizania aquatica L.	−30·9
Arundineae	*Phragmites communis* Trin.	−26·6
Stipeae	*Stipa spartea* Trin.	−27·8
	S. comata Trin & Rupr	−24·8
	Oryzopsis hymenoides (Roem & Schult.) Ricker	−28·0
Festuceae	*Festuca rubra* L.	−27·3
	Bromus kalmii A. Gray	−30·3
	B catharticus Vahl	−30·7
Hordeae	*Elymus canadensis* L.	−27·1
	E mollis Trin.	−27·6
	Agropyron intermedium (Host) Beauv	−28·3
Aveneae	*Ammophila breviligulata* Fern	−27·7
	Agrostis perennans (Walt) Tuckerm.	−30·0
	A scabra Willd	−28·7
Aeluropodeae	*Distichlis spicata* (L.) Greene	−15·0
Spartineae	*Spartina cynosuroides* L.	−14·4
	S pectinata Link	−13·4
Eragrosteae	*Eragrostis cilianensis* Link	−13·3
	Sporobulus poiretti (R & S) Hitchc.	−13·7
	Muhlenbergia schreberi J F. Gmel.	−13·0
Chlorideae	*Bouteloua gracilis* (H B. K) Lag.	−12·7
	B curtipendula (Michx) Torr	−14·2
	Buchloe dactyloides (Nutt.) Engelm.	−14·2
	Cynodon dactylon L	−15·3
Paniceae	*Panicum amarulum* Hitchc. & Chase	−13·4
	P. virgatum L.	−13·7, −14·8
	P dichotomiflorum Michx.	−13·2
	P pacificum Hitchc & Chase	−33·2
	P. leibergii (Vasey) Scrib	−26·4
	P scribnerianum (Nash) Fern.	−32·1
	Echinochloa crus-galli (L) Beauv.	−13·1
Andropogoneae	*Andropogon glomeratus* (Walt) BSP	−14·2

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TABLE 2. MASS SPECTROMETRIC ANALYSES OF PLANTS FROM OTHER FAMILIES

Family	Genus and species	$\delta^{13}C$‰ relative to PDB
Typhaceae	*Typha angustifolia* L.	−31·0
Cyperaceae	*Scirpus cespitosus* L.	−28·1
	S. olneyi Gray	−29·1
	S. validus Vahl	−28·0
	S. robustus Pursh	−26·9
	Cladium jamaicensis Crantz	−28·6
	Eriophorum angustifolium Honckeny	−27·6
	Carex stricta Lam.	−27·0
	C. lacustris Willd	−27·8
	Eleocharis parvula (R. & S.) Link	−21·6
	Cyperus odoratus L.	−13·2
	C. filiculmis Vahl	−13·3
Commelinaceae	*Tradescantia ohiensis* Raf.	−26·1
Juncaceae	*Juncus roemerianus* Scheele	−28·6
	J. effusus L.	−30·1
Liliaceae	*Yucca filamentosa* L.	−27·1
	Aloe arborescens Mill. Gard. Dict.	−24·5
	Sanseveria fasciata Poupion	−17·1
Polygonaceae	*Oxyria digyna* (L.) Hill	−27·2
Chenopodiaceae	*Atriplex patula* L	−31·6
	A. rosea L.	−19·4
	A. hastata L.	−32·1
	Chenopodium album L.	−28·1
	Haloxylon salicornicum Birge	−14·5
	Salsola foetida Del	−12·4
	Suaeda fruticosa (L.) Forsk.	−12·2
Amaranthaceae	*Aerva javanica* Juss.	−13·3
	A. pseudotomentosa Blatt	−14·5
	Pupalia lappacea Juss	−24·4
	Amaranthus blitoides S. Wats.	−14·1
	A. retroflexus L.	−13·3
Portulacaceae	*Portulaca oleracea* L.	−12·1
	P. grandiflora Hook	−11·4
Crassulaceae	*Sempervivum calcareum* Ford	−16·8
	Sedum spurium Bieb	−27·6
	S. spectabile Bor	−26·6
	S. rubrotinctum Clausen	−13·8
	Kalanchoe tubiflora Hamet	−14·2
	Echeveria cilva White	−18·1
	Crassula tomentosa L	−18·9
Leguminosae	*Glycine soja* (L.) Sieb. & Zucc	−28·9
	Lupinus perennis L.	−28·5
	Amorpha canescens Pursh	−27·9
Euphorbiaceae	*Euphorbia pulcherrima* Willd. ex Klotzsch	−29·4
	E. corollata L.	−26·1
	E. tirucalli L.	−15·3
	Pedilanthus tithymaloides var. *cucullatus* L.	−15·9
	Ricinus communis L.	−30·2
Cistaceae	*Hudsonia tomentosa* Nutt	−28·4
Cactaceae	*Opuntia strobiliformis* A. Berg.	−15·7
	O. humifusa Raf.	−13·8
	Cereus peruvianus Hort. ex Foerst	−15·2
Umbelliferae	*Eryngium yaccifolium* Michx.	−27·5
Ericaceae	*Cassiope tetragona* D. Don	−27·9
Asclepiadaceae	*Asclepias syriaca* L.	−28·6
	Hoya carnosa R. Br.	−16·7
	Stapelia semota N. E. Brown	−17·6

TABLE 2.—*cont.*

Family	Genus and species	$\delta^{13}C$‰ relative to PDB
Convolvulaceae	*Convolvulus sepium* L.	−25 7
Solanaceae	*Physalis heterophylla* Rydb.	−27·2
Compositae	*Tagetes* sp.	−28 9
	Artemisia tridentata Nutt.	−28·8
	A abrotanum L.	−29·7
	Senecio gregori (S Moore) Jacobsen	−19·5
	Tragopogon pratensis L.	−29·1

cycle.[8,9] *P. leibergii*, also with a low $\delta^{13}C$ value, is a species of the same Dichanthelium subgenus as the preceding two grasses.

The species of the other subfamilies of the Gramineae are depleted in ^{13}C; grasses of these subfamilies were found to be Calvin cycle plants.[6]

Several species of the Chenopodiaceae and Amaranthaceae show enrichment in ^{13}C. *Atriplex rosea*, which is known to follow the C_4-dicarboxylic acid pathway,[9] is in the enriched group; *A. hastata*[9,10] and *Chenopodium album*[11] are Calvin cycle plants and are depleted in ^{13}C. One other example of a known Calvin cycle plant is *Glycine soja*[6] whose $\delta^{13}C$ value is in the expected low range.

Photosynthetic studies on the Cyperaceae[11] showed that there were plants with both types of photosynthetic pathway in this family. While the mass spectrometric analyses are of different species than those used in the photosynthetic studies, two groupings of $\delta^{13}C$ values have been found.

A number of succulent plants and halophytes in the Portulacaceae, Crassulaceae, Cactaceae, and Asclepiadaceae were also studied and found to be enriched in ^{13}C. These are families in which the Crassulacean acid metabolism has been demonstrated.[12]

DISCUSSION

The results show a consistent difference in ^{13}C values in the two groups of plants which follow the different pathways in photosynthesis and confirm the suggestion made previously. These determinations show a range for $\delta^{13}C$ of −10 to −20‰ for plants which follow the C_4 pathway and −22 to −33‰ for Calvin cycle plants. In general when comparing plants of the two photosynthetic patterns which were grown in the same environment, there was an average difference of 12–14‰ between the two groups. The *Ammophila* and *Panicum amarulum* which were grown in government Maryland research grounds differ by 14·3‰; the *Atriplex rosea* and *A. hastata*, which are in the very low range of the two $\delta^{13}C$ groups, were both from Canada and the difference between them is 12·7‰. The average of the Wisconsin samples in the enriched group is −13·5, in the depleted group −27·7‰.

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It is of interest to note that $\delta^{13}C$ values for two other Calvin cycle plants,[6] the banana (Musaceae)[13] and *Nicotiana tabacum*[14] have been published as −24·6 and −28·5‰ respectively.

The two groups of plants which differ in photosynthetic pathway have already been shown to differ in other characteristics such as leaf anatomy,[15] maximum photosynthetic rates,[16] the effect of oxygen on photorespiration,[17] and the effect of oxygen concentration on photosynthesis.[18] It is not surprising then that the carbon isotope content of the plant groups is also different since a different sequence of reactions is involved in the utilization of atmospheric carbon dioxide.

Analogies between plants which show the Crassulacean acid metabolism followed by many succulent plants and those which follow the C_4 photosynthetic pathway have already been published.[10,15] The mass spectrometric analyses indicate an additional similarity. A large number of the plants enriched in ^{13}C are plants of tropical origin such as the tropical grasses, halophytes such as *Haloxylon* and *Salicornia*, and tropical succulents. Other workers have noted ^{13}C enrichment in succulent xerophytes.[19] This is in agreement with the hypothesis of Laetsch[15] that the biochemistry and structure of the plants are adaptations to facilitate carbon fixation in arid environments.

It is clear from these results that terrestrial plants may have $\delta^{13}C$ values in either of two groupings and it appears that the difference in the two groups is related to physiological variations. Because an emphasis has often been placed on the distinction between the $\delta^{13}C$ values of land and marine plants,[3,4,20,21] the plants analysed for this study were obtained from varying environments and in particular a few examples were obtained from marine environments such as coastal salt marshes, and a few examples were from the arctic. The *Phragmites*, *Juncus*, *Cladium* and *Typha* were all grown in Louisiana salt marshes but all are depleted in ^{13}C, while the *Panicum virgatum* was enriched to the same extent whether grown in Wisconsin or in a salt marsh. Small differences in δ value between species can be attributed to differences in growing conditions but the present results clearly suggest the large differences in values are due to physiological variations and not to variations in microclimate or environment.

EXPERIMENTAL

The carbon isotope ratios were measured with a precision of ±0 1‰ in $\delta^{13}C$ on CO_2 produced by combustion of the dried plant samples in a Parr bomb. The isotope measurements were made with the Nuclide Corporation RMS6-60 isotope ratio mass spectrometer of the University of Wisconsin Department of Chemistry The results are corrected for the O^{17} contribution to the mass 45 peak, cross contamination of the sample and standard gas, and for the fraction of mass 44 peak falling on mass 45 The corrections are those described in detail by Craig [22]

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