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Oxygen and Hydrogen Isotope Ratios of Water from Photosynthetic Tissues of CAM and C_3 Plants[1]

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ABSTRACT

Water samples from photosynthetic tissues of C_3 and Crassulacean acid metabolism (CAM) plants that grew together in the field were extracted and the stable oxygen and hydrogen isotope ratios determined. During the day, $^{18}O/^{16}O$ and deuterium/hydrogen (D/H) ratios of water from CAM plants were lower than those observed in water from C_3 plants. The patterns of diurnal variation (or lack thereof) in isotope ratios of plant water are consistent with the gross anatomical and physiological characteristics of the plants studied here. Our observations support the previously advanced hypothesis that high D/H ratios in cellulose nitrate prepared from CAM plants relative to those for C_3 plants are not caused by greater deuterium enrichment in the water in CAM plants, but rather by isotopic fractionations associated with different biochemical reactions in the two types of plants.

It is now well established that the stable isotopic composition of the nonexchangeable hydrogen in cellulose (measured as cellulose nitrate) is related to a plant's photosynthetic mode (7, 8, 11, 13). Cellulose nitrate prepared from CAM plants has more deuterium than that from C_4 and C_3 plants that grew under the same climatic regime and used the same water source. These isotopic differences have been explained by two models. The first is that evapotranspiration enriches the leaf water in CAM plants in deuterium more so than in non-CAM plants. This enrichment is then passed on to cellulose, with isotopic fractionation occurring during its synthesis not being different in CAM and non-CAM plants (13). According to the second model, deuterium-enrichment in CAM plants is caused not by isotopic fractionations occurring during evapotranspiration but rather by isotope effects, associated with biochemical reactions leading to cellulose synthesis, that are unique to CAM plants (7).

If the first model were correct, the occurrence of deuterium-enrichment in nonexchangeable hydrogens of cellulose from CAM plants would be accompanied by enrichment of ^{18}O in their cellulose relative to C_3 plant cellulose (7). No such co-enrichment occurs (7, 8, 11). Sternberg *et al.* (9) have also shown that aquatic CAM plants, which do not transpire, display deuterium-enrichment in their cellulose nitrate relative to that prepared from aquatic non-CAM plants with which they grew. Both observations are inconsistent with the suggestion that isotopic effects associated with evapotranspiration are responsible for the difference in deuterium concentrations in CAM and non-CAM plants.

A measurement that would test these two models directly has not been reported. This test involves isotopic analysis of leaf water in terrestrial CAM and non-CAM plants that grew near one another. If the first model is correct, leaf water from CAM plants should be enriched in deuterium relative to that from non-CAM plants. If no such deuterium enrichment in water of CAM plants occurs, the alternate model, involving isotopic fractionations during biochemical reactions, must be the correct one.

In this study we measured the hydrogen and oxygen isotope ratios of water in photosynthetic tissues of C_3 and CAM plants. These plants differ in two physiological aspects besides photosynthetic mode that could also affect the isotope ratios of their water. First, because CAM plants are typically more succulent than C_3 plants, a larger proportion of leaf water might not be subject to isotopic fractionations caused by transpiration. Second, CAM plants transpire only at night when the RH is higher than during the day, when C_3 plants transpire. Thus the isotope measurements reported here not only test the two models discussed above but also provide insight into the water budgets for these two types of plants.

MATERIALS AND METHODS

Small amounts of leaf or phylloclade tissue were collected on September 22, 1984, at dawn and in the late afternoon from *Fraxinus greggii*, *Prosopis glandulosa*, *Selaginella lepidophylla*, *Opuntia edwardsii*, *Agave lechequilla*, and *Echinocereus triglochidiatus* growing together near the Pecos River in Val Verde County, Texas. Weather conditions on that day were clear with a morning temperature of 14.5°C and RH of 45%. The late afternoon temperature was 26°C with 65% RH. After collection, samples were immediately inserted into test tubes that were then closed with a rubber septum and sealed with Parafilm. Samples were frozen until processed for isotopic analysis.

Individual *A. lechequilla* and *O. edwardsii* specimens collected as described above in Val Verde County, Texas, were uprooted and suspended in air in a growth chamber programmed to have a daytime temperature of 35°C and RH of 30% and a night temperature of 23°C with 80% RH. Light level in the growth chamber during the daily light period, which lasted 12 h, was 1400 μE. Leaf or phylloclade samples were collected as described above at the beginning of this experiment and 1 and 2 months later.

The outer epidermis and inner cortex of phylloclades of *O. edwardsii* from the Val Verde County site, which had been subjected to drought for 2 months in a growth chamber as described above and of *O. linheimeri* from a population in

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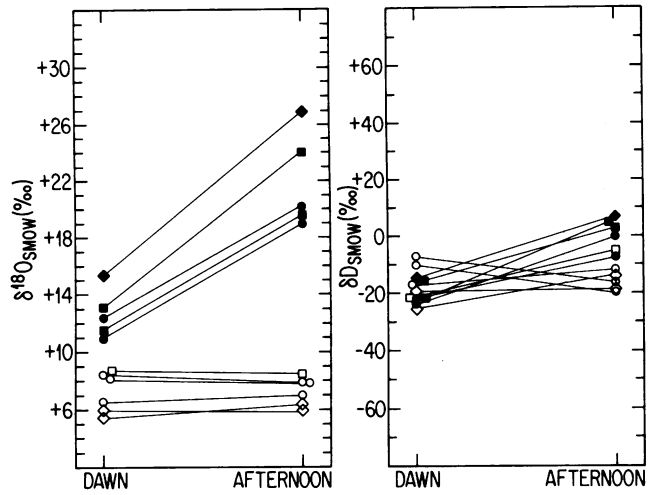

FIG. 1. $\delta^{18}O$ and δD values of water in leaves or phylloclades of C_3 and CAM plants collected in Val Verde County, Texas, at dawn and in the late afternoon of September 22, 1984. Symbols are: ● for *F. greggii* (C_3), ◆ for *P. glandulosa* (C_3), ■ for *S. lepidophylla* (C_3), ○ for *O. edwardsii* (CAM), □ for *A. lechequilla* (CAM), and ◇ for *E. triglochidiatus* (CAM).

Table I. *δD and $\delta^{18}O$ Values (Given in ‰) of Water in Epidermal Tissue and Inner Cortex of Two Species of Opuntia*

Opuntia Species	Tissue	Time	$\delta^{18}O$	δD
O. linheimeri	Epidermal	AM	+4.0	−5
O. linheimeri	Cortex	AM	+4.3	−3
O. linheimeri	Epidermal	PM	+3.4	−10
O. linheimeri	Cortex	PM	+4.5	−10
O. edwardsii	Epidermal	PM	+13.5	+22
O. edwardsii	Cortex	PM	+13.1	+18

Temple, Texas, freshly collected in the early morning and late afternoon of September 22, 1984, were separated and sealed into sample tubes as described above.

The water in leaf and phylloclade samples was recovered by puncturing the rubber septum with a needle attached to a vacuum system, evacuating the test tube, heating it at 100°C, and freezing the evolved water at liquid N_2 temperatures (10). Water oxygen isotope ratios were determined by equilibrating CO_2 with the water at 25°C prior to isotopic analysis of the CO_2 (4). Hydrogen isotope ratios were determined by the method of Bigeleisen *et al.* (2), in which small amounts of water are passed over uranium turnings at 750°C and the released hydrogen gas collected with a Toepler pump for isotopic analysis. Isotope ratios are expressed as δ values, where

$$\delta = [(R_{sample}/R_{standard}) - 1] \times 1000 \text{ per mil}$$

and R is $^{18}O/^{16}O$ for oxygen and D/H for hydrogen. The precision of isotopic analysis was ±0.2‰ for $\delta^{18}O$ values and ±2‰ for δD values. δ values are expressed relative to the SMOW[4] standard.

RESULTS AND DISCUSSION

Water in all C_3 plants increased in δD values from dawn to afternoon, while that of CAM plants in the afternoon had δD values that remained constant, decreased or increased relative to the values observed at dawn (Fig. 1). Our measurements indicate that during the day, when photosynthesis occurs, δD values of water from CAM plants were equal to or lower than those observed for C_3 plants. Previous measurements on plants from the same site as those whose water δD values are presented in Figure 1 show that cellulose nitrate from CAM plants had δD values about 100‰ higher than δD values of cellulose nitrate from C_3 and C_4 plants (8). Thus the model involving the proposal that cellulose from CAM plants is enriched in deuterium relative to that of non-CAM plants because of greater deuterium-enrichment during transpiration is not correct. The alternate model, involving differences in the isotopic fractionations occurring during biochemical reactions in the two types of plants, must be the correct one.

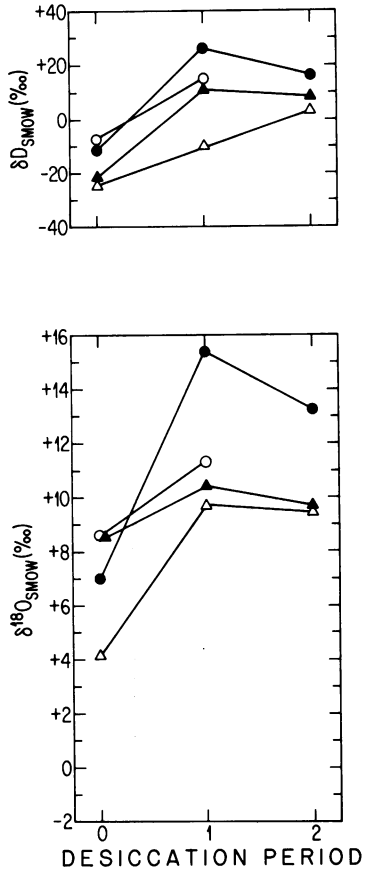

FIG. 2. $\delta^{18}O$ and δD values of water in leaves or phylloclades of CAM plants that were suspended in air in a growth chamber for the indicated period (1 and 2 months). Individual specimens are indicated by ● and ○ for *O. edwardsii*, and ▲ and △ for *A. lechequilla*.

The difference between the patterns of diurnal variations of oxygen isotope ratios of water from CAM and C_3 plants is similar to that observed for δD values but much more pronounced (Fig. 1). Water from C_3 plants increased in ^{18}O concentrations from dawn to afternoon (Fig. 1), which is consistent with other observations (6). Water from CAM plants, in contrast, does not become enriched in ^{18}O from dawn to afternoon. This is consistent with the lack of daytime transpiration in CAM plants. We expect that water from CAM plants would become enriched in ^{18}O and deuterium during the night, when they are transpiring.

There are two likely explanations for the overall lower $\delta^{18}O$ and δD values in water from CAM plants relative to that from C_3 plants during the day. The first is that, on a daily basis, CAM plants transpire less than C_3 plants since the former transpire predominantly at night when RH is higher than during the day (12). The second is that CAM tissues, being succulent, may have a higher proportion of water that is not subject to heavy isotope enrichment occurring during transpiration. Leaney *et al.* (5)

[4] Abbreviations: SMOW, standard mean ocean water.

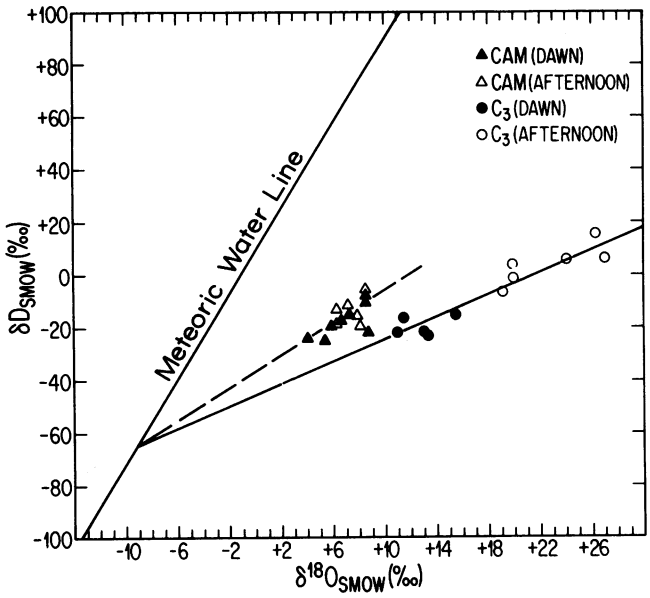

FIG. 3. The relationship between δD and $\delta^{18}O$ values of water in leaves or phylloclades of C_3 and CAM plants collected at dawn and in the late afternoon, as described in Figure 1. The meteoric water line is the relationship between the δD and $\delta^{18}O$ values of groundwater available for plant growth throughout the world, as described by Craig (3). The equation for the linear regression line (solid line) for C_3 plant water is $\delta D = 2.2 \times \delta^{18}O - 45.9‰$ ($R = 0.96$). The dashed line is not a linear regression line. It was drawn to pass through the midst of the CAM plant water data and to have the same intercept on the meteoric water line as the C_3 plant water linear regression line.

suggested a similar explanation to account for isotopic differences in leaf water of C_4 plants relative to that of C_3 plants. The second explanation does not seem to apply here. We measured $\delta^{18}O$ and δD values of water in carefully peeled outer epidermal layers (presumably containing water that undergoes isotopic fractionation during evapotranspiration) and in the internal tissue of CAM leaves (presumably containing water that is not directly exposed to the atmosphere and thus not undergoing isotopic fractionation during evapotranspiration) and found no substantial isotopic differences between these two types of waters (Table I). The differences observed between isotope ratios of water from *O. linheimeri* and *O. edwardsii* (Table I) are due to the fact that *O. edwardsii* was subjected to drought conditions for 2 months before waters in its tissues were collected. Thus we conclude that the $\delta^{18}O$ and δD values of water in CAM plants are lower than those of water in C_3 plants because of differences in diurnal transpiration patterns.

It has also been suggested that CAM plants, by maintaining metabolic activity during periods of drought when C_3 plants cease metabolic activity, have high cellulose nitrate δD values because they synthesize cellulose using hydrogen from leaf water that presumably becomes very enriched in deuterium during such periods (13). To test the extent of deuterium-enrichment that can occur in water from CAM plants under drought conditions, we uprooted *Agave* and *Opuntia* specimens and left them suspended in air in a growth chamber for a period of 2 months. Enrichment of tissue water in deuterium and oxygen-18 occurred only during the first month. Thereafter, no further enrichment occurred (Fig. 2). The increase in δD values of leaf water that occurred during desiccation of CAM plants was at most 40‰. Thus, even if CAM plants synthesized their cellulose only under drought conditions, the increase in water δD values accompanying desiccation would be inadequate to account for the observed differences in δD values of up to 100‰ between cellulose nitrate from CAM and C_3 plants that grew together (8). The pattern of isotopic variation in desiccated leaf or phylloclade water observed here is consistent with the physiology of CAM plants. When CAM plants are subject to water stress, they undergo CAM-idling, in which the stomata are closed both day and night and transpiration is minimal (12). CO_2 from respiration is probably recycled via CAM into the Calvin cycle during the day (11). The enrichment of CAM water in deuterium and oxygen-18 ended after the first month of drought stress (Fig. 2) because presumably at that point the CAM plants entered into the CAM-idling phase, after which only negligible amounts of water were lost through evapotranspiration.

Figure 3 shows the relationship between the δD and $\delta^{18}O$ values of leaf and phylloclade water in the C_3 and CAM plants we collected in Val Verde County on September 22, 1984. For C_3 plants, the linear regression line of δD values on $\delta^{18}O$ values, which is significant at the $P = 0.01$ level, has a slope of 2.2. Similar slopes, ranging from 1.2 to 3.6, have been reported for this relationship in leaf waters of C_3 plants (1, 4). In addition, values for leaf waters from C_4 and C_3 plants that grew together lie along the same line on δD-$\delta^{18}O$ value plots (1, 6). The data for CAM plants are too clustered to allow for meaningful linear regression analysis. However, the CAM leaf and phylloclade water points clearly do not lie on the line occupied by the C_3 leaf waters and may lie on a line with a steeper slope (dashed line on Fig. 3). This observation, based on the first determination of isotope ratios of water in CAM plants, may represent yet another isotopic difference between CAM plants and C_3 and C_4 plants. To confirm this, it will be necessary to analyze leaf waters of CAM and non-CAM species collected over a 24-h period so that changes in evapotranspiration (in response to daily and nightly changes in humidity and temperature conditions) will produce a greater range in the δD and $\delta^{18}O$ values of leaf water in both types of plants.

The results presented here provide conclusive evidence that during the day, when photosynthesis occurs, the δD values of water from terrestrial CAM plants are not higher than those of water from C_3 plants growing at the same site. Thus, elevated δD values of cellulose nitrate from CAM plants relative to those for C_3 plants cannot be due to evapotranspiration effects. We conclude, as we have done previously based on other lines of experimental evidence (7–9, 11), that the difference in the isotope ratios of the nonexchangeable hydrogens of cellulose from CAM and non-CAM plants are caused by isotopic fractionations occurring during biochemical reactions. At present, the identity and nature of these reactions have not been elucidated. In addition, the results presented here show that the dynamics of isotopic variations in leaf water differ for CAM and C_3 plants. These differences can be explained on the basis of the differences between the evapotranspiration regimes of the two types of plants.

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