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Carbon, oxygen and hydrogen isotope abundances in *Stylites* reflect its unique physiology

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Summary. Natural abundances of oxygen-18, carbon-14, carbon-13 and deuterium were measured in *Stylites andicola,* a terrestrial vascular plant lacking stomates, and two terrestrial plant species having stomates that grew nearby. No substantial differences in oxygen-18 and carbon-13 abundances were observed among the three species. The deuterium concentration in *Stylites* was much higher than in the other species, confirming a previous report of CAM in *Stylites. Stylites* was depleted in carbon-14 relative to the present day atmosphere, consistent with the proposal that it fixes CO_2 derived from decomposing peat.

A recent issue of *Nature* contains a report on the unusual photosynthetic mode of *Stylites andicola* (Keeley et al. 1984). This fern ally has no stomates and thus leaf absorption of CO_2 and transpiration are minimal (Keeley et al. 1984). Transport of minerals in these plants probably occurs in a manner similar to that observed for rooted vascular aquatic plants (Hutchinson 1975). The results of labeling experiments demonstrated that carbon dioxide is absorbed through the roots (Keeley et al. 1984). Subsequently, the CO_2 is metabolized via the CAM pathway, as evidenced by a diurnal flux of titratable acidity and malic acid, with higher acidity in the morning and lower acidity at sunset (Keeley et al. 1984). However, the $\delta^{13}C$ values for *Stylites* (see Materials and Methods for definition of δ) are in the range of those of C_3 plants, being more negative than those of other plants with CAM (Keeley et al. 1984). To explain this discrepancy, the authors proposed that *Stylites,* which commonly grows on peat, absorbs CO_2 derived from peat decomposition. This CO_2 would have $\delta^{13}C$ values lower than that of atmospheric CO_2, and consequently, its fixation and subsequent incorporation into plant biomass would lead to lower $\delta^{13}C$ values than are observed in CAM plants that fix atmospheric CO_2.

In order to test the proposal that *Stylites* uses CO_2 derived from peat decomposition as a carbon source, we investigated the ^{14}C content of *Stylites* and the peat in which it grew, since any significant contribution of peat-derived CO_2 should produce lower ^{14}C concentrations in *Stylites* compared with contemporary plants that utilize atmospheric CO_2 as their carbon source. We also measured the stable oxygen, hydrogen, and carbon isotope ratios of *Stylites* and two stomate-bearing species that grew nearby. In doing so, we hoped to obtain isotopic evidence for the occurrence of CAM in *Stylites.* This approach is based on the observation that CAM is recorded in the hydrogen isotope ratios of plants, even in cases in which the carbon isotope ratios are not diagnostic of the presence of this photosynthetic mode (Ziegler et al. 1976; Sternberg et al. 1984a, b). Our results are consistent with proposals put forth by Keeley et al. (1984), namely that *Stylites* fixes a significant fraction of its carbon from CO_2 derived by decomposition of peat and that the CO_2 is processed via CAM.

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Materials and methods

Stylites andicola Amstutz (Isoetaceae), *Plantago rigida* H.B.K. (Plantaginaceae), *Distichia muscoides* Nees et. Megen (Juncaceae), and the peat in which *Stylites* and the two other plant species were growing, were collected in December, 1982 in the vicinitiy of Lago de Junin (4,135 m elevation) in the Peruvian Andes as previously reported (Keeley et al. 1984). The plants grew within 10 m of one another. Peat samples were collected about 500 cm below the peat surface. For radiocarbon analysis, samples were treated with hydrochloric acid to remove carbonates, then with sodium hydroxide to remove humic substances, combusted, and assayed in a CO_2 proportional counter for a minimum of 50 h. For stable isotope analysis, plant material was dried at 50° C and ground in a Wiley mill. Cellulose was extracted and nitrated as described previously (DeNiro 1981). Cellulose oxygen isotope ratios and hydrogen and carbon isotope ratios of cellulose nitrate were determined as in previous studies (Sternberg et al. 1984a, b).

Isotope ratios are expressed as

$$\delta = \left[\frac{R_{SAMPLE}}{R_{STANDARD}} - 1 \right] 1{,}000\ (‰)$$

where R represents $^{14}C/(^{12}C + ^{13}C)$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, or D/H. The standards are the NBS oxalic acid standard for $\delta^{14}C$ values, standard mean ocean water (SMOW) for $\delta^{18}O$ and δD values, and the Peedee belemnite carbonate (PDB) for $\delta^{13}C$ values. The precision of isotopic analysis was $\pm 20‰$ for $\delta^{14}C$ values, $\pm 2‰$ for δD values, $\pm 0.5‰$ for $\delta^{18}O$ values and $\pm 0.2‰$ for $\delta^{13}C$ values.

residual water in the leaf enriched in $H_2{}^{18}O$ (Dongman et al. 1974). Thus, we expected that the $^{18}O/^{16}O$ ratios of leaf cellulose from the stomate-bearing plants, which transpire, would be higher than that of *Stylites*, which does not contain stomates and does not transpire. Results of oxygen isotope analysis (Table 1), however, show only a small difference in the expected direction between stomatal and non-stomatal species. A possible explanation for this observation follows. During the wet period in this environment, growing plants are exposed to high relative humidity. During this time transpiration would be minimal, so that leaf water and consequently cellulose of the transpiring species would not be substantially enriched in ^{18}O relative to *Stylites*. (In an analogous situation, DeNiro and Epstein (1981) observed that transpiring floating leaves of *Nymphoides aquaticum*, which are growing in a high humidity situation, have cellulose $\delta^{18}O$ values essentially identical to those of submerged leaves from the same plant, which do not transpire.) During the dry periods, transpiring plants may be dormant, so that the oxygen-18 enrichment of leaf water due to transpiration would not be recorded in the cellulose.

Conclusions

Taken together, our isotope abundance measurements indicate that *Stylites andicola* is taking up CO_2 from decomposing peat through its roots and that this CO_2 is processed via the CAM pathway.

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Results and discussion

The $\delta^{14}C$ value of the peat was 36‰ above the value of the NBS oxalic acid standard used in radiocarbon laboratories (Table 1). Thus, the peat we sampled was formed at some time in the mid-1950s, just at the beginning of the period when the atmosphere became heavily contaminated with carbon-14 from nuclear bomb testing. In fact, the carbon-14 content of the atmosphere in 1963/64 virtually doubled (i.e. $\delta^{14}C$ values approaching +1,000‰) when compared with the oxalic acid standard (Berger, Fergusson and Libby 1965; Nydal, Corseth and Gullicksen 1979). *Stylites*, like any other plant, cannot be older than its substrate, so that the formation of its biomass began at the earliest in the mid-1950s. Of course, incorporation of carbon into its biomass continued until the plant was collected in December, 1982. Table 1 shows $\delta^{14}C$ values for several tree rings which grew between 1960 and 1970 as reported by Cain (1979). Large amounts of bomb-produced carbon-14 were incorporated into the tree biomass. Others have measured the same increase in ^{14}C for other trees and different localities (Wilson 1961; Cain and Suess 1976). Using the relationship between $\delta^{14}C$ value versus year of ring formation reported by Cain (1979), we calculate that any plant growing continuously from 1955 to 1982, fixing only atmospheric carbon dioxide, should have a $\delta^{14}C$ value of about +370. Any plant which started to grow after 1955, continued to grow until 1982, and fixed only atmospheric CO_2 would not have not have a $\delta^{14}C$ value lower than +235, since the $\delta^{14}C$ values of the atmosphere between 1955 and 1982 were, for the most part, higher than that observed for 1982. The $\delta^{14}C$ value of *Stylites* was only +142‰ relative to the oxalic acid standard (Table 1), much less than what would be observed for a plant fixing atmospheric carbon dioxide since 1955, or any time after 1955, until 1982. We thus conclude that *Stylites* must be obtaining a large part of its carbon by fixing carbon dioxide derived from the ^{14}C-depleted decomposing peat. Further, since atmospheric mixing would quickly affect the radiocarbon content of ambient CO_2, the low level of radiocarbon can only be associated with the soil atmosphere.

The carbon isotope ratios for the three plants we analyzed (Table 1) were in the range of values observed for C_3 plants, (e.g. Sternberg and DeNiro 1983) *P. rigida* and *D. muscoides* have their leaves close to the ground. However, it is unlikely that they are refixing respired CO_2 since the environment where they were collected was relatively open, with air turbulence, and this allows for rapid intermixing of peat respired CO_2 with the atmospheric CO_2. Although Keeley et al. (1984) report CAM in *Stylites*, our stable carbon isotope analysis (Table 1) shows no meaningful differences in $\delta^{13}C$ values between *Stylites* and the two non-CAM species. The stable carbon isotope ratio of *Stylites* is difficult to interpret because there may be several different isotopic sources of carbon dioxide for *Stylites* (Keeley et al. 1984). The problem of determining photosynthetic mode with $\delta^{13}C$ values for *Stylites* is akin to that encountered with submerged aquatic plants, which have several isotopically variable sources of CO_2 and variable diffusional resistances not encountered in terrestrial plants (Keeley and Busch 1984). Recently, Ziegler et al. (1976), Sternberg and DeNiro (1983) and Sternberg et al. (1984a, b) have demonstrated that hydrogen isotope ratios of cellulose nitrate in terrestrial as well as submerged aquatic plants are also indicative of photosynthetic mode. For example, Sternberg et al. (1984a) noted that submerged aquatic CAM plants are enriched in deuterium relative to non-CAM plants, while, for the same plants, no difference was observed in carbon isotope ratios. The δD value of cellulose nitrate from *Stylites* was 36‰ and 56‰ higher than those for the two C_3 plants growing nearby (Table 1). The elevated δD value of *Stylites* is thus consistent with the report of its having CAM. We note here that the elevated δD value in *Stylites* relative to the two other species cannot be due to enrichment of plant water in deuterium, caused by transpiration (Wershaw et al. 1966), since *Stylites* has no stomates and transpiration is negligible (Keeley et al. 1984). Further, the similarity of the oxygen isotope ratios of cellulose from the three species of plants (Table 1) indicates that there were no substantial differences in leaf water $\delta^{18}O$ values (and hence δD values) between *Stylites* and the two non-CAM species (Sternberg and DeNiro 1983). The elevated δD values in *Stylites* must be due to fractionations occurring during biochemical reactions, as has been concluded for other CAM plants (Sternberg and DeNiro 1983; Sternberg et al. 1984a).

It has been shown previously that the $\delta^{18}O$ value of plant cellulose is determined primarily by the oxygen isotope ratio of the water at the site of cellulose synthesis (Epstein et al. 1977; DeNiro and Epstein 1981). Thus $\delta^{18}O$ values of leaf cellulose are determined by $\delta^{18}O$ values of leaf water (DeNiro and Epstein 1979). The oxygen isotope ratio of leaf water in terrestrial plants is a function of two factors: the isotope ratio of groundwater available for plant growth and the amount of transpiration a leaf undergoes (Dongman et al. 1974). Leaves which are undergoing transpiration have water with higher $\delta^{18}O$ values than leaves which are not. This is because $H_2{}^{16}O$, having a higher vapor pressure than $H_2{}^{18}O$, evaporates preferentially, leaving the

Table 1. Isotope abundances in atmospheric CO_2; growth rings in a spruce from Nestuca Bay, Oregon; and peat, *Stylites andicola*, *Plantago rigida*, and *Distichia muscoides*, all from Lago de Junin, Peru. The peat and the plants from Peru were collected in December, 1982

	δ^{14} (‰)	$\delta^{18}O$ (‰)	$\delta^{13}C$ (‰)	δD (‰)
Atmospheric CO_2 (December, 1982)	~ +235[a]		~ − 7.0[b]	
Peat	+ 36[c]		−26.6[d]	
Growth rings from Spruce				
1960	+250[e]			
1965	+900[e]			
1970	+470[e]			
Stylites andicola	+142[c]	+18.5[f]	−22.5[g]	− 47[g]
Plantago rigida		+19.6[f]	−24.5[g]	− 83[g]
Distichia muscoides		+19.1[f]	−22.2[g]	−103[g]

[a] Berger et al. (1985)
[b] Keeling et al. (1979)
[c] Total organic matter
[d] Total organic matter, from Keeley et al. (1984)
[e] Total organic matter, from Cain (1979)
[f] Cellulose
[g] Cellulose nitrate